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Foodborne pathogens

Hazards, risk analysis and control

Edited by
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Part I

Risk assessment and management in the food chain

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1

Introduction

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1.1 Trends in foodborne disease

Foodborne disease continues to be a common and serious threat to public health all over the world and is a major cause of morbidity. Both industrialised and developing countries suffer large numbers of illnesses and the incidence, on a global basis, appears to be increasing. Most foodborne illnesses are mild, and are associated with acute gastrointestinal symptoms such as diarrhoea and vomiting. Sometimes foodborne disease is much more serious and is life-threatening, particularly in children in developing countries, and infection can also be followed by chronic sequelae or disability. In many countries where information on foodborne diseases is collected, the total number of cases has been increasing over the past 20–30 years (Käferstein *et al*., 1997). For example, in the UK, figures have risen from just under 10 000 cases recorded in 1977, to more than 90 000 cases in 1998. Most European countries have reported a doubling of salmonellosis cases between 1985 and 1992 (Anon, 1995b). In the UK, for instance, there were 12 846 infections (23 per 100 000) in 1981 compared with 33 600 (58 per 100 000) in 1994 (Anon, 1995a). In North America, there has been a notable increase in infections caused by *Salmonella* Enteritidis since the late 1980s (St Louis *et al*., 1988; Levy *et al*., 1996). Some of the increases recorded are undoubtedly due to improved systems for information collection and reporting, better diagnoses and a greater awareness of food safety, but these changes do not explain the general increases observed.

In recent years, the increased awareness of food safety, changes in regulatory and educational measures and changes in practice in food production have led to decreases in incidence of particular foodborne diseases in some regions. For example, in the year 2000, salmonellosis in the UK was at its lowest level

since 1985, with a 54% decrease in the number of reported cases compared with the previous year. A decrease in the number of cases of salmonellosis has also been observed recently in the US (Olsen *et al*., 2001). These particular decreases in salmonellosis have been attributed to vaccination programmes for poultry and other changes that have been implemented in these regions. Decreases in the number of cases of listeriosis have also been observed in the UK and the US in recent years. However, for other pathogens such as campylobacters, numbers of associated cases continue to rise at a steady rate in many countries.

1.2 Incidence of foodborne disease

Accurate estimates of the yearly incidence of foodborne disease are difficult and sometimes impossible, depending on the reporting systems in different countries. Foodborne disease statistics in some European countries and the Americas, where reporting systems are better than some other regions, are dominated by cases of salmonellosis and campylobacteriosis. In other regions, however, foodborne disease statistics tend to rely on outbreak information only, and in some cases, other organisms are identified as leading causes of illness. For example, in Taiwan, 74% of outbreaks in 1994 were caused by bacterial pathogens of which *Vibrio parahaemolyticus* (56.7%), *Staphylococcus aureus* (20.3%), *Bacillus cereus* (14.9%) and salmonellas (8.1%) were the major agents identified (Pan *et al*., 1996). In a study of diarrhoeal disease in south eastern China between 1986–87, the overall incidence of diarrhoeal illness was 730 episodes per 1000 population (Kangchuan *et al*., 1991). The most commonly isolated organisms in order of frequency of occurrence were enterotoxigenic *Escherichia coli*, *Shigella* species, enteropathogenic *E. coli*, *Campylobacter jejuni*, vibrios and enteroinvasive *E. coli*. These organisms and *Entamoeba histolytica* are typical causes of diarrhoea in developing countries (DuPont, 1995). It is important to remember that foods will only be one of a number of possible sources of infection in these cases, but the lack of good epidemiological data in these regions leads to the role of food being poorly acknowledged (Käferstein *et al*., 1997).

In the US, it has been estimated that foodborne diseases cause approximately 76 million illnesses, 325 000 hospitalisations and 5000 deaths each year, with known pathogens accounting for 14 million illnesses, 60 000 hospitalisations and 1800 deaths (Mead *et al*., 1999). In this study, estimates were made using data from a number of sources, including outbreak-related cases and passive and active surveillance systems. The organisms identified as causing the largest number of foodborne-related cases of illness were Norwalk-like viruses, followed by campylobacters, salmonellas, *Clostridium perfringens*, *Giardia lamblia*, staphylococci, *Escherichia coli* and *Toxoplasma gondii*, respectively. Incidence of foodborne disease in different countries is often difficult to compare because of different reporting systems.

1.3 Foodborne disease surveillance

Quantifying numbers of reported foodborne illnesses, identification of emerging pathogens and elements that increase the risk of disease can all be determined with surveillance systems. These systems include laboratory based reporting of specific pathogens, illnesses reported by physicians, outbreak investigations and active surveillance. Information from such systems is used to determine the priorities for food safety actions, including development of new or modified policies and procedures, monitoring efficacy of programmes, identifying new hazards, educating and training those involved in food manufacturing, handling and preparation, including consumers. Each surveillance system has its drawbacks and strengths and focuses on different aspects of foodborne disease investigation. Many systems are not able to determine the true incidence of foodborne illness, because of the reporting systems used and various other reasons, such as underreporting due to methodologies unable to determine the actual causes of outbreaks. It is estimated that for every case of salmonellosis reported to the Centers for Disease Control and Prevention in the US, between 20 and 100 cases go unreported (Tauxe, 1991). Experts on foodborne disease estimate that most cases of foodborne illness in the US originate from foods prepared in the home (IFT, 1995). Surveys of consumer practices and perceptions (Altekruse *et al*., 1995; Fein *et al*., 1995) tend to demonstrate that awareness of the major causes of foodborne illness such as salmonellas and campylobacters is extremely poor and emphasise the need for and importance of effective education and training.

Different surveillance systems are reviewed in a series of studies published by Guzewich, Bryan and Todd (Guzewich *et al*., 1997; Bryan *et al*., 1997a, b; Todd *et al*., 1997). It is critical that surveillance systems share common information across national boundaries and where possible exchange information on outbreaks of foodborne disease utilising the power and capability of modern telecommunication facilities. An example of such a system is Enter-net, which is used to provide early recognition and subsequent comprehensive investigation of outbreaks of salmonellosis and vero cytotoxin-producing *E. coli* O157 in Europe (Fisher and Gill, 2001). International networks such as Enter-Net are important tools considering the large-scale production of food and globalisation of food trade. Development in nucleic acid-based techniques has had a major impact on disease surveillance, enabling rapid pathogen detection and characterisation. This has resulted in 'sporadic cases' being linked, sometimes over large geographical areas, and identified as outbreaks, with a common source of infection. The 'new outbreak scenario' resulting from low-level contamination of widely distributed food products or ingredients is described in detail by Tauxe (1997) and attributed to changes in the way food is produced and distributed.

1.4 Emerging foodborne disease and changing patterns in epidemiology

In recent years, the epidemiology of foodborne diseases has been changing as new pathogens have emerged. 'Emerging diseases' are described as those that have increased in prevalence in recent decades or are likely to do so in the near future (Altekruse and Swerdlow, 1996), so it is not necessary for an emerging pathogen to be newly evolved. Foodborne diseases that are regarded as emerging include illness caused by enterohaemorrhagic *Escherichia coli* (EHEC or vero cytotoxigenic, VTEC particularly serovar O157:H7), *Campylobacter jejuni*, *Salmonella* Typhimurium Definitive Type (DT) 104. In some cases, disease has been associated with food vehicles only relatively recently. Examples of these pathogens include *Listeria monocytogenes*, *Cryptosporidium parvum* and *Cyclospora cayetanensis.* Many of these foodborne pathogens have a non-human animal reservoir, and are termed zoonoses, but they do not necessarily cause disease in the animal. Previously, animal or carcass inspection was used as a method of preventing zoonotic diseases being transferred through food, but this can no longer be relied upon.

Both *E. coli* O157:H7 and *S.* Typhimurium DT 104 are found in cattle and are examples of relatively newly evolved pathogens. According to Whittam (1996), *E. coli* O157:H7 probably evolved from an enteropathogenic O55:H7 ancestor through horizontal gene transfer and recombination, and a stepwise evolutionary model has been proposed by Feng *et al*. (1998). When outbreaks of vero cytotoxigenic *E. coli* associated illness were first identified, many were associated with undercooked beef, such as burgers. More recently, the list of food vehicles associated with EHEC is becoming longer and longer, and an increasing number of infections are being linked to fresh produce such as vegetables and fruit. Enterohaemorrhagic *E. coli* O157:H7 and other EHEC have changed the 'rule book' in a number of ways, primarily because they are able to cause illness in relatively low numbers and infection can result in very serious illness or even death. Some foods that were traditionally regarded as 'safe', such as apple juice and fermented meats, have caused haemorrhagic colitis and more serious illness associated with EHEC. Growth of the organism in foods is not necessary to cause infection, so any contamination, even at very low levels, may have serious consequences. Worryingly, a new sub-clone of a second group of EHEC (primarily comprising O26:H11 and O11:H8 serotypes) has emerged in Europe and this clone shares the same prominent virulence factors of O157:H7 and is common in the bovine reservoir (Donnenberg and Whittam, 2001). These organisms, and others like them, may well emerge as important foodborne pathogens in the future.

Genetic promiscuity is facilitated by a range of genetic elements including plasmids, transposons, conjugative transposons and bacterophages. The ability to evolve through horizontal gene transfer and acquire 'foreign' DNA, has resulted in novel phenotypes and genotypes emerging, and this is causing confusion among some microbiologists who prefer to group organisms according to one

or two characteristics. For example, there are six pathotypes of diarrhoeagenic *E. coli*, characterised usually on the basis of disease caused and also on presence of mainly non-overlapping virulence factors. There is, however, an increasing number of studies describing *E. coli* isolates associated with diarrhoeal disease that possess previously unreported combinations of virulence factors. In some cases, virulence factors are encoded on large DNA regions termed pathogenicity islands, and these are shared amongst different pathogenic organisms, contributing to microbial evolution (Hacker *et al*., 1997). Some genetic elements, such as bacteriophages, as well as being important vectors for transmission of virulence genes, also serve as important precursors for the expression of bacterial virulence, as shown in *Vibrio cholerae* (Boyd *et al*., 2001). It has also been shown that some members of the Enterobacteriaceae carry defects in the *mutS* gene, which directs DNA-repair processes (LeClerc *et al.*, 1996). The formation of deletions also plays a major role in so-called 'genome plasticity' and can contribute to development of organisms with improved functionality (Stragier *et al*., 1989). Fortunately, new techniques determining DNA sequences specific to particular regions should allow investigation of evolutionary mechanisms that allow development of new pathogens, and will facilitate identification and characterisation of these organisms. Such techniques have recently been used to show that old lineages of *E. coli* have acquired the same virulence factors in parallel, indicating that natural selection has favoured an ordered acquisition of genes and the progressive build-up of molecular mechanisms that increase virulence (Reid *et al*., 2000).

Adaptation to particular environments appears to have played a part in the recent emergence of *S.* Typhimurium DT 104. A number of researchers have proposed that the use of antibiotics in human health, agriculture and aquaculture has resulted in the selection of *Salmonella* strains, notably DT 104, that are resistant to multiple antibiotics. There is increasing evidence that 'stresses' imposed by an organism's environment can modulate and enhance virulence, providing there is a potential driving force promoting adaptive mutations that may serve to select strains that are even more virulent (Archer, 1996). Factors associated with demographics, consumer trends and changes in food production have also been put forward as possible contributors to the emergence of new pathogens that have appeared in different areas of the globe simultaneously. Many of these shifts have magnified the potential impact of a single source of infection.

1.4.1 Demographics

An increasing world population places increased pressure on global food production and the question 'will supply meet expected demand?', especially in developing countries, cannot be answered with any certainty (Doos and Shaw, 1999). Fuelled by urbanisation and higher incomes, there are likely to be changes in the pattern of food consumption. For example, there is likely to be a major increase in consumption of meat in the developing world and this will place more pressure on animal production systems (van der Zijpp, 1999).

Demographic changes occurring in industrialised nations have resulted in an increase in the proportion of the population with heightened susceptibility to severe foodborne infections. Growing segments of the population have immune impairment as a consequence of infection with HIV, ageing or underlying chronic disease (Slutsker *et al*., 1998).

1.4.2 Consumer trends

There are several consumer trends that may have an impact on foodborne disease. There is a trend towards 'more natural' and 'fresh' food with less preservation and processing. This has manifested itself in increasing consumption of fresh fruits and vegetables and the number of outbreaks associated with these types of foods has also increased. Anecdotes about the health properties of raw foods may also be interfering with health messages about the risks associated with eating some raw or lightly cooked foods (Slutsker *et al*., 1998).

Another consumer trend is the increase in the percentage of spending on food eaten away from home. This places greater importance on the safe operation of catering establishments for the control of foodborne disease. By the 1990s, for example, foodborne outbreaks that occurred outside the home accounted for almost 80% of all reported outbreaks in the United States (Slutsker *et al*., 1998). It is also suggested that this situation is compounded by a decrease in home food hygiene instruction, particularly in light of other important health concerns tackled in schools, e.g. substance abuse, HIV infection and obesity (Slutsker *et al*., 1998).

International travel has increased dramatically during the last century. Travellers may become infected with foodborne pathogens that are uncommon in their nation of residence and may transmit the pathogen further when they return home. International travel is also one of the drivers for an increasing demand for international foods in local markets, and this in turn fuels the international trade in foods.

Immigration has also contributed to the epidemiology of foodborne disease, as some reports of foodborne illnesses involve transmission through foods consumed primarily by immigrant groups, an example of this being the increase in parasite infections in the United States (Slutsker *et al*., 1998).

1.4.3 Trends in food production

There is a trend towards global sourcing of raw materials and processing in large, centralised facilities and distribution of product over large geographical areas using longer and more complex supply chains. As a result, there are now many more potential points at which pathogens, including those that might otherwise not have been considered, can be introduced into the supply chain and spread within a country and across regional and national borders. However, on the positive side this provides large companies with the opportunity to

significantly reduce foodborne disease globally by focusing their resources on identifying hazards, assessing risks and implementing effective preventative and control measures.

In contrast to this centralisation of food processing there is a trend for increasing numbers of localised catering and food preparation operations, driven by consumer demand for out-of-home food consumption. This leads to greater challenges in order to provide a unified standard of food safety in this food sector.

As the global demand for food grows there will be an increasing need for intensification of agriculture and this will have dramatic impacts on the diversity, composition and functioning of the world's ecosystems (Tilman, 1999). The likelihood for the proliferation of human pathogens in more intensive and centralised forms of animal and crop production, and potential contamination of water supplies, will be greater and will require effective management.

1.5 Control of foodborne disease

The complexity of the global food market means that the control of foodborne disease is a joint responsibility and requires action at all levels from the individual to international groups, and at all parts of the supply chain from the farm to the fast-food restaurant. The tools used and approaches taken to ensure control require different emphasis, depending on a number of factors such as where food materials have come from, how they have been processed and handled and how they are stored. The risk of foodborne illness can be reduced by using existing technologies, such as pasteurisation and refrigeration, and by adopting some simple precautions such as avoiding cross contamination by separation of raw and cooked foods and employing good hygienic practices.

Although the onus is on prevention of foodborne disease, valuable lessons can be learned by reviewing food poisoning statistics and incidents. This in turn can provide a focus for effective control measures to help reduce food poisoning (Bryan, 1988). Ranking the factors that contribute to outbreaks of foodborne diseases can indicate trends and also differences in the different foodborne pathogens reflecting their association with raw material and physiological properties.

For many foodborne diseases, multiple choices for prevention are available, and the best answer may be to apply several steps simultaneously, for example measures both to eliminate organisms during the food process and to reduce the likelihood of the organisms being present in the first place. A better understanding of how pathogens persist in animal reservoirs (such as farm herds) is also critical to successful long-term prevention. In the past, the central challenge of foodborne disease lay in preventing contamination of human food with sewage of animal manure. In the future, prevention of foodborne disease will increasingly depend on controlling contamination of feed and water consumed by the animals themselves (Tauxe, 1997).

1.6 Rationale for this book

Ultimately, the control of foodborne pathogens requires the understanding of a number of factors including the knowledge of possible hazards, their likely occurrence in different products, their physiological properties, the risks they pose to the consumer and the availability and effectiveness of different preventative/intervention measures. This aim of this book is to help provide this understanding.

While there are good reference texts for the microbiologist on foodborne pathogens, there are less that relate current research to practical strategies for hazard identification, risk assessment and control. This text takes this more applied approach. It is designed both for the microbiologist and the non-specialist, particularly those whose role involves the safety of food processing operations.

Part 1 looks at general techniques in assessing and managing microbiological hazards. After a review of analytical methods and their application, there are chapters on modelling pathogen behaviour and carrying out risk assessments as the essential foundation for effective food safety management. The following chapters then look at good management practice at key stages in the supply chain, starting with farm production and ending with the consumer. In between there are chapters on hygienic plant design and sanitation, and safe process design and operation. These provide the foundation for what makes for effective HACCP systems implementation.

This discussion of pathogen control then provides a context for Part 2 which looks at what this means in practice for major pathogens such as pathogenic *E. coli*, *Salmonella*, *Listeria* and *Campylobacter*. Each chapter discusses pathogen characteristics, detection methods and control procedures. Part 3 then looks at non-bacterial hazards such as toxigenic fungi, viruses and parasites, as well as emerging potential hazards such as *Mycobacterium paratuberculosis* and the increasingly important area of chronic disease.

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2

Detecting pathogens in food

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2.1 Introduction

The detection and enumeration of microorganisms either in foods or on food contact surfaces form an integral part of any quality control or quality assurance plan. Microbiological tests done on foods can be divided into two types: (a) quantitative or enumerative, in which a group of microorganisms in the sample is counted and the result expressed as the number of the organisms present per unit weight of sample; or (b) qualitative or presence/absence, in which the requirement is simply to detect whether a particular organism is present or absent in a known weight of sample.

The basis of methods used for the testing of microorganisms in foods is very well established, and relies on the incorporation of a food sample into a nutrient medium in which microorganisms can replicate thus resulting in a visual indication of growth. Such methods are simple, adaptable, convenient and generally inexpensive. However, they have two drawbacks: firstly, the tests rely on the growth of organisms in media, which can take many days and result in a long test elapse time; and secondly, the methods are manually oriented and are thus labour intensive.

Over recent years, there has been considerable research into rapid and automated microbiological methods. The aim of this work has been to reduce the test elapse time by using methods other than growth to detect and/or count microorganisms and to decrease the level of manual input into tests by automating methods as much as possible. These rapid and automated methods have gained some acceptance and application within the food industry.

Microbiology methods are fundamental to Quality Control (QC), but with the inexorable move towards a Quality Assurance (QA) approach to food safety they

Table 2.1 A comparison of Quality Assurance and Quality Control

Factor	QA	QC
Approach	Preventative	Reactive
Reliance for delivering safety	Central standards and processes	Measurement
Focus	Consumer	Legal and commercial issues

Source: From Kilsby (2001).

have been the brunt of much denigrating. However, microbiological testing, even with all its limitations, is now being seen as an essential tool as part of this assurance, albeit with a shift in application and emphasis.

This chapter considers the application of microbiological methods in the identification of hazards, the assessment of risk and hazard control, as well as providing a comprehensive overview of the principles behind both conventional and rapid and automated methods.

2.2 A comparison of Quality Control and Quality Assurance

QC and QA are two different approaches to deliver safety; both systems share tools, but the emphasis is very different (Table 2.1). Both approaches are legitimate but they need totally different organisations, structures, skills, resource and ways of working (Kilsby, 2001).

QC is a reactive approach influenced by the pressures in the external world. In a QC organisation the emphasis is on measurement, which needs to be robust and statistically relevant, and the focus is on legal and commercial issues. In contrast, QA is a preventative approach driven by the company's internal standards. The emphasis is on operational procedures, which must be robust and regularly reviewed, and the focus is on the consumer.

2.3 Use of microbiology methods in a Quality Control system

In a QC system, measurement is relied upon to deliver quality and safety. This means that microbiological methods must be robust and the results that are produced must be statistically relevant. This, in turn, places great importance on the use of sampling plans, which are covered briefly later. Raw materials and finished products have to be tested on a regular basis, often according to the risk they pose. For raw materials the onus is on the buyer to analyse samples and

Table 2.2 Factors that may influence the choice of microbiological method

Factor	Considerations
Performance	Sensitivity, specificity, accuracy, precision, reproducibility, repeatability
Time	Total test time (presumptive/confirmed results), 'hands-on' time, time constraints
Ease of use	Complexity, automation, robustness, training requirement, sample throughput, result interpretation
Standardisation	Validation, accreditation, international acceptance
Cost	Cost/test, capital outlay/equipment running cost, labour costs

reliance is place on positive release rather than supplier assurance for compliance with standards.

Microbiology methods can differ widely in their comparative advantages and disadvantages. These relative benefits and limitations may influence the choice of microbiological method for a particular task (Table 2.2). For example, for products with a short shelf-life, rapidity of test result may be an important factor. However, when maximising the volume of material sampled is crucial, sample throughput and low cost/test may be higher on the priority list. In recent years a plethora of rapid test kits has become available that, to a greater or lesser extent, have helped to expedite, simplify, miniaturise and automate methodology. The drive for standardisation, validation and international acceptance of methods, with regard to good laboratory practice and accreditation, means that this is often a constraint on method selection.

There are several problems associated with relying on testing for product safety assurance (van Schothorst and Jongeneel, 1994). In order to apply any statistical interpretation to the results, the contaminant should be distributed homogeneously through the batch. As microbiological hazards are usually heterogeneously distributed this means that there is often a major discrepancy between the microbiological status of the batch and the microbial test results (Anon, 1986). Even if the microbial distribution is homogeneous, it still may be prohibitive to test a sufficient number of sample units for all the relevant hazards to obtain meaningful information. Microbiological testing detects only the effects and neither identifies nor controls the causes.

2.4 Sampling

Although this chapter deals with the methodologies employed to test foods, it is important for the microbiologist to consider sampling. No matter how good a method is, if the sample has not been taken correctly and is not representative of the batch of food that it has been taken from, then the test result is meaningless.

It is useful to devise a sampling plan in which results are interpreted from a number of analyses, rather than a single result. It is now common for microbiologists to use two or three class sampling plans, in which the number of individual samples to be tested from one batch are specified, together with the microbiological limits that apply. These types of sampling plan are fully described in Anon. (1986).

Once a sampling plan has been devised then a representative portion must be taken for analysis. In order to do this the microbiologist must understand the food product and its microbiology in some detail. Many chilled products will not be homogeneous mixtures but will be made up of layers or sections: a good example would be a prepared sandwich. It must be decided if the microbiological result is needed for the whole sandwich (i.e. bread and filling), or just the bread, or just the filling; indeed in some cases one part of a mixed filling may need to be tested, when this has been decided then the sample for analyses can be taken, using the appropriate aseptic technique and sterile sampling implements (Kyriakides *et al*., 1996). The sampling procedure having been developed, the microbiologist will have confidence that samples taken are representative of the foods being tested and test methods can be used with confidence.

2.5 Use of microbiology methods in a Quality Assurance system

Owing to the difficulty of assuring microbiological safety through testing alone there is now widespread adoption of the quality assurance approach using the Hazard Analysis Critical Control Point (HACCP) system. Successful implementation of a fully validated HACCP study means that the supposed reliance on microbiological testing, with all its sampling limitations, is relinquished and this should enable a significant reduction in the volume of testing. Some in the food industry went so far as to surmise that microbiological testing would become obsolete (Struijk, 1996). In reality, however, microbiology testing has continued albeit with a shift in application and emphasis.

Microbiological methods are needed within a HACCP-based programme for risk assessment, the control of raw materials, the control of the process line and the line environment, and for validation and verification of the HACCP program (de Boer and Beumer, 1999). It has also been pointed out that although in spite of meticulous adherence to HACCP-based good practices occasional human, instrumental or operational hiatuses can and will occur (Struijk, 1996). Microbiological methods are still required for trouble shooting and forensic investigation in order to identify the cause of the contamination and rectify it.

2.5.1 Hazard analysis

The HACCP process comprises seven principles (see Table 8.1), which are further broken down into series of stages. The first principle is to conduct a hazard analy-

sis and the use of microbiological tests may be required by the HACCP team to gather relevant data. This may involve determining the incidence of pathogens or indicator organisms in raw materials, the efficacy of equipment cleaning procedures, the presence of pathogens (e.g. *Listeria*) in the environment, and microbial loads in foods and on equipment (Stier, 1993).

The use of molecular characterisation techniques has further increased the microbiologist's armoury and epidemiological tracking of strains can provide a more in-depth knowledge of the food process. This may enable the determination of sites of cross-contamination, or sites where strains appear and disappear, thus pinpointing the positions contributing to the final flora of the product, permitting more precise identification of critical control points (CCPs) (Dodd, 1994).

2.5.2 Monitoring CCPs

The HACCP process requires the establishment of systems to monitor all identified CCPs. Most microbiology methods are too slow for monitoring of CCPs, but a notable exception to this is the application of ATP bioluminescence for checking the cleaning of equipment. Results from these methods can be obtained in only a few minutes, which allows sufficient time for equipment to be recleaned before production begins if they are found to be contaminated. Although care in the application of these methods is required to prevent being lulled into a false sense of security (Stier, 1993), the methodology can have a beneficial impact in demonstrating to staff responsible for cleaning the importance of their role.

In the context of HACCP, microbiological specifications and criteria play a role in the monitoring of CCPs in food processing and distribution (Hall, 1994) and both conventional and rapid methods have a role to play in the checking of raw materials and monitoring of supplies. Receipt of raw materials is often identified as a CCP, and intake testing may be identified as one of the preventative measures for its control. However, if this is done it is often in the context of verifying the supplier's own microbiology assurance procedures.

2.5.3 Validation of HACCP

Validation of the technical accuracy of the hazard analysis and effectiveness of the preventative measures is important before the HACCP study is finalised and implemented. Examples where microbiology methods may be used for validation include pre-operation checks of cleaning and sanitising, screening of sensitive raw materials, challenge testing and monitoring of critical sites for microbiological build-up during processing (Hall, 1994). For safe product design a defined reduction (e.g. 5 or $6 \log_{10}$) of target microorganisms may be required, delivered either in one CCP or over a series of process steps. Quantitative data may be required to demonstrate that the process can deliver the defined level of microbial kill or that the end-product meets the specification for safety and/or stability.

Microbial methods, particularly molecular characterisation ones, can be useful in answering questions that may arise as part of the HACCP validation exercise.

For example, if a hazardous organism appears in a product at a point in the production line beyond the CCP designed to control it, does this mean failure of the CCP, or does it indicate post-process contamination (Dodd, 1994).

2.5.4 HACCP verification and review

Part of the HACCP process involves establishing procedures for verification to confirm that the HACCP system is working effectively. Once a HACCP plan is operational, finished product testing can be one of the means by which its successful implementation is verified. In addition, microbiological data can provide valuable sources of information for trend analysis and statistical process control. In theory, a well-functioning HACCP plan should only require occasional testing as part of the verification process. However, sometimes local legislation, customer requirements or the company's own standards demand a higher level of testing (Stier, 1993).

HACCP is a living system and as such new hazards may need to be considered and risk assessed. In addition, changes or proposed changes to a process may require that microbiological data is generated to ensure that sufficient control is maintained.

2.5.5 Microbiological specifications and criteria

Regardless of whether HACCP is used, microbiological specifications and criteria are still applied to foods. They can serve as a determinant of the acceptability of an ingredient, finished product or process with regard to microbiological safety and/or microbiological quality. In practice, microbiological specifications typically are used both as an internal tool by the manufacturer to judge acceptability against pre-determined standards and as an external measure against customer or governmental standards (Hall, 1994).

Increasing international trade and the potential for disputes places further emphasis on the need for agreed and reliable methodologies. This checking of conformance to specifications may mean that raw materials and finished products are held pending the results of microbiological tests. In these cases, faster techniques can help to determine the fate of products more quickly.

2.5.6 Risk assessment

One important area within the food industry where methodology is raising its profile is quantitative risk assessment. Risk assessment is very much tied in with microbiological data and microbiological examinations of samples of ingredients and end-products may be necessary (de Boer and Beumer, 1999). Risk assessment methods can identify gaps in our knowledge that are crucial to providing better estimates of risk and this may in fact lead to an increase in the level of microbiological testing. Assessing the risk posed by a 'new' or 'emerging'

organism may also highlight deficiencies in current methodology requiring the need for method development.

2.6 Conventional microbiological techniques

As outlined in the introduction, conventional microbiological techniques are based on the established method of incorporating food samples into nutrient media and incubating for a period of time to allow the microorganisms to grow. The detection or counting method is then a simple visual assessment of growth. These methods are thus technically simple and relatively inexpensive, requiring no complex instrumentation. The methods are however very adaptable, allowing the enumeration of different groups of microorganisms.

Before testing, the food sample must be converted into a liquid form in order to allow mixing with the growth medium. This is usually done by accurately weighing the sample into a sterile container and adding a known volume of sterile diluent (the sample to diluent ratio is usually 1 : 10); this mixture is then homogenised using a homogeniser (e.g. stomacher or pulsifier) that breaks the sample apart, releasing any organisms into the diluent. The correct choice of diluent is important. If the organisms in the sample are stressed by incorrect pH or low osmotic strength, then they could be injured or killed, thus affecting the final result obtained from the microbiological test. The diluent must be well buffered at a pH suitable for the food being tested and be osmotically balanced. When testing some foods (e.g. dried products) which may contain highly stressed microorganisms, then a suitable recovery period may be required before the test commences, in order to ensure cells are not killed during the initial phase of the test procedure (Davis and Jones, 1997).

2.6.1 Conventional quantitative procedures

The enumeration of organisms in samples is generally done by using plate count, or most probable number (MPN) methods. The former are the most widely used, whilst the latter tend to be used only for certain organisms (e.g. *Escherichia coli*) or groups (e.g. coliforms).

Plate count method

The plate count method is based on the deposition of the sample, in or on an agar layer in a Petri dish. Individual organisms or small groups of organisms will occupy a discrete site in the agar, and on incubation will grow to form discrete colonies that are counted visually. Various types of agar media can be used in this form to enumerate different types of microorganisms. The use of a non-selective nutrient medium that is incubated at 30 °C aerobically will result in a total viable count or mesophilic aerobic count. By changing the conditions of incubation to anaerobic, a total anaerobe count will be obtained. Altering the incubation

temperature will result in changes in the type of organism capable of growth, thus showing some of the flexibility in the conventional agar approach. If there is a requirement to enumerate a specific type of organism from the sample, then in most cases the composition of the medium will need to be adjusted to allow only that particular organism to grow. There are three approaches used in media design that allow a specific medium to be produced: the elective, selective and differential procedures.

Elective procedures refer to the inclusion in the medium of reagents, or the use of growth conditions, that encourage the development of the target organisms, but do not inhibit the growth of other microorganisms. Such reagents may be sugars, amino acids or other growth factors. Selective procedures refer to the inclusion of reagents or the use of growth conditions that inhibit the development of non-target microorganisms. It should be noted that, in many cases, selective agents will also have a negative effect on the growth of the target microorganism, but this will be less great than the effect on non-target cells. Examples of selective procedures would be the inclusion of antibiotics in a medium or the use of anaerobic growth conditions. Finally, differential procedures allow organisms to be distinguished from each other by the reactions that their colonies cause in the medium. An example would be the inclusion of a pH indicator in a medium to differentiate acid-producing organisms. In most cases, media will utilise a multiple approach system, containing elective, selective and differential components in order to ensure that the user can identify and count the target organism.

The types of agar currently available are far too numerous to list. For details of these, the manuals of media manufacturing companies (e.g. Oxoid, LabM, Difco, Merck) should be consulted.

MPN method

The second enumerative procedure, the MPN method, allows the estimation of the number of viable organisms in a sample based on probability statistics. The estimate is obtained by preparing decimal (tenfold) dilutions of a sample, and transferring sub-samples of each dilution to (usually) three tubes of a broth medium. These tubes are incubated, and those that show any growth (turbidity) are recorded and compared to a standard table of results (Anon., 1986) that indicate the contamination level of the product.

As indicated earlier, this method is used only for particular types of test and tends to be more labour and materials intensive than plate count methods. In addition, the confidence limits are large even if many replicates are studied at each dilution level. Thus the method tends to be less accurate than plate counting methods but has the advantage of greater sensitivity.

2.6.2 Conventional qualitative procedures

Qualitative procedures are used when a count of the number of organisms in a sample is not required and only their presence or absence needs to be determined. Generally such methods are used to test for potentially pathogenic microorgan-

isms such as *Salmonella* spp., *Listeria* spp., *Yersinia* spp. and *Campylobacter* spp. The technique requires an accurately weighed sample (usually 25 g) to be homogenised in a primary enrichment broth and incubated for a stated time at a known temperature. In some cases, a sample of the primary enrichment may require transfer to a secondary enrichment broth and further incubation. The final enrichment is usually then streaked out onto a selective agar plate that allows the growth of the organisms under test. The long enrichment procedure is used because the sample may contain very low levels of the test organism in the presence of high numbers of background microorganisms. Also, in processed foods the target organisms themselves may be in an injured state. Thus the enrichment methods allow the resuscitation of injured cells followed by their selective growth in the presence of high numbers of competing organisms.

The organism under test is usually indistinguishable in a broth culture, so the broth must be streaked onto a selective/differential agar plate. The microorganisms can then be identified by their colonial appearance. The formation of colonies on the agar that are typical of the microorganism under test are described as presumptive colonies. In order to confirm that the colonies are composed of the test organism, further biochemical and serological tests are usually performed on pure cultures of the organism. This usually requires colonies from primary isolation plates being restreaked to ensure purity. The purified colonies are then tested biochemically by culturing in media that will indicate whether the organism produces particular enzymes or utilises certain sugars.

At present a number of companies market miniaturised biochemical test systems that allow rapid or automated biochemical tests to be quickly and easily set up by microbiologists. Serological tests are done on pure cultures of some isolated organisms, e.g. *Salmonella*, using commercially available antisera.

2.7 Rapid and automated methods

The general interest in alternative microbiological methods has been stimulated in part by the increased output of food production sites. This has resulted in the following:

1. Greater numbers of samples being stored prior to positive release – a reduction in analysis time would reduce storage and warehousing costs.
2. A greater sample throughput being required in laboratories – the only way that this can be achieved is by increased laboratory size and staff levels, or by using more rapid and automated methods.
3. A requirement for a longer shelf-life in the chilled foods sector – a reduction in analysis time could expedite product release thus increasing the shelf-life of the product.
4. The increased application of HACCP procedures – rapid methods can be used in HACCP verification procedures.

There are a number of different techniques referred to as rapid methods and most have little in common either with each other or with the conventional procedures that they replace. The methods can generally be divided into quantitative and qualitative tests, the former giving a measurement of the number of organisms in a sample, the latter indicating only presence or absence. Laboratories considering the use of rapid methods for routine testing must carefully consider their own requirements before purchasing such a system. Every new method will be unique, giving a slightly different result, in a different timescale with varying levels of automation and sample throughput. In addition, some methods may work poorly with certain types of food or may not be able to detect the specific organism or group that is required. All of these points must be considered before a method is adopted by a laboratory. It is also of importance to ensure that staff using new methods are aware of the principles of operation of the techniques and thus have the ability to troubleshoot if the method clearly shows erroneous results.

2.7.1 Electrical methods

The enumeration of microorganisms in solution can be achieved by one of two electrical methods, one measuring particle numbers and size, the other monitoring metabolic activity.

Particle counting

The counting and sizing of particles can be done with the 'Coulter' principle, using instruments such as the Coulter Counter (Coulter Electrics, Luton). The method is based on passing a current between two electrodes placed on either side of a small aperture. As particles or cells suspended in an electrolyte are drawn through the aperture they displace their own volume of electrolyte solution, causing a drop in d.c. conductance that is dependent on cell size. These changes in conductance are detected by the instrument and can be presented as a series of voltage pulses, the height of each pulse being proportional to the volume of the particle, and the number of pulses equivalent to the number of particles.

The technique has been used extensively in research laboratories for experiments that require the determination of cell sizes or distribution. It has found use in the area of clinical microbiology where screening for bacteria is required (Alexander *et al*., 1981). In food microbiology, however, little use has been made of the method. There are reports of the detection of cell numbers in milk (Dijkman *et al*., 1969) and yeast estimation in beer (MaCrae, 1964), but little other work has been published. Any use of particle counting for food microbiology would probably be restricted to non-viscous liquid samples or particle-free fluids, since very small amounts of sample debris could cause significant interference, and cause aperture blockage.

Metabolic activity

Stewart (1899) first reported the use of electrical measurement to monitor microbial growth. This author used conductivity measurements to monitor the putre-

faction of blood, and concluded that the electrical changes were caused by ions formed by the bacterial decomposition of blood constituents. After this initial report a number of workers examined the use of electrical measurement to monitor the growth of microorganisms. Most of the work was successful; however, the technique was not widely adopted until reliable instrumentation capable of monitoring the electrical changes in microbial cultures became available.

There are currently four instruments commercially available for the detection of organisms by electrical measurement. The Malthus System (IDG, Bury, UK) based on the work of Richards *et al*. (1978) monitors conductance changes occurring in growth media as does the Rabit System (Don Whitley Scientific, Yorkshire, UK), whilst the Bactometer (bioMeriéux, Basingstoke, UK), and the Batrac (SyLab, Purkersdorf, Austria) (Bankes, 1991) can monitor both conductance and capacitance signals. All of the instruments have similar basic components: (a) an incubator system to hold samples at a constant temperature during the test; (b) a monitoring unit that measures the conductance and/or capacitance of every cell at regular frequent intervals (usually every 6 minutes); and (c) a computer-based data handling system that presents the results in usable format.

The detection of microbial growth using electrical systems is based on the measurement of ionic changes occurring in media, caused by the metabolism of microorganisms. The changes caused by microbial metabolism and the detailed electrochemistry that is involved in these systems has been previously described in some depth (Eden and Eden, 1984; Easter and Gibson, 1989; Bolton and Gibson, 1994). The principle underlying the system is that as bacteria grow and metabolise in a medium, the conductivity of that medium will change. The electrical changes caused by low numbers of bacteria are impossible to detect using currently available instrumentation, approximately 10^6 organisms/ml must be present before a detectable change is registered. This is known as the *threshold of detection*, and the time taken to reach this point is the *detection time*.

In order to use electrical systems to enumerate organisms in foods, the sample must initially be homogenised. The growth well or tube of the instrument containing medium is inoculated with the homogenised sample and connected to the monitoring unit within the incubation chamber or bath. The electrical properties of the growth medium are recorded throughout the incubation period. The sample container is usually in the form of a glass or plastic tube or cell, in which a pair of electrodes is sited. The tube is filled with a suitable microbial growth medium, and a homogenised food sample is added. The electrical changes occurring in the growth medium during microbial metabolism are monitored via the electrodes and recorded by the instrument.

As microorganisms grow and metabolise they create new end-products in the medium. In general, uncharged or weakly charged substrates are transformed into highly charged end-products (Eden and Eden, 1984), and thus the conductance of the medium increases. The growth of some organisms such as yeasts does not result in large increases in conductance. This is possibly due to the fact that these

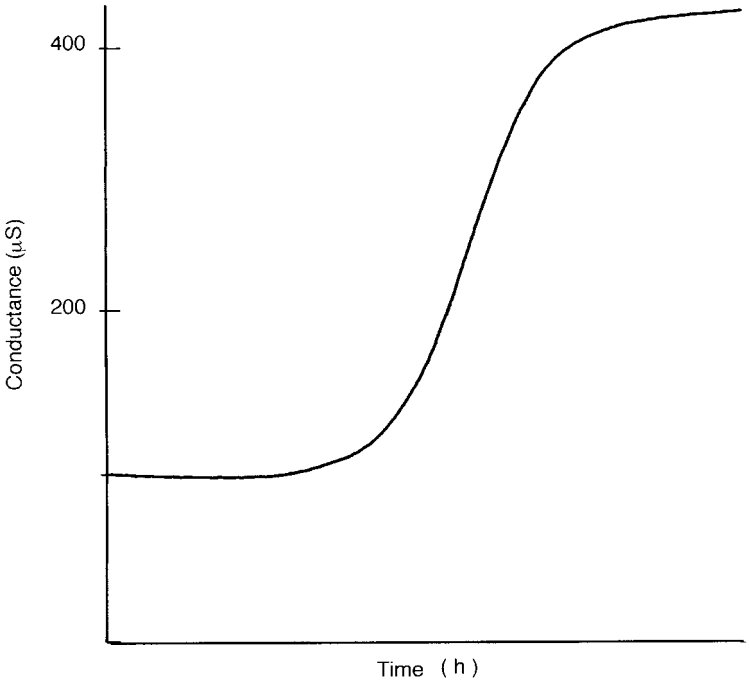

Fig. 2.1 A conductance curve generated by the growth of bacteria in a suitable medium.

organisms do not produce ionised metabolites and this can result in a decrease in conductivity during growth.

When an impedance instrument is in use, the electrical resistance of the growth medium is recorded automatically at regular intervals (e.g. 6 minutes) throughout the incubation period. When a change in the electrical parameter being monitored is detected, then the elapsed time since the test was started is calculated by a computer; this is usually displayed as the detection time. The complete curve of electrical parameter changes with time (Fig. 2.1) is similar to a bacterial growth curve, being sigmoidal and having three stages: (a) the inactive stage, where any electrical changes are below the threshold limit of detection of the instrument; (b) the active stage, where rapid electrical changes occur; and (c) the stationary or decline stage, that occurs at the end of the active stage and indicates a deceleration in electrical changes.

The electrical response curve should not be interpreted as being similar to a microbial growth curve. It is accepted (Easter and Gibson, 1989) that the lag and logarithmic phases of microbial growth occur in the inactive and active stages of the electrical response curve, up to and beyond the detection threshold of the instrument. The logarithmic and stationary phases of bacterial growth occur during the active and decline stages of electrical response curves.

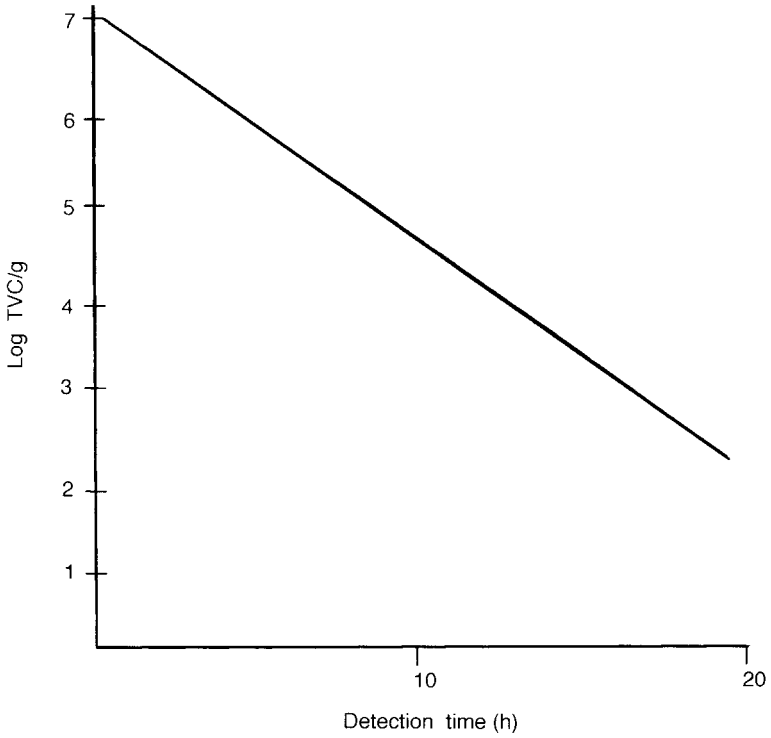

Fig. 2.2 Calibration curve showing changes in conductance detection time with bacterial total viable count (TVC).

In order to use detection time data generated from electrical instruments to assess the microbiological quality of a food sample, calibrations must be done. The calibration consists of testing samples using both a conventional plating test and an electrical test. The results are presented graphically with the conventional result on the *y*-axis and the detection time on the *x*-axis (Fig. 2.2). The result is a negative line with data covering 4 to 5 log cycles of organisms and a correlation coefficient greater than 0.85 (Easter and Gibson, 1989). Calibrations must be done for every sample type to be tested using electrical methods; different samples will contain varying types of microbial flora with differing rates of growth. This can greatly affect electrical detection time and lead to incorrect results unless correct calibrations have been done.

So far, the use of electrical instruments for total microbial assessment has been described. These systems, however, are based on the use of a growth medium and it is thus possible, using media engineering, to develop methods for the enumeration or detection of specific organisms or groups of organisms. Many examples of the use of electrical measurement for the detection/enumeration of specific

organisms have been published; these include: Enterobacteriaceae (Cousins and Marlatt, 1990; Petitt, 1989), *Pseudomonas* (Banks *et al*., 1989), *Yersinia enterocolitica* (Walker, 1989), yeasts (Connolly *et al*., 1988), *E. coli* (Druggan *et al*., 1993) and *Campylobacter* (Bolton and Powell, 1993). In the future, the number of types of organisms capable of being detected will undoubtedly increase. Considerable research is currently being done on media for the detection of *Listeria*, and media for other organisms will follow.

Most of the electrical methods described above involve the use of direct measurement, i.e. the electrical changes are monitored by electrodes immersed in the culture medium. Some authors have indicated the potential for indirect conductance measurement (Owens *et al.*, 1989) for the detection of microorganisms. This method involves the growth medium being in a separate compartment to the electrode within the culture cell. The liquid surrounding the electrode is a gas absorbent, e.g. potassium hydroxide for carbon dioxide. The growth medium is inoculated with the sample and, as the microorganisms grow, gas is released. This is absorbed by the liquid surrounding the electrode, causing a change in conductivity, which can be detected.

This technique may solve the problem caused by microorganisms that produce only small conductance changes in conventional direct conductance cells. These organisms, e.g. many yeast species, are very difficult to detect using conventional direct conductance methods, but detection is made easy by the use of indirect conductance monitoring (Betts, 1993). The increased use of indirect methods in the future could considerably enhance the ability of electrical systems to detect microorganisms that produce little electrical change in direct systems, thus increasing the number of applications of the technique within the food industry.

2.7.2 Adenosine triphosphate (ATP) bioluminescence

The non-biological synthesis of ATP in the extracellular environment has been demonstrated (Ponnamperuma *et al*., 1963), but it is universally accepted that such sources of ATP are very rare (Huernnekens and Whiteley, 1960). ATP is a high-energy compound found in all living cells (Huernnekens and Whiteley, 1960), and it is an essential component in the initial biochemical steps of substrate utilisation and in the synthesis of cell material.

McElroy (1947) first demonstrated that the emission of light in the bioluminescent reaction of the firefly, *Photinus pyralis*, was stimulated by ATP. The procedure for the determination of ATP concentrations utilising crude firefly extracts was described by McElroy and Streffier (1949) and has since been used in many fields as a sensitive and accurate measure of ATP. The light-yielding reaction is catalysed by the enzyme luciferase, this being the enzyme found in fireflies causing luminescence. Luciferase takes part in the following reaction:

$$\text{Luciferase} + \text{Luciferin} + \text{ATP} \rightarrow \text{Mg}^{2+}\ \text{Luciferase} - \text{Luciferin} - \text{AMP} + \text{PP}$$

The complex is then oxidised:

$$\text{Luciferase} - \text{Luciferin} - \text{AMP} + O_2 \rightarrow (\text{Luciferase} - \text{Luciferin} - \text{AMP} = O) + H_2O$$

The oxidised complex is in an excited stage, and as it returns to its ground stage a photon of light is released:

$$\text{Luciferase} - \text{Luciferin} - \text{AMP} = 0 \rightarrow (\text{Luciferase} - \text{Luciferin} - \text{AMP} = 0) + \text{Light}$$

The light-yielding reaction is efficient, producing a single photon of light for every luciferin molecule oxidised and thus every ATP molecule used (Seliger and McElroy, 1960).

Levin *et al*. (1964) first described the use of the firefly bioluminescence assay of ATP for detecting the presence of viable microorganisms. Since this initial report considerable work has been done on the detection of viable organisms in environmental samples using a bioluminescence technique (Stalker, 1984). As all viable organisms contain ATP, it could be considered simple to use a bioluminescence method to rapidly enumerate microorganisms. Research, however, has shown that the amount of ATP in different microbial cells varies depending on species, nutrient level, stress level and stage of growth (Stannard, 1989; Stalker, 1984). Thus, when using bioluminescence it is important to consider:

1. the type of microorganism being analysed; generally, vegetative bacteria will contain 1 fg of ATP/cell (Karl, 1980), yeasts will contain ten times this value (Stannard, 1989), whilst spores will contain no ATP (Sharpe *et al*., 1970);
2. whether the cells have been subjected to stress, such as nutrient depletion, chilling or pH change. In these cases a short resuscitation may be required prior to testing;
3. whether the cells are in a relatively ATP-free environment, such as a growth medium, or are contained within a complex matrix, like food, that will have very high background ATP levels.

When testing food samples one of the greatest problems is that noted in 3 above. All foods will contain ATP and the levels present in the food will generally be much higher than those found in microorganisms within the food. Data from Sharpe *et al*. (1970) indicated that the ratio of food ATP to bacterial ATP ranges from 40 000 : 1 in ice-cream to 15 : 1 in milk. Thus, to be able to use ATP analysis as a rapid test for foodborne microorganisms, methods for the separation of microbial ATP were developed. The techniques that have been investigated fall into two categories: either to physically separate microorganisms from other sources of ATP, or to use specific extractants to remove and destroy non-microbial ATP. Filtration methods have been successfully used to separate microorganisms from drinks (LaRocco *et al*., 1985; Littel and LaRocco, 1986) and brewery samples (Hysert *et al*., 1976). These methods are, however, difficult to apply to particulate-containing solutions as filters rapidly become blocked. A potential way around this problem has been investigated by some workers and

utilises a double filtration system/scheme (Littel *et al*., 1986), the first filter removing food debris but allowing microorganisms through, the second filter trapping microorganisms prior to lysis and bioluminescent analysis. Other workers (Baumgart *et al*., 1980; Stannard and Wood, 1983) have utilised ion exchange resins to trap selectively either food debris or microorganisms before bioluminescent tests were done.

The use of selective chemical extraction to separate microbial and nonmicrobial ATP has been extensively tested for both milk (Bossuyt, 1981) and meat (Billte and Reuter, 1985) and found to be successful. In general, this technique involves the lysis of somatic (food) cells followed by destruction of the released ATP with an apyrase (ATPase) enzyme. A more powerful extraction reagent can then be used to lyse microbial cells, which can then be tested with luciferase, thus enabling the detection of microbial ATP only.

There are a number of commercially available instruments aimed specifically at the detection of microbial ATP; Lumac (Netherlands), Foss Electric (Denmark), Bio Orbit (Finland) and Biotrace (UK) all produce systems, including separation methods, specifically designed to detect microorganisms in foods. Generally, all of the systems perform well and have similar specifications, including a minimum detection threshold of 10^4 bacteria (10^3 yeasts) and analysis times of under one hour.

In addition to testing food samples for total viable microorganisms, there have been a number of reports concerning potential alternative uses of ATP bioluminescence within the food industry. The application of ATP analysis to rapid hygiene testing has been considered (Holah, 1989), both as a method of rapidly assessing microbiological contamination, and as a procedure for measuring total surface cleanliness. It is in the latter area that ATP measurement can give a unique result. As described earlier, almost all foods contain very high levels of ATP, thus food debris left on a production line could be detected in minutes using a bioluminescence method, allowing a very rapid check of hygienic status to be done. The use of ATP bioluminescence to monitor surface hygiene has now been widely adopted by industry. The availability of relatively inexpensive, portable, easy to use luminometers has now enabled numerous food producers to implement rapid hygiene testing procedures that are ideal for HACCP monitoring applications where surface hygiene is a critical control point. Reports suggest (Griffiths, 1995) that all companies surveyed that regularly use ATP hygiene monitoring techniques note improvements in cleanliness after initiation of the procedure. Such ATP-based test systems can be applied to most types of food processing plant, food service and retail establishments and even assessing the cleanliness of transportation vehicles such as tankers.

One area that ATP bioluminescence has not yet been able to address has been the detection of specific microorganisms. It may be possible to use selective enrichment media for particular microorganisms in order to allow selective growth prior to ATP analysis. This approach would, however, considerably increase analysis time and some false high counts would be expected. The use of specific lysis agents that release ATP only from the cells being analysed have

been investigated (Stannard, 1989) and shown to be successful. The number of these specific reagents is, however, small and thus the method is of only limited use. Perhaps the most promising method developed for the detection of specific organisms is the use of genetically engineered bacteriophages (Ulitzur and Kuhn, 1987; Ulitzur *et al*., 1989; Schutzbank *et al*., 1989).

Bacteriophages are viruses that infect bacteria. Screening of bacteriophages has shown that some are very specific, infecting only a particular type of bacteria. Workers have shown it is possible to add into the bacteriophage the genetic information that causes the production of bacterial luciferase. Thus, when a bacteriophage infects its specific host bacterium, the latter produces luciferase and becomes luminescent. This method requires careful selection of the bacteriophage in order to ensure false positive or false negative results do not occur; it does, however, indicate that, in the future, luminescence-based methods could be used for the rapid detection of specific microorganisms (Stewart, 1990).

In conclusion, the use of ATP bioluminescence in the food industry has been developed to a stage at which it can be reliably used as a rapid test for viable microorganisms, as long as an effective separation technique for microbial ATP is used. Its potential use in rapid hygiene testing has been realised and the technique is being used within the industry. Work has also shown that luminescence can allow the rapid detection of specific microorganisms but such a system would need to be commercialised before widespread use within the food industry.

2.7.3 Microscopy methods

Microscopy is a well-established and simple technique for the enumeration of microorganisms. One of the first descriptions of its use was for rapidly counting bacteria in films of milk stained with the dye methylene blue (Breed and Brew, 1916). One of the main advantages of microscope methods is the speed with which individual analyses can be done; however, this must be balanced against the high manual workload and the potential for operator fatigue caused by constant microscopic counting.

The use of fluorescent stains, instead of conventional coloured compounds, allows cells to be more easily counted and thus these stains have been the subject of considerable research. Microbial ecologists first made use of such compounds to visualise and count microorganisms in natural waters (Francisco *et al*., 1973; Jones and Simon, 1975). Hobbies *et al*. (1977) first described the use of Nuclepore polycarbonate membrane filters to capture microorganisms before fluorescent staining, while enumeration was considered in depth by Pettipher *et al*. (1980), the method developed by the latter author being known as the direct epifluorescent filter technique (DEFT).

The DEFT is a labour-intensive manual procedure and this has led to research into automated fluorescence microscope methods that offer both automated sample preparation and high sample throughput. The first fully automated instrument based on fluorescence microscopy was the Bactoscan (Foss Electric, Denmark), which was developed to count bacteria in milk and urine. Milk

samples placed in the instrument are chemically treated to lyse somatic cells and dissolve casein micelles. Bacteria are then separated by continuous centrifugation in a dextran/sucrose gradient. Microorganisms recovered from the gradient are incubated with a protease to remove residual protein, then stained with acridline orange and applied as a thin film to a disc rotating under a microscope. The fluorescent light from the microscope image is converted into electrical impulses and recorded. The Bactoscan has been used widely for raw milk testing in continental Europe, and correlations with conventional methods have reportedly been good (Kaereby and Asmussen, 1989). The technique does, however, have a poor sensitivity (approximately 5×10^4 cells/ml) and this negates its use on samples with lower bacterial counts.

An instrument-based fluorescence counting method, in which samples were spread onto a thin plastic tape, was developed for the food industry. The instrument (Autotrak) deposited samples onto the tape, which was then passed through staining and washing solutions, before travelling under a fluorescence microscope. The light pulses from the stained microorganisms were then enumerated by a photomultiplier unit. Tests on food samples using this instrument (Betts and Bankes, 1988) indicated that the debris from food samples interfered with the staining and counting procedure and gave results that were significantly higher than corresponding total viable counts.

Perhaps the most recent development in fluorescence microscope techniques to be used within the food industry for rapid counting is flow cytometry. In this technique the stained sample is passed under a fluorescence microscope system as a liquid in a flow cell. Light pulses caused by the light hitting a stained particle are transported to a photomultiplier unit and counted. This technique is automated, rapid and potentially very versatile. Of the microscope methods discussed here, the DEFT has perhaps the widest usage, while flow cytometry could offer significant advantages in the future. These two procedures will therefore be discussed in more detail.

DEFT

The DEFT was developed for rapidly counting the numbers of bacteria in raw milk samples (Pettipher *et al*., 1980; Pettipher and Rodrigues, 1982). The method is based on the pretreatment of a milk sample in the presence of a proteolytic enzyme and surfactant at 50 °C, followed by a membrane filtration step that captures the microorganisms. The pretreatment is designed to lyse somatic cells and solubilise fats that would otherwise block the membrane filter. After filtration the membrane is strained with the fluorescent nucleic acid binding dye acridine orange, then rinsed and mounted on a microscope slide. The membrane is then viewed with an epifluorescent microscope. This illuminates the membrane with ultraviolet light, causing the stain to emit visible light that can be seen through the microscope. As the stain binds to nucleic acids it is concentrated within microbial cells by binding to DNA and RNA molecules; thus any organisms on the membrane can be easily visualised and counted. The complete pretreatment and counting procedure can take as little as 30 minutes.

Although the original DEFT was able to give a very rapid count, it was very labour intensive, as all of the pre-treatment and counting were done manually. This led to a very poor daily sample throughput for the method. The development of semi-automated counting methods based on image analysis (Pettipher and Rodrigues, 1982) overcame some of the problems of manual counting and thus allowed the technique to be more user friendly.

The early work on the uses of DEFT for enumerating cells in raw milk was followed with examinations of other types of foods. It was quickly recognised that the good correlations between DEFT count and conventional total viable counts that were obtained with raw milk samples did not occur when heat-treated milks were examined (Pettipher and Rodrigues, 1981). Originally this was considered to be due to heat-inducing staining changes occurring in Gram-positive cocci (Pettipher and Rodrigues, 1981); however, more recent work (Back and Kroll, 1991) has shown similar changes occur in both Gram-positives and Gram-negatives. Similar staining phenomena have also been observed with heat-treated yeasts (Rodrigues and Kroll, 1986) and in irradiated foods (Betts *et al*., 1988). Thus the use of DEFT as a rapid indication of total viable count is mainly confined to raw foods.

The type of food with which DEFT can be used has been expanded since the early work with raw milk. Reports have covered the use of the method with frozen meats and vegetables (Rodrigues and Kroll, 1989), raw meats (Shaw *et al*., 1987), alcoholic beverages (Cootes and Johnson, 1980; Shaw, 1989), tomato paste (Pettipher *et al*., 1985), confectionery (Pettipher, 1987) and dried foods (Oppong and Snudden, 1988) and with hygiene testing (Holah *et al*., 1988). In addition, some workers (Rodrigues and Kroll, 1988) have suggested that the method could be modified to detect and count specific groups of organisms.

In conclusion, the DEFT is a very rapid method for the enumeration of total viable microorganisms in raw foods and has been used with success within the industry. The problems of the method are a lack of specificity and an inability to give a good estimate of viable microbial numbers in processed foods. The former could be solved by the use of short selective growth stages or fluorescently labelled antibodies; however, these solutions would have time and cost implications. The problem with processed foods can be eliminated only if alternating straining systems that mark viable cells are examined; preliminary work (Betts *et al*., 1989) has shown this approach to be successful, and the production and commercialisation of fluorescent viability stains could advance the technique. At present the high manual input and low sample throughput of DEFT procedures has limited the use of the procedure in the food industry.

Flow cytometry

Flow cytometry is a technique based on the rapid measurement of cells as they flow in a liquid stream past a sensing point (Carter and Meyer, 1990). The cells under investigation are inoculated into the centre of a stream of fluid (known as the sheath fluid). This constrains them to pass individually past the sensor and enables measurements to be made on each particle in turn, rather than average

values for the whole population. The sensing point consists of a beam of light (either ultraviolet or laser) that is aimed at the sample flow and one or more detectors that measure light scatter or fluorescence as the particles pass under the light beam. The increasing use of flow cytometry in research laboratories has largely been due to the development of the reliable instrumentation and the numerous staining systems. The stains that can be used with flow cytometers allow a variety of measurements to be made. Fluorescent probes based on enzyme activity, nucleic acid content, membrane potential and pH all have been examined, while the use of antibody-conjugated fluorescent dyes confers specificity to the system.

Flow cytometers have been used to study a range of eukaryotic and prokaryotic microorganisms. Work with eukaryotes has included the examination of pathogenic amoeba (Muldrow *et al*., 1982) and yeast cultures (Hutter and Eipel, 1979), while bacterial studies have included the growth of *Escherichia coli* (Steen *et al*., 1982), enumeration of cells in bacterial cultures (Pinder *et al*., 1990) and the detection of *Legionella* spp. in cooling tower waters (Tyndall *et al*., 1985).

Flow cytometric methods for the food industry have been developed and have been reviewed by Veckert *et al*. (1995). Donnelly and Baigent (1986) explored the use of fluorescently labelled antibodies to detect *Listeria monocytogenes* in milk, and obtained encouraging results. The method used by these authors relied on the selective enrichment of the organisms for 24 hours, followed by staining with fluorescein isothiocyanate labelled polyvalent *Listeria* antibodies. The stained cells were then passed through a flow cytometer, and the *L. monocytogens* detected. The author suggested that the system could be used with other types of food. A similar approach was used by McClelland and Pinder (1994) to detect *Salmonella* typhimurium in dairy products.

Patchett *et al*. (1991) investigated the use of a Skatron Argus flow cytometer to enumerate bacteria in pure cultures and foods. The results obtained with pure cultures showed that flow cytometer counts correlated well with plate counts down to 10^3 cells/g. With foods, however, conflicting results were obtained. Application of the technique to meat samples gave a good correlation with plate counts and enabled enumeration down to 10^5 cells/g. Results for milk and paté were poorer, the sensitivity of the system for paté being 10^6 cells/ml, whilst cells inoculated into milk were not detected at levels in excess of 10^7 ml. The poor sensitivity of this flow cytometer with foods was thought to be due to interference of the counting system caused by food debris and it was suggested that the application of separation methods to partition microbial cells from food debris would overcome the problem.

Perhaps the most successful application of flow cytometric methods to food products has been the use of a Chemunex Chemflow system to detect contaminating yeast in dairy and fruit products (Bankes *et al*., 1991). The procedure used with this system calls for an incubation of the product for 16–20 hours followed by centrifugation to separate and concentrate the cells. The stain is then added, and a sample is passed through the flow cytometer for analysis. An evaluation of the system by Pettipher (1991), using soft drinks inoculated with yeasts, showed that it was reliable and user friendly. The results obtained indicated that cytome-

ter counts correlated well with DEFT counts, however, the author did not report how the system compared to plate counts.

Investigations of the Chemflow system by Bankes *et al*. (1991) utilised a range of dairy and fruit-based products inoculated with yeast. Results indicated that yeast levels as low as 1 cell/25 g could be detected in 24 hours in dairy products. In fruit juices a similar sensitivity was reported: however, a 48 hour period was required to ensure that this was achieved. The system was found to be robust and easy to use. The Chemflow system has now been adapted to detect bacterial cells as well as yeasts, and applications are available for fermentor biomass and enumeration of total flora in vegetables. The Chemflow system has been fully evaluated in a factory environment (Dumain *et al*., 1990) testing fermented dairy products. These authors report a very good correlation between cytometer count and plate count ($r = 0.98$), results being obtained in 24 hours, thus providing a time saving of three days over classical methods.

In conclusion, flow cytometry can provide a rapid and sensitive method for the rapid enumeration of microorganisms. The success of the system depends on the development and use of (a) suitable staining systems, and (b) protocols for the separation of microorganisms from food debris that would otherwise interfere with the detection system. In the future a flow cytometer fitted with a number of light detection systems could allow the analysis of samples for many parameters at once, thus considerably simplifying testing regimes.

Solid phase cytometry

A relatively new cytometric technique has been developed by Chemunex (Maisons-Alfort, France) based on solid phase cytometry. In this procedure samples are passed through a membrane filter which captures contaminating microorganisms. A stain is then applied to the filter to fluorescently mark metabolically active microbial cells. After staining, the membrane is then transferred to a Chemscan RDI instrument, which scans the whole membrane with a laser, counting fluorescing cells. The complete procedure takes around 90 minutes to perform and can detect single cells in the filtered sample. The Chemscan RDI solid phase cytometry system is an extremely powerful tool for rapidly counting low levels of organisms. It is ideally suited to the analysis of waters or other clear filterable fluids, and specific labelling techniques could be used to detect particular organisms of interest. Foods containing particulate materials could, however, be problematic as organisms would need to be separated from the food material before filtration and analysis.

2.7.4 Immunological methods

Antibodies and antigens

Immunological methods are based on the specific binding reaction that occurs between an antibody and the antigen to which it is directed. *Antibodies* are protein molecules that are produced by animal white blood cells, in response to contact

with a substance causing an immune response. The area to which an antibody attaches on a target molecule is known as the *antigen*. Antigens used in immunochemical methods are of two types. The first occurs when the analyte is of low molecular weight and thus does not stimulate an immune response on its own; these substances are described as haptens and must be bound to a larger carrier molecule to elicit an immune response and cause antibody production. The second type of antigen is immunogenic and is able to elicit an immune response on its own.

Two types of antibody can be employed in immunological tests. These are known as *monoclonal* and *polyclonal* antibodies. *Polyclonal* antibodies are produced if large molecules such as proteins or whole bacterial cells are used to stimulate an immune response in an animal. The many antigenic sites result in numerous different antibodies being produced to the molecule or cell. *Monoclonal* antibodies are produced by tissue culture techniques and are derived from a single white blood cell; thus they are directed towards a single antigenic site. The binding of an antigen is highly specific. Immunological methods can therefore be used to detect particular specific microorganisms or proteins (e.g. toxins). In many cases, when using these methods a label is attached to the antibody, so that binding can be visualised more easily when it occurs.

Labels

The labels that can be used with antibodies are of many types and include radiolabels, fluorescent agents, luminescent chemicals and enzymes; in addition agglutination reactions can be used to detect the binding of antibody to antigen.

Radioisotopes have been extensively used as labels, mainly because of the great sensitivity that can be achieved with these systems. They do, however, have some disadvantages, the main one being the hazardous nature of the reagents. This would negate their use in anything other than specialist laboratories, and certainly their use within the food industry would be questioned.

Fluorescent labels have been widely used to study microorganisms. The most frequently used reagent has been fluorescein. However, others such as rhodamine and umbelliferone have also been utilised. The simplest use of fluorescent antibodies is in microscopic assays. Recent advances in this approach have been the use of flow cytometry for multiparameter flow analysis of stained preparations, and the development of enzyme-linked immunofluorescent assays (ELIFA), some of which have been automated.

Luminescent labels have been investigated as an alternative to the potentially hazardous radiolabels (Kricka and Whitehead, 1984). The labels can be either chemiluminescent or bioluminescent, and have the advantage over radiolabels that they are easy to handle and measure using simple equipment, while maintaining a similar sensitivity (Rose and Stringer, 1989). A number of research papers have reported the successful use of immunoluminometric assays (Lohneis *et al.*, 1987); however, none has yet been commercialised.

Antibodies have been used for the detection of antigens in precipitation and agglutination reactions. These assays tend to be more difficult to quantify than

other forms of immunoassay and usually have only a qualitative application. The assays are quick and easy to perform and require little in the way of equipment.

A number of agglutination reactions have been commercialised by manufacturers and have been successfully used within the food industry. These methods have tended to be used for the confirmation of microbial identity, rather than for the detection of the target organisms. They offer a relatively fast test time, are easy to use and usually require no specialist equipment, thus making ideal test systems for use in routine testing laboratories.

Several latex agglutination test kits are available for the confirmation of *Salmonella* from foods. These include the Oxoid *Salmonella* Latex Kit (Oxoid) designed to be used with the Oxoid Rapid *Salmonella* Test Kit (Holbrook *et al.*, 1989); the Micro Screen *Salmonella* Latex Slide Agglutination Test (Mercia Diagnostics Ltd); the Wellcolex Colour *Salmonella* Test (Wellcome Diagnostics) (Hadfield *et al.*, 1987a, b); and the Spectate *Salmonella* test (Rhone Poulenc Diagnostics Ltd.) (Clark *et al.*, 1989). The latter two kits use mixtures of coloured latex particles that allow not only detection but also serogrouping of *Salmonella*. Latex agglutination test kits are also available for *Campylobacter* (Microscreen, Mercia Diagnostics), *Staphyloccocus aureus* (Staphaurex, Wellcome Diagnostics), *Shigella* (Wellcolex Colour *Shigella* Test, Wellcome Diagnostics) and *Escherichia coli* 0157 : H7 (Oxoid). Agglutination kits have also been developed for the detection of microbial toxins, e.g. Oxoid Staphylococcal Enterotoxin Reverse Passive Latex Agglutination Test (Rose *et al.*, 1989; Bankes and Rose, 1989).

Enzyme immunoassays have been extensively investigated as rapid detection methods for foodborne microorganisms. They have the advantage of specificity conferred by the use of a specific antibody, coupled with coloured or fluorescent end-points that are easy to detect either visually or with a spectrophotometer or fluorimeter. Most commercially available enzyme immunoassays use an antibody sandwich method in order initially to capture and then to detect specific microbial cells or toxins. The kits are supplied with two types of antibody: capture antibody and conjugated antibody. The capture antibody is attached to a solid support surface such as a microtitre plate well. An enriched food sample can be added to the well and the antigens from any target cells present will bind to the antibodies. The well is washed out, removing food debris and unbound microorganisms. The enzyme conjugated antibody can then be added to the well. This will bind to the target cell, forming an antibody sandwich. Unbound antibodies can be washed from the well and the enzyme substrate added. The substrate will be converted by any enzyme present from a colourless form, into a coloured product. A typical microplate enzyme immunoassay takes between two and three hours to perform and will indicate the presumptive presence of the target bacterial cells. Thus positive samples should always be confirmed by biochemical or serological methods.

There are a number of commercially available enzyme immunoassay test kits for the detection of *Listeria*, *Salmonella*, *Escherichia coli* O157, staphylococcal enterotoxins and *Bacillus* diarrhoeal toxin, from food samples. The sensitivity of

these systems is approximately 10^6 cells/ml, so that a suitable enrichment procedure must be used before analysis using the assay. Thus, results can be obtained in two to three days, rather than the three to five days required for a conventional test procedure.

Over recent years a number of highly automated immunoassays have been developed; these add to the benefit of the rapid test result, by reducing the level of manual input required to do the test. Automation of enzyme immunoassays has taken a number of forms; a number of manufacturers market instruments which simply automate standard microplate enzyme-linked immunosorbent assays (ELISAs). These instruments hold reagent bottles and use a robotic pipetting arm, which dispenses the different reagents required in the correct sequence. Automated washing and reading completes the assay with little manual input needed. At least two manufacturers have designed immunoassay kits around an automated instrument, to produce very novel systems.

The Vidas system (bioMérieux, Basingstoke) uses a test strip, containing all of the reagents necessary to do an enzyme-linked immunosorbent assay (ELISA) test: the first well of the strip is inoculated with an enriched food sample, and placed into the Vidas instrument, together with a pipette tip internally coated with capture antibody. The instrument then uses the pipette tip to transfer the test sample into the other cells in the strip containing various reagents needed to carry out the ELISA test. All of the transfers are completely automatic, as is the reading of the final test result. Vidas ELISA tests are available for a range of organisms including *Salmonella*, *Listeria*, *Listeria monocytogenes*, *E. coli* 0157, *Campylobacter* and staphylococcal enterotoxin. Evaluations of a number of these methods have been done (Blackburn *et al.*, 1994; Bobbitt and Betts, 1993) and indicated that results were at least equivalent to conventional test methods.

The EIAFOSS (Foss Electric, Denmark) is another fully automated ELISA system. In this case the instrument transfers all of the reagents into sample containing tubes, in which all of the reactions occur. The EIAFOSS procedure is novel as it uses antibody coated magnetic beads as a solid phase. During the assay these beads are immobilised using a magnet mounted below the sample tube. EIAFOSS test kits are available for *Salmonella*, *Listeria*, *E. coli* 0157 and *Campylobacter*, and evaluations have indicated that these methods operate well (Jones and Betts, 1994).

The newest immunoassay procedure that has been developed into a commercial format is arguably the simplest to use. Immunochromatography operates on a dipstick, composed of an absorbent filter material which contains coloured particles coated with antibodies to a specific organism. The particles are on the base of the dipstick and when dipped into a microbiological enrichment broth, they move up the filter as the liquid is moved by capillary action. At a defined point along the filter material lies a line of immobilised specific antibodies. In the presence of the target organism, binding of that organism to the coloured particles will occur. This cell/particle conjugate moves up the filter dipstick by capillary action until it meets the immobilised antibodies where it will stick. The build up of coloured particles results in a clearly visible coloured line, indicating a positive test result.

A number of commercial kits are based around this procedure including the Oxoid *Listeria* Rapid Test (Jones *et al.*, 1995a) and the Celsis Lumac Pathstik (Jones *et al.*, 1995b) have been developed and appear to give good results. The immunochromatography techniques require an enrichment in the same way as other immunoassays; they do not, however, require any equipment or instrumentation, and once the dipstick is inoculated, need only minutes to indicate a positive or negative result.

Immunoassay conclusions

In conclusion, immunological methods have been extensively researched and developed. There are now a range of systems that allow the rapid detection of the specific organism to which they are directed. Numerous evaluations of commercially available immuno-based methods have indicated that the results generally correlate well with conventional microbiological methods. Enzyme immunoassays in particular appear to offer a simple way of reducing analysis times by one or two days; automation or miniaturisation of these kits has reduced the amount of person time required to do the test and simplified the manual procedures considerably.

The main problem with the immunological systems is their low sensitivity. The minimum number of organisms required in an enzyme immunoassay system to obtain a positive result is approximately 10^5/ml. As the food microbiologist will want to analyse for the presence or absence of a single target organism in 25 g of food, an enrichment phase is always necessary. The inclusion of enrichment will always add 24–48 hours to the total analysis time.

2.7.5 Nucleic acid hybridisation

Nucleic acids

The specific characteristics of any organism depend on the particular sequence of the nucleic acids contained in its genome. The nucleic acids themselves are made up of a chain of units each consisting of a sugar (deoxyribose or ribose, depending on whether the nucleic acid is DNA or RNA), a phosphorus-containing group and one of four organic purine or pyrimidine bases. DNA is constructed from two of these chains arranged in a double helix and held together by bonds between the organic bases. The bases specifically bind adenine to thymine and guanine to cytosine. It is the sequence of bases that make different organisms unique.

The development of nucleic acid probes

Nucleic acid probes are small segments of single-stranded nucleic acid that can be used to detect specific genetic sequences in test samples. Probes can be developed against DNA or RNA sequences. The attraction of the use of gene probes in the problem of microbial detection is that a probe consisting of only 20 nucleotide sequences is unique and can be used to identify an organism accurately (Gutteridge and Arnott, 1989).

In order to be able to detect the binding of a nucleic acid probe to DNA or RNA from a target organism, it must be attached to a label of some sort that can easily be detected. Early work was done with radioisotope labels such as phosphorus (^{32}P) that could be detected by autoradiography or scintillation counting. Radiolabels, however, have inherent handling, safety and disposal problems that make them unsuitable for use in food laboratories doing routine testing. Thus the acceptance of widespread use of nucleic acid probes required the development of alternative labels.

A considerable amount of work has been done on the labelling of probes with an avidin–biotin link system. This is based on a very high binding specificity between avidin and biotin. The probe sequence of nucleic acid is labelled with biotin and reacted with target DNA. Avidin is then added, linked to a suitable detector, e.g. avidin-alkaline phosphatase, and binding is detected by the formation of a coloured product from a colourless substrate. These alternative labelling systems proved that non-radiolabelled probes could be used for the detection of microorganisms. However, the system was much less sensitive than isotopic procedures, requiring as much as a 100-fold increase in cell numbers for detection to occur, compared with isotope labels.

In order to develop non-isotopic probes with a sensitivity approaching that of isotope labels, it was necessary to consider alternative probe targets within cells. Probes directed toward cell DNA attach to only a few sites on the chromosome of the target cell. By considering areas of cell nucleic acid that are present in relatively high copy number in each cell and directing probes toward these sites, it is possible to increase the sensitivity of non-isotopic probes considerably. Work on increasing probe sensitivity centred on the use of RNA as a target. RNA is a single-stranded nucleic acid that is present in a number of forms in cells. In one form it is found within parts of the cell protein synthesis system called ribosomes. Such RNA is known as ribosomal RNA (rRNA), and is present in very high copy numbers within cells. By directing nucleic acid probes to ribosomal RNA it is possible to increase the sensitivity of the assay system considerably.

Probes for organisms in food

Nucleic acid hybridisation procedures for the detection of pathogenic bacteria in foods have been described for *Salmonella* spp. (Fitts, 1985; Curiale *et al.*, 1986), *Listeria* spp. (Klinger *et al.*, 1988; Klinger and Johnson, 1988), *Yersinia enterocolitica* (Hill *et al.*, 1983b; Jagow and Hill, 1986), *Listeria monocytogenes* (Datta *et al.*, 1988), enterotoxigenic *Escherichia coli* (Hill *et al.*, 1983a, 1986), *Vibrio vulnificus* (Morris *et al.*, 1987), enterotoxigenic *Staphylococcus aureus* (Notermans *et al.*, 1988), *Clostridium perfringens* (Wernars and Notermans, 1990) and *Clostridium botulinum* (Wernars and Notermans, 1990).

The first commercially available nucleic-probe-based assay system for food analysis was introduced by Gene Trak Systems (Framingham, MA, USA) in 1985 (Fitts, 1985). This test used *Salmonella*-specific DNA probes directed against chromosomal DNA to detect *Salmonella* in enriched food samples. The format of the test involved hybridisation between target DNA bound to a membrane filter

and phosphorus 32-labelled probes. The total analysis time for the test was 40–44 hours of sample enrichment in non-selective and selective media, followed by the hybridisation procedure lasting 4–5 hours. Thus the total analysis time was approximately 48 hours. The *Salmonella* test was evaluated in collaborative studies in the USA and appeared to be at least equivalent to standard culture methods (Flowers *et al.*, 1987). Gene Trak also produced a hybridisation assay for *Listeria* spp., based on a similar format (Klinger and Johnson, 1988).

The Gene Trak probe kits gained acceptance within the United States and a number of laboratories began using them. In Europe, however, there was a reluctance among food laboratories to use radioisotopes within the laboratory. In addition, ^{32}P has a short half-life, which caused difficulties when transporting kits to distant sites. In 1988 Gene Trak began marketing non-isotopically labelled probes for *Salmonella*, *Listeria* and *Escherichia coli*. The detection system for the probes was colorimetric. In order to overcome the reduction in sensitivity caused by the use of non-isotopic labels, the target nucleic acid within the cell was ribosomal RNA. This nucleic acid is present in an estimated 500 to 20 000 copies per cell.

The colorimetric hybridisation assay is based on a liquid hybridisation reaction between the target rRNA and two separate DNA oligonucleotide probes (the capture probe and the reporter probe) that are specific for the organism of interest. The capture probe molecules are extended enzymatically with a polymer of approximately 100 deoxyadenosine monophosphate residues. The reporter probe molecules are labelled chemically with the hapten fluorescein.

Following a suitable enrichment of the food under investigation, a test sample is transferred to a tube and the organisms lysed, releasing rRNA targets. The capture and detector probes are then added and hybridisation is allowed to proceed. If target rRNA is present in the sample, hybridisation takes place between the probes and the target 16s rRNA. The solution containing the target probe complex is then brought into contact with a solid support dipstick, containing bound deoxythymidine homopolymer, under conditions that will allow hybridisation between the poly-deoxyadenosine polymer of the capture probe and the poly-deoxythymidine on the dipstick. Unhybridised nucleic acids and cellular debris are then washed away, leaving the captured DNA–RNA complex attached to the surface of the dipstick. The bound fluoresceinated reporter probe is detected by the addition of an antifluorescein antibody conjugated to the enzyme horseradish peroxidase. Subsequent addition of a chromogenic substrate for the enzyme results in colour development that can be measured spectrophotometrically.

Results of the colorimetric assays (Mozola *et al.*, 1991) have indicated a good comparison between the probe methods and conventional cultural procedures for both *Salmonella* and *Listeria*. The sensitivity of the kits appeared to be between 10^5 and 10^6 target organisms/ml, and thus the enrichment procedure is a critical step in the methodology. Since the introduction of the three kits previously mentioned, Gene Trak have marketed systems for *Staphylococcus aureus*, *Campylobacter* spp. and *Yersinia enterocolitica*, although the latter assay is not currently available.

Commercially available nucleic acid probes for the confirmation of *Campylobacter*, *Staphylococcus aureus* and *Listeria* are available from Genprobe (Gen Probe Inc., San Diego, USA). These kits are based on a single-stranded DNA probe that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the organism, the labelled DNA probe combines with it to form a stable DNA : RNA hybrid. The hybridised probe can be detected by its luminescence.

The assay method used is termed a hybridisation protection assay and is based on the use of a chemiluminescent acridinium ester. This ester reacts with hydrogen peroxide under basic conditions to produce light that can be measured in a luminometer. The acridinium esters are covalently attached to the synthetic DNA probes through an alkylamine arm. The assay format is based on differential chemical hydrolysis of the ester bond. Hydrolysis of the bond renders the acridinium permanently non-chemiluminescent. When the DNA probe which the ester is attached hybridises to the target RNA, the acridinium is protected from hydrolysis and can thus be rendered luminescent. The test kits for *Campylobacter* and *Listeria* utilise a freeze-dried probe reagent. The *Campylobacter* probe reacts with *C. jejuni*, *C. coli* and *C. pylori*; the *Listeria* probe reacts with *L. monocytogenes*. In both cases a full cultural enrichment protocol is necessary prior to using the probe for confirmation testing.

An evaluation of the *L. monocytogenes* probe kit (Bobbit and Betts, 1991) indicated that it was totally specific for the target organism. The sensitivity required approximately 10^6 *L. monocytogenes* to be present in order for a positive response to be obtained. The kit appeared to offer a fast reliable culture confirmation test and had the potential to be used directly on enrichment broth, thus reducing test times even further.

Probes – the future

The development and use of probes in the food industry have advanced little in recent years. The kits that are currently available show great promise but are not as widely used as immunoassays. Microbiologists must always consider the usefulness of analysing the genetic information within cells, for example to detect the presence of genes coding for toxins could be detected, even when not expressed, and screening methods could be devised for pathogenicity plasmids, such as that in *Yersinia enterocolitica*. It may be, however, that the advances in molecular biology mean that the best way to test for such information is by using nucleic acid amplification methods such as the polymerase chain reaction (PCR).

Nucleic acid amplification techniques

In recent years, several genetic amplification techniques have been developed and refined. The methods usually rely on the biochemical amplification of cellular nucleic acid and can result in a 10^7-fold amplification in two to three hours. The very rapid increase in target that can be gained with nucleic acid amplification methods makes them ideal candidates for development of very rapid microbial

detection systems. A number of amplification methods have been developed and applied to the detection of microorganisms:

- Polymerase chain reaction (PCR) and variations, including nested PCR, reverse transcriptase (RT) PCR and multiplex PCR.
- Q Beta Replicase.
- Ligase Amplification Reaction (LAR).
- Transcript Amplification System (TAS), also known as Self Sustained Sequence Replication (3SR) or Nucleic Acid Sequence Based Amplification (NASBA).

Of these amplification methods only PCR has been commercialised as a kit-based procedure for the detection of foodborne microorganisms. Much research has been done with NASBA and there are a number of research papers outlining its use for detecting food pathogens but, as yet, no commercially available kits are on the market.

Polymerase chain reaction (PCR)
PCR is a method used for the repeated *in vitro* enzymic synthesis of specific DNA sequences. The method uses two short oligonucleotide primers that hybridise to opposite strands of a DNA molecule and flank the region of interest in the target DNA. PCR proceeds via series of repeated cycles, involving DNA denaturation, primer annealing and primer extension by the action of DNA polymerase. The three stages of each cycle are controlled by changing the temperature of the reaction, as each stage will occur only at particular defined temperatures. These temperature changes are accomplished by using a specialised instrument known as a thermocycler. The products of primer extension from one cycle, act as templates for the next cycle, thus the number of target DNA copies doubles at every cycle.

Reverse transcriptase PCR
This involves the use of an RNA target for the PCR reaction. The PCR must work on a DNA molecule; thus initially reverse transcriptase is used to produce copy DNA (cDNA). The latter is then used in a conventional PCR reaction. The RT-PCR reaction is particularly applicable to certain microbiological tests. Some foodborne viruses contain RNA as their genetic material; thus RT-PCR must be used if amplification and thus detection of these viruses are necessary. A second use of RT-PCR is in the detection of viable microorganisms. One of the problems associated with PCR is its great sensitivity and ability to amplify very low concentrations of a target nucleic acid. Thus, if using PCR to detect the presence or absence of a certain microorganism in a food, PCR could ‘detect’ the organism, even if it had been previously rendered inactive by a suitable food process. This could result in a false positive detection. A way to overcome this problem is to use an RT-PCR targeted against cellular messenger RNA, which is only produced by active cells and once produced has a short half-life. Thus a detection of

specific mRNA by an RT-PCR procedure is indicative of the presence of a viable microorganism.

NASBA

NASBA is a multi-enzyme, multicycle amplification procedure, requiring more enzymes and reagents than standard PCR. It does, however, have the advantage of being isothermal, therefore all stages of the reaction occur at a single temperature and a thermocycler is not required. Various research papers have been published which use NASBA to detect foodborne pathogens (e.g. Uyttendaele *et al.*, 1996); however, the procedure has yet to be commercialised.

Commercial PCR-based kits

Currently there are three manufacturers producing kits based on PCR for the detection of foodborne microorganisms. BAX (Qualicon, USA) utilises tableted reagents and a conventional thermocycler, gel electrophoresis-based approach. Positive samples are visualised as bands on an electrophoresis gel. BAX kits are available for *Salmonella* (Bennett *et al.*, 1998), *Listeria* genus, *Listeria monocytogenes*, *E. coli* 0157 : H7; the tests for *Salmonella* and *E. coli* 0157 have been through an Association of Official Analytical Chemists Research Institute (AOACRI) testing procedure and have gained AOACRI Performance Tested Status.

The second of the commercially available PCR kits is the Probelia kit (Sanofi, France); this uses conventional PCR followed by an immunoassay and colorimetric detection system. Kits are available for *Salmonella* and *Listeria*.

The final commercial PCR system is the TaqMan system (Perkin Elmer, USA). This uses a novel probe system incorporating a TaqMan Label. This is non-fluorescent in its native form, but once the probe is bound between the primers of the PCR reaction, it can be acted upon by the DNA polymerase enzyme used in PCR to yield a fluorescent end-product. This fluorescence is detected by a specific fluorescence detection system. TaqMan kits are available for *Salmonella* and under development for *Listeria* and *E. coli* 0157. Perhaps one of the most interesting future aspects of TaqMan is its potential to quantify an analyte. Currently PCR-based systems are all based on presence/absence determinations. TaqMan procedures and instrumentation can give information on actual numbers. Therefore the potential for using PCR for rapidly counting microorganisms could now be achieved.

Separation and concentration of microorganisms from foods

In recent years there has been considerable interest in the potential for separating microorganisms from food materials and subsequently concentrating them to yield a higher number per unit volume. The reason for this interest is that many of the currently available rapid test methods have a defined sensitivity, examples are: 10^4/ml for ATP luminescence, 10^6/ml for electrical measurement, 10^5–10^6/ml for immunoassay and DNA probes and approximately 10^3/ml for current PCR-based kits. These sensitivity levels mean that a growth period is usually required

before the rapid method can be applied and this growth period may significantly increase the total test time.

One way in which this problem can be addressed is by separating and concentrating microorganisms from the foods, in order to present them to the analytical procedure in a higher concentration. An additional advantage is that the microbial cells may be removed from the food matrix, which in some cases may contain materials which interfere with the test itself. A simple example of the use of concentration, is in the analysis of clear fluids (water, clear soft drinks, wines, beers, etc.). Here contamination levels are usually very low, thus large volumes are membrane filtered to concentrate the microorganisms onto a small area. These captured organisms can then be analysed. A thorough review of separation concentration methods has been given by Betts (1994). They broadly fall into five categories:

1. filtration
2. centrifugation
3. phase separation
4. electrophoresis
5. immuno-methods.

Of these categories only one has reached commercialisation for use in solid foods, that is the immuno-methods. Immunomagnetic separation relies on coating small magnetic particles with specific antibodies for a known cell. The coated particles can be added into a food suspension or enrichment, and if present, target cells will attach to the antibodies on the particles.

Application of a magnetic field retains the particles and attached cells allowing food debris and excess liquid to be poured away, thus separating the cells from the food matrix and concentrating them. This type of system has been commercialised by Dynal (Norway), LabM (England) and Denka (Japan), an automated system incorporating the procedure is produced by Foss Electric (EIAFOSS). The various companies produce kits for *Salmonella*, *Listeria*, *E. coli* 0157, other verocytotoxin producing *E. coli* and *Campylobacter*. Immunomagnetic separation systems for detecting the presence of *E. coli* 0157 have been very widely used and become accepted standard reference methods in many parts of the world.

Identification and characterisation of microorganisms

Once an organism has been isolated from a food product it is often necessary to identify it; this is particularly relevant if the organism is considered to be a pathogen. Traditionally, identification methods have involved biochemical or immunological analyses of purified organisms. With the major advances now taken in molecular biology, it is now possible to identify organisms by reference to their DNA structure. The sensitivity of DNA-based methods will in fact allow identification to a level below that of species (generally referred to as characterisation or sub-typing). Sub-typing is a powerful new tool that can be used by food microbiologists not just to name an organism, but also to find out its origin.

Therefore it is possible in some cases to isolate an organism in a finished product, and then through a structured series of tests find whether its origin was a particular raw material, the environment within a production area or a poorly cleaned piece of equipment.

A number of DNA-based analysis techniques have been developed that allow sub-typing, many of these have been reviewed by Betts *et al.* (1995). There is, however, only one technique that has been fully automated, and made available to food microbiologists on a large scale, and that is Ribotyping through use of the Qualicon RiboPrinter (Qualicon, USA). This fully automated instrument accepts isolated purified colonies of bacteria, and produces DNA band images (RiboPrint patterns), that are automatically compared to a database to allow identification and characterisation. The technique has successfully been used within the food industry to identify contaminants, indicate the sources and routes of contamination and check for culture authenticity (Betts, 1998).

2.8 Future trends

The food industry has the responsibility to produce safe and wholesome food and providing this assurance is ultimately the microbiological goal. A microbiology test that could analyse a batch of food non-destructively, on-line and with the required accuracy, sensitivity and specificity is the 'Holy Grail' and would provide this assurance. However, our current technical capabilities fall well short of this ideal situation.

Conventional microbiological methods have remained little changed for many decades. Microbiologists generally continue to use lengthy enrichment and agar-growth-based methods to enumerate, detect and identify organisms in samples. As the technology of food production and distribution has developed, there has been an increasing requirement to obtain microbiological results in shorter time periods.

The rapid growth of the chilled foods market, producing relatively short shelf-life products, has led this move into rapid and automated methods, as the use of such systems allows: (a) testing of raw materials before use; (b) monitoring of the hygiene of the production line in real time; and (c) testing of final products over a reduced time period. All of these points will lead to better quality food products with an increased shelf-life.

All of the methods considered in this chapter are currently in use in Quality Control laboratories within the food industry. Some (e.g. electrical methods) have been developed, established and used for a considerable time period, while others (e.g. PCR), are a much more recent development. The future of all of these methods is good; they are now being accepted as standard and routine, rather than novel. Some users are beginning to see the benefits of linking different rapid methods together to gain an even greater test rapidity, e.g. using an enzyme immunoassay to detect the presence of *Listeria* spp., then using a species-specific nucleic acid probe to confirm the presence or absence of *L. monocytogenes*.

One of the problems of many of the rapid methods is a lack of sensitivity. This does in many cases mean that lengthy enrichments are required prior to using rapid methods. Research on methods for the separation and concentration of microorganisms from food samples would enable microorganisms to be removed from the background of food debris and concentrated, thus removing the need for long incubation procedures. The developments in DNA-based methods for both detection and identification/characterisation have given new tools to the food microbiologist, there is no doubt that these developments will continue in the future giving significant analytical possibilities that are currently difficult to imagine.

In summary, methods have an important place in our armoury against the threats posed by microorganisms in food. Owing to the diversity of applications and user requirements and the shift from QC to QA, methodology still plays a key role in assuring food safety and new methods still have the potential to bring benefits.

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3

Modelling the growth, survival and death of bacterial pathogens in foods

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3.1 Introduction

Simple mathematical models have been used in food microbiology since the early 1920s when much work was done to support the safe operation of canning processes. To calculate the process time needed to produce 'sterility' in canned foods, Bigelow *et al*. (1920) came up with a lethal rate curve to relate the reciprocal of the time needed to destroy all the spores present in the food to the process temperature. Ball (1923) introduced the term '*z*' to describe the slope of this curve with the value of the slope equal to the temperature change needed to effect a 1 log change in the time. He also presented formulae and graphical methods to calculate the total lethality of a thermal process including come-up and cooling times. At about the same time, the classic work of Esty and Meyer (1922) on thermal resistance of toxigenic spores defined the processing conditions needed to ensure destruction of *Clostridium botulinum* spores and gave rise to the so-called 'botulinum cook', or minimum process lethality required to destroy 10^{12} spores of *C. botulinum* in foods with pH greater than 4.5. The familiar term '*D*' to define the time to cause a 1 log reduction in microbial population at a given temperature was not introduced until later (Stumbo *et al*., 1950), that concept having previously been represented by ζ (zeta), giving rise to some confusion between *z* and ζ.

According to Lambert and Johnston (2000) similar models were used even earlier for assessment of chemical disinfection. The fermentation industries developed more sophisticated models for growth rates, product yield, mass

transfer requirements, etc., in the interests of designing more efficient processes (Pirt, 1975). However, the widespread acceptance of mathematical modelling in food microbiology has gained ground slowly only since the early 1980s.

Applications in food microbiology include models that predict the growth rate of bacterial pathogens in response to product or environmental factors such as water activity (a_w), temperature or pH (Buchanan and Phillips, 1990; Gibson *et al*., 1988; McClure *et al*., 1997; Sutherland and Bayliss, 1994). These can help food processors to produce safe products with less reliance on laboratory testing. Growth models can be used to design safe product formulations, to set appropriate storage conditions and to explore the maximum interval between cleaning and sanitising for process equipment. Models that can predict the rate of death of pathogens or spoilage organisms can be used to design safe and effective processes.

The potential of models to help improve product safety was identified by the UK and US governments in the 1980s and both funded sophisticated programmes to develop models for growth, survival and death of foodborne pathogens. The success of those programmes resulted in suites of models that are available for consultation. The UK government models reside in a software package called Food MicroModel available from the Food MicroModel subsidiary of Leatherhead Food Research Association (see section 3.11 Sources of further information). The approach to developing those models was described by McClure *et al.* (1994a). The US government made its models freely available and they can be downloaded from the US Department of Agriculture website (see Sources of further information) using a personal computer with Internet access.

While the science and applications of modelling in microbiology are becoming ever more sophisticated (Baranyi and Roberts, 2000), this chapter is designed to be a practical guide to modelling, supported by references to primary sources of modelling information. Our aim is to give readers an appreciation of the principles involved in creating useful models and help them to identify soundly based models.

3.2 Approaches to modelling

3.2.1 Principles

By 'model' we mean an equation that describes or predicts the growth, survival or death of microorganisms in foods. In food microbiology, these models are empirical. In other words they simply relate the microbial growth, survival or death responses to the levels of the controlling factors throughout the experimental design space. They tell us nothing about the physiological mechanisms or biological, chemical or physical principles that drive the responses. For this reason they are sometimes known as 'black-box' models. Empirical models should not be used outside the range of the factors used to create them because there is no underlying principle on which to base extrapolation. Hence, we must

carefully consider the range over which they will be used before beginning experimentation. The ranges may be decided from our knowledge of the conditions found in relevant product categories and/or knowledge of the likely microbial responses, e.g. minimum a_w or pH for growth of the organism of interest. Microbial responses to single factors are well documented (ICMSF, 1996) but responses to factors acting together are still much less well characterised.

A simplifying common approach is to experiment in liquid laboratory media that are homogeneous and less complex than the foods that we are ultimately interested in. This restricts the range of controlling factors to a relatively few, such as a_w, pH, temperature, acid concentration, salt concentration or preservative concentration. As long as appropriate factors are chosen they will predict the greatest part of the microbial response. Normally we select the three or four factors most relevant to the intended use of the model based on previous experience with the organism of interest. Experimentation with more factors is possible but rapidly becomes more complex and expensive as the number of factors increases. Where previous experience is limited, as with a new or emerging pathogen, or with combinations of factors acting together, screening experiments can help to identify the range of conditions to test and estimate the response.

In experimenting prior to model building our objective is to obtain high-quality data. The range of conditions must be covered in sufficient detail to see the full range of responses in the conditions of interest based on the intended final use of the model. There should be at least some replication to minimise the impact of variability inherent in the responses. The variability in response is usually highest in the most 'marginal' conditions, i.e. those closest to the limits permitting growth or causing death. This requires careful attention to the details of the experimental design and implies that considerable attention should be paid to the 'marginal' conditions. Unfortunately for experimenters the 'marginal' conditions are usually the hardest to work in! Several authors have given detailed descriptions of experimental design for modelling in food microbiology (Davies, 1993; McMeekin *et al*., 1993; Ratkowsky, 1993). Guidelines for data collection and storage were put together by the protocols group of the UK Food MicroModel programme (Kilsby and Walker, 1990) and discussed by Walker and Jones (1993).

3.2.2 Types of models

Perhaps the easiest way of considering the different types of models is in terms of their use either to assess the increase of a hazard (e.g. growth models) or the reduction of a hazard (inactivation or survival models). In this way, models can be classified based on the nature of the microbial response.

Growth models are concerned with responses where at least part of the range of conditions permits growth to occur and can describe the increase in numbers with time (kinetic), the conditions allowing growth or no growth (boundary) or the chance of growth (probabilistic).

- *Kinetic growth models* are usually based on growth curves over a range of conditions and can allow growth rates to be predicted. These models can also predict the time needed to go from the starting condition to the final condition of interest, e.g. hours for 3 log increase in numbers. If desired, they can predict a complete growth curve for the conditions of interest.
- *Boundary models* describe the limits of a set of conditions that permit or do not permit growth of the organism. This type of models predicts a time to growth based on data collected as a qualitative response, i.e. growth or no growth, at intervals over a specified time period. These models are useful for predicting growth times in terms of weeks or months and can be used to identify new treatment conditions that do not allow growth of the microorganisms of concern. Boundary models are complementary to kinetic growth models, which typically predict growth in terms of hours or days. They do not predict growth curves. A feature of the data sets used to build these models is that the no-growth observations, known as 'censored' data, tell only that growth was not observed during the experiment. Owing to the censoring, these data cannot be handled by standard regression modelling approaches. Rather, statistical techniques designed for 'survival analysis' must be used.
- *Probability or probabilistic models* describe the likelihood of a particular response being observed or the time until an event occurs. Such models are appropriate when a binary response (i.e. either 'growth' or 'no growth') is available and a model for the probability of growth at a defined point in time desired.

Death or inactivation models are designed to predict for conditions where a lethal process is deliberately applied. In consequence, microbial death is relatively rapid. Most of these models were developed for thermal processes but there are some for other deliberately lethal conditions including irradiation treatment and high-pressure processing. They are usually kinetic in nature and describe inactivation with time of lethal treatment.

Survival models relate to transitional conditions between growth and death. Typically death occurs relatively slowly in conditions where growth is prevented but no deliberately lethal treatment is applied (e.g. at ambient temperature and low a_w).

Models may naturally fit into both approaches to classification, e.g. kinetic death models or growth boundary models.

3.3 Kinetic growth models

A good kinetic growth model requires high-quality growth curves across the range of conditions of interest. Good growth curves typically have 10 or more data points, though the placement of points can be more important than the number in order to identify regions of rapid change (Fig. 3.1). For good estimates of the length of the lag phase and the rate of growth in the logarithmic phase it

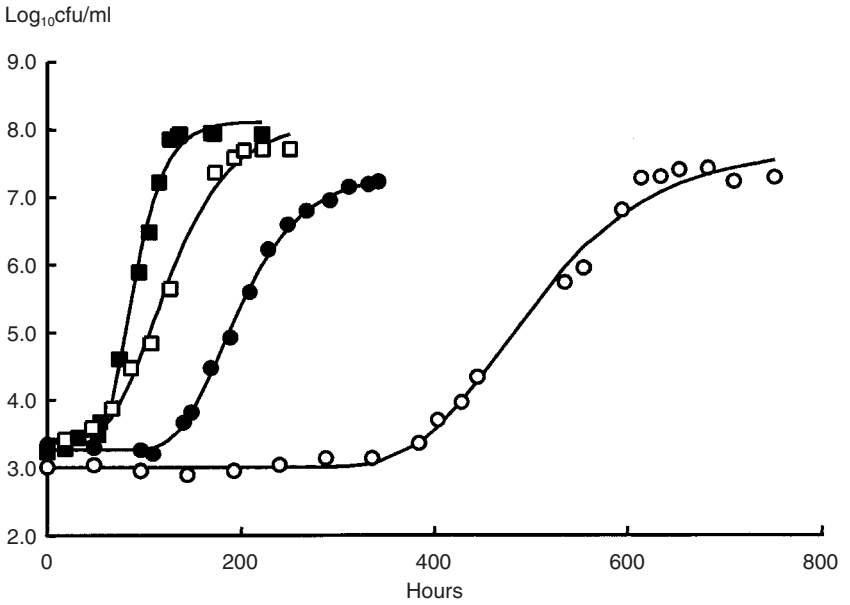

Fig. 3.1 Growth curves for *Aeromonas hydrophila* under different conditions showing that fitted modified Gompertz curves give good descriptions of growth when based on high-quality colony count data in sufficient amounts. In this case the data are for growth in nutrient broth, pH 7.0 at 5 °C with NaCl concentrations of 0.5 ■, 1.5 □, 2.5 ● and 3.5 ○ %w/v. Redrawn from McClure *et al.* (1994b) with permission.

is important to have data points close to the point of inflection that marks the transition between these two phases. Data points are needed close to the point of inflection between the logarithmic growth and stationary phases if the final population density is of interest. Data from a collection of good-quality growth curves are used to build the model. There is no simple rule for the number of growth curves required. A model based on three controlling factors probably needs 70–100 curves but with careful consideration of the placement of curves in the experimental design based on prior knowledge and experience, and a little luck, fewer may be sufficient.

3.3.1 Experimental design

In this chapter we use the following terms:

- *Factor:* a factor is an independent variable, in other words a design condition such as temperature or pH, that takes more than one value.
- *Treatment:* a treatment is a unique combination of factors and their levels. For example, pH 6.5 and 25 °C.
- *Response:* sometimes known as a dependent variable, the response is the thing we are measuring and modelling, e.g. growth rate or viable count.

- *Parameter:* a parameter is a term in a model that is applied to the value of a factor to obtain the prediction. For example in $\ln N = \ln N_0 + \mu t$, N is the dependent variable (cell numbers), N_0 is the initial value of the response, μ is a parameter (in this case growth rate) and t is the independent variable (in this case time).

The first consideration when designing kinetic growth modelling experiments is the intended use of the model. We can never consider all the factors involved in food composition so we must select those that are most important for controlling microbial growth in the foods that we are concerned with. Significant factors are commonly a_w, pH and temperature and there is good evidence that these account for the largest part of the microbial response in many foods. Careful consideration should be given both to relevant factor levels and to how they are achieved.

Salt (NaCl) is the humectant most commonly used to control a_w because it is cheap, convenient and used in preparation of a wide range of foods. However, different growth responses can be observed at a single a_w when it is controlled by different humectants (Slade and Levine, 1988). For practical purposes, a_w is frequently good enough to guide decision making but by itself it is not enough to accurately predict the limits of growth of any microorganism. For example, the minimum a_w for growth of proteolytic *Clostridium botulinum* is given as 0.96 with NaCl as the humectant and 0.93 with glycerol (Lund and Peck, 2000). There is a growing understanding of the influence of the humectant on the physical properties of the system and the physiological effects that together influence the microbial growth response. For example, NaCl is an ionic humectant that has specific effects on membrane transport systems but does not form an aqueous glass. It can place both osmotic and ionic stresses on bacterial cells. Glycerol is a non-ionic humectant that passively permeates the cell membrane and has no direct effect on ion transport systems. It forms an aqueous glass that reduces the molecular mobility of the system and affects the amount of osmotic stress placed on the bacterial cells (Slade and Levine, 1988). Each glass-forming solute has a characteristic glass transition temperature (T_g). The role of T_g in control of microbial growth is currently controversial, but it is sensible to approach differences in humectant with caution when designing models to support safety-critical decisions (Stewart *et al*., 2001).

The acidulant used to adjust pH can also influence the microbial response observed. The simplest approach is to use hydrochloric acid as the acidulant. This minimises the influence of undissociated organic acids and ignores the effects of pH buffering that can be seen in foods. Models based on HCl as the acidulant tend to predict faster growth than models based on organic acids. Where organic acids are significant components of the foods of interest, for example in fermented foods and pickles, it is important to be able to determine the concentration of undissociated acid.

Other factors that may be important include the type and concentration of any added preservatives (e.g. nitrite, sorbate, benzoate, propionate), the composition of the atmosphere around the product and the structure of the food. In these cases

we need to be aware that the number of experimental treatments rises rapidly with the number of factors. It may be necessary to recognise a trade-off between the number of factors and the number of factor levels. Alternatively we may substitute an approach such as growth boundary modelling that is more amenable to the use of automation, for example automated turbidimetry or automated conductance or impedance measurements.

We require that models predicting the growth of pathogens should be 'fail safe'. That is they should err on the side of safety and not over-predict the time to the final condition of interest. For this reason it is more efficient to use cocktails of representative strains than single strains. A cocktail helps to overcome the variability between strains and increase the chance of detecting the fastest growth at different points in the experimental matrix. This helps to create a 'leading-edge' model, without repeating the experiment many times using different strains. A representative cocktail is likely to contain a blend of laboratory strains, for example stored isolates from relevant food-poisoning outbreaks or well-characterised experimental strains, and isolates from relevant foods. Strains should, as far as possible, be reasonably typical of those likely to be encountered in the foods that represent the intended use of the model. The value of models is significantly reduced if they become unrealistically conservative as a result of using rare strains with unusually fast growth rates, or extreme tolerance to one or more of the controlling factors.

Inoculum size and condition are also important. For kinetic growth studies, inoculation to give an initial concentration of 10^2–10^3 cfu/ml is ideal because it allows counts to be measured during the lag phase but reduces the risk of unrealistically raising the probability of growth occurring as can happen with high inoculation levels. Low-level inoculation also approximates the concentration of pathogens expected in foods. The culture history in terms of growth and storage conditions, etc., can significantly affect the length of the lag phase so preparation of the inoculum must be standardised to minimise its influence on variability between repeat experiments. The method of inoculum preparation should tend to minimise the length of the lag phase.

It is important to be aware that some pathogens may produce toxins even when growth is very limited. For example, it is commonly considered that *Clostridium botulinum* may form toxin in association with only 1–2 log increase in numbers. For such pathogens, the methods used to measure growth must be sensitive enough to detect all relevant amounts of growth. Toxin tests on media from marginal conditions at the end of experimentation can be used to confirm 'no-growth' observations.

A number of formal statistical designs may be used to help design microbiological modelling experiments (Davies, 1993; McMeekin *et al*., 1993; Ratkowsky, 1993). Even so, for the microbiologist beginning a modelling study there is no substitute for involving a good statistician or mathematician at all stages of experimental design, experimentation and data analysis. Whatever design is chosen, it should be flexible enough to be modified in the light of results as they are obtained. Some principles to consider are as follows.

Levels of factors

We are usually interested in modelling second order behaviour, i.e. quantifying any curvature in the response. For this we need a minimum of three different levels spanning the range of relevant conditions and more treatments than degrees of freedom for the model to be built. For simple three-factor models, e.g. pH, a_w and temperature, a basic matrix of five levels of each factor is better. This gives the potential for 5^3, or 125, different treatments in the matrix requiring growth curves to be measured. In practice, considerably fewer (perhaps 70 to 100) will be sufficient because some of the original 125 treatments will involve combinations of factors that do not support growth. By dropping certain treatment conditions, the design becomes 'unbalanced' and the ability to gauge the effect of each factor is lost. Since the main objective here is to develop predictive models, this is not an important concern.

For most factors, equal linear spacing of levels is initially preferred. However, geometric spacing may be preferred when this leads to more uniform changes between levels in the size of the microbial response. For example, equal spacing in the level of hydrogen ion concentration $[H^+]$ may be better than equal intervals of pH (which give logarithmic changes in $[H^+]$. Also the use of a zero level for a factor requires careful consideration, e.g. for a preservative the levels might be 0, 500 and 1000 ppm. The use of a zero level is potentially risky because there may be a discontinuity in the response as the level of a factor goes from presence (however small) to complete absence. When subject matter expertise indicates that such a discontinuity is unlikely, the experimenter should feel free to use zero level designs.

Placement of treatments is also important. The microbial responses change most rapidly, and the variance is highest, as the limits for growth are approached. Placing treatments closer together in these conditions is valuable.

Replication

Replication is used to improve the estimate of the response through averaging. Classical training in microbiology emphasises the need to repeat all experiments and replicate all points because microbiological data usually exhibit large amounts of variability. The cost of labour and materials to follow this ideal becomes prohibitive when attempting to create and validate kinetic microbial growth models. To make progress we need to make some pragmatic compromises and recognise that we have done so. The principle is to build in independent tests of individual results by comparing them with the results from similar conditions, rather than simply repeating them. Just as individual points on a curve contribute to defining the shape of the curve, so individual treatments in a matrix contribute to defining the overall shape of the response surface described by the model. Hence the first emphasis is on populating the experimental matrix as thoroughly as possible with treatments, even at the expense of limited repeats. The second emphasis is on independent repeats of selected treatments in the matrix where variability is known to be particularly high. These treatments are those in the most

marginal growth conditions. The repeats use media made on different occasions and are run on different days, preferably by different experimenters. Sample replicates, i.e. multiple samples from each treatment, are also useful when the design includes a densely populated experimental matrix and ample repeats but make the smallest contribution to the overall performance of the model. The common practice of counting colonies on duplicate plates to reduce measurement error can itself contribute some information on variability, particularly if duplicate plates are identified and specifically recorded when the difference between them falls outside a predetermined range. These steps are all taken on the understanding that the model will be validated against data produced in foods (ideally in a different laboratory) to give as independent a test as can be arranged of the performance of the model. None of these comments is intended to imply that replication is not useful or valuable, merely that, as with other elements of experimental design, there can be a trade-off between costs and benefits.

Growth curves
The point of growth curves for kinetic modelling is to allow the length of the lag phase and the slope of the logarithmic growth phase to be estimated accurately. It is hard to achieve this consistently with fewer than 10–12 points, though as few as 7 or 8 well-placed points can give good curves if the points of inflection at the beginning and end of the logarithmic growth phase are clearly defined.

3.3.2 Experimentation

Screening experiments
To produce large numbers of growth curves is laborious. The investment of time and effort is rewarded when a high proportion of the experimental data can be included in the data set used for model building. Screening experiments can help to define the experimental space before the final design is completed. For many of the factors controlling the growth of microorganisms the limits of growth given in the literature are defined in terms of only a single factor unless modelling studies have already been done. When two or more factors are studied together, the limits can be radically different from published values based on single factors. It is also hard to find good estimates of the lag phase at non-optimal values even of single factors. Both of these limitations make the planning of modelling studies harder. One solution is to run screening experiments over a wide range of the factors of interest in the intended combinations. Laboratory instruments based on automated readings of turbidity, conductance or capacitance, metabolite production or other markers of microbial growth can be particularly valuable when the data can be plotted as ‘pseudo-growth curves’. These results can indicate where to place growth curves and how frequently to take samples for counting. However, such instruments are costly. Simple visual inspection of flasks or tubes for turbidity, indicating that growth has occurred, repeated at intervals, can

exclude 'no-growth' conditions and indicate a range of lag times to expect under different non-optimal growth conditions.

Order of experiments
It is tempting to begin working at conditions that are close to the limits for growth (as indicated by screening experiments) on the grounds that the experiments at these conditions must be run for the longest time, or even to try to work on all conditions at once. The disadvantage of these approaches is that they can lead to a high proportion of relatively poor growth curves or experiments in 'no-growth' conditions. It is probably more efficient to begin working at conditions near the optimum for growth where work progresses quickly. Good growth curves can be obtained in only a few 'all-night' experiments and the results can be used to refine the plans for work with less optimal combinations of factors. As experiments work out towards the limits for growth it may become apparent that the position of growth curves should be modified to obtain the best definition of the rapidly changing responses in the most marginal conditions.

Note that this selective ordering of treatments flies in the face of the usual notions of randomisation. For reasons outlined above, such restricted randomisation can be tolerated. We must be especially careful, though, to ensure that extraneous factors do not affect the experimental results.

Stability of treatments
Treatments may change during the course of an experiment, particularly if growth is permitted, as a result of microbial metabolism that may cause the pH to drop and the a_w to rise in response to fermentation of sugars in the medium, for example. In kinetic modelling the volumes of media are usually relatively large, the water activities relatively high and the duration of experiments relatively short, such that changes in a_w caused by evaporation are likely to be negligible. Even so, it is good practice to make measurements of the experimental variables during and at the end of an experiment. These measurements can be useful in planning follow-up studies and interpreting unexpected results.

Traceability of measurements
One assumption underlying the application of models is that the results are independent of the laboratory in which the model was created. This may never be true, but will be most closely approached when the accuracy of all measurements is known. Although this sounds obvious, it is not simple to achieve because all measuring devices (thermometers, pH meters, balances, etc.) and control equipment (incubators, waterbaths, etc.) introduce errors. Formal quality management systems include those based on the ISO 9000 series, ISO Guide 25 or the principles of Good Laboratory Practice (Wood *et al*., 1998). They can help to ensure the accuracy of measurements through a chain of evidence linking laboratory measurements to national or international standards of measurement. Use of such systems is still not common in research laboratories. At the very least, all measurements of weight, volume, temperature, pH and a_w (or relative humidity)

should be made using equipment with calibration traceable to the relevant national standard.

Temperature of incubation should ideally be monitored continuously. Where this is not feasible some indication of the range of temperatures experienced during incubation should be obtained, e.g. using a maximum and minimum thermometer.

Time can be measured using clocks or timers with traceable calibration and this practice is encouraged. However, modern electronic clocks are generally so accurate that any errors attributable to the clock would be insignificant compared with other sampling and recording errors. When the interval between observations is long and the record is of time to an event (e.g. time for optical density to exceed some threshold value) it is important also to record the last time at which the event was *not* observed. This is likely to be more of a concern in boundary modelling than in kinetic modelling.

3.3.3 Data analysis

To generate the model(s) that relate the growth responses to the experimental treatments the first step is to plot the growth curves for each experimental treatment and inspect them. Reject those where no growth was seen or where the growth curves are clearly inadequate because of known laboratory errors. Continue with the remaining high-quality data sets. Fit growth curves for individual treatments, extract the values of the parameters that describe each curve, then fit the model that relates the changing values of the growth parameters to the levels of the experimental factors. This is often called the two-step approach. Selection of growth curves for modelling does not imply arbitrary discarding of data. For example, significant variability in the observed response, often termed 'noisy data', will lead to a model having higher error associated with its predictions. However, the variability may itself be an important feature of the response and an estimate of that variability can be valuable.

With access to appropriate statistical software, it is possible to fit a response surface to define the microbial count in terms of the independent variables and time. This may be called a one-step approach. One-step fitting can be particularly helpful when the data set contains incomplete growth curves because it captures more of the information in the data than the two-step approach. But it is more demanding both of software and statistical expertise than the two-step approach.

Handling variance

To obtain the best fit of a model to the data the variance should be independent of the value of the response variable (in this case the microbial count). However, in microbial count data the variance tends to increase with the count. Transforming the data by taking the logarithm of the counts normalises the variance, i.e. makes it independent of the value of the count. Kilsby and Walker (1990) discussed some considerations relevant to the most appropriate counting technique.

Fitting growth curves

Several different approaches give reasonable descriptions of sigmoid bacterial growth curves (i.e. typical growth curves exhibiting lag, log and stationary phases) and examples are shown in Table 3.1. The fitness of any particular approach is partly dependent on the objective underlying model building. Van Gerwen and Zwietering (1998) discussed some advantages of different approaches when the objective is to develop models for risk assessment purposes. Among the approaches more commonly used are the modified Gompertz equation, the Baranyi equation and the logistic equation (Baranyi *et al*., 1993; Gibson *et al*., 1988). Combinations of linear models for exponential, lag and exponential or lag, exponential and stationary phases have also been used (Buchanan *et al*., 1997a) though the last of these is controversial (Baranyi, 1997; Garthright, 1997). Appropriate statistical software is used to find the parameters that allow the equation to predict the line that gives the best fit to the data. For Gompertz, Baranyi and logistic equations this involves non-linear regression analysis.

More than one curve-fitting approach may give acceptable models for a particular data set (Fig. 3.2). Selection of the best model is to some degree subjective and the choice may be simply pragmatic based on model validation, a view of the principles underlying the different modelling approaches or other relevant criteria.

The Baranyi model has advantages over the modified Gompertz equation in that it describes a growth curve where the lag phase has a slope equal to zero and a logarithmic growth phase with a slope that is practically a straight line. It is, however, computationally somewhat more difficult to use.

Modelling the response of growth parameters to levels of experimental variables

The process here is to use a regression modelling tool (available in many statistical data analysis packages such as the Statistical Analysis Systems (SAS) software (SAS Institute Inc., Cary, NC) to relate changes in the growth parameters to the levels of the experimental variables. A common strategy uses a polynomial model, though alternative models are available (Table 3.2). A polynomial model has the form:

$$\text{Response} = C_0 + C_1V_1 + C_2V_2 + C_3V_1V_2 + C_4(V_1)^2 + C_5(V_2)^2$$

where all Cs are constants and all Vs are variables. Start with a general model that includes terms for all the variables' main effects (V_1, V_2, V_3, etc.), their interactions, e.g. ($V_1 \times V_2$), and quadratics, e.g. $(V_1)^2$. Main effects are known as first order terms; interactions and quadratics are known as second order terms. Not all of the terms are equally influential. Regression coefficient estimates for each term in the model can be used to judge the relative importance of each term. The least influential terms are progressively removed and the regression modelling procedure is re-run in a series of iterations until the most parsimonious model, i.e. the model with the fewest possible terms that accurately matches the observations, is achieved. The principle of parsimony is observed because there is uncertainty

Table 3.1 Commonly used models that describe microbial growth curves

Model	Equation	Where:
Exponential	$\ln(n) = \ln(n_0) + \mu t$	n = count/g; n_0 = count/g when t = zero; t = time (hours) μ = specific growth rate (per hour)
Lag-exponential	$\ln(n) = \ln(n_0)$, for $t < \lambda$ $\ln(n) = \ln(n_0) + \mu(t - \lambda)$, for $t \geq \lambda$	n = count/g; n_0 = count/g when t = zero; t = time (hours) μ = specific growth rate (per hour); λ = lag time (hours)
Modified Gompertz	$L(t) = A + C\exp\{-\exp[-B(t - M)]\}$	$L(t) = \log_{10}$ bacterial count at time t; B = the relative maximum growth rate (/h) M = the time at which maximum growth rate occurs (h); A = the lower asymptotic $\log_{10}$ bacterial count as t decreases indefinitely; C = the difference between A and the upper asymptotic $\log_{10}$ bacterial count as t increases indefinitely. Also: Lag = $M - (1/B)$ Growth rate = $[B \times C \times \ln(10)]/\exp(1)$ Peak population density = $\log_{10}(A + C)$ Generation time = $\log_{10}(2)\exp(1)/(B \times C)$
Baranyi	$y(t) = y_0 + \mu_{max} A_n(t) + \ln\left\{1 + \frac{\exp[\mu_{max} A_n(t)] - 1}{\exp(y_{max} - y_0)}\right\}$	μ_{max} = maximum specific growth rate; $y(t)$ = ln population concentration at time t; y_0 = ln initial population density; y_{max} = ln maximum population density; A_n is an adjustment function as defined by Baranyi *et al.* (1993), considered to account for the physiological state of the cells. Its role is to define the lag phase.
Jones and Walker	$N = N_0 2^{[G(t) - M(t)]}\ t \geq 0$	$G(t) = A\left\{1 - \left[1 + (t/B) + \frac{1}{2}(t/B)^2 + \frac{1}{6}(t/B)^3\right]\exp(-t/B)\right\}$ $M(t) = \exp[(t - D)/\mathrm{C}] - \exp[-(t - D)/C] - \exp(-D/C) + \exp(D/C)$ A, B, C and D are constants, t represents time and N_0, the count at time 0, and N are actual counts not log counts.
Logistic	$\ln(n) = \ln(n_0) + \frac{a}{1 + \exp(b - ct)}$	n = count/g; n_0 = count/g when t = zero; t = time (hours); a, b and c are all fit parameters; t = time (hours)

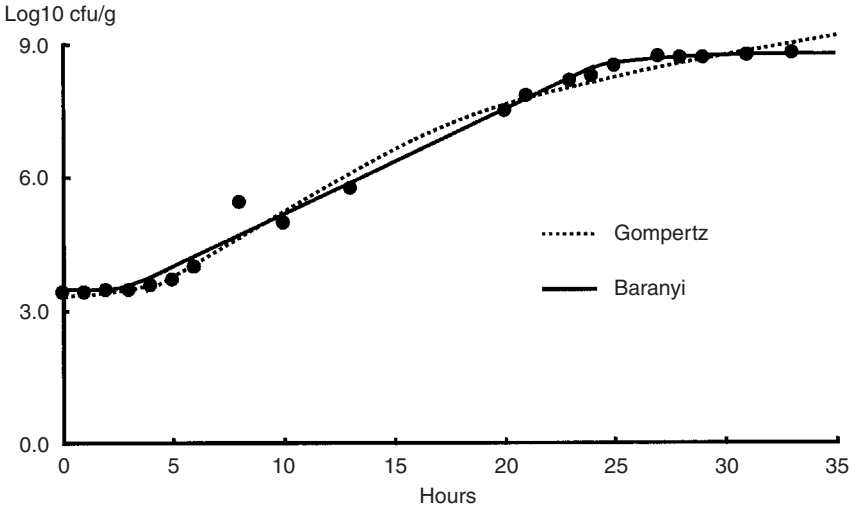

Fig. 3.2 Comparison of the fits given by Gompertz and Baranyi models on viable count data (adapted from Baranyi *et al.*, 1993), illustrating that the two models give similar results.

associated with the estimate of each term. A model based on the few most influential terms minimises the influence of this uncertainty while retaining most of the predictive power of the underlying factors. At each iteration of the process it is useful to plot the experimental observations against the surface predicted by the model and look for outlying observations that reduce the overall goodness of fit and 'leverage points' that have a disproportionate influence on the model because of their position. It is sensible to discard outliers if, upon re-examination, they are shown to be due to some identifiable laboratory error.

The modelling process can sometimes be done in fewer iterations and with an improvement in the overall goodness of fit if some of the inputs can be held constant. For example, we might use the mean initial $\log_{10}$ cfu/ml from all experimental treatments as the starting value for all growth curves rather than the actual measured value for each treatment. This makes sense because differences between treatments in the starting count are more likely to be a function of inoculation error than of any influence of the experimental factors. In this example we lose essentially nothing and gain some simplicity. Another example would be to fix the population density at stationary phase. Here the price of simplicity might be to miss some real differences in the final population density. This might be acceptable if we are mainly interested only in the length of lag phase and the growth rate.

Diagnostic tests (mathematical tests) for model evaluation

A range of mathematical and visual diagnostic tests can be used to help evaluate models (Baranyi *et al.*, 1999). A good place to start is to plot observed growth

Table 3.2 Some secondary models for growth parameters. Other examples are given in, e.g. McMeekin *et al*. (1993) and van Gerwen and Zwietering (1998)

Model type	Equation	Where	Comments
Polynomial	$\text{Response} = C_0 + C_1V_1 + C_2V_2 + C_3V_1V_2 + C_4(V_1)^2$	All *C*s are constants and all *V*s are variables	Straightforward to apply by multiple linear regression. No knowledge of process needed. But no theoretical foundation, no biological meaning to parameters
Square root	$\sqrt{\mu} = b(T - T_{\min})\sqrt{(V_1 - V_{1\min})}\sqrt{(V_2 - V_{2\min})}$	μ is specific growth rate (/h); T is temperature in kelvin; V_1 and V_2 are independent variables; and b is a fit parameter	Parameters claimed to be biologically interpretable and can calculate relative effects of each variable. But non-linear regression involved if pH and/or a_w are included. No theoretical foundation. Parameters extrapolated to growth limits
Arrhenius-type	$\mu = A\exp(-E_a/RT)$	μ is specific growth rate (/h); A is the 'collision factor'; E_a is the 'activation energy' of the system; R is the universal gas constant (8.31 J/mol/K^{-1}); T is temperature in kelvin	Theoretically grounded for chemical reactions involving collisions between simple models but purely empirical for biological systems. Describes temperature response only but has been modified to include other factors (e.g. a_w, Davey, 1989)

against the growth curves predicted by the model for the same conditions. Inspection of the plots quickly establishes how well the model predicts across the range of conditions. Plots of observed versus predicted values for length of lag, growth rate and time to a defined increase in numbers for all the conditions used in building the model help to visualise its overall performance. Normal probability plots of residuals test the performance of the model across the whole range of conditions. Regression statistics such as the R^2 value can also help to quantify how well the model describes the variance in the data. This statistic can initially be troubling because R^2 varies inversely with the number of points and the variability in the data. Hence values in the region of 0.8 to 0.9 (or even lower) are not unusual for models, but look alarmingly low compared with the 0.99 or so that we are accustomed to seeing for individual curves.

Another possible method for model selection is to apply each model to a separate, unmodelled 'hold-out' sample. A hold-out sample is one that was included in the original design and run within the model-building experiment for the purpose of comparison with the model. The result from the hold-out sample is not included in the statistical analysis for model-building purposes. Rather it is compared with a prediction from the model for the conditions relevant to the hold-out sample. For each sample, calculate the mean squared prediction error by computing a prediction and then averaging the squared prediction error. The model with the smallest squared prediction error is deemed the best.

Model acceptance

Examination for biological sense

Once the model has been generated and the statistical diagnostic tests have shown that it gives a good description of the data it should be examined for 'biological sense'. This examination tests that predictions from the model behave as expected on the basis of microbiological experience. It is often easiest to assess biological sense using contour or surface plots of predictions for a matrix of conditions similar to that used to create the model (Fig. 3.3).

Validation

The final step in building confidence in the model is to validate it by comparing predictions from the model to observed growth responses in relevant foods. In some cases it is possible to extract validation data from the literature. Unfortunately, data in the literature are often too incomplete to use and we must resort to experimentation. In most cases we need analytical measurements of the relevant factors (salt, pH, a_w, etc.) in the food. Then we compare actual growth data points with predicted growth curves (Fig. 3.4). It is less critical to catch the points of inflection than it is with the model-building experiments and four to six well-spaced points per curve can be enough. However, more points can allow growth parameters to be derived from the food data and this can facilitate comparison with the model predictions. Good agreement between predicted and observed responses helps to build confidence in the model. The comparison is often shown

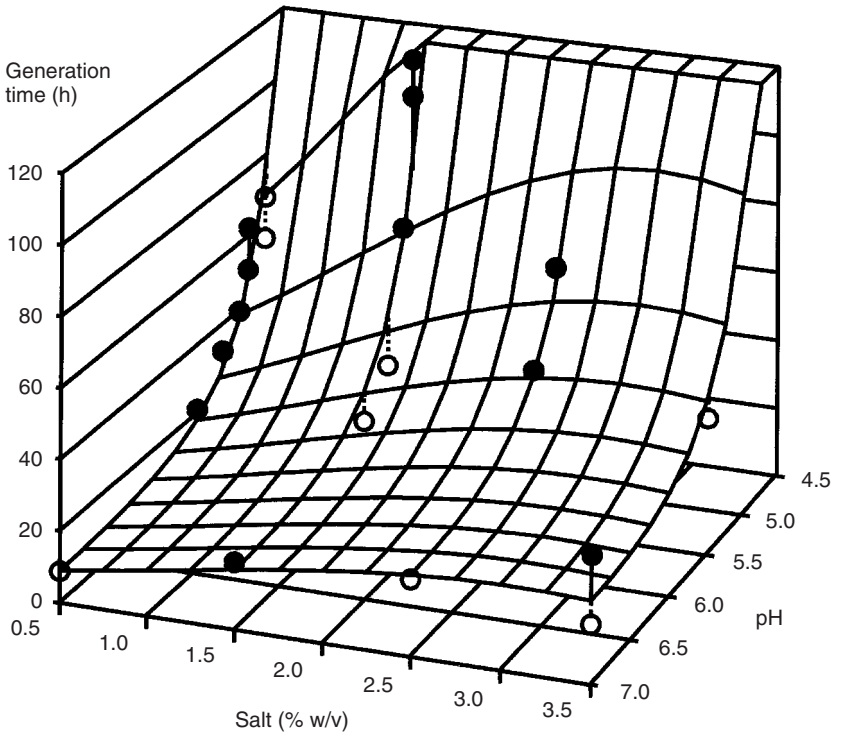

Fig. 3.3 Quadratic response surface for generation time of *Aeromonas hydrophila* with respect to pH and salt concentration (adapted from McClure *et al*., 1994b). Open points lie below the surface and solid points are above it. This illustrates a model that makes 'biological sense' with generation times becoming longer as conditions become more inhibitory.

as a plot of observed against predicted values (Fig. 3.5) in which the responses observed in foods should be no faster than those predicted by the model for maximum confidence. Data should be obtained for all relevant food types (e.g. meat, poultry, fish, dairy, cereal, eggs) for which we intend to use the model to support decision making.

Perhaps the greatest difficulty in working with foods is that they are not naturally sterile. Irradiation of foods for validation studies can simplify recovery of the pathogen of interest from inoculated foods. Where (more usually) we have no convenient access to irradiation facilities, it may be necessary to use selective media to pick the species of interest out from the general microbial population in the food. In this case it is important to be sure that the recovery on selective media is comparable with that on the non-selective media used for counting during model building. We should also consider whether to count all organisms of the relevant species, or only those that have grown from the strains used to inoculate the food. If the latter, and if the incidence of the organism in the food

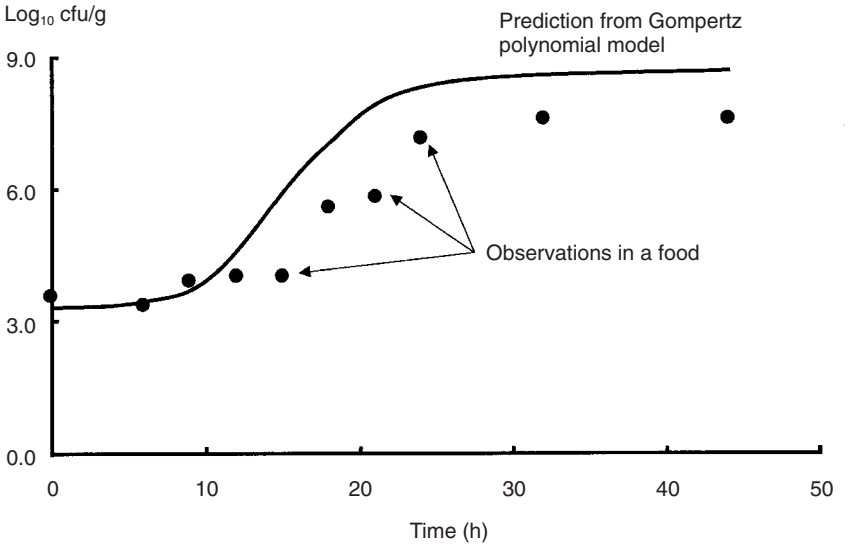

Fig. 3.4 An example of food validation data plotted against a predicted growth curve. Predictions and observations were for *Bacillus licheniformis* in custard at pH 6.14, NaCl 0.3% and 28 °C. The prediction is from the Food MicroModel *B. licheniformis* model and the data were from J. D. Legan, P. A. Voysey and P. S. Curtis (unpublished observations).

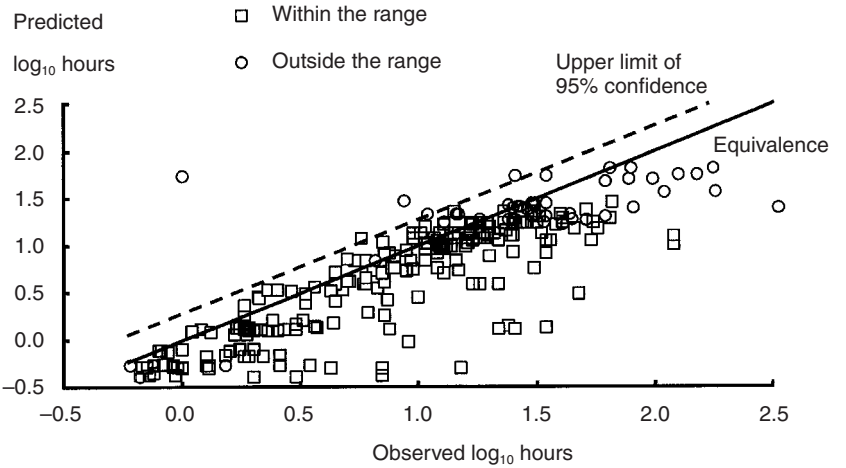

Fig. 3.5 An example of model validation across a range of conditions for predictions from Food MicroModel for growth of *L. monocytogenes* compared with literature data (adapted from McClure *et al.*, 1994b).

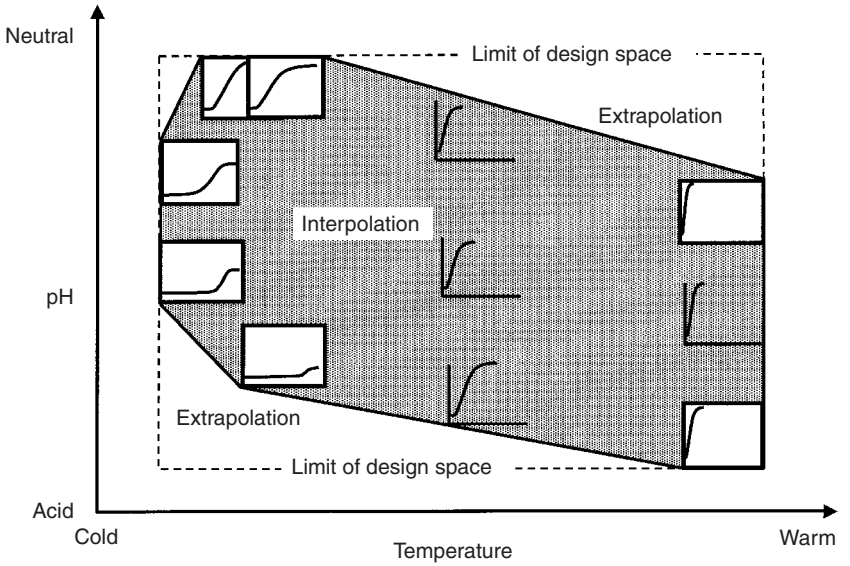

Fig. 3.6 Schematic of the domain of validity of a model (adapted from Baranyi, 1999). The shaded area shows the minimum complex polyhedron that bounds the region of strict interpolation. It is defined by the extreme combinations of conditions where data were gathered, indicated by the growth curves in boxes. All conditions outside the shaded region involve extrapolations unsupported by experimental data and are less reliable, even though they may be within the limits of the experimental design space.

of interest is significant, it may be necessary to use variants of the model-building strains selected for antibiotic markers (Curtis *et al*., 1995). In this case it is important to be sure that the marked variants behave similarly to the parent strains.

Domain of validity

Predictions from models have high degrees of confidence only for those conditions that involve interpolation within the conditions used to build the model. This range includes only those conditions within the limits where good experimental data were obtained and may not be the same as that included in the design matrix. Establishing the range of interpolation helps to define the useable range of the model (Fig. 3.6; Baranyi, 1999).

Fluctuating conditions

Real foods experience a range of temperatures throughout their shelf-life caused by normal events. Predicting growth in these circumstances is somewhat more involved than predicting for constant conditions but can be achieved by time/temperature integration or other approaches (Baranyi *et al*., 1995).

Combining data sets
It is rarely appropriate to combine models. However, when there are several different data sets for the organism of interest, these may be combined and remodelled together. This allows models to be strengthened by using data produced in different laboratories. The range of existing models can also be extended by including additional data. In these circumstances it is particularly important to be aware of the domain of validity.

3.4 Growth boundary models

3.4.1 Experimental design

Many of the design considerations such as intended use of the model, choice of experimental variables and their levels, method of establishing levels, etc., are similar to those for kinetic growth models and have already been discussed. There are, however, some important differences.

The goal of a boundary model is primarily to determine the borderline conditions that prevent growth of the target organism without specific inactivation processes, such as heat, being applied. To develop this type of model we need many points, ideally close to and either side of the boundary, with approximately 50% of the conditions allowing growth and 50% not allowing growth. This gives us the best opportunity to find the position of the boundary and important advantages in data analysis. We need many replicates because the area of greatest interest contains the most marginal conditions for growth. In these conditions the microorganisms are highly stressed and the variability in their growth response is at its highest.

Experimentation for boundary modelling often makes use of automated equipment that detects growth by changes in optical density, conductance or impedance or production of metabolites because we are only interested in if, when and under what conditions growth occurs (or does not occur). Specific quantification of growth (e.g. colony counts) is not needed. However, careful consideration must be given to the appropriate growth 'threshold value' (e.g. the optical density level) defining the borderline between growth and no growth. The threshold should be the lowest realistic value of the measured response that is clearly greater than the noise seen over time at no-growth conditions. If the threshold is in doubt, more than one value can be selected and models created based on each. Comparison of the models may resolve the selection of the response threshold. We should be aware that the automated method might correlate better with total microbial biomass than with colony count, particularly when changes in environmental stresses cause a change in the size or shape of the cells.

Automated methods may only be sensitive to the presence of 10^5 cfu/ml or higher and this may influence the inoculum level. Ideally we would still inoculate at a level of 10^2–10^3 cfu/ml to avoid inducing growth through the presence of an unrealistically high initial level. The difference in time to detect growth between an automated method and one based on plate counting will be relatively

small because the length of the lag phase has much more effect on the time to any threshold population density than the growth rate. However, when working with toxin-forming bacteria we may choose to use a higher inoculum to ensure that the amount of growth needed to cross the detection threshold of the automated method is less than that associated with toxin formation.

In other disciplines, such as engineering, central composite designs are commonly used for developing response surface models. For microbiological modelling, however, these designs have serious limitations and should be avoided. Central composite designs concentrate treatments in the centre of the design space and have fewer treatments in the extreme regions where biological systems tend to exhibit much greater variability. Furthermore, given the availability of automated laboratory equipment for detection of growth, constraints on the number of experimental treatments are less rigid. For this reason, full factorial experiments are recommended. In a factorial experiment, every factor level is run with every other factor level. If there are regions in the design space in which either (1) conditions are so harsh that no growth is expected, or (2) conditions are so favourable that rapid growth is ensured, then treatments in these areas may be omitted without adversely affecting the model, provided that the proportion of growth to no-growth conditions remains approximately equal. A consequence of not using all combinations of factors is that the experimental design may become unbalanced. This could be important in a screening experiment to identify the important factors within the range of possible factors because the ability to estimate the importance of each factor declines as the design becomes more unbalanced. When experimenting primarily for the purpose of developing predictive models it is less of a problem because, presumably, we have already decided which are the important factors based on prior knowledge or experience.

As always, the experimenter should try to minimise the impact of external, uncontrollable factors on the outcome of the experiment through randomisation. Complete randomisation is the ideal but often cannot be achieved in practice. In lieu of complete randomisation, the experimenter should randomise wherever practical and should always be wary and observant of external factors that might influence outcomes.

There are some considerations that may help to determine the number of replicates (duplicate samples from the same treatment), repeats (duplicate preparations of a treatment) and additional treatment combinations in the experiment. The most important consideration is to create the experimental design so that, as stated above, 50% of the conditions allow and 50% do not allow growth. Second order models are desired, so three to five equally spaced levels of each factor should be run. Finally, three to five sample replicates should be run at each treatment condition to check that results are reasonable and aid in detecting outliers. If using an automated method, a full factorial design is ideal and can be handled with relative ease. The most labour-intensive part of the experiment is making, inoculating and dispensing the media for the many treatments in even a simple factorial experiment. With careful planning, media can be made over a period of several days by one person and refrigerated as appropriate until all the treatments

are ready. Inoculation and dispensing are then done in a single day ready to begin incubation.

3.4.2 Experimentation

Order of experimentation
In boundary modelling the emphasis is on simply detecting growth, without the need to quantify it. Therefore, less effort is involved in monitoring during boundary modelling experiments than in kinetic modelling. Hence the balance of advantage favours setting up 'marginal' and 'no-growth' treatments first because these treatments will run for the longest time (possibly several months). Those conditions in which growth is expected to be relatively quick can be set up last because they only need monitoring until growth is detected. When automated methods are used it may be most effective simply to start all treatments at the same time. To minimise the chance for systematic error, the order of treatments should be randomised as far as practicable during preparation, dispensing, inoculation and reading and position in the laboratory equipment, if relevant.

Stability of treatments
As boundary-modelling experiments can span several months, particular care must be taken to ensure that the initial conditions of interest do not change over time solely as a result of an uncontrolled interaction with the laboratory environment. Changes resulting from microbial activity may, however, be an important part of the mechanism leading to growth initiation and we would not wish to stabilise the system at the expense of growth that would naturally occur in a food. For example, gain or loss of moisture can significantly change the a_w (or relative humidity, RH) of the system with a consequent change in its ability to support growth. The RH around the treatment can be stabilised, for example by incubating in a sealed container over saturated salt slurries that give the same RH as the medium (Weast *et al*., 1984). In contrast, maintaining the initial pH over time is typically neither possible nor practical, even in buffered media. In fact, allowing a change in pH due to growth of the organism more closely mimics what would happen in a food product than maintaining the initial pH over time.

Data analysis: initial inspection of data
The first step is to inspect the raw data for quality and consistency and investigate the cause of any observations that seem inconsistent with the total data set. Various plots or tabulations may help to show patterns in the data. Then we derive the time to growth from the records of the first time that growth was observed and the last time that it was not. For example, if monitoring growth via optical density, the OD time series is used to determine the time to growth for each sample. This determination can be made 'by eye' with small data sets. If the data set is large it may be more efficient to identify a suitable interpolation method to

determine time to growth for the majority of observations. Interpolation using a geometric time interval is theoretically more accurate than if linear time intervals are used but differences in any particular circumstances may be small.

Modelling growth parameters

The final data set ready for boundary modelling usually contains a high proportion of results where growth was not observed before the end of the experiment. These are usually presented as 'time to growth > t_{max}', where t_{max} is the maximum duration of the experiment. Results of this type are known as 'censored data' because they tell us nothing about what might have happened if the experiment had continued. Least squares regression analysis is not applicable in these circumstances. Instead, we need to use a statistical package that is capable of 'survival analysis' using maximum likelihood estimation methods. A number of packages are available, but not all offer the same range of capability.

The SAS LIFEREG procedure (SAS Institute Inc, Cary, NC) is one survival analysis tool that has often been used for microbiological modelling. By default, the procedure fits a polynomial model to the log of the dependent variable (in this case, time). The resulting model can easily be transformed to a regular time scale. The result is a regression equation of the form:

Table 3.3 Example of parameters derived from a SAS LIFEREG output table for a time to growth boundary model for *Staphylococcus aureus* based on relative humidity (RH), pH and calcium propionate (cal) (Stewart *et al.*, 2001). In the first iteration of modelling, the quadratic term cal^2 was excluded because it was not significant ($p = 0.6247$) whereas all the other p values were <0.0001 (see $p >$ Chi-square column)

Variable	Degrees of freedom	Estimate	Standard error	Chi-square	$p >$ Chi-square
Intercept	1	2.2496	0.0319	4983.5007	<0.0001
RH	1	–3.7032	0.0562	4338.8734	<0.0001
pH	1	–1.4881	0.0327	2069.5765	<0.0001
cal	1	0.4176	0.0301	192.5908	<0.0001
RH^2	1	1.5717	0.0474	1101.7569	<0.0001
pH^2	1	0.3430	0.0220	243.5212	<0.0001
RH × pH	1	0.8145	0.0300	746.1667	<0.0001
RH × cal	1	–0.1305	0.0302	18.7282	<0.0001
pH × cal	1	–0.2209	0.0208	112.2248	<0.0001
Scale	1	0.2000	0.0082		

$$\ln(\text{time to growth}) = C_0 + C_1V_1 + C_2V_2 + C_3V_1V_2 + C_4(V_1)^2 + C_5(V_2)^2$$

where all *C*s are constants and all *V*s are variables. It is important to remember that the LIFEREG procedure assumes an event will occur at some time and therefore inclusion of censored data where no growth would ever occur would bias results.

LIFEREG outputs a table of regression coefficient estimates and approximate chi-squared (x^2) distribution p-values for each factor in the model. The relative

importance of each factor can be judged by the *p*-value: factors with small *p*-values are most influential and predictive of the time to growth (TTG; Table 3.3). Starting with a general model that includes all the variables' main effects (V_1, V_2, V_3, etc.), their interactions, e.g. ($V_1 \times V_2$), and quadratic terms, e.g. (V_1^2), those factors that do not have a significant effect are progressively excluded through a series of iterations and the data reanalysed. The final model is parsimonious, i.e. it gives the best description of the data in the fewest possible terms. As with kinetic modelling, relating the data to the model at each iteration and looking for outliers and leverage points can help both to improve the quality of the final model and identify any limitations on its application.

LIFEREG allows the user to specify the error distribution to account for the variation in TTG not explained by the regression model. Several distributions, including Weibull, log-normal and log-logistic, typically give good fits to survival data. All should be considered to identify the one giving the best fit. Since these are not all from the same class of distributions, it is not possible to formally test for goodness of fit using likelihood ratio tests. However, comparison of the sample log-likelihood can informally be used and that with the largest log-likelihood should be considered to give the best fit. Jenkins *et al*. (2000) and Stewart *et al*. (2001) give more detailed descriptions of boundary modelling using SAS LIFEREG. Survival analysis packages other than SAS LIFEREG may not offer such a wide range of error distributions.

Model acceptance: validation

The principles of model acceptance are essentially the same as those already discussed under kinetic modelling but with an additional consideration relevant to toxin-producing microorganisms. If toxin formation could be associated with an amount of growth less than the difference between the inoculum level and the sensitivity of the technique used to determine growth, the boundary conditions should be confirmed by either plate counts, direct microscopic counts or toxin assays. This confirmation must be done before using the model to support safety-critical decisions.

Diagnostic tests (mathematical tests)

As the adage goes, all models are wrong; some are simply less wrong than others. Diagnostic testing helps in the selection of the best model. One approach for assessing model quality is to do graphical checks of model predictions and residuals. Residuals are the difference between model prediction and the observed TTG. Useful graphs include plots of predicted TTG vs observed TTG, residual vs observed TTG, and residuals vs. individual factor levels. Another important plot is a probability plot of the residuals. Such plots indicate whether the data might be a better fit with a different error distribution. If patterns are detected in these plots, the model might be improved by addition/deletion of factors or scale transformation. Most regression books provide detailed discussions of residual diagnostics and possible remedies. Another useful metric of model quality is the

sample log-likelihood. This is automatically calculated by most survival analysis software. Depending on the distributions being considered, we can do a generalised likelihood ratio test to compare models (Allison, 1995). Generally, models with relatively large sample likelihoods are preferred.

3.5 Death models

3.5.1 Experimental design

As with growth modelling, the first consideration is the intended use of the model. We must select the most important factors that influence the rate of microbial death in the food categories and processes that we are concerned with. In particular, we must select realistic levels for factors that can either protect organisms from the lethal agent or increase their sensitivity to it. We should also consider that conditions to kill bacterial spores are more extreme than conditions to kill vegetative cells.

We must be open-minded about the nature of the response that will be seen and place points to allow the true nature of the microbial response to the lethal agent to be determined. Ideally this involves 10–12 points over a 6–7 log (or greater) reduction in population size, which implies an inoculation level of at least 10^8–10^9 cfu/ml. Points should be spaced to allow any curvature in the response to be described. A zero time point is essential and time intervals increasing geometrically between samplings can be beneficial.

To develop realistic yet 'fail-safe' models, strains with above average, but not abnormal, resistance to the lethal agent are preferred. Experiments to generate death data should be done using single strains. Use of strain cocktails can lead to death responses that give complex curves that are difficult to interpret.

In some cases it is easier to kill bacteria than to destroy their toxins. For example, enterotoxins produced by *Staphylococcus aureus* are known to be extremely heat-stable. Much less is known about the effects of novel processes including irradiation, pulsed electric field technology, high-intensity light treatment or high-pressure processing on inactivation of microbial toxins. In applying models to help to design safe processes it is important to keep this in mind.

3.5.2 Experimentation

In death modelling, the response (reduction in viable microbial count) occurs over a relatively short time, typically a few seconds (or less) to minutes. Hence errors in measuring the exposure time are likely to be far more significant than in the case of growth modelling. Accordingly, the lethal agent is usually applied, and removed, rapidly to allow the exposure time to be accurately determined. Sometimes, the microbial response to the rate of change of the lethal agent is the factor of interest (Stephens *et al*., 1997a). In these cases it is important to be aware of the conditions that can allow a change in resistance to develop. For example, slow inactivation may allow adaptation to occur. In any case we must be aware of the

likely effect of culture history on the resistance of the organism to ensure that resistance is at least equal to that in the food or process of concern.

3.5.3 Data analysis

As with growth modelling, the approach to discovering the model(s) that relate the death responses to the experimental treatments is to fit death curves for individual treatments and extract the values of the parameters that describe each curve. Next we fit the model that relates the changing values of the fitted parameters to the levels of the experimental factors.

The first step is to plot the inactivation curves for each experimental treatment and inspect them. Reject those where the curves are clearly inadequate because of insufficient points, known laboratory errors, etc., and continue with the remaining high-quality data sets.

If microbial death data are approached with an open mind, several different approaches can give reasonable descriptions of microbial inactivation curves and Table 3.4 shows examples. The traditional approach using log-linear models of microbial death began in the 1920s (Ball, 1923; Bigelow *et al*., 1920). Sometimes called the mechanistic theory (Anderson *et al*., 1996), the explanation usually given for these first order death kinetics is that all cells or spores in a population have identical heat resistance and that death is caused by the denaturation of a single gene. The resulting linear plots of log survivors against time allow simple calculations and comparisons of thermal process equivalencies to be made. However, deviations from linearity in log-linear models have been repeatedly noted. An alternative view of microbial death considers that individual microorganisms within a population show a range of resistances to inactivation, caused by a permanent distribution of sensitivity to heat or any other means of inactivation. Sometimes known as the vitalistic theory (Anderson *et al*., 1996), this view sees non-linearity arising from the underlying distribution of sensitivities. It is compatible with the approach used in other areas of biology where a lethal stress is measured in terms of the dose needed to kill 50% of the population (the LD_{50}). In these days of inexpensive computers and statistical software, non-linear curve fitting avoids the need to prejudge the microbial response and allows more objective analysis of the data. The drawbacks of using non-linear models to make predictions outside the range of the experimental data are quite apparent (Fig. 3.7). The fact that extrapolation of linear models can be equally flawed is often overlooked.

Fitting death curves

There are many non-linear models that can be used to describe microbial death curves and examples are shown in Table 3.4. Among the more commonly used are the Gompertz and logistic equations: van Gerwen and Zwietering (1998) discussed some advantages of these approaches. Peleg and Cole (1998) discussed the use of the cumulative Weibull function ($\mathrm{Log}\, S = -bt^n$). This is particularly flexible because it can describe curves with upwards ($n < 1$) or downwards

Table 3.4 Commonly used models that describe microbial death curves

Model	Equation	Where
Exponential	$\log(n) = \log(n_0) - kt$	n = count/g at time t t = time (usually in seconds) k = a rate constant Also: $1/k = D$ = time needed for 1-log reduction in count/g Note, assumes log-linear death kinetics.
Logistic	$\ln(n) = \ln(n_0) + \frac{a}{1+\exp(b-ct)}$	n = count/g; n_0 = count/g when t = zero; t = time (hours); a, b and c are all fit parameters; t = time (hours) Note: fits shoulders and tails but 'expects' survival curves to be sigmoid.
Cumulative Weibull	$s = \exp(-bt^n)$ or, $\log(s) = -bt^n$	s = surviving fraction (n/n_0) at time t b is the scale parameter n is the shape parameter It is generally preferable to work with the second, semi-logarithmic form to capture detail in the tail of the curve. Note: this model fits shoulders or tails, but not both.
Gompertz	$L(t) = A + C\exp\{-\exp[-B(t-M)]\}$	$L(t) = \log_{10}$ bacterial count at time t; B = the relative maximum death rate (/h); M = the time at which death growth rate occurs (h); A = the upper asymptotic $\log_{10}$ bacterial count as t decreases indefinitely; C = the difference between A and the lower asymptotic $\log_{10}$ bacterial count as t increases indefinitely. Note, fits shoulders and tails but 'expects' survival curves to be sigmoid.

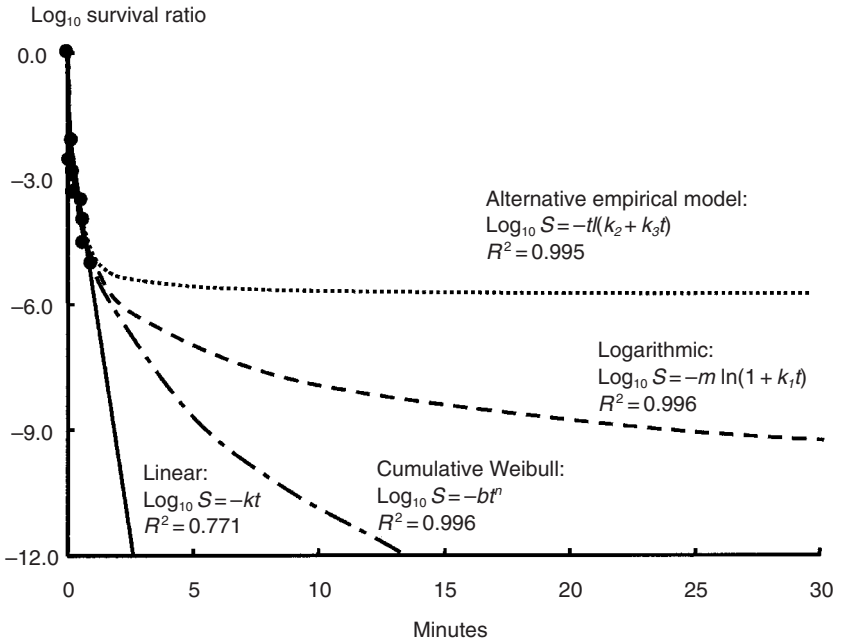

Fig. 3.7 Experimental death curves of *Clostridium botulinum* spores at 119 °C (Anderson *et al.*, 1996) fitted with different models, adapted from Peleg and Penchina (2000). The curves are all similar when interpolating between points but give drastically different values when extrapolated to a 12 log reduction in numbers. In the equations S is survival ratio, t is time, b, k, k_1, k_2, k_3, m and n are fit parameters and ln indicates natural logarithm.

($n > 1$) concavity and accommodate log-linear death curves simply as the special case where $n = 1$.

More than one curve-fitting approach may give acceptable models of a particular data set. Selection of the best model is somewhat subjective and the choice may be simply pragmatic based on model validation, a view of the principles underlying the different modelling approaches or other relevant criteria. The most important consideration is that any model selected should clearly describe the observed data and not merely be forced to fit, or be fitted to, an arbitrarily selected subset of the data. With this in mind, we strongly urge authors of future publications concerning microbial inactivation to present raw data and show how well their chosen model describes it. Far too many research papers present only derived D and/or z values and make comparisons between conditions based on these quantities. This has two damaging consequences. Firstly, without evidence that the chosen model gives an adequate description of the data the validity of any comparisons are in doubt. Secondly, without the underlying data it is impossible for others to compare different modelling approaches and valuable opportunities for future improvement are lost.

Modelling death parameters

At this stage the principles are essentially the same as when modelling growth parameters. The simplest approach is to fit a polynomial regression model that relates the parameters describing the individual death curves to the factors (pH, a_w, etc.) that define the experimental treatments. However, other models have been used for this stage and examples are given in Table 3.5.

Validation

The final step in building confidence in a death model is to compare its predictions with observations in foods. Validation data may come from the literature, but more commonly we need to collect experimental observations. Individual death data points obtained in foods are compared with death curves predicted for relevant values of the controlling factors (pH, a_w, temperature, etc). This checks that the model properly represents the shape of the death curves in foods. Four to six well-spaced points per curve should be enough, though more points allow more detailed comparisons. When individual observed and predicted death curves look similar for foods matching conditions across the model's range, summary plots can be constructed. These could, for example, show observed versus predicted time to some required total lethality such as a 4 or 6 $\log_{10}$ reduction in numbers. Summary plots allow the performance of the model at many different conditions to be easily compared. For a conservative model the observed time to the required lethality should be no longer than the predicted value. Validation data should include observations for samples of all the different food types for which a model is designed. For a toxin-forming organism, it is additionally important to remember that the conditions needed to destroy toxin may be quite different from those needed to kill cells or spores. Validation says nothing about the ability of any process to destroy preformed toxin, even if it gives large reductions in pathogen numbers.

Varying conditions

An important application for death models is as a tool for evaluation of food processes. A characteristic of the 'kill-step' in most food processes is that the intensity of the lethal agent varies with time, for example due to heating and cooling times. To calculate microbial survival under such conditions we must integrate the momentary lethal effects throughout the process (e.g. temperature profile). Standard techniques for performing this calculation based on log-linear kinetic models are well documented (Texeira, 1992; Toledo, 1998). Though widely accepted, these approaches are technically invalid when survival curves are not log-linear. Approaches for calculating microbial survival in varying conditions have been proposed by Peleg and Penchina (2000) and others (Körmendy and Körmendy, 1997). The Peleg and Penchina approach has been shown to be relatively easy to use and effective (Mattick *et al*., 2001; Peleg *et al*., 2001) and certainly is worthy of further investigation.

Table 3.5 Some examples of secondary models for death parameters. Other examples are given in, e.g., van Gerwen and Zwietering (1998)

Model type	Equation	Where	Comments
Polynomial	Response = $C_0 + C_1V_1 + C_2V_2 + C_3V_1V_2 + C_4(V_1)^2$	All *C*s are constants and all *V*s are variables	Straightforward to apply by multiple linear regression. No knowledge of process needed. But no theoretical foundation, no biological meaning to parameters
Arrhenius-type	$k = A\exp(-E_a/RT)$	k is death rate constant; A is the 'collision factor'; E_a is the 'activation energy' of the system; R is the universal gas constant (8.31 J/mol/K); T is temperature in kelvin	Parameters have no biological meaning. Has been adapted to include other parameters (e.g. pH) but does not predict limiting values for variables (Davey *et al.*, 1995)
z concept	$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}}$	z is temperature for a 10-fold change in D value; D is time for 10-fold reduction in survivors; T_1 is lower temperature; T_2 is higher temperature	Uses linear fitting by eye or by regression. Assumes a first-order death kinetic

3.6 Survival models

Survival models are generally concerned with non-thermal inactivation under relatively mild conditions, often just outside the limits for growth. Some pathogens can remain viable for many months or even years in non-growth conditions. An outbreak of food poisoning caused by *Salmonella* Napoli in chocolate occurred in 1982 after such an occurrence and the infectious dose was judged to be as few as 50 organisms per patient (1.6 cfu/g of chocolate; Greenwood and Hooper, 1983). Survival models can help us to understand the limits of survival in different food systems. They are less common than growth or death models because of some of the practical constraints on their creation but there are examples in the literature (Buchanan *et al*., 1994; Little *et al*., 1994; Whiting *et al*., 1996; Zaritzky *et al*., 1999).

3.6.1 Experimental design

Most of the design considerations are similar to those already discussed under growth and death models. The conditions of interest often include some that support growth while others lead to death. Accordingly the inoculation level is usually moderate (10^5–10^6 cfu/ml) to allow changes in either direction to be followed. Choice of single strains or cocktails is harder here because of the range of responses that we may see. Use of single strains simplifies interpretation of the inactivation kinetics but puts a particular emphasis on selecting strains with representative characteristics. Cocktails may allow us to compensate for variability between strains at the risk of survival curves that are hard to interpret.

3.6.2 Laboratory work

Automated methods such as automated turbidimetry, conductance or capacitance measurement, are not effective for following population death. This means that laboratory work for survival models involves colony counting and is similar to that for kinetic growth models, with similar resource constraints and design considerations.

3.6.3 Data analysis

The approach to data analysis for survival models follows the principles already outlined, though the selection of models for curve fitting may be somewhat different (Jones and Walker, 1993).

3.7 Applications of models: product and process design, product shelf-life

3.7.1 Product and process design

Improving food quality to meet consumer needs or requirements often takes processes and food characteristics closer to the conditions where

microbiological hazards can occur, e.g. using milder heat treatment to improve the taste and appearance of the product, or reducing the preservative levels to meet new legislation. However, to ensure microbiological safety, the food process must be controlled a safe distance away from the hazardous conditions. Therefore, to meet both safety and quality requirements, process conditions should be as close as safely possible to the hazardous conditions (quality requirement), while sufficiently far away to minimise the risks. Our ability to move towards the boundary between safe and hazardous conditions with confidence is a measure both of how well the process is controlled and of how well the position of the boundary is known. If there is neither good control nor good definition of the boundary between safe and hazardous conditions, the process must be operated well away from the boundary in order to ensure a safe product. If process parameters are well defined and controlled, the variability associated with the process will be smaller. Consequently the process may be modified to improve quality while ensuring that the process parameters remain within the safe processing area. The achievable improvement in quality is then restricted by how well the boundary of the hazard conditions is known. If the boundary is not well known the process parameters must remain outside a large area of uncertainty. Better definition of the limits of the hazardous conditions reduces the area of uncertainty. This may identify new, milder conditions available for safe processing. Predictive models allow product and process designers to explore the conditions of the product and the process that will allow growth, survival or death of the organisms of concern. In other words, they help to determine more accurately the limits between safe and hazardous processing conditions. In this respect predictive models are more useful than traditional challenge tests. This is not to say that challenge tests should be abolished. After obtaining a prediction from a model, it may still be necessary to validate its usefulness by some challenge tests in food.

3.7.2 Determination of product shelf-life

Time/temperature integration can help to assess the amount of growth in chill distribution chains (Fig. 3.8), or establish safe cooling times after cooking a bulk product.

3.8 Applications of models: hygienic equipment design, HACCP systems

3.8.1 Hygienic design of equipment

Numerical simulation of flow and temperature combined with microbial growth models can be used to predict microbial numbers in closed processes and give guidance on hygienic design of equipment. For example, if equipment contains dead ends, models can be used to see if microorganisms can grow in the dead end and then recontaminate the flow.

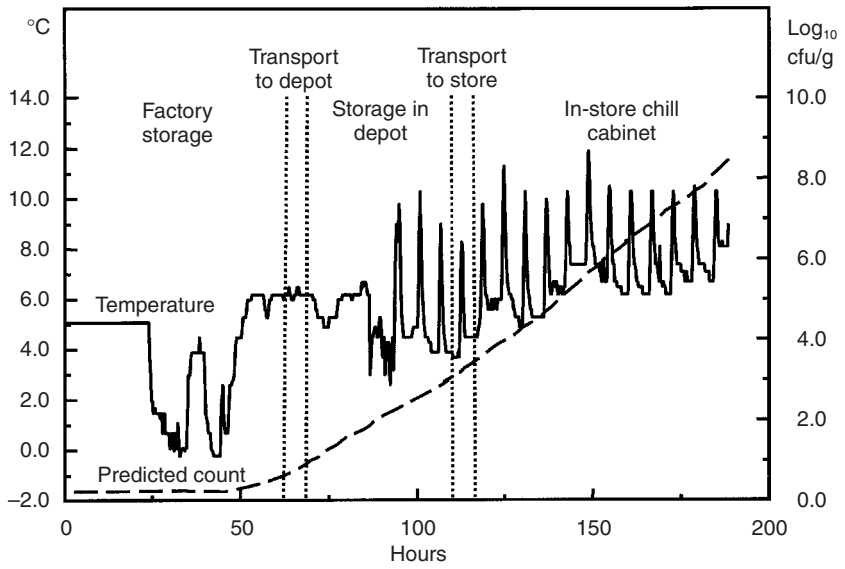

Fig. 3.8 Schematic of predicted count for a spoilage organism in a chilled food at different points of manufacture, transport and in-store display based on the temperature profile of the food. This type of prediction can be used, for example, to estimate product shelf-life provided that the level of organisms coinciding with unacceptable quality is known.

3.8.2 Hazard Analysis Critical Control Point (HACCP)

Models allow us to predict the behaviour of microorganisms during food processes. Hence, models can have a significant input into HACCP (Baker, 1995). Significant progress has been made in recent years in developing the use of predictive modelling for risk assessment and HACCP (Notermans *et al.*, 1994). Predictive models can help to decide if a particular micro-organism should be a concern for a particular food product in the risk analysis. They can also help to determine the Critical Control Points (CCPs) in HACCP studies by providing information on the likely growth or survival of the microbiological hazards at each point in the process. Once the CCPs have been identified, predictive models can help to define the critical limits at the CCPs by exploring the microbial responses to different critical limits. Models can help to calculate the parameters of any corrective action necessary (e.g. temperature and time for repasteurisation; Walker and Jones, 1994). Models can also be used to determine parameters for new or intended processes by comparing the predicted potential for microbial growth with that for existing processes (Armitage, 1997).

3.9 Applications of models: risk assessment, food safety objectives

3.9.1 Risk assessment

Predictive microbial models are essential tools in quantitative risk assessment. They can be used in hazard identification to establish whether a particular hazard should be considered under a given set of conditions; for example, to determine whether a particular pathogen can grow in a product or process before considering it further in the risk assessment. Models can help in disease characterisation, e.g. to understand the behaviour of newly recognised pathogens, and in dose–response assessment to assess the risk of infection (Foegeding, 1997). Microbial models are probably most important, though, in the exposure assessment stage of a risk assessment. Exposure assessment determines the probability of consuming a biological agent and the amount consumed. Microbial risk assessment is generally more complex than chemical risk assessment as the hazard (the microorganism) has the potential to increase (through growth) or decrease (through inactivation or dilution) throughout the production of a food. Reliable predictive models are essential for estimating the likely increases or decreases in the level of a hazard throughout complex food production, manufacture, storage and distribution chains (Baker *et al*., 1997; Buchanan and Whiting, 1996; Cassin *et al*., 1998; ICMSF, 1998; Marks *et al*., 1998; Whiting and Buchanan, 1997).

3.9.2 Validation of control measures to meet food safety objectives

A key provision of the World Trade Organization Sanitary and Phytosanitary (SPS) Agreement is the requirement for countries to provide risk assessments to facilitate resolution of disputes with trading partners that involve food safety issues. The risk assessments help to quantify whether the risks faced by consumers are significant or if the levels of assurance required from the exporting country are greater than those mandated by the importing country for its equivalent domestic industry (ICMSF, 1998). This is a significant advance on the previous requirement for risk to be 'as low as reasonably possible' because technological capabilities vary between countries and even between companies within the same country. The perception of 'reasonable' also differs between countries with 'acceptable risk' being culturally defined. Developments in quantitative risk assessment and validated microbial models that can accurately predict the growth, death or survival of the major foodborne pathogens have made it possible to link the exposure assessment of a pathogen to probable public health outcomes (Buchanan *et al*., 1997b; Whiting and Buchanan, 1997).

The International Commission on Microbiological Specifications for Foods (ICMSF) has proposed a scheme for managing microbiological risks for foods in international trade in which the Food Safety Objective (FSO) is a functional link between risk assessment and risk management. The FSO is defined as 'a statement of the frequency or maximum concentration of a microbiological

hazard in a food considered acceptable for consumer protection' (van Schothorst, 1998) and allows the equivalence of different control measures to be established. Control measures include destroying microorganisms during processing, preventing their growth by using appropriate storage conditions and restricting their initial incidence and/or concentration by the application of suitable acceptance sampling schemes. In order to compare the equivalence of different control measures, it is necessary to be able to relate their performance in terms of achieving an FSO, in other words, in terms of frequency or concentration of a microbiological hazard. The ICMSF proposal outlines five steps for managing food safety:

1. Conduct risk assessment.
2. Conduct risk management option assessment.
3. Establish the food safety objective.
4. Confirm that the food safety objective is achievable through good hygiene practices and HACCP.
5. Establish acceptance procedures.

The following definitions of terms are useful in understanding how FSOs are achieved (van Schothorst, 1998):

- *Food safety objective* (FSO): a statement of the frequency or maximum concentration of a microbiological hazard considered acceptable for public protection. For example, the amount of staphylococcal enterotoxin in cheese must not exceed 1 µg/100 g or the level of *Listeria monocytogenes* in ready-to-eat foods should not exceed 100 cfu/g at the time of consumption.
- *Performance criterion*: the required outcome of a step or a combination of steps that can be applied to ensure an FSO is met. For example, a performance criterion could be a $6 \log_{10}$ reduction in the target organism.
- *Step*: a point, procedure, operation or stage in the food chain including raw materials from primary production to final consumption.
- *Process criterion*: the control parameters of a step or combination of steps that can be applied to achieve the performance criterion. An example of a process criterion could be heating for 2 minutes at 70 °C or high-pressure treatment at 500 MPa for 7.5 minutes.

When establishing performance criteria, consideration must be given to the initial level of a hazard and changes in the hazard during production and processing, distribution, storage, preparation and use. A performance criterion can be defined by the equation:

$$H_0 + \Sigma R + \Sigma I \leq \text{FSO}$$

where:

H_0 = initial level of the hazard (elimination);
ΣR = the cumulative (total) decrease of the hazard – R is negative by definition (reduction);

ΣI = the cumulative (total) increase of the hazard – I is positive by definition (prevention, increase);
$\leq$ = preferably less than, but at worst equal to.
FSO, H_0, R and I are expressed in $\log_{10}$ units.

This scheme offers flexibility for the food industry in terms of allowing the use of alternative, equivalent means for reaching the same FSO. Achieving the FSO can be done by controlling the initial concentration of the target organism in the raw materials (H_0), controlling the total reduction of the target microorganism by the process(es) (ΣR), and/or controlling the total amount of growth of the target organism throughout production and distribution (ΣG). The sum of $H_0 + \Sigma R + \Sigma G$ must be less than or equal to the FSO. The ΣG and ΣR can be determined using respectively kinetic growth or inactivation models as described previously in this chapter. This framework is gaining ground internationally for establishing processes and food safety systems. Predictive microbial models make a significant contribution to risk assessment through their ability to explore new conditions rapidly and quantitatively and can be powerful tools to help microbiologists and others make risk management decisions. Together with acceptance sampling schemes of quantifiable performance (Legan *et al*., 2001) they will transform the way in which the microbiological safety of foods is managed.

3.10 Future trends

Although there are some excellent validated models for growth of pathogens there is still a need to develop further models for survival and inactivation of pathogens under different conditions. Changes in the nature of food poisoning, including the emergence of more highly infectious pathogens and a general increased susceptibility of the population, have meant that emphasis in modelling has shifted. Kinetic growth models that predict the likely increase of pathogens to high numbers are giving ground to boundary models that will predict where growth and no growth occurs and inactivation models that predict the reduction of pathogens.

In order to establish safe and optimised process criteria required for a pathogen reduction step (ICMSF, 1998) it is important to examine the inactivation kinetics of the target organism or organisms. In the traditional first order approach to describing bacterial inactivation, it is assumed that all of the cells or spores in a population are identical in their sensitivity to the lethal agent. It is now becoming more widely accepted that this assumption is incorrect. New modelling approaches based on an assumption that, for any population of cells or spores, there will be a distribution of sensitivities are now being employed (Anderson *et al*., 1996; Cole *et al*., 1993; Little *et al*., 1994; Peleg and Cole, 1998). It will be important to understand the microbial risk implications of non-first order inactivation kinetics.

In the last few years there has been growing research and commercial interest especially in Europe and the US in non-thermal or cold pasteurisation techniques such as ultra high-pressure, pulsed electric field treatments and ionising irradiation. Interest in such technologies has been fuelled by a continuing consumer desire for foods that are more fresh-like but convenient and safe. A decontamination step that does not significantly alter the organoleptic qualities of the food would have obvious advantages. Cold pasteurisation technologies offer the promise of foods that have freshness, flavour, colour, texture and nutritional value closer to non-heated products while, at the same, time exhibiting enhanced microbiological safety.

Commercialisation of non-thermal technologies has been slow, for several reasons. One of the main reasons is that there is no 'processing continuum' for these technologies in which industry can make production decisions based on the product type and microorganism(s) of concern in a particular food.

For non-thermal technologies to be widely commercialised, there is a need to obtain systematic inactivation kinetics data. It is nearly impossible, from existing literature, to determine appropriate processing parameters for commercial food production utilising new technologies such as high pressure (HP) and pulsed electric field (PEF) processes because the data are scattered, with limited exceptions (Ritz *et al*., 2000; Zook *et al*., 1999). There is an immediate need for systematic data from which food processes can be developed. Unfortunately, we cannot apply thermal inactivation kinetics to inactivation of the same organisms by other processes because heat resistance does not directly correlate to pressure resistance. *Staphylococcus aureus* is an excellent example, being quite heat sensitive yet pressure resistant. Development of death kinetic models for these technologies, using methods described earlier in this chapter, will be critical for the successful use of new preservation technologies such as HPP and PEF in commercial production of food products.

3.10.1 Biovariability

An important consideration for both growth and inactivation modelling is to obtain better estimates of inherent within and between strain variability (biovariability). This will be essential for the application of predictive models to very low pathogen levels. This appreciation of natural biovariability has been termed quantum or quantal microbiology (Bridson, 1997; Bridson and Gould, 2000) and compares our current understanding of microbiology to the level of understanding in physics during the Newtonian years. For the last 120 years the study of microorganisms, as brought to us by Koch and others, has assumed that a pure subculture of a microorganism will contain identical cells. In the future, mathematical models will have to account for natural biovariability as described above for inactivation kinetics and in the area of modelling the fate of small numbers of cells. Single cell techniques such as image analysis (Billon *et al*., 1997), flow cytometry (Ueckert *et al*., 1995) and automated turbidimetry (Stephens *et al*., 1997b) will be invaluable for this.

3.10.2 Mechanistic modelling

To this point, we have discussed only empirical models, which simply describe experimental observations by some convenient mathematical relationship but have nothing to say about underlying physiological or physical processes. Experience has shown that such models are adequate for many practical purposes in food safety management, but they provide no secure basis for extrapolation outside the range of the experimental data.

Mechanistic, or deterministic, models, in contrast, are built upon a theoretical understanding of the system and can help to confirm or improve our basic understanding of the underlying processes. They have the potential to give more accurate predictions than empirical models and can explain why the response follows the variables in the way that is observed. Mechanistic models also provide a better basis for extrapolation outside the range of the experimental data because it is the mechanism controlling the response that provides the foundation for the model. This added predictive capability is extremely valuable in suggesting regions where further experimentation may be valuable but extrapolation without validation may still be dangerous because the mechanism itself may change, or prediction errors may become very large (Box *et al*., 1978).

Many 'quasi-mechanistic' models have been developed (Bazin and Prosser, 1992; McMeekin *et al*., 1993; Ross, 1999) and have certainly proved useful for developing and testing hypotheses. The mechanisms postulated include rates of reaction between enzymes and nutrients, rates of protein denaturation in response to temperature changes and rates of enzyme synthesis by ribosomes. These models have all indicated linkages between the putative mechanisms and the observations of growth responses used in empirical models. However, in all cases the 'key enzyme' is unknown and a 'mechanistic' model whose parameters cannot be determined experimentally cannot be considered truly mechanistic (Heitzer *et al*., 1991). Despite much progress, the observation by van Dam *et al*. (1988) remains essentially true:

> Much is known empirically about rates of growth and substrate consumption for different microorganisms growing on various substrates. At the same time the biochemistry and molecular biology of the organisms is known in considerable detail. However, the question of how growth (and death) kinetics are related to the physiology of microorganisms is generally not well understood.

A rare example of a truly mechanistic model linking these elements is the work of Cayley *et al*. (1992) that relates the growth rate of *Escherichia coli* K12 under osmotic stress to the intracellular accumulation of betaine and proline and the thermodynamics of osmoprotection.

Box *et al*. (1978) commented that judgement is needed in deciding when and when not to use mechanistic models. They indicated that a mechanistic approach is justified whenever a basic understanding of the system is essential to progress or when the state of the art is sufficiently advanced to make a useful mechanistic model easily available. Clearly the latter is not yet true in microbiology, but

basic understanding is being actively pursued. As Cole (1991) observed: 'researchers in the field of predictive microbiology are striving to develop models for microbial growth and death based upon an understanding of cell variability and physiology and that could be used to extrapolate to other conditions'. We are confident that truly mechanistic models will be developed in time as these activities help to develop our understanding of the links between microbial physiology and growth (and death) responses to environmental conditions.

3.11 Sources of further information and advice

The US pathogen models are available from the USDA website at http://www.arserrc.gov/mfs/pathogen.htm

Information on the UK models in Food MicroModel is given at http://www.foodmicromodel.com

Further information on ICMSF can be found at its website at http://www.dfst.csiro.au/icmsf.htm

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4

Risk assessment and pathogen management

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4.1 Introduction

There are many pathogens that can potentially contaminate foods and many more scenarios by which that contamination could arise. End-product testing is time consuming, expensive, often invasive and largely ineffective at ensuring the level of food safety required. Consumer desire for minimally processed yet safe foods creates a paradox for the food industry, particularly because food must remain 'affordable'. Meeting these competing demands poses a challenge to the food industry and its regulators alike. It is important to be able to recognise hazards and their potential impact on public health and to allocate resources where they will have greatest effect in assuring public health.

While hazard analysis is implicit in the HACCP system, there is no formal procedure within HACCP for differentiation of trivial or unlikely hazards in foods from those that pose a serious threat to public health. The concept of 'risk' embodies both the likelihood of a hazard occurring and the severity of the consequences if it does, and enables a means of identifying hazards that most require control.

Risk assessment is one element of a formal and objective approach to the analysis of risks associated with specific activities. The overall framework has been termed 'risk analysis', and is considered also to include the elements 'risk management' and 'risk communication'.

Risk assessment techniques have been used for decades in fields including the insurance industry, financial market analysis, budgeting for large construction projects, threats to human and environmental health from industrial developments, and injury risks from mechanical failure of equipment or machinery. More

recently the techniques have been applied to biological risks related to quarantine issues, to drinking water and, most recently, to the microbiological safety of foods. During that time, the techniques for risk assessment have evolved and improved from qualitative analyses to the current situation where highly quantitative descriptions of risk from a specified source are possible. Formal risk assessment of foodborne pathogens is being actively promoted by the food industry and regulators both at national and international levels.

This chapter describes the principles of microbial food safety risk assessment and their application to managing the risk of foodborne pathogens through integration with existing tools including HACCP and predictive microbiology. HACCP is widely regarded as the best strategy for the management of microbial food safety and this chapter will demonstrate that risk assessment has an important role in the development of optimum HACCP plans. Data needs and sources are identified and methods appropriate to different levels of risk assessment discussed. Some tools to simplify food safety risk assessments are presented, and future needs identified and prospects discussed.

4.2 The development of risk assessment

4.2.1 Risk and risk perception

In the 1990s attention focused on the use of formal risk assessment techniques to improve the microbiological safety of foods. This seems to have originated from concern about the safety of the food supply after a series of catastrophic and well-publicised microbial foodborne disease outbreaks. Related to this was a perception by some scientists that, despite the great amount of effort expended to show that certain chemicals in foods *might* be toxic or carcinogenic, microbial hazards *do* make tens of thousands of people ill, and kill hundreds every year even in 'developed' nations such as the USA (Mead *et al.*, 1999).

Some risks are perceived as being greater than others because they are well publicised. Some risks, though unlikely, are 'dreaded' so that perception of the risk is out of proportion to its real likelihood, e.g. the risk of dying in a plane crash compared with that of dying from cigarette smoking. In some cases greater vulnerability is felt from a risk that people believe they have no control over and, in consequence, are much more sensitive if they are exposed to that risk agent. In addition, there is a perception that there are many risks, each of which needs to be considered and eliminated, and which compete for attention.

Table 4.1 presents an example of the discrepancy that sometimes exists between perceptions of the risks associated with foods. Similar disparity has been found among the opinions of US consumers and public health officials. It should be recognised that those perceptions can change over time, and as issues become topical or receive media attention.

The objectivity of risk assessment was promoted as a means of refocusing the attention of risk managers responsible for public health protection to promote food safety research where it was most needed and would provide the greatest benefit (e.g. CAST, 1994).

Table 4.1 Ratings of food safety risks as assessed by experts and as perceived by consumers

Actual risk (expert opinion)	Risk factor	Perceived risk (consumer opinion)
High	Microbiological contamination	Low
↑	Packaging failure	↓
	Distribution failure	
	Pesticide residues	
	Biotechnology	
	Food additives	
Low	Food irradiation	High

Source: after Hudson (1991).

A significant factor leading to the sudden increase of quantitative risk assessment methods in all fields was the availability of software for personal computers which enabled sophisticated probability, or 'stochastic', modelling techniques to be performed by users without high-level mathematical and programming skills. This brought quantitative risk assessment within reach of many fields of study. Morgan (1993) wrote:

> Only a few years ago . . . detailed study of risk required months of custom programming and days or weeks of mainframe computer time. Today a variety of powerful, general-purpose tools are available to make calculations involving uncertainty. These programs, many of which run on personal computers, are revolutionising the field. They enable accomplished analysts to complete projects that just a decade ago were considered beyond the reach of all but the most sophisticated organisations. Although such software require training, they could democratise risk assessment and make rigorous determinations far more widely available.

A further impetus was the desire of nations to facilitate international trade in foods, in particular the removal of 'artificial' barriers to trade, e.g. tariffs. In 1993 the Uruguay round of the General Agreement on Tariffs and Trade (GATT) resolved that barriers to international trade in food could only be science-based, including the protection of public health. Risk assessment was the method agreed for demonstration of the hygienic equivalence of foods originating in different nations and under different production systems and regulatory environments.

4.2.2 Microbial food safety risk assessment

Definitions and terminology

Despite the promotion of risk assessment methodology by various national governments and international trade and political organisations (CAC, 1996; Kindred, 1996; Craun *et al.*, 1996; Buchanan, 1997), currently there are no

universally agreed methods and terminology. Nonetheless, the approaches and terminology suggested to date are not greatly divergent. For convenience, we adopt the approach of the Codex Alimentarius Commission (CAC, 1996), which is representative.

Risks and hazards

In common usage, the words 'risk' and 'hazard' or 'risky' and 'hazardous' are often used interchangeably. In the formal discipline of risk assessment 'risk' and 'hazard' have specific meanings. Codex defines a hazard as 'a biological, chemical, or physical agent in, or a condition of, food with the potential to cause an adverse health effect' and risk as 'a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food'. In simple terms, a hazard is considered to be the possibility of an undesirable effect, e.g. that a food might contain viable pathogens at the time it is eaten and make someone sick. *Risk*, however, is the combination of the fact that a hazard exists, the *likelihood* of it happening, and the *severity* of the consequences if it does.

Risk analysis

Codex describes risk analysis as a process involving three aspects: risk assessment, risk management and risk communication. One interpretation of the interplay between these three aspects of risk analysis is shown in Fig. 4.1.

Risk assessment is the scientific evaluation of the probability of occurrence and severity of known or potential adverse health effects resulting from human exposure to foodborne hazard.

Risk management involves the weighing of policy options in the light of results of risk assessment and, if required, selection and implementation of appropriate control options. Whereas risk assessment aims to be a completely objective process, risk managers must take into account cultural and economic considerations, as well as technological feasibility.

Risk communication is the exchange of information and opinion interactively among risk assessors, risk managers and other interested parties. It seeks both to understand the perceptions of risk of all those affected by the hazard ('stakeholders'), to communicate to stakeholders all factors relevant to the risk, and to advise the basis of risk management decisions taken.

Risk assessment is considered to consist of four steps:

1. Hazard identification.
2. Hazard characterisation or dose–response assessment.
3. Exposure assessment.
4. Risk characterisation.

These are explained below.

Hazard identification

This step establishes the causal relationship between a pathogenic agent, an illness and a food as a vector of that illness. In microbial food safety risk assess-

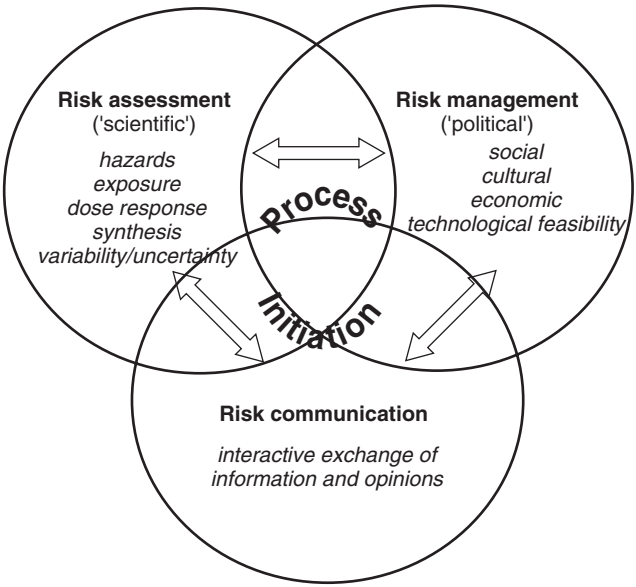

Fig. 4.1 A schematic representation of the interplay between risk management, risk communication and risk assessment. The risk assessment may be initiated from any source, but its conduct will typically be under the control of a risk manager who will coordinate the process, oversee exchange of information, and turn the results of the assessment into a plan of action. The diagram also emphasises that the risk analysis process is not sequential, but interactive and iterative. Modified from McNab *et al.* (1997).

ments the hazard is often unambiguous in comparison to the risks posed by chemical toxins. Many microbiological hazards are already known and the relationship between human illness, the pathogen and a food as a vehicle is well known. There is clear epidemiological and medical evidence of the hazard associated with many foodborne microbial pathogens in the scientific and medical literature.

It may also be possible to assess exposure to a suspected pathogen but, by definition, a risk assessment cannot be completed unless the causal relationship between exposure to the pathogen and human illness is known (see 'Hazard characterisation' below). Conversely, even if a specific pathogen has never been linked to a specific food as a vector, the potential for it to do so can be assessed. There are a number of decision support 'tools' to assist in determining whether a pathogen is, or could be, an important hazard in a given food/food process combination. These include various semi-quantitative scoring systems, decision trees and expert systems (see, e.g. Notermans and Mead, 1996; Todd and Harwig, 1996; ICMSF, 1996; van Gerwen *et al.*, 1997; van Schothorst, 1997), such as the one in Fig. 4.2. Decision trees enable the experience of others to be shared and can assist in decision making by presenting a structured series of

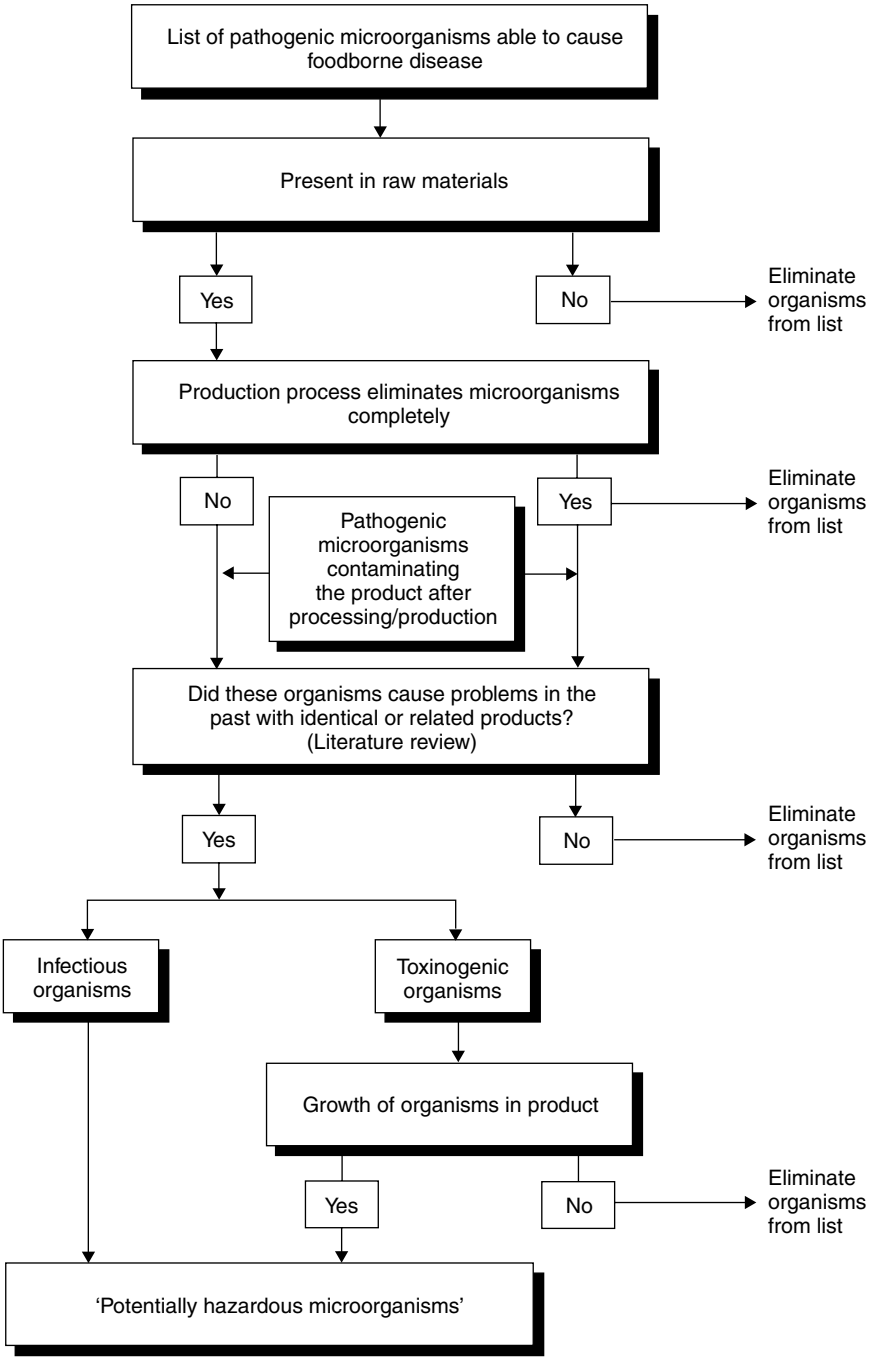

Fig. 4.2 A decision tree to aid the identification of microbial hazards in finished foods. Reproduced from Notermans and Mead (1996).

questions relevant to the decision being made. In essence, the structured approach of risk assessment offers the same assistance for more complex decision processes.

Exposure assessment

Exposure assessment seeks to estimate the following:

1. How often consumers become exposed to a hazardous agent in food.
2. How heavily contaminated that food is.
3. How much of the food is eaten.

This information is rarely available directly, and usually must be inferred from knowledge of the contamination level at some earlier time in the history of the product. It requires knowledge of the changes that the product has undergone since the point of contamination, whether through processing, storage, transport or preparation for eating. This includes quantification of inactivation, concentration, dilution or amplification of the frequency and concentration of pathogens in foods and their ingredients.

Data on the duration and environmental conditions during processes and handling on the fate of pathogens in foods are required to assess exposure.

Translation of this knowledge into an estimate of the numbers of pathogens in the product at the time of consumption is also required. This task is the province of predictive microbiology, which provides part of the scientific underpinning of microbial food safety risk assessment. Predictive microbiology is discussed in greater detail in Chapter 3. Many commentators have noted the synergy between predictive microbiology and quantitative microbial risk assessment (Buchanan and Whiting, 1996; Foegeding, 1997; Lammerding, 1997; McNab, 1998; van Gerwen and Zwietering, 1998; McMeekin *et al.*, 2000; Lammerding and Fazil, 2000).

Equally, knowledge of patterns of food consumption is required. These data can be obtained from several sources. The most widely used are human nutrition surveys but there are several limitations of these studies as sources of consumption data of specific commodities. In consumption studies foods are usually grouped according to nutritional properties of the foods (e.g. protein, salt, fat, vitamin, calorie levels), which often bear little relationship to the microbial ecology of the product.

Other than age and gender, food consumption surveys often do not collect demographic information to enable discrimination of consumers who are in different risk categories, e.g. immunocompromised owing to a health condition. In addition, important details about the form in which the food is eaten, or the storage conditions of the food prior to consumption, are often not available. More specific consumption data can be derived from the individual records of each consumer surveyed. These data are kept by some survey authorities but are not publicly released for reasons of confidentiality. They may be obtained under some circumstances or with special restrictions, however, and may be used to determine more accurately the consumption patterns of at-risk groups. For example,

the Australian National Nutrition Survey (ABS, 1995) included a health status survey.

Another source of data, complementary to that of the consumption surveys, is the inventory databases of food retailers. While these databases cannot identify the details of purchasers that affect their susceptibility to pathogens, they can provide very specific data on the number of units of every product type sold. Most large retailers have estimates of their market share and 'shrinkage'[1], and, from this, estimates of specific consumption levels from national to local levels can be derived. Commercial confidentiality may restrict access to these data. Similar information is available from market research companies that determine consumer preferences and sales volumes.

For a food manufacturer undertaking a risk assessment of their own products and processes, consumption data should be readily available. However, the manufacturer will still need to know who will consume the product, and how it will be handled prior to consumption.

Hazard characterisation (dose–response characterisation)

Dose–response characterisation attempts to relate the probability and severity of illness to the dose of the pathogen ingested. The paucity of published dose–response data is compounded by the relevance (or lack of it) to normal human populations. The few studies that have been performed have usually involved healthy adult males (typically prison inmates, or soldiers) who would be expected to have higher intrinsic resistance than the young, old, pregnant or immunocompromised (YOPI) groups. Dose–response information for enteric pathogens has been summarised by Teunis *et al.* (1996) and Kothary and Babu (2001).

In the assessment of chemical toxins, studies typically have relied on a laboratory animal model. This approach has also been applied in microbial risk assessment but can be criticised because of the uncertain relationship between effects observed in generally healthy, genetically homogeneous laboratory animals, and a heterogeneous human population.

Another approach to discerning microbial dose–response relationships uses epidemiological data, particularly that arising from identifiable foodborne disease outbreaks. To characterise the dose–response relationship for *Listeria monocytogenes*, Buchanan *et al.* (1997) used data on the incidence and level of *Listeria* contamination in a single food product in Germany, and compared it with the incidence of listeriosis in the German population. A similar approach was taken by Lindqvist and Westöö (2000). Buchanan *et al.* (1997) attempted to derive a conservative value, i.e. the lowest reasonable value, for the ID_{50} (i.e. that dose of *Listeria monocytogenes* that would cause 50% of people who ingested it to become ill). A criticism of this approach, and perhaps of risk assessment approaches in general, is that it tends to 'blur the details' by grouping

[1] Shrinkage is a term used to describe product not sold because it is spoiled or beyond its stated use-by date.

all types of people together, the average value obtained for the ID_{50} is heavily biased towards the response of the majority, healthy, population. The dose–response relationship ideally should reflect not only the probability of an average person becoming ill, but should identify the susceptibility of various subpopulations.

At the time of writing there is debate about which of several competing mathematical models of dose–response relationships is most appropriate. The two that are most widely used are the beta-Poisson and the exponential models (Haas, 1983). There are no reliable data sets by which to evaluate those models fully, and it is generally considered unlikely that these data will become available in the near future owing to ethical considerations involved with working with human subjects.

The exponential model is the simplest dose–response model. It assumes that there is no threshold for infection, i.e. each virus or cell has the potential to cause infection, however remote the probability. The exponential model also assumes that there is a direct proportionality between the dose of the pathogen and the risk of infection, up to an asymptotic level above which the probability of infection does not further increase. A consequence of the exponential model is that for a wide range of pathogen concentrations, the model predicts that the distribution of the pathogens in the food does not change the risk estimate, i.e. 100 cells in one unit of the food represents the same risk as one cell in each of 100 units of the food.

Dose–response relationships were derived by 'expert elicitation' for 13 foodborne pathogens by Martin *et al.* (1995). Those authors concede that the information collected from the expert elicitation process cannot substitute for the scientific data needed to accurately estimate dose–response relationships and their variance. Martin *et al.* (1995) considered, nonetheless, that 'given the difficulty of collecting experimental data on harmful pathogens, this information can provide a characterisation of scientific judgements of relative risk to the population from these microbial pathogens'.

Buchanan *et al.* (2000) and WHO/FAO (2000a) provide detailed discussion of the approaches to, and problems of, preparing dose–response models for microbial pathogens in food and water.

Risk characterisation

Risk characterisation is a synthesis of the exposure assessment and dose–response characterisation, i.e the combination of all the data gathered and their interrelationships. It results in a 'qualitative'[2] or quantitative estimation of the likelihood

[2] There is philosophical debate about whether any truly qualitative assessment of risk can be prepared. It is difficult to envisage a truly qualitative expression of risk that would convey any useful meaning, because the terms used would be completely subjective. Even qualitative terms such as 'high' or 'low' risk have some quantitative aspects, because they beg the question: ''high' or 'low' relative to what?', or rely on some implicit understanding of 'normal' or 'acceptable' risk. Even the terms 'better' or 'worse' can only be understood relative to some known level.

of occurrence, and severity, of the consequences to the health of a defined population due to the hazardous product/process/pathogen combination.

Risk characterisation can be performed and communicated in various ways:

1. Absolute measures of risk (e.g. frequency of human illness due to the product/pathogen combination of interest) or the risk relative to some known or existing level of risk.
2. A risk estimate based upon a series of point estimates, e.g. averages.
3. Average, worst-case and conservative estimates based on a series of average, worst-case and conservative estimates for each variable in the assessment to generate an estimate of the range of possible outcomes as well as one considered most likely.
4. An estimate derived by combining the frequency distribution of possible values of most or all of the variables in the system to give as complete a picture as possible of the range of possible outcomes and the likelihood of each.

Until the advent of stochastic simulation modelling software, most public health risk assessments involved the assumption and combination of a series of conservative, average and worst-case values to derive a point estimate that was presumed to be conservative and protective of public health. One weakness of that approach, however, is termed the 'problem of compounding conservatism', in which the combination of a sequence of conservative estimates leads to an overall assessment which is *too* conservative, i.e. that greatly overestimates the risk. The stochastic simulation modelling approach is the option currently preferred by most workers. However, some workers who have attempted fully quantitative risk assessments and have been frustrated by the lack of data to support that approach are seeking simpler methods for risk assessment. Semi-quantitative methods might enable simpler assessments to be undertaken more rapidly and less expensively yet still provide the benefits of the systematic approach to problem solving that formal risk assessment methods offer. Simpler methods can also be used to screen hazards to determine which are most important and may require more detailed and quantitative assessment (see Section 4.4).

4.3 Risk assessment methodology

The basic steps involved in performing a risk assessment may be considered as follows:

1. Problem formulation.
2. Data gathering.
3. System description (modelling).
4. Data and model synthesis (risk characterisation).
5. Model validation/evaluation.

4.3.1 Problem formulation

The principal purpose of risk assessment is to support decisions. The risk assessment process should begin by identifying specifically the problem to be addressed or the decision to be made. This leads to identification of the information that the risk manager needs to make that decision, and to explain the basis of that decision to others. Risk assessment should provide a structured ordering and synthesis of information relevant to the risk management decision.

Depending on the problem, not all risk assessments need be equally comprehensive or detailed. Stating the reason for doing the assessment will help to define the parameters of the assessment, and should reflect the importance of the activity, and the resources available to undertake the assessment. The resources required to provide the 'decision support' should not exceed the magnitude of the problem.

Of the spectrum of approaches available for risk assessment, the most suitable approach will depend on the problem to be investigated, and the availability of data. Furthermore, semi-quantitative approaches may be used as a means to screen a range of hazards to the industry or consumers, to discern which of them require further, more detailed (e.g. quantitative) risk assessment (van Gerwen *et al.*, 1997, 2000). Figure 4.3 shows a flow diagram of the process of risk assessment and indicates several decision points in the risk assessment process that help to define the scope and focus of the assessment.

4.3.2 Data collation

Obtaining the data to enable the risk assessment to be performed is probably the most time-consuming aspect of the overall task. It is hoped that, as the formal risk assessment approach is more widely adopted the value of data will be realised and that, once collected, data will be collated in a form that facilitates data retrieval in other forms and for other uses and users. Several forums have proposed the creation of such data 'clearing houses', which are already established in the chemical and pharmaceutical industry. In the USA a microbial food safety risk assessment 'clearing house' is established within the Joint Institute for Food Safety and Applied Nutrition (http://www.foodriskclearinghouse.umd.edu/) to 'capture' data generated and collated within risk assessments so that they are more readily available for subsequent assessments. The WHO/FAO (2000b) also recommended that the feasibility of establishing an international repository for microbial food safety risk assessment data be investigated.

Owing to the cost involved in generating data, specific data to enable assessment of risk often cannot be obtained *de novo*, and appropriate data or related data that might be used as proxies will have to be found in the published scientific literature. Useful data may also be obtained from industry records and surveys, government agencies, etc. if they can be accessed. With industry records confidentiality issues may arise. Much epidemiological data is also available via the Internet (see Section 4.7 Sources of further information and advice).

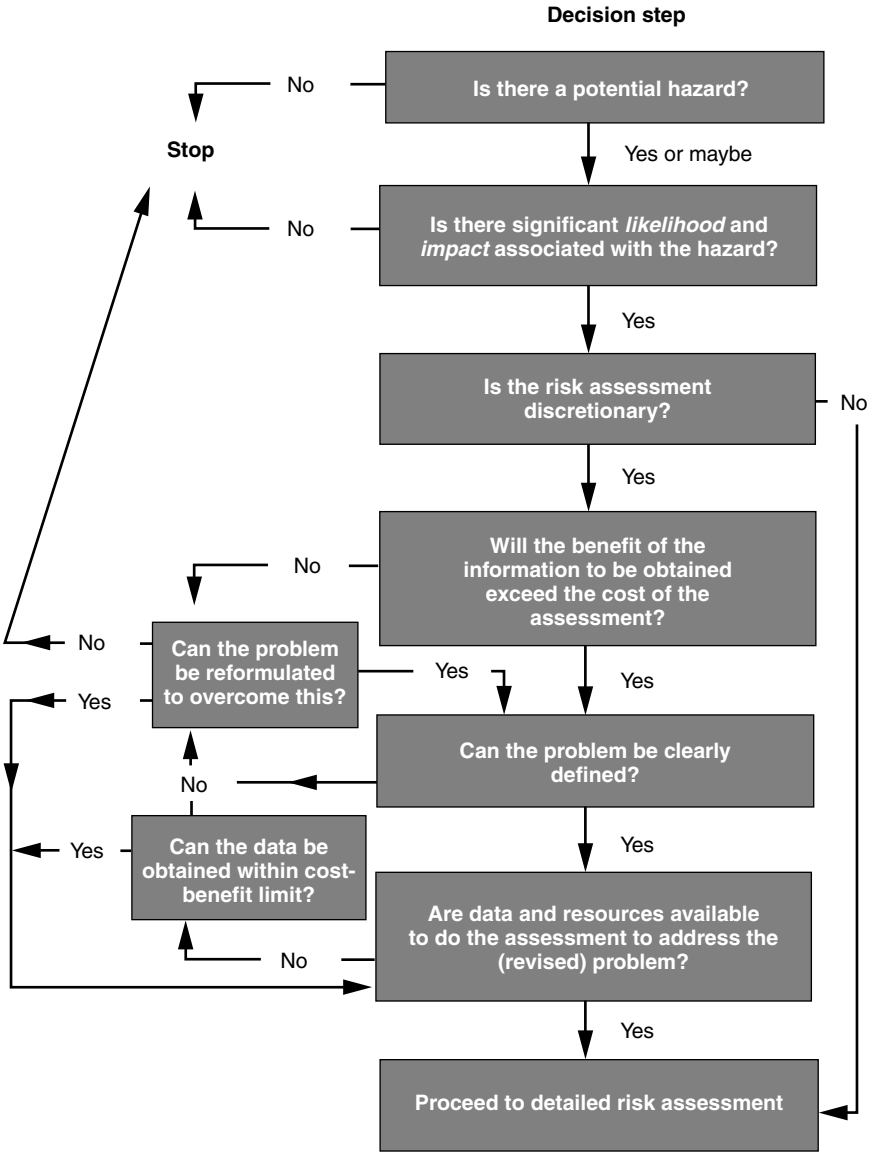

Fig. 4.3 A decision tree for microbial risk assessment projects. The same decision tree process can be applied for the first stage of the HACCP process.

'Experts' provide another source of data. There is a range of techniques that can be used systematically and objectively to obtain knowledge from experts and collate it so that it can be incorporated into risk assessments. While it is preferable to avoid the limitations of elicitation of expert opinion, a number of commentators (Hathaway, 1997; Lammerding, 1997; Whiting and Buchanan, 1997;

Vose, 1998) consider that microbial food safety risk assessment will have to rely to some extent on qualitative methods or expert opinion-based estimates of distributions at least for the foreseeable future.

4.3.3 System description

Conceptual model

Hazards can arise at any stage during the harvest, processing, distribution and preparation of a food, and microbial hazards, in particular, are probably affected by subsequent handling steps. Thus, the system under analysis is a continuum, often from the point of production (farm) to the point of consumption, and hazards at one point in the chain cannot be considered in isolation of the system as a whole. To assess risk it is necessary to *understand* where in that system the risks arise, how the risk changes and the interactions between risk-affecting factors. The system can be described in a number of ways, but it is often easiest to start the process using diagrams such as flow charts to show the origin of hazards and the relationships and operations that can change the risk during the life of the product. Fault trees and event trees can aid critical thinking in this phase, by highlighting the factors that are likely to be important inputs to the final risk, and that need to be included in the risk assessment. This description of the process is termed a 'conceptual model'. For microbial food safety risk assessment, a formulation of the conceptual model that traces the evolution and likelihood of risk in the product from harvest to consumption was developed and described as a 'process risk model' (Cassin *et al.*, 1998). While a food processing business must develop pathogen management strategies that apply specifically to its stage in the commercial food chain, it will have to understand how the product will be handled by distributors, retailers and food preparers to ensure that pathogens that may be present in the product do not reach dangerous levels prior to consumption. Thus, even an individual business developing pathogen management strategies appropriate to its own operations will often need to use a holistic conceptual model. This is discussed in more detail in Section 4.4.

Mathematical model

A refinement of the conceptual model is to construct a mathematical model of the relationships. In principle, the entire system and the relationships between all variables could be explicitly defined by expressing the relationships mathematically, i.e. using algebraic notations and equations. By substituting data or values based on expert opinion for the variables in the model, the equations describing the origin and amount of the pathogen in the food and the factors that impinge upon it can, in principle, be solved to yield a numerical estimate of exposure.

It is now possible to model very complex and variable systems easily – the so-called stochastic 'spreadsheet models'. Whiting and Buchanan (1997) first presented that approach in the food microbiology literature to assess the risk from

Salmonella enteritidis in liquid pasteurised eggs. However, while it is easy to develop spreadsheet models, it is also possible to introduce errors that are not immediately obvious, i.e. to develop models that are mathematically or logically incorrect. A common problem is to fail to include in the model relationships between variables, so that combinations of conditions that could never occur in practice are included in the model. For example, the range of storage times and storage temperatures for a food could be described independently by separate distributions. If the relationship between these factors were not explicit in the model, the model could generate predictions based on long storage times and high temperatures, situations unlikely to be observed in practice because higher temperatures are usually associated with shortened shelf-life for perishable foods. This problem arises generally in the area of quantitative risk assessment (Morgan, 1993; Vose, 1996), not only food safety risk assessment.

When solving exposure assessment models a decision has to be made regarding the value of the variables to be used in the model. Typically, the factors in a system that affect exposure do not have single, fixed, values but are characterised by a range of possible values. The most obvious method is to characterise the variable quantity by its most common (mode), or average, value. Thus, the mathematical model would produce an estimate of the risk characterised by the most commonly occurring scenario. However, this might ignore important but unusual circumstances. Exposure to low levels (e.g. <100 cfu/g) of *L. monocytogenes* in ready-to-eat (RTE) foods, is common. For the average person the likelihood of serious illness from this exposure would be predicted to be virtually nil. It is known, however, that the risk to susceptible members of the population is considerably higher, both in terms of the likelihood of infection from low doses, and also the consequences of that infection. Thus, a risk assessment in which average values were used to represent the variables could severely underestimate the true risk. Accordingly, some risk assessors have characterised variables by some values taken to characterise the risk better, e.g. 90th or 95th percentiles. The problem of 'compounding conservatism' (Cassin *et al.*, 1996) was discussed earlier.

The use of point estimates of parameters determining the probability of an adverse event cannot give a complete assessment of risk (Whiting and Buchanan, 1997) because users will also need to have an understanding of the level of certainty in the model predictions. The confidence intervals of model predictions will be affected by two factors, variability and uncertainty which are discussed later. To provide a measure of prediction confidence, many workers have advocated the use of stochastic modelling techniques for exposure and risk assessment to reflect the range and likelihood of predicted outcomes arising from all possible combinations of factors.

Stochastic modelling

In practice, the values of factors that affect microbial food safety risks form a continuous spectrum of values, some of which are more likely to occur than others, i.e. they form a *distribution*. Many distributions can be described by a

unique mathematical equation, and those mathematical equations can be used instead of the fixed variable values in the mathematical conceptual model. This approach generates 'probabilistic' or 'stochastic' models.

The answer obtained by solving a stochastic mathematical model is called the *explicit* solution. The explicit solution is itself a distribution of values, which is based on all the possible combinations of circumstances and thus shows the range of possible outcomes, as well as the probability of each of those outcomes. Each combination of factors can be considered a 'scenario', and the overall outcome of the model is based on a 'scenario set'. The explicit solution offers a complete 'picture' of the range of consequences and likelihood of all scenarios, and provides much more insight than a calculation based on average values. In most cases, however, the calculations required to reach the explicit solution become so complicated so quickly that they could not be solved for anything but the simplest models.

Typically simulation modelling software (e.g. @Risk, Crystal Ball, Analytica) is used to analyse complex systems or processes for which explicit mathematical models do not exist or are difficult, if not impossible, to solve. Instead, the software automates calculation of possible combinations of factors by calculating the answer many times sequentially. Each time is called an iteration and represents one scenario. Typically tens of thousands of iterations are performed. At each iteration a value is selected from each variable range (at random but according to the probability distribution describing that variable), and the outcome is evaluated for that set of circumstances. This technique is called Monte Carlo simulation. All of those values are collated to generate a distribution of possible outcomes, i.e. there is a range of outcomes, some of which will occur more often than others.

Simulation modelling is a powerful tool, but like many tools that appear easy to use, it is often less easy to use *correctly* and its limitations and operation must be understood to get a valid and reliable result. The results of an exposure assessment by simulation modelling are dependent on the model and the data ranges and distributions that were used, and may be dependent on any assumptions included in the model. When used appropriately, simulation software can provide a way of identifying and ranking factors that contribute to risks, quantify levels of risk, and identify strategies and information needed to control or minimise risk.

A full discussion of potential pitfalls in simulation modelling is beyond the scope of this chapter but Morgan (1993), Burmaster and Anderson (1994), Vose (1996) and EPA (1997) provide discussion and guidelines for simulation modelling in risk assessment.

4.3.4 Risk characterisation and its uses

Hazard identification, hazard characterisation and exposure assessment are for presentation and evaluation of data only, but do not present or provide conclusions. In the risk characterisation step the information is combined to provide an integrated summary.

The synthesis of the available data with the conceptual model to yield an assessment of risk can take many forms. A distinction is often made between qualitative and quantitative risk assessments. Qualitative assessments tend to use a range of 'descriptors' for levels of risk, e.g. 'low', 'moderate', high', 'extreme', but, as discussed earlier, such qualitative descriptors are inherently semi-quantitative, or else completely subjective and meaningless. A ranking of risk along some arbitrary scale can also be used, when insufficient data are available for a numerical estimate of risk. Some semi-quantitative examples are given in Section 4.4. In a fully quantitative microbial food safety risk assessment using stochastic modelling, the risk estimate is presented as a distribution of estimates of cases of human illness. Examples are shown in Fig. 4.4.

Variability and uncertainty

Acceptance of a degree of uncertainty and variability is fundamental to an estimation of risk in any model. Uncertainty refers to information that is required for completion of the assessment but that is not available and has to be assumed, or inferred, e.g. simplification of complex processes into mathematical models; small sets of scenarios are generalised to all scenarios of importance, best guesses of ranges of values where primary data are simply not available. Variability is an inherent property of some systems. There is natural variability (heterogeneity) among the constituents of a population whether people or numbers of pathogens in a sample, in strain virulence, in the susceptibility of consumers and their eating patterns, etc.

All assumptions, their scientific basis, and their implications should be clearly stated within the risk assessment, so that they are understood by the risk manager. Expert judgements that influence the risk assessment and the overall degree of confidence in the assessment should also be clearly identified.

Uses of risk characterisation

In many cases a numerical estimate of risk is of academic interest only. Often, risk managers require only a measure of risk compared to some other known level of risk in order to prioritise management strategies and actions. In many other situations, a system is modelled so that the merits of proposed risk reduction strategies can be assessed. For example: 'does a 1°C reduction in the average temperature of the chill chain reduce the risk of salmonellosis more than reducing the prevalence of *Salmonella* contaminated ready-to-eat foods by 90%?'

As stated earlier it is often difficult to obtain the data needed so that a risk assessment will be meaningful. A further application is in deciding the importance of obtaining additional data to complete the risk assessment. If a good model is developed, different assumptions about the range and distributions of the data can be tested, and sensitivity analysis used to determine how important the data are to the outcomes. From this analysis the *value* of a particular set of information can be deduced, and can be used to make decisions about research investments and priorities.

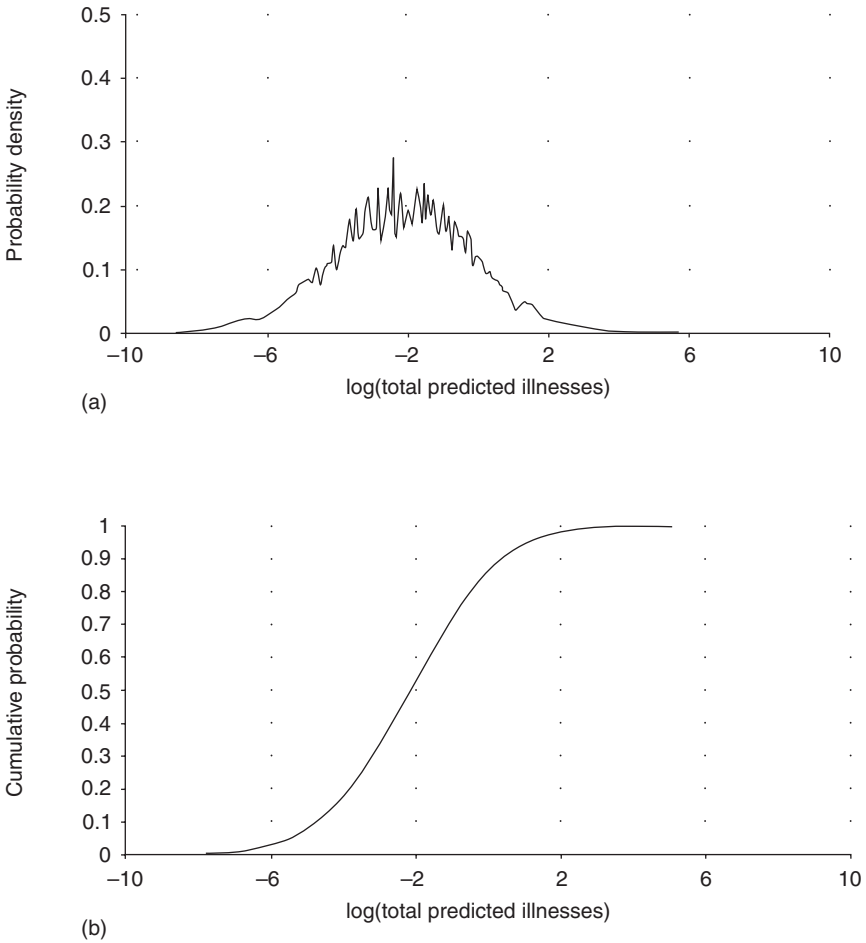

Fig. 4.4 Two presentations of a risk assessment for human health outcomes using stochastic modelling approaches. In the upper figure (a probability density plot) the *x*-axis presents the range of illnesses, while the height of the plot represents the likelihood of each outcome being observed. The lower figure is a cumulative density function. It presents the same information, but the *y*-axis is read as the likelihood that the level of illness is below the corresponding value on the *x*-axis.

Sensitivity analysis

As it goes through the iterations the software 'keeps track' of the relationship between the magnitude of the input values and the magnitude of the output. If the two values usually increase in parallel, or usually move in opposite directions, it suggests that the input strongly affects the output, and is thus a critical factor in determining the outcome. Sensitivity, or 'importance', analyses are a powerful tool within simulation software for identifying those variables in the

model that most affect the risk, and identifying them as potential targets for process control and risk management. It should be noted, however, that sensitivity of the model can be affected by the range of values that a variable is allowed to take in the model, i.e. a factor may be judged unimportant simply because it does not vary widely. For example, temperature will be very important to the growth of pathogens and hence the predicted risk, but if temperature is well controlled within narrow limits it may not be an important variable in that situation.

Scenario analysis
Scenario analysis identifies combinations of the independent variables that lead to desired levels of the dependent variable, i.e. scenario analysis tries to identify groupings of inputs that cause certain outputs to occur. This can be used to identify particularly favourable or unfavourable combinations of factors, and can aid in development of management strategies.

4.3.5 Model validation

Currently, it is not possible to develop food safety risk models without making some assumptions, because some data are not available and some possibly never will be. Clearly, there must be a 'feel' for the importance of the assumptions that are made. Some assumptions may have little effect on the output, while others may be critically important and will need to be replaced by hard data. There is a need for a 'quality control' or validation process, before the results of risk assessment can be trusted and used.

A pragmatic method is to do a simple 'reality check', i.e. do the predictions of the simulation model agree with the available data and experience that we have? Development of other, more sophisticated, techniques is required to evaluate and differentiate the effects of variability and uncertainty on model outcomes, particularly if data to verify the model's prediction are not available.

4.4 Risk assessment tools

Some schemes to assist qualitative risk assessments have already been developed. Two are presented and contrasted here.

4.4.1 Qualitative schemes

There are a number of schemes (e.g. Corlett and Pierson, 1992; NACMCF, 1992) which suggest a two-step process for risk assessment in the context of HACCP. In those approaches hazard characteristics and risk-contributing factors are formulated into statements, and the number of positive answers to the questions determines the risk ranking. Huss *et al*. (2000) adopted and adapted this approach and we consider their scheme as an example.

In the scheme, the risk of foodborne disease is determined by the number of positive answers to the following questions:

1. Is there epidemiological evidence that the particular type of food product has been associated with foodborne disease many times, or with very serious disease?
2. Does the production process *not* include any Critical Control Points for at least one identified hazard?
3. Is the product subject to potentially harmful recontamination after processing and before packaging?
4. Is there substantial potential for abusive handling in distribution or in consumer handling that could render the product harmful when consumed?
5. Is there potential for growth of pathogens in the product?
6. Is there *no* terminal heat process after packing or during preparation in the home?

If the product/pathogen combination is positive for four or more of the above questions the risk is considered to be high. Less than four positive characteristics is considered to be a low risk.

There are a number of deficiencies in the above approach when used to assess risk. First, there is no differentiation of likelihood and severity. Secondly, because of the simplicity of the scheme it does not discriminate well between different levels and sources of risk. For example severity of illness and probability of illness and/or exposure (as suggested by epidemiological data) are combined in a single question. A further difficulty in answering the question about disease severity is that it varies with host susceptibility factors. Only two levels of risk are predicted, which provides very little decision support. Clearly risk is a continuum. In addition, there is no consideration of the probability of contamination or the volume of product consumed.

The schemes are also implicitly geared toward bacterial pathogens which cause infections or produce toxins. This leads to some inconsistencies. For example, temperature abuse is only relevant if a pathogen could grow in the product to an infectious or toxic dose level, but is irrelevant to the risk if the answer to the next question ('is there potential for growth?') is negative. Other risks, e.g. due to algal toxins, or viruses, are not well predicted by the above scheme. Each factor is considered to have equal importance, which can also lead to inconsistencies because of the lack of discrimination. For example, for the risk due to *L. monocytogenes* in cold smoked salmon, the score would be: − + + + + +, suggesting that this is a very hazardous product even though there is practically no epidemiological evidence to implicate this product/pathogen combination (FAO, 1999). Conversely, enteric viruses in oysters are a well-documented source of disease. Using the above scheme, the score would be: + + − − − +, suggesting that this is less of a risk. Overall, such schemes are really intended to determine whether a perceived microbial hazard is worthy of consideration in a HACCP plan, rather than determining whether an unacceptable risk exists.

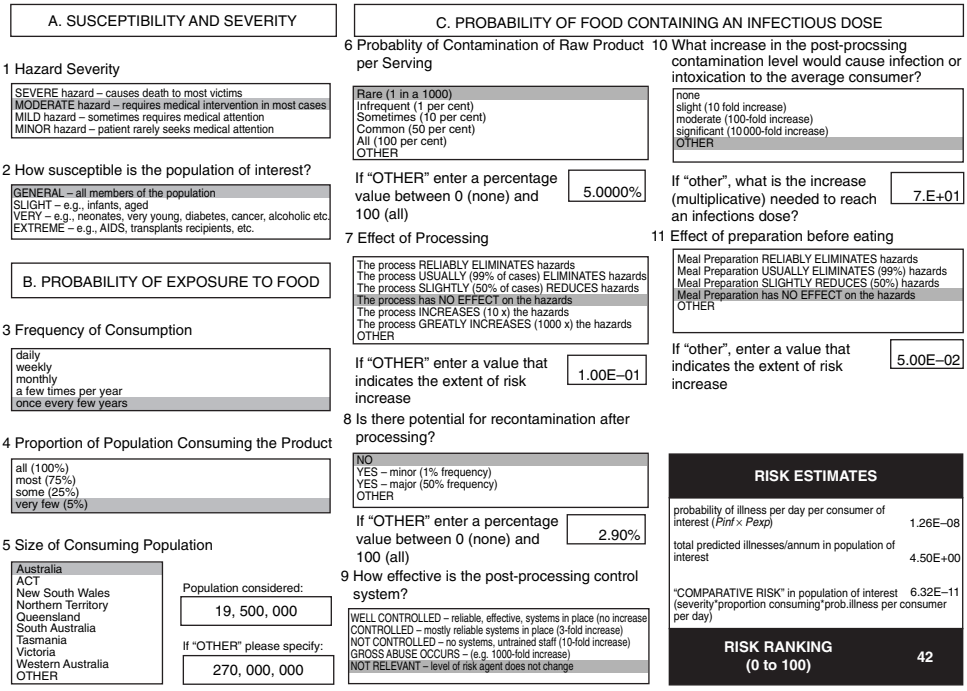

Fig. 4.5 A proposed interactive risk food safety risk assessment tool developed in spreadsheet software. Details of its use and source are described in the text.

4.4.2 Semi-quantitative schemes

Building on earlier approaches such as that above, Ross and Sumner (in press) developed a novel risk calculation tool to aid determination of relative risks from various product/pathogen/processing combinations. The tool is intended to assist risk managers, or others, without extensive experience in risk modelling to provide a first estimate of relative risk and for food safety risk management prioritisation. Additionally, the model is intended to be simple, generic and robust, to include all factors that could affect food safety risks, and to be consistent with the formal risk assessment approach described earlier.

The model is presented and automated in spreadsheet software (*see* Fig. 4.5). The user mouse-clicks on the appropriate descriptor in each box (selections are highlighted automatically) in response to 11 questions, and can nominate some specific numerical values. As a value is changed, the risk estimates (lower right) are automatically recalculated. To assist users to make selections, and to improve 'transparency' of the model, some of the weighting factors are specified in the list of descriptors.

The underlying model translates these descriptors, using relatively simple mathematical relationships, into a range of risk estimates. Some estimates con-

sider only the probability of illness, while others also consider the severity to estimate the risk of the illness and the numbers affected.

The model is based on a series of multiplicative factors that increase or decrease the estimate of the probability of the hazard occurring or the estimate of risk. Some factors, such as processing or cooking, have been assigned a value of zero, i.e. they are modelled to eliminate the risk. The model also recognises that even if a process completely eliminates the risk re-contamination may occur, however, and re-introduce the risk. The risk estimate is 'truncated' so that no more than one illness *per* consumer *per* day is predicted.

Some of the multiplicative factors are derived from fixed relationships, e.g. the risk of daily consumption compared to monthly or less frequent consumption. Similarly, the risk will depend on the size of the exposed population, and the proportion of them consuming the food. The susceptibility of the population includes values for relative risk of infection/intoxication for a variety of hazards and is based on epidemiological data. Currently, the hazard severity is arbitrarily weighted by factors of ten for each increasing level of severity.

The frequency of contamination ('probability of contamination') and the implications of subsequent processing and handling are also considered. The concentration of the hazard is included indirectly in question 10 in Fig. 4.5. In Section 4.2.2, it was stated that the distribution of the total pathogen load in the food system has little effect on the calculated risk. Differentiation of the concentration and prevalence of pathogens is not included in the tool. The risk ranking value is scaled logarithmically between 0 and 100, where 0 represents no risk and 100 represents the opposite extreme situation, i.e. where every day every member of the population eats a meal that contains a lethal dose of the hazard.

The spreadsheet, while providing estimates of risk, also helps to focus attention on the interplay of factors that contribute to the risk of foodborne disease, and can be used to explore the effect of different risk reduction strategies. Users must remember that some of the weighting factors are arbitrarily derived, however, and that the predicted effect of those management options may reflect only the assumptions on which the model is based. Nonetheless, weightings can be changed easily if data are available to indicate a more appropriate weighting.

The scheme is preliminary and is an aid, not a definitive model. It can be criticised on several grounds, but contains all elements required to estimate risk from foods and can be modified to suit the specific question of the risk assessor or risk manager. Tools such as these can help managers to think about how risks arise, and change, and to help to decide where interventions might be applied with most likelihood of success.

4.5 The role of risk assessment in pathogen management: food safety objectives and HACCP systems

For many pathogens and production systems the presence of the pathogen, even in very low numbers, can lead to unacceptable public health risks. This limits the

effectiveness of microbiological examination in assessing the safety of food. The uneven distribution of pathogens within foods further compounds the problem (Harris *et al*., 1995).

The Codex Committee on Food Hygiene (FAO/WHO, 1996) stated that microbiological safety of foods is principally assured by control at the source, product design and process control, and the application of good hygienic practices during production, processing, and handling, distribution, storage, sale, preparation and use. This philosophy is the basis of the HACCP, and similar, approaches. Such preventive systems are now almost universally considered to offer more control of food safety than end-product testing, and to provide the best mechanism for developing and implementing pathogen management strategies.

Many pathogens can *potentially* be present on or in many foods. Pathogen management must concentrate resources on those that represent the greatest risk to public health, whether due to the likelihood of the pathogen being present at dangerous levels, the probable number of people affected, or the severity of the illness caused. Pathogen management must also concentrate on preventing the presence of pathogens from becoming an unacceptable risk.

As other chapters will indicate, there are many strategies to reduce the levels and frequencies of occurrence of microbial hazards in foods at the time of consumption. Rational and optimal pathogen management strategies must discriminate between significant and trivial hazards. This requires knowledge of the pathogen, the food process and consumers of the product. Knowledge underpins effective pathogen management. That knowledge includes scientific knowledge, e.g. of pathogen physiology and ecology in foods, of virulence and pathogenicity mechanisms and the range of human responses, and industrial/commercial knowledge of processing, handling and distribution practices.

4.5.1 The ICMSF's proposal for the management of microbiological safety of foods

The International Commission for the Microbiological Specification for Foods (ICMSF) states that the first step in pathogen management is a risk assessment to determine the need for microbiological food safety objectives (FSO), and to provide the scientific basis for subsequent risk management decisions (van Schothorst, 1998). The second step identifies the strategies available for managing those risks and may lead to the specification of a FSO (Step 3). A food safety objective specifies the maximum level of microbiological hazard in a food that is considered acceptable for consumer protection. The ICMSF stresses that food safety objectives must be technologically feasible through the application of good hygienic practices and HACCP because 'these are the only tools available'.

Step 4 is proposed as the development and documentation of strategies to satisfy the FSO and which include quantifiable and verifiable performance criteria. Those performance criteria are defined as the required outcome or a step or combination of steps that can be applied to ensure that the FSO is met. The criteria need to address initial levels of contamination, reduction of the hazard

during production, manufacturing, distribution and end-use preparation steps. Process criteria (the CCPs of HACCP) are the control parameters that can be applied to achieve the performance criterion. Step 5 involves the establishment of microbiological criteria, if appropriate. These criteria can be applied to assure the incoming levels of ingredients in foods, or to verify process and performance criteria.

These five steps implicitly indicate the relationship between HACCP, risk assessment, knowledge and the task of pathogen risk management for consumer protection. The interplay of these tools in developing pathogen management strategies has been discussed by many commentators (Buchanan, 1995; Buchanan and Whiting, 1996, 1998; Elliott, 1996; Armitage, 1997; Hathaway and Cook, 1997; Mayes, 1998; Mossel *et al.*, 1998; Unterman, 1998).

4.5.2 Synthesising risk assessment and HACCP

The risk assessment framework provides a tool for synthesising and ordering knowledge so that hazards and their potential consequences can be compared. In some cases the creation of a process risk model may not generate new knowledge, but simply provide a tool to aid structured thinking and communication. If properly formulated, however, a process risk model can help to identify those potential interventions that most significantly reduce food safety risk. Additionally, if a quantitative model is used, it can be used to set critical limits for critical control points. For pathogens, predictive microbiology offers to provide a sound scientific underpinning to meet this need (see, e.g. Ross and McMeekin, 1995; Buchanan and Whiting, 1996; Hathaway and Cook, 1997; McNab, 1998; Coleman and Marks, 1999). The desire for minimally processed foods, in particular, creates a challenge that requires a quantitative approach.

Risk assessment is often seen as a high-level activity, frequently undertaken at government level. Provided, however, that outcome-based rather than prescriptive regulations are imposed by regulators the same methods can be used by a private organisation to identify the most useful pathogen control options, including specification of incoming materials quality and pathogen loads.

Microbiological limits at the point of consumption can be specified from knowledge of infectious doses. Frequencies of contamination consistent with an acceptable level of risk represent a more subjective decision. Combined, these limits can be used to set a food safety objective.

Once the target level at the point of consumption has been identified, the tools of risk assessment in combination with predictive microbiology can be used to establish end-product microbiological criteria that will satisfy the FSO. For products that offer potential for pathogen growth between the point of manufacture and consumption, the processor must consider the handling of the product after it leaves their control, i.e. manufacturers need to take a holistic approach to setting end-product criteria.

The process risk model should describe the handling of product after it leaves the processor's control, and estimate the potential for growth of pathogens to

unacceptable levels under various scenarios. The amount of growth during subsequent handling can be estimated from a knowledge of product formulation, and times, temperatures and conditions of distribution and storage using predictive models. Thus, by 'working backwards', an end-product specification can be established, leading naturally to the formulation of an end-product sampling scheme to test compliance with that specification. It should be stressed that the sampling is to test the efficacy of the HACCP system, not the individual units of product. Occasional failures would be expected and tolerated and should be part of the criterion, as is the case with three-class sampling schemes, as long as they have been demonstrated to represent an insignificant risk to consumers. ICMSF (1986) proposes 15 categories of food hazard and suggests two- and three-class sampling schemes appropriate to each of those hazard levels.

Once end-product microbiological criteria are established, the conceptual model can also be used to characterise the potential for growth or inactivation *during the process* from times, temperatures and composition of the product using predictive models. Thus, the incoming material quality can similarly be specified by 'working backwards' from the desired end-product level and understanding the effect of processing on any pathogens initially present.

Pathogen management strategies are most effectively translated into reality using HACCP or analogous approaches, i.e. HACCP is the mechanism by which food safety strategies are implemented. The conceptual risk model can also be used to assess the probable effectiveness of different strategies for pathogen management. As stated earlier, the use of sensitivity analysis can help to identify those steps in the process at which a small change will have a large effect on the outcome. Such steps are good candidates for CCPs.

Similarly, for pathogens where any level of contamination is considered unacceptable, the conceptual model, if properly formulated, will help to identify those steps in the process at which the risk can be prevented or eliminated. Figure 4.6 depicts the interplay of HACCP, risk assessment and knowledge in the development of pathogen management strategies to satisfy the overall objective of 'safe' food.

Implicit in the above is acceptance of a statistical or probabilistic approach to food safety. While risk can be minimised by pathogen management strategies, there is no zero risk. Inevitably, pathogen management is about reducing risks to acceptable levels and balancing the 'benefits' of the minimisation of food-borne disease risk against 'costs' such as increased production costs, decreased nutritional value and loss of organoleptic quality.

There will be variability and uncertainty associated with the predictions of risk assessment models which may require that 'safety margins' are added to risk estimates when setting microbiological criteria based on those estimates. Uncertainty requires the procurement of additional information, to produce a more reliable estimate of risk. Variability can be accommodated by setting criteria that encompass some selected level of safety, e.g. some percentile level on the cumulative probability curve (see Fig. 4.4b) commensurate with the required level of protection of public health. Similarly, stochastic models enable the effect of variability in individual steps in the human food chain to be quantified. This allows

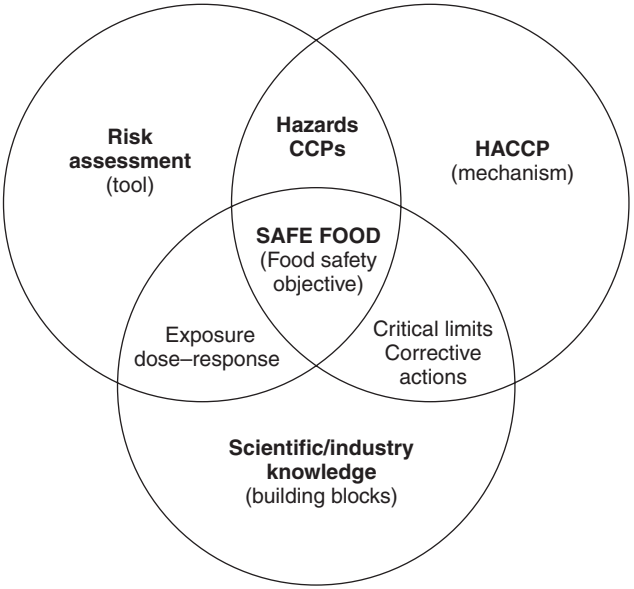

Fig. 4.6 Fundamental elements of pathogen management, showing the interplay of knowledge, decision support tools (risk assessment methods) and HACCP as the mechanism for translating quantitative, risk-based, food safety strategies into practical pathogen management systems to achieve the overall objective of 'safe' food.

the specification of meaningful critical limits for those steps, and of appropriate actions when process deviations occur, e.g. whether the deviation is so large that it will have a serious effect on risk to the consumer, and what an appropriate corrective action might be under those circumstances.

4.6 Future trends

There is a bewildering diversity of microbial pathogens and scenarios by which they, or their products, can lead to foodborne disease. There is an equally bewildering array of potential strategies for eliminating or minimising those risks. Development of optimal pathogen management strategies requires knowledge of the pathogen, the consumer, how the food becomes a vehicle for disease transmission and the differentiation of risks and hazards. The risk assessment paradigm provides a sound basis for understanding and communicating where risks arise, and how they are altered, in the human commercial food chain. This assists in developing optimal strategies that can achieve levels of safety while minimising the cost or the effects on the organoleptic qualities of the food.

Methods for microbial food safety risk assessment are still evolving, however, and will continue to do so to meet the challenges and variety of needs of risk

assessment. Those challenges include insufficient data defining the human host response to different levels of pathogens and bio-toxins, and good knowledge of microbial physiology and responses related to processing and subsequent handling of foods. Initiatives such as data repositories, however, can help to make risk assessment a progressive, cumulative and cooperative process.

Risk assessment methods can also identify which sets of information are crucial to providing better estimates of risks, and will serve as a means of prioritising research needs, or steps in the food chain where adherence to strict limits is essential, i.e. identification of critical control points and specification of critical limits and monitoring requirements for those steps. It is to be hoped that research to fill the most critical data gaps will be supported by public health authorities nationally and internationally. In the interim, in response to imperfect data, risk assessors are also working towards creating more accessible models, such as that shown in Fig. 4.5, which can help to screen apparent risks from different product/pathogen/pathway combinations, and to focus attention on those most requiring management action. This will make the benefits of the risk assessment approach for providing support for decisions available to a wider range of users concerned with pathogen management and for a wider range of situations.

In essence, a risk assessment is never completed because new data become available, systems can change and new pathogens can emerge, so that the model must continue to be developed and refined. Uncertainty is reduced as more knowledge is incorporated into the model and confidence in the reliability of the models predictions is increased. Also, models can be created as a series of connected modules so that once a stage or process in the food chain is modelled its structure or data or both can be re-used in other risk assessments. Thus, the model itself can be seen as a 'living' and structured repository of data and knowledge that can assist understanding of the complex commercial food chain and that can facilitate objective microbial food safety management decisions.

4.7 Sources of further information and advice

The preceding sections have identified many reviews and commentaries of the use of risk assessment methods and HACCP in developing pathogen management plans. Many additional resources are available from government and private Internet sites around the world, and are readily located using Internet search engines. Some sites of particular interest are presented below.

Risk World provides many links to risk analysis-related sites:

http://www.riskworld.com/

Risk analysis and HACCP:

http://haccpalliance.org/alliance/foodsafety.html

The Society for Risk Analysis has a Food and Water Special Interest Group:

http://members.tripod.com/Cristina704/Foodrisk/

A food safety risk assessment bibliography has been compiled by the USDA and is available from:

http://www.nal.usda.gov/fnic/foodborne/risk.htm

The Food and Agriculture Organization and the World Health Organization microbial food safety risk assessment activities and major reports are documented at

http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/pagerisk/riskpage.htm

and

http://www.who.int/fsf/mbriskassess/index.htm

The Center for Disease Control and Prevention (USA) web site:

http://www.cdc.gov/

provides much information on the incidence of foodborne disease, and links to other sites.

Other communicable disease resources are as follows.

Centre for Food Safety and Applied Nutrition (USA):

http://vm.cfsan.fda.gov/list.html

Public Health Laboratory Service (UK)

http://www.phls.co.uk/index.htm

and Communicable Diseases – Australia

http://www.health.gov.au/pubhlth/cdi/cdihtml.htm

provide links to many international communicable disease sites.

Useful texts include:

Vose, D. (2000) *Risk analysis: a quantitative guide*, John Wiley and Sons, Chichester, UK.

Volumes 36 (2–3) and 58 (3) in 1997 and 2000 respectively of *International Journal of Food Microbiology* featured exclusively papers concerning microbial food safety risk assessment and its applications as does the *Journal of Food Protection*, 1996 Supplement.

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5

HACCP in farm production

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5.1 Introduction

The current use of the terms 'farm to table', 'stable to table' and 'plough to plate' clearly identifies the farm as one part of the production chain which must be considered in terms of food safety. Safe food produced on a farm, whether from animal or vegetable origin, should be free from pathogens that infect people and from contamination with poisons and residues. Farming practices, in particular the apparent reliance in recent years on intensive farming systems, have been linked with the rise in foodborne illness in humans. The production of meat, milk and eggs, regardless of new technology or changes in production methods, cannot be expected to achieve zero bacterial or chemical risk. There is, however, the need to reduce the risk and, where possible, eliminate it at the farm level.

Hazard Analysis Critical Control Point (HACCP) offers a risk and management system or tool that can be implemented prospectively, unlike other programmes such as animal herd health schemes on the farm that usually work retrospectively. With the need for food manufacturers to show due diligence throughout the food chain, as a defence the HACCP system has become the recognised standard and is increasingly being extended to encompass the entire farm-to-table continuum. In the United States, for example, the 1996 USDA food safety HACCP regulations which deal with slaughterhouses are seen to have an inevitable impact on farm production practices.[1]

5.2 Planning the HACCP system

The HACCP system derived from Codex Alimentarius 1992 consists of seven principles. The sequence of applying HACCP as described by Noordhuizen and Welpelo[2] comprises 12 steps. Use of these 12 steps in relation to farming can be seen in Table 5.1.

Before a HACCP programme can be implemented in any system it is essential that all personnel be committed to the same goals. Farm resources must be sufficient to achieve the correct monitoring steps. The hazards will depend on the

Table 5.1 Steps in applying HACCP

Steps		Examples and specification
Step 1	Identification of persons involved	Farmer and employees. External experts.
Step 2	Description of products	Animals, meat, eggs, milk, wool.
Step 3	Identification of intended use	Disease agents the herd should be free of.
Step 4	Construction of flow diagram	Description of animal production process as communication tool.
Step 5	On site verification of flow diagram	Allows specific adjustments and first review of potential hazards.
Step 6	Listing of hazards at each process element [Principle 1]	Check hazards for severity and probability risk quantification needed.
Step 7	Application of a HACCP decision tree [Principle 2]	Selection of CCP for each hazard.
Step 8	Establish target levels and tolerance for each CCP [Principle 3]	Animal replacement: free of specific disease agents. Diagnostic tests: antigen-testing vs. serology.
Step 9	Establish a monitoring system [Principle 4]	CCPs are linked to a monitoring system. Monitoring aims at detecting loss of control at an early stage, and at providing information for correction action.
Step 10	Establish corrective actions [Principle 5]	Needed for each CCP selection. Correction also needed when monitoring indicates trend towards loss of control.
Step 11	Verification of the application [Principle 6]	Check correct functioning with respect to steps 6–10 necessary for introducing and maintaining system.
Step 12	Documentation [Principle 7]	Relevant processes, demonstrable control, certification and insurance.

individual farm production system which will vary between farms and within one farm, in both the species kept and the production system used. It is therefore impossible to design one HACCP plan that can be applied to all farms. There are also a number of differences between the kinds of HACCP system used in food processing and those feasible on farms:

- The people involved with Step 1 are the farm staff, usually consisting of a farmer or farm manager and in most cases only one to three members of staff, if any, who are often members of the farmer's family. In addition there are external advisers who need to be consulted, such as the farm's veterinarian and the animal feed specialist.
- In Step 2 the product is the slaughter animal (more specifically the meat that will be derived from that animal), milk or eggs.
- The intended use (Step 3) of the HACCP process is to ensure good health for the herd or flock and refers to disease agents or other hazards that the individual animal should be free of to ensure that carcase meat and offal can include, in addition to the healthy, people who are at greater risk, e.g. the immunocompromised, children, the elderly, pregnant women, and people with allergies to pharmaceutical compounds such as penicillin.
- The construction of a flow diagram in Step 4 is important as it helps to identify all the aspects of the farm production process that influence product quality as well as animal and human health.
- In Step 5, while farmers are often unfamiliar with many concepts of food safety and hygiene, it is critical that they are consulted to make adjustments to the flow diagram as they have a fundamental understanding of their farm and how it operates. A good method of doing this is to construct a flowchart for each species showing where all potential hazards may occur. The flowchart should identify all biological, chemical and physical risks that can occur, and assess them in terms of Critical Control Points (CCP).
- Effective implementation of Step 5 provides a good foundation for Steps 6 and 7.

5.3 Problems with hazard and CCP identification

As identified by Noordhuizen and Welpelo,[2] herd or flock health management requires the identification of specific disease hazards and their related preventative measures concerning the occurrence and spread of undesired disease agents. Risk assessment and risk management achieves this. It is important to understand the limitations of the HACCP system on farms. Mitchell[3] highlights the major reasons for failure which in relation to the farm would be as follows:

- Failure to establish relevant monitoring systems (Principle 4).
- Failure to establish proper corrective actions (Principle 5), despite monitoring systems highlighting the need for correction.

- Failure to consider all hazards appropriate to the farm.
- Difficulty in implementing theoretical aims practically in the farm environment.
- Over-complication of HACCP plan leading to failure of compliances.
- The farm system is not yet ready for the HACCP system.

Some of these problems may be traced to difficulties in applying HACCP to the farm environment. There are a number of factors that create these difficulties:

- Pathogens can never be entirely eliminated from a farm. With animals disease is inevitable. In addition, the very environment in which animals are reared will always have a bacterial load with some level of pathogens.
- Detecting pathogens is often difficult. Perfectly healthy animals may be disease carriers and asymptomatic excretors of pathogens. The human pathogen *Escherichia coli* 0157, which is particularly associated with ruminants, demonstrates the problem of an organism that has a highly variable presence, is able to maintain itself in the herd, yet has a transient nature of shedding influenced by a complex mix of factors such as feeding, weather and transport.[4]
- Many of the most important controls required to control pathogenic hazards are not CCPs but good working practices such as the overall standard of husbandry.

5.4 Good working practices

The type and standard of husbandry are critical to pathogen control in a number of ways. As an example, the most certain way to reduce or remove the risk of introducing disease organisms to animals is to use biosecure housing. This, of course, is contrary to the trend towards more extensive systems where there is the inevitable exposure to wildlife and vermin which are vectors of a number of important pathogens. The use of production systems that have biosecure housing does allow an 'all in all out' policy, followed by thorough cleaning and disinfection of the house before restocking. The original method was to apply this practice to each house on the site as it was emptied of animals or birds. More recently this practice has been extended to involve all animal accommodation on the site, every unit being emptied of livestock, then all cleaned and disinfected before any unit on that site is restocked.

In addition to keeping animals healthy, a critical part of husbandry is also to make sure they are kept visibly clean. This is of particular importance to reduce the possibility of contamination of milking animals and animals destined for slaughter so that they do not have dirty outer coats. A major influence on the cleanliness of the animals is the type of housing, the material used as bedding and, if the animals are kept outside, the underfoot conditions. There is a variety of housing systems used in practice, including straw bedded or deep litter yards, cow cubicles with straw, sand, rubber mats or even waterbeds as bedding, and

sheds with slatted floors, or a combination of these. Straw bedding is a much-favoured system for comfort and cleanliness but is only satisfactory if the existing bedding is regularly replaced with clean straw. Failure to use good-quality straw or empty out the yards regularly, as dung builds up, will lead to a problem with environmental organisms. This is of major concern for dairy cows housed in such a system, where failure to change the bed completely at regular intervals results in clinical mastitis caused by the environmental organisms. In some regions straw may not be available locally, which requires it to be transported from arable areas. A major factor in the effectiveness of any system in keeping the animals clean is the standard of management. Failure to attend to detail will lead to an increase in environmental organisms and inevitably also pathogens. The stockman therefore has a crucial role to play from both the animal health and public health perspectives.

5.5 Critical Control Points

This foundation of good working practices can be combined with specific controls at particular points in livestock production which can be identified as CCPs. In livestock production there are a number of points where such controls can be applied. The first is at the birth of the animal, or at hatching in the case of poultry, and extends through all stages of animal production and includes the foodstuffs fed to the animals. The aim should be to have the young born fit and healthy with good levels of maternal immunity. In addition to their appropriate use in the neonate, vaccines can be given to the pregnant dam, such as the bovine combined rotavirus and K99 *E. coli* vaccine for calf scours, to help to protect the young in the first weeks of life.

The bringing on to the farm of new animals, whether as replacement breeding stock or as animals to be fattened for slaughter, is frequently a way by which diseases are introduced. In most cases the major impact will be from diseases that affect animals but frequently such infections can include zoonotic organisms. It is of the utmost importance that incoming animals are kept separate from those already on the farm for the necessary period of quarantine and where possible that they come from a farm with a known health history.

Foodstuffs that are fed to animals must be free from both pathogens and undesirable residues. The role of animal feed in food safety has been highlighted in relation to both *Salmonella*, in particular *S.* enteritidis phage type 4 in poultry,[5,6] and bovine spongiform encephalopathy (BSE) in cattle[7] and more recently dioxins in animal feeds in Belgium.[8,9] Following the BSE epidemic, the long-established practice of using recycled animal protein has been questioned, with a ban on the use of ruminant- or mammalian-derived protein in animal feeds in some countries. Animal feeds are compounded from both home-grown and imported ingredients most frequently produced as a compounded, nutritionally balanced ration from commercial feed mills. The farmer may well prepare the feeds on the farm using either home-grown or purchased forage and cereals. It

has been well documented that the ingredients for animal feeds may carry pathogens. The process of producing some forms of compounded feed, such as pelleted feed, requires a heat treatment stage which is effective against bacterial pathogens, but subsequent handling stages may allow recontamination. The farmer has a role to play in making sure the feed is stored in a manner that prevents contamination from external influences such as wildlife on the farm.

One of the easiest and perhaps more clearly defined parts of the farming operation to which the HACCP concept can be applied is the use of medications. This must include the decision-making process on whether to use, and if so which, medication as well as the mechanics of delivering the medications to the animals. While the treatment of bacterial disease in humans and companion animals is invariably directed to the individual patient, the treatment of food-producing animals, especially pigs and poultry, is generally applied on the group or herd basis.[10] The main reasons for antibiotic use in animals are therapy, prophylaxis or strategic medication, and in farm animals performance enhancement. Therapy usually involves individual animals or a defined group of diseased animals for treatment of a previously identified disease. Prophylaxis or strategic medication is usually to contain the spread of infection and prevent illness in advance of clinical signs. Prophylactic treatment involves the medication of a herd or group of animals following the diagnosis of illness in one or more animals in the group, or on the basis of previous experience, usually when a number of animals are diseased during a defined period and the probability of most, or all, animals getting infected is high. The animal diseases requiring the most extensive use of antimicrobials for therapy or prophylaxis are respiratory and enteric diseases, especially of pigs and cattle, and mastitis in dairy cattle.

The use of antibiotics, without veterinary prescription, for the purposes of increasing growth in food and animal production started in the early 1950s. Following an outbreak of food poisoning due to multi-drug resistant salmonella, an expert committee chaired by Professor Swann reviewed the use of antibiotics in agriculture. Its report in 1969[11] resulted in significant changes in the use of antibiotics, including their use for growth promotion purposes. More recently there has again been considerable concern about the use of antibiotics, especially for growth promotion purposes, in animals and specifically about food being a vector of antibiotic resistance from animals to humans. This has led to a number of reports from groups of experts, nationally and internationally, considering the use of antibiotics in animals, in humans and for plant protection purposes.[12,13]

There is agreement that there should be prudent use of antibiotics in veterinary and human medicine with little justification for the uncontrolled use of antibiotics at subtherapeutic levels to promote growth. The major concern is if there is evidence of medical equivalence for the antibiotic, either where the same drug is used in people and in animals or if there is known antibiotic resistance. This is particularly relevant if there is a possible impact on the effectiveness of important antibiotics used in human medicine, especially when the antibiotic is one of last choice for life-threatening infections. Debate on growth promotion will undoubtedly continue, but already there is evidence of sectors of the indus-

try stopping the use of antibiotic growth promoters as part of their production system.[14] It is easy to say that there should be no use of these products just to sustain cheap food production systems and make animals grow faster. However, use of some of the very same 'antibiotic growth promoters' appears to reduce disease in the animals, and stopping their use would require a greater use of therapeutic antibiotics. There is a balance, which can be achieved between the two schools of thought, that requires the husbandry systems to be changed to reduce the need for use of antibiotics in any form. The issue of consumption of residues in food of animal origin is perhaps of less concern, as there is mandatory testing for residues and a requirement only to use drugs that are licensed for use in food-producing species within EU member states.

5.6 Documentation

Farms need to provide documentation that verifies what steps are taken and what controls are in place. An effective monitoring system will also enable management to take timely decisions before a process gets out of control. Critical to food safety is the recording of the following:

- Data on the herd as a whole, including information about administration of medicinal products and immunisation programmes.
- Data on the health status, including information from the disease records and the general body condition of the animals going to transport.
- Data on the performance of each group, and the herd as a whole, e.g. daily liveweight gain, mortality and morbidity figures.
- Knowledge about farm environmental factors, which are crucial for a good result of fattening, including data on buildings.
- Feed quality control at the farm level, including feed supplier quality assurance.
- Traceability of individual animals and groups of animals at all times, including movements on to the unit.

To this information must be added the 'feedback information' from the slaughter plant or the processing plant. This would include findings at post-mortem meat inspection from the slaughterhouse, including any effects of transport such as the presence of Pale Soft Exudative (PSE) or Dark Firm Dry (DFD) meat or other defects such as injuries, filthiness, fatigue or stress. The monitoring of pathogens and residues, identified and agreed as being appropriate to the production system and to the geographical region in which the animals are produced, is part of the necessary epidemiological surveillance. The slaughter plant and further processing can provide valuable data from routine testing programmes for zoonotic pathogens such as *Salmonella* and *Trichinella spiralis* as well as for residues. For the link to be effective there must be baseline data from the live animal stage.

It is perhaps easier for the farm to apply the HACCP concept when tracking and documenting residues. This will include residues from the use of medications and from other sources. The legislative requirement for medications to be used on food-producing animals is a major factor on the safety of that food from the animal(s) but care has to be exercised to ensure residues from other sources do not enter the food chain. The assessment of risk must therefore consider all obvious sources of residues and also recognise the risk from unintentional access to source of a potential residue or to residues following an illegal act or operation.

5.7 HACCP plans: the examples of meat and dairy production

The combination of good working practices (or good manufacturing practices – GMPs) with CCPs will form the basis of a HACCP system. This can be illustrated by HACCP systems for cattle production. The various stages of cattle production are illustrated in Table 5.2. These may be compared with the detailed HACCP system for cattle production shown in Section 5.10 at the end of this chapter.

5.7.1 Beef cattle

The beef farm may raise the animals on the farm as a suckler herd followed by the fattening stage. Animals may be sold on for the final stages of the fattening process. The farmer may have no breeding animals and rear through to fat animals bought in as baby or weaned calves. Whichever system is used, it is crucial that each animal is identified, and full records of any movements between farms, auction markets and the abattoir must be kept. Cattle going for slaughter are graded by conformation criteria. In addition to deciding that the cattle are ready for slaughter, they must be inspected to ensure they do not have any condition making them unfit for human consumption. To avoid contamination of the carcass during the slaughter process the animals should be unsoiled on leaving the farm and not become soiled during transport or at auction.

5.7.2 Dairy unit

The dairy industry has had many years of experience of working to high standards of milk quality and safety. This has been helped, at least for milk from cows, by a combination of financial inducement for high standards or financial penalty for failure(s) along with legislative control. Although there is the single raw product, milk, which is consumed or will go for processing, it can be from a number of species of animal. The main milk-producing species is cattle, with sheep and goats also milked commercially. Milk can also be harvested from less

Table 5.2 Summary of production stages for cattle

Procedure	Problem	Prevention
Replacement breeding animals or purchased for fattening	Buying in disease, e.g. salmonella, tuberculosis, pneumonia	Purchase from known disease-free source – check identification. Do not introduce to herd until certain they are not carriers or excretors
Vaccination	Viral diseases, pneumonia, possibly clostridial rotavirus/*E. coli*	Vaccination of breeding stock to ensure maximum passive immunity transfer to calves and before risk period
Feed	Contamination of incoming feed and when in store with enteric bacteria and moulds. Transmissible spongiform encephalopathy	Vermin-proof stores; good quality hay and silage. No mammalian-derived protein in feed
Environment	Spread of disease by direct contact between cattle discharges, aerosol or by handler	Use good quality straw for bedding. Clean pens using 'all in all out' principle. Good ventilation if housed
Use of medicines	Injection site abscess Residues in meat Antibiotic resistance	Sterile needles and good technique. Withdrawal periods adhered to. Avoid need for antibiotics by good husbandry, clean environment and good colostrum intake by neonate
Pasture contamination	Waterlogged pasture encourage coccidia and fluke	Adequate drainage or fence off and use of coccidiostat and flukicide
	Nematode infestation	Pasture management and use of anthelmintic
	Hydatid, *C. bovis*, infestation	Regular worming of dogs and appropriate exclusion period if sludge applied
Foot care	Welfare Arthritis possible	Early recognition and treatment. Routine foot trimming and dipping
Housing during fattening	Build up of faeces on hide	Good housing and husbandry to avoid soiling. May be necessary to wash or clip before dispatch for slaughter
Housing before slaughter	Cattle coming off wet fields or fodder crops can be very soiled	Put out deep, clean, dry straw bedding for a few days or until suitable to go for slaughter

common animals such as camels, buffalo and horses and may be done so commercially in the future.

The hazards in milk are mainly from faecal and environmental contamination of the teat and udder, but both chemical and microbiological hazards can be present in the milk within the udder. The chemical contaminants may be due to feeding practices (aflatoxins, dioxins, nitrates), from husbandry practices (pesticides), from veterinary medicines and from pollution (heavy metals, radioactive elements). Microbiological hazards include the zoonotic organisms present in the milk as contaminants of the milking process, and organisms that are excreted in the milk from the udder.[15–18] The form of the milk-producing animal does not help, with the udder at the rear of the animal and under the anus. The major microbiological risk is from faeces, in particular when the faeces are soft or very liquid. Sheep and goat faeces are typically voided as pellets which reduces to some degree the faecal soiling of the animal and the hands of the milker. The relevant aspects of milk production relevant to control of the hazards include routine milking schedules, the importance of an efficient, well-maintained milking machine, management of the housing, and mastitis control. Bacteria reach the milk from contamination of the udder surface, from within the mammary gland and from the inner surfaces of the milking equipment, including the bulk storage tank. Milk from a cow with clinical mastitis can easily have 10^6 organisms per millilitre, which if allowed to pass into the bulk tank could have serious consequences. Subclinical mastitis is a problem for the farmer, not only for the health of the udder but also as the presence of an increase in the somatic cell count of the milk lowers the quality of the product, particularly for manufacturing.

The process of milking animals can be divided into a number of defined actions which impact on the product and the well-being of the animal. There are few control points, with most actions being good practice (Table 5.3). The factors that impact on milk hygiene are shown in Fig. 5.1.

5.8 Summary: the effectiveness of HACCP on the farm

The potential benefits of on-farm HACCP for improving the health status of livestock, for reducing or controlling foodborne pathogens and for quality assurance has been commented on by several authors.[5,19–24] With regard to cattle and sheep, most attention has been focused on dairy cattle, particularly with regard to antibiotic residues in milk.[21,25] Food safety is critical for any farmer, farm group or national industry to maintain or increase market share. The implementation of the HACCP system on the farm has real potential to improve both public health, food quality and animal health and welfare.

Few commentators give examples of actual on-farm HACCP plans for cattle or sheep farms or consider the practicality of implementing such a system in a non-intensive farming environment. The poultry industry has applied HACCP-like principles as part of the *Salmonella* reduction programme. Noordhuizen and

Table 5.3 Actions taken during the milking process

Milking procedure	Action
Identify cow on entry to milking parlour	GMP
Ensure correct ration given	GMP
Remove cows with signs of mastitis	CCP
Dry wipe or wash teats (pre-milk disinfection possible)	CP
Apply cluster immediately after preparation	GMP
Remove cluster at end of milking or use automatic cluster removal to avoid over-milking	GMP
Teat end disinfection	GMP
Milking staff should wear rubber/vinyl gloves	CP
Clean and disinfect plant after each milking session	CCP
Prompt cooling of milk	CCP
On-farm pasteurisation	CCP
Test for Somatic Cell Counts and Total Bacterial Counts	CP
Test for residues	CCP

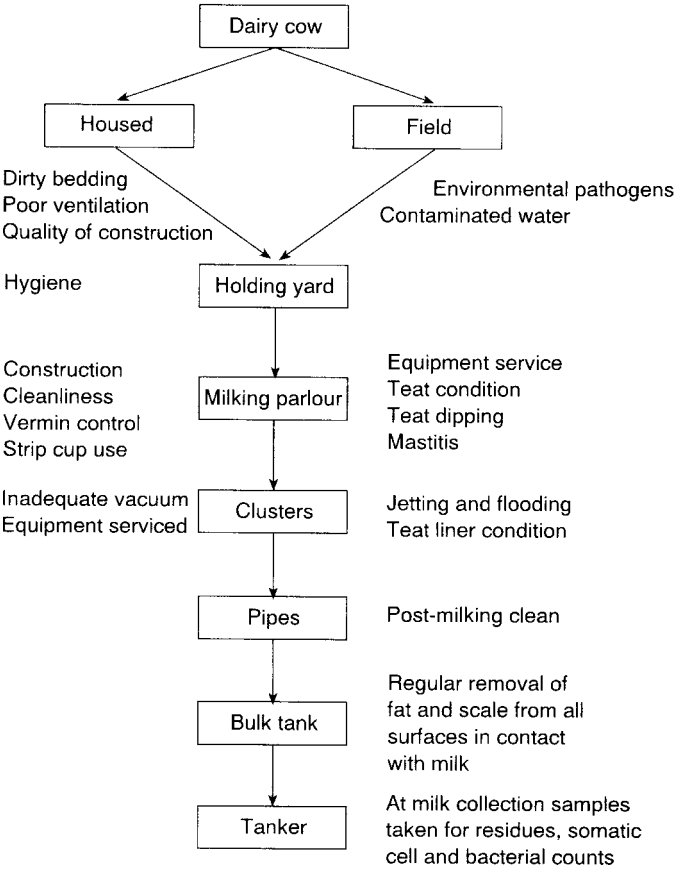

Fig. 5.1 Factors that impact on clean milk production.

Frankena[23] give an example of a generic HACCP-like approach to the control and prevention of salmonellosis on pig farms but again not for a specific farm. Furthermore Pierson[19] stated in 1995 that the animal and feed production HACCP plans that he had come across were essentially good manufacturing practice (GMP) plans in a HACCP format without any true CCPs in place. There needs to be greater provision of actual examples of farm-level HACCP plans and discussion of the practicalities, strengths and weaknesses of such plans.

Farm-level factors and their impact on health of animals intended for slaughter must have flexibility to take into account regional risks but must be part of a vertically integrated production and slaughter chain. There has been in recent years an increase in the number of farm-assured producer schemes and, through such schemes, direct influence by the major retailers on the agricultural practices of their suppliers. These farm quality assurance programmes stress the importance of a strong working relationship between producers and their customers and emphasise that efficient management practices on the farm are an important way of improving the safety of the food supply. However, it is important that the wider focus of these quality assurance schemes do not dilute the specific management of food safety.

To implement a HACCP system successfully the farm should already be observing all GMPs. There must also be a real commitment from the management to develop a HACCP system with effective communication with and training of the farm staff and others involved in any way with the farm operation. The success of any scheme for any farm or unit requires the following, as a minimum:

- Surveillance of possible diseases or risks.
- Appropriate measures for necessary actions put in place.
- Active supervision at all levels.
- Appropriate documentation.
- Investigation of all possible, or actual, problems or variations from the normal.

A cornerstone of future assurance to consumers, the EU and the rest of the world will be that proper supervision and checks are being carried out on the farm with adequate records being maintained. To provide this assurance the minimum aim must be 100% compliance with current legislation with evidence available that this level of compliance is being maintained.

The international use of the HACCP system by food manufacturers and producers is a logical progression enabling harmonisation of international food safety regulations and the removal of non-tariff barriers to trade arising from food safety.[26] The considerations must also apply to foods and animals imported from countries where the controls, for example, on antimicrobial use will not always be as rigorous as in the UK. In this respect, the consumer must recognise that there is a cost to all the improvements to farm production. If the controls placed on the industry are too stringent, there will be such an increase in the cost of production that the result will be increased imports of produce from countries where the standards of husbandry and slaughter are lower than in, for example, the UK. Increasingly agricultural produce, the raw material on which the rest of the food

industry relies, is a commodity on a worldwide market. There is increasing competition among producers of beef and lamb as well as pork/pigmeat and poultry meat. An example could be the banning of sow stalls on welfare grounds in the UK with a significant extra cost to the UK pig producer, which has not been applied in all other countries. Equally of concern at this time are the increasing reports of animal medicines available illegally, even by mail order, with suggestions that they are 'on the Internet'. They must be very tempting to farmers at this time of economic crisis in farming, not least when they are at less cost than the veterinary surgeon can purchase the same drug.

The role for food from non-traditional species must also be considered in the future. World supplies of animal-derived protein are limited and in some parts of the world under considerable pressure. It is possible to harvest more from the wild provided care is taken while drawing on wildlife reserves. Already game farming and fish farming in particular have changed the availability of different types of meat.

In conclusion the application of HACCP 'behind the farm gate' is still in its early stages; however, as consumer demand for good quality, disease-free products increases the need for the implementation of such control systems will be higher. The aim is to produce animals in a manner concurrent with these aims, with the minimum of medical/pharmaceutical intervention. This will include improvements to husbandry, appropriate use of vaccines if available, even changes to the management of the farm. While on-farm HACCP is not a panacea that will remove foodborne pathogens and other health risks from food of animal origin, it is a system with widely understood principles for identifying significant risks and their control. HACCP allows implementation of an effective documented system that will eliminate or reduce the likely occurrence of foodborne hazards. In addition HACCP is an internationally recognised system for quality assurance that is understood and accepted by the rest of the food industry, including livestock producers and customers.

Disease control in animals is multi-faceted and the more traditional 'fire-brigade' responses without consideration of preventative measures are no longer acceptable. In professional hands with diligent attention to good veterinary practice they are valuable, versatile and safe components with a vital and specific role to play in control of bacterial disease in animals. The success of animal production practices cannot be based only on an increase or a reduction of human foodborne disease. There must be a gathering of information relating to animal production, including the influence of changes in management practices that may play a role in pathogen prevalence. Epidemiological surveillance will enable the prediction or projection of risk factors and of emerging issues so that perception can be replaced by reality based on scientifically reliable data. As an example, production and health information from poultry units has been used for a number of years to target the level of post-mortem meat inspection necessary for each batch of broilers delivered to the slaughter plant. There is a strong possibility that all inspection systems will change to one based on an analysis of risk. An important part of any new system will be the monitoring of salmonella on the farms of

origin. Studies of the type by Edwards *et al*.[27] and Fries *et al*.[28] are required to provide the basis for any alternative system of integrated meat inspection. Such studies will provide the basis for an integrated production system, involving the farm, with a net benefit to consumer health protection.

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5.10 Appendix: model HACCP system for cattle

Process step	Risk: H, M, L	Control	Criteria	Control measures	Monitoring	Corrective action	Records
1. Breeding female	In poor health and/or carriers of disease/parasites. Susceptible to *Salmonella* infection (H)	GMP	All animals in good health. Free from signs of clinical infection	Therapeutic treatment of animals suffering from infections. If suspect *Salmonella* isolate animal(s) from other livestock and seek veterinary advice	Daily inspection of all animals by specified person	Veterinary advice with clinical infections or unknown causes of ill health	Medicines book. Diary of illness in animals entered into database weekly
		GMP	Improve herd/ flock resistance to clinical and sub-clinical disease	Minimize risk of disease by optimum husbandry including, e.g. control of parasitic infestations	Daily inspection. Feedback of meat inspection data from abattoir	Seek veterinary advice if prophylaxis appears to be ineffective, e.g. parasite infestation detected during PM meat inspection	Medicines book. Diary of illness. Keep record of all meat inspection results. Enter into database daily
		GMP	Good body condition	Maintain ideal condition score (CS)	Daily inspection of all animals in flock by specified person	If condition score incorrect adjust diet appropriately	Keep record of CS and diet on database
2. Breeding male, in addition to 1 above	Can introduce disease onto farm (H)	GMP	If rented or bought in should be disease free and in good health	Do not rent sires. Quarantine new sires after purchase, appropriate vaccination and prophylaxis	Specified person to inspect	If bought in sires show signs of ill health isolate immediately and seek veterinary advice	Keep record of all movements on database

3. General	Animals in poor health and/or carriers of disease/parasites. Susceptible to *Salmonella* infection (H)	GMP	All animals in flock in good health. Free from signs of clinical infection	Therapeutic treatment of sick animals. Isolation of animals which are ill or abort. Cull barren animals or those with history of mastitis	Daily inspection by specified person. Pregnancy diagnosis	Veterinary advice with clinical infections or unknown causes of ill health	Movement records. Diary of illness and results of pregnancy diagnosis. Enter into database weekly. Medicines book up to date
		GMP	Animals kept in good conditions	Provide high standard of husbandry	Monitoring of staff performance	Train staff before start job and update as necessary	Document training
4. Parturition							
Cleaning and disinfection of pens	Environmental build up of *Salmonella* (M)	GMP	No environmental contamination with *Salmonella*	Pens cleaned between groups on an all in all out basis	Weekly visual inspection of pen cleanliness by management	If cleaning insufficient, repeat cleaning process	Keep record of pen disinfection and cleaning
Newborn	Poor passive immunity. Risk of infection with Enterobacteriaceae (H)	GMP	Ensure sufficient quantity and quality of colostrum within first 6 hours	Help to suckle if having difficulty. Store colostrum to feed if extra colostrum not available from dam	Designated person to check whether neonate has fed within first 5 hours after birth	Feed with mother's, bought in or stored colostrum using stomach tube	Keep record of when colostrum given
	Hypothermia (H)	GMP	Ensure adequate colostrum received within first 8 hours	Help to suckle if having difficulty. Store colostrum to feed if extra colostrum required	Designated person to check whether neonate has fed within first 5 hours after birth	Feed with mother's or stored colostrum using stomach tube	Keep record of when lamb receives colostrum

Appendix: Continued

Process step	Risk: H, M, L	Control	Criteria	Control measures	Monitoring	Corrective action	Records
		GMP	Temperature between 39 and 40 °C	Temp. 37–39 °C: ensure fed, place below warming lamp. Temp. below 37 °C: place in warming box, give intraperitoneal injection of glucose solution	Designated person to check and take temperature if suspect hypothermia	If neonate has hypothermia carry out control measures	Keep record of animals treated for hypothermia
		GMP	Sufficient teats and milk	Check udder and number of teats	Check before parturition	Foster extra piglets, lambs	Record reason for fostering
Bedding in pens	Build up of infective material on surface layer of bedding	GMP	Clean dry bedding (straw) in pen	Place large quantities of fresh good-quality straw bedding to all pens every day, twice per day when weather wet. Individual mothering pens: add fresh straw before every new ewe and lamb(s)	Designated person to check cleanliness of bedding in pens daily	If bedding in pen not clean, add sufficient straw to cover pen surface	Keep record of number of straw bales used per day
Place mother and newborn in mothering pen	Poor bond between neonate and mother leading to poor performance/ health in lamb due to rejection	GMP	Good bond between mother and neonate	Place in mothering pen for 48 hours if mother does not accept progeny	Designated person to check for rejected newborn	If rejected, place mother and progeny into foster pen or feed artificially	Record all rejected neonates and success of fostering

Identificaton	Difficult to determine which newborn belongs to which mother	GMP	Mother and progeny should be clearly identifiable	Apply visible ID such as marker spray soon after birth. Use ear tags for individual animal identification	Daily management observation to ensure that staff identify lambs correctly	Apply identification to lambs or ewes that are unmarked or incorrectly marked. Replace identification equipment if necessary	Record all lamb and ewe identification marks, ear tags, etc.
Castration, disbudding of calves and tailing of lambs	Stress reduces ability to resist infection (M)	GMP	Disbud, castrate and tail with minimum of pain and suffering	To be carried out by competently trained individual fully conversant with legal requirements	Management (or designated person) to check daily whether castration and tailing done correctly	Advise person carrying out tailing/castration if incorrect procedure being used	Record date of birth and time of disbud, tailing or castration
5. Put out into field							
Grazing	Contamination with pathogens (M)	CCP_2	Do not allow pasture to be grazed when untreated faecal material has been applied	No grazing on land which has had sewage sludge, slurry or manure applied unless within the guidelines for application	Check records weekly to ensure sheep or cattle are not grazing grassland or forage that has not been sufficiently rested	If animals are grazing land which has not been sufficiently rested move them to a different field	Date of sludge or manure application on all fields. Sludge treatment method
		CCP_2	Contamination of pasture by geese	Bird scare device to deter geese from grazing pasture	Weekly observation by management for signs of geese. Weekly inspection of bird scare device by designated member of staff	If geese present use additional bird scare or use shooting as a control measure. Repair or replace faulty bird scare	Keep record of bird scare inspection. Keep record of sightings of geese

Appendix: Continued

Process step	Risk: H, M, L	Control	Criteria	Control measures	Monitoring	Corrective action	Records
Drinking water	Contamination with *Salmonella*, *Campylobacter*, cryptosporidia (M)	CCP_2	Drinking water free from pathogens	Use mains water only. Clean drinking troughs thoroughly annually	Sample water troughs annually and test for *Salmonella*	If trough is positive for *Salmonella* clean and disinfect immediately. Retest and if still positive, re-clean, disinfect and test water supply. Identify source of contamination	Record results of all water samples
			Clean drinking water	Ensure drinking troughs are cleaned out regularly	Daily visual inspection by designated member of staff of all drinking troughs in use	Removal of visible contamination. Empty and clean if contaminated with faeces, birds, etc.	Record findings of daily visual inspection
6. Prior to housing or parturition	Pneumonia, clostridial infection (L)	GMP	Animals free from pneumonia or clostridial infection	Vaccination prior to housing or parturition. Check ventilation of buildings	Specified person to ensure that vaccination is at correct time	If not vaccinated do so at next opportunity	Diary and medicines book
7. Control of parasites	Infestation with helminths (H) and ectoparasites (L)	GMP	Free from clinical and subclinical helminth and ectoparastic infestation	Administration of appropriate anthelmintic, depending on helminth species of concern, e.g. if	Daily inspection of all animals by specified person. Look for signs of helminth infestation such	Immediate treatment if symptoms of infestation. Seek veterinary advice if treatment	Keep livestock medicines book up to date. Keep record of meat inspection results for lambs, if

				wet grazing need to use flukicide	as diarrhoea and ectoparasites, e.g. hair loss. Post-mortem results of meat inspection	appears ineffective. Helminth infestation: modify prophylaxis if necessary	possible to obtain
8. Weaning	Post-weaning stress	GMP	–	Careful handling to minimise stress	Implement further inspection the week following weaning	Prompt treatment of animals showing signs of ill health	Records of any ill health or treatment
9. Over-wintering outside	Animals develop poor condition, ill health due to adverse conditions	GMP	Animals have dry area to shelter from wind, rain and snow	Provide shelter such as straw bales by hedge or other temporary windproof structure. Provide dry lying areas within shelter with tarpaulin, tin or other suitable cover to protect from rain or snow	Designated person to inspect shelter daily. Designated person to observe to determine if some animals are not gaining shelter	If shelter is insufficient or damaged provide additional and/or replacement shelter	Record of daily inspections of shelter
		GMP	Provision of clean water	Ensure water troughs are clean and that water is not frozen	Designated person to inspect water supply in fields daily	If supply frozen break ice and clad pipes if necessary. If water in danger of freezing increase checks to 3 times daily. If water contaminated clean trough	Record of daily inspections of water troughs

Appendix: Continued

Process step	Risk: H, M, L	Control	Criteria	Control measures	Monitoring	Corrective action	Records
			Correct nutrition for weather conditions	Ensure that sufficient roughage is available and that nutrition is at desired level	Designated person to inspect daily	If in poor condition provide supplementary feed or house	Record of daily inspections
10. General precautions							
Drinking water	Contamination with enteric pathogens (H)	CCP_2	Drinking water free from enteric pathogens	Use mains water whenever possible. Clean the drinking bowls and buckets once every month	Sample water bowls prior to housing and test for *Salmonella*	If water bowl is positive for *Salmonella* clean and disinfect immediately. Retest if still positive, re-clean, disinfect and test water supply	Record results of all water samples
			Clean drinking water	Clean and disinfect all drinking bowls and buckets before and after housing of sheep	Daily visual inspection by designated member of staff of all drinking bowls and buckets in use	Removal of visible contamination. Empty and clean if contaminated with faeces	Record findings of daily visual inspection
Clean feed	Contamination of feed with *Salmonella* (H)	CCP_2	Ensure feed is stored under clean and dry conditions	Store feed in closed bins that are dry and vermin proof. Bagged feed cover with bird-proof	Specified person to check integrity of feed bins/feed store once per week	If feed bins are damaged move any feed to a new bin and repair or replace damaged feed bin	Record findings of weekly feed bin/ feed store checks

				sheeting. Use blower so that loose feed does not come into contact with ground. Ensure feed store is dry and clean			
Rat/mice population	Infection with, e.g., *Salmonella*, *Leptospira* (H)	CCP_2	Control rat and mouse population	Poison baits around buildings. Seek advice of specialist pest-control contractor	Weekly inspection of baits by specialist contractor	Replacement of baits and poison if necessary by contractor	Keep records of all dead rats and mice found
			Have 3 metres of open ground surrounding livestock building and feed storage area	Keep whole farm tidy. Do not stack pallets or leave farm machinery by livestock buildings/feed storage	Weekly visual inspection by management	Removal of rubbish and proper storage of equipment, farm materials and machinery	Record of rubbish or equipment requiring removal
Staff	Spread of *Salmonella* from other livestock (H)	CCP_2	Clean clothes and boots	Staff must change protective clothing and use disinfectant foot dips before and after entering areas	Managerial observation	Enforcement of measure by management	Record of occasions when hygiene measure requires enforcement
Visitors	Introduction of *Salmonella* (H)	GMP	Minimise presence of visitors	Vehicles parked away from buildings	Managerial/staff checking of enforcement of these measures	Ask unauthorised visitors to immediately leave farm area. Remove vehicles from vicinity of buildings	Visitors book. Visitors should sign in and out

Appendix: Continued

Process step	Risk: H, M, L	Control	Criteria	Control measures	Monitoring	Corrective action	Records
		GMP	All visitors to wear clean protective clothing	Changing facilities near housing	Managerial/staff checking of enforcement of these measures	Ask visitors to immediately go and change into protective clothing	Record use of protective clothing. Sign clothing in and out
		CCP_2	Ensure visitors/ staff do not tread infectious agents into farm buildings	Obligation for staff and visitors to use disinfectant foot dip before entering into livestock buildings. Designated person to change foot dips weekly	Weekly managerial inspection of foot dips	Replenishment of foot dips when necessary	Record use of foot dip solution. Record inspection of foot dips
Wild birds	Infection with *Salmonella*, *Campylobacter* (H)	CCP_2	Minimise birds roosting in building roof	Use bird scare such as bird of prey silhouette or sonic bird scare	Daily visual inspection of building by specified person	Shoot pigeons	Keep records of all dead birds found
		GMP		Remove spilt, waste feed	Daily visual inspection of building by specified person	Clean up any spilt, waste feed	Keep record of spilt feed and disposal

6

Hygienic plant design and sanitation

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6.1 Introduction: hygienic design

Hygienic design and operation of a food processing plant provide the foundation of safe food manufacture. This chapter considers the hygienic design of the factory and methods of cleaning. It complements the following chapter which considers the design and operation of the manufacturing processes that take place within the factory. The chapter looks first at key issues in hygienic plant design. It then considers how these principles may be translated into practice by appropriate construction techniques. Finally, it looks at the maintenance of a hygienic factory environment by the use of correct sanitation methods.

The primary aim of hygienic plant design should be to set up effective barriers to microbial and other contamination. Factories should be constructed as a series of barriers that aim to limit the entrance of contaminants. Figure 6.1 shows that there are up to three levels of segregation that are typical for food plants:

- Level 1 represents the siting of the factory.
- Level 2 represents the factory building which should separate the factory from the external environment.
- Level 3 represents the internal barriers that are used to separate manufacturing processes of different risk, e.g. pre- and post-decontamination.

Sections 6.2–6.4 look at these different sets of barriers.

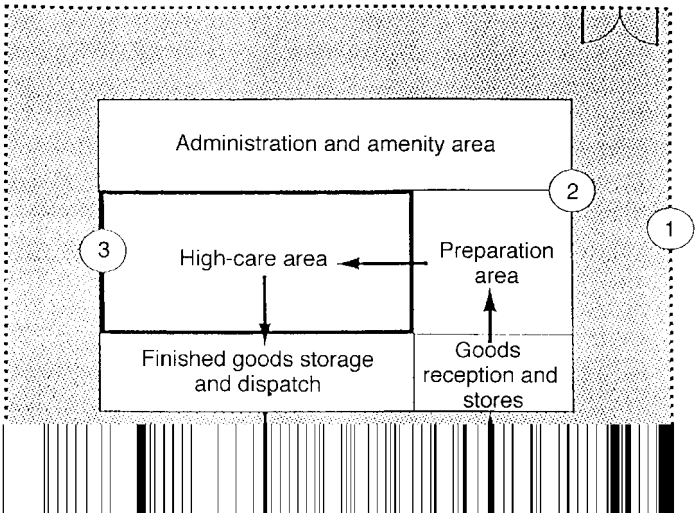

Fig. 6.1 Schematic layout of a factory site showing 'barriers' against contamination: (1) perimeter fence; (2) main factory buildings; (3) walls of high-care area.

6.2 Level 1: the factory site

Attention to the design, construction and maintenance of the site surrounding the factory provides an opportunity to set up the first (outer) of a series of barriers to protect production operations from contamination. It is a sound principle to take all reasonable precautions to reduce the 'pressures' that may build up on each of the barriers making up the overall protective envelope. A number of steps can be taken. For example, well-planned and properly maintained landscaping of the grounds can assist in the control of rodents, insects, and birds by reducing food supplies and breeding and harbourage sites.

The use of two lines of rodent baits located every 15–21 m along the perimeter boundary fencing and at the foundation walls of the factory, together with a few mouse traps near building entrances is advocated by Imholte (1984). Both Katsuyama and Strachan (1980) and Troller (1983) suggest that the area immediately adjacent to buildings be kept grass-free and covered with a deep layer of gravel or stones. This practice helps weed control and assists inspection of bait boxes and traps.

The control of birds is important, otherwise colonies can become established and cause serious problems. Shapton and Shapton (1991) state there should be a strategy of making the factory site unattractive by denying birds food and harbourage. They stress the importance of ensuring that waste material is not left in uncovered containers and that any spillages of raw materials are cleared up promptly.

Shapton and Shapton (1991) state that many insects are carried by the wind and therefore are inevitably present in a factory. They point out the importance

of preventing the unauthorised opening of doors and windows and the siting of protective screens against flying insects. Imholte (1984) considers such screens present maintenance problems. These authors draw attention to lighting for warehouses and outdoor security systems attracting night-flying insects and recommend high-pressure sodium lights in preference to mercury vapour lamps. Entrances that have to be lit at night should be lit from a distance with the light directed to the entrance, rather than lit from directly above. This prevents flying insects being attracted directly to the entrance. Some flying insects require water to support part of their life cycle, e.g. mosquitoes, and experience has shown that where flying insects can occasionally be a problem, all areas where water could collect or stand for prolonged periods of time (old buckets, tops of drums, etc.) need to be removed or controlled.

Good landscaping of sites can reduce the amount of dust blown into the factory, as can the sensible siting of any preliminary cleaning operations for raw materials such as root vegetables, which are often undertaken outside the factory. Imholte (1984) advocates orientating buildings so that prevailing winds do not blow directly into manufacturing areas. The layout of vehicular routes around the factory site can affect the amount of soil blown into buildings. Shapton and Shapton (1991) suggest that for some sites it may be necessary to restrict the routes taken by heavily soiled vehicles to minimise dust contamination.

6.3 Level 2: the factory building

The building structure is the second, major, barrier, providing protection for raw materials, processing facilities and manufactured products from contamination or deterioration. Protection is in part from potential sources of environmental contamination, including rain, wind, surface runoff, delivery and dispatch vehicles, dust, pests and uninvited people. Protection is also internally from microbiological hazards (e.g. raw material cross-contamination), chemical and physical hazards (e.g. service functions such as power, water and air supply). While protecting against these sources of contamination, the factory buildings should also be designed and constructed to suit the operations carried out in them and should not place constraints on the process or the equipment layout. If they do, they may compromise subsequent internal barriers against contamination (see Section 6.4).

The type of building, either single- or multi-storey, needs to be considered. Imholte (1984) comments that the subject has always been a controversial one and describes the advantages and disadvantages of both types of buildings. He also suggests a compromise may be achieved by having a single-storey building featuring mezzanine floors to allow gravity flow of materials, where this is necessary. Single-storey buildings are preferred for the majority of high-risk (e.g. chilled food) operations and generally allow the design criteria for high-risk areas to be more easily accommodated. However, it should be appreciated that where production is undertaken in renovated buildings, it may not be possible to capitalise on some of the advantages quoted by Imholte (1984). Of particular concern

in multi-storey buildings is leakage, of both air and fluids, from areas above and below food-processing areas. The authors have undertaken investigative work in a number of factories in which contamination has entered high-risk areas via leakage from above, through both floor defects and badly maintained drains. In addition, on a number of occasions the drainage systems have been observed to act as air distribution channels, with air from low-risk areas (both above and below) being drawn into high risk. This can typically occur when the drains are little used and the water traps dry out.

The factory layout is paramount in ensuring both an economic and safe processing operation. Straight line flow minimises the possibility of contamination of processed or semi-processed product by unprocessed or raw materials and is more efficient in terms of handling. It is also easier to segregate clean and dirty process operations and restrict movement of personnel from dirty to clean areas. While ideally the process line should be straight, this is rarely possible, but there must be no backtracking and, where there are changes in the direction of process flow, there must be adequate physical barriers. These issues are discussed in more detail in Chapter 7 on process design.

The layout should also consider that provision is made for the space necessary to undertake the process and associated quality control functions, both immediately the factory is commissioned and in the foreseeable future. Space should also be allowed for the storage and movement of materials and personnel, and for easy access to process machinery. Imholte (1984) states 915 mm (3.0 feet) should be considered as the bare minimum of space surrounding most processing units. He recommends 1830 mm (6.0 feet) as a more practical figure to allow production, cleaning and maintenance operations to be undertaken in an efficient manner.

In addition to process areas, provision may have to be made for a wide range of activities including storage of raw materials and packaging; water storage; plantroom; engineering workshop; cleaning stores; microbiology, chemistry and quality control (QC) laboratories; test kitchens; pilot plant; wash-up facilities; changing facilities; restrooms; canteens; medical rooms; observation areas/viewing galleries; and finished goods dispatch and warehousing.

Other good design principles given by Shapton and Shapton (1991) are:

- The flow of air and drainage should be away from 'clean' areas towards 'dirty' ones.
- The flow of discarded outer packaging materials should not cross, and should run counter to, the flow of either unwrapped ingredients or finished products.

Detailed information on the hygienic design requirements for the construction of the external walls or envelope of the factory is not easily found. Much of the data available are understandably concerned with engineering specifications, which are not considered in this chapter. Shapton and Shapton (1991), Imholte (1984) and Timperley (1994) discuss the various methods of forming the external walls and give a large amount of advice on pest control measures, particularly for rodents. A typical example of a suitable outside wall structure is shown in Fig. 6.2. The diagram shows a well-sealed structure that resists pest ingress and is

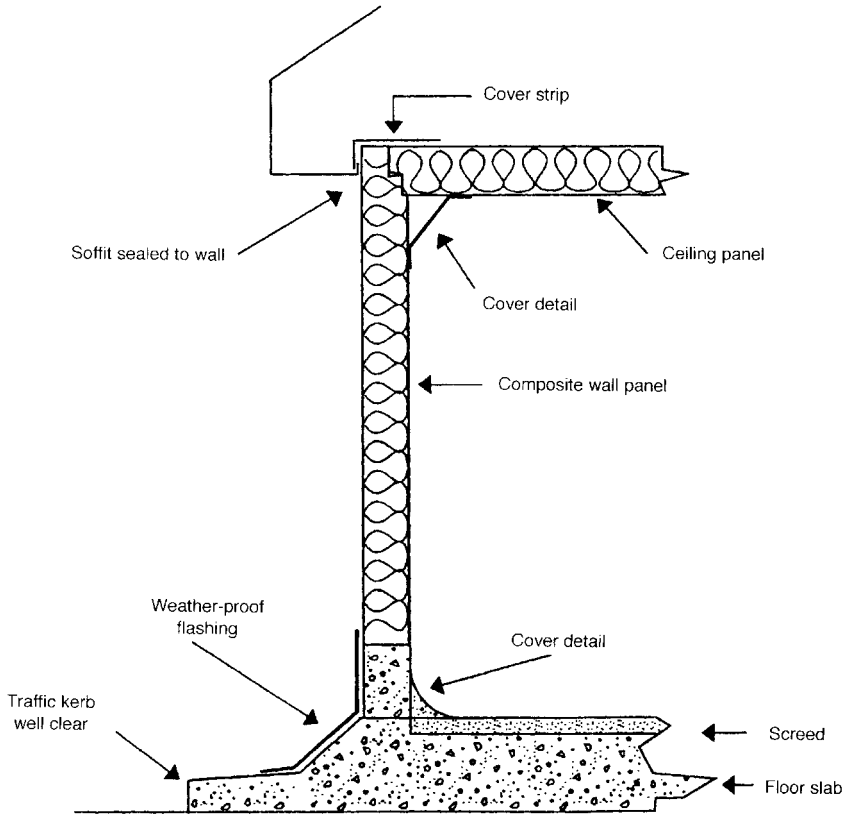

Fig. 6.2 Outside wall configuration showing a well-sealed structure with elevated factory floor level.

protected from external vehicular damage. The ground floor of the factory is also at a height above the external ground level. By preventing direct access into the factory at ground floor level, the introduction of contamination (mud, soil, foreign bodies, etc.), particularly from vehicular traffic (forklift trucks, raw material delivery, etc.) is restricted.

In addition, the above references provide considerable information on the hygienic requirements for the various openings in the envelope, particularly doors and windows. Points of particular interest are as follows:

- Doors should be constructed of metal, glass reinforced plastic (GRP) or plastic, self-closing, designed to withstand the intended use and misuse and be suitably protected from vehicular damage where applicable.
- Exterior doors should not open directly into production areas and should remain closed when not in use. Plastic strip curtains may be used as inner doors.

- If possible, factories should be designed not to have windows in food-processing areas. If this is not possible, e.g. to allow visitor or management observation, windows should be glazed with either polycarbonate or laminated. A glass register, detailing all types of glass used in the factory, and their location, should be composed.
- Metal or plastic frames with internal sills sloped (20°–40°) to prevent their use as 'temporary' storage places and with external sills sloped at 60° to prevent bird roosting, should be used.
- Opening windows must be screened in production areas and the screens be designed to withstand misuse or attempts to remove them.

6.4 Level 3: internal barriers separating manufacturing processes

The final set of barriers to contamination are those within the factory itself. Two levels of barriers are required:

- The first level to separate processing from non-processing areas.
- The second to separate 'high-risk' from 'low-risk' processing areas.

The design of any food-processing area must allow for the accommodation of five basic requirement, i.e.

- Raw materials and ingredients.
- Processing equipment.
- Staff concerned with the operation of such equipment.
- Packaging materials.
- Finished products.

All other requirements should be considered as secondary to these five basic requirements and, wherever possible, must be kept out of the processing area. These secondary requirements are:

- Structural steel framework of the factory.
- Service pipework for water, steam and compressed air; electrical conduits and trunking; artificial lighting units; and ventilation ducts.
- Compressors, refrigeration/heating units and pumps.
- Maintenance personnel and equipment associated with any of these services.

Ashford (1986) suggests building a 'box within a box' by creating insulated clean rooms within the structural box of the factory, with the services and control equipment located in the roof void above the ceiling. Equipment and ductwork are suspended from the structural frames and access to all services is provided by catwalks, as shown diagrammatically in Fig. 6.3. This arrangement, if properly undertaken, eliminates a major source of contamination from the process area.

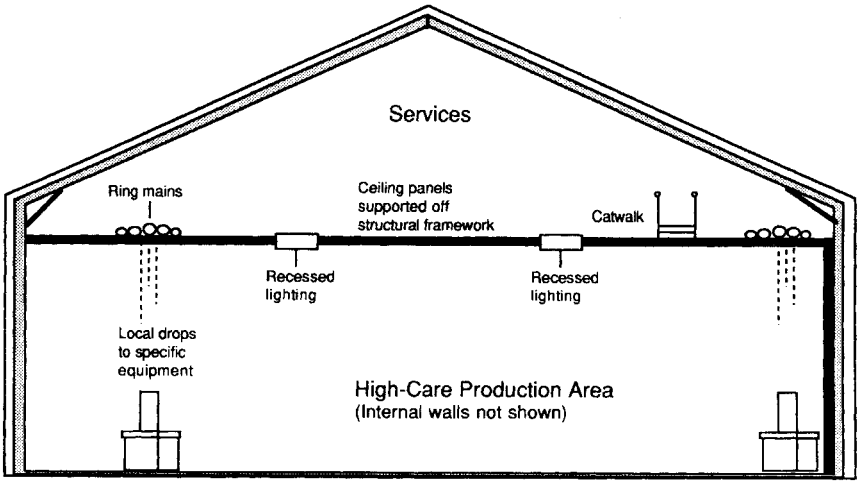

Fig. 6.3 Basic design concepts – the separation of production from services and maintenance operations.

Within the overall manufacturing area, a further, final set of barriers is required between 'high-risk' and 'low-risk' processing areas. High-risk (high-hygiene or high-care) areas may be broadly defined as areas processing food components that have undergone a decontamination or preservation process and where there is a risk of product recontamination between decontamination/preservation and a final process, for example pack sealing, which removes the immediate risk of further contamination. In contrast, low-risk areas refer to those processes dealing with food components that have not yet undergone a decontamination/preservation process. Some experts make a further distinction, for example, between 'high-risk areas' (HRAs) and 'high-care areas' (HCAs). The UK Chilled Food Association, for example, uses both terms (Anon., 1997a). In general the requirements in both these type of areas dealing with decontaminated product are the same. It is important also to note that the distinction between high- and low-risk areas does not mean that lower overall standards are acceptable in 'low-risk' areas, for example raw material reception or final product storage or distribution. Unsatisfactory practices in 'low-risk' areas may put greater pressure on the barriers separating the two, either increasing the level of initial contamination or increasing the risk of recontamination, for example through poor storage or damage to the packaging of the final product.

The final barrier between high- and low-risk processing areas is composed of a number of sub-barriers designed to control contamination from a number of routes:

- The point at which the product leaves the preservation/decontamination process and enters the high-risk area.
- The movement of other materials in to and out of the high-risk area (e.g. waste, packaging).

- The air.
- The movement of employees and equipment in to and out of high-risk areas.

Some of these potential sources of contamination may be controlled by appropriate procedures, for example governing movement of personnel and materials, which are discussed in the following chapter. The principal areas where hygienic design is the critical factor are:

- The interface between preservation/decontamination and the high-risk area.
- The air.
- Appropriate facilities to support the movement of personnel in to and out of the high-risk area.

6.4.1 The interface between preservation/decontamination and high-risk areas

Decontamination/preservation equipment must be designed such that as far as is possible a solid, physical barrier separates the low- and high-risk areas. Where it is not physically possible to form a solid barrier, air spaces around the equipment should be minimised and the low-/high-risk floor junction should be fully sealed to the highest possible height. The fitting of devices that provide heat treatment within the structure of a building presents two main difficulties. Firstly, the devices have to be designed to load product on the low-risk side and unload on the high-risk side. Secondly, the maintenance of good seals between the heating device surfaces, which cycle through expansion and contraction phases, and the barrier structure, which may have a different thermal expansion, is problematical. Of particular concern are ovens:

- Some ovens have been designed such that they drain into the high-risk area. This is unacceptable since it may be possible for any pathogens present on the surface of product to be cooked to fall to the floor through the melting of the product surface layer (or exudates on overwrapped product) at a temperature that is not lethal to the pathogen. The pathogen could then remain on the floor or in the drain of the oven in such a way that it could survive the cook cycle. On draining, the pathogen would then subsequently drain into the high-risk area. Pathogens have been found at the exit of ovens in a number of food factories.
- Problems have occurred with leakage from sumps under the ovens into the high-risk area. There can also be problems in sump cleaning where the use of high-pressure hoses can spread contamination into the high-risk area.
- Where the floor of the oven is cleaned, cleaning should be undertaken in such a way that cleaning solutions do not flow from low-risk areas to high-risk areas. Ideally, cleaning should be from the low-risk area with the high-risk area door closed and sealed. If cleaning solutions have to be drained into the high-risk area, or in the case of ovens that have a raining water cooling system, a drain should be installed immediately outside the door in the high-risk area.

6.4.2 Personnel

Within the factory building, provision must be made for adequate and suitable staff facilities and amenities for changing, washing and eating. There should be lockers for storing outdoor clothing in areas that must be separate from those for storing work clothes. Toilets must be provided and must not open directly into food-processing areas, all entrances of which must be provided with handwashing facilities arranged in such a way that their ease of use is maximised.

In high-risk operations, personnel facilities and requirements must be provided in a way that minimises any potential contamination of high-risk operations. The primary sources of potential contamination arise from the operatives themselves and from low-risk operations. This necessitates further attention to protective clothing and, in particular, special arrangements and facilities for changing into high-risk clothing and entering high-risk areas. Best practice with respect to personnel hygiene is continually developing and has been recently reviewed by Guzewich and Ross (1999), Taylor and Holah (2000) and Taylor *et al*. (2000).

High-risk factory clothing does not necessarily vary from that used in low risk in terms of style or quality, though it may have received higher standards of laundry, especially related to a higher temperature process, sufficient to reduce microbiological levels significantly. Indeed some laundries now operate to the same low-/high-risk principles as the food industry such that dirty laundry enters 'low risk', is loaded into a washing machine that bridges a physical divide, is cleaned and disinfected and exits into 'high risk' to be dried and packed.

All clothing and footwear used in the high-risk area is colour coded to distinguish it from that worn in other parts of the factory and to reduce the chance that a breach in the systems would escape early detection. High-risk footwear should be captive to high-risk areas, i.e. it should remain within high-risk areas, operatives changing into and out of footwear at the low-/high-risk boundary. This has arisen because research has shown that boot baths and boot washers are unable adequately to disinfect low-risk footwear such that they can be worn in both low and high risk and decontaminated between the two (Taylor *et al*., 2000). In addition, boot baths and boot washers can both spread contamination via aerosols and water droplets that, in turn, can provide moisture for microbial growth on high-risk floors. Bootwashers were, however, shown to be very good at removing organic material from boots and are thus a useful tool in low-risk areas both to clean boots and help prevent operative slip hazards.

The high-risk changing room should provide the only entry and exit point for personnel working in or visiting the area and is designed and built both to house the necessary activities for personnel hygiene practices and to minimise contamination from low-risk areas. In practice, there are some variations in the layout of facilities of high-risk changing rooms. This is influenced by, for example, space availability, product throughput and type of products, which will affect the number of personnel to be accommodated and whether the changing room is a barrier between the low- and high-risk operatives or between operatives arriving from outside the factory and high risk. Generally higher construction standards

are required for low-/high-risk barriers than outside/high-risk barriers because the level of potential contamination in low risk, both on the operatives hands and in the environment, is likely to be higher (Taylor and Holah, 2000). In each case, the company must evaluate the effectiveness of the changing-room layout and procedure to ensure the high-risk area and products prepared in it are not being put at risk. This is best undertaken by a Hazard Analysis Critical Control Point (HACCP) approach, so that data are obtained to support or refute any proposals regarding the layout or sequence.

Research at the Campden and Chorleywood Food Research Association (CCFRA) has also proposed the following hand hygiene sequence to be used on entry to high-risk areas (Taylor and Holah, 2000). This sequence has been designed to maximise hand cleanliness, minimise hand transient microbiological levels, maximise hand dryness yet at the same time reduce excessive contact with water and chemicals that may both lead to dermatitis of the operatives and reduce the potential for water transfer into high-risk areas.

1. Remove low-risk or outside clothing.
2. Remove low-risk/outside footwear and place in designated 'cage' type compartment.
3. Cross over the low-risk/high-risk dividing barrier.
4. WASH HANDS
5. Put on in the following order:
   - high-risk captive footwear;
   - hair net – put on over ears and cover *all* hair (plus beard snood if needed) – and hat (if appropriate);
   - overall (completely buttoned up to neck).
6. Check dress and appearance in the mirror provided.
7. Go into the high-risk production area and apply an alcohol-based sanitiser.
8. Draw and put on disposable gloves, sleeves and apron, if appropriate.

A basic layout for a changing room in shown in Fig. 6.4 and has been designed to accommodate the above hand hygiene procedure and the following requirements:

- An area at the entrance to store outside or low-risk clothing. Lockers should have sloping tops.
- A barrier to divide low- and high-risk floors. This is a physical barrier such as a small wall (approximately 60 cm high), that allows floors to be cleaned on either side of the barrier without contamination by splashing, etc., between the two.
- Open lockers at the barrier to store low-risk footwear.
- A stand on which footwear is displayed/dried.
- An area designed with suitable drainage for bootwashing operations. Research has shown (Taylor *et al*., 2000) that manual cleaning (preferably during the cleaning shift) and industrial washing machines are satisfactory bootwashing methods.

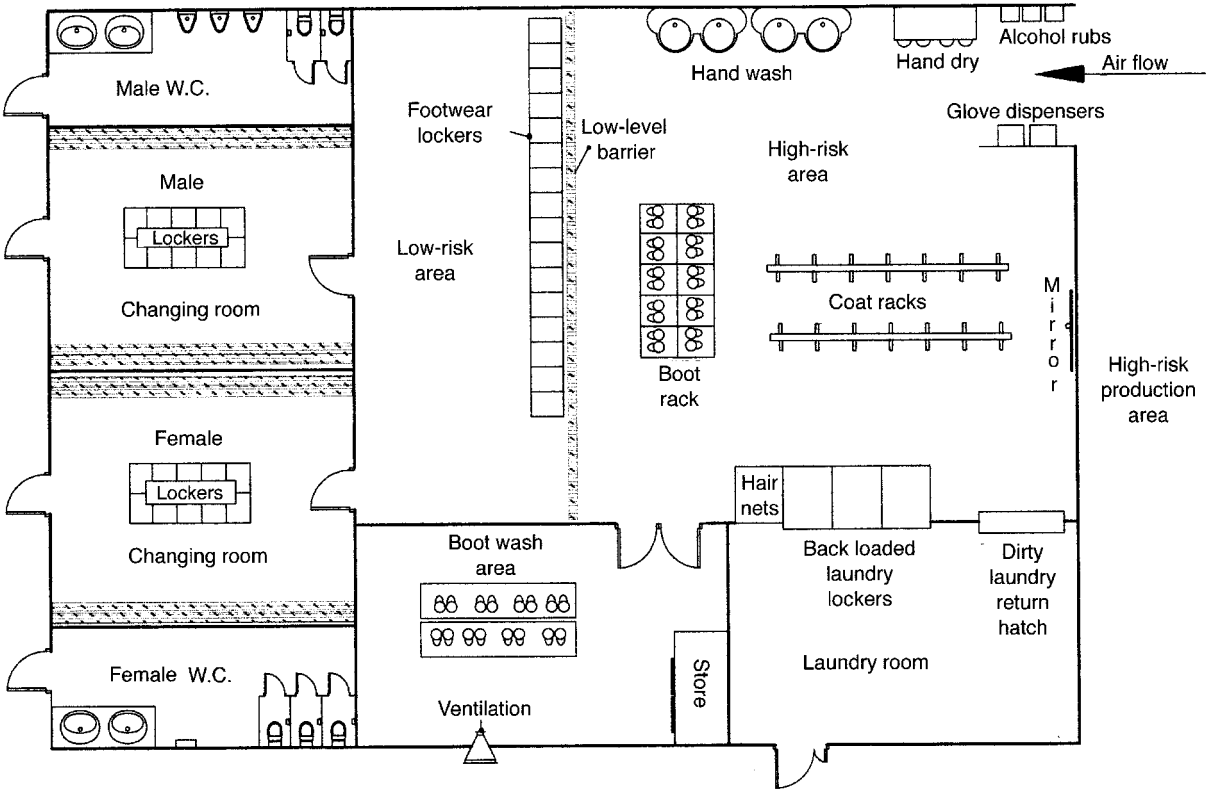

Fig. 6.4 Schematic layout for a high-risk changing room.

- Hand wash basins to service a single hand wash. Handwash basins must have automatic or knee/foot operated water supplies, water supplied at a suitable temperature (that encourages hand washing) and a waste extraction system piped directly to drain. It has been shown that hand wash basins positioned at the entrance to high-risk areas, which was the original high-risk design concept to allow visual monitoring of hand wash compliance, gives rise to substantial aerosols of staphylococcal strains that can potentially contaminate the product.
- Suitable hand-drying equipment, e.g. paper towel dispensers or hot-air dryers, and, for paper towels, suitable towel disposal containers.
- Access for clean factory clothing and storage of soiled clothing. For larger operations this may be via an adjoining laundry room with interconnecting hatches.
- Interlocked doors are possible such that doors only allow entrance to high-risk areas if a key stage, e.g. hand washing, has been undertaken.
- Closed-circuit television (CCT) cameras as a potential monitor of hand wash compliance.
- Alcoholic hand rub dispensers immediately inside the high-risk production area.

There may be the requirement to site additional handwash basins inside the high-risk area if the production process is such that frequent hand washing is necessary. As an alternative to this, Taylor *et al*. (2000) demonstrated that cleaning hands with alcoholic wipes, which can be done locally at the operative's work station, is an effective means of hand hygiene.

6.4.3 Air

The air is an important potential source of pathogens and the intake into the high-risk area has to be controlled. Air can enter the high-risk area via a purpose-built air-handling system or can enter into the area from external uncontrolled sources (e.g. low-risk production operations). For high-risk areas, the goal of the air-handling system is to supply suitable filtered fresh air, at the correct temperature and humidity, at a slight overpressure to prevent the ingress of external air sources.

The cost of the air-handling systems is one of the major costs associated with the construction of a high-risk area, and specialist advice should always be sought before embarking on an air-handling design and construction project. Following a suitable risk analysis, it may be concluded that the air-handling requirements for high-care areas may be less stringent, especially related to filtration levels and degree of overpressure. Once installed, any changes to the construction of the high-risk area (e.g. the rearrangement of walls, doors or openings) should be carefully considered as they will have a major impact on the air-handling system.

Air quality standards for the food industry were reviewed by a CCFRA Working Party and guidelines were produced (Brown, 1996). The design of the air-handling system should consider the following issues:

- Air flow and movement.
- Degree of filtration of external air.
- Overpressure.
- Temperature requirements.
- Local cooling and barrier control.
- Humidity requirements.
- Installation and maintenance.

Air flows

The main air flows within a high-risk area are shown in Fig. 6.5. A major risk of airborne contamination entering high-risk areas is from low-risk processing operations, especially those handling raw produce, which is likely to be contaminated with pathogens. The principal role of the air-handling system is thus to provide filtered air to high-risk areas with a positive pressure with respect to low-risk areas. This means that wherever there is a physical break in the low-/high-risk barrier, e.g. a hatch, the air flow will be through the opening from high to low risk. Microbial airborne levels in low-risk areas, depending on the product and processes being undertaken, may be quite high (Holah *et al*., 1995) and overpressure should prevent viable pathogenic microorganisms entering high-risk areas.

In addition to providing a positive over-pressure, the air-flow rate must be sufficient to remove the heat load imposed by the processing environment (processes and people) in maintaining the desired temperature in the high-risk area. It must also provide operatives with fresh air. Generally 5–25 air changes per hour are adequate, though in a high-risk area with large hatches/doors that are frequently opened up to 40 air changes per hour may be required.

Joint work undertaken since 1995 by CCFRA and the Silsoe Research Institute, sponsored by the UK Ministry of Agriculture, Fisheries and Food (MAFF, now DEFRA), has looked at the control of airborne microbial contamination in high-risk food production areas. The work has resulted in the production of a best practice guideline on air flows in high-risk areas published by MAFF in 2001 (Anon., 2001a). The work has centred on the measurement of both air flows and airborne microbiological levels in actual food factories. Computational fluid dynamics (CFD) models have been developed by Silsoe to predict air and particle (including microorganism) movements. The work has led to innovations in two key areas:

- Firstly, the influence on air flows of air intakes and air extracts, secondary ventilation systems in, e.g., washroom areas, the number of hatches and doors and their degree of openings and closing, can readily be visualised by CFD. This has led to the redesign of high-risk areas, from the computer screen, such that air-flow balances and positive pressures have been achieved.
- Secondly, the CFD models allow the prediction of the movement of airborne microorganisms from known sources of microbial contamination, e.g. operatives. This has allowed the design of air-handling systems which provide

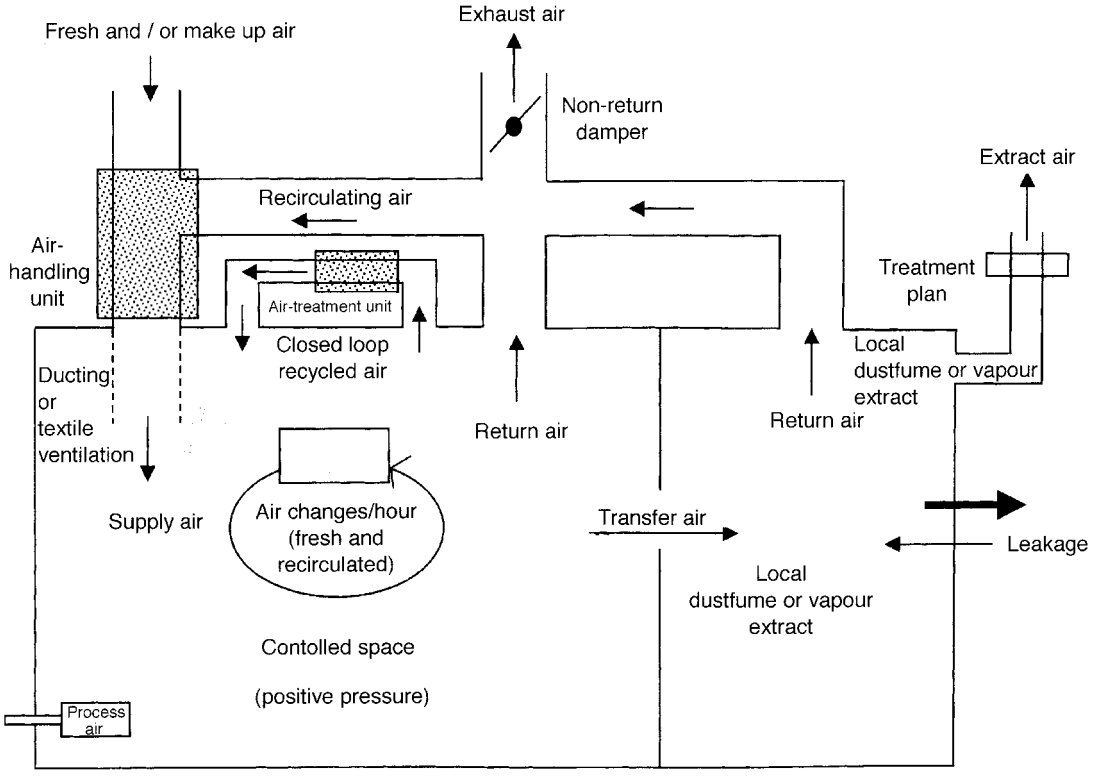

Fig. 6.5 Schematic diagram showing the air flows within a high-risk or high-care production area.

directional air that moves particles away from the source of contamination, in a direction that does not compromise product safety.

Filtration
To aid the performance of the air-handling system, it is also important to control potential sources of aerosols, generated from personnel, production and cleaning activities, in both low- and high-risk areas. Filtration of air is a complex matter and requires a thorough understanding of filter types and installations. The choice of filter will be dictated by the degree of microbial and particle removal required and filter types are described in detail in the CCFRA guideline (Brown, 1996).

Overpressure
To be effective, the pressure differential between low- and high-risk areas should be between 5 and 15 pascals. The desired pressure differential will be determined by both the number and size of openings and also the temperature differentials between low- and high-risk areas. For example, if the low-risk area is at ambient temperature (20 °C) and the high-risk area at 10 °C, hot air from the low-risk area will tend to rise through the opening while cold air from the high-risk area will tend to sink through the same opening, causing two-way flow. The velocity of air through the opening from the high-risk area may need to be 1.5 m/s or greater to ensure one-way flow is maintained.

Humidity
The choice of relative air humidity is a compromise between operative comfort, product quality and environmental drying. A relative humidity of 55–65% is very good for restricting microbial growth in the environment and increases the rate of equipment and environment drying after cleaning operations. Low humidities can, however, cause drying of the product with associated weight and quality loss, especially at higher air velocities. Higher humidities maintain product quality but may give rise to drying and condensation problems that increase the opportunity for microbial survival and growth. A compromise target humidity of 60–70% is often recommended, which is also optimal for operative comfort.

Chilled foods operations
Chilled foods manufacturers have traditionally chosen to operate their high-risk areas at low temperatures, typically around 10–12 °C, both to restrict the general growth of microorganisms in the environment and to prevent the growth of some (e.g. *Clostridium perfringens*) but not all (e.g. *Listeria*) food pathogens. Chilling the area to this temperature is also beneficial in reducing the heat uptake by the product and thus maintaining the chill chain. Moreover, chilled food manufacturers have to ensure that their products meet legislative requirements such as those governing temperature control in food processing in the UK (Anon., 1995) as well as those imposed by their retail customers. However, there is a need to balance these requirements with operator comfort. Recommendations on achieving an appropriate balance are provided, for example, by *Guidance on Achieving*

Reasonable Working Temperatures and Conditions during Production of Chilled Foods (Brown, 2000).

Another joint CCFRA/Silsoe, MAFF-sponsored, project, has examined the use of localised cooling with the objectives of:

- Providing highly filtered (H11–12), chilled air directly over or surrounding product. This could reduce the requirement to chill the whole of the high-risk area to 10 °C (13 °C would be acceptable), and reduce the degree of filtration required (down to H8–9). The requirement for positive pressure in the low-risk area is paramount, however, and the number of air changes per hour would remain unchanged.
- Using the flow of the air to produce a barrier that resists the penetration of aerosol particles, some of which would contain viable microorganisms.

An example of such a technology has been reported in Burfoot *et al*. (2000).

Equipment and installation

Air is usually supplied to high-risk areas by either ceiling grilles or textile ducts (socks), usually made from polyester or polypropylene to reduce shrinkage. Ceiling grilles have the advantage that they are cheap and require little maintenance but have limitations on velocity and flow rate without high noise levels or the potential to cause draughts. With respect to draughts, the maximum air speed close to workers to minimise discomfort through 'wind chill' is 0.3 m/s. Air socks have the ability to distribute air, at a low draught-free velocity with minimal ductwork connections, though they require periodic laundering and spare sets are required. Ceiling-mounted chillers that cool and recirculate the air are suitable for high-care operations only if additional air supplies are used to maintain positive pressures.

Finally, air-handling systems should be properly installed such that they can be easily serviced and cleaned and, as part of the commissioning programme, their performance should be validated for normal use. The ability of the system to perform in other roles should also be established. These could include dumping air directly to waste during cleaning operations, to prevent air contaminated with potentially corrosive cleaning chemicals entering the air-handling unit, and recirculating ambient or heated air after cleaning operations to increase environmental drying.

6.5 Hygienic construction

Once the design of a factory has been agreed, the next stage is construction. This section considers the following aspects of hygienic construction:

- Floors.
- Drainage.
- Walls.
- Ceilings.

6.5.1 Floors

The floor may be considered as one of the most important parts of a building because it forms the basis of the entire processing operation. It is thus worthy of special consideration and high initial capital investment. Guidelines for the design and construction of floors have been prepared by Timperley (1993).

Unsatisfactory floors increase the chances of accidents, cause difficulties in attaining the required hygiene standards and increase sanitation costs. The failure of a floor can result in lengthy disruptions of production and financial loss while repairs are completed. Design specifications for floors should cover:

- The structural floor slab.
- The waterproof membrane, which should extend up walls to a height above the normal spillage level.
- Movement joints in the subfloor and final flooring, around the perimeter of the floor, over supporting walls, around columns and machinery plinths.
- Drainage, taking into account the proposed layout of equipment.
- Screeds, either to give a flat enough surface to accept the flooring or to form the necessary falls when these are not incorporated in the concrete slab.
- Floor finish, either tiles or a synthetic resin.
- Processing considerations including trucking; impact loads from proposed operations, and equipment and machinery to be installed; degree of product spillage and associated potential problems with corrosion, thermal shock, drainage requirements; types of cleaning chemicals to be used and requirements for slip resistance.

The choice of flooring surfaces can be broadly grouped into three categories:

- Concretes.
- Fully vitrified ceramic tiles.
- Seamless resin screeds.

Concrete flooring, including high-strength granolithic concrete finishes, although suitable and widely used in other parts of a factory, is not recommended for food processing areas. This is because of its ability to absorb water and nutrients, allowing microbial growth below the surface where it is extremely difficult to apply effective sanitation programmes.

Pressed or extruded ceramic tiles have been used by the food industry for many years and are still extensively used in processing areas. In recent years they have been partially replaced on grounds of cost by the various seamless resin floors now widely available. Provided tiles of a suitable specification (fully vitrified ceramics) are selected and properly laid – an important prerequisite for all types of flooring – they are perfectly suitable for food production areas and give a long-life floor.

Tiles are laid on sand and cement mortar-bonded to the subfloor (thin bed), or on a semi-dry sand and cement mix (thick bed). A tile thickness of approximately 20 mm will provide adequate strength with either of the bedding methods. Thinner tiles (12 mm) are used for bedding into a resin bed by a vibratory method. Tile

surfaces may be smooth, studded or incorporate silicon carbide granules to improve slip resistance. Studded tiles are not recommended because of the greater difficulty of cleaning such surfaces. Ideally, surfaces that offer the greatest ease in cleaning should be used. However, in practice, the requirements for anti-slip conditions cannot be ignored and as a result the final choice should reflect a balance of the relevant factors and the emphasis placed on them.

Joints should be grouted as soon as practical, otherwise the joint faces may become contaminated. Cementitious grouts are not considered suitable for hygienic applications and resin grouts are normally used. These should not be applied for at least three days after the tiles have been laid, so that water from the bed can evaporate. Epoxy resins are widely used for grouting but have limited resistance to very high concentrations of sodium hypochlorite and soften at temperatures above 80 °C. Polyester and furan resins are more resistant to chemical attack. Shapton and Shapton (1991) cite data for the chemical resistance of different resins given by Beauchner and Reinert (1972). The grouting material should fill the joints completely to a depth of at least 12 mm and be finished flush with the tile surface. Thinner joints (1 mm) are achieved when the tiles are vibrated into a resin bed. The procedure ensures a flat plane and reduces the possibility of damage to the tile edges in use. One advantage of tile floors that is not always fully appreciated is that sections or local areas of damaged surface can be replaced and colour-matched with relative ease, so that the overall standard and appearance of the floor can be maintained.

Resin-based seamless floors offer a good alternative means of attaining a hygienic surface provided they are laid on a sound concrete base. The choice of finish can be made either from various resin-based systems (primarily epoxy or polyurethane) or from polymer-modified cementitious systems. The resin-based systems can be broadly grouped under three headings:

- *Heavy duty:* heavily filled trowel-applied systems 5–12 mm thick. Such screeds are of high strength and are normally slip-resistant.
- *Self-levelling:* 'poured and floated' systems applied at 2–5 mm thickness. These systems are sometimes more correctly described as 'self-smoothing'. They generally give smooth glossy surfaces.
- *Coatings:* usually 0.1–0.5 mm thick. They are not recommended for high-risk or other production areas because of their poor durability. Failures of such floors have been associated with microbial contamination, including *Listeria monocytogenes*, becoming trapped under loosened areas where the coating has flaked.

A further aspect that needs to be considered is whether the proposed floor meets legislative requirements. Statements in UK and EU legislation are of a general nature but do call for floors to be 'waterproof' or 'impervious' and 'cleanable'. Work at the CCFRA (Taylor and Holah, 1996) has developed a simple technique to assess the water absorption of flooring materials and materials can be quickly accepted or rejected on any water uptake recorded. Water uptake is unacceptable because if fluids are able to penetrate into flooring materials, microorganisms can

be transported to harbourage sites that are impossible to chemically clean and disinfect. Cleanability is more difficult to interpret but both Taylor and Holah (1996) and Mettler and Carpenter (1998) have proposed suitable test methods in which the cleanability of attached microorganisms is assessed. When considering the selection of flooring materials, therefore, evidence for imperviousness and cleanability should be sought. The floor should be coved where it meets walls or other vertical surfaces such as plinths or columns as this facilitates cleaning.

6.5.2 Drainage

Ashford (1986) states that drainage is often neglected and badly constructed. Detailed consideration of the drainage requirements is an important aspect of floor design. Ideally, the layout and siting of production equipment should be finalised before the floor is designed to ensure that discharges can be fed directly into drains. In practice, this is not always possible, and in the food industry in particular there is a greater chance that the layout of lines will be frequently changed. Equipment should not be located directly over drainage channels as this may restrict access for cleaning.

Discharges from equipment, however, should be fed directly into drains to avoid floor flooding. Alternatively, a low wall may be built around the equipment from which water and solids may be drained. Where the channels are close to a wall they should not be directly against it to avoid flooding of the wall to floor junction. An indirect advantage of channels near a wall is that the siting of equipment hard up to the wall is prevented, thus providing access for cleaning.

Satisfactory drainage can be achieved only if adequate falls to drainage points are provided. A number of factors should be taken into consideration when establishing the optimum or practical fall, for example:

- *Volume of water:* wet processes require a greater fall.
- *Floor finish:* trowelled resin surface finishes require a greater fall than self-levelling ones. Otherwise 'puddles' created by small depressions in the surface may remain.
- *Safety:* falls greater than 1 in 40 may introduce operator safety hazards and also cause problems with wheeled vehicles.

Timperley (1993) states that floors should have a fall to drain of between 1 in 50 and 1 in 100, depending upon the process operation and surface texture while Cattell (1988) suggests a compromise figure of 1 in 80 for general purposes and safety.

The type of drain used depends to a great extent upon the process operation involved. For operations involving a considerable amount of water and solids, channel drains are often the most suitable. For operations generating volumes of water but with little solids, aperture channel drains are more favourable (Fig. 6.6).

In most cases, channels should have a fall of at least 1 in 100, have round bottoms and not be deeper than 150 mm for ease of cleaning. They must be provided with gratings for safety reasons. The channel gratings must be easily

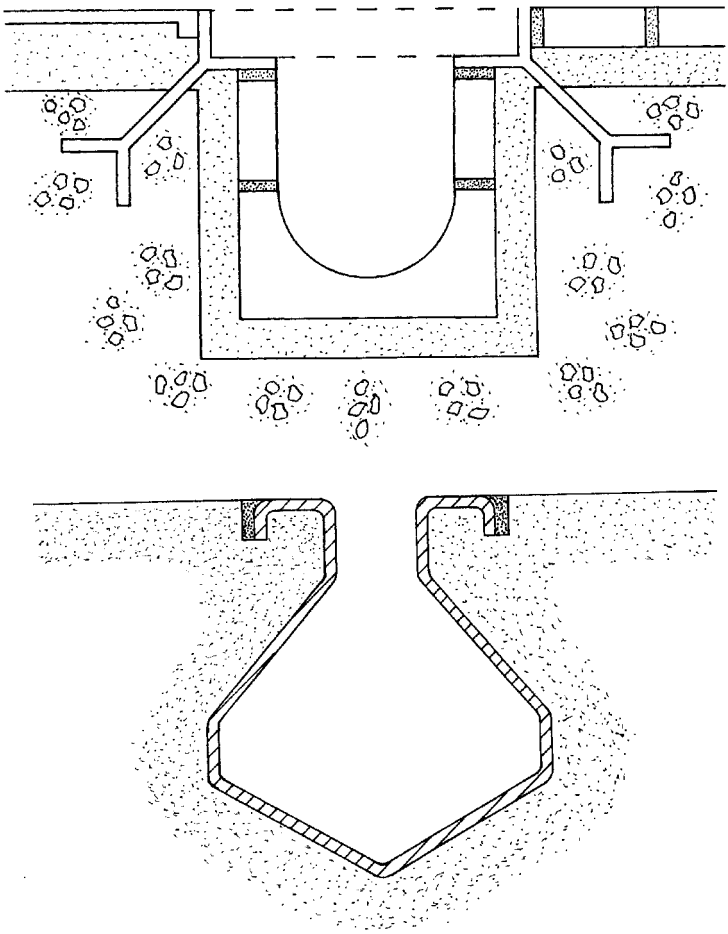

Fig. 6.6 Half-round drainage channel with reinforced rebate for grating and stainless steel aperture channel drain.

removable, with wide apertures (20 mm minimum) to allow solids to enter the drain. In recent years there has been a marked increase in the use of corrosion-resistant materials of construction, such as stainless steel for drain gratings. Stainless steel is also finding a wider use in other drain fittings, e.g. various designs of traps, and for the channels of shallower (low-volume) drainage systems. The profile of aperture channel drains is such that all internal surfaces can be easily cleaned.

The drainage system should flow in the reverse direction of production (i.e. from high to low risk) and, whenever possible, backflow from low-risk to high-risk areas should be impossible. This is best achieved by having separate low- and high-risk drains running to a master collection drain with an air-break between each collector and master drain. The drainage system should also be

designed such that rodding points are outside high-risk areas. Solids must be separated from liquids as soon as possible, by screening, to avoid leaching and subsequent high effluent concentrations. Traps should be easily accessible, frequently emptied and preferably outside the processing area.

6.5.3 Walls

Guidelines for the design and construction of walls, ceilings and services have been prepared by Timperley (1994). A number of different types of materials may be used to construct walls forming the boundaries of a high-risk area and of the individual rooms within the area. When considering the alternative systems, a number of technical factors such as hygiene characteristics, insulation properties and structural characteristics need to be taken into consideration.

Modular insulated panels are now used very widely for non-load-bearing walls. The panels are made of a core of insulating material between 50 and 200 mm thick, sandwiched between steel sheets, which are bonded to both sides of the core. Careful consideration must be given, not only to the fire retardation of the wall insulation or coating material, but also to the toxicity of the fumes emitted in the event of a fire as these could hamper a fire-fighting operation. The steel cladding is generally slightly ribbed to provide greater rigidity and can be finished with a variety of hygienic surface coatings, ready for use. The modules are designed to lock together and allow a silicone sealant to provide a hygienic seal between the units. The modules can be mounted either directly (in a U-shaped channel) onto the floor or on a concrete upstand or plinth (Fig. 6.7). The latter provides useful protection against the possibility of damage from vehicular traffic, particularly fork-lift trucks. However, it should be appreciated that this arrangement reduces the possibility of relatively easy and inexpensive changes to room layout to meet future production requirements. Sections fixed directly onto the floor must be properly bedded in silicone sealant and coved to provide an easily cleanable and watertight junction. As with wall-to-floor joints, it is also good practice to cove wall-to-ceiling junctions to assist cleaning.

To ensure continuity in the appearance and surface characteristics of walling throughout a food processing area, thin sections (50 mm) of insulated panel are sometimes used to cover external or load-bearing walls. When such a practice is adopted, there is a possibility of introducing harbourage sites for pests between the two walling materials. The chances of problems occurring are greatly increased if openings for services are made in the insulated panels without effective sealing.

In the UK, load-bearing and fire-break walls are often constructed from brick or blockwork. Walls made from such materials do not generally provide a smooth enough surface to allow the direct application of the various types of coating. A common practice is to render the brickwork with a cement and sand screed to achieve the desired surface smoothness for the coating layer. The walls may be covered by other materials such as tiles or sheets of plastics. The former is preferred, provided each tile is fully bedded and an appropriate resin is used for

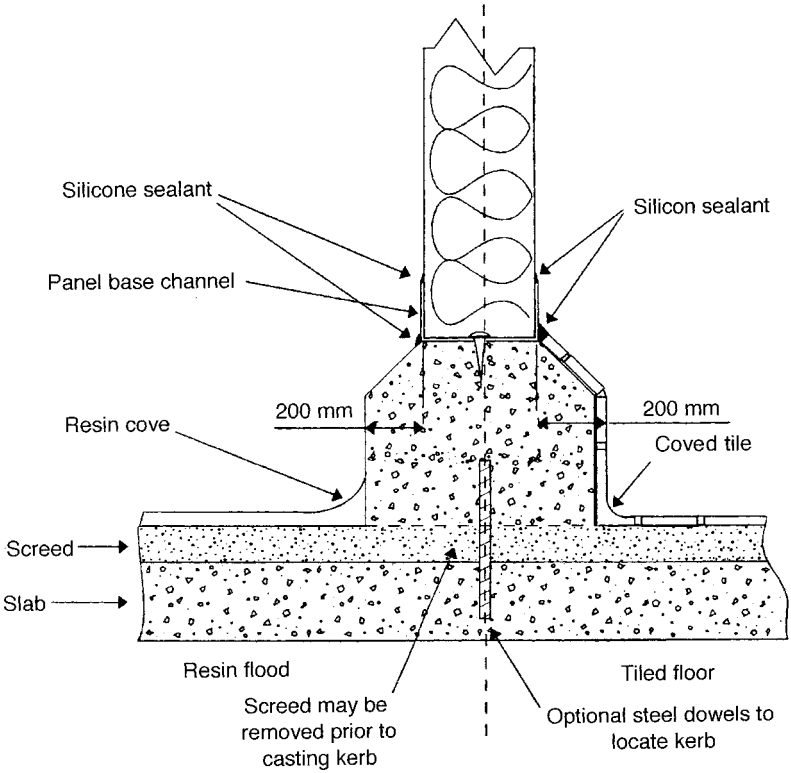

Fig. 6.7 Modular insulated panel located in U-channel and fixed to a concrete plinth.

grouting. In very wet or humid areas, where there is a strong possibility of mould growth, the application of a fungicidal coating may be considered; there is evidence that some such coatings remain effective for many years.

Hygiene standards for walls as defined in various EC Directives require that they must be constructed of impervious, non-absorbent, washable, non-toxic materials and have smooth crack-free surfaces up to a height appropriate for the operations. For high-risk areas the standard of construction and finish must apply right up to ceiling level. The same hygienic assessment techniques as described for flooring materials are also directly applicable to wall coverings and finishes.

Openings in the walls of the high-risk area need to be limited and controlled and openings for product, packaging and personnel have already been considered. In addition:

- Emergency exits: such doors must be fitted with 'out-only' operating bars. The doors must remain closed except in the case of an emergency.
- Larger 'engineering' doors required for the occasional access of equipment in and out of high-risk areas: these doors must also remain closed and should be sealed when not in use.

6.5.4 Ceilings

When considering the basic design concepts for high-risk areas, the idea of using ceilings to separate production and service functions was discussed. In practice this is often achieved by either using suitable load-bearing insulation panels or suspending sections of insulated panels, as used for the internal walls, from the structural frame of the building. The use of such insulated panels meets legislative requirements by providing a surface that is easily cleanable and will not shed particles.

It is important to ensure that drops from services passing through the ceiling are sealed properly to prevent ingress of contamination. Cables may be run in trunking or conduit but this must be effectively sealed against the ingress of vermin and water. All switchgear and controls, other than emergency stop buttons, should, whenever possible, be sited in separate rooms away from processing areas, particularly if wet operations are taking place.

Lighting may be a combination of both natural and artificial. Artificial lighting has many advantages in that, if properly arranged, it provides illumination over inspection belts and a minimum of 500–600 lux is recommended. Fluorescent tubes and lamps must be protected by shields, usually of polycarbonate, to protect the glass and contain it in the event of breakage. Suspended units should be smooth, easily cleanable and designed to the appropriate standards to prevent the ingress of water. It is suggested that lighting units are plugged in so that in the event of a failure the entire unit can be replaced and the faulty one removed from the processing areas to a designated workshop for maintenance. Ideally, recessed lighting flush with the ceiling is recommended from the hygienic aspect but this is not always possible and maintenance may be difficult.

6.6 Hygienic equipment design

The manufacture of many foods involves some element of batch or assembly operations or both. The equipment used for such operations is predominantly of the open type, that is, it cannot be cleaned by recirculation (clean-in-place, CIP) procedures, and must be of the highest hygienic design standards. Hygienic equipment design provides three major benefits to food manufacturers:

1. Quality – good hygienic design maintains product in the main product flow. This ensures that product is not ‘held-up’ within the equipment where it could deteriorate and affect product quality on rejoining the main product flow. Or, for example in flavourings manufacture, one batch could not taint a subsequent batch.
2. Safety – good hygienic design prevents the contamination of the product with substances that would adversely affect the health of the consumer. Such contamination could be microbiological (e.g. pathogens), chemical (e.g. lubricating fluids, cleaning chemicals) and physical (e.g. glass). Prevention of contamination of products with potentially allergenic agents through proper cleaning, etc., is also important.

3. Efficiency – good hygienic design reduces the time required for an item of equipment to be cleaned. This reduction of cleaning time is significant over the lifetime of the equipment such that hygienically designed equipment which is initially more expensive (compared to similarly performing poorly designed equipment), will be more cost-effective in the long term. In addition, savings in cleaning time may lead to increased production.

Texts on hygienic design include Anon. (1983a), Timperley and Timperley (1993), the European Hygienic Design Group (EHEDG 1995), Timperley (1997) and Holah (1998a). Within Europe (the EHEDG) and the USA (the 3-A Standards and the National Sanitation Foundation – NSF), a number of organisations exist to foster consensus in hygienic design and the use of these organisations' guidelines can have a quasi-legal status. It should be noted that in Europe, hygienic design guidelines tend to be more generic in nature than the more prescriptive requirements American readers may be familiar with.

In the EU, the Council Directive *on the approximation of the laws of Member States relating to machinery* (89/392/EEC) was published on 14 June 1989. The Directive includes a short section dealing with hygiene and design requirements which states that machinery intended for the preparation and processing of foods must be designed and constructed so as to avoid health risks and consists of seven hygiene rules that must be observed. These are concerned with materials in contact with food; surface smoothness; preference for welding or continuous bonding rather than fastenings; design for cleanability and disinfection; good surface drainage; prevention of dead spaces which cannot be cleaned and design to prevent product contamination by ancillary substances, e.g. lubricants. The Directive requires that all machinery sold within the EU shall meet these basic standards and be marked accordingly to show compliance (the 'CE' mark).

Subsequent to this Directive, a European Standard EN 1672-2 *Food processing machinery – Safety and hygiene requirements – Basic concepts – Part 2; Hygiene requirements* (Anon., 1997b) has recently been adopted to further clarify the hygiene rules established in 89/392/EEC. In addition to this, a number of specific standards on bakery, meat, catering, edible oils, vending and dispensing, pasta, bulk milk coolers, cereal processing and dairy equipment are in preparation. The basic hygienic design requirements as presented in EN 1672-2 can be summarised under eleven headings and are described below:

1. Construction materials. Materials used for product contact must have adequate strength over a wide temperature range and a reasonable life, and must be non-tainting, corrosion and abrasion resistant, easily cleaned and capable of being shaped. Stainless steel usually meets all these requirements and there are various grades of stainless steel which are selected for their particular properties to meet operational requirements, e.g. Type 316 which contains molybdenum is used where improved corrosion resistance is necessary.
2. Surface finish. Product contact surfaces must be finished to a degree of surface roughness that is smooth enough to enable them to be easily cleaned.

Surfaces will deteriorate with age and wear (abrasion) such that cleaning will become more difficult.

3. Joints. Permanent joints, such as those which are welded, should be smooth and continuous. Dismountable joints, such as screwed pipe couplings, must be crevice-free and provide a smooth continuous surface on the product side. Flanged joints must be located with each other and be sealed with a gasket because, although metal/metal joints can be made leak tight, they may still permit the ingress of microorganisms.
4. Fasteners. Exposed screw threads, nuts, bolts, screws and rivets must be avoided wherever possible in product contact areas. Alternative methods of fastening can be used where the washer used has a rubber compressible insert to form a bacteria-tight seal.
5. Drainage. All pipelines and equipment surfaces should be self-draining because residual liquids can lead to microbial growth or, in the case of cleaning fluids, result in contamination of product.
6. Internal angles and corners. These should be well radiused, wherever possible, to facilitate cleaning.
7. Dead spaces. As well as ensuring that there are no dead spaces in the design of equipment, care must be taken that they are not introduced during installation.
8. Bearings and shaft seals. Bearings should, wherever possible, be mounted outside the product area to avoid possible contamination of product by lubricants, unless they are edible, or possible failure of the bearings due to the ingress of the product. Shaft seals must be so designed that they can be easily cleaned and if not product-lubricated, then the lubricant must be edible. Where a bearing is within the product area, such as a foot bearing for an agitator shaft in a vessel, it is important that there is a groove completely through the bore of the bush, from top to bottom, to permit the passage of cleaning fluid.
9. Instrumentation. Instruments must be constructed from appropriate materials and if they contain a transmitting fluid, such as in a Bourdon tube pressure gauge, then the fluid must be approved for food contact. Many instruments themselves are hygienic but often they are installed unhygienically.
10. Doors, covers and panels. Doors, covers and panels should be designed so that they prevent the entry of and/or prevent the accumulation of soil. Where appropriate they should be sloped to an outside edge and should be easily removed to facilitate cleaning.
11. Controls. These should be designed to prevent the ingress of contamination and should be easily cleanable, particularly those that are repeatedly touched by food handlers to allow process operation.

The potential for well-designed and constructed equipment to be operated in a hygienic manner may be easily compromised by inadequate attention to its location and installation. Timperly (1997), when considering the accessibility of

equipment, recommended that it is more effective to consider complete lines instead of individual items of equipment and recommended the following:

- There should be sufficient height to allow adequate access for inspection, cleaning and maintenance of the equipment and for the cleaning of floors.
- All parts of the equipment should be installed at a sufficient distance from walls, ceiling and adjacent equipment to allow easy access for inspection, cleaning and maintenance, especially if lifting is involved.
- Ancillary equipment, control systems and services connected to the process equipment should be located so as to allow access for maintenance and cleaning.
- Supporting framework, wall mountings and legs should be kept to a minimum. They should be constructed from tubular or box section material which should be sealed to prevent ingress of water or soil. Angle or channel section material should not be used.
- Base plates used to support and fix equipment should have smooth, continuous and sloping surfaces to aid drainage. They should be coved at the floor junction. Alternatively, ball feet should be fitted.
- Pipework and valves should be supported independently of other equipment to reduce the chance of strain and damage to equipment, pipework and joints.

6.7 Sanitation: introduction

Provided that the process environment and production equipment have been hygienically designed, cleaning and disinfection (referred to together as 'sanitation') are the major day-to-day controls of the environmental routes of food product contamination. When undertaken correctly, sanitation programmes have been shown to be cost-effective and easy to manage, and, if diligently applied, can significantly reduce the risk of microbial contamination. Given pressure from customers, consumers and legislation for ever-increasing hygiene standards, sanitation demands the same degree of attention as any other key process in the manufacture of safe and wholesome foods.

This discussion is concerned with the sanitation of 'hard' surfaces only – equipment, floor, walls and utensils. Sanitation is undertaken primarily to remove all undesirable material (food residues, microorganisms, foreign bodies and cleaning chemicals) from surfaces in an economical manner, to a level at which any residues remaining are of minimal risk to the quality or safety of the product. Such undesirable material, generally referred to as 'soil', can be derived from normal production, spillages, line-jams, equipment maintenance, packaging or general environmental contamination (dust and dirt). To undertake an adequate and economic sanitation programme, it is essential to characterise the nature of the soil to be removed.

Product residues are readily observed and may be characterised by their chemical composition, e.g. carbohydrate, fat, protein or starch. It is also important to

be aware of processing and/or environmental factors, however, as the same product soil may lead to a variety of cleaning problems dependent primarily on moisture levels and temperature. Generally, the higher the product soil temperature (especially if the soil has been baked) and the greater the time period before the sanitation programme is initiated (i.e. the drier the soil becomes), the more difficult the soil is to remove.

Microorganisms can either be incorporated into the soil or attach to surfaces and form layers or biofilms. There are a number of factors that have been shown to affect attachment and biofilm formation such as the level and type of microorganisms present, surface conditioning layer, substratum nature and roughness, temperature, pH, nutrient availability and time available. Several reviews of biofilm formation in the food industry have been published including Pontefract (1991), Holah and Kearney (1992), Mattila-Sandholm and Wirtanen (1992), Carpenter and Cerf (1993), Zottola and Sasahara (1994), Gibson *et al*. (1995) and Kumar and Anand (1998). Following HACCP principles, if the food processor believes that biofilms are a risk to the safety of the food product, appropriate control steps must be taken. These would include providing an environment in which the formation of the biofilm would be limited, undertaking cleaning and disinfection programmes as required, monitoring and controlling these programmes to ensure their success during their operation and verifying their performance by a suitable (usually microbiological) assessment.

6.8 The principles of sanitation

Within the sanitation programme, the cleaning phase can be divided up into three stages, following the pioneering work of Jennings (1965) and interpreted by Koopal (1985), with the addition of a fourth stage to cover disinfection. These are described below:

1. The wetting and penetration by the cleaning solution of both the soil and the equipment surface.
2. The reaction of the cleaning solution with both the soil and the surface to facilitate: digestion of organic materials, dissolution of soluble organics and minerals, emulsification of fats and the dispersion and removal from the surface of solid soil components.
3. The prevention of redeposition of the dispersed soil back onto the cleansed surface.
4. The wetting by the disinfection solution of residual microorganisms to facilitate reaction with cell membranes and/or penetration of the microbial cell to produce a biocidal or biostatic action. Dependent on whether the disinfectant contains a surfactant and the disinfectant practice chosen (i.e. with or without rinsing), this may be followed by dispersion of the microorganisms from the surface.

To undertake these four stages, sanitation programmes employ a combination of four major factors as described below. The combinations of these four factors vary for different cleaning systems and, generally, if the use of one energy source is restricted, this shortfall may be compensated for by utilising greater inputs from the others.

- Mechanical or kinetic energy.
- Chemical energy.
- Temperature or thermal energy.
- Time.

Mechanical or kinetic energy is used to remove soils physically and may include scraping, manual brushing and automated scrubbing (physical abrasion) and pressure jet washing (fluid abrasion). Of all four factors, physical abrasion is regarded as the most efficient in terms of energy transfer (Offiler, 1990), and the efficiency of fluid abrasion and the effect of impact pressure has been described by Anon. (1973) and Holah (1991). Mechanical energy has also been demonstrated to be the most efficient for biofilm removal (Blenkinsopp and Costerton, 1991; Wirtanen and Mattila-Sandholm, 1993, 1994; Mattila-Sandholm and Wirtanen, 1992; Gibson *et al*., 1999).

In cleaning, chemical energy is used to break down soils to render them easier to remove and to suspend them in solution to aid rinsability. At the time of writing, no cleaning chemical has been marketed with the benefit of aiding microorganism removal. In chemical disinfection, chemicals react with microorganisms remaining on surfaces after cleaning to reduce their viability. The chemical effects of cleaning and disinfection increase with temperature in a linear relationship and approximately double for every 10 °C rise. For fatty and oily soils, temperatures above their melting point are used to break down and emulsify these deposits and so aid removal. The influence of detergency in cleaning and disinfection has been described by Dunsmore (1981), Shupe *et al*. (1982), Mabesa *et al*. (1982), Anderson *et al*. (1985) and Middlemiss *et al*. (1985). For cleaning processes using mechanical, chemical and thermal energies, generally the longer the time period employed, the more efficient the process. When extended time periods can be employed in sanitation programmes, e.g. soak-tank operations, other energy inputs can be reduced (e.g. reduced detergent concentration, lower temperature or less mechanical brushing).

Soiling of surfaces is a natural process which reduces the free energy of the system. To implement a sanitation programme, therefore, energy must be added to the soil over time to reduce both soil particle–soil particle and soil particle–equipment surface interactions. The mechanics and kinetics of these interactions have been discussed by a number of authors (Jennings, 1965; Schlussler, 1975; Loncin, 1977; Corrieu, 1981; Koopal, 1985; Bergman and Tragardh, 1990), and readers are directed to these articles since they fall beyond the scope of this chapter. In practical terms, soil removal is often rapid initially and then declines. The reasons for this are unclear, though initially, unadhered, gross soil

is usually easily removed (Loncin, 1977) while, ultimately, soils held within surface imperfections, or otherwise protected from cleaning effects, would be more difficult to remove (Holah and Thorpe, 1990).

Routine cleaning operations are never, therefore, 100% efficient, and over a course of multiple soiling/cleaning cycles, soil deposits (potentially including microorganisms) will be retained. As soil accumulates, cleaning efficiency will decrease and soil deposits may for a period grow exponentially. The timescale for such soil accumulation will differ for all processing applications and can range from hours (e.g. heat exchangers) to typically several days or weeks. Periodic cleans are employed to return the surface-bound soil accumulation to an acceptable base level (Dunsmore *et al.*, 1981) and are achieved by increasing cleaning time and/or energy input, e.g. higher temperatures, alternative chemicals or manual scrubbing. A typical example of a periodic clean is the 'weekend clean down' or 'bottoming'.

6.9 Sanitation chemicals

Within the sanitation programme it has traditionally been recognised that cleaning is responsible for the removal of not only the soil but also the majority of the microorganisms present. Mrozek (1982) showed a reduction in bacterial numbers on surfaces by up to 3 log orders whilst Schmidt and Cremmling (1981) described reductions of 2–6 log orders. The results of work at the CCFRA on the assessment of well-constructed and competently undertaken sanctions programmes on food-processing equipment in eight chilled food factories is shown in Table 6.1. The results suggest that both cleaning and disinfection are equally responsible for reducing the levels of adhered microorganisms. It is important, therefore, not only to purchase quality cleaning chemicals for their soil removal capabilities but also for their potential for microbial removal.

Unfortunately no single cleaning agent is able to perform all the functions necessary to facilitate a successful cleaning programme; so a cleaning solution, or detergent, is blended from a range of typical characteristic components:

- Water.
- Surfactants.

Table 6.1 Arithmetic and log mean bacterial counts on food processing equipment before and after cleaning and after disinfection

	Before cleaning	After cleaning	After disinfection
Arithmetic mean	1.32×10^6	8.67×10^4	2.5×10^3
Log mean	3.26	2.35	1.14
No. of observations	498	1090	3147

Table 6.2 Solubility characteristics and cleaning procedures recommended for a range of soil types

Soil type	Solubility characteristics	Cleaning procedure recommended
Sugars, organic acids, salt	Water-soluble	Mildly alkaline detergent
High protein foods (meat, poultry, fish)	Water-soluble Alkali-soluble Slightly acid-soluble	Chlorinated alkaline detergent
Starchy foods, tomatoes, fruits	Partly water-soluble Alkali-soluble	Mildly alkaline detergent
Fatty foods (fat, butter, margarine, oils)	Water-insoluble Alkaline-soluble	Mildly alkaline detergent; if ineffective, use strong alkali
Heat-precipitated water hardness, milk stone, protein scale	Water-insoluble Alkaline-insoluble Acid-soluble	Acid cleaner, used on a periodic basis

Source: Modified from Elliot (1980).

- Inorganic alkalis.
- Inorganic and organic acids.
- Sequestering agents.

For the majority of food-processing operations it may be necessary, therefore, to employ a number of cleaning products, for specific operations. This requirement must be balanced by the desire to keep the range of cleaning chemicals on site to a minimum so as to reduce the risk of using the wrong product, to simplify the job of the safety officer and to allow chemical purchase to be based more on the economics of bulk quantities. The range of chemicals and their purposes is well documented (Elliot, 1980; ICMSF, 1980, 1988; Russell *et al*., 1982; Hayes, 1985; Holah, 1991; Koopal, 1985; Anon., 1991) and only an overview of the principles is given here.

A general-purpose food detergent may, therefore, contain a strong alkali to saponify fats, weaker alkali 'builders' or 'bulking' agents, surfactants to improve wetting, dispersion and rinsability and sequestrants to control hard water ions. In addition, the detergent should ideally be safe, non-tainting, non-corrosive, stable, environmentally friendly and cheap. The choice of cleaning agent will depend on the soil to be removed and on its solubility characteristics, and these are summarised for a range of products in Table 6.2.

Because of the wide range of food soils likely to be encountered and the influence of the food manufacturing site (temperature, humidity, type of equipment, time before cleaning, etc.), there are currently no recognised laboratory methods for assessing the efficacy of cleaning compounds. Food manufacturers have to be satisfied that cleaning chemicals are working appropriately, by conducting suitable field trials.

6.10 Disinfectants

Although most of the microbial contamination is removed by the cleaning phase of the sanitation programme, there are likely to be sufficient viable microorganisms remaining on the surface to warrant the application of a disinfectant. The aim of disinfection is therefore to further reduce the surface population of viable microorganisms, via removal or destruction, and/or to prevent surface microbial growth during the inter-production period. Elevated temperature is the best disinfectant as it penetrates surfaces, is non-corrosive, is non-specific to microbial types, is easily measured and leaves no residue (Jennings, 1965). However, for open surfaces, the use of hot water or steam is uneconomic, hazardous or impossible, and reliance is, therefore, placed on chemical biocides.

While there are many chemicals with biocidal properties, many common disinfectants are not used in food applications because of safety or taint problems, e.g. phenolics or metal-ion-based products. In addition, other disinfectants are used to a limited extent only for specific purposes, e.g. peracetic acid, biguanides, formaldehyde, glutaraldehyde, organic acids, ozone, chlorine dioxide, bromine and iodine compounds. Of the acceptable chemicals, the most commonly used products are:

- Chlorine-releasing components.
- Quaternary ammonium compounds (QAC).
- Amphoterics.
- Quaternary ammonium/amphoteric mixtures.

For the disinfection of dry processing areas and for mid-shift cleaning and disinfection in high-risk areas, alcohol-based products are commonly used. This is primarily to restrict the use of water for cleaning during production as a control measure to prevent the growth and spread of any food pathogens that penetrate the high-risk area barrier controls. Ethyl alcohol (ethanol) and isopropyl alcohol (isopropanol) have bactericidal and virucidal (but not sporicidal) properties (Hugo and Russell, 1999), though they are only active in the absence of organic matter, i.e. the surfaces need to be wiped clean and then alcohol reapplied. Alcohols are most active in the 60–70% range, and can be formulated into wipe- and spray-based products.

The efficacy of disinfectants is generally controlled by five factors:

- Interfering substances (primarily organic matter).
- pH.
- Temperature.
- Concentration.
- Contact time.

To some extent, and particularly for the oxidative biocides, the efficiency of all disinfectants is reduced in the presence of organic matter. Organic material may react chemically with the disinfectant such that it loses its biocidal potency, or spatially such that microorganisms are protected from its effect. Other

interfering substances, e.g. cleaning chemicals, may react with the disinfectant and destroy its antimicrobial properties, and it is therefore essential to remove all soil and chemical residues prior to disinfection.

Disinfectants should be used only within the pH range as specified by the manufacturer. In general, the higher the temperature the greater the disinfection. For most food manufacturing sites operating at ambient conditions (around 20 °C) or higher this is not a problem as most disinfectants are formulated (and tested) to ensure performance at this temperature. This is not, however, the case in the chilled food industry. Taylor *et al*. (1999) examined the efficacy of 18 disinfectants at both 10 °C and 20 °C and demonstrated that for some chemicals, particularly quaternary ammonium-based products, disinfection was much reduced at 10 °C and recommended that in chilled production environments, only products specifically formulated for low-temperature activity should be used.

In practice, the relationship between microbial death and disinfectant concentration is not linear but follows a sigmoidal curve. Microbial populations are initially difficult to kill at low concentrations, but as the biocide concentration is increased, a point is reached where the majority of the population is reduced. Beyond this point the microorganisms become more difficult to kill (through resistance or physical protection) and a proportion may survive regardless of the increase in concentration. It is important, therefore, to use the disinfectant at the concentration recommended by the manufacturer. Concentrations above this recommended level may thus not enhance biocidal effect and will be uneconomic while concentrations below this level may significantly reduce biocidal action.

Sufficient contact time between the disinfectant and the microorganisms is perhaps the most important factor controlling biocidal efficiency. To be effective, disinfectants must find, bind to and traverse microbial cell envelopes before they reach their target site and begin to undertake the reactions that will subsequently lead to the destruction of the microorganism (Klemperer, 1982). Sufficient contact time is therefore critical to give good results, and most general-purpose disinfectants are formulated to require at least five minutes to reduce bacterial populations by 5 log orders in suspension. For particularly resistant organisms such as spores or moulds, surfaces should be repeatedly dosed to ensure extended contact times of 15–60 minutes.

Ideally, disinfectants should have the widest possible spectrum of activity against microorganisms, including bacteria, fungi, spores and viruses, and this should be demonstrable by means of standard disinfectant efficacy tests. The currently available disinfectant test methods were reviewed by Reybrouck (1998) and fall into two main classes, suspension tests and surface tests. Suspension tests are useful for indicating general disinfectant efficacy and for assessing environmental parameters such as temperature, contact time and interfering matter such as food residues. The current European food industry disinfectant test methods of choice for bactericidal and fungicidal action in suspension are EN 1276 (Anon., 1997c) and EN 1650 (Anon., 1998a) respectively. A surface test is more appropriate in testing effectiveness against particular microorganisms remaining on surfaces after cleaning. A harmonised surface test has recently been agreed for

the European food industry (Anon., 2001b). However, it can be argued that in reality, surface tests do not consider the environmental stresses the organisms may encounter in the processing environment prior to disinfection (action of detergents, variations in temperature and pH and mechanical stresses), which may affect susceptibility. Both suspension and surface tests have limitations, however, and research-based methods are being developed to investigate the effect of disinfectants against adhered microorganisms and biofilms *in situ* and in real time. Such methods have been reviewed by Holah *et al.* (1998). Because of the limitations of disinfectant efficacy tests, however, food manufacturers should always confirm the efficacy of their cleaning and disinfection programmes by field tests either from evidence supplied by the chemical company or from in-house trials.

As well as having demonstrable biocidal properties, disinfectants must also be safe (non-toxic) and should not taint food products. Disinfectants can enter food products accidentally, e.g. from aerial transfer or poor rinsing, or deliberately, e.g. from 'no rinse status' disinfectants. The practice of rinsing or not rinsing has yet to be established. The main reason for leaving disinfectants on surfaces is to provide an alleged biocide challenge (this has not been proven) to any subsequent microbial contamination of the surface. It has been argued, however, that the low biocide concentrations remaining on the surface, especially if the biocide is a QAC, may lead to the formation of resistant surface populations.

6.11 Sanitation methodology

Cleaning and disinfection can be undertaken by hand using simple tools, e.g. brushes or cloths (manual cleaning), though as the area of open surface requiring cleaning and disinfection increases, specialist equipment becomes necessary to dispense chemicals and/or provide mechanical energy. Chemicals may be applied as low-pressure mists, foams or gels while mechanical energy is provided by high- and low-pressure water jets or water or electrically powered scrubbing brushes. These techniques have been well documented (Marriot, 1985; Anon., 1991; Holah, 1991) and this section considers their use in practice.

The use of cleaning techniques can be described schematically following the information detailed in Fig. 6.8. The figure details the different energy source inputs for a number of cleaning techniques and shows their ability to cope with both low and high (dotted line) levels of soiling. For the manual cleaning of small items a high degree of mechanical energy can be applied directly where it is needed and with the use of soak tanks (or clean-out-of-place techniques) contact times can be extended and/or chemical and temperature inputs increased such that all soil types can be tackled.

Alternatively, dismantled equipment and production utensils may undergo manual gross soil removal and then be cleaned and disinfected automatically in tray or tunnel washers. As with soak tank operations, high levels of chemical and thermal energy can be used to cope with the majority of soils. The siting of tray

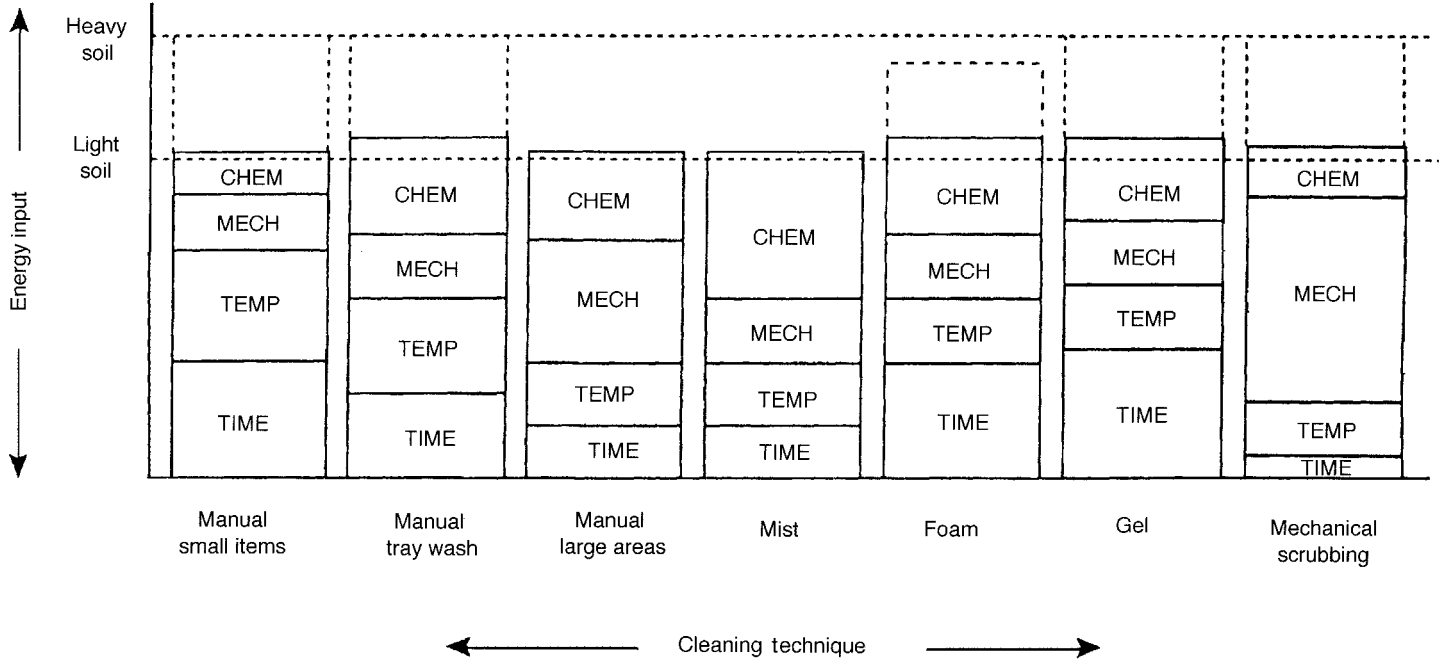

Fig. 6.8 Relative energy source inputs for a range of cleaning techniques. (Modified from Offiler, 1990.)

washes in high-risk chilled production areas should be carefully considered, however, as they are prone to microbial aerosol production which may lead to aerial product contamination.

In manual cleaning of larger areas, for reasons of operator safety, only low levels of temperature and chemical energy can be applied, and as the surface area requiring cleaning increases, the technique becomes uneconomic with respect to time and labour. Labour costs amount to 75% of the total sanitation programme and, for most food companies, the cost of extra staff is prohibitive. Only light levels of soiling can be economically undertaken by this method.

The main difference between the mist, foam and gel techniques is in their ability to maintain a detergent/surface contact time. For all three techniques, mechanical energy can be varied by the use of high- or low-pressure water rinses, though for open surface cleaning, temperature effects are minimal. Mist spraying is undertaken using small hand-pumped containers, 'knapsack' sprayers or pressure washing systems at low pressure. Misting will only 'wet' vertical smooth surfaces; therefore only small quantities can be applied and these will quickly run off to give a contact time of five minutes or less. Because of the nature of the technique to form aerosols that could be an inhalation hazard, only weak chemicals can be applied, and so misting is useful only for light soiling. On cleaned surfaces, however, misting is the most commonly used method for applying disinfectants.

Foams can be generated and applied by the entrapment of air in high-pressure equipment or by the addition of compressed air in low-pressure systems. Foams work on the basis of forming a layer of bubbles above the surface to be cleaned which then collapses and bathes the surface with fresh detergent contained in the bubble film. The critical element in foam generation is for the bubbles to collapse at the correct rate: too fast and the contact time will be minimal; too slow and the surface will not be wetted with fresh detergent. Gels are thixotropic chemicals that are fluid at high and low concentrations but become thick and gelatinous at concentrations of approximately 5–10%. Gels are easily applied through high- and low-pressure systems or from specific portable electric pumped units and physically adhere to the surface.

Foams and gels are more viscous than mists, are not as prone to aerosol formation and thus allow the use of more concentrated detergents, and can remain on vertical surfaces for much longer periods (foams 10–15 minutes, gels 15 minutes to an hour or more). Foams and gels are able to cope with higher levels of soils than misting, although in some cases rinsing of surfaces may require large volumes of water, especially with foams. Foams and gels are well liked by operatives and management, because of the nature of the foam, a more consistent application of chemicals is possible and it is easier to identify areas that have been 'missed'.

Fogging systems have been traditionally used in the chilled food industry to create and disperse a disinfectant aerosol to reduce airborne microorganisms and to apply disinfectant to difficult to reach overhead surfaces. The efficacy of fogging was recently examined in the UK and has been reported (Anon., 1998b).

Providing a suitable disinfectant is used, fogging is effective at reducing airborne microbial populations by 2–3 log orders in 30–60 minutes. Fogging is most effective using compressed air-driven fogging nozzles producing particles in the 10–20 μm range. For surface disinfection, fogging is effective only if sufficient chemical can be deposited onto the surface. To reduce inhalation risks, sufficient time (45–60 minutes) is required after fogging to allow the settling of disinfectant aerosols before operatives can re-enter the production area.

Cleaning equipment is prone to contamination with *Listeria* spp. and other pathogenic microorganisms and, by the nature of its use, provides an excellent way in which contamination can be transferred from area to area. Cleaning equipment should be specific to high-risk areas and after use, equipment should be thoroughly cleaned and, if appropriate, disinfected and dried. The potential for cleaning equipment to disperse microbial contamination by the formation of aerosols has been reported (Holah *et al.*, 1990b) and it was shown that all cleaning systems tested produced viable bacterial aerosols from test surfaces contaminated with attached biofilms.

6.12 Sanitation procedures

Sanitation procedures are concerned with both the stage at which the sanitation programme is implemented and the sequence in which equipment and environmental surfaces are cleaned and disinfected within the processing area. Sanitation programmes are so constructed as to be efficient with water and chemicals, to allow selected chemicals to be used under their optimum conditions, to be safe in operation, to be easily managed and to reduce manual labour. In this way an adequate level of sanitation will be achieved, economically and with due regard to environmental friendliness. The principal stages involved in a typical programme are described below.

1. *Production periods.* Production staff should be encouraged to consider the implications of production practices on the success of subsequent sanitation programmes. Product should be removed from lines during break periods and this may be followed by manual cleaning. In high risk areas this is usually undertaken by wiping with alcohol (to avoid the use of water during production periods). Production staff should also be encouraged to operate good housekeeping practices (this is also an aid to ensuring acceptable product quality) and to leave their work stations in a reasonable condition. Soil left in hoppers and on process lines, etc., is wasted product! Sound sanitation practices should be used to clean up large product spillages during production.
2. *Preparation.* As soon as possible after production, equipment should be dismantled as far as is practicable or necessary to make all surfaces that microorganisms could have adhered to during production accessible to the cleaning fluids. All unwanted utensils/packaging/equipment should be covered or removed from the area. Dismantled equipment should be stored

on racks or tables, not on the floor! Machinery should be switched off, at the machine and at the power source, and electrical and other sensitive systems protected from water/chemical ingress. Preferably, production should not occur in the area being cleaned, but in exceptional circumstances if this is not possible, other lines or areas should be screened off to prevent transfer of debris by the sanitation process.

3. *Gross soil removal.* Where appropriate, all loosely adhered or gross soil should be removed by brushing, scraping, shovelling or vacuum, etc. Wherever possible, soil on floors and walls should be picked up and placed in suitable waste containers rather than washed to drains using hoses.
4. *Pre-rinse.* Surfaces should be rinsed with low-pressure cold water to remove loosely adhered small debris. Hot water can be used for fatty soils, but too high a temperature may coagulate proteins.
5. *Cleaning.* A selection of cleaning chemicals, temperature and mechanical energy is applied to remove adhered soils.
6. *Inter-rinse*. Both soil detached by cleaning operations and cleaning chemical residues should be removed from surfaces by rinsing with low-pressure cold water.
7. *Disinfection.* Chemical disinfectants (or occasionally heat) are applied to remove and/or reduce the viability of remaining microorganisms to a level deemed to be of no significant risk. In exceptional circumstances and only when light soiling is to be removed, it may be appropriate to combine stages 5–7 by using a chemical with both cleaning and antimicrobial properties (detergent-sanitiser).
8. *Post-rinse.* Disinfectant residues should be removed by rinsing away with low-pressure cold water of known potable quality. Some disinfectants, however, are intended to be left on surfaces until the start of subsequent production periods and are thus so formulated to be both surface-active and of low risk, in terms of taint or toxicity, to foodstuffs.
9. *Inter-production cycle conditions.* A number of procedures may be undertaken, including the removal of excess water and/or equipment drying, to prevent the growth of microorganisms on production contact surfaces in the period up until the next production process. Alternatively, the processing area may be evacuated and fogged with a suitable disinfectant.
10. *Periodic practices.* Periodic practices increase the degree of cleaning for specific equipment or areas to return them to acceptable cleanliness levels. They include weekly acidic cleans, weekend dismantling of equipment, cleaning and disinfection of chillers and sanitation of surfaces, fixtures and fittings above two metres.

A sanitation sequence should be established in a processing area to ensure that the applied sanitation programme is capable of meeting its objectives and that cleaning programmes, both periodic and for areas not cleaned daily, are implemented on a routine basis. In particular, a sanitation sequence determines the order in which the product contact surfaces of equipment and environmental

surfaces (walls, floors, drains, etc.) are sanitised, such that once product contact surfaces are disinfected, they should not be recontaminated.

6.13 Evaluating the effectiveness of sanitation programmes

Assessment of the effectiveness of the sanitation programme's performance is part of day-to-day hygiene testing and, as such, is linked to the factory environmental sampling plan. The control of the environmental routes of contamination is addressed through the development of a thorough risk analysis and management strategy, typically undertaken as part of the factory HACCP study, resulting in the development of the factory environmental sampling plan. The development of environmental sampling plans has recently been established by a CCFRA industrial working party and is reported in Holah (1998b).

Environmental sampling is directly linked with both process development and product manufacture and, as such, has three distinct phases:

1. Process development to determine whether a contamination route is a risk and assessing whether procedures put in place to control the risk identified are working.
2. Routine hygiene assessment.
3. Troubleshooting to identify why products (or occasionally environmental samples) may have a microbiological count that is out of specification or may contain pathogens.

Routine hygiene testing is an important aspect of due diligence and is used for two purposes, monitoring to check sanitation process control, and verification to assess sanitation programme success. Monitoring is a planned sequence of observations or measurements to ensure that the control measures within the sanitation programme are operating within specification and are undertaken in a time frame that allows sanitation programme control. Verification is the application of methods in a longer time frame to determine compliance with the sanitation programme's specification.

Monitoring the sanitation programme is via physical, sensory and rapid chemical hygiene testing methods. Microbiological testing procedures are never fast enough to be used for process monitoring. Physical tests are centred on the critical control measures of the performance of sanitation programmes and include, for example, measurement of detergent/disinfectant contact time; rinse water, detergent and disinfectant temperatures; chemical concentrations; surface coverage of applied chemicals; degree of mechanical or kinetic input; cleaning equipment maintenance and chemical stock rotation.

Sensory evaluation is usually undertaken after each of the sanitation programme stages and involves visual inspection of surfaces under good lighting, smelling for product or offensive odours, and feeling for greasy or encrusted sur-

faces. For some product soils, residues can be more clearly observed by wiping the surface with paper tissues. Rapid hygiene methods are defined as monitoring methods whose results are generated in a time frame (usually regarded as within approximately 10 minutes) sufficiently quickly to allow process control. Current methodology allows the quantification of microorganisms (ATP), food soils (ATP, protein) or both (ATP). No technique is presently available which will allow the detection of specific microbial types within this time frame. ATP has been successfully used to monitor the hygiene of surfaces for approximately 15 years and many references are available in the literature citing its proficiency and discussing its future potential, e.g. Bautista *et al*. (1992), Poulis *et al*. (1993), Bell *et al*. (1994), Griffiths *et al*. (1994) and Hawronskyi and Holah (1997). Techniques have also been developed that use protein concentrations as markers of surface contamination remaining after cleaning operations. As these are dependent on chemical reactions, they are also rapid but their applicability is perhaps less widespread as they can only be used if protein is a major part of the food product processed.

Verification of the performance of the sanitation programmes is usually undertaken by microbiological methods though ATP levels are also used (especially in low-risk areas). Microbiological sampling is typically for the total number of viable microorganisms remaining after cleaning and disinfection, i.e. total viable count (TVC), both as a measurement of the ability of the sanitation programme to control all microorganisms and to maximise microbial detection. Sampling targeted at specific pathogens or spoilage organisms, which are thought to play a major role in the safety or quality of the product, is undertaken to verify the performance of the sanitation programme designed for their control. Microbiological assessments have also been used to ensure compliance with external microbial standards, as a basis for cleaning operatives' bonus payments, in hygiene inspection and troubleshooting exercises, and to optimise sanitation procedures.

The choice of sampling site will relate to risk assessment. Where there is the potential for microorganisms remaining after (poor) cleaning and disinfection, through, for example, direct product contact, to infect large quantities of product, these sources would require sampling much more frequently than other sites which, although they may be more likely to be contaminated, pose less of a direct risk to the product. For example, it is more sensible, and gives more confidence, to sample the points of the equipment that directly contact the product and that are difficult to clean than to sample non-direct contact surfaces, e.g. underneath the equipment framework.

As part of the assessment of sanitation programmes, it is worthwhile looking how the programme is performing over a defined time period (weekly, monthly, quarterly, etc.) as individual sample results are only an estimate of what is happening at one specific time period. This may be to ensure that the programme remains within control, to reduce the variation within the programme or, as should be encouraged, to try to improve the programme's performance. An assessment of the performance of the programme with time, or trend analysis, can be undertaken simply, by producing a graphical representation of the results on a time

basis, or can be undertaken from a statistical perspective using Statistical Process Control (SPC) techniques as described by Harris and Richardson (1996). Generally, graphical representation is the most widely used approach, though SPC techniques should be encouraged for more rigorous assessment of improvement in the programme's performance.

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7

Safe process design and operation

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7.1 Introduction: product and process design

Product and process designs will always be a compromise between the demands for safety and quality on the one hand, and cost and operational limitations in the supply chain on the other. The development of a design represents the most important opportunity for a producer to turn a concept into a safe product. Unless designs are sound and capable of providing safe food, even the best controlled process cannot succeed in making a safe product. The goal is to produce attractive food, while preventing or reducing safety risks in order to protect consumers and brands by meeting their expectations on shelf-life, quality, safety and regulatory requirements. A multi-disciplinary team (e.g. quality assurance (QA), development, production, engineering and marketing) is normally the best means of doing this.

The increased globalisation of trade in foodstuffs increases the challenge to effective identification and control of microbiological hazards because it introduces a wider range of hazards and usage conditions. The degree of safety built into product designs will always be constrained by the demands of the market-place for particular sensory properties (e.g. milder preservation systems or freedom for preservatives (such as salt, nitrite or sorbate), shelf-life and less obvious processing). The skill of the product designer is to balance these competing demands and decide where an acceptable balance lies. The techniques of risk assessment and risk management may be used to guide the manufacturer in achieving a predictable and acceptable balance between safety, quality and cost. Hazards may be identified in various ways including: previous identification in the food chain, disease surveillance, other monitoring information, knowledge of production practices (such as any process or procurement

innovations or awareness of lack of compliance with standards) or consumer complaints.

Many safety systems are built-up during manufacture, by ingredient selection, processing and packaging. Usually more than one step will contribute to microbiological safety and the capability of the process, formulation and control options chosen should be considered when manufacturing plant is identified. In broad terms manufacturing should eliminate infectious pathogens and control the numbers and/or inhibit the growth of toxigenic species, so that harmful amounts of toxin are not present in the food at the time of consumption. The safety of some foods, for example chilled foods, with no inherent preservative properties, additionally depends on suitable conditions being maintained throughout the distribution and home storage parts of the chain (e.g. refrigeration temperatures and times). The growth of pathogens or spoilage microorganisms may be prevented during storage by the action of extrinsic factors (e.g. chilling or freezing) and/or intrinsic factors (e.g. adjusting pH, A_w; adding preservatives) or employing microbiological competition (e.g. lactic acid bacteria). Usually, a combination of options will be more effective in reducing risks than single factors.

A product designer or raw material buyer may reduce risks by avoiding materials with a substantiated history of contamination or toxicity. The aim of the supply chain should be to prevent or minimise contamination and/or the introduction of pathogens with raw materials. At least, subsequent processing should ensure that any pathogens can be eliminated (e.g. by cooking, acidification). Rather than relying on consumer cooking, buyers acting with suppliers can contribute to safety by reducing the levels of specific pathogens in primary production (e.g. *Salmonella* in poultry).

Successful process designs must consider not only contamination risks, but also the impact of shelf-life and anticipated storage conditions imposed by distributors, retailers or customers (CFDRA, 1990). Labuza and Fu (1995) have proposed the use of time/temperature integrators (TTI) for monitoring temperatures and the extent of abuse in the distribution chain. These indicators are not widely used because of the difficulty of making their response kinetics match those of pathogens and also ensuring that the temperatures they measure accurately reflect conditions in the food. For foods where storage conditions and preparation by the customer are an integral part of the safety chain, risks may be increased by consumer mishandling or misuse. A manufacturer should account for such risks in the design of products whose safety relies on correct customer use. Brackett (1992) has pointed out that chilled foods, for example, contain few, if any, additives to prevent growth of pathogenic microorganisms and are susceptible to inadequate refrigeration that may allow pathogen growth. He also highlights related issues such as over-reliance on shelf-life as a measure of quality and the need to consider the needs of sensitive groups (such as immunocompromised consumers) in the product design. If the product design relies on customer cooking to free the product of infectious pathogens, such as salmonellae, it is important that helpful, accurate and validated heating and cooking instructions are provided by

the manufacturer and that use of these instructions also results in high product quality.

7.2 Modelling and product/process design

The risks associated with any particular products can be investigated either by practical trials (such as challenge testing) or by the use of predictive modelling. Modelling the fate of microorganisms can improve supply chain management. The interaction of time and temperature in determining growth, survival or (process) lethality based on D and z values are examples of models. In the UK, Food MicroModel (www.foodmicromodel.com) and in the US, the Pathogen Modelling Program (www.arserrc.gov/mfs/pathogen.htm) are computer-based predictive microbiology databases applicable to food products. Panisello and Quantick (1998) used FMM to make predictions on the growth of pathogens in pâté in response to variations in the pH and salt content and specifically the effect of lowering the pH. Zwietering and Hasting (1997) have taken this concept a stage further and developed a modelling approach to predict the effects of processing on microbial growth during food production, storage and distribution. Their process models were based on mass and energy balances together with simple microbial growth and death kinetics, illustrated using meat product and burger processing lines. Such models can predict the contribution of each individual process stage to the level of microbes in a product.

Zwietering *et al*. (1991, 1994a, b) have also modelled the impact of temperature, time and shifts in temperature during processing on the growth of *Lactobacillus plantarum*. Such predictive models can, in principle, be used for suggesting the conditions needed to control microbial growth or indicate the extent of the microbial 'lag' phase during processing and distribution where temperature fluctuations may be common and could allow growth. Impe *et al*. (1992) have also built similar models describing the behaviour of bacterial populations during processing in terms of time and temperature, but have extended their models to cover inactivation at temperatures above the maximum temperature for growth. A model has been developed by Augustin *et al*. (2000) to describe the effect of temperature history and duration of pre-incubation period on the regrowth lag time of *Listeria monocytogenes*. Their model takes into account the influence of prolonged starvation conditions and physiological state of the cells on lag time before re-growth. Giffel *et al*. (1999) have applied mathematical models to predict the growth of spoilage and pathogenic microorganisms in food production chains and point out the potential use of such models for product development, HACCP analysis and risk assessment. Two examples are provided – one to predict the growth of *Listeria monocytogenes* and hence determine the critical control points in the production of sliced, cooked ham. The other describes the use of a model to predict adherence, growth and release of thermoresistant streptococci during whey processing in a cheese factory.

Adair and Briggs (1993) have proposed the development of expert systems, based on predictive models to assess the microbiological safety of chilled food. Such systems could be used to interpret microbiological, processing, formulation and usage data to predict the microbiological safety of foods. However, to be realistic, models are only as good as the inputs and at present there is both uncertainty and variability associated with the data available. Betts (1997) has also discussed the practical application of growth models to the determination of shelf-life of foods and points out the usefulness of models in speeding up product development and the importance of validating the output of models in real products. Modelling technology can offer advantages in terms of time and cost, but is still in its infancy (Pin and Baranyi, 1998). Usefulness is currently limited by variations in the microbial types present in raw materials and products, their activities and interactions, other factors altering growth or survival rates or the rate of production of metabolites causing spoilage.

Walls and Scott (1997) surveyed scientists in the food industry to determine their views on the value of predictive microbiology. They took as examples assessment of the risks of foodborne illness from a cooked meat product contaminated with *Staphylococcus aureus* and a hamburger contaminated with *Salmonella*. They found that over 80% of the respondents had used predictive microbiology software and 36% had developed predictive models in their companies. There was support for using models to determine Critical Control Points in a Hazard Analysis plan. Based on the examples, users concluded that more data were needed for reliable microbiological risk assessments and that predictions should not be limited to single-point estimates. To take account of variability, a Monte Carlo simulation of the data distribution should be used to produce a range of risk estimates, e.g. best, average or worst.

7.3 Safety management tools: good manufacturing practice (GMP), HACCP and risk assessment

7.3.1 Good manufacturing practice

Good manufacturing practice (GMP) or prerequisites cover the principles needed to design plant layouts, equipment and procedures for the production of safe food. Good hygiene practice (GHP) focuses attention on the hygienic measures that are a prerequisite for other management techniques, such as Hazard Analysis Critical Control Point (HACCP). Inappropriate or unhygienic factory processing will lead to microbial survival or cross-contamination; in conjunction with temperature or time abuse during storage it will certainly lead to the growth of pathogenic and spoilage microorganisms. GMP codes and the hygiene requirements for the hygienic manufacture of foods may be formally specified for example by the Codex Alimentarius Committee on Food Hygiene (FAO/WHO; also see Anon., 1984, 1986). They may also be developed by the food industry, often acting in collaboration with food inspection and control agencies or other groups (Jouve *et al.*, 1998). Generally GHP/GMP requirements cover the following:

- The hygienic design and construction of food manufacturing premises.
- The hygienic design, construction and proper use of machinery.
- Cleaning and disinfection procedures (including pest control).
- General hygienic and safety practices in food processing including
  - the microbiological quality of raw materials;
  - the hygienic operation of each process step;
  - the hygiene of personnel and their training in hygiene and the safety of food.

7.3.2 HACCP

The HACCP system is a food safety management system that uses the approach of identifying hazards and controlling their fate at fixed points in the supply chain (Critical Control Points, CCPs) to prevent food safety problems. It can be used to ensure food safety in all scales and types of food manufacture. The widespread introduction of HACCP has promoted a shift in emphasis from end-product inspection and testing to the preventative control of hazards during production, especially at the CCPs. The technique is ideal for processes where many elements contribute to chemical, physical and microbiological safety. Control and monitoring of compliance all along the supply chain offers the consumer better protection than testing products for pathogens. End-product testing cannot ensure safety, as at practical levels there is a very low probability of detecting product that is hazardous because it contains pathogens. The delay to await results of microbiological testing also uses up shelf-life.

For steps in the manufacturing process that are not recognised as CCPs, the use of GMP/GHP is essential to provide assurance that suitable controls and standards are present. The identification and analysis of hazards within the HACCP programme will provide information to interpret GMP/GHP requirements and indicate staff training needs for specific products or processes. A range of guidance is available for different products and processes. For example, the Microbiology and Food Safety Committee of the National Food Processors Association (NFPA, 1993) has considered HACCP systems for chilled foods produced at a central location and distributed chilled to retail establishments. Chicken salad was used as a model to propose critical control points and give practical advice on HACCP planning (i.e. development of a supply chain flow diagram, hazard identification, establishing critical limits, monitoring requirements; and verification procedures to ensure the HACCP system is working effectively). There are also US Department of Agriculture recommendations and outline HACCP flow diagrams for other processes, such as cook-in-package and cook-then-package (Snyder, 1992). Testing against microbiological criteria (regulations, standards or advisory criteria) has been widely used to determine product safety and verify the effectiveness of HACCP plans. In a HACCP plan, microbiological criteria retain their value as tools for judging the implementation and effectiveness of HACCP (verification), validating control measures and investigating problems.

7.3.3 Risk analysis

Ensuring the microbiological safety and wholesomeness of food requires formal or informal identification of realistic hazards and their means of control (risk assessment). The ability of a food producer to assess the impact of a proposed design or changes to a process, product and market on the level of risk and the type of hazard is important to the assurance of consistent standards of food safety. The effect of supply chain changes on realistic hazards and their risks need to be identified. Important changes include the development of new products and processes, different sources of raw materials, or the targeting of new customer groups, such as children. Food producers have always assessed these risks using either empirical or experiential approaches. As causal links have been established between foodborne illness and the presence, or activities (toxigenisis), of food-poisoning microorganisms, so control targeted at identified hazards has progressively become the means of ensuring food safety. These practical approaches have now developed into formal systems with well-defined procedures and are known as Microbiological Risk Assessment (MRA) and Risk Management. They are described in Microbiological Risk Assessment; an interim report (ACDP, 1996) or by the Codex scheme and *Proposed Principles and Guidelines for the Conduct of Microbiological Risk Management* (Codex Alimentarius Commission, 2000).

The overall aim of risk analysis is to reduce risk by:

- identifying realistic microbiological hazards and characterising them according to severity;
- examining the impact of raw material contamination, processing and use on the level of risk;
- communicating clearly and consistently, via the output of the study, the level of risk to the consumer.

When risk assessment is put together with risk communication (distribution of information on a risk and on the decision taken to combat a risk) and used to promote sound risk management (actions to eliminate or minimise risk), a risk analysis is produced (ACDP, 1996). Risk assessment has been reviewed (Jaykus, 1996) and applied to specific problems; listeriosis (Miller *et al*., 1997), the role of indicators (Rutherford *et al*., 1995) and links with HACCP. More details on risk assessment can be found in Chapter 4.

7.4 Principles of process design

The manufacture of safe, high-quality products relies on the use of established process principles within an organised framework specifying target microorganisms, raw material quality, heat processes or other decontamination procedures and the prevention of recontamination. The target pathogens that need to be controlled or eliminated from food products by processing, or consumer usage include the following:

- Infectious types – salmonellae especially enteritidis and Typhimurium, enterohaemorrhagic *Escherichia coli*, *Vibrio parahaemolyticus*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Listeria monocytogenes*.
- Toxic types – *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus* and *Bacillus cereus* (*subtilis* and *licheniformis*).

While these are a minimum range of targets, designs should also control spoilage microorganisms that may be more resistant. Where a decision is taken to accept limited spoilage to achieve a product with a certain character or shelf-life, the developer must be certain that a safety risk is not created. For example, vacuum or modified atmosphere packaging may be used to slow the growth of pseudomonads or moulds, but may create anaerobic conditions favouring the growth of anaerobic pathogens, such as the clostridia.

Because of the critical effect of ingredients and dimensions on the rate of heating of portions or containers, and hence on the heat treatment, the product design must recognise any factors (such as coatings or particles in a liquid stream) affecting the rate of heating. Measures specified to control this variability must be realistic and operating targets may be off-set (to the safe side) from minimum, based on a knowledge of process, ingredient or supplier variability. For example, if a product requires a tight specification (e.g. of particle size) for a safe heat treatment, but this critical feature is variable, then the largest particle size must be used as a basis in the process design. This must also apply to the minimum temperatures (e.g. chilled or frozen) used for setting heating or frying conditions and should identify any means for controlling them (e.g. tempering or thawing) besides highlighting the probable coldest and slowest heating ingredients found in production (e.g. frozen). Heating rates may vary in response to different ingredients, temperatures and times in the factory kitchen. Thickeners such as starch or proteins will affect product heating or flow characteristics and should be specified by the design. Allowances for safety must not be allowed to exert a cumulative and detrimental influence on quality by leading to over-severe processes. If heat treatments have been derived, or substituted, by calculation (e.g. using D and z), it may be necessary to conduct tests on the initial commercial production runs to validate the process conditions. QA and development staff involved in trials should understand the limitations of any predictions and tests. Assessing the effects of changes, e.g. quantity of ingredients, product composition, method of product make-up, size or shape or coating of a material, must be the responsibility of a suitably qualified person in the management team, e.g. QA or development manager.

The highest risk foods are those made from materials likely to contain pathogens and intended to be consumed without sufficient heating, e.g. prepared meals or salads. In other cases (e.g. beef and poultry) consumers may not make the link between thorough cooking and safe food. Some domestic cooking appliances such as microwave ovens may not be capable of uniformly achieving pasteurising temperatures and hence microwave products should be designed with

this in mind. Therefore, the design principles employed in the manufacture, distribution and sale of such foods should be primarily designed to minimise the risks of them containing harmful concentrations of food-poisoning bacteria, whose growth during ambient or chilled distribution and storage will increase any risk. The control of spoilage microbes should always be a secondary consideration, although it may often require the application of more severe processes or conditions of hygiene than the control of safety. Sometimes the control of spoilage cannot be achieved without prejudicing the sensory quality of a food product; in this case there must be a commercial decision on the acceptable balance between a controlled loss of quality and the frequency of spoilage in the market-place. However, microbiological safety standards should never be compromised to improve sensory quality. If the required processing conditions cannot ensure safety against the background of realistic consumer usage, then the product should not reach the market-place (see Gould, 1992; Walker and Stringer, 1990).

Product character and consumer usage will usually dictate essential process requirements. The aim of factory kitchen and downstream processing will be to make a product that is ready for consumption by the customer with a minimum of further preparation. For many products this may include factory cooking to eliminate pathogens. The minimum heat treatments specified by the design must effectively eliminate or control hazards and in some cases, ensure compliance with local legislation (e.g. milk pasteurisation). The combination of ingredients, supply chain conditions and use instructions should ensure that products are safe if they are used according to the pack instructions. Any information necessary to achieve this should be presented in the product design and manufacturing specification, e.g. portion dimensions or raw materials quality.

Ready-to-eat products need to be free of pathogens, hence the plant layout needs to be specified to minimise chances of cross- or recontamination after decontamination. Operating procedures must ensure that quality and safety aspects of the product design are consistently delivered. These procedures may include rates of heating, cooling or freezing and specified temperatures and times. The process design should specify heat treatments and take account of process variability when operational process conditions are defined. Procedures must be in place to ensure consumer safety when processes go wrong.

Wherever possible there should be forward flow of material in a process area, with physical or operational segregation of the pre- and post-cooking areas, with appropriate high levels of hygiene in all the areas handling cooked product. Hygiene, operational practices and cleaning, in areas handling decontaminated product (e.g. sterilised cans), should minimise the chances of recontamination. For example, the design and operation of areas handling blanched vegetables for salads may need to ensure that they are not contaminated or re-contaminated after blanching. In many cases existing equipment is unhygienic and the emphasis must be placed on operational procedures, such as cleaning, for ensuring safety. If a product is made from a mixture of cooked and uncooked material, then it should be handled, treated and labelled as uncooked.

Table 7.1 Risk classes of chilled foods

Risk class[a]	Typical shelf-life	Critical hazard	Relative risk	Required minimum heat treatment	Required manufacturing class[b] MA	HA	HCA
1	1 week	Infectious pathogens	High	Customer cook (minimum 70 °C, 2 min)	✓	(✓)	
2	1–2 weeks	Infectious pathogens	Low	Pasteurisation by manufacturer (minimum 70 °C, 2 min)	✓	✓	✓
3	>2 weeks	Infectious pathogens and spore-formers	Low	Pasteurisation by manufacturer (minimum 90 °C, 10 min)	✓		✓
4	>2 weeks	Spore-formers	Low	Pasteurisation by manufacturer (minimum 90 °C, 10 min)	✓	✓	

[a] Class 1: Raw chill-stable foods, e.g. meat, fish; Class 2: Products made from a mixture of cooked and low-risk raw components; Class 3: Products cooked or baked and assembled or primary packaged in a high-care area; Class 4: Products cooked in-pack.
[b] MA: Manufacturing area; HA: Hygienic area; HCA: High-care area.

Some products may be incidentally heated as part of their processing (e.g. blanching). This type of heating is not intended to produce microbiologically safe products; and although it may be sufficient to cause decontamination, minimum heating may not be ensured. Any handling or packaging procedures and equipment used after heat treatment must be designed to be hygienic and operated to prevent recontamination. At its most extreme, this approach is used in aseptic packaging.

7.4.1 Product grouping and process design

Chilled foods, for example, may be categorised according to their chances of carrying pathogens after processing (Table 7.1). Some are made entirely from raw ingredients (Class 1) and will require storage conditions that prevent growth and toxin production plus cooking by the customer to eliminate infectious pathogens. Others are mixtures of raw and cooked components (Class 2), processed and packaged to ensure a satisfactory shelf-life. These may not require cooking, but may contain infectious pathogens (e.g. *L. monocytogenes* or *Salmonella*). Hence the manufacturer can control product safety by minimising numbers of pathogens on the raw

materials (e.g. by careful choice of suppliers) and preventing contamination of products during storage and processing. Shelf-life and storage temperatures should not allow pathogen numbers to increase and ensure that only 'safe' numbers are present if foods are stored for their full indicated shelf-life.

Other chilled foods may contain only cooked or decontaminated components (Class 3), or may be cooked by the manufacturer within their primary packaging (Class 4). If manufactured under well-controlled conditions such foods will be free of infectious pathogens and spoilage microbes and can have a long shelf-life (up to 42 days at chilled temperatures), as they will not be subject to rapid microbial spoilage. Foods for ambient distribution and storage often have shelf-lives of a year or more. So they must either be preserved (e.g. acidified or low A_w) to prevent the growth of pathogens and spoilage microorganisms or decontaminated (e.g. sterilised or pasteurised) and packaged (e.g. cans, jars or aseptic packaging) to prevent re-contamination.

7.5 Process flow and equipment

From a microbiological point of view, processes should be designed to control the presence, growth and activity of target pathogens, while producing products of good quality. Designs for safety should concentrate on unit operations that will eliminate or reduce numbers of bacteria or provide opportunities for recontamination or growth. Raw material type, product design and shelf-life/storage requirements, and even factory hygiene and layout, will determine realistic target pathogens for each process stage. At the beginning of the supply chain, agricultural produce can act as a reservoir of food-poisoning bacteria (e.g. *Salmonella*, *Campylobacter*, *E. coli* O157, *Staphylococcus aureus* and the harmful *Bacillus* and *Clostridia*). Therefore it is important that conditions during handling and processing can control this initial contamination. The extent of precautions needed will be proportional to the hazard severity, occurrence of the pathogens and the complexity and scale of the supply chain. This should be examined by risk assessment.

7.5.1 Process flow

Process flow, plant layout and equipment controls are key contributors to product safety. Designs will have different priorities and criteria depending on the type of product being made. In designing and operating process flows, it should be assumed that unprocessed materials may contain pathogens, making the forward flow of materials essential to prevent contamination and ensure that packaged goods have not missed a critical stage, e.g. sterilisation. Major routes for recontamination are raw materials, food debris and food-handlers, therefore layouts and procedures should be designed to minimise these risks. Food residues remaining in a machine or processing area after cleaning can become a major source of contamination. Airborne contaminants are a relatively minor source of conta-

mination. Process flows should be designed for easy operation and access for cleaning and to help this processing equipment should provide a narrow and predictable range of residence times for product.

Manufacturing unpreserved (low acid) ambient-stable or extended shelf-life (chill) products accentuates microbiological hazards, especially from toxigenic spore-forming bacteria (such as clostridia), hence processes need to eliminate them. Because spores may survive mild heating processes that destroy infectious pathogens or spoilage microorganisms, they should only be used for foods with preservation systems designed to inhibit spore outgrowth (e.g. pH < 4.6) under the conditions of storage. Where this is not the case, products should be sterilised (e.g. ultra-heat treatment, UHT) either in-pack or in-line as part of an aseptic process. Minimum heating requirements for ambient stability are specified in many countries ($F_0 \geqslant 3$). In the case of chilled products, 90 °C × 10 min is accepted as an adequate heat treatment to destroy spores of cold-growing strains of *Clostridium botulinum*.

There is still no general agreement on the risks of botulism from unpreserved chill-stored foods, but there is evidence that, in spore-inoculated model systems, growth occurs and toxin can be produced at temperatures representing commercial conditions (Notermans *et al*., 1990). However, there is an absence of evidence from epidemiological and survey data that these foods really constitute a realistic botulinum hazard. Spoilage of both ambient and chill-stable foods may be caused by the survival and outgrowth of *Bacillus* or clostridial spores.

If heating of chilled foods has not been done in the primary packaging and unwrapped, heat-treated components are used, and these should be handled and assembled in high-care areas to prevent recontamination with spores and infectious pathogens.

7.5.2 Equipment

Many of the critical quality and safety attributes of foods are determined by the hygiene and technical performance of the equipment and control systems used for cooking, cooling, cutting, shaping or packaging. Therefore the correct design and reliable operation of these stages is most important to product safety. Lag periods and growth rates of any contaminants will be influenced by prior process conditions, including cooling rates and storage temperatures and should be controlled by operational procedures. During storage growth may be influenced by factors such as the distribution of preservatives, such as curing salts and acidulants, gas packaging, sealing or seaming machines.

7.6 Manufacturing areas

7.6.1 Raw material and packaging delivery areas

Most factories will have designated areas for deliveries and designs for these areas should ensure they are suitable for the type of vehicles arriving and they

can be divided according to material storage requirements, e.g. frozen, chilled or ambient-stable. They should be operated to minimise the opportunities for cross-contamination, especially if the materials handled are for direct use in finished products (such as packaging). Separation may also be governed by legislative requirements. Delivery areas should allow the efficient and rapid unloading of vehicles with a minimum of temperature change and damage to packs, or packaging, and the direct removal of materials to storage areas. Areas should have facilities for the inspection, coding and maintenance of raw material batch integrity. There should be facilities for the efficient removal and disposal of secondary packaging, such as cardboard boxes. If product is delivered unpacked, e.g. vegetables, clean containers or conveyors may be required for handling, sorting and storage prior to use.

Delivery areas must be designed for effective cleaning and should not be used for storage. If materials are to be taken directly into hygienic or high-hygiene areas, it may be necessary to disinfect their outer packaging before entry. To minimise risks, these materials should be delivered to a separated area handling only low-risk ingredients and not to areas handling raw materials likely to contaminate them.

7.6.2 Storage areas

Food raw materials differ in their storage requirements although all should be stored so that contamination and premature spoilage are prevented. A factory may therefore require a number of different storage areas controlled for time, temperature (chilled, 0–5 °C or frozen, below −12 °C) and hygiene. Temperature-controlled areas should be fitted with reliable control devices, monitoring systems (to provide a record of conditions in the store) and an alarm system indicating loss of control or failure of services. A low humidity store may be required for dry ingredients and packaging materials and the residence time and rotation (first in first out, FIFO) of stock should also be controlled, with batches labelled so that their use-by dates and approval for use are clear. Layout should allow easy access to all stored items and effective stock rotation, to ensure that particular deliveries or production batches can be traced or identified.

Operation of the store and control of the means of access (such as self-closing doors) should ensure that the specified conditions (such as 2–4 °C in chills) can be maintained during the working day. Refrigeration equipment should have sufficient capacity to maintain product temperatures during high outside temperatures or peak demand. All storage areas should be easily cleanable, using either wet or dry methods, as appropriate. The layout of racking and access to floors, walls and drains should allow easy cleaning, racking should not be made of wood.

7.6.3 Raw material preparation and cooking areas

Preparation and cooking areas receive ingredients from storage areas and are used to convert them into ingredients by a variety of techniques, such as cutting,

mixing or cooking. Preparation and cooking may take place in a single area for cook-in-pack products, but if long chilled shelf-life and prevention of contamination are important, cooking may be done in a separate area to prevent cross-contamination. For sterilised or aseptically packed products maintenance of 'initial temperature' for processing may be a key consideration for kitchen and process design and vessel (batch) sizing, to prevent excessive cooling.

Where the cooked product is taken to a hygienic area for cooling and packaging, it is essential that the layout, operation and access from the preparation area prevent recontamination after cooking. If space permits, cooking operations are ideally carried out in areas separated from preparation, to minimise the chances of contamination by airborne particles, dust, aerosols or personnel. Where physical separation cannot be achieved, cooked product should not be handled by personnel or equipment that have been in contact with uncooked material. In rooms or areas where cooking is done, the vessels or ovens may be sited to form a barrier between 'dirty' areas, i.e. those handling uncooked material, and 'clean' areas. Air flow should be from 'clean' to 'dirty' areas, and the supply of air to extraction hoods should ensure that condensation does not contaminate cooked product. The effective extraction of steam from cooking areas is very important to prevent the recontamination of cooked product by water droplets. If single-door ovens or autoclaves (for sterilisation) are used there is an increased risk of cross-contamination, as it is not possible to segregate raw (pre-process) and cooked (processed) material effectively. Care should be taken to ensure that *Staphylococcus aureus* cannot grow in processing areas where nutritionally rich materials (such as egg or dairy sauces, or batters) are produced at ambient temperatures. The entry/exit areas to these heat-processing areas need to be kept clean, with loading and unloading done by separate staff, so that the opportunities for product contact are minimised. Pre- and post-process product should be clearly identifiable to minimise the chances of unprocessed material being sold.

Short shelf-life raw products and products containing components that have not been decontaminated may contain infectious pathogens. If cooking is used to provide a 90 °C/10 min heat treatment for long-life chilled products, then stringent precautions must be taken to prevent re-contamination with clostridial spores. These include a forward flow, physical separation of process stages and control of air flow away from the de-contaminated product. Typical process routes for short and long shelf-life chilled products are shown in Figs 7.1–7.4.

7.6.4 Thawing of raw materials

Prior to use, it may be necessary to thaw frozen ingredients. This should be done under conditions that minimise pathogen growth, i.e. the maximum surface temperature of the ingredient should not be within their growth range (i.e. below 10–12 °C). If this is not possible, then thawing times should be minimised to prevent growth and quality change. Special thawing equipment such as

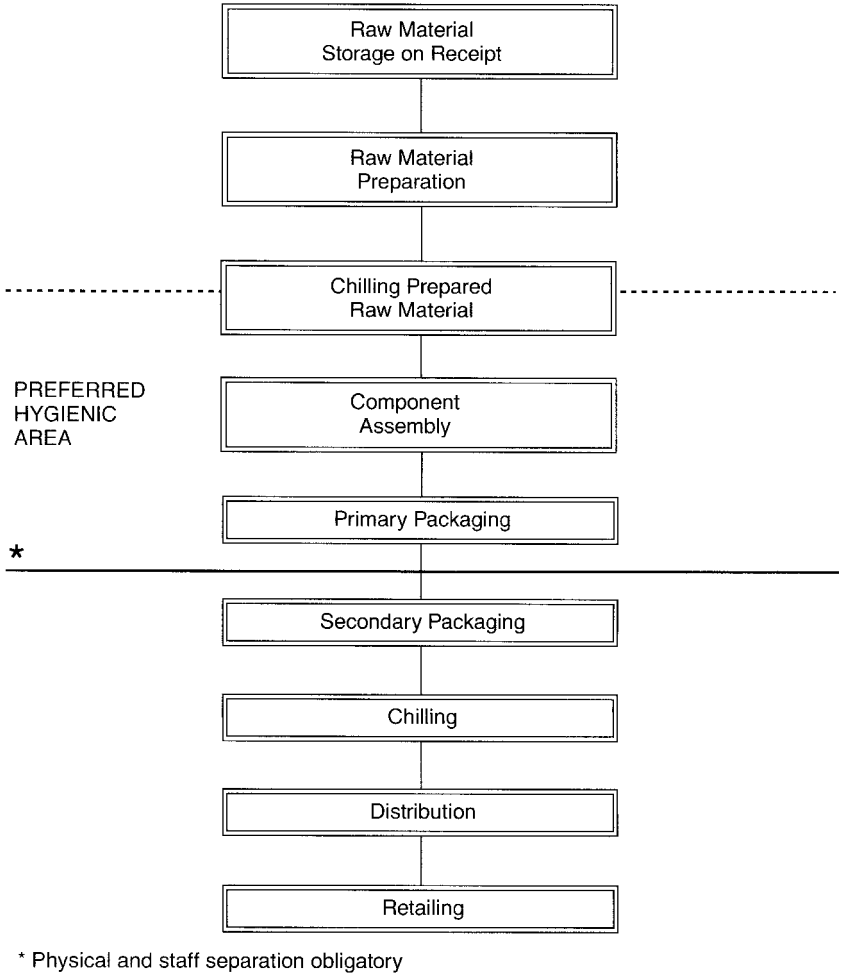

Fig. 7.1 Typical flow diagram for the production of chilled foods prepared from only raw components.

microwave tempering units, running-water thawing baths or air thawing units may be used for microbiologically safe thawing, these should be operated according to a technically justifiable specification. Thawing at ambient temperatures can lead to the uncontrolled and unrecognised growth of pathogens. Where frozen ingredients are to be heat processed (e.g. pasteurised or sterilised), it is important that materials and especially particles entering the heating stages have a uniform size and controlled minimum temperature so that cold spots, which will be insufficiently heated, are not accidentally created. This is essential when products are being sterilised.

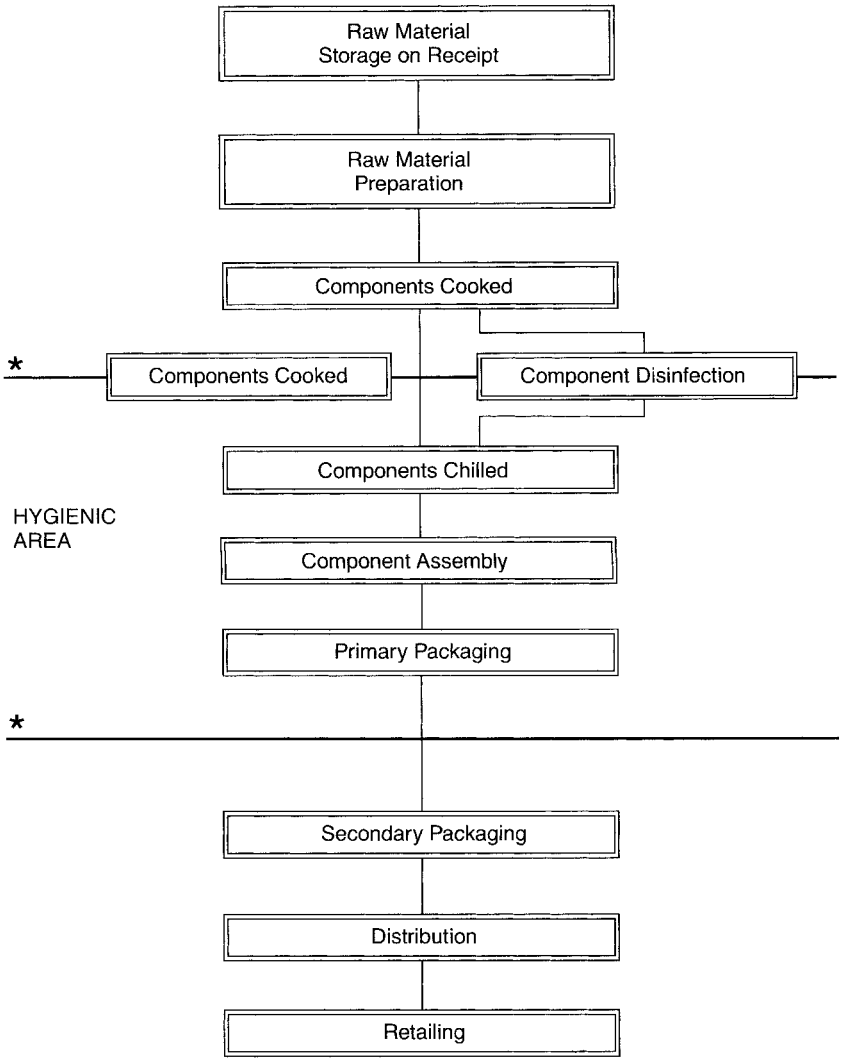

Fig. 7.2 Typical flow diagram for the production of chilled foods prepared from both cooked and raw components.

7.6.5 Hygienic areas

For the preparation of chill-stable, or ready-to-eat foods, hygienic areas providing different levels of risk may be used. Areas offering the lowest level of protection are used for raw (Class 1), or foods made with mixed raw and cooked components (Class 2). These areas should be designed and operated to prevent infectious pathogens becoming established in or introduced to them, based on

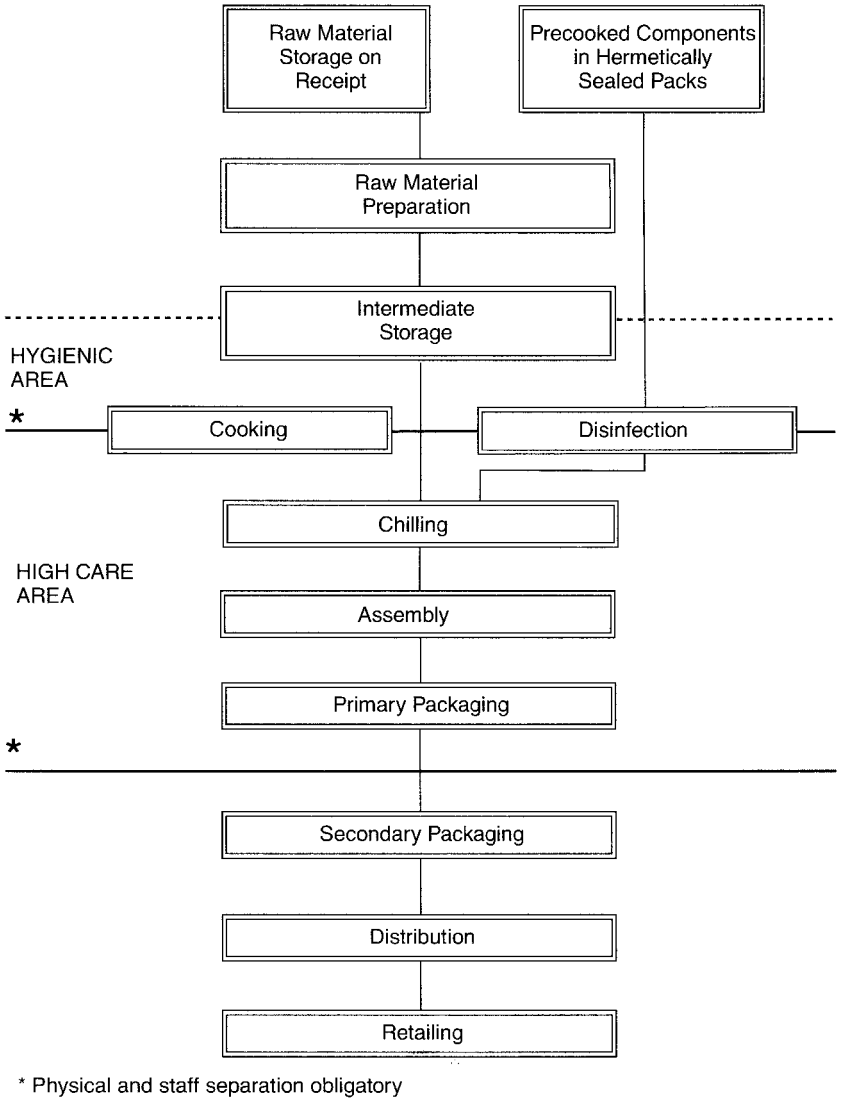

Fig. 7.3 Typical flow diagram for the production of pre-cooked chilled meals from cooked components.

the requirements below. The design of hygienic areas is further discussed in Chapter 6. Operational procedures and staff training should be designed to minimise three things:

- Carriage of pathogens into production and storage areas.
- Opportunities for their growth in the production and storage areas.
- The number of environments allowing survival.

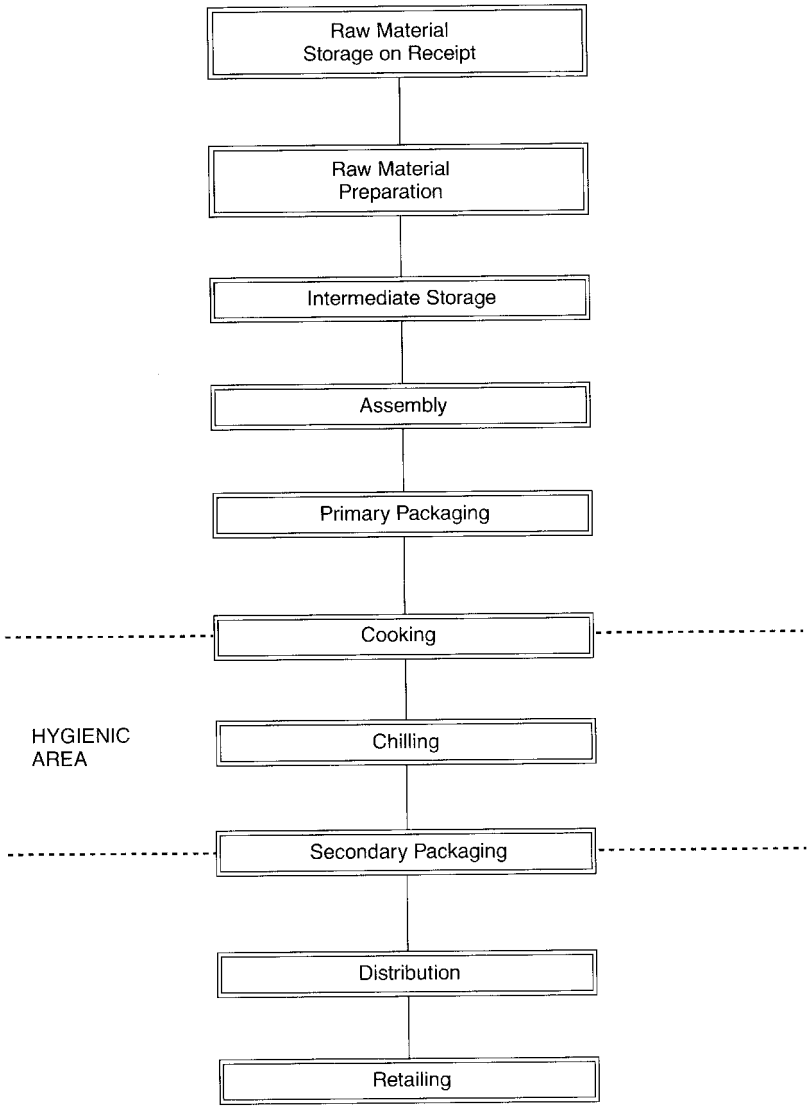

Fig. 7.4 Typical flow diagram for the production of chilled foods cooked in their own packaging prior to distribution.

It is worth remembering that between cleaning and production (e.g. at weekends), areas may not be chilled and, depending on conditions, *Listeria* and other pathogens may grow in food residues left on equipment. Layout and production procedures should minimise opportunities for cross-contamination. The production planning system should provide opportunities for cleaning at suitable intervals and this is critical if allergenic materials are handled and products not labelled as 'at risk'.

Where ready-to-eat products are made from mixtures of raw and cooked components, any components known to have a high risk of containing pathogens should be excluded from areas handling unwrapped product unless they have been effectively decontaminated; thus prior processing may mean they can be used safely. It is important to identify pathogens that can survive any decontamination procedures used (see listings by Doyle, 1988). Examples of higher-risk materials are prawns, other shellfish from warm waters and untreated herbs and spices, which may carry *Salmonella*. It must be accepted that from time to time these products and their components will contain pathogens, and hence storage conditions, hygiene in manufacturing areas, use instructions and coding practices should be designed with this in mind.

7.6.6 High-care areas

High-care areas are designed for the post-cook handling, cooling and assembly of ready-to-eat products made entirely of cooked (or otherwise decontaminated) components. These areas should be designed and operated to prevent microbiological contamination of products. They are used for handling cooked components for chilling, assembly, or further processing and then packaging (e.g. sliced cooked meat products, such as pâté). The shelf-life and safety of ready-to-eat products relies very largely on the prevention of recontamination, though a few products do have preservation systems capable of halting the growth of pathogens. High-care areas may be used for the handling of long shelf-life chill stable products made from decontaminated (e.g. free of cold-growing strains of *Clostridium botulinum*) components by prior processing. There are a number of specific requirements for high-care areas.

Physical separation

Sometimes these areas may have a higher standard of hygiene than is strictly necessary to prevent pathogen contamination. This is done to limit recontamination with spoilage microorganisms (such as yeasts, moulds and lactic acid bacteria) and to minimise spoilage risks. Only foodstuffs and packaging that have been reliably decontaminated and handled to prevent recontamination should be admitted to these high-care areas.

Surfaces

All the food contact surfaces in a high-care area should be impervious to water and capable of being easily cleaned, disinfected and kept dry. After cleaning and disinfection, surfaces should have fewer than 10 microorganisms per 9 cm^2 and Enterobacteriaceae should not be recoverable.

Chillers and cooling

Chillers are an integral part of high-care areas, designed to cool components (blast chills; James *et al*., 1987) or to maintain temperature in previously cooled components. Cooling of hot products should be started as soon as practicable after

cooking and containers should be dimensioned to ensure rapid cooling. Minimum cooling rates/times should be designed to prevent the growth of surviving spore-forming bacteria. Because these chillers may receive 'naked' product, the quality of cooling air and environmental hygiene are critical to safety (see below). When warm products are placed in the chillers, condensation may occur on their surfaces and this should be ducted away to prevent contamination. The design, hygiene and operating temperature of the cooling elements in fan-driven evaporator units play a critical role in limiting product recontamination by aerosols. If the temperature of the heat exchanger coils is too high, water will condense on them without freezing and be blown onto the products by the fan. Many evaporator units are designed so that the condense tray does not drain and is inaccessible for cleaning, hence such units can harbour *Listeria* and cause contamination.

If water is used as a direct cooling medium as a spray, a shower or in a bath (for products in hermetically sealed containers), chlorination or other disinfection procedures should be used to ensure that the product will not be recontaminated. Stringent cleaning and disinfection systems should be used to ensure the hygiene of any circulation or recirculation systems, including heat exchangers. Packs should be dried as soon as possible after cooling and manual handling of wet packs prevented.

Air supply

The air supply to high-care areas should be filtered to remove particles in the 0.5–50 μm range and its flow should be from clean to dirty. This is often achieved by means of a slight overpressure in the clean area, to prevent the ingress of untreated air. Air supply and heating and ventilation systems should be designed for easy access for inspection and cleaning.

7.6.7 Waste disposal

The efficient removal and disposal of waste from manufacturing areas is an essential part of hygiene. Suitable separate storage facilities and distinctive containers should be provided to prevent product contamination. Any equipment or utensils used for the handling of waste should not be used for the manufacture or storage of products; it should be maintained and cleaned to the same standards as the area in which it is used.

7.7 Handling and processing products

For convenience, many process designs give emphasis to inactivation rates of pathogens and the design of equipment to do this. However, other equipment is also important in ensuring safety. During manufacture a temperature range rather than a single temperature will be experienced by the food, hence the importance of modelling to predict risks in a supply chain. When heating is a key step in safety, attention has to be paid to the time taken for a pack or vessel to achieve

the process temperature and then to cool. In practice, liquid or pumpable foods will heat most rapidly because their flow properties permit the use of efficient heat exchangers. For this reason, high-temperature short time (HTST) and UHT treatments can be used for soups, sauces and milk. Solid or viscous foods are usually packed and heated in containers (e.g. cans, jars or pouches). Because of their geometry and the fact that heat is transferred by conduction from the outside, these containers will have slowest heating and cooling points. The rates of temperature change at the cold spot will often determine the time–temperature combination used for processing. This principle is equally applicable to sterilisation for ambient stability and the design of trays for cooling ready meals.

7.7.1 Working surfaces for manual operations

Many preparation or product assembly operations will be carried out on tables or other flat surfaces. These should be hygienic and technically efficient. For example, stainless steel is not suitable for cutting on, although it is easy to keep clean. Cutting surfaces should be made of hygienic materials such as nylon, polypropylene or occasionally Teflon. Working surfaces should be easy to remove for cleaning or be completely cleanable without removal. It should be possible to restore the integrity of working surfaces by machining or some other process, because cut surfaces with uncleanable crevices are likely to be a potent source of contamination. The use of triclosan impregnation of surfaces has been recommended to provide a degree of antimicrobial protection. However, Cowey (1997) recommends that this is effective only as an additional line of defence, not as a replacement for hygiene procedures.

7.7.2 Cutting and slicing

Many products such as meats and pâtés are prepared and cooked as blocks. Automated slicing or cutting after cooking is an integral part of their conversion into consumer packs. Slicers can be potent sources of contamination, because they are usually mechanically complex, providing many inaccessible sites that can harbour bacteria. Bacteria, including pathogens, live in the debris produced by slicing, which is often not completely removed by cleaning. The effectiveness of cleaning procedures may be further reduced if cleaning operatives try to avoid wetting sensitive parts of machines (e.g. electronic controls, motors and sensors), which may be rendered inoperative by water penetration, especially if high-pressure cleaning is used. An integral part of the hygienic design of such machines is good access for cleaning and inspection, and effective water-proofing. An insight into the routes for microbial recontamination of product can be gained by auditing the machines for product and debris flow during operation, so that the sources of recontamination can be identified. After production, when the equipment has been cleaned, machines should be re-examined to identify difficult-to-clean parts and areas where product is still present. Effective cleaning schedules for them can be developed, implemented and monitored.

The difficulty of controlling the hygiene in such machines typifies the much more widespread problem of recontamination by process equipment. Many forming machines handling cooked product operate in chilled areas both to improve their technical performance and to slow or halt the growth of micro-organisms by minimising temperatures in retained product debris. Temperature auditing of machines will show that they contain many sources of heat that are not effectively controlled by environmental or dedicated cooling (e.g. motors and gearboxes). In a well-designed machine, heat is conducted away without causing significant temperature rises; however, many machines have localised hot spots in contact with food or debris, where microbial growth can occur. It is not uncommon for some parts of these machines to operate at temperatures well above the design temperature of the area and within the growth range of pathogens. For example, in the area of the main cutter shaft bearing and motor of a slicer, temperatures of 25 °C+ could be found when material at below 4 °C is being processed in an area operating at 7–10 °C. During operation, these warm spaces may fill with product debris, which is retained, warmed and incubated, then released back into or onto product. Ideally, hot spots should be eliminated by machine design, but in practice in many existing machines, these risks can only be minimised. For safe, hygienic operation, warm areas retaining product must be identified, and then controlled by cleaning, cooling or other preservation techniques, to ensure that microbial growth and product contamination are minimised. In some cases, simple engineering modifications can improve the hygiene of machines by reducing the quantities of product retained and its temperature.

7.7.3 Transport and transfer of product

From the time that a product is cooked, it may be transferred between production areas before being put into its final, primary packaging and further contamination prevented.

Containers

In the simple process lines, products cooked in open vessels will be unloaded into trays for cooling. Whatever type of container is used, it should not contaminate the product, and its shape, size and loading should ensure that rapid cooling is achieved. Generally, stainless steel or aluminium trays are more hygienic and cool faster than plastic ones. Plastic becomes more difficult to clean as surfaces become scratched in use.

Belts

In complex production lines, transfer or conveyor belts may be used for transporting both unwrapped product or product in intermediate wrappings from one process stage to another. Belts may also be an integral part of tunnel or spiral equipment, such as cookers, ovens and coolers. Although many types of belting material are used, they can be split into two broad groups:

- Fabric or solid.
- Mesh or link.

For hygienic operation, fabric conveyors should not absorb liquids and have a smooth surface finish, so that they are easy to clean and disinfect. They are generally used only for transport under chill or ambient conditions, as they are not often heat-resistant. Solid belts for heating or cooling, they are usually made of (stainless) steel, which is hygienic and can be heated or cooled directly or indirectly. Belts can be cleaned in-place by in-line sprays, providing both cleaning and rinsing. A drying stage may be incorporated, using an air knife or vacuum system. The hygienic problems of conveyor belts are usually associated with wear or unhygienic design of drive axles, beds or supporting frames or poor maintenance.

Mesh or plastic link belts are used because they can carry heavier loads and form corners or curves. Metal mesh belts are widely used in ovens and chillers where circulation of hot or cold air is a part of the process, for example in spiral coolers or cookers. Belts that are regularly heated, or pasteurised, do not present a hygiene problem, as any material retained between the links will be freed of viable microbes by heating. If these belts are used in coolers, or for the transport of unwrapped product at ambient temperatures, special attention must be paid to their potential for recontamination via retained debris. Cleaning systems should be devised to remove the debris from between the links (for example, by high-pressure spray cleaning on the return leg of the belt). After cleaning, the belt should be disinfected or preserved, for example by chilling or drying, so that microbial growth does not occur during the processing period.

Hygienic performance becomes more difficult to achieve if routine engineering maintenance is not carried out correctly and belts become damaged or frayed. If specialised belts are incorrectly set up, or used with fragile products, this can increase the quantity of product waste generated by damage, so that even a properly designed and operated cleaning system will not keep the system hygienic. Product and pack characteristics, such as stickiness and crumbliness, should therefore be considered when transfer systems are designed, so that the generation of debris is minimised.

7.7.4 Dosing and pumping

Most food products are sold in weight or volume-controlled packs, often with individual ingredients in a fixed ratio to one another, for example meat and sauce. Where ingredients are liquid or include small (*ca* 5 mm) particles suspended in a liquid, they may be dosed using filling heads or pump systems. If this type of equipment is used for dosing decontaminated materials, then its hygienic design and operation are critical to product safety and shelf-life.

Dosing and filling systems may be operated at cold (below 8–10 °C), intermediate (20–45 °C) or hot (above 60 °C) temperatures. The most hazardous operations are those run at intermediate temperatures, within the growth range of

food-poisoning bacteria, and unless the food producer is completely confident of the decontamination of the feed-stock, and hygiene of equipment, and is prepared for frequent cleaning/disinfection breaks, intermediate temperatures should not be used. Where hot filling is the preferred option, control of minimum temperatures is critical to safety. Often, target temperatures will be set well above the growth maximum of foodborne pathogens (*ca* 55–65 °C) to allow for cooling during dosing, and especially if there are stoppages and breaks in production, when the flow of product may be halted. If product is supplied to the filling heads by pipework, it is necessary to ensure that an unacceptable temperature drop (leading to temperatures in the growth range) does not occur, recirculatory loops returning to heated tanks or vessels may be used to prevent this, but their hygiene must be carefully monitored. Dosing equipment is often cleaned by CIP (clean-in-place) systems and it is essential that the pumps, valves and couplings used are suitable for this type of cleaning as well as for production. If in-pack pasteurisation is used, consistent dosing plays a major role in ensuring that packs with uniform heating characteristics, headspaces and closing characteristics are produced.

7.7.5 Packaging

Primary packaging

Most food products are sold packaged. The functions of packaging are to prevent contamination and retain the product. Packing materials may be chosen for a variety of technical reasons, such as cost, appearance, machinability and heat or freeze resistance. From a microbiological safety point of view, the most important attributes of packing materials are their physical strength to provide pack integrity and exclude bacteria (especially at seams or seals) and their provision of gas and moisture barrier properties. If foods are intended for a long storage life, these properties must be maintained during the time involved. This is most important if packaging forms part of the preservation system. For example the pack must retain its integrity during processing (e.g. during heat sterilisation at 121 °C), and thereafter exclude contaminants. Filling equipment is important if headspace determines heating characteristics: a vacuum may be essential to ensure that a cap remains in place and food trapped in the seal area can prevent a hermetic seal being formed. A major cause of pack failure is soiling of the seal area of the pack with product during filling. If residues remain in the seal area during heat sealing, then the two layers of plastic of the lid and base cannot be welded together by the sealing head, an analogous fault can occur during the double seaming of cans. Sealing problems are encountered especially when flat or unprofiled heat sealing heads are used, as these can trap material under them during sealing. Some heads are profiled to move residues out of the seal area during the sealing cycle; their effectiveness is determined by head profile and product flow characteristics. This may not always be an effective solution to a sealing problem, if food remains in the seal area, preventing formation of a

continuous weld or causing a bridge to be formed across the seal. Problem fills are fat, water (which turns to steam on heating) and hard cellulosic fibres, such as celery.

Modified atmosphere packs

Some products designed for chilled storage rely on the gas (Modified Atmosphere – MA) surrounding the product forming part of the preservation system (e.g. low P_{O_2}, high P_{N_2} and CO_2). This can give a considerable extension of shelf-life at chilled temperatures, for example with chilled meats. Aerobes may be inhibited if oxygen is removed from the headspace by vacuum packing and then diffusion back prevented by the barrier properties of the packaging film. Limited microbial metabolism in the pack may lead to the build-up of CO_2. In such packs the potential for growth of anaerobic pathogens must always be accounted for in the design of shelf-life and distribution temperatures. The efficiency of flushing and replacement of oxygen with a gas mixture, control of vacuum level, and the frequency with which leaking packs are produced are key operational parameters for this type of packing. Colour indicators have been advocated to indicate leaking packs (Ahvenainen *et al*., 1997).

Vacuum or MA packs that are not gas-tight because of a faulty seal (leakers) will have a shortened shelf-life and present increased risks of contamination. This fault may be caused by incorrect choice of packaging film, or equipment faults (e.g. mis-setting of the sealing head temperature, pressure alignment or dwell time). The fault can be seen, or felt, soon after manufacture as the pack may feel soft, if squeezed. If packs with an incorrect headspace volume or with weakened seals are processed in systems (such as retorts or ovens) that generate a pressure differential between the pack and its surroundings, bursting or pack weakening may result.

Other types of pack

Some short shelf-life products do not need bacteria-tight packs. Packs with a crimped seal between the lid and the base container (which may be made of aluminium or C-PET) may be used to provide a container for oven cooking or reheating by the customer. These packs carry an increased risk of product contamination, unless overwrapped.

Any packaging materials coming into direct contact with the products must not contaminate them either chemically, physically or microbiologically. There is legislation governing the materials that may be used for food contact packaging. Toxicological clearance is linked to usage, as temperature plays a major role in determining migration of chemicals from packaging into food. Packs for microwave or oven heating carry very different risks to a pack used for the distribution of chilled meat, as they will be subjected to different conditions during use. Overwrapping of the primary or food contact packaging and its handling within the storage and production areas should be designed to minimise the chances of contamination and pack damage.

Secondary packaging
Once the product is in its primary packaging, it is generally protected from recontamination. Primary packs sometimes have additional, secondary packaging. This packaging may be decorative, or may protect the pack from damage or stress during handling or transport. For this latter function, control of the secondary pack characteristics should be part of the factory QA system.

For any product, the strength of the primary and secondary packs must be able to maintain primary pack integrity and prevent damage during automated handing and transport on pallets. When chilled products are handled or marketed either in boxes or closely packed on pallets, there is only a limited opportunity for temperature change – as the surface area to volume ratio is unfavourable to rapid temperature changes, and rates of heat penetration through product and packaging are low. Therefore it is essential that product in its primary packaging is at the target temperature prior to secondary packaging and palletisation.

7.8 Control systems

7.8.1 Instrumentation and calibration

Wherever control limits and targets are specified in the HACCP plan, it is essential that reliable instrumentation or measurement procedures are present, correctly located and calibrated. Their function is to ensure that the process is kept within pre-set limits, so that the designed standards of product safety and quality are met. Sensors need to be prepared, installed and monitored, to prevent errors in readings (Sharp, 1989). Outputs can be used either for the control of process conditions (such as during sterilisation, pasteurisation, chilling or storage) or for monitoring compliance with specifications. Key outputs include temperature, time and pressure measurements. For specialised pieces of equipment, such as retorts and freezers, other measurements may be important (e.g. water level, heat exchange medium flow, product flow or pack rotation). Sensors and their associated instruments may be in-line (e.g. oven, heat exchanger or fridge thermometers and timers or time/temperature recorders), at the side of the line (e.g. pack seam measurement, drained weight apparatus, salt or pH meters) or in the laboratory (e.g. colour measurement, gas composition or nitrogen determination). Wherever the instrument is situated, it needs to be maintained, with its sensor kept free of product debris – as this may produce erroneous signals. All sensors and equipment need regular calibration and operatives need to have training and a measurement or recording procedure.

7.8.2 Process monitoring, validation and verification

Manufacturing, storage and distribution operations in the supply chain should be controlled and monitored to ensure that the whole chain performs within agreed limits. Wherever possible the data from control systems should be recorded and

used to produce information for management and operative control and trend analyses. Often responsibilities for the safety and quality of ingredients or a range of products will be shared between several suppliers and producers, who all require compatible levels of control. The increasing concentration of food businesses on their core activities (such as component manufacture – packaging, flavours, etc.) means that vertical integration within the supply chain is uncommon, therefore product safety and quality systems rely on effectively managed and specified customer–supplier relationships. This trend is leading to the establishment of partnerships, rather than looser arrangements where suppliers are played off against each other. Even if safe process and product design principles from the HACCP study cover realistic hazards, unsafe products can result if the requirements noted in the plan are not carried out correctly, are not working effectively or have not been reviewed to take account of changed circumstances or a new hazard. Review of the scientific and technical content of a HACCP plan is an essential part of its maintenance.

Verification of a HACCP plan is a routine auditing activity that systematically analyses its working and implementation. It is done by examining process and product-related data, making comparisons with specifications and any technical agreements that, although they may not be part of the HACCP plan, form the customers' requirements. In-house QA departments, regulatory authorities, auditors and those inspecting suppliers on behalf of customers, are usually responsible for verification. They act as systems experts working over a period of months or years to establish compliance with systems, procedures and the other outputs of the HACCP plan, or in some cases ISO 9000 series documentation.

As a minimum, verification should focus on data showing the producer's performance at each CCP. It should analyse existing procedures, systems and records, supplemented if necessary with sample data, record inspection and auditing to form an assessment of the consistency of product quality or how well the process is controlled. To establish the data for verification, the steps in the HACCP plan (i.e. hazard analysis, identification of CCPs), control criteria and critical limit values and monitoring of the CCPs, should be considered. Process stages where the risks of microbial contamination, survival or growth are significant should always be covered to show how well the workforce and management are complying with the requirement of the HACCP plan. Operational risks investigated by verification include poor training and management procedures, poor hygiene, inadequate management of heating, cooling, segregation or packaging and occurrence of defective products.

Reliable verification is based on validation, which examines the scientific basis of the HACCP plan and the range of hazards covered. It should be done prior to implementation of the plan and regularly during its operation, as part of the review procedure. Validation determines whether realistic hazards have been identified, and suitable process control, hygiene and monitoring measures implemented, along with safe remedial actions for use when processes go outside their control limits. For many foods, the specification of correct heat processing conditions, suitable packaging, prevention of recontamination and assurance

of storage temperatures and times are essential activities and should be directed at specific hazards. Because precautions are directed at specified hazards, it is essential that hazards are regularly reviewed to take account of changes in the origin of raw materials or new markets. Unsuitable process features that should be identified by validation include unhygienic equipment, working practices, targets, controls or layouts that may lead to product contamination or the design of unstable preservation or distribution systems. Changes in equipment performance shown by verification procedures should be examined by re-validation.

7.8.3 Process and sample data

For foods with a short shelf-life, distributed immediately after manufacture, microbiological results cannot be used for assurance of safety for two reasons. Firstly, sufficient numbers of portions cannot be sampled to provide any confidence that processing and ingredient quality were under control for the duration of processing. Secondly, the time for a microbiological result may be longer than the pre-despatch time in the factory, even when rapid methods are used.

The results of microbiological testing should be used only for supplier monitoring, trend analysis of process control and hygiene and for 'due diligence' purposes. In some cases they may be used to supplement process data (verification) and provide additional criteria for release. Data showing the control of CCPs should be taken at a defined frequency and kept for a period equal to at least the shelf-life plus the period of use of the product, to verify the performance of the supply chain. Process control records may be generated and retained according to the framework proposed in the ISO 9000 documents on quality management.

Conformance samples may be taken to track long-term changes in quality. This is not a problem with long shelf-life products, but with short shelf-life products, some manufacturers retain frozen samples and accept the quality changes caused by freezing. Others take end-of-shelf-life samples and score their sensory attributes against fixed scales or parameters.

7.8.4 Lot tracking

Packs of food product should be coded to allow production lots or batches of ingredients to be identified. This is a requirement of the EU General Hygiene Directive (93/43). Documentation and coding should allow any batch of finished product to be correlated with process time and batches of raw materials and packaging used for manufacture and with corresponding process data and laboratory records. In practice, the better defined the lot tracking system, the better the chances of identifying and minimising the impact of a manufacturing or ingredient fault on the amount of product potentially at risk. Consideration should be given to allocating each batch of ingredients or process period a reference code to identify it in processing and storage. Deliveries of raw materials, any re-work and packaging should be stored so that identities do not become lost. If there is

a fault, or re-call, or good coding and tracking procedures will facilitate responding to complaints and ensuring customer safety.

7.8.5 Training, operatives, supervisors and managers

All staff involved in the manufacture of food products should be adequately trained because they make an essential contribution to the control of product safety. The best way of achieving this is by a period of formal or standardised training at induction, which will enable them to make their contribution to assuring the safe manufacture of high-quality products (Mortimore and Smith, 1998; Engel, 1998). After training they should at least understand the critical aspects of hygiene for food handlers, product composition, presentation, process control and the prevention of recontamination. Many manufacturing operations will be done in lines and will involve teamwork; therefore all staff must be trained in, and understand, the reasons for specified hygiene and process control standards and procedures. Staff and supervisors should have defined responsibilities to ensure that risks of unsafe product are minimised. This is critical if products are ready to eat, or will only receive minimal heat processing by the customer or if they are low-acid canned foods. The role of external HACCP consultants has been identified (White, 1998) as being especially useful to small food companies in helping them put together effective HACCP programmes and staff training. Health screening may be required to ensure that personnel have the standard of health and personal cleanliness required for the job.

7.8.6 Process auditing

Auditing is the collection of data or information about a process or factory by a visit to the premises involved. It may be used to determine whether the HACCP plan is correctly established, effectively implemented and can achieve its objectives. It is especially important where product safety relies on many aspects of a process (van Schothorst, 1998; Sperber, 1998). An integral part of an audit is to see the line operating; effective auditing does more than inspect records. Topics covered by inspection of paperwork should include an assessment of the company structure, company policy on quality and safety, the capability and management of the product development department, in-take storage; and handing of raw and packaging materials. Design, control and operation of manufacturing should be assessed, including the safe design of products and processes, matching the products to consumer use and the equipment and layout available. Lastly, the operational aspects of manufacturing should be examined – production, hygiene and housekeeping. Because of their importance distribution, logistics should be considered. The contribution of the QA department and laboratory to the management of the operation and training should be assessed.

Internal or external auditors may do the auditing, a checklist may be used and often a scoring system or noting of non-compliances may be used for assessment in a customer–supplier relationship. A more recent development is supplier self-

auditing in a supplier–customer relationship with a partnership basis. This starts with a clear statement of trading objectives and the resources and products involved. Next an evidence package covering specification and records is agreed between the supplier and customer. Because quality and process control may vary, a mechanism for managing it must also be agreed, to prevent false rejection of product and the realistic management of any safety risks or non-compliances. Successful operation of such systems relies on the identification and allocation of responsibilities and ownership of critical technical factors by both organisations, so that the person best placed to know and make decisions can always be identified.

This type of audit system has a better cost–benefit than the traditional audit visit and produces information for decision making on a continuing basis. Externally accredited auditors may do this, but their ability to examine a process and derive the optimum system can be limited by access to commercially sensitive data on processing. The basis of product safety is a continuing supply of data, and if properly handled it provides a better level of customer protection than end-product sampling and testing. Procedures for managing process breakdowns and other deviations must be developed and audited to ensure that risks to customers are minimised.

7.9 Conclusions

A major expansion in the sale of processed food products, such as ready-prepared foods and ingredients, continues globally to meet the constantly increasing requirements of consumers for convenience, variety and less severe processing and preservation. The supply chain is now supplied, and sells, on a global basis, introducing new microbiological hazards and providing outlets with different degrees of control over handling plus the challenge of customers using products in different ways. Foods are processed industrially and at home using less heat; in many countries the link between cooking and food safety has been lost in the minds of consumers. Foods also contain lower levels of preservatives and these changes are not immediately compatible with an improvement in microbial stability or safety. Indeed most changes have led to a lessening of the intrinsic preservation or stability of foods and an increase in reliance on external factors, such as the cold chain. Hence there is greater reliance on safe design and operation of all the unit operations along the supply chain. Conscientious review of the design of products and processes against new microbiological challenges and different consumer needs and expectations is the only way to provide continuing supply of safe food and clear specifications for processing and distribution.

The assurance of food safety is an essential consumer requirement and therefore of paramount importance for the producer. It is here that modern design, processing and distribution techniques backed up by risk assessment and management techniques, such as HACCP (Mayes, 1992), properly applied, can more than compensate for the stability and safety that otherwise could be lost. Although

the management of safety and prevention of new hazards is complex in detail, as indicated above, the principles of effective and safe design and processing of food products are few. They include:

- The reliable identification of hazardous microorganisms, so that design conditions will allow control of them.
- Clear identification of the criteria required for safe products, e.g. numbers or presence of pathogens or process/product conditions ensuring their numbers are below a harmful level.
- Controlled and predictable raw material quality and processing leading to the reliable elimination, inactivation or inhibition of microorganisms by processes, storage conditions, product formulations and consumer use.
- Control and management of process stages critical to the control of pathogens in the product and the assurance of traceability, to minimise risks in the event of a process or material problem.
- The prevention of recontamination and cross-contamination after decontamination.
- Control of the survival or persistence of any microorganisms or toxins that remain in the food.
- Provision of robust packaging systems able to prevent contamination or loss of preservation conditions during distribution, in all markets.
- The provision of clear consumer use instructions that are compatible with customer expectations and habits.

Safe, effective processing and distribution of any food product depends on consistent, confident and robust control of these elements by the food industry. Although these principles are effective if properly carried out, it is likely that their practical effectiveness can be further improved in the future (e.g. by better implementation of HACCP) and this may become necessary to meet consumer expectations and match their perception of safety.

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8

The effective implementation of HACCP systems in food processing

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8.1 Introduction

'It is widely acknowledged that, although absolute safety in food production is unattainable, effective Hazard Analysis Critical Control Point (HACCP) implementation is the surest way of delivering safe food'. (Mayes and Mortimore, 2001). The development of HACCP from its initial conception by Pillsbury working with Natick is well documented. Initially developed as a means of ensuring the microbiological safety of foods used in the space programme, its wider potential for ensuring the control of microbiological and other food safety hazards in a broad range of foods in manufacturing, distribution, food service and retail situations was soon realised. Initially the HACCP concept developed in a somewhat piecemeal fashion, and it was not until the mid- to late-1980s that HACCP development began to accelerate in a coordinated way. The HACCP concept was endorsed by the WHO/FAO as an effective way of controlling foodborne disease in 1983 when the Joint FO/WHO Expert Committee on Food Safety advised that HACCP should replace traditional end-product testing approach to food safety assurance. Since then there have been many guidelines issued on the principles of HACCP implementation (e.g. ICMSF, 1988; US National Advisory Committee on Microbiological Criteria for Foods, 1992; ILSI, 1997; Campden & Chorleywood Food Research Association, 1997).

It was, however, only during the late 1980s and early 1990s that there was a concerted international attempt to harmonise HACCP principles and terminology. The current Codex Alimentarius HACCP document (CAC, 1997) can be seen as the first authoritative, internationally agreed document both agreeing HACCP principles and providing guidelines for their implementation in practice. This

Table 8.1 Seven principles of the HACCP system

Principle 1	Conduct a hazard analysis
Principle 2	Determine the Critical Control Points (CCPs)
Principle 3	Establish critical limit(s)
Principle 4	Establish a system to monitor control of the CCP
Principle 5	Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control
Principle 6	Establish procedures for verification to confirm that the HACCP system system is working effectively
Principle 7	Establish documentation concerning all procedures and records appropriate to these principles and their application

Table 8.2 Guidelines for the application of the HACCP system

Task 1	Assemble the HACCP team
Task 2	Describe product
Task 3	Identify intended use
Task 4	Construct flow diagram
Task 5	On-site verification of flow diagram
Task 6	List all potential hazards, conduct a hazard analysis, determine control measures
Task 7	Determine CCPs
Task 8	Establish critical limits for each CCP
Task 9	Establish a monitoring system for each CCP
Task 10	Establish corrective action for deviations that may occur
Task 11	Establish verification procedures
Task 12	Establish record keeping and documentation

document identifies seven principles underlying the HACCP system and provides guidelines listing 12 tasks for the implementation of these principles (Tables 8.1 and 8.2). The document is widely seen as the international benchmark for effective HACCP implementation.

The seven principles, and in particular the 12 'guidelines' tasks listed in Table 5.2 outline the minimum practical steps needing to be taken to implement HACCP. Although not specifically listed in the above table, the CAC guidelines make much of the philosophy behind HACCP plan and the need to have the correct balance of skills and experience in the team that prepares the HACCP plan and the commitment needed to successfully implement and maintain the plan. Although there are some differences in emphasis on the guidelines for HACCP implementation in some publications (see later) the CAC document is widely seen as the international benchmark for effective HACCP implementation. While HACCP is relatively well developed in some sectors, notably larger food processors in developed countries, it is still far from widespread in others, particularly small and medium-sized enterprises (SMEs), most notably in the

service and catering sectors, and in developing countries in particular. The problems of implementing HACCP systems in the SME sector have, for example, been identified by the WHO (WHO, 1993, 1999). Studies in the UK, for example, confirm a relative lack of investment by SMEs in HACCP (Gormley, 1999; Mortlock *et al.*, 1999; Panisello *et al.*, 1999). However, even where it has been established longest, among larger food manufacturers in Europe and the United States for example, the quality of implementation has varied (Kane, 2001).

What makes for effective HACCP implementation? Based on the experience of those who have implemented HACCP systems in practice, this chapter outlines the key issues in HACCP implementation identified by HACCP practitioners and how they can be addressed in building a truly effective food safety regime.

8.2 HACCP methodology and implementation

Although there is broad agreement on basic HACCP methodology, some differences in emphasis can be seen when comparing guidelines on HACCP implementation from three leading authorities:

- The Codex Alimentarius (CAC, 1997).
- Campden and Chorleywood Food Research Association (CCFRA) (1997).
- Those provided by one of the most respected texts in this field: Mortimore and Wallace's *HACCP: A Practical Approach* (1998).

These approaches are compared in Table 8.3. For the purposes of comparison, only the basic headings provided by Codex and the CCFRA are given. These are then related to the detailed step-by-step approach in *HACCP: A Practical Guide*. While the central stages covering HACCP planning and design are broadly similar across all three sets of guidelines, it is noticeable that *HACCP: A Practical Guide* lays particular emphasis on the preparatory work required such as training in HACCP methodology and conducting a baseline audit. The importance of effective preparation is endorsed, for example, by the New Zealand government which suggests the need to agree the scope of the HACCP plan and to set appropriate food safety objectives (Lee, 2001: p. 146). As the following sections show, the experience of HACCP practitioners confirms the importance of such preparation, whether in the importance of training, defining the scope of a HACCP study (for example what should be covered by prerequisite programmes, PRPs, and whether quality issues should be covered), or conducting a baseline audit of a company's existing operations.

There are a number of areas that influence effective HACCP implementation:

- Motivation.
- Knowledge.
- Resources and planning.
- Prerequisite systems.
- HACCP teams and analysis.

Table 8.3 Approaches to HACCP implementation[a]

Codex guidelines	Mortimore and Wallace	CCFRA manual
	Stage 1 Preparation and planning	
1 Assemble HACCP team	• understanding HACCP concept • identifying and training HACCP team • baseline audit • project planning (incl. improving prerequisite systems)	2 Assemble HACCP team
	Stage 2 HACCP studies and planning	
2/3 Describe product and intended use	• terms of reference • describe product and intended use	1 Define terms of reference 3/4 Describe product and intended use
4/5 Construct and verify flow diagram	• construct process flow diagram	5/6 Construct and verify flow diagram
6 Conduct hazard analysis, identify control measures	• hazard analysis • identify CCPs • establish critical limits	7 Conduct a hazard analysis, identify control measures
7 Determine CCPs	• identify monitoring procedures	8 Identify CCPs
8 Establish critical limits	• establish corrective action procedures	9 Establish critical limits
9 Establish monitoring procedures	• validate HACCP plan	10 Establishing monitoring procedures
10 Establish corrective action procedures		11 Establish corrective action procedures
	Stage 3 Implementing the HACCP plan	
12 Establish records and documentation	• determine implementation method • set up implementation team	13 Establish documentation and record-keeping
11 Establish verification procedures	• agree actions and timetable (incl. training, equipment, record-keeping) • confirm implementation complete • verify implementation through audit	12 Verification

Table 8.3 Continued

Codex guidelines	Mortimore and Wallace	CCFRA manual
	Stage 4 Maintaining the HACCP system • defined standards and regular audit • ongoing maintenance • data analysis and corrective action • HACCP plan re-validation • update	14 Review the HACCP plan

[a] The sequence of steps given by Codex and the CCFRA has been altered to fit the sequence suggested by Mortimore and Wallace for ease of comparison. The original sequences are indicated by the numbering of the steps in each case.

- Implementation.
- Maintenance.

These areas are discussed in the following sections.

8.3 Motivation

As with any significant new system within a company, there needs to be the requisite motivation to take on the challenge and follow it through. This is particularly important in the case of HACCP because motivation lies at the heart of any HACCP system. HACCP requires a proactive approach at all levels of an organisation. The impetus to develop a HACCP system typically comes from a number of drivers, including:

- Customer pressure.
- Regulatory requirements.
- Desire for self-improvement.

Many SMEs in particular are inherently suspicious of HACCP for a range of reasons. They may be ignorant of food safety issues and have a low perception of the severity of hazards arising from their day-to-day operations. It has also been suggested that the early association of HACCP with larger manufacturers, who were the first to implement HACCP systems, encouraged the assumption that HACCP was a complex system only feasible for big companies with the appropriate skills and resources (Barnes, 2001: p. 211). This combination of a low perception of need and a fear of the difficulties of implementation is a potent barrier. In developing countries, consumers may not regard food safety as a major issue, reducing the commercial rationale for HACCP implementation in relation to the

costs involved (Suwanrangsi, 2001: p. 190). In addition, the risk of prosecution for food safety offences may be low. In many cases, businesses face major weaknesses in infrastructure, such as poor quality in the supply of raw materials, inadequate facilities and equipment, and an unreliable distribution system (Marthi, 2001: pp. 82–4). In these circumstances the demands of HACCP implementation can seem prohibitive in relation to the advantages it gives the business.

For businesses in developing countries and in the SME sector generally, the impetus for HACCP implementation may, therefore, come most strongly from external regulatory or customer pressure. A particularly important motor for change is pressure from customers, whether these be larger manufacturers supplied with semi-finished goods by a network of smaller suppliers, or retailers stocking a business's food products for sale to the consumer (Route, 2001: p. 32). As global trade has increased, businesses in developing countries seeking to export to regions such as North America or the EU have needed to develop HACCP systems to meet both import requirements and the supplier quality assurance (SQA) standards set by their retail and manufacturing customers. Such customers often have well-developed SQA schemes requiring suppliers to develop HACCP systems which are then regularly audited either by internal audit teams or accredited third-party auditors.

Whilst such external pressures have been an important way of disseminating the HACCP concept, such a pathway into HACCP can have significant disadvantages. If a business has been forced reluctantly to embrace HACCP because of pressure from outside, it is unlikely to have the level of commitment required for effective implementation. In a study of HACCP implementation in the Thai seafood export industry, for example, businesses developing HACCP systems in reaction to the demands of their overseas customers initially relied on often conflicting customer advice and undiscriminating use of generic HACCP models. This reactive approach resulted in ill-considered, poorly understood and ineffective systems that did not reflect the particular requirements of the individual business. The real change in momentum came when businesses themselves came to see HACCP as essential for competitive success in export markets and as a basis for future growth and profitability. The best HACCP systems are developed by businesses that are self-driven, though a mandatory approach may be necessary to accelerate progress and ensure a more consistent approach and standards (Lee, 2001: pp. 142–3). Key reasons for HACCP to be embraced positively by a business include the following:

- Acknowledgement of the importance of food safety and awareness of the rising trends in foodborne illness and their implications for both consumers and businesses.
- Understanding the business benefits of HACCP in terms of customer reassurance, competitive advantage and process improvement.
- Seeing HACCP for what it is: a 'minimal' system allowing businesses to understand and focus on the critical safety areas and use resources more effectively.

Once they have committed themselves to HACCP implementation, many businesses have realised the benefits it brings and have retained and improved their HACCP systems in preference to alternative systems. One study of Australian companies showed that, after three years, only half had retained their quality assurance (QA) systems while all had retained their HACCP systems (Sumner, 1999). SMEs in the UK have confirmed the value of HACCP as a more cost-effective alternative to the accreditation process for a quality standard such as ISO 9001 (Route, 2001: p. 33). This enthusiasm for HACCP systems derives from the real, and sometimes unexpected, advantages it can bring. Among these, those with practical experience of implementing HACCP systems have noted the following:

- Increased sales because of assured product quality.
- A reduced level of auditing by customers with increased trust in a business's own systems.
- Improved process control leading to reduced product recall and rework, improved productivity and lower costs.
- Greater confidence in extending the range of products manufactured.
- A more proactive culture throughout the business generally with all staff more committed and more ready to suggest system improvements.

Examples of particular savings noted by individual businesses include over $200 000 a year at the US firm Cargill, and a 47% increase in productivity in one of Hindustan Lever's ice cream plants in India owing, in part, to fewer line shutdowns and less defective product (McAloon, 2001: pp. 77–8; Marthi, 2001: pp. 91–2).

The issue of motivation remains central at all stages of HACCP implementation. As an example, at some point ownership of a HACCP system has to transfer to the line staff appointed as CCP monitors. The system will stand or fail on their commitment and understanding in monitoring and taking corrective action where required. Ways of motivating line staff are discussed later in the chapter. It is important that all senior managers are fully briefed on the importance of HACCP so that their cooperation and support are secured, particularly given the demands that HACCP planning and implementation will have on staff and resources (Killen, 2001: p. 122). As soon as the senior management team has recognised the value of HACCP to the organisation and decided to proceed, this commitment needs to be communicated outwards to other staff. As a first step, the appointment of a senior person as HACCP 'champion' sends the right signal in disseminating a sense of importance and value of HACCP within the organisation. Another step is to integrate HACCP implementation into existing sets of objectives for management, setting HACCP-related goals which are monitored as part of a company's existing system for appraisal of its managers. This might even be reinforced through bonus schemes for good performance in safety management. The US firm Cargill, for example, encouraged awareness campaigns and newsletters at both corporate and plant level to emphasise the importance of food safety to all employees (McAloon, 2001: pp. 63–4). Changes

such as this help to integrate HACCP into an organisation's existing management culture.

8.4 The knowledge required for HACCP

One reason that SMEs in particular give for their caution in approaching HACCP implementation is a lack of knowledge and expertise. Effective HACCP implementation depends on the following kinds of knowledge:

- Understanding of HACCP methodology.
- Knowledge of key hazards.
- Expertise in the organisation's processes and technologies.

While the concept of HACCP is relatively straightforward, a full understanding of HACCP methodology is more demanding. Experience of implementing HACCP systems in practice suggests a number of common areas of misunderstanding:

- A failure to distinguish between prerequisite and HACCP systems.
- Difficulty in separating out safety from other quality issues, and in prioritising hazards for attention.
- Problems in identifying which control points are genuinely critical.

Such basic misunderstanding of HACCP methodology, combined with limited knowledge of relevant hazards and their control, produces inappropriate, overcomplex and unmanageable HACCP systems. Such systems tackle too many 'hazards' (for example spoilage and other quality problems which do not present a hazard to consumers, or pathogens which are not a problem for the particular sector in which the business operates) and are overloaded with control points, only a few of which are CCPs. These inappropriate systems have given rise to the idea among some companies in the food industry that HACCP systems in general are complex, bureaucratic and burdensome.

Lack of expertise in hazard analysis is often one of the most significant problems in effective HACCP implementation. A UK study of SMEs in the ready meals sector, for example, identified that the employment of experienced technically qualified staff was the most important single factor in successful HACCP implementation (Holt, 1999). However, few companies can call on specialised microbiological expertise within the organisation, and a thorough knowledge of microbiological hazards and their methods on control can take years to acquire. Common problems resulting from lack of expertise in this area include the following:

- Difficulty in prioritising risks, for example physical versus microbiological risks.
- An inability to identify which pathogens present a significant risk.
- Identifying which control points are genuinely critical in controlling hazards.

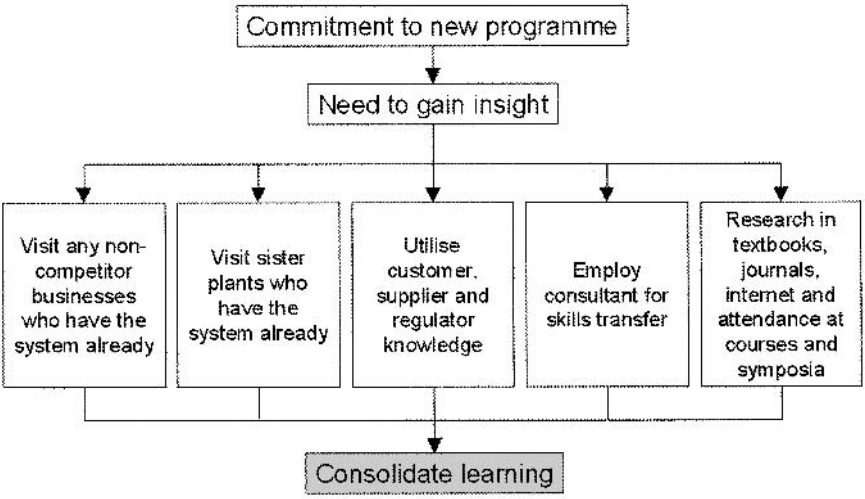

Fig. 8.1 Sources of knowledge and skills transfer.

Ignorance of which hazards are most important and how they should be tackled either produces systems that fail to identify and control key hazards, or over-complex and unwieldy systems that attempt to tackle all possible hazards. In one UK example, a technical manager from an SME initially identified over 140 'critical' control points on a process line, subsequently reduced to 4 CCPs after training (Taylor, 2001: p. 18). Such misconceived systems prove impossible to manage, generating confusion, overload and dilution of control.

Knowledge of its own products, processes and procedures should ideally present few problems for a business. However, even here knowledge may be diffused through various levels of an organisation. In many cases processes have evolved over a number of years and procedures developed piecemeal with no central or formal record. Informal practices often develop, which differ significantly from those standards that do exist.

8.5 Initial training and preparation

There are many ways of finding out about HACCP systems and what they involve. A range of sources of information and advice which can help to get a business started is summarised in Fig. 8.1. Among these, effective training provides a first step in gaining a full understanding of HACCP methodology and can be a particularly worthwhile investment in making HACCP implementation successful. As an example, it can demonstrate the importance of distinguishing between prerequisite and HACCP systems. Recognising the relationship between the two, and building up strong prerequisite systems first as a foundation for HACCP implementation, has been proved to make the latter a simpler

and more successful process. Key training requirements are summarised in Table 8.4. There are a number of factors determining the effectiveness of training, notably:

- The quality of training provision or advice.
- How effectively it is delivered.
- Its relevance to the circumstances of the business.

Although, at present, there is no recognised international body that can certify training providers, there have been various national initiatives, for example in New Zealand and the UK, to establish standards against which to assess training programmes, develop accredited qualifications in HACCP implementation, and recognised training materials (Anon., 1999b; Royal Institute of Public Health and Hygiene, 1995a, b; UK Steering Group on HACCP Training Standards, 1999). These initiatives provide a benchmark against which to assess the quality of the range of training on offer. WHO has developed a standard HACCP training package that is both internationally recognised and designed to be easily translated (Motarjemi and Van Schothorst, 1999). As well as the quality of the provider, various issues affect how effective training delivery is. Some of these are summarised in Table 8.5.

There are various ways of disseminating training through an organisation. The US firm Cargill, for example, set up a Corporate Food Safety Department (CFSD) to build up a central pool of expertise in food safety, initially using external consultants to train staff in key HACCP skills (McAloon, 2001: pp. 64–6). With the help of the CFSD, each Cargill business set up its own business management team which included a CFSD member of staff. These teams were then responsible for individual plant Food Safety Committees (FSCs) which, again, included a CFSD representative and, where possible, the business management team coordinator. CFSD and the business management team coordinator assumed responsibility for training each plant FSC in HACCP methodology. In this way key skills were cascaded from external consultants through the CFSD and business management teams to individual plant teams. This structure also allowed Cargill to set common standards in HACCP implementation across its individual businesses and, through the CFSD, business teams and regular liaison between plants, share knowledge between plant teams. The CFSD also built up a central resource of materials in such areas as training. A similar approach has been taken by Hindustan Lever in India (Marthi, 2001: pp. 87–90).

8.6 Building knowledge and expertise

Training is a crucial first step in building knowledge and skills. Businesses can then use this foundation to use some of the other sources of information outlined earlier. In the case of one UK SME, the HACCP team used existing customer audits to help identify key issues and start HACCP planning, followed by visits to key customer staff for advice on particular issues. In some cases customers

Table 8.4 Possible training objectives for different groups of staff

Group	Training objective
Senior management	1. Understand the general principles of HACCP and how they relate to the food business. 2. Demonstrate an understanding of the training and knowledge requirements for HACCP team members and the workforce as a whole. 3. Demonstrate an understanding of the links between HACCP and other quality management techniques and programmes and how a combined product management system can be developed. 4. Understand the need to plan the HACCP system and develop a practical timetable for HACCP application in the whole operation.
HACCP team leaders	*HACCP system and its management*[a] 1. Demonstrate an up-to-date general knowledge of HACCP. 2. Explain how a HACCP system supports national and international standards, trade and legislative requirements. 3. Describe the nature of prerequisite programmes and their relationship with HACCP. 4. Demonstrate the ability to plan an effective HACCP system. 5. Demonstrate a knowledge of how to lead a HACCP team. 6. Demonstrate an understanding of the practical application of HACCP principles. 7. Demonstrate the ability to design, implement and manage appropriate programmes for verification and maintenance of HACCP systems. 8. Explain the methods to be used for the effective implementation of HACCP. *Additional topics* 1. Demonstrate an understanding of the nature of hazards and how they are manifested in food products/operations and give relevant examples. 2. Demonstrate an understanding of the intrinsic factors governing the safety of product formulations and methods that can be used to assess safety of new products. 3. Carry out the steps to identify significant hazards relevant to the operation and determine effective control measures, i.e. assessment of risk (likelihood of occurrence and severity). 4. Demonstrate an understanding of the training and knowledge requirements for HACCP team members and the workforce as a whole. 5. Develop appropriate training programmes for CCP monitoring personnel. 6. Demonstrate an understanding of the links between HACCP and other quality management techniques and how a combined product management system can be developed.
HACCP team members	*HACCP system*[b] 1. Justify the need for a HACCP system.

Table 8.4 Continued

Group	Training objective
	2. Show how the legal obligations on food business proprietors to analyse food hazards and identify critical steps in the business activities should be met in their appropriate industries. 3. List and explain the importance of the principles of HACCP. 4. Describe the method by which hazard analysis may be carried out and appropriate control measures ascertained to assess the practical problems. 5. Identify CCPs including critical limits to ensure their control. 6. Develop suitable monitoring procedures for critical points and explain the importance of corrective action procedures. 7. Verify the HACCP system by the use of appropriate measures. 8. Carry out the steps to introduce and manage a fully operational HACCP system. *Additional topics* 1. Demonstrate an understanding of the nature of hazards and how they are manifested in food products/operations and give relevant examples. 2. Demonstrate an understanding of the intrinsic factors governing the safety of product formulations and methods that can be used to assess safety of new products. 3. Carry out the steps to identify significant hazards relevant to the operation and determine effective control measures, i.e. assessment of risk (likelihood of occurrence and severity). 4. Develop appropriate training programmes for CCP monitoring personnel.
CCP monitors	1. Understand the general principles of HACCP and how they relate to the food handler's role. 2. Perform CCP monitoring tasks, record results and initiate appropriate actions.
Auditors of HACCP systems	*HACCP and regulatory auditing*[c] 1. Provide up-to-date general knowledge of HACCP and its relationship with national and international standards, trade requirements and legislative requirements. 2. Examine the role of good hygiene practices as a foundation for HACCP-based food safety management systems. 3. Provide a comprehensive revision of the application of HACCP principles for the development of HACCP-based systems for food businesses. 4. Consider the design and management requirements associated with the application and implementation of HACCP-based food safety management systems in food businesses. 5. Enhance the skills required for the assessment of HACCP-based food safety management systems. 6. Consider the tools available to educate food business operators in the principles of HACCP and to provide advice and support during development and implementation of food safety management systems.

Table 8.4 Continued

Group	Training objective
	Additional topics 1. Understand the need for audit preparation including the development of suitable check-lists. 2. Perform HACCP audits using sampling, questioning, observation and assessment skills. 3. Construct audit reports giving clear indication of findings and corrective action needed.
General workforce	1. Understand the general principles of HACCP and how they relate to the food handler's role.

Sources: [a] = UK Steering Group on HACCP Training Standards (1999)
[b] = Royal Institute of Public Health and Hygiene (1995a)
[c] = UK Food Standards Agency (2000)

supplied sample documentation and advice on the emerging HACCP plan (Route, 2001: p. 35). Such cooperation between customers and suppliers, and the readiness of retail and manufacturing customers to support their suppliers, can provide an important source of advice in such areas as:

- Selecting a HACCP team and team leader.
- Constructing process flow charts.
- Information on microbiological hazards.
- Procedures for classifying the severity of hazards and isolating CCPs.
- Methods for auditing a HACCP plan.

However, organisations need to be careful in assessing the information and advice they get from outside sources. A number of those with direct experience of HACCP implementation highlight the limitations in advice provided by inexperienced regulatory and retail audit staff with an ill-informed approach reflecting confusion about HACCP methodology and a business's operations. Some government agencies are themselves relatively inexperienced in HACCP methodology (McEachern *et al*., 2001: pp. 177–8; Mayes and Mortimore, 2001: p. 269). In a number of cases, for example, businesses have been advised to add additional CCPs which subsequently were accepted as not critical (Taylor, 2001: pp. 27–8; Route, 2001: pp. 35–6). In the UK, retailers have recognised that they have sometimes given conflicting advice to businesses supplying more than one retail customer (Kane, 2001: p. 44).

Using external consultants in either training or subsequently in HACCP planning has not always proved successful for SMEs in particular. Problems have included the variable quality of consultant advice, the unfamiliarity of SMEs with the key issues on which advice was needed, and the consultant's unfamiliarity with the business's operations and requirements. A UK study of the use of consultants by SMEs in HACCP development showed that none of the

Table 8.5 Reasons for ineffective training

Reason	Possible solution
Lack of motivation	Ensure that the trainees are briefed prior to undertaking the training. They need to know what their role is in relation to the topic and why they are being sent on the course. When they are on the course it should be made relevant to them.
Lack of understanding of the type of training needed	It is necessary to understand the task to be achieved following the training. For example, if a trainee is charged with leading a HACCP programme then a half-day 'Introduction to HACCP' course will not produce an expert who is up to job. The other aspect here is the learning approach to take. A cognitive approach combined with experiential learning is likely to be appropriate. Operatives are often not used to taking decisions or using their initiative, and a behavioural approach to training would not encourage this.
Training the wrong people or training at the wrong time	A typical mistake would be to train a HACCP team who then do not begin the project until 12 months later.
Lack of follow-up	Reinforcement after training is a well-documented requirement if the learning is to be cemented in the minds of the trainees.
Poor-quality training	This can encompass a lack of understanding on the part of the trainer, a lack of ability to transfer knowledge effectively, a poor scope and quality of training materials and inappropriate learning styles. These will all contribute to a poor outcome. There are advantages in using in-house trainers. As a result of developing in-house trainers you also create HACCP champions and, as a greater level of expertise is required to run a training course, the overall skill base within the company reaches a higher level. As there is no recognised standard for HACCP training certification, when using external trainers it can be difficult to assess the quality of trainer beforehand. Businesses have to choose between the good and the bad – and cost is not necessarily a good indicator.
Coverage – not training enough people	This can be a problem, particularly for SMEs who cannot afford to send many (if any) people on external training courses. The benefit to having in-house trainers is that more people can be trained.

resulting HACCP systems was adequate when independently audited (Holt, 1999).

These problems can be overcome in part by better initial training for SMEs, equipping them to respond more critically to the advice given to them. Where cost is an issue, some governments have made financial support available to subsidise training and have suggested approved providers. This initial training allows companies to do more of the groundwork before approaching a consultant, for example, using consultants to develop and help validate a HACCP plan rather than employing them right from the start. The UK government, for example, has provided a service to SMEs involving initial HACCP training followed by subsidised access to an approved consultant. Other initiatives in the UK have included setting up local HACCP resource centres to provide a range of services including the following:

- Details of suitably qualified HACCP consultants and trainers.
- Sources of funding (often not accessed by SMEs).
- Information sources and advice.
- The opportunity for SMEs to meet to share problems and solutions.
- IT facilities with access to the Internet and HACCP software.

UK retailers and manufacturers have also recognised the importance of establishing a common approach to auditing HACCP systems, moving from independent auditing to the use of accredited third-party auditors and establishing minimum audit standards through, for example, the British Retail Consortium Technical Standard (Dillon and Griffith, 2001: p. 2).

In developing countries, government agencies have provided similar support, particularly for their export industries which are both economically important and face demanding requirements from importing countries and businesses. In India, for example, the Agricultural Products Export Development Authority (APEDA) provides training, guidance and financial support for HACCP implementation. In the fisheries sector, the Central Institute for Fisheries Education (CIFE) provides training materials (in the appropriate language) and support, while the Marine Products Exports Development Agency (MPEDA) advises exporters on the range of international standards they must meet (Marthi, 2001: p. 85). Given the range of international standards, guidance in this area is particularly important for exporting countries (Suwanrangsi, 2001: pp. 192–3). In developing countries such as India there is a particular need to build up more information on microbiological hazards in such countries, given that the majority of such information relates to pathogens within developed countries.

In its report on SMEs and HACCP, the WHO suggests that generic plans may be a useful starting point in HACCP planning (WHO, 1999). The New Zealand and Thai governments, for example, have produced generic HACCP plans for specific sectors of the meat and seafood industries (Lee, 2001: 141; Suwanrangsi, 2001: 194). It is important, however, that businesses take a generic plan only as a starting point in developing a customised HACCP plan for the business. In Thailand, for example, government inspectors assessed the amount of reworking

of generic HACCP models and used this as a measure of business commitment to and understanding of effective HACCP implementation.

In general, while governments have responded in different ways to the needs of their food industries in HACCP implementation, there has been an overall increase in the level of support provided, particularly for SMEs which often lack the resources and expertise to develop a HACCP system on their own. The New Zealand government, for example, has developed generic HACCP plans as a starting point for businesses in particular sectors, information on microbiological and other hazards to support businesses in HACCP planning, and, in collaboration with industry, HACCP competency qualifications for both industry and regulatory personnel (Anon., 1997, 1999a, b). Similar developments have been noted in the UK and Thailand. The WHO (1996) has recommended that national governments develop national food safety strategies which identify key food safety issues and then develop a programme including the following:

- Strengthening food safety legislation and enforcement.
- Promoting industry food safety management systems such as HACCP.
- Providing education and advice in food safety.
- Developing resources such as microbiological data.

However, the effective use of the support and advice available to an organisation will always depend on the organisation taking responsibility for developing its own HACCP system and adopting a critical approach. An essential element in this process is the quality of the HACCP team and team leader, reinforcing the importance of initial training and the motivation to make HACCP implementation a central activity for the organisation. An important starting point is the development of process flow diagrams, an area in which a business will have the most expertise. This will provide a sound foundation for moving on to hazard and CCP analysis.

8.7 Resources and planning

The time and material resources required for HACCP implementation depend on how well a business has been run before HACCP implementation, its size and the complexity of its products and processes. It has been estimated that a well-run business will already meet 80–90% of HACCP requirements (McAloon, 2001: p. 68). In planning effectively, businesses often underestimate the time and resources required in two areas in particular:

- Getting PRPs right can often be the most costly and time-consuming part of the process, particularly for businesses in developing countries. Even in the case of larger manufacturers in the US and Europe, improvements to PRPs can take six months or more.
- The amount of training required, whether in building up basic hygiene skills for PRPs, HACCP implementation, or in maintaining the HACCP system in the long term, is often underestimated.

Setting clear objectives and priorities is a critical first step. In the case of the US company Cargill, for example, the company undertook a risk assessment to identify where the greatest potential risks to food safety were located. Other larger manufacturers have adopted the same approach, targeting immediate food safety problems identified through a baseline audit and then concentrating on high-risk products (Rudge, 2001: 101–2). Cargill focused first on training and supporting their priority plants in HACCP implementation (in meat, poultry and eggs where risks were greatest). They allowed a year to complete implementation within these plants, and two to three years for implementation within other lower-risk sectors of the business (McAloon, 2001: p. 65). This timetable compares to 18 months for implementation across the various plants at Hindustan Lever in India (Marthi, 2001: p. 87).

Cargill also decided to introduce HACCP systems within one plant at a time rather than simultaneously, despite the extra work and time involved. This more cautious approach proved effective in ensuring HACCP plans were tailored closely to the requirements of each plant, were fully 'owned' by each plant team and benefited from cumulative experience as the implementation programme moved from plant to plant. The same approach was used at Hindustan Lever where one factory was chosen for pilot implementation, based on risk assessment, size of operations and level of preparedness.

The approach at one of Heinz's largest facilities in Europe was to concentrate on one production line at a time because this did not overstretch their resources and gave them the benefit of cumulative experience and expertise as they moved from line to line (Killen, 2001: p. 123). Product-specific issues were then tackled within the analysis of each process step. The complete HACCP programme for all 12 production lines took three years in total, including completion of longer-term changes such as new equipment installation as part of improving PRPs. Another large European manufacturer, Kerry, adopted a different approach, looking at products individually in one of its Polish subsidiaries in hazard identification, using HACCP plans developed in sister plants, and then at common processes for these products in identifying CCPs (Rudge, 2001: pp. 105 and 107).

In the case of SMEs, it has been suggested that a developmental approach is best, concentrating in turn on the following:

- The installation of sound PRPs.
- HACCP studies to identify specific areas that need additional control, followed by development of CCP control measures and monitoring regimes.
- Finally, developing appropriate systems of verification and review.

Such a process might need to be phased in over a period of up to two years (Taylor, 2001: p. 29). This approach has also been recommended for businesses in developing countries. The examples above clearly indicate that it is possible to implement HACCP successfully using more than one approach. The approach selected must be that which is the best fit for the business circumstances. Whichever route is chosen, it is essential to plan it thoroughly with clear priorities and objectives, and to learn the lessons of experience.

8.8 Prerequisite programmes (PRPs)

The term prerequisite programme (PRPs), covers the general (i.e. non-product specific) policies, practices and procedures that control the operational conditions within a food establishment allowing conditions favourable for the production of safe food. PRPs cover a range of general measures designed to promote food safety, including but not limited to:

- Hygiene training, equipment and procedures for personnel.
- Cleaning and disinfection procedures for plant and equipment.
- Pest control.
- Water and air control.
- Waste management.
- Equipment maintenance.
- Raw materials purchasing/supplier quality assurance.
- Transportation.
- Product rework and recall procedures.
- Labelling and traceability systems.

Although definitions differ, the concept of Prerequisite programmes does not differ significantly from what may be termed Good Manufacturing Practice (GMP) or Good Hygienic Practice (GHP). In effect the concept refers to a base level of practice and procedure necessary for the production of safe food. The most successful implementation of HACCP is done within an environment of well-managed prerequistie programmes (Codex, 1997a, 1997b). Whilst it is in theory possible for an establishment to develop PRPs and HACCP at the same time, this is not the preferred option. In practice however many businesses may need to work on the PRP systems and HACCP development at the same time. If so, it can be helpful to set up separate project teams, both of which report to the HACCP team leader.

The vast majority of experienced HACCP practitioners will state that HACCP must be built on a sound base of effective PRP implementation and this is certainly the view of the authors. The need to assess quality of PRP within business through a base-line audit is recommended by Mortimore and Wallace (1998) as well as by others with practical experience of HACCP implementation. The US Food and Drug Administration (FDA), for example, has issued detailed guidance which sets benchmarks for building effective PRPs and how HACCP controls can then be built on the foundation they provide (Taylor, 2001: 22).

In many cases PRPs will already be in place when establishments begin to introduce HACCP, but may just not be termed as such or standardised. They may often have been built up piecemeal in response, for example, to customer requests or changing legislative requirements. Once sound PRPs are in place most businesses realize that they need a relatively simply HACCP system with few CCPs. Although GMPs/GHPs cannot substitute for a CCP, collectively they can minimise the potential for hazards to occur, thus eliminating the need for a CCP. The implementation of effective PRPs will control ‘general’ or ‘establishment’

hazards that would otherwise have to be controlled by a CCP. This will leave the HACCP Plan, and inherent CCPs, to concentrate on the control of product/process specific hazards and thereby ensure that the HACCP Plan contains only those CCPs that are essential to the safety of the product. Failure to have PRPs in place will inevitably lead to a large number of CCPs in the HACCP Plan covering both 'general/establishment' hazards and product specific ones.

8.9 HACCP teams

The quality of HACCP analysis depends critically on effective training, and the composition and leadership of the HACCP team. HACCP teams need to be multifunctional. Those with experience of managing such teams suggest keeping them to a maximum of five or six, to ensure a sufficient pool of expertise while preventing teams from becoming unwieldy, and keeping meetings to no more than 2–3 hours (Killen, 2001: pp. 124 and 127). It may be sensible to co-opt occasional team members to contribute at particular points where they have specific expertise. Co-opting staff may also be a way of using particularly busy members of the company who might not be free to attend all HACCP meetings. Preparation is key, for example in having line layout charts prepared in advance and in team leaders making their own preliminary analysis of key issues so as to be able to anticipate issues within meetings. In the case of SMEs, it may not be possible to set up a formal multi-disciplinary team but alternative routes to ensure that the right mix of skills and knowledge can be taken. Some SMEs enlist the help of local regulators, customers or suppliers in order to extend the resources available to them.

An essential element in making HACCP teams effective is involving operational staff and motivating them to contribute, particularly in putting together process flow diagrams in the early stages of HACCP planning (McAloon, 2001: p. 68). Their early involvement has a number of advantages:

- They know the relevant processes best.
- They are closest to how systems really work rather than how they may look on paper.
- They can become HACCP champions on shop floor.
- They can help translate HACCP plan into systems and documentation their colleagues can use easily and effectively.

It is possible to secure their motivation by reinforcing their key role in the safety of products for the consumer. To be most effective, it is best to involve line staff where they have the most to contribute, particularly in describing processes and in designing CCP procedures and documentation, rather than in more unfamiliar areas such as hazard analysis.

In this respect, SMEs may be particularly well suited to HACCP teamwork. The relatively small number of employees makes it easier for the organisation to

be well represented, enabling greater ownership to develop. It is also likely that many managers have worked their way up from the factory floor (and may continue to work there when production demands are high). This first-hand knowledge of operational procedures and empathy for the workforce speeds up the work of constructing a process flow diagram and CCP identification. The subsequent control strategies are more likely to be both practical and effective.

8.10 Hazard analysis

Hazard analysis has been identified as one of the most demanding tasks for a HACCP team. The approach at Cargill was to break down hazards into one of four areas:

- Hazards controlled or minimised by CCPs.
- Hazards controlled or minimised by GMPs.
- Hazards considered to be of such low risk that no control is needed.
- Hazards that can be designed out of the process.

This process significantly reduced the number of hazards that needed to be included in the HACCP study. The last option is not always considered but can be the best way of eliminating a hazard. HACCP practitioners suggest that it is often best to start with physical hazards as the easiest kind of hazard to understand and manage (Rudge, 2001: 111). However, in some respects this is not so easy as debates rage, for example, about what size and shape of metal constitutes a significant food safety hazard? The most common problem in hazard analysis identified by practitioners in both the larger manufacturing and SME sectors was the tendency to confuse quality (e.g. spoilage) and safety issues (Mortimore and Mayes, 2001: 255). This problem can be resolved by setting clear objectives at the start of the HACCP programme during the 'terms of reference' stage (see Table 5.3) where clarification of two key issues should be sought:

- is the HACCP study going to cover just product safety hazards, or also quality issues?
- what group of hazards will be considered (e.g. microbiological and/or physical and/or chemical hazards for safety studies).

Even when the above questions have been answered study teams still have difficulties sometimes selecting which specific hazards within one of the groups above should be considered during the study. As an example, which microorganisms represent a significant hazard to the product/process? In this case, and in many others, a broad representation of skills and expertise in the study team will help in decision making. Where such skills are lacking internally, the study team can co-opt outside experts to help, or use one of the many published sources of information on significant hazards that can be found on the internet. There are three important rules to follow in hazard analysis:

- Always be sure of the terms of reference for the study.
- Ensure that the team selects all the significant hazards relevant to the study.
- Start simply – do not try to cover all hazard groups at once unless the team is already experienced.

8.11 HACCP implementation

In larger plants it may be necessary to employ someone full-time on HACCP implementation: experience suggests this can halve the time required for implementation (McAloon, 2001: p. 69). However, the most critical element is securing the commitment of supervisors and operatives. A HACCP system requires CCP monitors to be proactive and show initiative in spotting if a critical limit has been exceeded and taking the appropriate corrective action. In many cases this does not just involve learning new skills but assuming a new level of responsibility in taking important decisions for themselves. Persuading operational staff to take on this role can be difficult with a suspicion of management, a 'seen it all before' attitude and a conservatism about adopting new work practices. Such resistance may be compounded by a prescriptive management culture which restricts the independence of more junior staff. It is essential that these issues be resolved because failures by CCP monitors to understand and perform their duties is one of the biggest common weaknesses in HACCP implementation (Mortimore and Mayes, 2001: pp. 256–7). SMEs with less formal management structures and simpler channels of communication are often at an advantage in motivating line staff.

As has been noted, these problems can be resolved by active involvement of supervisors and line staff at an early stage in working in HACCP teams, writing or, at least reviewing, the HACCP plan and assisting in the design of HACCP documentation. This involvement means that the HACCP plan has the benefit of their knowledge of actual operations on the production line, and that the system takes account of their needs. Line staff are more likely to 'own' the system, to embrace the new responsibilities a HACCP system brings and to champion it among colleagues.

Effective training of line staff is also critical to their success as CCP monitors. Such training will be successful if it addresses not just what staff need to do but why. Explaining the implications of a failure in food safety for consumers and what role employees play in preventing such failures is a proven way of gaining employee commitment. It is then important to balance the information on hazards and CCPs so that operators know enough without being swamped by unnecessary detail. Involving them in the design of documentation and procedures will help to achieve this balance. It may also be necessary to review operator knowledge of and skills in operating processes and conduct refresher training.

One of the main criticisms levelled at HACCP systems by SMEs in particular is the requirement for documentation. However, research suggests that

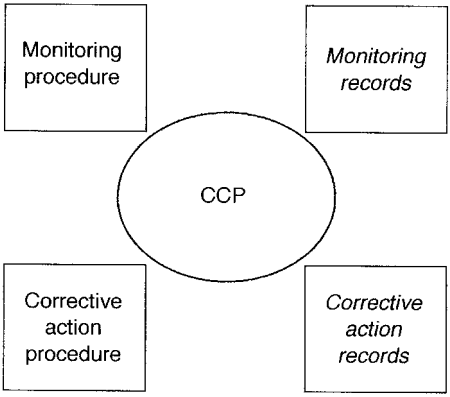

Fig. 8.2 The essential documentation required for each CCP.

excessive documentation is usually associated with poor design of HACCP systems (Taylor, 2000). If properly designed, a HACCP system usually has relatively few CCPs which require a basic system of record-keeping (Fig. 8.2). The US firm Cargill developed a flexible approach to document design, setting an overall standard specifying basic content as a framework for staff to develop their own documentation.

The Irish firm Kerry reviewed all its existing documentation and consolidated it into a single system. As an example, internal non-conformity and finished product reject documents from the business's existing quality system were combined with the corrective action documentation required by the HACCP system to produce a single non-conformity report. The design of the report was made as simple as possible so that it could be readily understood and completed. The company also computerised the record-keeping system to improve its efficiency and flexibility (Rudge, 2001: 115).

8.12 Maintenance

The failure to verify that a HACCP system is working as planned is another commonly reported cause of HACCP systems failure. Effective and regular company audit procedures are the best solution. Such audits are best focused on CCP monitoring and documentation to ensure that staff genuinely understood their responsibilities and that CCPs are properly monitored (McAloon, 2001: pp. 74–5; Killen, 2001: pp. 130–1). The benefit of such regular verification activity in these companies was that HACCP system efficiency not only improved but that staff were prompted to start suggesting improvements of their own to make HACCP documentation, for example, easier to manage. Internal auditors require a high degree of expertise and experience, and should, if possible, be drawn from those

with direct experience of production operations. The HACCP audit must be carried out at regular intervals, (typically not longer than 12 months), and cover the entire HACCP system. Many companies will select to audit part of the system on a monthly basis, reporting findings to the HACCP team. Internal audits also help companies to prepare effectively for, and make best use of external audits, whether from ogvernment agencies, 3rd party certification bodies, or from customer audit staff.

Audits form only part of verification activities. Review of CCP records is vitally important and is usually linked to a positive release programme, i.e. review of records by a trained HACCP reviewer prior to release of product. Microbiological testing can also be useful as part of a verification programme, i.e. to confirm that the product meets its microbiological specification limits. Consumer complaint data will also provide information, particularly with regard to physical hazard control. Some companies will set up a HACCP Maintenance Team to manage and mutually review the activities which form the HACCP verification programme. Validation, which is often referred to as part of verification, but is in reality an exercise to determine that the HACCP Plan is technically and scientifically sound, should be carried out prior to HACCP Plan implementation, and following receipt of new information (e.g. emerging hazards) that affect the HACCP Plan (ILSI, 1999). If the HACCP Plan is not valid (i.e. not technically correct) then the whole plan is suspect.

It is also important to have an ongoing training programme to maintain awareness of food safety and HACCP skills. Food safety needs to be made an integral part both of new employee training and regular training programmes or briefing sessions for existing staff. Regular monthly training sessions of 15–20 minutes can be effective, as can simple reward systems to recognise good food safety management. At Heinz in the UK, employees are issued with training 'passports' that record all the training they have undergone. Employees are independently checked on their understanding after training to verify its effectiveness (Killen, 2001: pp. 131–2). Experience at large firms such as Cargill suggests the value of having back-up staff to cover the absence of key staff together with standard procedures such as checklists that can be used by a colleague when a member of staff is on holiday or sick leave (McAloon, 2001: p. 76).

8.13 HACCP and globalised production

As production has become more global, HACCP systems have been adopted in most countries involved in international trade in food products. However, many of the published materials, and most knowledgeable experts and longest-established HACCP systems are from developed countries such as the United States, Australia, New Zealand and those in Europe. There can be significant problems in transferring this expertise to other countries where translated materials do not exist and HACCP experts are not fluent in the local language. Practical solutions to such hurdles include the following:

- Using a bilingual expert in HACCP methodology to translate materials rather than a non-expert translator who may well produce misleading and confusing documentation. Where possible, use someone from the company, though it is best to avoid using staff from marketing or sales (who are most likely to have language skills), given their lack of subject expertise.
- Training a bilingual member of staff to help in teaching, which is likely to be more successful than using a third-party expert to train via an interpreter.
- Basing initial discussion around translated versions of the process flow diagrams. Local personnel will be able to relate to these much more easily.
- Allowing extra time to ensure that key issues are properly understood, particularly if discussions have to proceed via a translator. Where possible, try to check understanding independently of the translator.

There can also be significant cultural differences to negotiate. In some cultures, an overtly critical and challenging approach to problem-solving is considered rude. In one example, validation and verification procedures were initially viewed with suspicion by employees within a Chinese business since they appeared to question their competence (Route, 2001: p. 40). Such procedures had to be explained in terms that recognised these sensibilities. In some business cultures there are stronger traditions of hierarchy and paternalism with more junior staff expected, and expecting, to pass problems and decisions to their superiors. Those responsible for working with partner companies with such cultures need to be able to negotiate these differences. One UK manager advising a Chinese supplier on HACCP implementation, for example, won the support of senior staff by complimenting them on the quality of their staff in implementing an important new system for the company, and the support of staff by emphasising the trust senior management had in their abilities to carry out this major project on their behalf (Route, 2001: p. 39).

8.14 Future trends

Those involved in implementing systems in practice, and with auditing them, have identified a number of common problem areas in effective implementation, notably:

- Poor initial motivation and commitment.
- Ignorance of HACCP methodology.
- Lack of relevant technical expertise.
- A failure to define the scope of and plan effectively for HACCP implementation, notably in relationship to improvements to PRPs.
- Failures in effective CCP monitoring.
- A failure to audit and maintain the HACCP system.

As this chapter has shown, there are a range of practical solutions to all these problems open to businesses, whether they are large companies or SMEs, in

developed or developing countries. Indeed, SMEs, for example, can be at an advantage over larger companies in some aspects of HACCP implementation. It is also clear that where it is well implemented, the HACCP approach can make a significant difference to food safety. In the United States, for example, where HACCP systems became mandatory for larger meat and poultry plants by 1998 and for smaller plants by 2001, contamination levels in chicken, turkey and beef fell initially by as much as 45% in some cases (Crawford, 2000: p. 30). Where food safety incidents have occurred, it has been possible to trace them to specific failures to follow correct procedures within PRPs, for example. It is also evident, even in situations where HACCP implementation has been mandated, that effective implementation rates can be disappointingly low (Losikoff, 2000). It is clear that just mandating HACCP will not be sufficient on its own to reduce the number of cases of food poisoning – effective support, training in use and monitoring of performance will be necessary. The USA has taken the lead in the use of HACCP as a regulatory tool but many other countries (e.g. Canada, Australia/New Zealand, EU countries and others), now have HACCP as a part of their regulatory process. Not all countries intend to use HACCP in the same way as the USA has piloted, and that is quite appropriate, since the maturity of the HACCP legislation must reflect the maturity of the HACCP process.

HACCP needs to be seen as one essential element in effective food safety management. A typical criticism of HACCP is that it is not outcome oriented and that, as a result, it is difficult to measure its benefits (Orris and Whitehead, 2000). It has been suggested that HACCP systems should be linked to Food Safety Objectives based on risk assessment exercises which set specific targets or appropriate levels of protection (ALOPs) for target groups of consumers (Mayes and Mortimore, 2001: pp. 271–2). Although controversial, this development may help to give HACCP systems a clearer focus in the future.

It is also increasingly clear that, from its origins in manufacturing, HACCP needs to be applied across the entire food chain from 'farm to fork'. HACCP principles are applicable to all sectors of the food supply chain, though it is important to be flexible in how they are applied. In farm and retail settings, for example, CCPs may be difficult to identify and implement, and more emphasis needs to be given to sound PRPs in controlling hazards (Johnston, 2000). In this respect, the relationship between PRPs and HACCP systems needs greater clarification (ILSI, 1998).

In conclusion, what are the critical success factors for effective HACCP implementation?

- *Preparation Stage:*
  - Real management commitment
  - Knowledge and expertise:
    i) Hazard analysis
    ii) Pre-requisite programmes
    iii) The relationship between i) and ii)

- *Maintenance Stage*
  - Verification activities
  - Awareness of emerging hazards
  - Continued training
  - Keeping the momentum going

HACCP is a fantastic tool for food processors but only if used effectively. If it is implemented reluctantly because of regulatory or customer pressure, and in the absence of a real desire for self improvement, then it will probably add no real value to the business and will fail to ensure safe food. Used properly it can help to focus businesses in many, if not all, of its quality control programmes, from supplier quality assurance (SQA) to finished goods inspection. It can make a substantial contribution to efficient and effective food control.

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9

Good practices for food handlers and consumers

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9.1 Introduction

9.1.1 Food safety, industry and the consumer

Ensuring food safety is the responsibility of all links in the food chain, including consumers, and important others, such as the government (via legislation, health services, educational system) as well as the food and media industries. Their respective roles are summarised in Fig. 9.1.

The role of government includes education, health service provision, epidemiological and other research as well as legislation. The latter provides the correct framework or context in which all others will act. Increasingly, for political or other reasons, governments worldwide are forming units such as the UK Food Standards Agency, which can be seen as independent bodies to manage a government's food safety role. The European Commission has issued proposals for the formation of an independent European food authority in an attempt to ensure high food safety standards across the Community.[1] The work of this authority would include enforcement and legislation as well as the formation of a scientific reference point. Perhaps given the economic importance of food and recent political and international disagreements relating to food and trade it is inevitable that a legislative food framework will be debated and agreed at a supranational level.

Industry includes all links in the food chain from primary production through retailing to food service. Industry's role is to produce, market and label food of the best microbiological quality possible within moral, technical and financial constraints, although currently nutritional labelling has a higher profile than food safety labelling. Production of high-quality food requires a trained workforce and a food safety management system, implemented within an appropriate food safety

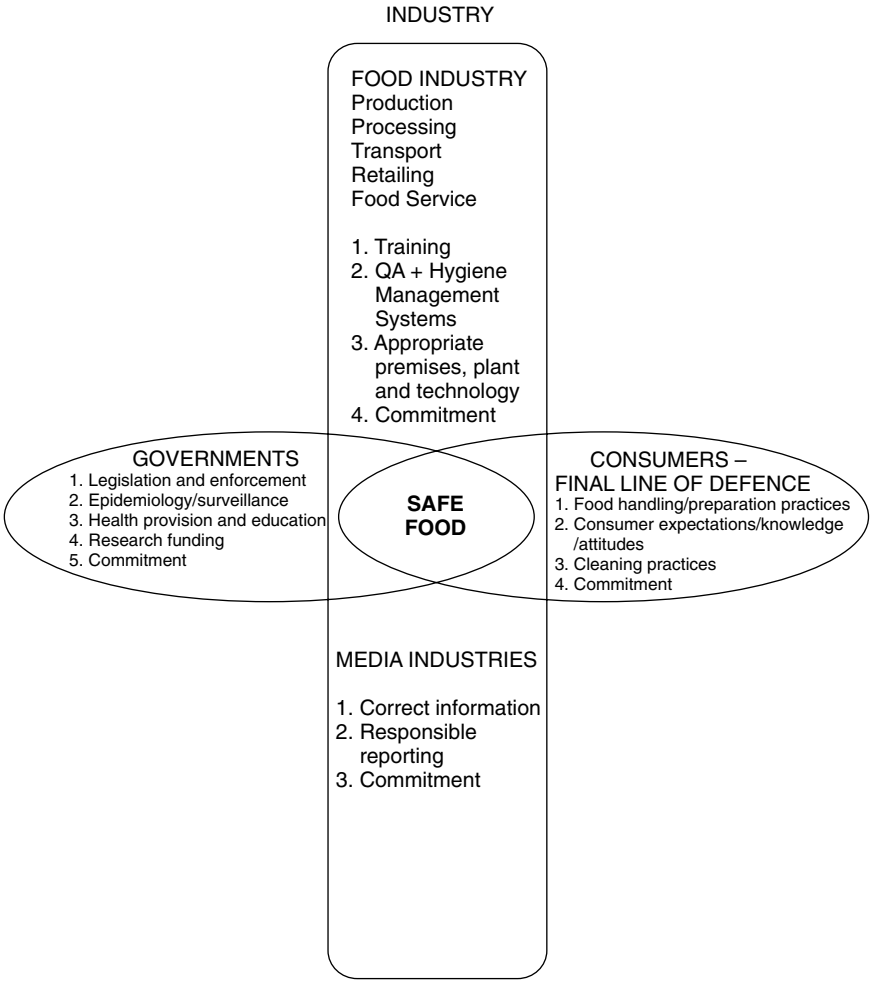


Fig. 9.1 Shared responsibility for food safety.

culture. It may be that industry also has a greater educational role to include more nutritional and food safety advice on labels and packaging. The media industries can also play an important role although one that perhaps they may not even recognise, let alone implement appropriately.

Elements of the mass media may argue their main role is to entertain, not to educate or inform, yet they should provide adequate, accurate food safety information in an unbiased way. It has been stated that the news media are an effective food safety watchdog but are guilty of exaggerating reports on food safety concerns.[2]

Consumers also have a responsibility. They may hope or anticipate that the food reaching them will be pathogen-free, but their responsibility is to handle

Table 9.1 The home as a location for food poisoning outbreaks

	1992	1993	1994	1995	1996
Ratio of general to family outbreaks	1 : 6.7	1 : 7.1	1 : 7.0	1 : 5.6	1 : 6.0
Percentage of general outbreaks of food poisoning occurring in the home	19%	14%	13%	11%	13%

Source: Food Hygiene '98 Conference.[6]

food as if it *were* contaminated. Consumers along with the food service industry and some food retailers represent the final line of defence, i.e. food passes from them directly for consumption and thus their actions are paramount to food safety. It is possible for previously uncontaminated food to be contaminated, with a variety of pathogens, at the point of final handling. For safe food, and thus safe eating, to be achieved all with a responsibility must have a commitment to safe food and this forms part of the prevailing food safety culture.

9.1.2 Epidemiology of foodborne diseases – 'eating out and eating in'

People must eat to survive yet they should not be ill as a consequence. Data concerning the incidence of foodborne illness are usually incomplete but suggest that it is a major problem in many countries. A recent UK study projected one in five members of the population in England and Wales would suffer infectious intestinal disease each year.[3] Previous data suggest that in general UK outbreaks of infectious intestinal disease, 20–40% are mainly foodborne.[4] Other US studies indicate a similar general figure, with variation depending upon pathogens involved.[5] If this is true it would indicate a significant morbidity due to food-transmitted illnesses. In broad terms the food eaten can be considered to be prepared either inside or outside the home. While large outbreaks that inevitably occur outside the home capture headlines, most cases of foodborne illnesses are sporadic, with food prepared in the home being an important vehicle of illness.[6]

It is difficult to determine accurately the number of cases occurring in a domestic setting; however, the probability is that the home is the single most important location for acquiring food poisoning. Table 9.1 indicates a UK ratio of general to family outbreaks of approximately 1 : 6. Although it cannot be assumed that all family outbreaks are acquired in the home, it is likely that the majority are. Furthermore, approximately 12% of all general outbreaks have their origin in a domestic setting and this seems to be more or less typical of other developed countries.[7–9] The data relate to notifications and while there are likely to be more cases reported per general outbreak than per family outbreak it is probable that the large majority of unreported cases occur in the home. Collectively this type of data suggest the home is likely to be a major location for the acquisition of

Table 9.2 Risk factors implicated by various studies in outbreaks of food poisoning

Contributory factor	US data 1995 (%)	England and Wales data 1995 (%)	England and Wales data 1996 (%)	US data 1994 (%)	England and Wales data 1998 (%)	England and Wales data 1998 (%)
Inappropriate storage	21.1	38.5	24.4	23.9	45	45
Preparation of food in advance	22.6	57.1	–	9.9	–	–
Inadequate heating	15.5	15.8	23.3	20.0	50	–
Inadequate re-heating	10.6	26.4	–	8.5	–	40
Inadequate hot-holding	16.6	–	–	17.3	–	–
Cross-contamination	5.4	6.4	22.0	8.9	39	36
Infected food handlers					12	21

– not reported
Source: adapted from Coleman and Griffith,[17] with additional information from Evans *et al.*[4]

food poisoning and highlights the need for a greater understanding of consumer food-handling behaviour and a need to educate the consumer. This need is supported by various studies of consumer knowledge, attitudes and practices.[9–14] When food poisoning does occur outside the home it is most likely to be associated with a food service establishment.[15] It has been estimated that in the UK 1 in 1500 food service establishments gives rise to a notified case of food poisoning every year,[16] although this is likely to be an underestimate.

The understanding of risk and the potential for causing illness is poorly understood both within food service establishments and by consumers (see Section 9.5.2).

9.1.3 Risk factors and errors associated with foodborne illnesses

Surveillance authorities around the world have attempted to identify factors contributing to outbreaks of foodborne illness. Unfortunately different methodologies are used to collect the data, with different approaches and categories used for presenting or grouping the results (see Table 9.2).[17] The rationale behind this approach is an attempt to understand and thus manage foodborne illness based upon previous malpractices and levels of risk. There is likely to be error, with particular malpractices especially cross contamination underreported, nevertheless this approach is useful, as far as it goes. An implicated malpractice, e.g. cross-contamination, can be owing to a number of different reasons, e.g. deliberate human action to ignore or break the rules (human error), lack of an identification of cross-contamination as a hazard coupled with lack of appropriate corrective action (system error) or failure to disinfect adequately owing to equipment/chemical factors (equipment error). A case of food poisoning represents failure

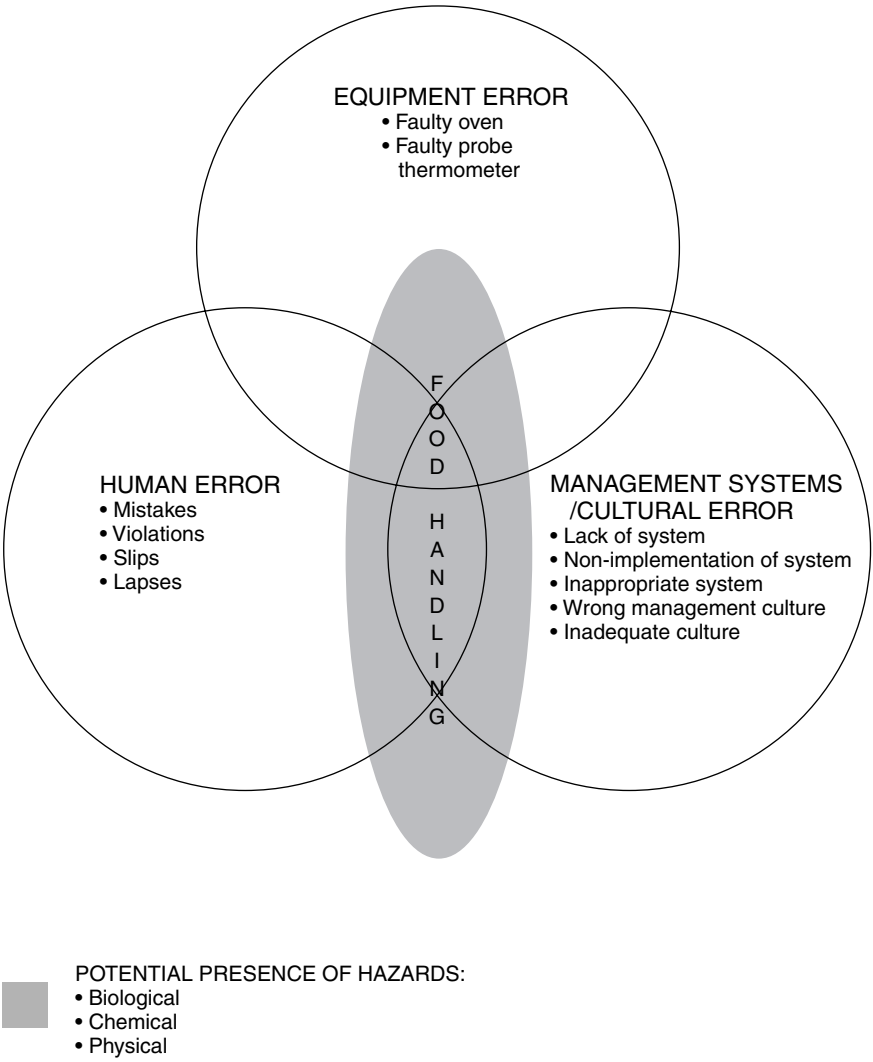


Fig. 9.2 Generalised scheme of error categories. Food handling with one or more errors, in the presence of hazards, can result in food poisoning.

and a generalised scheme of error categories which can, in the presence of hazards, result in illness is illustrated in Fig. 9.2.

It is suggested that human error is a factor in 97% of general outbreaks of food poisoning.[18] However, caution is needed. Although often perceived as separate, there is often considerable overlap between each of the categories. It may be difficult to allocate failure, in an outbreak, to a single error category and it will often be a combination of two or more.

This is illustrated by an outbreak of hepatitis A in a Washington subway sandwich store. An investigation revealed that one or more of the employees was infected with the hepatitis virus at the time of the outbreak. Upon initial examination it could be recorded as human operator error. However, subsequent action was taken, stating that 'the store operators were negligent in their selection, training and supervision of their employees and in failing to adequately instruct and supervise them and provide them with the necessary health and safety practices to prevent contamination of food with the virus'. This suggests the error was a management/systems error. Another illustration from the UK demonstrates this difficulty. It involved a chain of public houses/restaurants in which poor food-handling practices were publicised by a journalist before food poisoning occurred. Trained staff were being persuaded to deliberately implement poor food safety practices, using food past its shelf-life and recoding the use-by date on food, by a unit manager for economic reasons.

Superficially this could appear as human (food handler) error but in reality it was a management error at unit level, leading to the establishment of an inappropriate food safety culture. Relatively few outbreaks involve equipment failure, and the use of this type of generic approach would suggest that more information is needed about the human/cultural/systems/ management intermix of factors in food preparation and handling.

9.1.4 Aim of the chapter

Handling food at any stage in the food chain requires care and commitment but is especially important for foods that will receive no further processing likely to remove pathogens, prior to consumption. Investigations of outbreaks suggest that human and systems/cultural errors are of particular importance and the aim of this chapter is to inform the reader about factors influencing these errors, especially those close to the point of consumption.

9.2 Food safety management in manufacturing: HACCP and GMP

Food safety legislation around the world is being updated in an attempt to minimise foodborne illness. A key feature of the newer legislation is the incorporation of a risk-based approach to management requiring the identification and control of steps that are critical to food safety as well as, if necessary, the ability to demonstrate 'due diligence'. To fulfil the latter, an appropriate food safety management system is required, plus evidence that it is being effectively implemented. Failure to comply with legislation, which in the UK applies to all food businesses, can result in fines, closure or even jail.

All food businesses should therefore have a formal or informal (more likely in small companies) food safety management system. This can be defined as the

organisational structures, responsibilities, practices, procedures and appropriate resources to produce food safely.

Newer legislation focuses on hazard identification and risk management that requires the adoption of Hazard Analysis Critical Control Point (HACCP) principles. HACCP is a food safety management system that can be applied to even the smallest food operation;[19] however, it cannot be used in a vacuum and should be implemented in conjunction with a range of prerequisite programmes (PRPs). These are part of good manufacturing practice or GMP and cover the fundamental principles, procedures, running, design and construction of an environment for the production of food of acceptable quality. The good hygiene and manufacturing processes of a company form the foundation upon which HACCP plans can be built and provide the framework within which a company can develop a positive food safety culture. Organisational culture is an elusive concept that can be defined in various ways: most simply it can be described as 'the way we do things around here' and can help to establish subjective norms (see Section 9.5.1 and Fig. 9.4).

9.3 Safety management in the food service sector: GCP, ASC and SAFE

HACCP was originally designed for manufacturing environments where it has generally been implemented, although its application to all food operations has been advocated, it is less well implemented in non-manufacturing businesses.[20] The reasons for this are summarised in Table 9.3.

Because of the difficulties in introducing HACCP into smaller, less-developed food service businesses, adaptations of the HACCP approach in the form of a more 'user-friendly' format have been attempted. This has led to the development of Assured Safe Catering (ASC), Systematic Assessment of the Food Environment (SAFE) and generic HACCP approaches.[20]

Strategies designed to help smaller, less-developed businesses introduce HACCP have been reviewed[19] and include the development of sector-specific industry guides. These have been developed in a number of countries. The UK version[21] assists food businesses to comply with the legal requirement to analyse and control potential food hazards, as well as providing advice on training and good catering practice (GCP). GCP is analogous to GMP and deals with the effective management of food safety and quality in catering/food service businesses.

9.4 Domestic food preparation: GDKP

The domestic kitchen, unless used for business, is exempt from legislation. Hence there is no requirement for HACCP although its application has been recommended.[22] Implementing good domestic kitchen practice (GDKP) is likely

Table 9.3 Factors that could make it more difficult to implement HACCP in food service operations

Food manufacturers	Food service
Restricted range of food items	Wide range of food items
Better technical support	Less technical support (none in some cases)
Dedicated design and construction	'Ad hoc' use of premises Poorer design and construction
Production in relation to scheduling	Production in peaks and troughs to meet demand
Produce only	Produce and serve
Better trained labour force	Poorer trained, high turnover, often part-time labour force
Better facilities and equipment	Poorer facilities and equipment
Fewer, usually larger, businesses	Many more businesses usually much smaller
Greater business demand for HACCP, e.g. from retailers	Less business demand

to be the consumer's approach to food safety/hygiene and, being dependent upon the individual food handler (consumer), is extremely variable and likely to be informal.

The role of the consumer in food safety, ignored for many years, is currently the subject of investigation and research.[10–14] In general terms studies from around the world have indicated standards of GDKP are a cause for concern, with failure to implement even basic hygiene practices. This could be because consumers are unaware of all the practices that are needed to handle food safely or how to clean properly. There is evidence that domestic kitchens are more difficult to clean than their commercial equivalents.[23] They may be subject to contamination from non-food sources, e.g. pets, and may be used for a variety of non-food handling activities, e.g. gardening, motor maintenance.[24] It is not surprising that many food pathogens have been isolated from a range of kitchen sites[20] and the kitchen is thought to be more heavily contaminated than bathrooms and toilets.[25]

The problem is compounded by the fact that consumers often do not implement the food hygiene practices that they know. Further work is required to determine why this is so: some evidence suggests that people find implementation of hygiene practices too much trouble or too time-consuming.

The importance of the foodborne illness from in-home preparation has now been realised and a number of countries have, or are planning, food safety initiatives. These should raise the general awareness and importance of GDKP (food

hygiene) and should present the information within a framework based on hazard, risk and specific control measures.

9.5 Understanding food handlers

It has been suggested that poor food-handling practices contribute to 97% of food-borne illnesses in food service establishments and the home.[18] If this is correct, food-handling behaviour is the single most important factor affecting the control of food hazards and in managing risk. Although work on understanding aspects of human behaviour has been undertaken by psychologists there is very little information on why or how people implement food safety practices. Also lacking are data that link individual food handler behaviour to a company's food safety culture. A better understanding of both of these could help to facilitate correct food handling and identify appropriate behavioural change.

The need to understand the human aspects of food handling with the involvement of behavioural scientists has been recognised.[26] In one recent study of food handlers' beliefs, 62% of food handlers admitted to sometimes not carrying out all food safety behaviours on every appropriate occasion, with 6% admitting that they often did not.[27] Lack of time was the most quoted reason for failure to implement relevant practices. This could, in food service operations, be related to unreasonable demands made on food handlers by their employers.

Possible factors influencing human food safety behaviour are summarised in Fig. 9.3. However, it must be realised that food handlers, especially consumers, are not a homogeneous group: they are individuals and they will not all behave in the same way or necessarily in a way food safety experts might expect.

9.5.1 Behavioural and psychological models

In attempting to interpret, study or even predict behaviour it is helpful to use existing theoretical models. The simplest of these is the knowledge, attitudes, practice (KAP) model which is used as the basis for much hygiene training. This relies on the provision of information (knowledge) to modify food handlers' attitudes and then behaviour. This approach takes no cogniscance of the conditions, culture and environment in which the food handler may be working.

More sophisticated and successful models try to consider many more of the factors that can influence behaviour. Social cognition models start from the assumption that an individual's behaviour is best understood in terms of their perceptions of their social environment. Two of the most widely used models are the Theory of Planned Behaviour (TPB) and the Theory of Reasoned Action (TRA) (Fig. 9.4). These theories aim to measure behavioural intentions, recognising that certain uncontrollable factors can inhibit implementation and thus prediction of actual behaviour, e.g. an individual's intention is to wash their hands, but they arrive at the sink to find there is no soap. These models include the element of

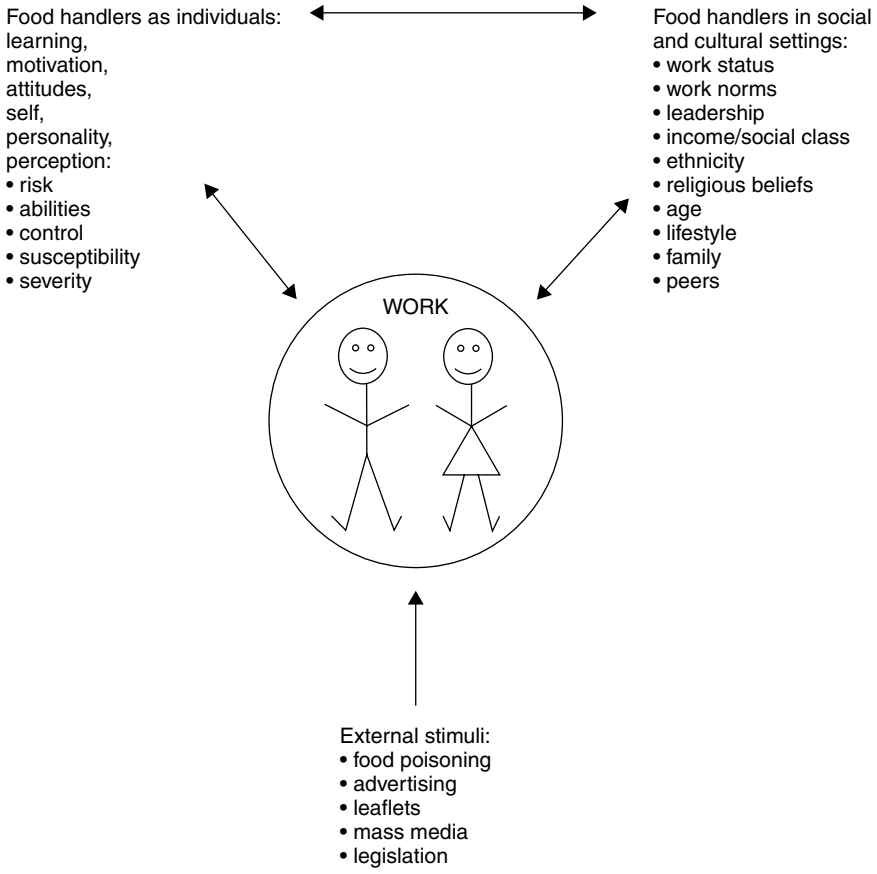
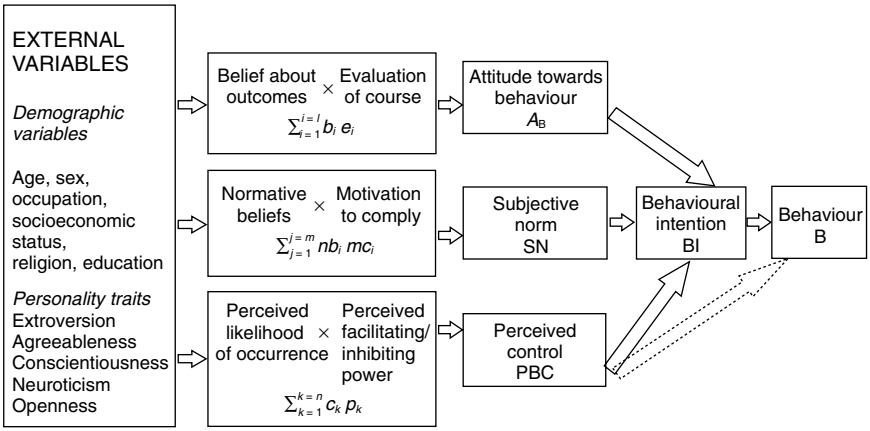


Fig. 9.3 Factors influencing food handlers' behaviour.

subjective norm which considers the perceived beliefs of others and the individual's desire to comply with those beliefs.

One model that has been applied to food hygiene behaviour is the Health Belief Model (HBM)[28] (Fig. 9.5). However, this model has been criticised as being more a 'catalogue of variables' rather than a model.[29] The Health Action Model[30] combines elements of the HBM with the TRA. Other models exist and although they vary in structure, they can provide a basis for understanding food hygiene behaviour and a list of target areas for interventions to focus upon. Considering these targets at the time of hygiene training could help to make the training more effective (see Section 9.6.3).

Another model that is attracting interest for use in consumer food hygiene education (see Section 9.6.1) is the trans-theoretical model of change of Prochaska and Diclemente which can be used as a precursor to social marketing and can help to identify who is likely to change their behaviour and thus who should be



Theory of Reasoned Action

This model emphasises the relationships between specific attitudes and specific behaviours, with 'intention' as the mediator between these two elements. Behaviour is a linear regression function of intention to behave and perceived control over that behaviour:

$B = w_1 BI + w_2 PBC$

where B = behaviour, BI = behavioural intention, PBC = perceived behavioural control, w_1 and w_2 = regression weights. Although this model has been used in food choice, there have been no publications to date of its practical application to food hygiene although several are in press.

Theory of Planned Behaviour

This model does not include the variable of perceived control and is used to understand habitual behaviour.

Fig. 9.4 Theory of reasoned action/planned behaviour.

targeted.[31] This model suggests that making a change to behaviour is not usually instantaneous but is part of a series of five decisions or stages (Fig. 9.6).

9.5.2 Risk communication and risk perception

There is evidence that the concept of risk is poorly understood within the food service industry with failure to believe or accept that catering operations present a food safety risk. Behavioural change is most likely to follow self-realisation of the level of risk posed and self-assessment checklists can be useful devices for achieving this.[17]

Given that zero risk with regard to food safety is unlikely then government and the agri-food industry have a responsibility for communicating information on risk and risk management to the consumer.[14] Risk communication will be an important element of the work of the Food Standards Agency in the UK[32] and it is vital to ensure transparency and, where possible, for consumers to participate in decision making. It is important to inform and advise them about risks in a way that includes how they have the ability to manage or control the risks.

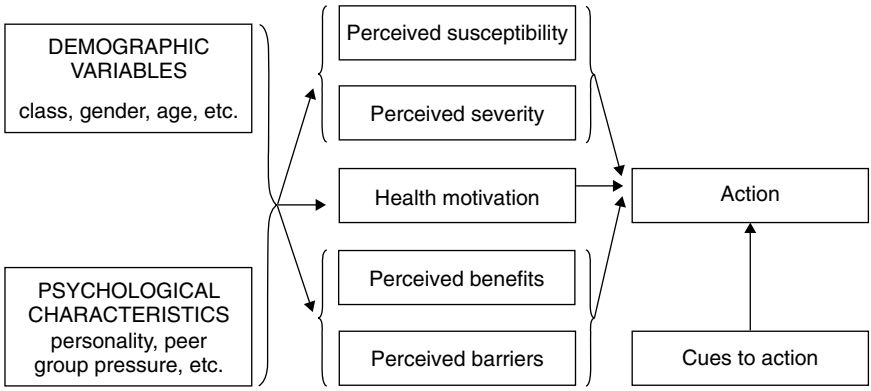


Fig. 9.5 The health belief model.

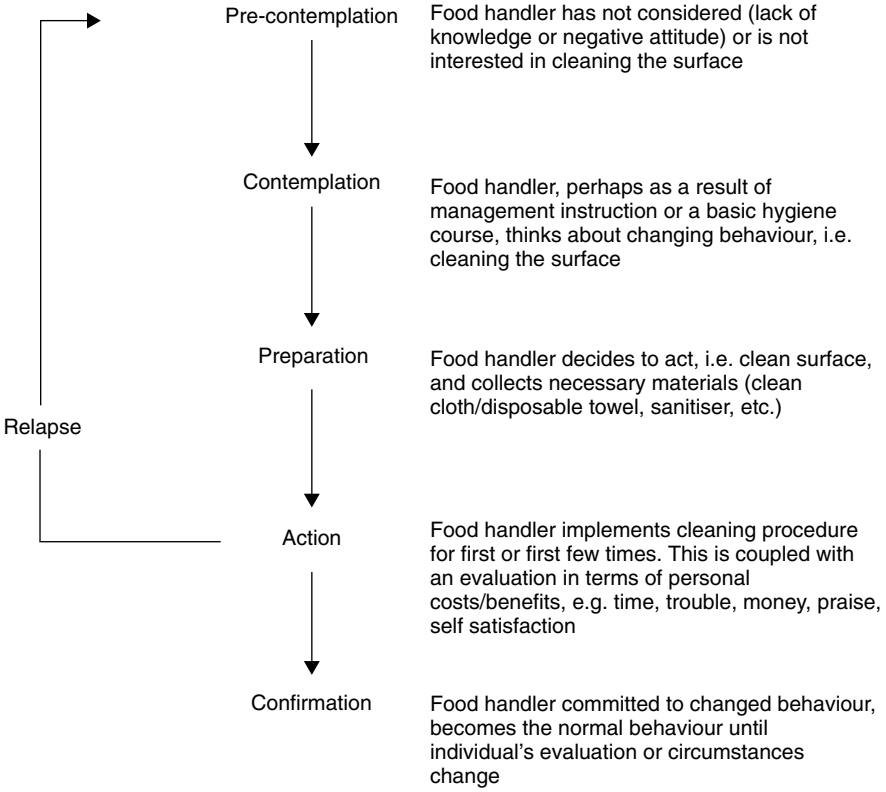


Fig. 9.6 Stages of change model applied to cleaning a work surface after contact with raw food.

This latter aspect could be of concern to specific sectors of the food industry. If there is a problem with the presence of pathogens in raw foods, e.g. *Campylobacter* in poultry, then risk needs to be communicated to consumers in a way that both alerts them to the problem and informs them of how they can reduce/manage the risk. This is known as the reassurance/arousal paradox. Failure by a sector of industry to inform consumers about risk associated with a food could lead to an increase in outrage (see Table 9.4). What is clear is that risk is not just about science but is heavily influenced by psychology, and experts and the public perceive risk in very different ways.[33]

In general terms, risk communication should enable the recipients to understand the nature of the problem, help or support decision making and be unbiased. There is debate over the precise role of the government in this process: should this be restricted merely to provision of information or should it include modifying or influencing behaviour? A detailed study of risk communication is beyond the scope of this chapter and readers should be aware of useful reviews;[33,34] however, it is worthwhile considering some of the pitfalls of or barriers to effective risk communication.

A common misconception is that consumers and experts view and consider risk in the same way. This applies to the levels of risk associated with food hazards and the probable response to them, i.e. the risks considered by experts to be important are not the ones that may concern the public. Experts believe all they have to do is present the public with current risk data and consumers will change their behaviour. When this does not happen a common response by experts is to believe that the public are stupid or at best ignorant. One explanation is that risk is a combination of hazard + outrage. Outrage concerns the emotive response by the public to information on hazards. It has been suggested that experts respond to hazard information, the public responds to outrage. Factors likely to increase outrage are outlined in Table 9.4.

Another problem is that while consumers may accept there is a risk to the public in general, they may not realise the risk applies to them. Known as unrealistic optimism or optimistic bias, this can apply to food poisoning in the home and in food service establishments. Food handlers may believe (perhaps incorrectly) that food poisoning is less likely from food prepared in their kitchens. A possible contributing explanation to this, and a factor that should be considered in education strategies, concerns the illusion of control. Food handlers see or hear messages about food handling and agree this is necessary information for the public. However, what can happen is that individual food handlers fail to realise that the message applies to them, erroneously believing that their own practices and behaviour are adequately hygienic and capable of controlling food hazards.

9.5.3 Sources of food safety information

In general terms larger food businesses have access to the latest food safety information. Increasing use of the Internet may assist smaller businesses and consumers, but there is evidence that they currently lack information on food

Table 9.4 Factors relating to hazards that are likely to increase consumer outrage

Acceptance/low outrage	Rejection/high outrage
Natural	Technological
Voluntary	Compulsory
Obvious consumer benefits	No obvious consumer benefits
Enjoyable	Not enjoyable
Consumer control	No consumer control
Information readily available/open	Secrecy, information not available, restricted
Familiar	Exotic
Trustworthy sources	Untrustworthy sources
Hazard has limited damage potential (severity or dread)	Widespread damage potential, can be passed through generations
Fair	Unfair

safety.[15] Improved channels of communications, possibly via Environmental Health Departments or by small business/trade associations could help small manufacturers/food services but not the consumer.

The consumer will acquire food safety information from three main sources: the home, school and others, e.g. television, magazines and food safety campaigns. Parents may not have the correct information to give to their children and there is evidence that consumers' (parents') knowledge about food safety is inadequate. Hygiene teaching in schools has suffered, certainly in the UK, as a result of a packed school curriculum. Recently food safety material specially designed for schools has been produced and its use could help to initiate good practice at an early age.[35] Elements of the mass media, especially television, offer a powerful means of influencing behaviour: unfortunately television does not always project the correct message on cookery programmes, with highly respected or admired chefs acting as role models who may illustrate bad practices.[36] Furthermore there are suggestions that the media present conflicting advice, causing confusion in consumers.[37] In spite of this in the USA, the majority of consumers depend on the media as their primary information source. Initiatives designed to inform the consumer need to use a wide variety of strategies, traditionally food safety health education has overly-depended upon the use of leaflets, although this is changing.[38]

9.6 Improving food-handling practices

9.6.1 Educating the consumer

Until recently, educating the consumer about food hygiene has been relatively ignored. The publicity concerning food poisoning in the UK in the late 1980s led to the issuing of leaflets with food safety advice.[36] However, findings from other

Table 9.5 The 4 Ps of marketing – marketing mix

Product	What is being offered must be acceptable and as tangible, accessible and attractive as possible. The changed behaviour must not be excessively expensive, time-consuming, totally impracticable, painful, etc.
Price	Decisions to act are a result of a consideration of costs and benefits. Ways to minimise the cost as well as the benefits need to be addressed in strategies for behavioural change.
Place	The means to implement new behaviour must be readily available and convenient, e.g. availability of soap for hand washing.
Promotion	The message needs to be 'advertised' in the most appropriate way. This could include leaflets/TV/cook books/magazines or individual advice, e.g. health visitors.

areas of health education suggest that although leaflets can play a role in raising awareness, they generally do not bring about the behavioural change.[26] Leaflets can provide people with the information enabling them to change, if they are motivated to and want to change. Other consequences of the publicity in the UK included the realisation that more information concerning knowledge, attitudes and behaviour was required and a food safety initiative was launched by the Food and Drink Federation (FDF).[36]

Additionally a number of research initiatives, for collecting information concerning consumer food safety, were launched although they mostly relied upon self-report of behaviour. A few studies have focused upon actual behaviour and these have indicated that often consumers do not know or understand hygiene advice and also that they often may not implement known hygiene practices.[13]

Difficulties in improving consumer food safety practices have been reviewed and more recently attempts using a social marketing approach have had limited success in improving behaviour.[38] Social marketing is the application of marketing techniques used in the commercial sector, to promote changes in socially important behaviours, e.g. drug use, and smoking. Social marketing starts and ends with the target customer – the person whose behaviour needs to be changed. This requires a careful evaluation of consumer behaviour and the identification of target risk groups using market segmentation. A precise message is identified and the target audiences' response to and beliefs about the message are assessed, as well as the likelihood they will change behaviour (see Fig. 9.6). Results from these preparatory stages are then used to plan the initiative based upon marketing principles (involving the 4 Ps or marketing mix, see Table 9.5). After implementation the initiative is evaluated, preferably based upon actual behavioural evaluation.

This type of approach is more comprehensive than an ordinary health educational approach. The latter is generally more concerned with imparting and receiving messages, with people learning facts. Social marketers argue that learning is important only if it results in the desired behavioural change. While there are differences in marketing commercial products compared to improved food safety handling, not least in terms of budgets available, there are potential

Table 9.6 Factors influencing training in the food service sector

Staffing	High staff turnover, Large numbers of part-time staff Low pay Low status Possible poorer educational background and language skills
Business size	Smaller businesses can not afford time/money for training
Type of operation	Poorer facilities to practice hygiene production and service

benefits in adopting the approach. Relatively small improvements or changes in behaviour could significantly reduce the cost of foodborne disease.

9.6.2 Training food handlers

Legislation and education have been advocated as part of a dual strategy to reduce food poisoning.[26] Current UK legislation now contains a requirement for handlers to be trained commensurate with work activities. This was introduced in 1995 but although industry considers training important, levels of training are variable, particularly with regard to type of employee and food industry sector.[39] The food service sector particularly lags behind, although there may be specific reasons for this (see Table 9.6). The legislation itself has been criticised.[40] The proprietor need not necessarily be trained and while guides to implementation provide a definition of a food handler they are not themselves legal documents.

It has been estimated that only 46% of food handlers have been trained,[39] but even if trained there is no guarantee that food handlers will behave differently. Generally there is a lack of information on the efficacy of training, although individual studies have produced mixed results: some showing an improvement in knowledge, others no improvement. In cases where knowledge improved this did not necessarily change behaviour. Therefore it has been suggested that training should consider behavioural theory.[26] In the final analysis while over 4 million people have been trained, despite vagueness with epidemiological data, there seems to have been no improvements on levels of food poisoning. This should act as a spur to make learning more effective and the need for improved food safety attitudes and culture.

Most studies of food hygiene learning have concentrated on formal learning as opposed to work-based learning (defined as learning at work, linked to a job requirement), although the two differ (see Table 9.7).[40] Companies should try to develop training strategies embracing both types of learning.

9.6.3 How to make training effective

Suggestions have been made to improve the quality of formal courses[40] including changes to syllabuses, examinations and pass marks. The following should be considered in delivering or purchasing formal training:

Table 9.7 Formal and informal work-based training

	Formal	Informal
Subject matter	Syllabus externally set, training courses	Content internally set, work notes/sheets/ manuals
Certification	Likely to be formally recognised	Less likely to be formally recognised
Attendance	Part-time study, often off-site, day release usually in class with others	During work, one to one Peer/managerial involvement
Evaluation	Written test/knowledge-based	Competency/behavioural-based
Value	Valued by company	Employee valued
Relationship to organisational culture	Greater chance of being remote	Part of work culture

- Training should be targeted and specific and geared to the needs of audiences, using work-related examples.
- Information should be delivered within a hazard/risk framework.
- The content should be simple, accurate and jargon-free, at an appropriate level and should promote understanding. It is important to cover theory and practice – what to do and why.
- Trainers should have the respect of the trainees, be considered trustworthy and reputable (and have 'street credibility').
- The trainer should examine, with trainees, likely barriers to implementation of good practice and how they can be overcome.
- Trainers should assess available facilities and equipment and ensure implementation is possible. If inadequate, management should be informed.
- The correct management culture should be in place. Trainees must work in an environment which expects implementation of all practices as the norm and establish that inappropriate practices will not be tolerated.
- A motivational framework should be provided to encourage training and implementation of learned behaviour.
- Training should address costs (including failure) as well as benefits. These may be financial, social or medical.
- Training is part of a regular programme with updating, associated with maintaining training logs and records.
- Training effectiveness should be evaluated and appropriate improvements made to training as a result.

9.7 Future trends

A substantial level of food poisoning is related to food service establishments and the home and the role of food handlers is paramount. Legislation will require food businesses to operate food safety management systems based upon good practice in conjunction with some form of specific risk management. This is less likely in the home, although elements of GDKP could be linked to risk-based advice.

Achieving desirable food-handling practices is likely to be of major importance to bodies such as the Food Standards Agency but this is not merely dependent upon the provision of information. Good food handling requires understanding the factors that influence human behaviour and greater efforts need to be made to promote good handling practices, including those of consumers. There have been considerable achievements with respect to food safety training in the UK but improvements need to be made. A greater percentage of the workforce requires training and this training needs to be made more effective. In the future, more training will be needed particularly with respect to HACCP; however, it is paramount that businesses provide the correct cultural framework and context for food safety to be practised. A company must not merely provide training but must allow the learning to be implemented. Ultimately failure to do so could cost a business money or even survival; however, of more importance is that it may cost someone their life.

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Part II

Bacterial hazards

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10

Pathogenic *Escherichia coli*

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10.1 Introduction

The involvement of *Escherichia coli* in human illness has been recognised virtually since its discovery in 1885. It has been associated with diarrhoea (particularly in children), haemorrhagic colitis, dysentery, bladder and kidney infections, surgical wound infection, septicaemia, haemolytic uraemic syndrome, pneumonia and meningitis; some of these conditions result in death.

Although the role of contaminated food and water in outbreaks of illness attributed to *E. coli* has been widely acknowledged for decades, it is only in more recent years that the food industry has re-focused attention on *E. coli* as a cause of significant morbidity and mortality in outbreaks of foodborne illness; Vero cytotoxin-producing (Vero cytotoxigenic) *E. coli* (VTEC) is of particular and major concern.

Since the early 1900s, *E. coli* has been viewed by public health microbiologists as an indicator of faecal contamination in water sources and milk. The inclusion of *E. coli* in many food product specifications today also recognises its value as an indicator of the hygienic status of many food types. Although there is still much to learn concerning the epidemiology of the organism, there are strategies that can be followed and actions which can be taken by those in the business of supplying food as primary producers, processors, distributors, caterers or retailers, to minimise the incidence and level of the organism in foods, thus improving their safety. This chapter aims to give the reader an overview of *E. coli*, particularly VTEC, in respect of the hazard they present to food products and the means for controlling these organisms.

Despite the fact that a vast amount of work has been carried out on all aspects of *E. coli* since it was first described, the organism continues to provide new

challenges to food safety because of the wide diversity of types within the species, ranging from harmless commensals to dangerous human pathogens. Although, in many countries including the UK and USA, *E. coli* O157:H7 is currently the most predominant foodborne VTEC, it is not the only VTEC associated with foodborne illness: *E. coli* O26, O103, O111, O118 and O145 and other VTEC are causing significant morbidity in many countries and such serogroups are increasingly being recognised as posing an equal or possibly greater threat to human health than *E. coli* O157. There is therefore a continuing need for information concerning sources, growth and survival characteristics, detection methods and effective, practical control measures that can be applied in the food industry for the control of all VTEC.

It is essential that a detailed and competent hazard analysis is carried out at an early stage in all new food product and process developments to ensure that relevant critical controls and monitoring systems can be put in place. This will help to minimise potential public health problems that could arise from the presence and outgrowth of all types of pathogenic *E. coli*, especially the Vero cytotoxigenic types. It is equally important to recognise that hazard analyses conducted for existing products and processes should be reviewed taking any 'new' pathogens such as VTEC into account so that any additional controls can be identified and implemented.

10.2 Characteristics of *Escherichia coli*

In 1885, Theodor Escherich described some organisms he had isolated from infant stools one of which he named *Bacterium coli commune.* Following a great deal of work on the phenotypic characteristics of bacteria, by the 1960s, the genus *Escherichia* was described as: Gram negative, non-sporing rods; often motile, with peritrichate flagella. It is easy to cultivate on ordinary laboratory media, aerobic and facultatively anaerobic. All species ferment glucose with the formation of acid or of acid and gas, both aerobically and anaerobically. All reduce nitrates to nitrites and are oxidase negative, catalase positive. Typically, they are intestinal parasites of humans and animals, though some species may occur in other parts of the body, on plants and in the soil and many species are pathogenic (Wilson and Miles, 1964). As bacterial classification systems have become more technically sophisticated, the conventional methods for distinguishing between strains of organisms based on biotype (Table 10.1) have been supplemented by, among others, serological techniques, phage typing and genotyping.

The DNA relatedness of *E. coli* to some other genera of the *Enterobacteriaceae*, particularly some notable human pathogens, has been established (Brenner, 1984) and, based on DNA homology, Jones (1988) indicates that *E. coli* and the four species of the genus *Shigella* should be considered a single species.

By the mid-1940s, a serogrouping scheme was developed that allowed *E. coli* to be divided into more than 170 different serogroups based on their somatic (O) antigens (Kauffmann, 1947). In addition, over 50 flagella (H) antigens and

Table 10.1 Some biotypic characteristics of *Escherichia coli*

Character	Reaction
Gram	negative
Cell morphology	non-sporing straight rod, 1.1–1.5 × 2.0–6.0 μm
Motility	+ by peritrichous flagellae or non motile
Aerobic growth	+
Anaerobic growth	+
Optimum growth temperature	37 °C
Catalase	+
Oxidase	–
D-Mannitol fermentation	≥90% +
Lactose 37 °C and 44 °C	≥90% +
D-Adonitol	≥90% –
D-glucose	acid produced
Indole 37 °C	≥90% +
Indole 44 °C	≥90% +
Methyl Red reaction	≥90% +
Voges–Proskauer reaction	≥90% –
Growth in Simmons' citrate	≥90% –
Urease, Christensen's	≥90% –
Phenylalanine deamination	≥90% –
Lysine decarboxylase	76–89% strains +
H_2S on TSI (triple sugar iron) medium	≥90% –
Growth in KCN (potassium cyanide) medium	≥90% –
Gelatin liquefaction (at 22 °C)	≥90% –

+ = positive reaction; – = negative reaction.
Source: adapted from Brenner (1984) and Ørskov (1984).

approximately 100 capsular (K) antigens are now also recognised and these are used to further subdivide *E. coli* into serotypes. Serogrouping and serotyping, together with other information such as biotype, phage type and enterotoxin production, now facilitate distinction between those strains able to cause infectious disease in humans and animals (Linton and Hinton, 1988). Some correlation has been established between the *E. coli* serogroup and virulence.

Depending on the virulence genes acquired, different types of pathogenicity are conferred to certain strains of *E. coli.* These strains are classified as enteropathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), entero-haemorrhagic *E. coli* (EHEC) and entero-aggregative *E. coli* (EAEC) (Nataro and Kaper, 1998). The different virulence factors expressed by the organism, e.g. colonisation factors, ability to invade epithelial cells of the small intestine, haemolysin production and toxin production, lead to the different strains of *E. coli* being associated with a wide variety of types of disease. Table 10.2 summarises information concerning the pathogenicity of some

Table 10.2 Pathogenicity and characteristics of foodborne illness caused by pathogenic *E. coli*

Pathogenic type of *E. coli*	Serogroup examples[a]	Summary of *E. coli*/host interaction	Time to onset of illness	Duration of illness	Range of symptoms
EPEC (enteropathogenic)	O18ab, O18ac, O26, O44, O55, O86, O114, O119, O125, O126, O127, O128, O142, O158	EPEC attach to intestinal mucosal cells causing cell structure alterations (attaching and effacing). EPEC cells invade the mucosal cells	17–72 hours, average 36 h	6 h to 3 days, average, 24 h	Severe diarrhoea in infants which may persist for more than 14 days. Also, fever, vomiting and abdominal pain. In adults, severe watery diarrhoea with prominent amounts of mucus without blood (main symptom) nausea, vomiting, abdominal cramps, headache, fever and chills
ETEC (enterotoxigenic)	O6, O15, O25, O27, O63, O78, O115, O148, O153, O159	ETEC adhere to the small intestinal mucosa and produce toxins that act on the mucosal cells	8–44 hours, average 26 h	3–19 days	Watery diarrhoea, low-grade fever, abdominal cramps, malaise, nausea. When severe, causes cholera-like extreme diarrhoea with rice water-like stools, leading to dehydration
VTEC (EHEC) (Vero-cytotoxigenic) (Entero-haemorrhagic)	O2, O4, O5, O6, O15, O18, O22, O23, O26, O55, O75, O91, O103, O104, O105, O111, O113, O114, O117, O118, O121, O128ab, O145, O153, O157,	EHEC attach to and efface mucosal cells and produce toxin	3–9 days, average 4 days	2–9 days, average 4 days	Haemorrhagic colitis: sudden onset of severe crampy abdominal pain, grossly bloody diarrhoea, vomiting, no fever Haemolytic uraemic syndrome (HUS): bloody diarrhoea, acute renal failure in children, thrombocytopaenia, acute nephropathy, seizures, coma, death. Thrombotic thrombocytopaenic

	O163, O168				purpura: similar to HUS but also fever, central nervous system disorders, abdominal pain, gastrointestinal haemorrhage, blood clots in the brain, death
EIEC (enteroinvasive)	O28ac, O29, O112ac, O121, O124, O135, O144, O152, O167, O173	EIEC invade cells in the colon and spread laterally, cell to cell	8–24 hours, average 11 h	Days to weeks	Profuse diarrhoea or dysentery, chills, fever, headache, muscular pain, abdominal cramps
Enteroaggregative EAggEC	O3, O44, O51, O77, O86, O99, O111, O126	EAggEC bind in clumps (aggregates) to cells of the small intestine and produce toxins	7–22 hours	Days to weeks	Persistent diarrhoea in children. Occasionally bloody diarrhoea or secretory diarrhoea, vomiting, dehydration
Diffusely adherent DAEC	O1, O2, O21, O75	Fimbrial and non-fimbrial adhesins identified	Not yet established	Not yet established	Childhood diarrhoea

[a] Only certain strains within a serogroup may be associated with human illness and full characterisation, e.g. serotype, phage type, is necessary to clearly identify the causative agent.
Note: there are an increasing number of strains that do not easily fit into the main groups described above.
Source: adapted from Bell and Kyriakides (1998) and Willshaw *et al.* (2000).

different serogroups together with the type of diseases and characteristics of the illnesses associated with these organisms. As can be seen, although *E. coli* is a common and harmless member of the normal commensal microflora of the distal (end or terminal) part of the intestinal tract of humans and other warm-blooded animals (constituting less than 1% of this flora in numbers ranging up to 10^8 per gram in humans; Smith, 1961), some strains are responsible for causing severe illness, sometimes resulting in death.

Production of cytotoxins (referred to as Shiga toxins) is a common feature of VTEC which may also be referred to as Shiga toxin-producing *E. coli* (STEC) and Entero-haemorrhagic *E. coli* (EHEC). There is still some debate as to an agreed terminology for these organisms and in this chapter the term VTEC will continue to be used.

Escherichia coli is acquired by infants within a very few days of birth. The organism is acquired predominantly from the mother by the faecal–oral route, but also from the environmental surroundings. Results from oral challenge experiments of adults suggest that levels of 10^5–10^{10} EPEC are required to produce diarrhoea, 10^8–10^{10} ETEC organisms are necessary for infection and diarrhoea and 10^8 cells of EIEC are required to produce diarrhoeal symptoms in adults. However, this may vary depending on the acidity in the stomach (Doyle and Padhye, 1989; Sussman, 1997). In complete contrast, however, epidemiological evidence suggests that the infective dose of VTEC can be very low, i.e. <100 cells of the organism (Advisory Committee on the Microbiological Safety of Food, 1995). The haemolytic uraemic syndrome (HUS), caused by VTEC and characterised by acute renal failure, haemolytic anaemia and thrombocytopaenia, usually occurs in young children (under 5 years of age). It is the major cause of acute renal failure in children in Britain and several other countries. Generally, about 5% of cases of haemorrhagic colitis caused by VTEC progress to HUS, in which the case fatality rate is approximately 10% (Anon., 1995a).

There are no specific treatments of the conditions caused by VTEC and each symptom is treated as it occurs in the individual (Advisory Committee on the Microbiological Safety of Food, 1995; Anon., 1995b). It should also be recognised that while this group of organisms can cause serious illness, it is also clear that some individuals exposed to the organism remain asymptomatic, a feature in common with a number of other enteric pathogens, e.g. *Salmonella* species. Stephan *et al.* (2000) reported the detection of the polymerase chain reaction (PCR) product for VT-encoding genes in 3.5% of over 5500 stool samples taken from healthy employees in the Swiss meat-processing industry, underlining the concern about asymptomatic human carriers in food-handling environments.

In some countries in which surveillance of foodborne infections has become routine and considered reliably indicative of trends, e.g. some western European countries, the USA and Canada, the numbers of cases of VTEC-related illness is being shown to be steadily increasing. Table 10.3 indicates this trend for the UK.

The very low infective dose of some VTEC, particularly VTEC O157 : H7, underlines the importance of ensuring that the highest possible standards are maintained in agricultural practice and that food processors consistently operate

Table 10.3 Trend in laboratory-confirmed cases of VTEC O157 in England and Wales

Year	Number of cases per year
1982	1
1983	6
1984	9
1985	50
1986	76
1987	89
1988	49
1989	119
1990	250
1991	361
1992	470
1993	385
1994	411
1995	792
1996	660
1997	1087
1998	890
1999	1084
2000	896 (Provisional data)

Source: adapted from Anon. (2000 and 2001).

well-designed and effective hygienic food production processes based on Hazard Analysis Critical Control Point (HACCP) assessments of each food process. In addition to attention to the detail of cleaning and hygiene procedures, the treatment and formulation of food products are important for controlling any residual *E. coli* and preventing their potential to cause harm to consumers. Table 10.4 indicates some of the key growth-limiting parameters for pathogenic *E. coli*. These and other physico-chemical factors, used either singly or in combination, can be effective in controlling the survival and growth of *E. coli* during processing and also in the finished food products (Bell and Kyriakides, 1998).

In the food industry, *E. coli* is commonly included in buying specifications relating to raw materials and finished food products as an indicator of the hygienic status of the food. It is also included in some industry guidelines and legislation but, currently, there is limited food-related legislation that refers to pathogenic *E. coli.* However, these will be included in the generic statements made in some food- and water-related legislation concerning microbiological safety requirements, e.g. all pathogens are required to be absent in mineral water (Anon., 1980), and in reference to 'all-milk products', all pathogens and their toxins 'must not be present in quantities such as to affect health' (Anon., 1992). In addition, the final text of the new European Water Directive states, among other requirements, that 'Member States shall take the measures necessary to ensure that water

Table 10.4 Growth-limiting parameters for pathogenic *E. coli*

	Minimum	Optimum	Maximum
Temperature (°C) *E. coli* (all types)	7–8	35–40	44–46
VTEC O157:H7	6.5[a]	37	44–45
pH Pathogenic *E. coli*	4.4[b]	–	9.0
a_w Pathogenic *E. coli*	0.95	–	–
Sodium chloride[c] (Glass *et al*., 1992) Pathogenic *E. coli*	Grows vigorously in 2.5% NaCl Grows slowly in 6.5% NaCl Does not grow in 8.5% NaCl		

[a] Kauppi *et al*. (1996).
[b] *E. coli* O157 is reported to survive at pH values below 4.4 and has also been shown to grow at pH 3.6 in apple juice, pH 3.58 in hydrochloric acid, pH 3.78 in lactic acid and pH 3.96 in citric acid.
[c] Glass *et al*. (1992).
Source: adapted from International Commission on Microbiological Specifications for Foods (1996).

intended for human consumption is wholesome and clean' and that this means the water must be free from any microorganism and parasites and from any substances which, in numbers or concentrations, constitute a potential danger to human health.

Escherichia coli O157 is specifically included in some food industry buying specifications but, when present, it is likely to be present only at a low frequency in the raw material or finished product (Table 10.5); therefore testing for the presence of pathogenic *E. coli* is likely to be of limited benefit. It will be the ability of the food producer or manufacturer to demonstrate well-structured and reliably operated procedures targeted to control *E. coli* O157 and other VTEC that will prove of most value.

10.3 Detecting *Escherichia coli*

Reliable methods for detecting and identifying VTEC are important in the support of properly developed and implemented HACCP systems for their control. Table 10.6 indicates the common conventional microbiological method used by food microbiologists to isolate and identify *E. coli* O157 and Table 10.7 gives some examples of the current range of microbiological techniques used for the detection of *E. coli* O157. When suspected positive results are obtained, further tests to characterise the organism may be necessary and these can include serological tests, tests for the production of Vero cytotoxins, phage typing and pulsed field

Table 10.5 Incidence of VTEC in some raw and processed meat products

Source of samples	Country	Occurrence (%)	Reference
Retail fresh meats and poultry	USA	6/164 (3.7) ground-beef 4/264 (1.5) pork 4/263 (1.5) poultry 4/205 (2.0) lamb All isolates *E. coli* O157:H7	Doyle and Schoeni (1987)
Retail chickens and sausages	UK	46/184 (25) pork sausages 0/71 (0) chickens All non-O157 VTEC	Smith *et al.* (1991)
Retail raw meats	Netherlands	2/770 (0.3) minced mixed beef and pork 0/1000 (0) raw minced beef 0/26 (0) minced pork 0/300 (0) poultry products Isolates confirmed as VTECs	Heuvelink *et al.* (1996)
Raw meat products	UK	3/89 (3.4) frozen beefburgers 1/50 (2.0) fresh minced beef 0/50 (0) sausages All isolates *E. coli* O157	Bolton *et al.* (1996)
Raw beef products	UK	36/3216 (1.1)	Chapman *et al.* (2000)
Raw lamb products	UK	29/1020 (2.9) of which lamb sausages 3/73 (4.1) lamb burgers 18/484 (3.7)	Chapman *et al.* (2000)
Mixed meat products	UK	7/857 (0.8) All isolates *E. coli* O157	Chapman *et al.* (2000)

gel electrophoretic (PFGE) pattern. These latter two forms of test, however, tend to be confined to use in public health and veterinary research laboratories for identifying and tracing outbreak strains of the organism and when investigating the epidemiology of pathogenic *E. coli*. A valuable summary of methods available has been prepared by Campden and Chorleywood Food Research Association (Baylis, 2000). More variations on these methods are still being developed and will undoubtedly become available but the more urgent need is for reliable methods to be developed for detecting and identifying other VTEC types of importance to food industry and public health microbiologists.

A wide variety of foods has been implicated in outbreaks of illness attributed to VTEC, particularly *E. coli* O157 (Table 10.8), and this has led to an increasing amount of work by both researchers and food industry scientists to answer questions concerning the specific survival characteristics of these organisms, to

Table 10.6 Common conventional method for the detection and identification of *E. coli* O157 in foods

Inoculate selective enrichment broth (Modified Tryptone Soya Broth with Novobiocin)[a]
⇓
Incubate 41.5 °C for 22 ± 2 hours
⇓
Capture cells with immunomagnetic beads[b] after 6 hours and 22 hours incubation
⇓
Plate out onto Sorbitol MacConkey Agar containing Cefixime and Potassium Tellurite (CT-SMAC) and Sorbitol MacConkey Agar containing Cefixime and Rhamnose (CR-SMAC)
⇓
Incubate 37 °C for 24 hours
⇓
Inspect plates Purify suspect colonies on nutrient agar
⇓
Incubate 37 °C for 24 hours Confirm the identity of the isolates, e.g.
⇓
O157 latex bead or antisera agglutination *if positive, then* Biochemical profile
⇓
Incubate 37 °C for 24 hours
⇓
Read reactions

[a] Stressed cells may not be detected by direct selective enrichment and may require periods of non-selective enrichment and/or longer enrichment times prior to subculture (Blackburn and McCarthy, 2000).
[b] Manufacturer's instructions must be followed.
Source: ISO (1999).

improve methods of detection and to develop controls aiming to minimise, if not eliminate, contamination of food by VTEC.

10.4 Control of pathogenic *Escherichia coli* in foods

Outbreaks of *E. coli* infection in developed countries, and particularly those caused by VTEC, continue to implicate bovine sources of contamination as a

Table 10.7 Different methods currently available for the detection and/or identification of *E. coli* O157 in foods (for a more extensive list see Baylis, 2000)

Test type/technique	Test target	Name of test	Approximate test time (hours)[a]	Supplier
Enzyme-linked immunosorbent assay (ELISA)	*E. coli* O157	Vidas *E. coli* O157	25	bioMérieux UK Ltd
	E. coli O157	EHEC-Tek incorporating immunocapture beads	18	Organon Teknika Ltd
	E. coli O157	Petrifilm-HEC	28	3M Healthcare Ltd
	E. coli O157:H7	*E. coli* O157:H7 Visual Assay	28	TECRA Diagnostics UK
Immunochromatography	*E. coli* O157:H7	Reveal for *E. coli* O157:H7	24	Neogen Corp.
	E. coli O157:H7	Reveal 8 for *E. coli* O157:H7	8	Neogen Corp.
	E. coli O157:H7	Visual Immunoprecipitate Assay VIP for EHEC	24	Bio Control Systems Inc.
	E. coli O157	EIAFOSS *E. coli* O157	26	Foss Electric
	E. coli O157	Pathstik	48	Celsis Ltd
Polymerase chain reaction	*E. coli* O157	BAX™ for *E. coli* O157	24	Qualicon
	E. coli O157:H7	PROBELIA™	24	Sanofi Diagnostics, Pasteur
	E. coli O157:H7	TaqMan® *E. coli* O157:H7 detection kit	24	PE Biosystems

[a] Using the enrichment protocols recommended by some manufacturers can lead to false-negative results particularly when cells are injured, and periods of non-selective enrichment may be required to improve detection rates (Blackburn and McCarthy, 2000).

Table 10.8 Some foods associated with outbreaks of illness caused by pathogenic *E. coli*

E. coli type	Year	Country	Suspected food vehicle	Cases (deaths)
EPEC O111:B4	1967	Washington DC, USA	Water	170(0)
ETEC O27:H20	1983	USA, also Denmark Netherlands Sweden	French Brie cheese	169(0) USA
ETEC O6:H16 and O27:H20	1983	UK	Curried turkey mayonnaise	27 (0)
EIEC O124	1947	UK	Canned salmon	47 (0)
EIEC O124:B17	1971	USA	French Brie and Camembert cheese	387 (?)
VTEC O157:H7	1982	Oregon and Michigan, USA	Hamburger patties in sandwiches	>47 (0)
VTEC O157	1985	Ontario, Canada	Undercooked beef patties	73 (17)
VTEC O157:H7 phage type 49	1991	UK	Yoghurt	16 (0)
VTEC O157:H7	1993	USA	Hamburgers	631 (3)
VTEC O111:NM	1995	South Australia	Uncooked, semi-dry fermented sausage	23 (?)
VTEC O157	1996	Sakai City, Japan	White radish sprouts	6309 (3)
VTEC O157 phage type 2	1996	Scotland, UK	Meat products	>272 (18)
VTEC O157:H7	1996[a]	USA – Connecticut and Illinois	Lettuce	>61 (0)
VTEC O157 phage type 2	1998[b]	UK	Unpasteurised cream	7 (0)
VTEC O157:H7	1999[c]	USA – New York State	Contaminated well water	>1000 (2)
VTEC O157 phage type 21/28 VT2	1999[d]	UK	Cheese made from unpasteurised milk	3 (0)
VTEC O157 phage type 21/28 VT2	2001[e]	UK	Cooked meats	>13 (?)

[a] Hilborn *et al*. (1999).
[b] Anon. (1998a,b).
[c] Charatan (1999).
[d] Anon. (1999a,b).
[e] Anon. (2001).
Source: adapted from Bell and Kyriakides (1998).

significant contributory factor. However, it is apparent that other animal species appear to be increasingly contaminated with these organisms and other routes of infection are also important, including contaminated water (Jackson *et al.*, 1998; Pebody *et al.*, 1999; Anon. 1999c), person-to-person spread and contact with contaminated animals, particularly at farms (Milne *et al.*, 1999). The controls outlined here principally focus on *E. coli* O157 and other VTEC because of the severity of these hazards and their increasing incidence but many of the controls equally apply to other pathogenic *E. coli*.

Historical outbreaks (Table 10.8) indicate that the factors likely to be associated with increased risk of *E. coli* infection include all of the following:

- Raw material or product exposed to contamination from bovine origin (meat or faeces).
- Product manufactured with no processing stage capable of destroying the organism, e.g. cooking.
- Product exposed to post-process contamination.
- Product sold as ready to eat.
- Contact with an infected individual or animals.

Escherichia coli O157 and other VTEC in particular do appear to be capable of surviving under adverse conditions in certain foods. Although these traits are also found in some other non-pathogenic *E. coli*, they have allowed VTEC to expose the frailties of products traditionally thought of as being safe, such as fruit juices, fermented meats and cheeses.

Control of *E. coli* and VTEC in food production is achieved through the application of effective control of raw materials, process conditions, post-process contamination and retail or catering practices. Indeed, it should be remembered that the general public also has a vital role to play in controlling the organism when handling and cooking products where it may be present, e.g. raw meat and beefburgers (Table 10.5).

10.5 Raw material control

A contaminated raw material is one of the most common reasons why *E. coli* is present in a final product. Where a product receives no further process capable of eliminating the organism, these contaminants will inevitably result in the sale of potentially hazardous foods. VTEC outbreaks associated with cheese made from unpasteurised milk (Anon., 1999a), unpasteurised apple juice (Besser *et al.*, 1993) and raw, fermented meats (Cameron *et al.*, 1995) have all implicated contaminated raw ingredients as significant contributory factors. However, it should also be remembered that contaminated raw materials used for the production of products intended for cooking have also caused outbreaks because of inadequate cooking. Such products have also caused outbreaks through cross-contamination of *E. coli* O157 from the contaminated raw product to a ready-to-eat product

(Ahmed, 1997). Bell and Kyriakides (1998) have extensively reviewed these outbreaks.

Effective control of the raw material to preclude or reduce the organism is absolutely critical to the safety of many food industry products (ILSI, 2001). The primary raw materials implicated in foodborne outbreaks include raw milk, raw beef and raw fruit. In all cases, the principal route of contamination to the material is from exposure to animal faeces, from bovine sources in particular.

10.5.1 Carriage of VTEC in animals

Escherichia coli O157 and other VTEC are believed to be carried asymptomatically by cattle, cows and sheep. For instance, Cerqueira *et al.* (1999) reported the results of a survey of healthy cattle in Brazil in which rectal swabs were taken from healthy animals from 10 dairy farms ($n = 121$), 4 beef farms ($n = 60$) and 1 beef slaughterhouse ($n = 16$); 99/121 (82%) of the dairy cattle and 40/76 (53%) of the beef cattle were positive for Shiga toxin gene sequences. However, the recent isolation of VTEC O26 from an 8-month old heifer suffering dysentery where attaching and effacing lesions were evident in the large intestine may also indicate that some strains are associated with illness in cattle (Pearson *et al.*, 1999).

Shedding of the organism in the faeces of animals is intermittent and can be exacerbated by a variety of factors (Garber *et al.*, 1999) including stress and feed types (Dargatz *et al.*, 1997; Kudva *et al.*, 1997), although the complete picture in relation to carriage and excretion is not fully understood. It is therefore unlikely to be possible to eliminate the organism from herds in the short term although application of current knowledge in relation to reducing shedding and spread through effective animal husbandry practices may be an important means of reducing the incidence of the organism in the faeces.

10.5.2 Raw milk

Raw milk is principally exposed to contamination with faecal pathogens from the faeces of the cow, which contaminate the udder and teats of the cow and pass into the milk during the milking stage. Clearly, once introduced onto milking equipment, the organisms can then either proliferate, if the milk is not stored under effective refrigeration (<8 °C) and/or spread to further batches through inadequate cleaning and sanitisation. It should be noted that some reports indicate the growth of *E. coli* O157 in raw milk at temperatures as low as 7 °C (Heuvelink *et al.*, 1998) although only a 1.5 log increase occurred over a 144 hour period. Similar findings were reported by Kauppi *et al.* (1996) and Katić and Radenkov (1998) who demonstrated slow growth in nutrient media milk at 6.5 °C and 7 °C, respectively. Controls that can reduce the introduction of faecal pathogens into the milk supply centre on effective milking parlour hygiene which includes cleaning and disinfection of udders and teats, together with cleaning and sanitisation of the milking equipment used for milking itself and subsequent milk storage systems. This should include all transfer pipes, including portable

hoses, which can often be overlooked as significant points for the build-up of contamination.

Raw milk should be subject to routine monitoring for indicators of contamination including *E. coli* or coliform bacteria. Where the raw milk is intended to be used in the manufacture of products without further bacterial reduction processes such as in the manufacture of raw milk cheeses, it is important to source milk from farms known to be operating very high standards of hygiene. The farmer should be aware of the intended use of the milk and it is common for manufacturers of such raw milk products to utilise incentive payment schemes using *E. coli* counts as a measure of hygienic status of the milk. Consistent supply of high-quality milk is rewarded with higher payment and poor-quality milk supply is penalised. It is clear that such approaches can achieve improved hygienic quality of the raw milk supply but it is also important to recognise that this cannot guarantee the absence of pathogens from the raw milk supply since low-level or low-frequency contamination is inevitable from time to time.

Microbiological surveys of raw milk frequently find a high incidence of bacterial indicators of faecal contamination, i.e. *E. coli*, and rarely find pathogenic strains. In a survey of raw milk in the UK, *E. coli* was detected (1/ml or greater) in 978 (58%) of 1591 phosphatase positive samples (Anon., 1998c). Some 0.4% samples had counts of 10^3/ml or greater, 3% samples were between 10^2/ml and $<10^3$/ml and 21% between 10 and $<10^2$/ml. The remaining samples had counts below 10/ml. *E. coli* O157 was not detected in any 25 ml sample in this or in any of three other surveys of 1011 samples of raw milk in the Netherlands (Heuvelink *et al.*, 1998), 42 samples in the USA (Ansay and Kaspar, 1997) and a variety of raw milk, dairy and associated samples in the UK (Neaves *et al.*, 1994). *E. coli* O157 was, however, detected in 3 out of 1097 samples of raw milk on sale in the UK (de Louvois and Rampling, 1998). In a survey of milk from dairy farms in Trinidad, 47.4% of bulk milk samples were contaminated with *E. coli* at levels between 8.4×10^3 and 2.0×10^5/ml (Adesiyun *et al.*, 1997). Some 27.7% and 18.5% of the *E. coli* strains isolated from the bulk milks were EPEC and VTEC, respectively.

10.5.3 Raw meat

Meat, and beef in particular, have been frequently implicated in outbreaks of foodborne *E. coli* O157 infection. Meat becomes contaminated through the transfer of faecal pathogens to the muscle tissue from faeces on the hide or from the intestine itself during the slaughtering operation. Minimising this contamination through the prevention of dirty animals entering the abattoir via effective farm handling, transport and lairage control is essential. The preclusion of dirty animals entering the slaughter line is part of the formal process used at inspection in UK abattoirs. Animal hide removal, evisceration and the handling of other parts of the animal carrying contaminants such as the hooves must be carefully controlled to prevent transfer from these areas to the muscle meat.

As well as effective operational procedures with well-defined process flows,

subsequent thorough cleaning and sanitisation of product contact surfaces particularly when cutting into primal joints is absolutely essential to prevent spread of any contaminants entering the plant. Like raw milk, it is not possible to preclude contamination with enteric organisms during raw meat processing and it is important to regularly monitor the hygienic status of the carcass meat. This is particularly important where the raw meat is subject to processes not capable of significantly reducing levels of enteric pathogens such as in the manufacture of fermented meats.

The inability to preclude such contamination has led many processors, particularly in the USA, to introduce steam pasteurisation plants, which effectively decontaminate the surfaces of the meat while maintaining the raw meat quality and appearance. These systems are capable of reducing contamination on the surface by up to 3 log units (Phebus *et al.*, 1997) and can make a significant contribution, together with effective animal husbandry and slaughter hygiene, to minimising the levels and frequency of contamination with these harmful organisms in raw meat.

Surveys for *E. coli* O157 have rarely found it to be present in poultry products and it is usually associated with ruminant animals such as cattle and sheep (Table 10.5).

10.5.4 Fruit, vegetables and other raw materials

An increasing number of outbreaks of VTEC infection have implicated fruit as a primary vehicle, usually when pressed to make unpasteurised fruit juice (Besser *et al.*, 1993; Mshar *et al.*, 1997). Vegetables and fresh produce have also been implicated in a variety of outbreaks throughout the world (Mermin *et al.*, 1997). In many cases, the source of the contamination has been found or is believed to have been from contamination of the fruit or vegetable with animal faeces in the field prior to harvesting. Clearly, many fruit and vegetables are grown in soil where animal manure is used to add nutrients and conditioning to the soil.

Many wild animals and birds are now known to be carriers of VTECs pathogenic to humans and these all serve to perpetuate the reservoir of these organisms in the environment. However, bovine faecal contamination is considered to be the primary source of VTEC in the environment and, if the incidence of *E. coli* O157 and other VTEC should increase in cattle and dairy herds, it follows that their incidence in animal wastes will also increase. Without effective treatment of the wastes prior to application to land, use of animal manure on soil has the potential to introduce enteric pathogens such as *E. coli* O157 to the soil where fruit and vegetables are grown.

Levels of pathogens remaining viable in the soil are subject to a variety of factors including exposure to sunlight, drying etc. However, *E. coli* O157 and other VTEC have been shown to survive for very long periods in animal wastes and in the soil. Fukushima *et al.* (1999) showed that *E. coli* O26, O111 and O157 survived in bovine faeces at 15 °C for 1 to 8 weeks when initially present at 10 colony-forming units (cfu)/g and for 3 to 12 weeks when present at 10^3 and

10^5 cfu/g. Maule (1997) reported the survival of *E. coli* O157 in soil cores stored under continuous illumination for over 120 days. Jones (1999) has usefully summarised data on the survival of *E. coli* O157 in the environment and considered the potential health risk associated with the persistence of the organism in agriculture.

Certain treatments of the animal waste such as composting can significantly reduce levels of contaminating pathogens owing to the high temperatures (*ca* 60 °C) achieved as a result of microbial growth and fermentation processes in the compost pile. Such approaches can make such materials safer to use. However, this is clearly dependent on effective composting practices with regular turning of the pile and breakdown of soil clumps. Much animal waste, however, is applied after only very little storage and consequently can remain potentially hazardous with respect to VTEC.

A practice often identified as contributory to outbreaks of VTEC infection attributed to unpasteurised fruit juices has been the use of 'drop fruit', i.e. fruit that has dropped to the ground and is picked up to avoid wastage. Contamination from pathogens present in or on the soil originating from the application of animal manure or via the deposition of these wastes by free-roaming animals may lead to the fruit subsequently carrying the organism into the processing plant, thereby entering the juice supply.

Proper treatment of animal wastes prior to application to soil, including long-term storage and composting, together with good agricultural practices that avoid the use of 'drop fruit' and ensure wastes are never applied to exposed crops intended for consumption without further processing, are simple, obvious but nevertheless effective control measures to reduce the chances of such materials being contaminated with VTEC.

Once introduced onto vegetables, *E. coli* O157 can survive for extended periods. Beuchat (1999) reported survival of *E. coli* O157 on lettuce for 15 days even when contaminated with low initial levels (1–10 cfu/g). Indeed, it has also been shown that *E. coli* O157 can become internalised in fruit such as apples by entering through the blossom end (Buchanan *et al.*, 1999) and through scar tissue.

Other potential sources of contamination should not be overlooked in relation to *E. coli* O157 including contaminated irrigation or processing water, other meat sources especially lamb, and seeds used for sprouting (Bell and Kyriakides, 1998). Any hazard analysis of a production process should examine the raw material sources, identify whether they are likely sources of the organism through historical knowledge or knowledge of the agricultural practices involved in their production. Controls should then be introduced to minimise the occurrence of the hazard. Ideally this should involve implementing and maintaining best practices in the production of the material, such as the introduction of a destruction stage but, in the absence of such options, the use of microbiological testing, appropriately applied, can be useful in indicating general hygienic quality of the material and can allow differentiation of poor supply points from those operating better standards. For example, sprouted seed salad producers, e.g. beansprouts, should routinely monitor the microbiological quality of the seed batches for the presence

of *Salmonella* and *E. coli* O157. It must be remembered, however, that such testing can never give assurance of a pathogen's absence.

10.6 Control in processing

The majority of food products are manufactured with some form of processing and some processes are capable of reducing or eliminating VTEC, if present. In such circumstances, it is the application of effective controls at critical stages of the manufacturing process that, if properly and consistently applied, will generate a safe finished product. Products such as cheese made from pasteurised milk, cooked meats and ready meals are all subject to processes in which the organism should be effectively eliminated.

Processes used to produce products such as prepared, ready-to-eat salads, raw, fermented meats together with some hard cheeses made from raw milk usually result in the reduction of contaminating pathogens, but survival may occur, particularly if initial contamination levels are high. And, of course, there are some products such as soft cheese made from raw milk or sprouted seeds such as beansprouts or alfalfa sprouts where the production process can allow growth and lead to elevation of pathogen levels if present originally in the raw material. However, even with these products it is possible to identify production methods that, if applied correctly, can reduce the risk associated with their consumption.

The application of effective processes and, hence, process controls necessitates some understanding of the effect of different process stages on the growth and survival of potential pathogens that might be present. Once understood, it is then possible to introduce relevant systems for monitoring the processes.

10.6.1 Cooking

Heat processing is probably the most effective means of eliminating *E. coli* O157 and VTEC. Cooked meats, pasteurised milk and milk products such as cheese and yoghurt, prepared ready-to-eat meals and a wide variety of other products rely on a heat process to eliminate the organism, if present, in the raw materials.

The effective application of the heat process is critical to the safety of the product and no matter what the product, appropriate validation of the process must be conducted to ensure the consistent delivery of correct cooking times and temperatures. Cooking validation should be carried out prior to the introduction of a new product or process and must take account of all variables likely to affect the heat process. Cooking validation is intended to identify the conditions that will consistently produce a safe product. All of the following factors must be considered when developing a safe heat process:

- Minimum ingoing temperature of the material.
- Largest size/piece size of the product.
- Cold spots in the oven/temperature distribution of the oven.
- Fill load of the container, if appropriate, i.e. air spaces will affect heat transfer.

- Oven load.
- Minimum time setting of the process.
- Minimum temperature setting of the oven.

In other words, for a cooked meat product, a process validation should establish that a cook of 70 °C for 2 minutes will be achieved throughout the product when the largest size product, entering the process at the lowest initial temperature, is cooked in the slowest heating position in the oven with a full oven load and where the oven is set at its minimum time and temperature.

Following the identification of effective process controls it is then necessary to apply systems to monitor the efficacy of the process and these should be built into the quality system of the manufacturing plant. Therefore, checks should be undertaken of the ingoing raw meat temperature, the size of the product and the process times and temperatures. It is usual to utilise a continuous chart recorder to monitor and record process times and temperatures. In addition, product is also temperature probed at the end of the cooking stage and it is common to check products from the top, middle and bottom of each rack of product being cooked.

Cooking temperatures applied to destroy *E. coli* O157 and VTEC vary depending on the product type. Pasteurised milk is heat processed in the UK to comply with legislative requirements achieving 71.7 °C for 15 seconds. Other cooked foods such as meat are heat processed to achieve a minimum process of 70 °C for 2 minutes. Studies have demonstrated that this process is sufficient to significantly reduce (>6 log cfu/g) the levels of contaminating enteric pathogens including VTEC O157 (Advisory Committee on the Microbiological Safety of Food, 1995). Based on studies in chicken homogenate, Betts *et al*. (1993) determined the D_{64} of a strain of *E. coli* O157 to be 0.36–0.46 minutes with a z value of 5.2–7.3 °C. Orta-Ramirez *et al*. (1997) reported a D_{68} of 0.12 minutes in ground beef (z value 5.59 °C). Stringer *et al*. (2000) collated data from different studies on the thermal destruction of *E. coli* O157:H7 and found Z-values ranged between 3.9 °C and 7.3 °C depending on meat type used. Such studies underline the need to carry out validation studies relating to specific products and processes to ensure product safety.

10.6.2 Fermentation processes

Products produced through a fermentation process such as raw, fermented meat and raw milk hard cheeses rely on the fermentation and drying process to reduce levels of contaminants present in the raw material. Although not as effective or controlled as a cooking process, these products can achieve some reduction in levels of enteric pathogens. It is essential that the conditions necessary to achieve such reductions are clearly understood by those manufacturing these products. Outbreaks occurring in these types of products have usually involved deficiencies in the fermentation or drying/maturation process, e.g. slow acid production or too short a drying period, or post-process hygiene conditions. As a result of some outbreaks it has also become evident that historically ‘safe’ processes have now been shown to be incapable of eliminating VTEC (Anon., 1996).

Soft cheese made from unpasteurised milk together with raw milk hard cheeses and raw, fermented meats undergoing only short maturation/drying periods are more vulnerable to outbreaks of *E. coli* O157 and VTEC. This is due to the ability of the organism to survive the fermentation stage (Getty *et al*., 2000) and, in the absence of significant drying stages, little further reduction of the organism occurs.

Challenge test studies should be undertaken, using research facilities, to identify the critical process points that require effective control to achieve a desirable reduction in *E. coli* O157 and VTEC. In general, an active fermentation process resulting in rapid acid production and associated pH reduction together with a long drying stage (several weeks) combine to achieve at least a 2–3 log reduction in *E. coli* O157 (Glass *et al*., 1992; Anon., 1996). Reduced drying times and reduced acidity development have been shown to result in less reduction of this organism in fermented meats. The processes employed for the production of these products are highly variable and it is for this reason that it is advised that such products should have process challenge tests conducted to determine whether the process achieves a sufficient reduction of VTEC and, if not, what modifications can be introduced to achieve this. This may include lengthening the maturation stage or perhaps adding antimicrobial factors such as salt.

10.6.3 Washing processes

Many production processes rely on simple washing stages to remove extraneous dust and soil and to reduce levels of contaminating organisms including pathogens, e.g. ready-to-eat salads and vegetables and fresh, unpasteurised fruit juices. A variety of outbreaks involving these products have highlighted the vulnerability of the minimal processing employed in their production. Salad vegetables and fruit for juicing are usually washed in chlorinated water prior to further processing. Chlorine levels used in commercial practice have historically been at $\leqslant$ 200 ppm. Studies have shown this level to be capable of reducing levels of contaminating pathogens by 1–2 log units.

Beuchat (1999) showed that *E. coli* O157 was reduced by *ca* 1–3 log units using a spray system to wash lettuce leaves with chlorinated water (200 ppm). However, rinsing in water alone achieved *ca* 1–2 log reduction and, at low levels of contamination, reductions were limited to *ca* 1 log unit with sprays of deionised water appearing as effective at reducing the organism as chlorinated water. When lettuce leaves were contaminated with *E. coli* O157 suspended in bovine faeces slurry at levels as low as 1.1 cfu/g, the organism could still be detected in 50 g samples of the product after spraying with chlorinated or deionised water.

Outbreaks associated with these product groups have, however, prompted processors to investigate much higher levels of chlorination or indeed the use of other sanitising agents in an attempt to achieve greater reductions (Taormina and Beuchat, 1999). Whatever method is used, it is important to ensure that the active ingredient, most often chlorine, is present in its active form on a continuous basis. Washing systems have just as much capacity to spread contamination as they have

for reducing it if the wash water is not regularly changed or the levels of active ingredient are not properly maintained. In addition, it is important to recognise that washing efficacy will also be dependent on facilitating good contact between the contaminant and the antimicrobial agent, and the surface structures of many vegetables and fruits can offer significant protection to microorganisms. Washing systems that incorporate means for agitation will clearly be helpful in reducing microorganisms on these plant material surfaces.

10.6.4 Sprouting processes

Sprouted seed vegetables have been implicated in a number of outbreaks caused by *E. coli* O157 (Gutierrez, 1996; Como-Sabetti *et al*., 1997) and other enteric bacteria such as *Salmonella* (O'Mahony *et al*., 1990; Taormina *et al*., 1999). These products are as vulnerable to microbial contamination as other prepared salads and fruits with one notable additional factor, germination in warm, wet conditions from seed. Because of this, any contaminating organisms, if present on the seed, are offered the opportunity for significant growth during the germination and growth of the sprouting seed. Levels of contamination on seed batches can increase by several orders of magnitude during the sprouting process (Jaquette *et al*., 1996; Hara-Kudo *et al*., 1997) and there is very little that can be done to prevent this from occurring if the organism is present initially on the seed. Taormina and Beuchat (1999) studied a variety of chemical treatments to reduce levels of *E. coli* O157 on alfalfa seed. Levels of 2000 ppm calcium hypochlorite, $Ca(OCl)_2$ gave a reduction of log 1.84 to $\geqslant$log 2.38 cfu/g after 3 minutes and log 1.83 to $\geqslant$log 2.5 cfu/g after 10 minutes. Treatment with 10 000 ppm and 20 000 ppm $Ca(OCl)_2$ gave reductions of $\geqslant$log 2.38 cfu/g after 3 minutes and $\geqslant$log 2.5 cfu/g after 10 minutes. These reductions were similar to those achieved with lower levels of hypochlorite. Although germination was not significantly reduced in comparison to water treatment, higher concentrations of hypochlorite reduced the rate of germination of the seed. It was also reported that *E. coli* O157, inoculated and then dried on alfalfa seeds at an initial level of log 3.04 cfu/g, were not significantly reduced when the seed was stored at 5 °C over a 20 week period and the organism could still be detected in 25 g samples of seed after 54 weeks.

Processors of these products usually employ extensive raw material control and testing programmes to monitor batches of seed for contamination. In addition, it is possible to test samples of spent water and germinated seed several days prior to harvesting and packing to gain an indication of contaminated production lots. Clearly, this cannot achieve total control of safety but in the absence of other suitable controls it is an important element of safety assurance for these types of product.

10.6.5 Hygiene and post-process contamination

A recurring factor often identified as a reason for outbreaks of *E. coli* O157 and VTEC infection is contamination of the product after application of the pathogen

reduction process. Outbreaks implicating pasteurised milk, cheese, cooked meats and prepared salads and fruit have all indicated this area as a source of product contamination.

If a process has a recognised pathogen reduction or destruction stage, it is essential to avoid contaminating the finished product after this stage. Contamination usually arises from two sources: exposure of the finished product to the raw material(s) or exposure of the product to contamination from the environment or people.

Facilities and procedures for segregation must be in place to prevent raw materials coming into contact with finished products. In the manufacture of cooked meat, ready meals or prepared salads this is usually achieved by separating the factory into two areas, the low- and high-risk areas. The pathogen reduction stage is used as the division between the two areas such as the building of an oven into a separating wall or the placing of a chlorinated wash water flume between high- and low-risk operations. Individuals on the low-risk side handle the raw product and then, after processing, it is removed by individuals dedicated to the high-risk side. The principles of segregation should be applied to all personnel moving from the low-risk side of the factory to the high-risk side, with appropriate coat and footwear changes and appropriate hand-washing procedures. An infectious disease policy must be in place for operatives handling ready-to-eat products, which should include notification of any instances of infectious disease and associated absence from work.

Effective cleaning and disinfection of all equipment is essential. It is particularly important to prevent the organism from building up in the equipment used for processing raw materials. High levels at these stages may result in spread of contaminants to other batches or excessive levels that may exceed the capacity of the subsequent process, e.g. cooking or fermentation to reduce initial levels of any bacterial pathogens to those resulting in a safe finished product. Cleaning efficacy can be effectively monitored using indicators of hygiene such as tests for coliform bacteria or ATP bioluminescence tests, which monitor the presence of residual levels of ATP from product residues and microorganisms.

Small manufacturers and butcher shops handle and process both raw and cooked meats in facilities that are not subject to the same level of sophisticated segregation controls in place at large manufacturers. However, the principles of separation between raw and cooked meat in processing and on display together with effective cleaning and sanitisation of surfaces and equipment are just as important to ensure product safety.

10.7 Final product control

Many products are sold in pre-packaged units that are not subject to further introduction of contaminants during distribution and retail display. However, other products are sold either loose or are subject to further preparation in the retail or catering outlet such as cooked meats that may be sliced. Outbreaks of *E. coli*

O157 have resulted from the poor segregation of raw meats from ready-to-eat products during storage and sale.

Effective segregation between raw products and those intended to be consumed as ready-to-eat must be operated in both the retail and catering environments. This includes using separate counters for displaying raw and cooked foods or having perspex/safety glass dividers in counters to keep them apart. Safe procedures for serving, slicing or weighing raw and ready-to-eat foods must be developed. Personnel carrying out such practices should be appropriately trained to understand the risk associated with cross-contamination from raw foods and from items coming into contact with raw foods such as equipment, surfaces and hands and they should be provided with the appropriate facilities to operate good hygienic practice e.g. hand washing basins and supplies.

10.7.1 Post-process additives

Some products have additives placed on them as a final garnish including pâtés and cooked meats. Clearly, the garnish or post-process additive must be subject to appropriate treatments and controls to ensure it will not add pathogens, including VTEC, to the garnished product. It is common for many garnish materials to be subject to some form of disinfection such as the washing of fresh herbs and/or they may be subject to a testing programme or requirement for supplier certification of compliance to a specification prior to use.

10.7.2 Labelling of products

The consumer often has a significant role to play in ensuring safety with respect to many foods. It is essential that appropriate information is given on the product packaging to provide guidance concerning safe handling and storage conditions and the preparation or cooking processes that need to be employed.

Food safety tips are often present on raw meat packaging as a reminder of the need to ensure separation of raw from ready-to eat foods during storage and preparation and also of the need for effective washing of hands, surfaces and utensils when handling raw and then ready-to-eat foods. In the USA this information is mandatory on raw meat products, but in the UK, some manufacturers and retailers choose to place this advice on products voluntarily.

Cooking instructions for raw foods such as beefburgers and meats are important to help guide consumers as to the approximate times and temperatures necessary to achieve an appropriate cook. The generation of this guidance needs to be subject to very careful assessment owing to the extremely wide variation in cooking temperature that can result from different cooking appliances and methods, etc. All the following variables should be carefully considered when generating cooking instructions for products:

- Heating appliance (gas, electric or microwave oven).
- Preheating of the heat source.
- Distance from the heat source.

- Number of products cooked together.
- Length of the cook.
- Temperature/power setting of the heat source.
- Turning frequency of the product.

Owing to the inherent variability of cooking processes in the home it is also common for cooking instructions to include general indicators for cooking efficacy such as 'ensure it is cooked until the centre is piping hot and until the juices run clear'.

Some products, even with all of the applied process controls, are not considered to be acceptably safe and are also provided with labels of warning or, indeed, advice to avoid their consumption. An example of this includes sprouting salad vegetables such as alfalfa sprouts in the USA which, because of the high number of serious outbreaks, the Food and Drug Administration issued advice to avoid the consumption of raw sprouts in order to reduce the risk of foodborne illness (Food and Drug Administration, 1999). Similarly, in the USA, unpasteurised fruit juice must be labelled with statements indicating that microbial dangers exist with the consumption of this type of product.

10.8 Future trends

It is clear that with the continued rise in VTEC infections, the combined conscientious effort of all those involved in producing, processing and selling food as well as those preparing food at home is required to ensure that food is ultimately kept safe for consumption from the hazard of VTEC. Governments should facilitate a positive partnership between all these groups to ensure that clear and consistently correct information about VTEC is available and acted upon.

10.9 References and further reading

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11

Salmonella

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11.1 Introduction

Salmonella species have been recognised for over 100 years now as the cause of illnesses ranging from mild to severe food poisoning (gastroenteritis), and even more severe typhoid (enteric fever), paratyphoid, bacteraemia, septicaemia and a variety of associated longer-term conditions (sequelae). Some of these severe conditions can incur high rates of morbidity and mortality and can occur in outbreaks involving large numbers of people, particularly in relation to typhoid outbreaks and septicaemic conditions.

From the time of probably the first laboratory confirmed outbreak of salmonellosis in 1888, when Gaertner isolated *Bacterium enteritidis* (later renamed *S. enteritidis*) from the meat of a cow and the organs of a man who consumed a large portion of the meat and who subsequently suffered fatal food poisoning (Topley and Wilson, 1929), *Salmonella* spp. have been considered some of the most important of the causal agents of foodborne illness throughout the world. The large number of outbreaks of foodborne salmonellosis that still occur in many countries are testimony to the importance of this bacterial genus in terms of morbidity and mortality.

Many foodborne incidents of salmonellosis are recognised as being sporadic in nature, occurring as apparently isolated cases. Improved methods of investigation of foodborne disease together with advancements in the collection and sharing of food poisoning statistics among developed countries has now allowed many of the sources of these organisms to be identified. This has also demonstrated quite clearly the importance of *Salmonella* spp. as some of the main causative agents of foodborne disease outbreaks, some of which have affected large numbers (up to many thousands) of individuals from a single food source.

Although a great deal is already known about *Salmonella* spp., these organisms continue to provide new challenges to food safety, particularly because of the evolution of new strains resulting from the acquisition of genes conferring characteristics such as multiple antibiotic resistance. There is, therefore, a continuing need for research and information concerning the origins and characteristics of these new strains so that practical control measures can be applied in the food industry. In addition, because of the ubiquitous nature of *Salmonella*, there is a need to further develop new production and processing methods to assist in the control of all *Salmonella* from all sources.

As for all foodborne bacterial pathogens, it is essential that a detailed and competent hazard analysis is carried out at an early stage in all food product and process developments to ensure that relevant critical controls and monitoring systems can be put in place. This will help to minimise potential public health problems that could arise from the presence and outgrowth of *Salmonella*.

This chapter aims to give an overview of *Salmonella* spp. in respect of the hazard they present to food products and the means for controlling these organisms.

11.2 Characteristics of *Salmonella*

In 1885, an American bacteriologist DE Salmon characterised the hog cholera bacillus causing 'swine plague' which, at that time, was named *Bacterium suipestifer* but later renamed as the type species of the genus named after him, *Salmonella cholerae-suis*. It was not until the 1960s, however, that the name *Salmonella* became the widely accepted name for this genus of the family Enterobacteriaceae.

Salmonella spp. are facultatively anaerobic, Gram negative, straight, small ($0.7–1.5 \times 2.0–5.0\,\mu m$) rods, which are usually motile with peritrichous flagella. Table 11.1 shows some key biochemical characteristics of *Salmonella.* Strains of *Salmonella* are antigenically distinguishable by agglutination (formation of aggregates/clumping) reactions with homologous antisera and the combination of antigens possessed by each strain, referred to as the antigenic formula, is unique to each *Salmonella* serotype. Extensive work on differentiation of the antigens on the surface of the bacterial cell where these are present, i.e. O, the somatic or outer membrane antigens; H, the flagella antigens and Vi, the capsular antigens has led to the current recognition of almost 2400 serotypes, a number that is increasing every year. Additional methods for differentiating strains of *Salmonella* serotypes are also used and phage typing (determined from the sensitivity of cells to the lytic activity of selected bacteriophages) has become a valuable method of particular importance in epidemiological studies and in tracing foodborne outbreaks of salmonellosis.

In recent years, the nomenclature of *Salmonella* has been the subject of considerable debate (Old, 1992; Old and Threlfall, 1997; Threlfall *et al*., 1999) and most *Salmonella* serotypes are now designated to one species, *S. enterica.* Seven subspecies of *S. enterica* have been recognised, although one, *S. enterica* subsp.

Table 11.1 Biochemical characteristics of *Salmonella*

Characteristic	Usual reaction
Catalase	+
Oxidase	–
Acid produced from lactose	–
Gas produced from glucose[a]	+
Indole	–
Urease produced	–
Hydrogen sulphide produced from triple-sugar iron agar	+
Citrate utilised as sole carbon source[a]	+
Methyl Red	+
Voges–Proskauer	–
Lysine decarboxylase	+
Ornithine decarboxylase	+

+ = positive reaction; – = negative reaction.
[a] an important exception is Typhi which is negative in these tests.
Source: Bell and Kyriakides (2002) from Brenner (1984) and Le Minor (1984).

bongori, is believed to represent a distinct species, *S. bongori.* Most of the previous *Salmonella* species associated with foodborne illness belong to the new subspecies *enterica*, i.e. *Salmonella enterica* subsp. *enterica*. Owing to the long names now assigned to the organisms, i.e. *Salmonella enterica* subsp. *enterica* serotype Typhimurium (previously *S. typhimurium*), these are now shortened to *S.* Typhimurium or, indeed, Typhimurium. The current practical nomenclature style of serotype names will be used in this chapter together with any additional descriptive information that is supplied for some of the more predominant *Salmonella* serotypes, e.g. provisional phage type (PT) or definitive phage type (DT), e.g. *S.* Typhimurium DT104.

Some *Salmonella* serotypes are specifically host-related, for example, *S.* Gallinarum and poultry, *S. Choleraesuis* and swine and *S*. Typhi and *S*. Paratyphi A and humans. Some strains, however, are more infectious to some animals, e.g. *S*. Choleraesuis to pigs and *S*. Dublin to cattle but they may still cause infections in humans. Uzzau *et al*. (2000) advocated referring to those serotypes that are almost exclusively associated with one particular host species as host restricted, e.g. *S.* Gallinarum and *S.* Typhi; serotypes that are prevalent in one host species but that can cause disease in other host species are referred to as host adapted, e.g. *S*. Dublin. Serotypes that cause infection in a broad range of unrelated species are referred to as unrestricted, e.g. *S.* Typhimurium and *S.* Enteritidis.

Salmonella spp. cause illness by means of infection. They multiply in the small

intestine, colonising and subsequently invading the intestinal tissues, producing an enterotoxin and causing an inflammatory reaction and diarrhoea. The organisms can get into the blood stream and/or the lymphatic system and cause more severe illnesses. Table 11.2 summarises the types of illness caused by different *Salmonella* serotypes. Although most people are vulnerable to salmonellosis, certain groups are more vulnerable to infection; young children, the elderly and those with underlying chronic illness or immunocompromised individuals being particularly vulnerable to salmonellosis. Although *Salmonella* can persist in faeces during convalescence following illness, excretion usually declines and eventually ceases. However, in a few cases, excretion can persist such that the individual becomes a chronic carrier of the organism. In such cases, the organism may be excreted only intermittently but it makes such individuals, particularly if employed as a food handler, a potential hazard to food safety and public health. Deaths resulting from salmonellosis (mild to moderate gastroenteritis) in outbreaks are rare although they do occur, and normally healthy individuals usually recover from salmonellosis with supportive treatment including fluid and electrolyte replacement and without the need for antibiotics.

In developing countries, infection by *Salmonella* more commonly results in severe gastroenteritis and in some outbreaks, up to 40% of cases may develop septicaemia and 30% of cases may die. Multiple-antimicrobial agent resistant strains of *Salmonella* are often involved in these outbreaks (Old and Threlfall, 1997). Strains of *S.* Typhimurium DT104 commonly resistant to at least five and sometimes more antibiotics are, at present, increasingly causing food-associated outbreaks of severe gastrointestinal infection (Anon., 2000a; Besser *et al*., 2000). The increasing occurrence of antibiotic-resistant *Salmonella* in cases of human illness is now of major concern.

The numbers of *Salmonella* infections recorded by the Public Health Laboratory Service for England and Wales between 1990 and 1997 ranged from 27 693 (1991) and 32 596 (1997) (Anon., 1999) but fell to 23 728 in 1998, to approximately 17 250 in 1999, 14 845 (provisional figure) in 2000 (Anon., 2000a, 2001) and then slightly increased to c. 16400 in 2001 (Anon., 2002a). These figures are recognised as being an underestimate of the real numbers of cases occurring; for every confirmed case there are estimated to be a further 3.2 cases in the community (Anon., 2000b). This recent reduction is probably as a result of public health and industry measures to control *Salmonella*, particularly in raw foods such as eggs and poultry.

The infective dose for *Salmonella* has, for decades, been considered to be in excess of 100 000 cells but a number of outbreaks have been recorded, particularly implicating products containing a high fat level, e.g. chocolate, cheese and salami, in which the infective dose was found to be very low, e.g. <10–100 cells. The realisation that such low levels could cause illness led to a reappraisal of industry controls which has taken place over the past two decades. The development and application of Hazard Analysis Critical Control Point (HACCP) assessments to each food process from raw materials through to expected consumer handling practices and the consequent implementation of relevant standards of

Table 11.2 Illnesses caused by *Salmonella*

Illness	Serotypes involved	Infective dose/cause	Characteristics of the illness
Gastroenteritis	Mainly members of *S. enterica* subsp. *enterica* (the majority of serotypes are in this subspecies, e.g. Agona, Dublin, Hadar, Enteritidis, Poona, Typhi, Typhimurium, Virchow. Also, members of *S. enterica* subsp. *arizonae*.	Usually high numbers (>10 000 cells) required to cause illness but where organisms are protected, e.g. in high-fat foods, low numbers (<100 cells) may cause illness.	Incubation 12–72 h, commonly 12–36 h. Lasts 2–7 days. Symptoms: diarrhoea (dehydration if this is severe), abdominal pain, vomiting, fever, sometimes fatal. Prolonged excretion may occur.
Enteric fever	*S.* Typhi and *S.* Paratyphi.	Infective dose may be <1000 cells.	Incubation 7–28 days, average 14 days. Typhoid. High fever, malaise, nausea, abdominal pain, anorexia, delerium, constipation in the early stages, diarrhoea in the late stages. Convalescence may take up to 8 weeks. Carrier state can last several months to years.
Septicaemia or bacteraemia	Members of *S. enterica* subsp. *enterica*.	Caused when *Salmonella* are present in the blood stream.	High fever, malaise, pain in the thorax and abdomen, chills and anorexia.
Sequelae	Members of *S. enterica* subsp. *enterica*.	Morbid condition that occurs as the result of a previous disease, e.g. salmonellosis.	Uncommon. A variety have been identified including arthritis, osteoarthritis, appendicitis, endocarditis, pericarditis, meningitis, peritonitis and urinary tract infections.

Source: adapted from Bell and Kyriakides (2002) using information from Anon. (1995) and ICMSF (1996).

agricultural practice and appropriate design and hygienic operation of all food production processes has helped to minimise the incidence and level of *Salmonella* at all stages of the food chain.

In any food production process, attention to the detail of in-process hygiene systems and procedures is important in the control of extraneous microbiological contamination. In addition, methods used in the actual manufacturing and handling processes and also the formulation of food products can be important in the control of any residual *Salmonella* in foods and preventing their potential to cause illness in consumers. Table 11.3 indicates some of the key growth-limiting parameters for *Salmonella*.

Salmonella is readily destroyed by heat in foods with a high water activity, e.g. $\geqslant$0.98 but in foods materials with a low water activity, e.g. high fat content, much higher temperatures are needed to kill the organism (Table 11.4). In frozen foods or those in which the water activity is low, *Salmonella* has been shown to survive for many months, even years (Table 11.5). Although the growth of *Salmonella* is believed to be controlled by low (refrigeration) temperatures and industry relies heavily on refrigerated storage of fresh foods to maintain their

Table 11.3 Limits for the growth of *Salmonella* under otherwise optimal conditions

Parameter (other conditions being optimal)	Minimum	Maximum
Temperature (°C)	5.2[a]	46.2
pH	3.8[b]	9.5
Water activity	0.94	>0.99

[a] Most serotypes will not grow at <7.0 °C.
[b] Most serotypes will not grow below pH 4.5.
Source: Bell and Kyriakides (2002), adapted from ICMSF (1996).

Table 11.4 *D*-values for *Salmonella* in some food substrates

Food substrate/ conditions	Temperature (°C)	*D*-value	Reference
Milk (sterile, homogenised)	68.3	0.28–10 s depending on serotype	ICMSF (1996)
Ground beef	63	0.36 min	
Milk chocolate	71	4.5–6.6 h depending on serotype	Lee *et al.* (1989)
Liquid whole egg	60	0.55–9.5 min depending on pH and serotype	D'Aoust (1989)

Table 11.5 Some survival data relating to different *Salmonella* serotypes

Condition	Serotype	Food	Temperature (°C)	Survival time	Reference
Freezing temperatures	Enteritidis Cholerae-suis Typhimurium Enteritidis Typhimurium	Poultry Minced beef Chow mein Ice cream	–18 –18 –25 –23	4 months 4 months 9 months 7 years	D'Aoust (1989)
Low water activity	Typhimurium PT10	Cheddar cheese	5	8 months	D'Aoust *et al*. (1985)
	Naturally contaminated with Typhimurium, Java, Blockley	Milk powder product samples		Up to 10 months	Ray *et al*. (1971)
	Infantis Typhimurium	Pasta		Up to 12 months	Rayman *et al*. (1979)
	Eastbourne	Milk chocolate (a_w 0.41)		>9 months	Tamminga *et al*. (1976)
	5-serotype culture: Agona Enteritidis Michigan Montevideo Typhimurium	Peanut butter (a_w 0.2–0.33)	5 21	up to 24 weeks up to 6 weeks	Burnett *et al*. (2000)

safety in relation to pathogen outgrowth, there have been some reports (summarised by D'Aoust, 1991) of growth of *Salmonella* in shell eggs at 4 °C and in minced meat and chicken parts at 2 °C. Some of these reports are, as yet, unconfirmed other than by observation of growth on microbiological media (ICMSF, 1996).

It is of concern to note that recent studies have shown that *S.* Enteritidis PT4 and *S.* Typhimurium DT104 increase in biomass in milk, chicken and microbiological media at 4 °C and in matrices at low water activities (0.93–0.98) due to filament formation rather than cell division (Phillips *et al*., 1998; ILSI, 2000; Mattick *et al*., 2000). Further work needs to be done to determine if there are any public health implications of this phenomenon. In the meantime, temperature, pH, water activity and other physico-chemical factors, used either singly or in combination, are still used effectively in the control of the survival and growth of *Salmonella* during manufacturing processes and also in the finished food products (Bell and Kyriakides, 2002).

Outbreaks of salmonellosis have been caused by foods produced commercially, by private caterers, e.g. for special events, and by domestically produced foods including hotels, homes for the elderly, hospitals, schools and individual households, and most, if not all, were preventable. In the commercial food maufacturing industry and large catering companies, *Salmonella* is commonly included in buying specifications relating to raw materials and finished food products so that there is some evidence from specific microbiological examinations that can be used for supporting both hazard control and due diligence requirements. The organism is also included in some industry guidelines, e.g. Code of hygienic practice based on HACCP for the prevention of *Salmonella* contamination in cocoa and chocolate and confectionery products, published in 1991 by the International Office of Cocoa, Chocolate and Sugar Confectionery, Brussels, Belgium, and in a number of microbiological standards written into food legislation, e.g. for egg products (Anon., 1989), live bivalve molluscs (Anon., 1991a, 1993a), fishery products (Anon., 1991b), raw milk, heat-treated milk and milk-based products (Anon., 1992) and minced meat and meat preparations (Anon., 1994a). In the UK, the Public Health Laboratory Service (PHLS) has published microbiological guidelines (Gilbert *et al*., 2000) that provide advice on the interpretation of results from certain types of microbiological examination of ready-to-eat food sampled from retail sale. Although having no statutory status, they could also be used in prosecution cases by Environmental Health departments. If *Salmonella* is found to be present in 25 g of any of the product groups described, then the advice given is to consider the food 'unacceptable – potentially hazardous'.

In the food manufacturing industry, confirmed detection of *Salmonella* in ready-to-eat products or those destined for vulnerable groups usually leads to complete removal of products from the distribution and retail system and, on occasion, to public recall and closure of the manufacturing line or unit pending full investigation and action (Bell and Kyriakides, 2002).

11.3 Detecting *Salmonella*

It is generally understood that, if *Salmonella* is present in a food, because there are likely to be only low numbers of heterogeneously distributed cells within the batch, a large number of samples must be taken to gain a high degree of confidence in the detection of the organism (ICMSF, 1986). This is especially important when foods are destined for vulnerable groups and it is common to find industry specifications requiring 'not detected in 250 g test portions' and several such test portions per batch being examined against this specification for materials such as milk powder destined to be used for baby food products. The buying specification for many other processed and ready to eat food materials and products such as herbs and spices, egg products, cooked meats and ready meals usually include criteria to be met for *Salmonella*; a common target level applied is 'not detected in 25 g' of three or five, 25 g test portions with no tolerance of acceptance, i.e. if one test portion is positive, the batch is withheld or rejected.

Useful sampling plans and recommended microbiological limits for *Salmonella* have also been published in relation to some foods in international trade (ICMSF, 1986).

Reliable methods for detecting and identifying *Salmonella* are important in the support of properly developed and implemented HACCP-based systems for their control. Table 11.6 indicates the common conventional microbiological method, which is widely used by food microbiologists to isolate and identify *Salmonella.* There are also a wide range of microbiological techniques available for the detection and characterisation of *Salmonella* and this range is increasing rapidly as the considerable investment in method/techniques development results in commercialisation of products. A valuable summary of methods together with validation information and key references has been prepared and is periodically updated by Campden and Chorleywood Food Research Association (Baylis, 2000).

There are a variety of miniaturised and automated biochemical kits/systems for distinguishing *Salmonella* from other members of the Enterobacteriaceae. However, some strains occasionally exhibit atypical biochemical reactions and some care is necessary particularly when assessing cultures on selective plates to ensure false negative results are minimised/avoided (Threlfall *et al*., 1983). Because of this and also because different strains of *Salmonella* may be sensitive to different combinations of inhibitory substances and high incubation temperatures, combinations of selective enrichment broths, selective plating media and incubation conditions are used to increase confidence in results from tests for detecting *Salmonella.* It is important to ensure that the methods used for the detection of *Salmonella* in specific food types are fully validated.

Following confirmation of the identity of *Salmonella*, it is important for food surveillance purposes and the investigation of outbreaks, to subtype or 'fingerprint' *Salmonella* serotypes. There are a variety of techniques available now that can allow confident traceability of strains in factory environments. These include biotyping, serotyping (including variation in H antigens), phage typing,

Table 11.6 Conventional method for the isolation and identification of *Salmonella* spp.

Pre-enrichment[a]	
Inoculate pre-enrichment medium e.g. buffered peptone water (1 part test portion + 9 parts medium)	Day 0
⇓ ⇓	Incubate 35 °C or 37 °C/16–20 h
Selective enrichment	
Subculture to 2 selective enrichment broths, e.g. Selenite-Cystine (SC) Medium (1 + 9), Rappaport Vassiliadis (RV) broth (1+ 100)	Day 1
⇓ ⇓	Incubate SC 37 °C/24 h + 24 h RV 42 °C/24 h + (if necessary) 24 h
Selective plating	
Streak onto 2 selective agars, e.g. Brilliant Green Agar (modified) Hektoen Enteric Agar XLD Agar	Days 2 and 3
⇓ ⇓	Incubate 35 °C or 37 °C/20–24 h and a further 18–24 h if necessary
Inspect plates for the presence of characteristic colonies and any primary biochemical reactions	Days 3/4 or 4/5
Confirmation of suspect colonies	
Purify suspect colonies on Nutrient Agar	Incubate 35 °C or 37 °C/18–24 h
⇓	Days 4–6
Serology 'O' & 'H' Inoculate media or test strips to obtain biochemical profile	
⇓ ⇓	Incubate according to the manufacturer's instructions, usually 35 °C or 37 °C/18–24 h
Read reactions	Days 5–7

[a] Alternative pre-enrichment systems may be required for some food types, e.g. chocolate and confectionery products, reconstituted Skim Milk Powder (10%w/v) with Brilliant Green dye (final concentration of 0.002%w/v) is commonly used; products that may contain inhibitory substances or products that may be osmotically active, e.g. some herbs and spices (oregano, cinnamon, cloves), honey, 1 : 100 dilution in Buffered Peptone Water, e.g. 25 g + 2475 ml may be used.
Source: Bell and Kyriakides (2002) based on Anon. (1998a).

antibiotic resistance patterns (resistotyping), various molecular typing methods including pulsed-field gel electrophoresis (PFGE) (Old and Threlfall, 1997) and ribotyping (Jones, 2000).

A wide variety of foods have been implicated in outbreaks of illness attrib-

uted to many different serotypes of *Salmonella.* Table 11.7 gives some indication of the wide involvement of *S*. Typhimurium in foodborne outbreaks and Table 11.8 the variety of *Salmonella* serotypes that have been implicated in outbreaks of illness associated with just salad and vegetable products. In addition, outbreaks of salmonellosis have implicated foods and food materials as diverse as potato salad, mustard dressing, black pepper, roast cuttlefish, savoury corn snack (yeast-based flavouring), infant cereal and toasted oats cereal, peanut butter and coconut. These continuing outbreaks of *Salmonella* foodborne illness involving most food types have continued to fuel extensive work programmes by both researchers and food industry scientists to further improve methods of detection and develop new controls aiming to eliminate contamination of food by *Salmonella*.

11.4 Control of *Salmonella* in foods

As has already been noted, a wide variety of food raw materials may be contaminated, albeit at low incidence and levels, by *Salmonella* and most food types have been implicated in outbreaks of salmonellosis. In particular, chicken and eggs, two of the most commonly consumed foods in many countries, are, year after year, among the most common food types implicated in food poisoning outbreaks reported in European countries, the USA and other industrialised countries (Evans *et al*., 1998; Trepka *et al*., 1999; Reporter *et al*., 2000).

Table 11.9 shows, from examples of a small number of outbreaks, how valuable information can be gained regarding factors that can be important for controlling the organism. Common underlying causes of foodborne outbreaks include:

- Contamination of the primary food raw materials, often from direct or indirect animal faecal contamination or cross-contamination from a contaminated source.
- Production processes with no stage that reduces or destroys the organism, e.g. cooking.
- Product is exposed to post-process contamination through poor personal or equipment hygiene practices.
- Product is consumed with no destruction/reduction process applied by the consumer, i.e. it is ready-to-eat.

It is probable that detailed and structured hazard analysis followed by the implementation and maintenance of all relevant controls at the critical points identified would have prevented most of the outbreaks attributed to commercially produced foods and everyone involved in the primary production, processing and sale of food should adopt a hazard analysis approach to food safety considering all relevant pathogens, including *Salmonella*. Indeed, this is a legal requirement in Europe (Anon., 1993b).

The International Commission on Microbiological Specifications for Foods,

Table 11.7 Examples of food-associated outbreaks of illness caused by *Salmonella* Typhimurium

Salmonella type	Year	Country	Suspected food vehicle	Cases
Typhimurium PT8	1953	Sweden	Raw meat	8845
Typhimurium	1974	USA	Apple cider	>200
Typhimurium PT204	1981	Scotland	Raw milk	654
Typhimurium PT10	1984	Canada	Cheddar cheese	>1500
Typhimurium	1985	USA	Pasteurised milk	16 284
Typhimurium	1985	Switzerland	Vacherin Mont d'Or cheese	>40
Typhimurium	1987	Norway and Finland	Chocolate products	361
Typhimurium DT124	1987–88	UK	Salami sticks	101
Typhimurium DT49	1988	UK	Mayonnaise	>76
Typhimurium DT12	1989	UK	Cooked meats	>545
Typhimurium DT141 and Enteritidis PT1A, PT4	1992	UK	Tiramisu (containing raw eggs)[a]	*ca* 98
Typhimurium	1995	Italy	Salami	83
Typhimurium 'DT12 atypical'	1997	France	Raw milk soft cheese – Morbier	113
Typhimurium DT104	1998	UK	Pasteurised milk	86
Typhimurium PT 135A	1999	Australia	Unpasteurised orange juice	>400

[a] Five separate outbreaks in the same year.
Source: adapted from Bell and Kyriakides (2002).

Table 11.8 Examples of produce-associated outbreaks of illness caused by different *Salmonella* serotypes

Salmonella serotype	Year	Country	Suspected food vehicle	Cases
Chester	1990	USA	Cantaloupe	295
Javiana	1990	USA	Fresh tomatoes	176
Poona	1991	USA	Cantaloupe	>400
Montevideo	1993	USA	Tomatoes	100
Bovismorbificans	1994	Finland	Sprouts	210
Stanley	1995	USA and Finland	Alfalfa sprouts	242
Newport	1995	USA and Canada	Alfalfa sprouts	133
Saphra	1997	USA	Cantaloupe	>20
Poona	2000	USA	Cantaloupe	>19
Enteritidis	2000	USA	Mung bean sprouts	45
Kottbus	2001	USA	Alfalfa sprouts	32*

* Anon., (2002b).
Source: adapted from Bell and Kyriakides (2002).

in a document prepared for the Codex Committee on Food Hygiene, indicated that, at present, *Salmonella* cannot be eliminated from most farms, slaughtering processes, raw milk, fruits and vegetables although good hygienic practices and properly implemented HACCP systems may minimise or reduce levels of contamination (Codex Alimentarius Commission, 1996). It is therefore prudent to expect that *Salmonella* will be present at some incidence and level in raw material foods and to ensure adequate control measures are in place to prevent the organism from becoming a hazard to consumer health.

The serotypes of *Salmonella* reported as the most prevalent causes of foodborne salmonellosis do change over the years. *S.* Typhimurium was the most common in the USA and UK up until the mid-1980s, then *S.* Enteritidis superceded *S.* Typhimurium because of a large rise, mainly in egg-related outbreaks, particularly involving *S.* Enteritidis PT4. Other serotypes have waxed and waned on the list of 'top ten' serotypes causing foodborne salmonellosis, and through epidemiological studies, particular food sources can be identified, e.g. in 1997, *S.* Enteritidis PT6 became the second most commonly reported phage type of *S.* Enteritidis in the UK (>1600 cases) and was attributed to shell eggs and poultry meat. In more recent years, cases attributable to multiple antibiotic-resistant *S.* Typhimurium DT104 have increased involving meat products, raw milk and products made from raw milk as well as direct contact with livestock.

Whatever the *Salmonella* serotype, effective controls for minimising/eliminating the hazard of *Salmonella* from foods are the same and involve control of the following:

- Raw materials.
- Personal, equipment and environmental hygiene.
- Manufacturing process conditions.

Table 11.9 Examples of foodborne outbreaks of illness caused by *Salmonella* and possible reasons for their occurrence

Outbreak	Organism	Product(s)	Possible reasons for occurrence
1973/74, USA/ Canada	*S.* Eastbourne	Chocolate balls and other chocolate novelty confectionery	• Raw cocoa beans contaminated with *Salmonella* and subsequent inadequate heat processing • Inadequate segregation of roasted beans from raw beans • Heat processing of subsequent chocolate liquor not adequate to destroy *Salmonella* due to the low water activity of the liquor • Consumption by vulnerable groups
1985, England	*S.* Ealing	Spray dried milk powder used for infant formula	• Contaminated water used to test wet-down integrity of spray drier • Water and bacterial contaminant leakage into the insulation cavity of the spray drier and milk powder contamination of the insulation cavity via a hole in the inner skin • Contaminants proliferate in the insulation cavity and are re-introduced into the spray drier • Consumption of the product by vulnerable groups
1994, USA	*S.* Enteritidis	Commercially produced ice cream	• Pasteurised ice cream premix transported in a trailer previously used for raw egg • Transport trailer inadequately cleaned/sanitised between loads • Raw material used in a product not subjected to a further pathogen destruction stage

Source: adapted from Bell and Kyriakides (2002).

- Post-process contamination.
- Retail and catering practices.
- Consumer handling.

11.5 Raw material control

Like many other enteric pathogens, contaminated raw materials are common routes by which *Salmonella* enters the food chain. Where such contaminated material is not subject to sufficient decontamination by the food processor or by the consumer, prior to consumption, or indeed, where cross-contamination occurs to ready-to-eat foodstuffs, outbreaks of food poisoning can occur.

A wide range of contaminated raw materials has been implicated in outbreaks of salmonellosis, although initial contamination is often traced back to human or animal sources. Key raw materials implicated in outbreaks include raw meat (poultry, cattle, pigs and sheep), raw milk, raw eggs, contaminated fruit and vegetables, contaminated water, and fish and shellfish. In most of these cases, it is cross-contamination of the organism from faeces (either animal or human) to the food material that presents the problem to the food processor or handler. Recognition of these sources of contamination and implementation of procedures to reduce or eliminate the organism is an important component of any strategy to deal with *Salmonella.*

11.5.1 Carriage of *Salmonella* in animals

Salmonella spp. can cause severe infections in animals and although some such strains are host restricted, i.e. *S.* Gallinarum (poultry), many strains cause little illness in the animal which nevertheless still serves to amplify the organism until its passage to the human host, in which more severe illness can occur.

A recent survey of rectal contents (1 g samples) of cattle and sheep presented at slaughter in Great Britain found only 2 out of 891 cattle and 1 out of 973 sheep rectal samples to be contaminated with *Salmonella* (Evans, 2000). The serotypes isolated from the cattle were *S.* Typhimurium DT193 and DT12 and from the sheep *S.* Typhimurium DT41. In contrast, a survey of caecal contents (10 g samples) from pigs taken in abattoirs in the UK found a 23% incidence of *Salmonella* (Davies, 2000), although clearly some of this difference must be attributable to the much larger sample size in comparison to the studies on cattle and sheep. The predominant isolates from the pig caeca were *S.* Typhimurium (11.1%) and *S.* Derby (6.3%) and the predominant phage types of *S.* Typhimurium were DT104 (21.9% of isolates of Typhimurium from caeca) and DT193 (18.7%).

Although *Salmonella* is most often associated with poultry, the incidence of the organism in live birds is not well documented as most effort is directed towards assessing contamination of the flock through combined samples of litter or, of the finished product through carcass sampling. Contamination can spread quickly through a poultry house as high levels build up in the chicken intestine.

Both feed and water and external body surfaces of the bird, i.e. feathers, feet, can become contaminated with faeces, thus spreading infection. Some strains such as *S.* Enteritidis are also invasive and can spread to the reproductive tissues of the bird through which they can pass to the egg and chick. Although rather a selective study, the data from UK statutory reports of *Salmonella* isolates from chickens (layers, broilers and breeders) in 1998 show many exotic serotypes including *S.* Mbandaka (10%), *S.* Senftenberg (8%), *S.* Montevideo (8%) as well as the more common *S.* Enteritidis (21%) and *S.* Typhimurium (7%) (Anon., 2000c).

Control of *Salmonella* in food animals and poultry is extremely difficult and is affected by diet, health, environmental factors and exposure to the organism in the first place. Clearly, contaminated feed can be a major source of the organism to these animals and considerable effort is required to prevent such sources of contamination, particularly where feeds are derived from industrial processes such as feed mills. Use of heat-treated feed, adequately pasteurised and where the raw ingredients are properly segregated from the finished product is preferable, particularly for breeding stock animals. When grazing animals, it is clearly preferable to avoid using grazing land that may be contaminated with potential sources of pathogens such as human and animal wastes. Where these are to be applied, this should be done using properly composted manure or treated waste to prevent recycling of pathogens. Clearly, there is very little that can be done to prevent natural deposition of faeces by animals onto land but if the animal is not infected in the first place then this source presents less of a risk.

Once introduced, *Salmonella* can spread quickly in conditions where animals are reared in close proximity such as poultry in sheds and every effort must be made to prevent the organism entering such sheds through control of the feed, water and environment. Pest control and control of human entry are key to a successful strategy for controlling *Salmonella*. Proper hand washing and boot changing/disinfection are essential to keep the organism out of poultry houses. A strategy that has achieved successful reduction of *Salmonella* and *S.* Enteritidis in particular in recent years, has been the introduction of vaccination. While this has been adopted widely for the laying flocks in the UK it has yet to gain a similar application to broiler flocks, which owing to the high cost per bird is unlikely to occur until the introduction of a live vaccine that could be given in the feed rather than the current dead vaccine injected into each bird.

Spread of the organism is also affected by animal husbandry and transportation practices where waste shed by one animal during transport to slaughter rapidly becomes spread to others owing to their close proximity in the vehicle and in the case of poultry where cages are often stored above each other. Reducing feed prior to transport (without causing undue starvation) together with means of preventing spread of waste are important considerations in preventing extensive contamination of live animals entering the slaughter house.

It is also important to recognise the role of effective cleaning and decontamination procedures for sheds, lairage and transportation cages/vehicles in avoiding the spread of contamination to successive batches of animals. It is possible

to reduce the incidence of pathogens such as *Salmonella* in animal herds and flocks through the application of known intervention measures but it must be recognised that such pathogens will be present in herds and flocks, from time to time, and subsequent processes must always be operated with this in mind.

11.5.2 Raw milk

Contamination of raw milk with *Salmonella* usually occurs as a result of the transfer of faeces from the animal to the milk via unclean teats and udders. Such contamination can pass into the milk during milking and once present on milking parlour equipment can readily proliferate and spread if such equipment is not adequately cleaned and sanitised. Notwithstanding the reports of growth/biomass increase at temperatures as low as 2–4 °C (D'Aoust, 1991; Phillips *et al*., 1998; ILSI, 2000; Mattick *et al*., 2000) growth in the milk should be limited by effective refrigeration (<8 °C). As described for other enteric pathogens (see Chapter 10), effective milking parlour hygiene (cleaning and disinfection of udders and teats), cleaning and sanitisation of milking equipment and subsequent milk storage systems are essential elements in preventing the spread of this organism.

Routine microbiological monitoring of the hygienic quality of raw milk should be employed using indicator bacteria such as *E. coli* or coliforms and incentive payment schemes should be considered where the milk is intended to be used without a bacterial destruction stage in the process, i.e. for raw milk cheeses, to encourage the adoption of high hygienic standards.

Microbiological surveys of raw milk for indicators of contamination have already been reviewed (see Chapter 10). Like many pathogens, *Salmonella* is not commonly found in surveys of raw milk owing to its relatively low incidence (usually <1%). Surveys of raw, bulk cows' milk in the USA and raw cows' drinking milk in the UK have found it to be present at 4.7% and 0.06%, respectively (McManus and Lanier, 1987; Anon., 1998b). When present, it is rarely found at enumerable levels, i.e. >1/ml.

11.5.3 Raw meat and eggs

Raw poultry (including birds such as chicken, duck and turkey), beef, lamb and pork are all commonly contaminated with *Salmonella* and, not surprisingly, have all been implicated in outbreaks of salmonellosis. Although some contamination of the deep muscle of meat is possible, particularly with some invasive strains of *Salmonella*, this is the exception to the general rule that most contamination occurs on the surfaces of the raw meat/carcass. Contamination occurs through the transfer of faeces containing the pathogen to the muscle tissue during slaughter and subsequent processing. In the case of large animals, e.g. sheep and cattle, this can occur during any stage from evisceration through to hide removal and subsequently during de-boning and cutting into joints. Preventing dirty animals (with excessive faecal/mud soiling of the hide) entering the abattoir is an important first stage in reducing contamination, which, together with controlled

procedures for hide removal, evisceration and the handling of other contaminated regions such as the hooves, can prevent transfer of pathogens to the muscle meat.

Many of the same principles apply to the slaughter and processing of pigs but the inclusion of a high-temperature singeing stage to remove hairs can reduce bacterial contamination on the pig-skin. Coupled with careful evisceration, as described for other animals, it is possible to minimise spread of intestinal contaminants.

In poultry processing, once a contaminated flock is introduced into the slaughter and process line, it is very difficult to prevent it spreading to other birds owing to the high throughput and use of common processing equipment. As a consequence of this it is common practice for poultry processors to test all flocks prior to processing in order to determine those that are contaminated with *Salmonella* so that these can be scheduled for processing at the end of the production day. In this way, flocks that are not infected stand a chance of remaining uncontaminated during their passage through the slaughter and processing plant. If present, *Salmonella* is readily spread through the scalding tank that operates at *ca* 60 °C to completely wet the feathers, facilitating effective defeathering. As the bird is yet to be eviscerated, faeces are shed into the tank and contamination is spread from bird to bird. This is further exacerbated in the defeathering equipment where rubber fingers pull the feathers out but can also spread contamination. After evisceration the bird is washed inside and outside with free-flowing clean water.

Strategies to reduce contamination on the final animal/bird carcass through carcass washing or, in the case of beef, through steam 'pasteurisation' have met with some success. Yang *et al*. (1998) demonstrated a 1.7 to 2.0 log cfu (colony-forming unit) reduction in *S.* Typhimurium inoculated onto chicken breast, the back and in the cavity when subject to washing with four different chemicals (10% tri-sodium phosphate (pH 12.3), 0.5% cetyl pyridinium chloride (pH 7.6), 2% lactic acid (pH 2.2) and 5% sodium bisulphate (pH 1.3). This compared to a 0.56 log cfu reduction when washing with water alone. Cutter *et al.* (2000) demonstrated nearly a 5–6 log reduction in *S.* Typhimurium when lean beef tissue was washed with 1% cetyl pyridinium chloride, although unacceptable residual levels of the chemical remained.

A large number of beef processors in the USA employ steam pasteurisation to decontaminate the external surface of the carcass after primary processing. The carcass enters a chamber where it is subject to steam that causes elevation of temperature at the beef surface. The carcass is then water-cooled and leaves the chamber for further processing. Such steam treatment has been shown to deliver a reduction approaching 3 log cfu on carcasses artificially inoculated with enteric organisms (Phebus *et al*., 1997).

It should always be remembered that effective cleaning and sanitisation of all equipment, surfaces and the environment in the slaughter and processing halls is essential for the control of enteric pathogens.

Raw meat and the associated environment should be subject to monitoring for indicators of process hygiene such as *E. coli* or coliforms/Enterobacteriaceae.

With the exception of poultry and perhaps pork, it is usually not worthwhile testing for *Salmonella* in raw meat because of its infrequent occurrence. However, where such material is frozen or is to be used for processes such as fermented or dried meat production, where minimal bacterial reduction occurs, it may be useful to monitor for the organism to ensure it is not present at an unusually high frequency/level in a batch. The use of indicators such as *E. coli*/coliforms may, however, give useful information about the hygienic quality of the material, and also deliver results faster.

Salmonella is found in all types of raw meat although the incidence varies considerably depending on the meat species and also on its processing and origin. A recent survey of 2509 carcass swab samples (0.1 m^2) taken from slaughtered pigs at abattoirs in the UK found 5.3% (135 samples) to be contaminated with *Salmonella* (Davies, 2000). The predominant serotypes were *S.* Typhimurium (2.1%) and *S.* Derby (1.6%). Older surveys of pork have reported a *Salmonella* incidence ranging from 0.4% in Sweden to over 75% in a Dutch survey (D'Aoust, 1989).

The incidence in beef is much lower than that in pork. A major study in the USA in 1992/93 found the average incidence to be 1% with levels on positive carcasses of 0.1 MPN/cm^2 (MPN is the most probable number; Anon., 1994b). Slightly higher incidence has been reported by Sofos *et al.* (1999).

Salmonella is a frequent isolate from poultry and poultry products. Although some countries have achieved a much lower incidence, notably Sweden, where the incidence is <1%, the majority of the rest of the developed world has reported incidences ranging from <10% to 100%. Sampling methodologies are not always easy to compare and vary from 25 g composite skin samples, i.e. neck flap, breast and thigh, to skin and meat composites to whole bird rinses. Nevertheless, it is rare to see surveys of raw poultry of any nature yielding an incidence of <5%. A survey of raw poultry on retail sale in the UK in 2001 found an average incidence of *Salmonella* of 5.8% (www.foodstandards.gov.uk, 2001). Personal experience indicates that the incidence can vary significantly from farm to farm and is very much dependent on the farming practices employed in the rearing of the birds and subsequently on the practices employed by the broiler processor. Nevertheless, it is now common to find UK fresh poultry with incidence regularly below 10% and some individual processors with <5% incidence. Chang (2000) reported a *Salmonella* incidence of 25.9% on raw broilers on sale in Korea (25 g samples of leg, thigh and breast of chicken meat and skin) with predominant serotypes being *S.* Enteritidis, *S.* Virchow and *S.* Virginia. Bryan and Doyle (1995) summarised results from a large number of surveys of raw chicken, duck and turkey which demonstrated an incidence varying from 2 to 100% with the median incidence of 30%. They also reported that the population of *Salmonella* on the carcass is typically 1–30 cells with occasionally as many as 10^4 cfu per 100 g of broiler skin.

The incidence of *Salmonella* in eggs also varies considerably depending on the sample size, time from laying i.e. fresh eggs or stored eggs and method of detection used. A survey carried out in Northern Ireland of 2090 packs of six eggs found an incidence of 0.43% *Salmonella* (Wilson *et al*., 1998) whereas a British

survey between 1992 and 1993 of eggshells and contents stored at 21 °C for five weeks found an incidence of 0.2% from a total of 7730 six-egg samples (de Louvois, 1994). Following the widespread introduction of egg layer vaccination and adherence to an industry code of practice (Lion code), the incidence of *Salmonella* in UK eggs is believed to be significantly lower than this now.

11.5.4 Fruit, vegetables and other raw materials

A large number of outbreaks have implicated produce as a potential vehicle for *Salmonella.* Outbreaks associated with sprouted salad vegetables, i.e. bean sprouts and alfalfa sprouts (Table 11.8) and fruit and unpasteurised fruit juice have served to focus attention on agricultural practices in this sector. Although the nature of the original contamination is not always clear in these outbreaks it is evident that contamination of these types of primary agricultural products can occur from a number of sources. Firstly, these crops are grown in soil, and much soil has animal waste applied to it to add nutrients and provide conditioning material. If animal waste handling and application is under poor control it could lead to the deposition of microbial pathogens, present in the waste, onto the land. Then, depending on the conditions in the soil and environment and the subsequent time to harvest of the crop, such organisms may either perish, persist or even proliferate in the soil, thus offering various risks of cross-contamination to the harvested crop.

Salmonella can be present in very large numbers in animal wastes, particularly if derived from infected herds or flocks (Nicholson *et al*., 2000) and can survive for periods up to 41 weeks in stored slurry depending on the slurry composition and time of year. Aeration of animal waste slurries can significantly reduce the levels of *Salmonella* (Heinonen-Tanski *et al.*, 1998).

Composting of animal wastes can achieve a reduction in levels of microbial pathogens owing to the high temperatures achieved in the pile due to microbial activity. Good management of the pile through regular turning can allow a safer waste material to be produced for spreading onto agricultural land. Even in the absence of composting, storage of liquid wastes for long periods to encourage microbial activity is a sensible approach that has been advocated to reduce the potential burden of pathogens on agricultural land (Nicholson *et al.*, 2000). Clearly, no animal wastes should be applied to land where there are growing crops as this will inevitably lead to contaminants entering the human food chain.

Irrigation water is another possible source of potential contamination of crops. Although the source of water is much more difficult to control as it is not usually under the direct authority of the farmer, it is important that known contamination sources are not used as irrigation water for growing crops.

A variety of wild animals including birds, reptiles and rodents can carry *Salmonella* (Ward, 2000) and although it is very difficult to control such pests in the field it is essential to prevent their entry into subsequent storage and processing areas of fruit and vegetable produce. Another practice that may also be increasingly associated with outbreaks of salmonellosis is the use of drop fruit; also the

potential for internalisation of pathogens in the fruit may be important. These have already been described in detail (see Chapter 10).

There are a large number of other possible sources of *Salmonella* including food materials such as herbs and spices, often subject to growing, harvesting and drying in poorly controlled conditions, together with raw material seeds such as mung or alfalfa seeds, eggs, a variety of shellfish, particularly those derived from aquaculture environments and cross-contamination sources such as many reptiles, amphibians and birds (Bell and Kyriakides, 2002).

All such hazards should be identified by the application of a thorough hazard analysis of any production process or new product development. In this way, suitable controls can be established to ensure the process and product are as safe as required.

11.6 Control in processing

For most processes, stages are identified where defined conditions are exerted that can reduce or eliminate enteric pathogen contamination, if present in the raw ingredients. Reliable identification and control of these stages are clearly critical to the safety of the finished product. In many processes the pathogen reduction stages are obvious, such as in cooking or washing, but in others, the reduction stage is less clear, as in raw fermented meats or raw milk cheese production. It is incumbent on the food producer to identify the stages and process conditions within those stages necessary to achieve a satisfactory reduction in pathogens to generate a safe product. This is best achieved by the application of hazard analysis-based approaches.

The descriptions here are short as many of the process and finished product controls relating to *Salmonella* are very similar to those for pathogenic *E. coli*, discussed in Chapter 10.

11.6.1 Cooking

The application of high temperatures in cooking or pasteurising foods is one of the best means by which to destroy *Salmonella*. The organisms are readily destroyed by moist heat and temperatures designed to destroy pathogenic *E. coli* will equally destroy (>6 log reduction) *Salmonella*, i.e. 70 °C for 2 minutes for meat and 71.7 °C for 15 seconds for milk. These heat processes generally confer a significant margin of safety in relation to most serotypes of *Salmonella*. For example, a review of heat-resistance studies by Doyle and Mazzotta (2000) concluded that it would take only 1.2 s at 71 °C to inactivate 1 log of *Salmonella* cells (z value 5.3 °C). Although this estimate excludes the most heat-resistant strain, *S.* Senftenberg 775W, even the milder milk pasteurisation would still deliver >4 log reduction in this strain (Bell and Kyriakides, 2002). Application of heat to destroy organisms in low moisture environments, i.e. chocolate, where water activity is very low requires much longer times or higher temperatures (Table 11.4).

The principles underpinning the consistent delivery of the correct temperature and subsequent segregation of cooked product to avoid recontamination after cooking have already been described in detail (see Chapter 10).

11.6.2 Fermentation processes

Many products are traditional in their manufacture and have been made in the same way for centuries. Although their manufacture is often considered to be a craft rather than a science, it is possible to use modern-day knowledge to identify and control the factors responsible for the production of a safe product. Raw fermented meat and raw dry-cured meats and raw milk cheeses are good examples of this. In all cases, it is the standard of hygiene in the production of the raw ingredient that is the first important control. This is then followed by the promotion of an effective fermentation stage by lactic microflora to both prevent the proliferation of pathogens and potentially cause their reduction. The addition of salt to both meat for fermented/dry-cured meat, and to the milk for cheese adds further microbial inhibitory compounds and the reduction in moisture achieved during the long drying periods for hard cheese and the raw dry-cured and fermented meats are also important factors in the pathogen reduction process. Reduction in *Salmonella* occurs both during the fermentation and maturation stages (Smith *et al*., 1975) and reduced fermentation and/or drying times have been reported as reasons for previous outbreaks of salmonellosis (Pontello *et al*., 1998). It is also for this reason that raw milk soft cheeses are often considered to represent a higher risk – they have fewer pathogen controls within the production process or intrinsic product pathogen protection characteristics than hard cheeses or fermented meat equivalent.

It is strongly recommended that challenge test studies be conducted in research facilities on these types of products to determine the process lethality and, together with tight control of the critical process stages, routine monitoring of the raw ingredients for pathogens and indicators of hygienic quality should be employed, i.e. *E. coli*/coliforms.

11.6.3 Washing processes

The washing of fruit and salad vegetables in preparation processes for their sale as ready to eat salads or fruit juice has already been described (see Chapter 10). The same levels of sanitisers are employed in respect of controls relating to both *Salmonella* and *E. coli*. However, given the recent number of outbreaks of salmonellosis associated with unpasteurised fruit juice, some additional discussion of this sector will be made here. It has already been mentioned that fruit used for juice extraction may sometimes be picked from the ground as ‘drop fruit’ and, as such, is more likely to be contaminated than that picked directly from the tree. Fruits are routinely washed prior to juice extraction and while chlorinated water

is often used for the washing process, a number of studies have been made to determine the efficacy of different washing treatments.

Pao and Davis (1999) assessed the reduction in *E. coli*, an indicator of enteric contamination, inoculated onto oranges using hot water treatment (1–4 minutes in water heated to 30, 60, 70 and 80 °C) and a number of chemical treatments; sodium hypochlorite (200 ppm chlorine), chlorine dioxide (100 ppm), acid anionic sanitiser (200 ppm), peroxy-acetic acid (80 ppm) and tri-sodium phosphate (2%). Chemical treatment for up to 8 minutes reduced *E. coli* levels by log 1 cfu to log 3.1 cfu, this varying according to the chemical and region of the inoculum (stem-scar *vs* non stem-scar region). In comparison, water reduced *E. coli* by log 0.6–2.0 cfu. Hot water immersion achieved a 5 log reduction in *E. coli* after treatment at 80 °C for 2 minutes although it was reported to result in a discernible difference in flavour of the extracted juice. Such information can be useful in relation to a consideration of the use of such treatments in the control of *Salmonella.*

It is already recognised that *Salmonella* can survive for extended periods under very acidic conditions. The lag time before *S.* Typhimurium even started to decline in numbers in orange juice at pH 4.1 and pH 3.8 was 16.15 days and 7.97 days, respectively, when stored at 4 °C (Parish *et al.*, 1997). Under conditions of less harsh pH, *Salmonella* will not only survive but can grow to extremely high numbers on some fruit. Golden *et al.* (1993) demonstrated the growth potential of a mixture of *Salmonella* serotypes when inoculated onto cantaloupe, watermelon and honeydew melons. When stored at 23 °C, levels increased by over 5 log cfu within 24 hours, although no growth was reported over the same time period when stored at 5 °C.

11.6.4 Sprouting processes

Sprouted seed vegetables have caused a number of salmonellosis outbreaks (O'Mahony *et al*., 1990; Taormina *et al*., 1999). The presence of *Salmonella* in the raw material seed represents a significant hazard to this process as the conditions of germination employed for their production encourage the rapid multiplication of any contaminating pathogens. Levels of *Salmonella* contamination on seed batches have been shown to increase by over 4 orders of magnitude during the sprouting process (Jaquette *et al*., 1996).

Studies on treatments to reduce *E. coli* O157 on the seed have already been reported (see Chapter 10) and they are likely to have a similar effect on *Salmonella.* Jaquette *et al.* (1996) demonstrated that treatment of contaminated alfalfa seeds with 100 ppm and 290 ppm active chlorine solution for 5 or 10 minutes achieved a significant reduction in contaminating *S.* Stanley, although this was still less than 1 order of magnitude. Increasing levels to 1010 ppm resulted in approximately a 2 log reduction of the organism.

As for *E. coli*, processors conduct extensive testing of raw material seed batches and production samples to monitor the effectiveness of controls in place and gain greater assurance of the microbiological safety of these products in relation to *Salmonella* spp.

11.6.5 Hygiene and post-process contamination

For details of this see Chapter 10.

11.7 Final product control

Final product control of *Salmonella* spp. is similar to that for pathogenic *E. coli*, discussed in Chapter 10.

11.8 General considerations

It is clear that there are common areas that need to be addressed in relation to the control of *Salmonella* regardless of the food materials being processed:

- Process flows should be designed to eliminate any possibility of cross-contamination between raw and heat-processed products.
- Procedures for effective personal, equipment and environmental hygiene must be carefully designed, implemented, maintained and monitored for ongoing efficacy.
- Product process conditions must be designed to consistently achieve the physico-chemical conditions required to control (reduce/eliminate) *Salmonella* at all stages of manufacture and the life of the finished product.

Where new technologies such as pulsed electric fields, ultrasonic energy, oscillating magnetic field pulses, high-pressure, high-intensity visible light and ultraviolet light and even old technologies not yet widespread in use, e.g. irradiation, are developed and exploited for use in food production processes, it is critical that they are fully assessed for safety in respect of specific pathogens including *Salmonella*.

Carefully designed challenge test studies can be useful for demonstrating effective control of *Salmonella* in relation to new and old technologies applied to many product types and have been widely used in the past to assess the survival of pathogens including *Salmonella* in foods/processes as diverse as bulk meat cooking processes, mayonnaise products and fermented dairy products. The results of such studies are valuable for helping to determine the critical controls and monitors required to effect safe processes in respect of the specific pathogen studied, e.g. *Salmonella*.

In addition to ensuring that appropriate production process practices and procedures are in place from the agricultural sector, through manufacturing and retailing processes, it is important to keep in mind that the caterer and home consumers can also play a significant part in maintaining the safety of foods. The basic rules for safe food handling by caterers and home consumers are well publicised but, it would seem, not well understood or applied by the consumer. The wealth of practical information available for consumers to use for playing their part in a safe food chain needs to be promulgated by a continuous education pro-

gramme for consumers of all ages. Such a programme must have a long-term benefit, both physical and economic, for all. Within such a programme, the food industry must continue to supply appropriate information on the product packaging that gives clear guidance concerning safe handling and storage conditions of food and the preparation or cooking processes that need to be employed to keep the food safe to eat.

11.9 Future trends

The ubiquitous nature of *Salmonella* and the wide diversity of foods that are associated with outbreaks of salmonellosis already cause many tens of thousands (probably millions) of cases of illness every year around the world. From the evidence to date, different serotypes or phage types of *Salmonella* will continue to 'emerge' as important causes of foodborne outbreaks of salmonellosis, go into decline and then another will 'emerge'. In addition, the increasing spread of antibiotic resistance in the environmental 'pool' of *Salmonella* may lead to longer persistence of 'emerged' serotypes and an increase in numbers of types emerging. Groisman and Ochman (1997, 2000) have suggested that virulence genes were transferred into *Salmonella* from related bacterial genera, e.g. *E. coli* or between strains of *Salmonella.* If such were needed, the prospects of further gene acquisitions/transfers resulting in a regular 'production' of new pathogens should be a clear indicator of the need to ensure that all the controls already known to be effective in the prevention of *Salmonella* contamination of foods should be actively encouraged and where necessary enforced by legislation.

The food industry should ensure that structured hazard analyses in respect of *Salmonella* are carried out of all food production processes from primary agriculture and aquaculture throughout the chain to the consumer. Any controls identified should be implemented, maintained and monitored for consistent efficacy. In addition, all food handlers and consumers should be provided with the necessary information and training (as applicable) in relation to food safety issues, by industry on product labelling and through structured government education programmes to ensure food is handled safely to final consumption. Such joint action should control even the, as-yet, 'unemerged' new salmonellas and significantly reduce the numbers of cases of salmonellosis that occur.

11.10 References and further reading

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12

Listeria monocytogenes

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12.1 Introduction

Listeria monocytogenes has been recognised as a significant foodborne pathogen only since the early 1980s when outbreaks of foodborne listeriosis demonstrated the severe nature of the illness with exceptionally high levels of mortality, particularly in the most vulnerable members of the community such as unborn babies, the elderly and the immunocompromised.

L. monocytogenes is widely distributed in the environment and occurs in almost all food raw materials from time to time. It is recognised that its presence in raw foods cannot be completely eliminated, but it is possible to reduce its incidence and levels in food products through the application of effective hygienic measures (Codex Alimentarius Commission, 1996).

12.2 Characteristics of *Listeria monocytogenes*

Currently, six clearly distinguishable species of *Listeria* are recognised: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. Proposals have also been made for two subspecies of *L. ivanovii*; *L. ivanovii* subspecies *ivanovii* and *L. ivanovii* subspecies *londoniensis* (Boerlin *et al.*, 1992). Table 12.1 indicates the common conventional tests used in the differentiation of *Listeria* spp. The most commonly occurring species in food are *L. innocua* and *L. monocytogenes* (Jay, 1996; Kozak *et al.*, 1996).

L. monocytogenes is the main human pathogen of the *Listeria* genus (Jones, 1990). The factors predisposing infection are not fully understood but include host immunity, level of inoculum and virulence including haemolytic activity of

Table 12.1 Common conventional tests used to differentiate *Listeria* spp.

Species	β-haemolysis	Production of acid from:			CAMP reaction with:	
		L-rhamnose	D-xylose	D-mannitol	*Staphylococcus aureus*	*Rhodococcus equi*
L. monocytogenes	+	+ (some strains –)	–	–	+	– (some strains +)
L. innocua	–	V	–	–	–	–
L. ivanovii	++	–	+	–	–	++
L. welshimeri	–	V	+	–	–	–
L. seeligeri	(+)	–	+	–	(+)	–
L. grayi	–	–	–	+	–	–

++ = Strong positive reaction.
+ = Positive reaction.
(+) = Weak positive reaction.
– = Negative reaction.
V = Variable reaction for different isolates.
Source: adapted from Bell and Kyriakides (1998).

the specific *L. monocytogenes* strain. The majority of large foodborne outbreaks of listeriosis have been attributed to one of two serogroups of *L. monocytogenes*, either 1/2a or, more frequently, 4b. The UK Public Health Laboratory Service define a case of listeriosis as a 'patient with a compatible illness from whom *L. monocytogenes* was isolated from a normally sterile site (usually blood or cerebrospinal fluid, CSF)' (Anon., 1997a). The serogroup of the organism is believed to be important when assessing the risk to an individual consuming a food containing *L. monocytogenes* and national authorities will need to consider the apparent differences in the potential of different serogroups to cause foodborne outbreaks in future risk assessments.

Individuals principally at risk from listeriosis have been reviewed by Rocourt (1996) and in order of descending risk are organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. *L. monocytogenes* can cause a variety of infections (Table 12.2) but listeriosis most commonly takes the form of an infection of the uterus, the bloodstream or the central nervous system which in pregnant women can result in spontaneous abortion, stillbirth or birth of a severely ill baby owing to infection of the foetus. Listeriosis may also be acquired by new-born babies owing to postnatal infection from the mother or other infected babies. The mother is rarely severely affected by listeriosis as the disease appears to focus on the foetus (Rocourt, 1996). Healthy non-pregnant adults may also suffer listeriosis, with the most vulnerable groups including the immunocompromised and the elderly because of the reduced competence of their immune systems. In such groups, listeriosis usually presents as meningitis and septicaemia.

Listeriosis is a rare but serious illness with an incidence of 2–3 cases per million of the total population of England and Wales (McLauchlin, 1993). Of 2449 cases of human listeriosis recorded in England and Wales between 1983 and 2000, a total of 739 (30.2%) were associated with pregnancy (Table 12.3), although significant variations can be seen from year to year (11–48%).

The symptoms of listeriosis do not usually resemble those of the more familiar types of food poisoning but there have been several recent episodes where the presence of extremely high levels of *L. monocytogenes* has resulted in the rapid onset of symptoms of vomiting and diarrhoea with few apparent cases of classical listeriosis (Salamina *et al*., 1996; Dalton *et al*., 1997; Aureli *et al*. 2000).

Listeria monocytogenes is transmitted via three main routes; contact with animals, cross-infection of new-born babies in hospital and foodborne infection. The latter two sources account for the majority of cases of listeriosis in humans. In the UK the levels of listeriosis have been relatively static except for a large rise in numbers of cases recorded for the period between 1987 and 1989 (Table 12.3) because of a contaminated food, pâté, from a single Belgian manufacturer (McLauchlin *et al*., 1991). The subsequent fall is believed to have been due to public health advice relating to the consumption of higher-risk food commodities including pâté and the concerted industry action at that time to control the organism (Gilbert, 1996).

The incidence of listeriosis in other countries is similar to that in England and

Table 12.2 Types of illness caused by *Listeria monocytogenes*

Type of illness	Incubation time	Symptoms
Zoonotic infection	1–2 days	Localised skin lesions that are mild and self-resolving
Neonatal infection: newborn babies infected from mother during birth or due to cross-infection from one neonate in the hospital to other babies	1–2 days (early onset) usually from congenital infection prior to birth	Can be severe, resulting in meningitis and death
Infection of the mother during pregnancy acquired from contaminated food. Infection is more common in the third trimester	Varies from 1 day to several months	Mild flu-like illness or asymptomatic in the mother but serious implications for the unborn infant including – spontaneous abortion, stillbirth and meningitis
Listeriosis acquired by non-pregnant people from contaminated food	Varies from 1 day to several months	Asymptomatic or mild illness which may progress to central nervous system infections such as meningitis. Most common and most severe in immunocompromised or elderly people
Listeria food poisoning caused by consumption of food containing very high levels (>10^7 per gram) of *L. monocytogenes*	<24 hours	Vomiting and diarrhoea, sometimes progressing to bacteraemia but usually self-resolving

Source: adapted from Bell and Kyriakides (1998).

Wales and similar decreases in infections to those noted in the UK have been reported in the USA following improvements in regulatory/industry control and the issuing of advice to susceptible groups (Tappero *et al.*, 1995). In spite of the relatively low incidence of disease, i.e. 2–4 cases per million in some western countries, listeriosis is a serious illness and this is reflected by the apparent high mortality rate in many cases, with fatalities averaging approximately 30% (Newton *et al.*, 1992).

Listeria species are widely distributed in the environment and the occurrence of *L. monocytogenes* in raw and processed foods has been extensively studied. Ben Embarek (1994) summarised many reports relating to raw and processed

Table 12.3 Cases of human listeriosis in England and Wales, 1983–99

Year	Total cases (pregnancy associated[a])
1983	111 (44)
1984	112 (35)
1985	136 (59)
1986	129 (42)
1987	238 (102)
1988	278 (114)
1989	237 (114)
1990	116 (25)
1991	126 (32)
1992	106 (25)
1993	103 (17)
1994	115 (26)
1995	87 (10)
1996	118 (17)
1997	124 (24)
1998	108 (22)
1999	106 (18)
2000	99 (13)

[a] Mothers and babies are counted as one case.
Source: adapted from Anon. (2000a).

seafoods in which prevalences up to 75% in lightly preserved (cold-smoked, hot-smoked, marinated) fish products are noted.

Beuchat (1996) summarised the results of studies of the prevalence of *L. monocytogenes* in raw vegetables including bean sprouts, cabbage, cucumber, potatoes, pre-packed salads, radish, salad vegetables, tomatoes; reported prevalences ranged from 1.1 to 85.7%.

When reviewing studies of the prevalence of *L. monocytogenes* in meat and poultry products, Jay (1996) combined the reported findings from a number of countries to give an overall prevalence in the different meat products; these are shown in Table 12.4 together with the incidence of the organism reported in some other surveys. In addition, 2–6% of healthy individuals are reported to be asymptomatic faecal carriers of *L. monocytogenes* (Rocourt, 1996).

It is clear that eliminating *L. monocytogenes* from most foods is both impractical and impossible but it is possible to reduce and control this hazard in foods, thereby minimising the risk presented to public health. In order to ensure the safety of food products in respect of potential foodborne bacterial pathogens, growing, harvesting, handling, storage, processing and associated food supply systems must be managed by food producers and processors in such a way as to reliably control the growth of *L. monocytogenes* which must be prevented from multiplying to potentially harmful levels (>100/g; Gilbert *et al*., 2000). To achieve

Table 12.4 Incidence of *Listeria monocytogenes* reported in some raw and processed foods

Samples	Incidence (%)	Reference
Soft and semi-soft cheeses	2/374 (0.5)	Farber *et al*. (1987)
Raw chicken portions	19/32 (59)	Arumugaswamy *et al.* (1994)
Raw beef	6/12 (50)	
Fresh prawns – raw	7/16 (44)	
Leafy vegetables	5/22 (23)	
Satay, ready to eat	11/39 (28)	
Squids, prawns, chicken and clams, ready to eat	6/27 (22)	
Raw caprine milk	37/1445 (2.56)	Gaya *et al*. (1996)
Read to use mixed vegetable salads	21/70 (30)	García-Gimeno *et al*. (1996)
Vegetable salads	1/63 (1.6)	Lin *et al*. (1996)
Raw milk	342/9837 (3.48)	Kozak *et al.* (1996)
Soft and semi-soft cheeses	20/333 (6)	Loncarevic *et al*. (1995)
Fresh and frozen pork (16 reports)	(20) $n = 1033$	Jay (1996)
Fresh and frozen beef and lamb (21 reports)	(16) $n = 1571$	
Fresh and frozen poultry (26 reports)	(17) $n = 7054$	
Processed meats (26 reports)	(13) $n = 5089$	
Ready to eat:	77/2113 (3.6)	Little *et al*. (1997)
Refrigerated salads, vegetables crudités	2/242 (0.8)	
Live shellfish	11/120 (9.2)	Monfort *et al*. (1998)
Raw cows' drinking milk	32/1591 (2)	Anon. (1998a)
Cold-smoked salmon	(34–43)	Jørgensen and Huss (1998)
Cold-smoked halibut	(45–60)	
Gravad fish	(25–33)	
Heat-treated seafood	(5–12)	
Cured seafood	(4)	
Prepared salads	15/146 (10.2)	de Simón and Ferrer (1998)
Prepared meals		
vegetables	8/217 (3.6)	
meat, poultry	16/324 (4.9)	
seafoods	2/137 (1.4)	
Preserved fish products – not heat treated	35/335 (10.4)	Nørrung *et al*. (1999)
Preserved meat products – not heat treated	77/328 (23.5)	

Table 12.4 *Continued*

Samples	Incidence (%)	Reference
Heat treated meat products	45/772 (5.8)	Norrung *et al.*, (1999)
Raw fish	33/232 (14.2)	
Raw meat	106/343 (30.9)	
Prepared retail foods including meat, fish, vegetable and cheese products		
1997 (*L. monocytogenes* present at >10/g)	760/7760 (9.8)	
1998 (*L. monocytogenes* present at >10/g)	670/7703 (8.7)	
Frozen, smoked mussels – imported in Korea	3/68 (4.4)	Baek *et al.* (2000)
Ice-cream – imported	8/132 (6.1)	
Domestic foods	111/1337 (8.3)	

this, it is necessary to understand the conditions and factors that affect growth and survival.

Attention to the detail of cleaning and hygiene practices throughout the food supply chain is the prime essential for minimising the levels of *L. monocytogenes* contaminating plant and animal crops and food production environments. Thereafter, it is the treatment, formulation and storage conditions of the food materials themselves that will determine how any residual contaminating population of *L. monocytogenes* develops.

Within food production processes, a variety of physico-chemical factors, used either singly or in combination can be effective in controlling the survival and growth of *L. monocytogenes* both during processing and in the finished food products. Table 12.5 indicates the growth-limiting temperature, pH and water activity for *L. monocytogenes*. It should be noted, however, that *L. monocytogenes* is known to grow at the temperatures used for refrigeration, although only slowly (Table 12.6). To control the growth of *L. monocytogenes* in chilled foods, it is crucial to operate and apply well-controlled chill holding and storage systems both within the production process for component or part-processed foods and for finished product storage and distribution. This and other physico-chemical factors can control the survival and growth of *L. monocytogenes* during the manufacturing process and the shelf-life of the finished food products (Bell and Kyriakides, 1998).

Although the pathogenicity of individual strains of *L. monocytogenes* and hence their relevance to public health is of great concern, in a detailed review of

Table 12.5 Growth-limiting parameters for *Listeria monocytogenes*

	Minimum	Maximum
Temperature (°C)	–0.4	45
pH	4.39	9.4
Water activity (a_w)	0.92	–

Source: adapted from ICMSF (1996).

Table 12.6 Growth rate and lag times for *Listeria monocytogenes* at different temperatures

Temperature (°C)	0–1	2–3	4–5	5–6	7–8	9–10	10–13
Lag time (days)	3–33	2–8	–	1–3	2	<1.5	–
Generation time (hours)	62–131	–	13–25	–	–	–	5–9

Source: adapted from Mossel *et al*. (1995).

the pathogenicity of *Listeria monocytogenes*, McLauchlin (1997) concluded 'in the interests of public safety and for considerations for public health purposes, all *L. monocytogenes*, including those recovered from food, should be regarded as potentially pathogenic'. It is also relevant to note that where *L. monocytogenes* is included in food-related legislation, guidelines or specifications, *L. monocytogenes* is not qualified as to serotype or other subdivision of the species. Indeed, the food industry always reacts to positive findings of the species, not specific serotypes of the species, and many manufacturers use the presence of *Listeria* spp. as a general indicator for the presence of *L. monocytogenes*. Industry guidelines and specifications reflect this approach.

The general approach taken to legislation in Europe and North America in the context of food safety is to indicate the clear responsibility of food business proprietors to produce and supply safe and wholesome foods using Hazard Analysis Critical Control Point (HACCP)-based approaches to the control and monitoring of food manufacturing processes, e.g. European Union Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. Some legislation, however, contains specific microbiological standards that include *L. monocytogenes* and compliance is compulsory, e.g. European Council Directive 92/46/EEC of 16 June 1992, laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. *Listeria monocytogenes* must,

of course, also be considered for inclusion in generic standards that state that pathogenic organisms must be absent in a specified quantity of food. In the USA, following surveys of the incidence of *L. monocytogenes* in foods and food-related samples, the Food and Drug Administration and Food Safety Inspection Service implemented a policy of 'zero tolerance' (negative in 25 g of food sample) for *L. monocytogenes* in cooked and ready-to-eat foods (Shank *et al*., 1996), which was reported to have contributed to a 40% decline in listeriosis in the United States (Tappero *et al*., 1995). It has also resulted in *L. monocytogenes* contamination accounting for the greatest number of food product recalls in the US between 1993 and 1998 (Wong *et al*., 2000).

The European Commission is also considering the need to strengthen controls in relation to *L. monocytogenes* in food. The European Commission Scientific Committee on Food recently supported the recommendation of the European Commission Scientific Committee on Veterinary Measures relating to Public Health (Anon., 1999a) to 'reduce significantly the fraction of foods with a concentration of *L. moncytogenes* above 100 cfu/g at the point of consumption' (Anon., 2000b).

L. monocytogenes is included in some food industry guidelines and many food company specifications relating to individual food products, particularly chilled, ready-to-eat foods, also include specific requirements for the 'absence' (non-detection) of *L. monocytogenes* in 25 g of food sample (Bell and Kyriakides, 1998).

The success or otherwise of any systems put in place to control *L. monocytogenes* is usually monitored by examining samples taken from various points in the process, e.g. incoming raw materials, food materials in process, e.g. after washing procedures, cooking procedures or slicing operations and finished products, for the presence of *Listeria* spp. and *L. monocytogenes* in particular.

12.3 Detecting *Listeria monocytogenes*

Reliable methods for detecting and identifying *L. monocytogenes* are important in the support of properly developed and implemented HACCP systems for control. Table 12.7 indicates the common conventional microbiological method used by food microbiologists to isolate and identify *Listeria monocytogenes* and Table 12.8 gives some examples of the current range of alternative microbiological techniques used for its detection and identification from foods and food-related samples. When suspected positive results are obtained, further tests to characterise the organism may be necessary and these can include serotyping, phage typing, production of listeriocins and their ability to inhibit the growth of selected indicator strains, ribotyping and pulsed field gel electrophoresis. A valuable summary of many methods available has been prepared by the Campden and Chorleywood Food Research Association (Baylis, 2000).

A wide variety of foods has been implicated in outbreaks of illness attributed to *L. monocytogenes* (Table 12.9). This has led to an increasing amount of work

Table 12.7 Common conventional method for the detection and identification of *Listeria monocytogenes* from foods

Inoculate selective enrichment broth e.g. FDA*, UVM1, half Fraser Broth	
⇓	
Incubate 30 °C for 24 hours (*FDA 48 hours incubation)	
⇓	
Sub-culture to secondary enrichment broth, e.g. Fraser Broth (*FDA medium does not require secondary enrichment stage)	
⇓	
Incubate 30 °C for 24 hours	
⇓	
Streak onto selective agars, e.g. Oxford Agar and Palcam Agar	
⇓	
Incubate 30 or 37 °C for 48 hours	
Inspect plates	
⇓	
Purify suspect colonies (Tryptone Soya Agar + 0.6%w/v Yeast Extract)	
Incubate 30 °C for 24 hours	
⇓	
Confirmatory tests	• Gram reaction • Catalase reaction • Tumbling motility at 22–25 °C • Carbohydrate utilisation, mannitol, rhamnose, xylose • β-haemolysis reaction • Christie, Atkins, Munch-Petersen (CAMP) test
⇓	
Read reactions	

Source: Based on EN ISO 11290 – 1: 1996 Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method. International Organisation for Standardisation, Geneva.

by both researchers and food industry scientists to determine the specific survival characteristics of this organism, to further improve methods of detection and to develop controls aimed at minimising, if not eliminating, contamination of food by *L. monocytogenes*.

12.4 Control of *Listeria monocytogenes* in foods

Despite the heightened profile of *L. monocytogenes* brought about by increases in incidence and outbreaks of listeriosis in the 1980s, the organism is still, regrettably, all too frequently implicated in foodborne disease outbreaks. Increased availability and consumption of ready-to-eat, extended shelf-life, chilled foods has clearly given this psychrotrophic organism greater scope to cause such problems if inadequately controlled. Outbreaks have implicated cooked meat-based

Table 12.8 Some methods for the detection and/or identification of *Listeria* spp. and *Listeria monocytogenes* in foods (for a more extensive list see Baylis, 2000)

Test type/technique	Name of test	Approximate test time (hours)	Supplier
Enzyme-linked immunosorbent assay (ELISA)	EIAFOSS *Listeria*	48	Foss Electric
	Transia plate *Listeria monocytogenes*	50	Diffchamb
	Listeria – Tek	50	Organon-Teknika
	Tecra *Listeria* Visual immunoassay	50	Tecra Diagnostics
Immuno-chromatography	Clearview *Listeria*	43	Oxoid Ltd.
	Reveal for *Listeria*	43	Neogen Corp.
Nucleic acid hybridisation	Gene Trak test for *Listeria* spp.	50	Gene Trak Systems
	Gene Trak test for *Listeria monocytogenes*	50	Gene Trak Systems
Polymerase chain reaction (PCR)	BAX™ for screening *L. monocytogenes*	48	Qualicon
	Foodproof® *Listeria monocytogenes*	48	Biotecon Diagnostics
	PROBELIA™ *Listeria monocytogenes*	48	Sanofi Diagnostics, Pasteur

products such as pâté and rillettes (Anon., 1999c), soft ripened cheese, cooked shellfish, coleslaw and milk shake (Table 12.9). Indeed, the range of products from which the organism has been isolated (Table 12.4) is significantly broader than those so far implicated in outbreaks of foodborne disease.

The factors that appear to elevate the risk in relation to the potential for a food product to cause outbreaks of listeriosis include all of the following:

- Raw material or product exposed to contamination.
- Product manufactured with no processing stage capable of destroying the organism, e.g. cooking.
- Product with little or no preservation factors e.g. neutral pH, low salt, high moisture.
- Product exposed to post-process contamination.
- Product sold with long shelf-life under chilled conditions.
- Product sold as ready-to-eat.

L. monocytogenes is not a special organism in terms of its ability to survive adverse conditions but the trait that has allowed it to exploit many food environments and cause outbreaks of illness is its capacity to grow at very low

Table 12.9 Some examples of foodborne outbreaks of listeriosis

Year	Country	Cases (deaths)	Food	Outbreak serotype	Reference
1980–81	Canada	41 (18)	Coleslaw	4b	Schlech *et al*. (1983)
1983	USA	49 (14)	Pasteurized milk	4b	Fleming *et al*. (1985)
1983–87	Switzerland	122 (34)	Vacherin cheese	4b	Bille (1990)
1985	USA	142 (48)	Mexican-style soft cheese	4b	Linnan *et al*. (1988)
1987–89	UK	>350 (>90)	Belgian pâté	4b	McLauchlin *et al*. (1991)
1992	New Zealand	4 (2)	Smoked mussels	1/2a	Baker *et al*. (1993)
1992	France	279 (85)	Pork tongue in aspic	4b	Goulet *et al*. (1993)
1994	USA	45 (0)	Chocolate milk	1/2a	Dalton *et al*. (1997)
1995	France	20 (4)	Raw-milk soft cheese	4b	Goulet *et al*. (1995)
1997	Italy	>1500 (0)	Corn and tuna salad	4b	Aureli *et al*. (2000)
1998	USA	>50 (8)	Hot dogs and delicatessen meats	4b	Anon. (1999b)
1998	USA	40 (4)	Cooked hot dogs	4b	Anon. (1998b)
1998–99	Finland	18 (4)	Butter	3a	Lyytikäinen *et al*. (1999)
1999–2000	France	26 (7)	Pork tongue in jelly	4b	Anon. (2000b)

temperatures in foods, i.e. as low as –0.4 °C. Together with its capacity to colonise factory environments, particularly moist/wet environments, the organism has been able to exploit chilled, ready-to-eat food products that have minimal preservative levels and long shelf-lives.

Control of *Listeria* spp. in foods is dependent on four key factors:

- Preventing contamination of raw materials or growth in raw materials, if present.
- Destroying or reducing it, if present in the raw materials.
- Preventing recontamination from the factory environment, equipment or personnel after applying a reduction or destruction stage.
- Minimising its growth, if present, during the shelf-life of the final product.

12.5 Raw material control

L. monocytogenes is ubiquitous and can be found in a wide variety of environments including soil, water and vegetation. As a consequence it will be present in most raw materials used in the food industry, which are not subject to a process that will kill the organism. Raw vegetables, meat and milk will be contaminated with the organism and, at best, controls at these stages will help to reduce the levels of contamination entering the process but cannot guarantee absence from most of these materials.

Clearly, the presence of *L. monocytogenes* represents the greatest hazard to those products whose raw materials are not subject to a subsequent process stage capable of reducing or eliminating the organism. Such products include cheeses made from raw milk, cold smoked fish and raw fermented meats. However, high levels of the organism in raw materials intended for products that only receive mild processing such as prepared salads and coleslaw, where the materials are only washed, can also represent a significant hazard. Evidence of this was seen in the large outbreak associated with coleslaw (sliced cabbage and carrots, without dressing) that occurred in Canada in 1981 (Schlech *et al*., 1983).

12.5.1 Raw milk

L. monocytogenes is a relatively frequent contaminant of raw milk. In a recent study of raw cows' milk on retail sale in England and Wales, 32 out of 1591 (2%) of samples contained *L. monocytogenes* in 25 ml samples (Anon., 1998a). None of the samples contained enumerable levels of the organism, i.e. >100/ml and, of the positive isolates, 10 were serotype 4 and 22 were serotype 1. Higher incidence of *L. monocytogenes* has been reported in surveys of raw cows' milk sampled from farm bulk tanks. O'Donnell (1995) reported the organism in 5% (102/2009) samples of raw milk in England and Wales between 1992 and 1993.

Listeria spp. most commonly gain access to the milk from the cows' udder

during milking. *L. monocytogenes* can be found in the faeces of animals and, indeed, can cause serious infection in ruminant animals, especially goats and sheep. Animals are most likely to become colonised through consumption of the organism in grass or feed and, in particular, silage. As with enteric contaminants, *Listeria* spp. can gain entry to the milk from faecal contamination of the udder. Hygienic milking practices involving udder and teat cleaning and sanitisation can help reduce contamination of the milk. However, equally important in the context of *Listeria* spp. is the cleaning and sanitisation regime applied to the milking and milk storage equipment.

Once introduced into the milking parlour or equipment, *L. monocytogenes* can readily colonise these moist environments. The capacity to grow at low temperatures also means that refrigerated storage, while useful in slowing the growth of the organism (Table 12.6), cannot prevent it from increasing in number completely. It is therefore essential to ensure that cleaning and disinfection are carried out properly. This must include the milking parlour environment and all the equipment, and must also take account of all transport vehicles and transfer pipes used in the movement of milk from the farm to the processing factory. Failure to do this will inevitably lead to increased incidence and levels of the organism in the raw milk itself. For the production of raw milk cheese, this could be the difference between a safe and unsafe product.

It is usual to subject farm milk used in the production of raw milk cheeses to routine monitoring for *Listeria* spp. and indicators of dairy parlour hygiene such as *Escherichia coli*. The latter is often used in incentive payment schemes where consistent high-quality milk is rewarded with higher payment and poor quality milk supplies are excluded from production of raw milk products.

12.5.2 Raw meat and fish

Listeria spp. are frequently present in/on raw meat of all types but appear to be less frequent in raw fish. Surveys have shown *L. monocytogenes* to be present at <1% to 20% in raw meat (Jay, 1996) where it comes from faecal or environmental contamination of the meat in the abattoir and during subsequent processing. It is not normal to find high levels of *L. monocytogenes*, i.e. >100/g, on carcass meat. Controls in the abattoir and meat processing rooms are not targeted at controlling *Listeria* spp. specifically but the controls in place to minimise contamination by faecal pathogens will also aid in the control of *Listeria* spp.

Raw fish used for manufacture of products such as smoked salmon or trout may occasionally be contaminated with *Listeria* species at an incidence of <5% and with only low levels, i.e. <100/g (Bell and Kyriakides, 1998). Contamination may arise on the fish farm, where most salmon used for such products are grown or, more commonly, arise and are spread during subsequent gutting and processing. Control of hygiene during the slaughtering and preparation stages is critical for minimising the level of contamination entering a cold smoked fish-processing unit. Results from studies of fish processing plants have revealed significant differences in the occurrence of *L. monocytogenes*. Autio *et al*. (1999) reported

only one sample (2%) of raw trout entering the processing unit to be contaminated with *L. monocytogenes* and none of the 49 samples of filleted fish, skinned fish or pooled skin were found to harbour the organism. However, Eklund *et al*. (1995) found the organism to be a common contaminant of raw, eviscerated salmon supplied to salmon processors, with the organism being isolated from 4/19 samples of slime, 30/46 skins, 8/17 heads and 1/15 belly cavity and belly flap trimmings.

Purchasers of raw meat for products such as fermented meat or raw fish for cold smoking where no cooking process takes place would normally monitor the material for incidence and levels of *Listeria* spp. in order to ensure high levels are not being routinely supplied on raw materials, thereby entering the process.

12.5.3 Fruit, vegetables and other raw materials

Listeria spp. will be present on many raw fruit and vegetables from time to time. They may be contaminated from soil and water sources, or indeed from animal wastes applied to land. The presence of *L. monocytogenes* is less of a concern in most fruits as the pH usually precludes its growth (Table 12.5) and, as high levels ($>10^3$) are normally required to cause infection and illness, any very low levels that may be present on fruit are not considered to represent a significant hazard. However, its presence on salad materials and vegetables intended for minimal further processing such as prepared salads is of more concern.

An outbreak of listeriosis has occurred because of extensive contamination of cabbages thought to be due to the use, on the growing field, of raw and composted sheep manure from animals with known recent incidence of listeriosis. In addition, many raw material vegetables are stored for long periods in cold stores to facilitate continued supply through winter months. Clearly, the use of animal wastes on land for crops intended to be consumed with little or no further processing, like many salads, needs to be carefully controlled. Animal wastes should be properly composted to ensure sufficient heat is generated to achieve a reduction in contaminating microorganisms before application to land. Wherever possible, artificial fertilisers should be employed.

Salad materials and vegetables used for direct consumption, if stored, should be stored under conditions that preclude or minimise the growth of *Listeria* spp. either at low temperatures or for short periods. Most vegetables will not support the growth of *Listeria* spp. if they are intact and if moisture is not available; dry storage conditions should be maintained and the produce protected from physical damage. If good conditions are not maintained, such produce can succumb to spoilage through rots or mould growth and in many situations, this serves as a useful ‘natural’ control of psychrotrophic pathogens and prevents potentially hazardous food materials from entering the food chain. However, the use of technologies involving modified atmosphere storage to extend shelf life by inhibition of ‘normal’ spoilage microflora must take account of the opportunity this provides for the growth of other contaminants such as *Listeria* spp. It is essential that process innovations to improve quality are carefully considered to avoid solving a spoilage problem at the expense of safety.

12.6 Control in processing

Control of raw material quality is an important element of many, if not all processes. However, many products are manufactured employing a stage that, if carried out properly and consistently, will deliver a significant reduction or achieve the destruction of *Listeria* spp. if present. Key to the safety of such products is an understanding of the process and the conditions required to achieve the desired reduction or destruction of the organism. Process validation studies allow a processor to understand where to apply the controls necessary to ensure the critical process is conducted correctly, thereby delivering a safety reduction or destruction of *L. monocytogenes* on every occasion. The proper application and operation of a HACCP-based system will naturally incorporate such approaches.

12.6.1 Cooking

Cooking is often used as a primary process to destroy *Listeria* spp. in the manufacture of a variety of products including cooked meats, ready meals, dairy desserts, etc. The controlling factors important in ensuring the consistent delivery of correct cooking processes have already been described in this book for pathogenic *E. coli* and include all of the following;

- Minimum ingoing temperature of the material.
- Largest size/piece size of the product.
- Cold spots in the oven/temperature distribution of the oven.
- Fill load of the container, if appropriate, i.e. air-spaces will affect heat transfer.
- Oven load.
- Minimum time setting for the load.
- Minimum temperature setting of the oven.

Process validation should be used to identify, for implementation, the process controls and checks that will ensure the process is delivered consistently on each occasion such as the monitoring of the time and temperature of the cook using thermographs (see Chapter 10).

L. monocytogenes is readily destroyed by pasteurisation temperatures applied to meat, which in the UK are specified as 70 °C for 2 minutes (Anon., 1992). This process is capable of delivering at least a 6-log reduction of the organism in a food. Studies with cooked chicken indicated a *D* value for *L. monocytogenes* of 0.133 minutes at 70 °C (Murphy *et al.*, 1999). Pasteurised milk is heat processed in the UK to comply with legislative requirements and achieves 71.7 °C for 15 seconds, significantly less than the process applied to meat but nevertheless still sufficient to achieve a 3–4 log reduction in *L. monocytogenes* (based on a *z* value of 5–7.5 °C). Concerns have been expressed relating to the potential protective effect afforded to *L. monocytogenes* by its capacity to invade somatic cells. However, the levels of contamination of *L. monocytogenes* in raw milk and the frequency with which this phenomenon occurs do not appear to have

resulted in *Listeria* spp. being found as common contaminants of pasteurised milk. It is not believed to be a significant safety consideration.

12.6.2 Fermentation processes

Products produced using a fermentation process such as raw, fermented meat and raw milk hard cheeses rely on the fermentation and drying process to reduce levels of microbial contaminants present in the raw material. It is apparent from the frequency with which *L. monocytogenes* occurs in raw, fermented meats (Anon., 1997b) that the organism can survive the traditional salami process. However, the low levels reported in many surveys indicate that growth is restricted.

Studies on the survival of *L. monocytogenes* in raw milk, mould-ripened, soft cheese, e.g. Camembert, indicate that growth is much greater during the ripening stage where levels can increase by 4–5 log units (Ryser and Marth, 1987). The growth coincides with the elevation in pH that occurs owing to the growth of mould on the surface of the cheese. The hazard of *L. monocytogenes* growth during ripening of mould-ripened soft cheeses relates just as much to pasteurised varieties as it does to raw milk varieties if the organism is introduced as a post-pasteurisation contaminant. This clearly can occur with both varieties during the ripening stages.

Hard cheeses such as Cheddar usually have a sufficiently low pH (*ca* 5.0) that, together with the low moisture and high aqueous salt content, serve to restrict the growth of *L. monocytogenes* during the ripening stages. These cheeses are therefore considered to represent less of a risk in relation to listeriosis. *L. monocytogenes* represents greatest risk in soft ripened cheeses and, indeed, other soft non-ripened cheese where the pH is not low e.g. mozzarella, mascarpone. Control is exerted by effective pasteurisation, if employed, and by the avoidance of post-process contamination particularly in the ripening and slicing stages. The safety of these products can only be ensured through the adoption of effective controls outlined above and, where necessary, by the restriction of shelf-life.

Wherever possible, the safety of these types of products should be validated using challenge test studies, using research facilities, to establish the measures necessary to preclude growth of the organism.

12.6.3 Washing processes

For many products including salads and vegetables only a simple washing stage is used in their preparation. Increasing quantities of these are sold as ready-to-eat products where washing by the consumer is not deemed or advised as necessary. Outbreaks of listeriosis implicating these products are rare, and it is probably the short shelf-life assigned to these products that is the principal reason for their apparent safety. Nevertheless, the washing stage for these products is essential to reduce levels of contaminating *Listeria* spp.

Prepared salad materials are washed in chlorinated water with levels normally

ranging from 50 to 200 ppm chlorine. Evidence from the incidence and levels of *Listeria* in washed produce indicates that these levels of chlorine are capable of achieving approximately a 1 log reduction in the organism. Zhang and Farber (1996) reported a 1.2 log reduction and 1.6 log reduction after 1 minute at 4 °C and 22 °C, respectively, in washed shredded lettuce, inoculated with *L. monocytogenes* and then washed in sodium hypochlorite (200 ppm chlorine, pH 9.24–9.31). Reductions were similar with cabbage and were not significantly greater when exposure to chlorine was extended to 10 minutes. At 100 ppm chlorine concentrations, reductions were reduced to <1 log after 1 minute.

Whatever the method used, it is important to ensure that the active ingredient, most often chlorine, is present in its active form on a continuous basis. Washing systems have just as much capacity to spread contamination as they have for reducing them if the wash water is not regularly changed or the levels of active ingredient are not properly maintained. It is therefore common to employ systems that continuously dose and/or continuously monitor the active concentration of the antimicrobial compound used.

12.6.4 Smoking process

Outbreaks of listeriosis have implicated smoked fish and shellfish. Two methods of smoking are usually employed: a cold smoking process at 18–28 °C and a hot smoking process at >68 °C. The capacity to achieve reductions in contaminating *L. monocytogenes* clearly differs between the two, with the latter process essentially representing a pasteurisation stage. Cold smoking takes place for *ca* 18 hours and in theory represents a significant opportunity for growth of contaminating *Listeria* species. The organism commonly contaminates the fish during slaughtering and subsequent processing in the gutting, filleting and salting stages. Reducing contamination through the operation of good hygienic practices at these stages is critical to the safety of this production process.

Smoking processes involve the burning of damp wood chips or wood shavings. Although there are reports of the antimicrobial effects of smoke on *Listeria* spp., it is evident that the nature of the smoke and the site of contamination are important in the potential for the survival and growth of *Listeria* spp. Eklund *et al.* (1995) showed the organism either did not grow or decreased in number if it was inoculated on the outside of the fish. However, levels increased by several orders of magnitude during the smoking process when inoculated into the flesh.

12.6.5 Hygiene and post-process contamination

There is probably no bacterial pathogen that exploits the food processing environment better than *Listeria monocytogenes.* Investigation of a large number of outbreaks throughout the world indicate that contamination of the product from the environment is a significant contributory factor. The organism survives well

in cold, wet environments and can exploit deficiencies in cleaning and hygiene practices.

Product contamination by *Listeria* spp. occurs from either the environment or from direct product contact surfaces, including utensils and equipment. The organisms are transferred from the environment to the product or via product contact surfaces from aerosols or from poor personnel handling practices. To date, it has not been common for listeriosis outbreaks to have implicated products that have been directly cross-contaminated from raw materials, although, clearly, this is an important potential route that should not be overlooked. It is, however, much more common for the organism to colonise the post-process environment and equipment and be a direct contaminant from such sources, e.g. slicers.

The main means for the effective control of *Listeria* spp., and *L. monocytogenes* in particular, is to reduce or eliminate it from the post-processing environment. The procedures necessary to achieve this are usually applied in the manufacture of those foods where its presence in the finished product represents a significant hazard. These include ready meals, cooked meats and sliced soft ripened cheeses. For these products, the raw material processing and finished product areas are segregated within the factory so that personnel or food materials cannot pass between the two areas without the application of procedures designed to prevent contaminants passing from one area to the other. The two areas, often referred to as 'low risk' and 'high risk', are separated by dividing walls and the pathogen reduction or destruction stage is constructed within the division, e.g. double entry cookers (see Chapter 10) or chlorinated water wash flumes. The food materials progress through cooking or washing from the low-risk to the high-risk side.

To further minimise entry of bacterial pathogens, a positive air pressure may be maintained on the high-risk side of the factory thereby ensuring air flows from the high- to the low-risk side of the factory. Such flows are also in place for drainage. Staff entering the high-risk side must change clothes in a segregated changing area, removing shoes and coats and putting on overalls/coats, shoes and hair covering, all dedicated to the high-risk side of the factory. Detailed guidance on such controls can be found in appropriate texts (CFA, 1997).

Effective cleaning and disinfection of the environment and equipment are absolutely essential to prevent the organism from building up and presenting a hazard to the product. All equipment such as slicers, mixing bowls, pipework and conveyor belts should be routinely dismantled or parts loosened for cleaning and disinfection as the organism readily colonises such equipment. 'Dead' spots such as redundant sample valves or poorly designed pipe ends, are often found in plants where the organism has persisted. Such areas should be designed out of the plant.

In addition to obvious product contact surfaces, it should be remembered that the organism may be found in 'reservoirs' throughout the factory on a wide variety of non-food contact surfaces. As these are often cleaned inadequately, the organisms can readily grow to high levels on residual product debris and then spread to product or product contact surfaces on the hands of personnel or by aerosols. Table ledges, the underside of tables, door handles, extraction hoods,

overhead pipes and many other areas should be routinely and properly cleaned and disinfected.

The organism is frequently found on floors, walls, in cracks and in drains. Although it is difficult to eliminate it completely from these sources, effective maintenance of floors and walls ensuring they are properly sealed and routinely cleaned and disinfected can contain the hazard. Cleaning practices themselves can also spread the organism, as in the use of power hoses to 'chase' food debris into drains, or if the cleaning utensils themselves are not cleaned or disinfected properly as is often the case with handles of brushes or spray guns, wheels and brush heads. Many of these factors have been reviewed in more detail in other texts (Bell and Kyriakides, 1998).

In addition to cleaning and disinfection, it is also important to employ routine monitoring of cleaning efficacy using both indicators of effective cleaning and tests for the organism itself. Indicators such as coliform bacteria or ATP bioluminescence tests, which monitor the presence of residual levels of product residues and microorganisms, can be useful, but effectively targeted tests for *Listeria* species can yield significant information about the organism and its control in the factory environment. Advice on sampling plans for such purposes is given by Bell and Kyriakides (1998).

12.7 Final product control

Finished products that are packed and sold as single units are protected from bacterial contaminants that may otherwise enter during distribution and retail display. It is common, however, for many items such as cooked meats, cheese, salads, etc., to be sold to retailers for slicing or open display on the delicatessen or food service counter. The potential for storage, slicing, preparation and display equipment and associated utensils to harbour *Listeria* spp. and subsequently contaminate products is high indeed. Contamination by *L. monocytogenes* on delicatessen counters and subsequent spread to other products was a contributory factor in a large outbreak of listeriosis in France; 279 people were affected, 63 died and 22 suffered abortions (Goulet *et al*., 1993). Cleaning and disinfection of utensils and surfaces in the retail environment are as important to product safety as the same disciplines in a manufacturing operation.

12.7.1 Shelf-life

Although it is possible to exclude *Listeria* spp. from products cooked in their containers or even hot-filled products, the organisms will be occasional contaminants of many other prepared foods. It may be possible to limit the frequency and level of contamination, but it is not always possible to exclude contamination completely. *L. monocytogenes* will be an occasional contaminant of chill stored products such as cooked sliced meats, ready meals, prepared salads and, ripened cheeses. Clearly, storage at low chill temperatures will reduce the rate of growth

of the organism, but as it can grow at low temperatures, the ultimate safety of the product rests on ensuring high levels of the organism are not allowed to develop in the product.

The addition of inhibitory factors such as salt or acid to the product can help reduce the growth of the organism but as most of these products have fairly neutral pH and only low salt levels, the only practical means of ensuring growth limitation is to restrict the product shelf-life. In neutral pH, high water activity, chilled products where *L. monocytogenes* may be a contaminant, e.g. ready meals, the organism can increase in numbers by several orders of magnitude over 10–12 days and shelf-lives should be set accordingly. Safe shelf-lives can be estimated using predictive models for the growth of *L. monocytogenes* in foods under varying physico-chemical conditions of the product. Although such models can assist in growth prediction, in many cases they may need to be supplemented with challenge tests in the actual product, under laboratory conditions, to determine the real growth potential of the organism in the food in question.

12.7.2 Post-process additives

A number of ready-to-eat products are garnished or have ingredients added after the main processing stage and prior to final packing. This may include the addition of cheese to ready meals or herbs to cooked pâté. It is normal for products entering the high-risk side of a factory to be dipped directly or contained in their packaging into a sanitiser such as chlorine to ensure they are not introducing contaminants into the high-risk side of the factory. The ingredients themselves should have been subject to some form of processing capable of reducing bacterial contamination loads including heating or washing and it is usual for such materials also to be subject to some form of testing programme on receipt or supplier certification of compliance against a specification prior to use.

12.7.3 Labelling of products

It is important to recognise that the consumer often plays an important role in maintaining the safety of products after their purchase from the retail store. In relation to *L. monocytogenes*, probably the most critical factor is effective temperature control. Products requiring refrigeration should be clearly marked ‘Keep Refrigerated’ and as the shelf-life of the product is often also critical to product safety, a clear durability indicator should also be printed on the pack. This is usually in the form of a ‘use-by’ date, which indicates when a product should be consumed by.

Some sectors of the population are more vulnerable to infection by *L. monocytogenes* than others, namely pregnant women, the young, the elderly and the immunocompromised. It is common practice to advise vulnerable groups of the need to avoid certain foods where the organism may be an occasional contaminant, e.g. soft cheeses, pâté. It is normal to do this using consumer advice leaflets or through media advertising and general awareness campaigns. Such advice may

also be found on the packaging of high-risk foods such as mould-ripened soft cheese made from unpasteurised milk. In such circumstances it is important that products are clearly labelled with suitable advice for the vulnerable groups to avoid eating such cheese.

Where cooking is recommended as an important control for the organism as in the case of ready meals for vulnerable groups, it is essential that the cooking guidance printed on the pack is clear and correct. Factors to take into consideration when establishing such guidance have already been described in Chapter 10.

12.8 Future trends

Much has been learnt since the 1980s about the factors necessary for the control of this organism in foods and food production environments. Listeriosis has generally been in decline in many countries, and certainly in the UK. However, it is apparent from the continued outbreaks occurring in processed foods in other developed countries that the main challenge remains the consistent implementation of the known control strategies. It is this that will make human listeriosis a disease of the past. However, the continued pressure for increased product shelf-life and less well-preserved foods can only be achieved safely by the elimination of the organism from the finished product through effective application of HACCP principles.

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13

Campylobacter and *Arcobacter*

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13.1 Introduction

Campylobacter species were first isolated from the stools of patients with diarrhoea in 1972 but were not commonly recognised as a cause of human illness until the late 1970s, after the development of selective media by Butzler and coworkers in Belgium and Skirrow in the UK. However, an association between micro-aerophilic *Vibrio*-like bacteria and diarrhoea in cattle and pigs resulted in the naming of *Vibrio jejuni* in the early 1930s and *Vibrio coli* in the late 1940s, respectively. Similar organisms were found in the blood of patients with diarrhoea as long ago as 1948. Véron and Chatelain (1973) published a comprehensive study on the taxonomy of these *Vibrio*-like organisms, proposing, among others, the names *Campylobacter jejuni* and *Campylobacter coli*, and after much confusion this was officially accepted and included in the 'Approved lists of bacterial names' (Skerman *et al*., 1980).

A group of aerotolerant campylobacters was identified in 1977, from the aborted foetuses of cattle, pigs and sheep. The organisms were also found in the milk of cows with mastitis and also in blood and faeces from humans with diarrhoea: *Campylobacter cryaerophila* was the name proposed for this group. Following this, the name *Campylobacter butzleri* was proposed for the group of strains most frequently isolated from human and non-human primates with diarrhoea. A number of detailed DNA : rRNA hybridisation studies followed and this resulted in a revision of the taxonomy of these organisms. *Arcobacter* was the name proposed for a new genus consisting of *C. cryoaerophila* and *C. butzleri*, among others. This genus is closely related to the genus *Campylobacter*.

Campylobacter jejuni subsp. *jejuni* (*C. jejuni*) is now regarded as the leading cause of bacterial foodborne infection in many developed countries and is

responsible for 80–90% of campylobacteriosis (Vandamme, 2000). Despite the huge number of *C. jejuni* cases currently being reported, the organism does not generally trigger the same degree of concern as some other foodborne pathogens, since it rarely causes death and is not commonly associated with newsworthy outbreaks of food poisoning. It is among the most common causes of sporadic bacterial foodborne illness. *Campylobacter jejuni* is associated with warm-blooded animals, but unlike salmonellae and *Escherichia coli* does not survive well outside the host. *Campylobacter jejuni* is susceptible to environmental conditions and does not survive well in food and is, therefore, fortunately relatively easy to control. *Campylobacter coli* is responsible for about 7% of human campylobacteriosis cases, but in some areas (e.g. Central African Republic and Zagreb) this number can be as high as 35–40%. *Campylobacter upsaliensis* and *C. lari* have been isolated from patients suffering diarrhoea, and are responsible for about 1% of human cases of illness associated with campylobacters.

Food-associated illness usually results from eating foods that are recontaminated after cooking or eating foods of animal origin that are raw or inadequately cooked. The organism is part of the normal flora intestinal flora of a wide variety of wild and domestic animals, and has a high level of association with poultry (Ketley, 1997). The virulence of the organism, as suggested by the relatively low infectious dose of a few hundred cells, and its widespread prevalence in animals are important features that explain why this relatively sensitive organism is a leading cause of gastroenteritis in people.

13.2 Characteristics of *Campylobacter* and *Arcobacter* species

Campylobacters are Gram-negative, micro-aerophilic, non-sporing, small vibroid (spiral-shaped) cells that have rapid, darting reciprocating motility. They reduce nitrate and nitrite (apart from *C. jejuni* subsp. *doylei* and *C. fennelliae*) and are unable to oxidise or ferment carbohydrates. *Campylobacter jejuni* is the most significant of the four thermophilic *Campylobacter* species and is one of 20 species and subspecies within the genus *Campylobacter* and family *Campylobacteraceae*, which also includes four species in the genus *Arcobacter*. The other thermophilic species include *C. coli*, *C. upsaliensis* and *C. lari*. The thermophilic species are characterised by their ability to grow best between 37 and 42 °C and their inability to grow at 25 °C. Strains of the two genera *Campylobacter* and *Arcobacter* have similar morphology and metabolism, and share several other genotypic and phenotypic features.

However, isolates are extremely diverse, compared to some other enteropathogens. There are more than 60 different heat-stable serotypes, more than 100 heat-labile serotypes, differences in adherence properties, invasive properties, toxin production, serum resistance, colonisation potential, aerotolerance and temperature tolerance. This diversity may be partly explained by the natural competency of campylobacters to take up DNA. The high levels of multiple-strain

colonisation and high frequency of incidence in mammals and birds mean there is substantial opportunity for exchange of genetic material. Genetic diversity, as evidenced by the different genotypes observed (through PFGE (pulsed field gel electrophoresis), RAPD (randomly amplified polymorphic DNA), ribotyping, AFLP (amplified fragment length polymorphism), etc.) is indicative of genomic plasticity – the order of genes on the chromosome is not conserved between isolates of the same species. Subtypes recognised by one phenotypic or genotypic method often do not correlate with other techniques.

Survival of *C. jejuni* outside the gut is poor, and the organism is sensitive to drying, freezing and low pH (pH ≤ 4.7). Survival kinetics tend to follow a rapid decline in numbers followed by a slower rate of inactivation. *Campylobacter jejuni* fails to grow in the presence of 2% NaCl. Studies investigating the survival of campylobacters show that the potential increases with decreasing temperature, with survival lasting a few hours at 37 °C and several days at 4 °C. Campylobacters can survive several weeks in groundwater, and survival is enhanced in the presence of other organisms and in biofilms (Buswell *et al.*, 1998). Survival in HCl solutions at pH values below 3.0 is poor while, at pH values of 3.6 and above, survival is unaffected. Protection against gastric acid may be afforded by ingestion with buffered foods, e.g. milk, or with water, which is rapidly washed-through. Chlorine is effective for the inactivation of campylobacters in water and other disinfectants are effective at commonly used concentrations. The decimal reduction time (or *D* value) for campylobacters is *ca* 1 min at 55 °C and the *z* value is about 5 °C (Park *et al.*, 1991).

Arcobacters are, generally speaking, much less well characterised than campylobacters. *Arcobacter* spp. are so named because of their bow-shape and are motile by a single unsheathed polar flagellum, exhibiting darting or corkscrew movement, whereas *Campylobacter* spp. may have a single flagellum at one or both ends. The growth range of *Arcobacter* spp. is between 15 and 37 °C, with the upper limit dependent on growth conditions (Mansfield and Forsythe, 2000). *Arcobacter* spp. do not generally show the same thermotolerance of *Campylobacter* spp. and arcobacters may grow aerobically at 30 °C. The pH range for growth is between 5.5 and 9.5 and the limited amount of information available on inactivation by irradiation suggests *Arcobacter* spp. are slightly more resistant than *Campylobacter* spp. *Arcobacter cryaerophilus* is divided into two subgroups, 1A and 1B, based on DNA–DNA hybridisation, cellular protein composition (using SDS-PAGE; sodium dodecyl sulphate polyacrylamide gel electrophoresis) and fatty acid analysis. However, there are no phenotypic analyses to differentiate these two groups. *Arcobacter butzleri* is closely related to *A. skirrowii*, and serotypes 1 and 5 are currently recognised as the main pathogenic strains (Lior and Woodward, 1991).

Campylobacter jejuni is regarded by some to be capable of forming so-called viable but non-culturable (VBNC) cells. Although these cells are metabolically active and show signs of respiratory activity, they are not able to be resuscitated by recovery through conventional culturing techniques. The VBNC state has been proposed as a survival strategy or as a moribund condition where cells become

progressively debilitated, until they finally 'die'. Induction of the VBNC state in *Campylobacter jejuni* comes about through exposure to sublethal adverse environmental conditions, such as prolonged exposure to water or freeze–thaw injury and recovery is effected by passage of the organism through a susceptible host (Rollins and Colwell, 1986; Jones *et al*., 1991; Saha *et al*., 1991). This probably reflects the inability of some culturing methods to provide suitable conditions for the resuscitation of 'injured' cells. The VBNC campylobacters sometimes form a coccoid shape, although non-coccoid VBNCs have also been described (Federighi *et al*., 1998). There have been examples of outbreaks of campylobacteriosis where cases were observed after attempts to culture the organism from the identified source were no longer positive (Palmer *et al*., 1983). Treatment of the water supply on one farm also resulted in the disappearance of a particular serotype that had colonised most of the chickens on the farm, although no campylobacters had been isolated from the chicken-shed water supply (Pearson *et al.*, 1988). The significance of the VBNC state is still unclear and reversion of coccoid cells is not easily initiated, requiring very specific conditions. The existence of this state may exert an important influence on considerations in the epidemiology of human and animal campylobacteriosis, and should not be ignored.

13.3 The nature of *Campylobacter* and *Arcobacter* infections

Campylobacter jejuni and *C. coli* are clinically indistinguishable and most laboratories do not attempt to distinguish between the two organisms. Campylobacteriosis in humans is usually characterised by an acute, self-limiting enterocolitis, lasting up to a week and clinical illness is often preceded by a prodrome of fever, headache, myalgia and malaise. The incubation period is usually 24 to 72 hours after ingestion, but may extend to 7 days (Skirrow, 1994). Although not all those exposed to the organism will develop symptoms, human volunteer studies indicate that the infectious dose can be low, of the order of a few hundred cells. Occurrence of water-borne outbreaks and person-to-person spread also implies that the infective dose can be low. A small proportion (5–10%) of affected individuals suffer relapses, possibly caused by an incomplete immune response since immunocompromised hosts often have severe, extra-intestinal and prolonged illness. Symptoms of disease often include abdominal pain and cramps (frequently severe) and diarrhoea, which may be inflammatory, with slimy/bloody stools, or non-inflammatory, with watery stools and absence of blood. In many regions, where antibiotics are used in the empirical treatment of *Campylobacter* infections, e.g. in the elderly, prolonged cases of enteritis, septicaemia or other extra-intestinal infections, some antibiotics such as fluoroquinolones are of limited use because of antibiotic resistance. Erythromycin is still the drug of choice. There is some evidence of protective immunity after infection from volunteer studies, and this may explain the higher incidence of disease in very young children. The efficacy of artificial immunogens is questionable since there is a

wide variety of virulence between different phenotypes and an absence in increase of specific antibody.

Reactive arthritis and bacteraemia are rare complications and infection with *C. jejuni* is also associated with Guillain–Barré syndrome (GBS), an autoimmune peripheral neuropathy causing limb weakness that is sometimes fatal. GBS has become the most common cause of acute, flaccid paralysis since the eradication of polio, in most parts of the world and campylobacter infection is now known as the single most identifiable antecedent infection associated with the development of GBS. This condition is thought to be associated with particular serotypes (e.g. O:19, O:4, O:5, O:41, O:2 and O:1) capable of producing structures (epitopes) that mimic ganglioside motor neurons. There are several pathological forms of GBS including demyelinating (acute inflammatory demyelinating polyneuropathy) and axonal (acute motel axonal neuropathy) forms. Host factors also play an important role in development of disease and it has been estimated that 1 of approximately every 1000 cases of *C. jejuni* infection is followed by GBS (Nachamkin *et al*., 1998). There are a number of pathogenicity determinants that have been suggested for *C. jejuni*, including motility, adherence, invasion and toxin production, but little is known about the mechanism causing disease in humans. Mortality rates of GBS have been reduced to 2–3% in the developed world but remain higher in much of the developing world. Incidence of bacteraemia and systemic infection in immunocompromised individuals indicates that the immune system is important for confining the disease associated with campylobacters to the intestine.

Prior to 1991, *Arcobacter butzleri* and *A. cryaerophilus* were known as aerotolerant *Campylobacter*. These organisms have been associated with abortions and enteritis in animals and enteritis in humans. Although both species are known to cause disease in people (i.e. clinical isolates have been reported in individuals with diarrhoea), most human isolates come from the species *A. butzleri*. There is very little known about the epidemiology, pathogenesis and real clinical significance of arcobacters, but it is thought that consumption of contaminated food may play a role in transmission of this group of organisms to people. Although arcobacters have never been associated with outbreaks of foodborne illness, they have been isolated from domestic animals, poultry, ground pork and water. Symptoms of *Arcobacter* infections are similar to those caused by campylobacters, and there are documented cases of extra-intestinal disease e.g. bacteraemia. Since primary screening procedures for campylobacters use high temperatures (42 °C) for recovery, *Arcobacter* spp. would be overlooked.

13.4 Risk factors for *Campylobacter*

Campylobacter is part of the normal intestinal flora of a wide variety of wild and domestic animals and has a high level of association with poultry (Shane, 1992). *Campylobacter jejuni* has become recognised worldwide as a leading cause of diarrhoeal disease and foodborne gastroenteritis, but the epidemiology of this

common infection is not well understood. Most cases are sporadic, as opposed to being associated with large outbreaks, and there is a high frequency in which the vehicle is not identified (Neal and Slack, 1997). Where identified, poultry, raw milk, contaminated drinking water, contact with pet animals and travel abroad have been recognised as the most common vehicles and/or risk factors associated with *Campylobacter* infection in humans (Schorr *et al*., 1994; Neal and Slack, 1997; Solomon and Hoover, 1999; Rautelin and Hanninen, 2000).

There is considerable evidence that poultry is the main vehicle for transmitting *Campylobacter* enteritis in humans and serotypes associated with poultry are also frequently associated with illness in humans (Shane, 1992). Consumption of poultry and poultry products have been frequently quoted as important risk factors for *Campylobacter* infection (Schorr *et al*., 1994; Solomon and Hoover, 1999; Rautelin and Hanninen, 2000). Other avian species, including geese, have been shown to be potential reservoirs for human and animal campylobacteriosis (Aydin *et al*., 2001).

Numerous surveys of retail raw poultry and raw poultry products have been made in different countries and *Campylobacter* contamination rates have ranged from 3.7% to 92.6% (Fernandez and Pison, 1996; Ono and Yamamoto, 1999; Osano and Arimi, 1999), although there has been a lack of association of the organism with shelled eggs (Zanetti *et al*., 1996). Where species identifications have been made, *C. jejuni*, *C. coli* and *C. laridis* have all been isolated but the predominant species has varied (Fernandez and Pison, 1996; Osano and Arimi, 1999). Where quantitative methods have been used, levels of *Campylobacter* of 10^2–10^4/g of poultry (McClure, 2000) and 10 to >230 campylobacters/100 ml chicken liver exudate have been reported (Fernandez and Pison, 1996).

Owing to the high level of *Campylobacter* on raw poultry many sporadic cases of campylobacter enteritis are probably caused by cross-contamination in the domestic environment. It has been shown that during preparation of chicken for cooking, *Campylobacter* became widely disseminated to hand and food contact surfaces (Cogan *et al*., 1999). Although *Campylobacter* is a relatively heat-sensitive vegetative bacterium, undercooking is another potential risk factor and a combination of inadequate cooking time and the use of large chicken pieces was the probable cause of a *Campylobacter* outbreak in a restaurant specialising in stir-fried food (Evans *et al*., 1998). Barbecues appear to present special hazards for infection because, in addition to the potential for undercooking, they permit easy transfer of bacteria from raw meats to hands and other foods (Butzler and Oosterom, 1991).

The high incidence of *Campylobacter* in retail poultry and poultry products is a direct reflection of the prevalence of the organism in live chickens and turkeys and the ease with which the organism can be spread during poultry processing. In surveys of broiler farms, *Campylobacter* spp. have been detected in 32–57% of flocks (Kapperud *et al*., 1993; van de Giessen *et al*., 1996; Hald *et al*., 2000) with the proportion of colonised flocks varying geographically and seasonally with a peak in the autumn (Kapperud *et al*., 1993). It has been estimated that when carrying the organism in their intestinal tract poultry typically has popula-

tions of 10^4–10^7 *Campylobacter*/g of intestinal content (Stern *et al*., 1995) with *C. jejuni* as the most prevalent species (Nielsen *et al*., 1997; Hald *et al*., 2000). Once *Campylobacter* colonise the poultry intestinal tract the subsequent shedding of the organism can result in environmental contamination, with a subsequent increased risk for the rest of the flock. In a study to determine the daily shedding pattern of *C. jejuni* in 24 artificially infected broiler chickens it was found that *C. jejuni* failed to colonise 16.6% of birds, whereas 12.5% of birds were observed to be chronic shedders (Achen *et al*., 1998). Throughout the sampling period from days 1 to 43, a cyclic pattern of shedding was observed in individual birds with *C. jejuni* excreted on an average of 25 out of 43 days. Enumeration of *C. jejuni* in the crop, jejunum and caecum on day 43 revealed that the caecum was the major colonisation site (Achen *et al*., 1998). In a separate study, the lack of colonisation of 1-day-old chicks by VBNC *C. jejuni* has been stated to cast serious doubts on the significance of the VNBC state in environmental transmission of *C. jejuni* (Medema *et al*., 1992).

Several studies have been made in order to identify the risk factors associated with the occurrence of, and increases in the incidence of, *Campylobacter* in broiler flocks. These risk factors have included: lack of a hygiene barrier when entering the broiler house, presence of other farm animals in the vicinity of the broiler house on farms with a missing hygiene barrier, dividing the flock into batches for staggered slaughter, the speed of catching and slaughtering of the flock and the use of undisinfected drinking water (Kapperud *et al*., 1993; van de Giessen *et al*., 1996; Hald *et al*., 2000, 2001). In poultry processing plants significant contamination of *C. jejuni* has been demonstrated in chicken carcasses, processing equipment and workers' hands and this contamination increased during the defeathering and evisceration processes (Nielsen *et al*., 1997; Ono and Yamamoto, 1999).

In contrast to poultry, there appears to be comparatively low incidence of *Campylobacter* in meat products with detection rates of up to 2% (Loewenherz-Luning *et al*., 1996; Ono and Yamamoto, 1999; Osano and Arimi, 1999). However, *Campylobacter* isolation rates of 47% and 46% have been found in cattle and swine, respectively, in slaughterhouses (Nielsen *et al*., 1997). *Campylobacter jejuni* was the most prevalent species associated with cattle while *C. coli* constituted the majority of isolates from swine. In one study *Campylobacter* spp. were isolated from 70 to 100% of the pigs on a pig farm (Harvey *et al*., 1999) with further evidence that *C. coli* predominates over *C. jejuni*. Concentrations of *C. coli* and *C. jejuni* ranged from 10^3 to 10^7 colony-forming units (cfu)/g of caecal content.

In cattle, where colonisation of dairy herds has been associated with drinking unchlorinated water, young animals are more often colonised than older animals, and feedlot cattle are more likely to be carriers than grazing animals (McClure, 2000). It has been proposed that *C. jejuni* is a cause of winter dysentery in calves and older cattle, and experimentally infected calves have shown some clinical signs of disease such as diarrhoea and sporadic dysentery. *Campylobacter jejuni* is a known cause of bovine mastitis, and the organisms associated with this

condition have been shown to cause gastroenteritis in persons consuming unpasteurised milk from affected animals. Drinking pasteurised milk from bottles with tops damaged by birds has been shown to be a risk factor (Neal and Slack, 1997). However, *Campylobacter* have not been associated with dairy products such as raw milk cheeses (Butzler and Oosterom, 1991; Federighi *et al*., 1999).

Shellfish, such as oysters, mussels and clams, can be contaminated by *Campylobacter* spp. and it has been presumed that they originate from faeces from gulls feeding in the growing or relaying waters (Butzler and Oosterom, 1991; Teunis *et al*., 1997; Federighi *et al*., 1999). The usual steaming process of mussels was found to completely inactivate *Campylobacter* spp. so that the risks associated with shellfish are restricted to consumption of the raw commodity (Teunis *et al*., 1997).

Contaminated drinking water is one of the vehicles for the transmission of *Campylobacter* infection and the possible role of viable but non-culturable forms of the organism in the large number of waterborne gastroenteritis outbreaks from which a disease agent cannot be isolated remains to be fully clarified (Thomas *et al*., 1999). Differences in the survival of *C. jejuni*, *C. coli* and *C. lari* in water together with their differing incidence in the sources of water contamination (e.g. birds, sewage effluent) are reflected in the isolation rates from surface waters (Korhonen and Martikainen, 1991; Obiri-Danso *et al*., 2001).

It is generally accepted that *Campylobacter* does not survive as well as other pathogens such as *Salmonella*, although it has been shown to survive in foods for longer at lower temperatures (Curtis *et al*., 1995). *Campylobacter* is probably very vulnerable to factors such as high temperature and dry environments and also to the presence of oxygen in atmospheric conditions. Therefore, it is assumed that the organism does not persist in products such a pelleted food, meals, egg powder and spices, which are often contaminated with *Salmonella* (Butzler and Oosterom, 1991).

13.5 Risk factors for *Arcobacter*

There is very little known about the epidemiology, pathogenesis and real clinical significance of arcobacters, but it is thought that consumption of contaminated food may play a role in transmission of this group of organisms to humans. Similar to *Campylobacter*, *Arcobacter* spp. have been isolated from poultry, cattle, swine, water and domestic animals (Mansfield and Forsythe, 2000; McClure, 2000). However, to date arcobacters have not been associated with outbreaks of foodborne illness, although person-to-person transmission has been reported (Mansfield and Forsythe, 2000).

The first description of *Arcobacter* spp. was from pigs in the late 1970s (Phillips, 2001) and pork products are routinely found to be contaminated with the organism (Wesley, 1996). In contrast, the first documented report of *Arcobacter* spp. in clinically healthy dairy cattle occurred more recently (Wesley *et al*., 2000). In this study, 71% of dairy operations and 14.3% of individual dairy cattle

faecal samples were positive for *Arcobacter*, compared with 80.6% of farm operations and 37.7% of individual dairy cattle faecal samples positive for *C. jejuni* and figures of 19.4% and 1.8%, respectively, for *C. coli*.

Arocobacter spp., including *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* (Atabay *et al*., 1998), have been isolated from poultry with recovery rates of 0–97%, with variations in isolation rate dependent on the environmental conditions of the originating flock, although eggs do not seem to be infected (Phillips, 2001). For example, *Arcobacter* spp. were detected in 53% of retail-purchased chicken samples (Gonzalez *et al*., 2000) and 77% of mechanically separated turkey samples, of which 74% were positive for *A. butzleri* (Manke *et al*., 1998). Despite its common isolation from poultry carcasses, *A. butzleri* is infrequently isolated from caecal samples, suggesting that arcobacters are probably not normal inhabitants of the poultry intestine and that contamination may be post-slaughter (Atabay and Corry, 1997; Phillips, 2001).

Water probably has a significant role in the transmission of *Arcobacter* spp. both to animals and to humans (Phillips, 2001) and it has been estimated that 63% of *A. butzleri* infection in humans is from the consumption of, or contact with, potentially contaminated water (Mansfield and Forsythe, 2000). Because of its sensitivity to chlorine, infection is probably due to improper chlorination procedures or post-treatment contamination (Phillips, 2001) and hence *Arcobacter* spp. may be more common in developing nations with inadequate water supplies (Mansfield and Forsythe, 2000).

Arcobacters have been isolated from drinking water reservoirs and treatment plants; canal water, river water, raw sewage and disinfected effluent (Mansfield and Forsythe, 2000) and it has been suggested that the land application of anaerobically digested sludge may cause high risk of infection (Phillips, 2001).

13.6 Control procedures for *Campylobacter*

Although *Campylobacter* has been demonstrated to be susceptible to a wide variety of antimicrobial treatments, food processing methods and environmental stresses (Solomon and Hoover, 1999; McClure, 2000), it still continues to cause an increasing level of human foodborne disease. It is obvious that there is no single approach to controlling this organism and a number of preventative measures are needed throughout the farm-to-table continuum in order to reduce the incidence of campylobacteriosis in humans.

Owing to the prevalence of *Campylobacter* in poultry, control measures to reduce infection and spread of the organism on broiler farms would reduce the risk of transmission to humans further down the food chain. Control measures that have been shown to be effective have included: strict hygienic routines when the farm workers enter the rearing room (washing hands, the use of separate boots for each broiler house and the use of footbath disinfection), disinfection of drinking water, and depopulation of broiler houses as quickly as possible and in one batch only (van de Giessen *et al*., 1996; Hald *et al*., 2000, 2001). In addition,

chicks dosed with anaerobic preparations of caecal mucus from *Campylobacter*-free adult hens were shown to be partly protected against *C. jejuni* (Mead *et al*., 1996). Vaccination and drug therapy have also been proposed (White *et al*., 1997) although the use of antibiotics has been a factor in the rapid emergence of antibiotic-resistant *Campylobacter* strains all over the world (Allos, 2001). This trend has further emphasised the need for appropriate and safe use of antibiotics in animal production (Pedersen *et al*., 1999).

When birds are sent for slaughter, there is a high risk that *Campylobacter* present will be transmitted from carrier birds to the carcasses being processed. This underlines the need for HACCP principles to be applied to processing plants, to minimise product contamination (Mead, 2000). Subsequent critical control points and good manufacturing practices that have been identified and implemented have included: temperature controls (washer and product), chemical interventions, water replacements, counter-flow technology in the scalder and chiller, equipment maintenance, chlorinated-water sprays for equipment and working surfaces, increase in chlorine concentrations in process water and removal of unnecessary carcass contact surfaces (Mead *et al*., 1995; White *et al*., 1997).

Campylobacter is relatively heat sensitive and so commercial heat processes within a HACCP framework should guarantee control of the pathogen. However, food handling at retail outlets and by the consumer often provides the last chance for control of this organism in the farm-to-table continuum. Adequate cooking and prevention of cross-contamination are probably the most important control measures in this environment. Following a prescribed cleaning procedure using hypochorite in addition to cleaning with detergent and hot water has been shown to reduce the level of contamination of *Campylobacter* in the domestic kitchen (Cogan *et al*., 1999). It is the effective promotion of good hygienic practices in such food preparation areas that could bring about the greatest benefits in terms of improved food safety, but this still presents an enormous challenge.

13.7 Control procedures for *Arcobacter*

The risk of transmission to humans of *Arcobacter* via properly cooked foods and chlorinated water is negligible as it is for *Campylobacter* (Wesley, 1996). As with all foodborne pathogens, effective implementation of HACCP is important in the control of *Arcobacter* (Phillips, 2001).

Although *Arcobacter* and *Campylobacter* are closely related this does not necessarily mean that all treatments are similarly effective (Phillips, 2001). For example, the fact that, unlike *Campylobacter*, arcobacters are probably not normal inhabitants of the poultry intestine (Atabay and Corry, 1997; Phillips, 2001) means that control measures should be focused on preventing contamination of, and proliferation in, the broiler environment and post-slaughter. Although *A. butzleri* is more resistant than *C. jejuni* to irradiation treatment, doses of irradiation currently allowed for pork in USA (0.3–1.0 kGy) provide an effective method of reducing, if not completely eliminating, *A. butzleri* from pork (Phillips,

2001). However, treatment with sprays of organic acids has been shown to be effective at reducing *Salmonella* and *Campylobacter* spp. on pork carcasses (Epling *et al.*, 1993), but there is evidence that this type of treatment may not be as effective against *A. butzleri* (Phillips, 1999).

Arcobacters also appear to be resistant to antimicrobial agents typically used in the treatment of diarrhoeal illness caused by *Campylobacter* spp., e.g. erythromycin, other macrolide antibiotics, tetracycline and choramphenicol and clindamycin (Mansfield and Forsythe, 2000).

13.8 Detection methods for *Campylobacter*

Campylobacter is a notoriously difficult organism to culture and maintain in the laboratory (Solomon and Hoover, 1999) and as a result there have been many methods developed, and method modifications proposed, for its detection in foods. As with the development of methods for other pathogens, media originally used for the isolation of the organism from faeces were used. Subsequent modifications have been required to enable the detection of low numbers of sublethally injured cells in the presence of higher numbers of competitor organisms and this has led to methods based on liquid enrichment prior to selective agar plating with colony identification. The history of the development of selective media for isolation of campylobacters, including the rationale for the choice of selective agents has been described by Corry *et al*. (1995). Most of the media include ingredients intended to protect campylobacters from the toxic effect of oxygen derivatives. Most commonly used are lysed or defibrinated blood; charcoal; a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate; and haemin or haematin.

A number of approaches have been taken to avoid the inhibitory effects of the toxic components in the media on sublethally injured cells including a preliminary period of incubation at reduced temperature and a delay in the addition of antibiotics. A 4 h pre-enrichment time at 37 °C was found to be optimal for allowing the recovery of low levels of *C. jejuni* while preventing competitive inhibition due to the outgrowth of the accompanying flora (Uyttendaele and Debevere, 1996). A delay of 4–8 h before adding antibiotics to broth was found to significantly increase the *Campylobacter* isolation rate from naturally contaminated river water compared with direct culture in selective broth. However, with chicken samples, significantly better results were obtained with selective broth as the primary medium (Mason *et al*., 1999). These findings probably reflect the varying degrees of sublethal injury of the *Campylobacter* cells in the different environments.

A number of enrichment broths for *Campylobacter* have been developed. In a recent study three of these broths – Bolton broth (BB), *Campylobacter* enrichment broth (CEB) and Preston broth (PB) – were compared for the isolation of *Campylobacter* from foods (Baylis *et al*., 2000). Both BB and CEB were better than PB for the isolation of *Campylobacter* from naturally contaminated foods,

although BB yielded more confirmed *Campylobacter* growth than CEB. The use of selective enrichment broths supplemented with the enzyme Oxyrase®, a membrane-bound enzyme derived from *E. coli*, has been used and shown to be effective in order to provide an oxygen-reduced atmosphere for optimal *Campylobacter* recovery (Abeyta *et al*., 1997). In contrast, a blood-free enrichment broth (BFEB) has been investigated under aerobic conditions and compared favourably with the US Food and Drug Administration's *Bacteriological Analytical Manual* (BAM) method for the recovery of *C. jejuni* from inoculated foods. The BFEB method had the advantage of not requiring the use of blood, Oxyrase® or special equipment (Tran, 1998).

Several agars have also developed for the isolation of *Campylobacter*. Three of the most commonly used agars are Butzler agar, charcoal cefoperazone desoxycholate agar (CCDA) and Preston agar and in a comparison for the detection of campylobacters in manually shelled egg samples and raw meat samples no substantial difference was seen (Zanetti *et al*., 1996) although CCDA was preferred. In this study the variable that had the most influence was the incubation temperature with a higher number of strains isolated at 42 °C than at 37 °C. Three isolation media (Karmali, Butzler and Skirrow agar) were compared for the detection of *Campylobacter* in 1500 samples of oysters, ready-to-eat vegetables, poultry products and raw milk cheeses following enrichment in Preston or Park and Sanders broths (Federighi *et al*., 1999). Park and Sanders enrichment and isolation on Karmali agar appeared to be the most efficient combination.

Cefoperazone amphotericin teicoplanin (CAT) agar was developed from charcoal cefoperazone deoxycholate (mCCD) agar by modification of the selective antibiotics in order to permit growth of strains of *Campylobacter upsaliensis* (Corry and Atabay, 1997). CAT agar supported the growth of a wider variety of *Campylobacter* species than mCCD agar, which was attributed to the level of cefoperazone in mCCD agar being inhibitory to some *Campylobacter* strains.

There is an international standard method for the detection of *Campylobacter* in food and animal feeding stuffs (Anon., 1995). This method involves enrichment in either Preston broth or Park and Sanders broth. If Preston broth is used, incubation is at 42 °C in a micro-aerophilic atmosphere for 18 h. If Park and Sanders broth is used the initial suspension is incubated in a micro-aerophilic atmosphere at 32 °C for 4 h, then antibiotic solution is added and incubated at 37 °C for 2 h followed by transfer to 42 °C for 40–42 h. The enrichment broth cultures are streaked out onto Karmali agar and a second selective agar from the following: modified Butzler agar, Skirrow agar, CCDA and Preston agar. Agar plates are incubated at 42 °C in a micro-aerophilic atmosphere for up to 5 days prior to a series of confirmatory tests on characteristic colonies.

In recent years, numerous rapid methods have been developed for the detection of *Campylobacter*, some of which have been evaluated for application with foods. However, very few of these methods have been commercialised, which probably reflects the fact that the food industry is not doing a lot of testing for *Campylobacter* and the market for rapid method test kits is therefore small. The reason for the lack of testing by the food industry is probably due to a number

of factors including: the organism does not grow in food under most normal storage conditions; it does not survive well and is relatively easily controlled in processed foods; it is prevalent in raw foods where the ultimate critical control is in the hands of the consumer; and the organism is fastidious and its detection and maintenance in the laboratory are not easy.

Latex agglutination tests for *Campylobacter* have been available for several years (Wilma *et al*., 1992). These tests enable convenient and easy visualisation of serological agglutination of *Campylobacter* and they are intended for confirmation of presumptive isolates. The concentration of cells needed for agglutination ranged from 10^6 to 10^8 cfu/ml and similar sensitivities were seen with the non-culturable coccoid forms of *Campylobacter*. In an attempt to improve the speed of the *Campylobacter* detection method, latex tests have been applied to enrichment broth cultures (Wilma *et al*., 1992). The Microscreen® *Campylobacter* test has been used after incubation in CCD broth (42 °C, 8 h), filtration (0.45 µm) and incubation in blood-free modified CCD broth (42 °C, 16–40 h) for the detection of *Campylobacter* in fresh and frozen raw meat (Baggerman and Koster, 1992). The Microscreen® *Campylobacter* latex kit has also been used for the testing of water samples following a physical enrichment (filtration and centrifugation) rather than a cultural enrichment (Baggerman and Koster, 1992).

More recently developed rapid methods for *Campylobacter* have been based on the polymerase chain reaction (PCR) technique (Nogva *et al.*, 2000; O'Sullivan *et al.*, 2000). A magnetic immuno-PCR assay (MIPA) was developed for the detection of *C. jejuni* in milk and chicken products (Docherty *et al*., 1996). Target bacteria were captured from the food sample by magnetic particles coated with a specific antibody and the bound bacteria then lysed and subjected to PCR. The MIPA could detect 420 cfu/g of chicken after 18 h enrichment, 42 cfu/g after 24 h and 4.2 cfu/g after 36 h. For artificially contaminated milk, 63 cfu/ml could be detected after 18 h and 6.3 cfu/ml after 36 h. Immunocapture has also been combined with PCR for the detection of *Campylobacter* in foods and this enabled detection of the pathogen without an enrichment step and took about 8 h to perform (Waller and Ogata, 2000). The assays were quantitative and the limit of detection was 1 cell/ml and this was not affected when tested in spiked milk samples and chicken skin washes.

Many food samples and enrichment media, e.g. the presence of charcoal and iron (Thunberg *et al*., 2000), are inhibitory to the PCR, thereby lowering its detection capacity. Sample preparation methods using buoyant density centrifugation (Uyttendaele *et al*., 1999; Wang *et al*., 1999) and heat treatment at 96 °C (Uyttendaele *et al*., 1999) have been used to overcome this inhibition. While Oxyrase™ has been shown to significantly enhance the growth of *C. jejuni*, it appeared not to improve the PCR detection of *C. jejuni* in naturally contaminated chickens (Wang *et al*., 1999).

A commercial kit, the API Campy, is available for the differentiation of *Campylobacter* spp., although identification of species within the family *Campylobacteriaceae* using standard biochemical tests can be problematical because of the variability and atypical reactions of some strains (Phillips, 2001). The

heat-stable serotyping system (the 'Penner' Scheme) has been used for *Campylobacter*. However, in a study in Denmark it was found that this system had limitations in that 16% of the stains were untypeable (Nielsen and Nielsen, 1999).

For epidemiological studies, molecular typing techniques have been preferred (Rautelin and Hanninen, 2000; Phillips, 2001). Pulsed-field gel electrophoresis was used for the first time in a *Campylobacter* outbreak in the USA, where a cafeteria worker was the probable source, and was critical in determining that community cases were not linked (Olsen *et al*., 2001). The use of PCR with a RAPD protocol has also proved a useful tool for the epidemiological analysis of *Campylobacter* (Hilton *et al*., 1997).

13.9 Detection methods for *Arcobacter*

Owing to only fairly recent interest in *Arcobacter* there are only a limited number of detection methods and a standardised method does not yet exist (Johnson and Murano, 1999b). Although *Arcobacter* strains are capable of aerobic growth, the optimum growth condition for primary isolation is micro-aerophilic (3–10% oxygen). Several different methods using both aerobic and micro-aerophilic conditions have been proposed based on media for *Campylobacter* and *Leptospira* (Mansfield and Forsythe, 2000; Phillips, 2001).

CAT agar was shown to support a wider range of arcobacters than mCCD agar and the sub-optimal growth on mCCD agar was considered to be due to the synergistic interaction between deoxycholate and cefoperazone (Corry and Atabay, 1997). A pre-enrichment stage in either CAT broth or *Arcobacter* enrichment broth together with a filter method onto mCCDA or CAT agar has been suggested for optimum isolation from chicken carcasses (Phillips, 2001). Another combination of pre-enrichment (in an *Arcobacter* selective broth) and plating onto a semi-solid *Arcobacter* selective medium has been used and in this case an isolation temperature of 24 °C was employed and piperacillin was added to prevent the outgrowth of *Pseudomonas* spp. from raw meats (Phillips, 2001).

The productivity of an *Arcobacter* enrichment medium (AM) [Oxoid] was compared with two *Campylobacter* enrichment media (Preston broth, Oxoid; LabM broth) (Atabay and Corry, 1998). Twenty strains of *Arcobacter* and *Campylobacter* were tested for growth. None of the *Campylobacter* spp. grew in AM and the medium supported good growth of all strains of *Arcobacter*.

An agar medium (JM agar) containing a basal nutrient mix along with 0.05% thioglycolic acid, 0.05% sodium pyruvate and 5% sheep's blood (pH 6.9 ± 0.2) was found to be the most effective formulation for the growth *of A. butzleri*, *A. cryaerophilus* and *A. nitrofigilis* (Johnson and Murano, 1999a). In addition to superior growth characteristics, a deep red colour around the colonies was also observed with this formulation. The use of an aerobic pre-enrichment in JM broth prior to plating on JM agar was subsequently found to be more effective at isolating arcobacters from broiler chicken samples than two existing methods (Johnson and Murano, 1999b).

Rapid method development for *Arcobacter* has centred on PCR-based methods. A multiplex PCR assay to identify *Arcobacter* isolates, and to distinguish *A. butzleri* from other arcobacters, has been developed (Harmon and Wesley, 1997). Upon PCR amplification all the *Arcobacter* isolates yielded a 1233 bp (base pair) product, whereas the *A. butzleri* exhibited an additional 686 bp product. It was claimed that the assay was specific, rapid and easy to interpret. A multiplex PCR method has also been developed to specifically detect both *C. jejuni* and *A. butzleri* in the same reaction tube (Winters and Slavik, 2000). The organisms were differentiated by 159 bp and 1223 bp products, respectively, and the method has been evaluated in a range of spiked foods.

Methods that have been used, or proposed, for the identification and characterisation of *Arcobacter* isolates have included: biochemical profiling, antimicrobial resistance testing, serotyping based on heat-labile antigens, SDS-PAGE of whole cell proteins, fatty acid profiles, PCR-based assays and ribotyping (Harrass *et al.*, 1998; Mansfield and Forsythe, 2000; Phillips, 2001). It has been suggested that some of these methods might represent valuable tools for epidemiological analyses of *Arcobacter* isolates. However, reliable commercially available tests are lacking. For example, the API Campy, although effective for the differentiation of *Campylobacter* spp., does not allow similar identification of *Arcobacter* at species level (Phillips, 2001).

13.10 Future trends

The reservoirs of *Campylobacter* spp. in the environment are extensive and include wild and domestic birds and animals. Despite this, there are examples of production facilities that rear infection-free stock, through use of strict hygiene and control procedures and practices, and through sourcing pathogen-free materials. In some countries, there has been sufficient motivation and resources, from government and the animal/poultry production industry, to realise *Campylobacter*-free production at the farm level. The public health impact of providing *Campylobacter*-free animal/poultry produce is unclear, however. In many regions where this produce is consumed, the incidence of campylobacteriosis remains high and there are likely to be other sources of infection that will still cause significant morbidity.

Public awareness of some of the hazards associated with foods has been increasing in the past 20 years. However, since large outbreaks of campylobacteriosis are relatively uncommon or go unrecognised, *Campylobacter* rarely hits the headlines in the popular press and public awareness of this pathogen is probably still relatively small. With better methods, particularly genotypic, for the characterisation of microorganisms (e.g. genetic 'fingerprinting') and common standardised approaches being used and synchronised in the investigation of foodborne diseases, there is likely to be an increasing number of links made between 'sporadic' cases of illness. The low biochemical activity of *Campylobacter* spp. and frequent variability made characterisation/identification on the

basis of phenotype very difficult. This difficulty has hampered epidemiological investigations and our understanding of the incidence and behaviour of *Campylobacter* spp. in the environment and animals. The development in molecular methods has changed bacterial diagnostics and taxonomy. At present the methods available have different pros and cons, and a combination of methods is needed to secure identification of all *Campylobacter* spp. These methods will continue to be developed and refined to enable more reliable characterisation/identification to be carried out in non-specialist laboratories.

With *Arcobacter* spp., there is currently little direct evidence of this group causing foodborne illness. However, since the organisms are known to cause disease in animals (including humans) and have been associated with meat and poultry products, there seems little doubt that they should be regarded as potential foodborne pathogens. Methods for characterisation/identification, as for *Campylobacter* spp., are being improved in terms of specificity and sensitivity. The trend towards use of molecular techniques has also enabled identification and direct detection of virulence genes, in isolates from different environments. This ability of linking pathogenicity markers (virulence factors) to isolates was not possible with the more classical characterisation techniques.

The impact of molecular approaches is also important for understanding pathogenesis. Since genotypic methods are becoming reliable and easily employed, genotypic analysis of isolates from different reservoirs should indicate whether animals (including humans) and birds share the same bacterial populations or whether there are sub-populations only pathogenic to susceptible groups or hosts. The genomic sequence of *C. jejuni* is now available and if homology with genes known to be responsible for virulence traits (e.g. invasiveness or toxin production) is identified, a better understanding of pathogenesis can be expected.

Another trend that deserves further elaboration is the change in surveillance strategies. Existing surveillance systems in many countries are limited and will only detect outbreaks of established and easily recognised pathogens. Both national and international surveillance strategies are now being enhanced by employing common, standardised sub-typing techniques and also by more active surveillance of populations. For example, in the USA, FoodNet has been designed as a platform to collect/collate information from different sources (e.g. case-control studies) so that true disease incidence can be determined. These efforts will result in identification of diffuse outbreaks that may have little impact on a local basis, but when taken together with other related cases, may have a much bigger impact. Immediate responses to these outbreaks will result in product recalls and longer-term activities will focus on prevention of future similar outbreaks. Environmental sampling and end-product testing is another trend that is increasing in some countries, and detection of campylobacters will be facilitated through development of new approaches and techniques.

The antibiotic resistance profile of isolates associated with foodborne illness is another feature that will be determined in surveillance activities. The development of antibiotic resistance in campylobacters has been attributed, at least in part, to the use of antibiotics in agriculture. This has led to the banning of par-

ticular types of antibiotics for some uses in agriculture and more restrictive controls are likely to be implemented both in veterinary and human medicine in the future.

With campylobacters, as for many other enteric pathogens, there are a number of measures that may be used for control. If used independently, these measures are often not sufficient to reduce the risk of infection to an acceptable level, and combinations of measures are necessary. For many national and international regulatory bodies, the emphasis is increasingly towards an integrated approach to food safety. For campylobacters, this means looking at reservoirs, where future control measures may include selective sourcing of 'clean' raw materials and feed, changing hygienic practices on the farm, modification of the slaughter process and consideration of alternative processing technologies, such as irradiation. Many of these changes and improvements come with a price and successful implementation will be dependent on an acceptance by the consumer and producers that these changes are a real benefit to them.

13.11 Sources of further information and advice

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14

Enterotoxin-producing *Staphylococcus*, *Shigella*, *Yersinia*, *Vibrio*, *Aeromonas* and *Plesiomonas*

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14.1 Introduction

The bacteria discussed in this chapter are diverse in terms of nature, physiology, ecology and diseases caused. These genera receive relatively little attention in comparison with other organisms, such as *Salmonella*, *Campylobacter* and verocytotoxigenic *Escherichia coli*, but their importance should not be underestimated. Under-reporting of foodborne disease is recognised as a significant problem in all countries, especially where symptoms are 'mild' and recovery rapid. The importance of *Staphylococcus aureus*, for example, has been reduced by improved control through refrigeration, but the organism remains a significant cause of foodborne morbidity. The emphasis placed on better recognised foodborne pathogens may also mean that the true role of other genera discussed in this chapter in the spectrum of foodborne disease has not yet been fully resolved.

14.2 Characteristics of enterotoxin-producing staphylococci

14.2.1 Introduction

The genus *Staphylococcus* comprises Gram-positive, catalase-positive, coccal-shaped bacteria. Metabolism of carbohydrates may be either oxidative or fermentative. Cells are small (*ca* 1 μm diameter) and often form characteristic clumps resembling bunches of grapes. Colonies on nutrient media are often pigmented, many strains of the most important foodborne species, *Staphylococcus aureus* producing a golden-yellow pigment.

Staphylococci are predominantly of animal origin, although isolation of some

species may be made from environmental sources. They may be present as part of the normal microflora of humans and other animals, *St. aureus*, being carried on skin and nasal cavities of *ca* 30% of the healthy human population.

Staphylococcal enterointoxication (staphylococcal food poisoning) results from ingestion of enterotoxins, synthesised during growth in foods. *St. aureus* is the species almost invariably involved, although enterotoxin production by several other species has been reported. Enterotoxin production is most common amongst *St. aureus* isolates of human origin and there is a strong correlation with production of the enzyme coagulase.

Occurrences of *St. aureus* intoxication are grossly under-reported (Stewart *et al*., 2001) and the importance consequently under-estimated. It has been estimated that consumption of foods contaminated by the bacterium is responsible for 185 060 illnesses, 1753 hospitalisations and 2 deaths per year in the United States (Mead *et al*., 1999). In the UK since 1980, numbers of reported cases have not exceeded 189 per annum, although this is only a small percentage of actual cases.

14.2.2 Nature of staphylococcal enterointoxications

Staphylococcal enterointoxication results from ingestion of foods containing 1 of 11 immunologically distinct enterotoxins, A, B, C_1, C_2, C_3, D, E, F, G, H and I. Most outbreaks involve SEA and SED, which are produced over a wider range of conditions. Staphylococcal enterotoxins (SEs) form a heterogeneous group of heat-stable, water-soluble, single-chain globular proteins with a molecular weight between 28 000 and 35 000 dalton. Enterotoxins are synthesised during growth of *St. aureus* in foods, SEA and SED being secreted during the logarithmic phase and SEB and SEC during the late logarithmic to early stationery phase. Extensive growth is required to produce sufficient toxin to cause illness. Toxin production is affected by a range of factors, including pH and a_w and is more readily inhibited than growth.

Symptoms of staphylococcal enterointoxication usually appear within 2–4 hours of ingestion, although periods as short as 30 minutes and as long as 8 hours have been reported. Nausea, usually followed by retching and vomiting, is the main symptom, although diarrhoea may also occur. Less common symptoms are headache, dizziness and weakness and, possibly, visual disturbances. Temperature is often subnormal, but low-grade fever can occur, possibly accompanied by chills and perspiration (Holmberg and Blake, 1984). Staphylococcal enterointoxication is self-limiting, symptoms rarely persist after 24 hours and recovery is usually rapid. Dehydration can be severe, leading in some cases to shock, which may require hospitalisation, usually short term. Death is rare, except in those at the extremes of life, or suffering from predisposing illness.

Staphylococcal enterotoxins do not act directly on intestinal cells and thus differ from classic enterotoxins, such as cholera toxin. The mode of action resembles that of a neurotoxin, the toxin-activating receptors in the abdominal viscera generating impulses, which travel via the vagus nerve and sympathetic afferents to the vomiting centre of the brain. The action of SEB, however, appears to differ

from that of other SEs in resembling a pseudoallergic reaction. For many years, minimum dose was considered to be 1 µg, or less (Bergdoll, 1972), but calculations made in the specific circumstances of an outbreak of SEA intoxication indicated that schoolchildren developed symptoms at levels of 94–184 ng (Varnam and Evans, 1996).

14.3 Risk factors, detection methods and control procedures

14.3.1 Risk factors

Although there is considerable person-to-person variation in sensitivity to SEs, there are no well-defined risk groups, with the exception of the old, the very young and those of generally poor health. Risk of staphylococcal enterointoxication is, therefore, defined primarily by likelihood of exposure. Although the bacterium may be isolated from a wide range of foods, its presence is usually of little significance. Exposure is associated with specific types of foods, primarily those whose production, or handling, has a significant risk of contamination with *St. aureus*, which supports rapid growth of the organism and which is stored at suitable temperatures for growth (usually above 15 °C). Main types of food involved are as follows:

- **Re-contaminated, heat-processed foods.** The same risk applies to foods which have been processed by ionising radiation, high pressures and other 'novel' treatments due to the absence of a competitive microflora. Examples of foods are sliced cooked meats, cream cakes, canned products. Risk is enhanced where a high level of handling is involved.
- **Fermented foods,** where slow acidification permits growth of *St. aureus* during fermentation. Examples are salamis and cheese. Risk is enhanced where starter cultures are not used, or are poorly controlled.
- **Dried and intermediate moisture products,** where growth of *St. aureus* is favoured at some stage in processing, or storage, by a combination of temperature and reduced a_w. Examples are dried milk powder, pasta, griddle bakery products.

14.3.2 Approaches to detection and enumeration

Examination for *St. aureus* may involve both enumeration of the microorganism itself and detection of SEs. This is necessary in full-scale investigation of outbreaks of food poisoning. The cost and complexity of SE assays, however, mean that, for quality control purposes, reliance is usually placed on enumeration of the organism. In most foods, this is considered a valid approach, although there is a counter-opinion that argues that only the toxin is of importance and the presence of cells in the absence of toxin is meaningless. This opinion, however, ignores the basic tenet of quality control; that action should be taken before a crisis is reached and that the presence of *St. aureus* at numbers significantly higher

than expected is indicative of control failure at some stage, irrespective of the presence, or absence, of toxin.

Exceptions occur in foods where toxin may be present in the absence of the producing cells. Examples include dried milk powder and salamis. In the case of salami, the use of the thermonuclease test, to act as an indicator of the presence of SEs has been proposed (NRC, 1985). Improved manufacture and control, however, has reduced problems and emphasis is now on application of HACCP systems, rather than retrospective testing.

Enumeration of *St. aureus* involves direct plating, enrichment being rarely used. Many selective media exist, of which Baird–Parker (egg yolk–glycine–tellurite–pyruvate; ICMSF, 1978) medium is most widely used and most effective. Colonies may not be typical and the coagulase test, or a commercial latex agglutination test, detecting protein A should be used as confirmation. Both *St. intermedius* and *St. hyicus*, which may be present in foods, also produce coagulase. *St. intermedius* may be differentiated on Baird–Parker medium by failure to reduce tellurite, but differentiation of *St. hyicus* from *St. aureus* requires biochemical identification for which a kit, such as the API Staph-Ident is convenient. Other species may produce small amounts of coagulase and only a strong positive reaction should be recorded as *St. aureus* (ICMSF, 1978). A variant of Baird–Parker medium, Rabbit Plasma Fibrinogen (RPF) medium, contains rabbit plasma and enables the coagulase reaction to be read directly on isolation plates. In practice, the coagulase reaction may be difficult to read and there is no means of differentiating *St. aureus* and *St. intermedius*.

Rapid methods for detecting cells of *St. aureus* have been developed, although work has largely concentrated on toxin detection. These have involved both serological and genetic techniques and include a novel method based on detection of protein A by a bioluminescent enzyme immunoassay (Fukuda *et al*., 2000). Rapid detection methods are not available commercially.

A number of serological methods have been developed for detection of enterotoxins in foods and commercial kits based on enzyme-linked assays or latex agglutination are available. There is also increasing interest in real-time analysis using biosensors and surface plasmon resonance has been suggested as a means of detecting SEs (Rasooly, 2001). A major difficulty, however, can be the extraction and purification of enterotoxins from the food substrate.

14.3.3 Control procedures

Elimination

Staphylococcus aureus is not heat resistant and is destroyed at temperatures normally used in food processing, including milk pasteurisation and the recommended process severity for meats. The organism is also destroyed at treatment levels proposed for most other means of processing food for safety, such as irradiation. *St. aureus* is relatively resistant to high-pressure processing and this method may be best applied in combination with nisin. Toxins are

stable and are not destroyed by processing of severity usually applied in food processing.

Prevention of contamination

It is unrealistic to attempt to exclude *St. aureus* from raw foods of animal origin, although good hygiene practice should minimise contamination. Stringent measures are required to minimise contamination of cooked foods. Staphylococcal enterointoxication is the only major form of food poisoning in which the handler plays a significant role (Gilbert, 1983) and clearly stated precautions must be maintained. Although up to 30% of the healthy population may be carriers of *St. aureus*, routine medical screening for the organism is not justified. Hands-on operations should be minimised, however, under all circumstances. While the risk of recontamination from raw foods is generally well recognised and prevention incorporated into Hazard Analysis Critical Control Point (HACCP) plans, it should also be appreciated that some strains of *St. aureus* are able to colonise equipment and the factory environment. Cleaning schedules should take account of this possibility. Prevention of post-process contamination of canned goods is dependent on good cannery practice, primarily in ensuring seam integrity, disinfection of cooling water and avoidance of handling of cans until cooling is complete and the can fully dried.

Prevention of growth

St. aureus is of widespread distribution and total elimination is practically impossible in some foods. In such circumstances, control of growth is essential, procedures differing in different types of food.

- *Recontaminated, heat-processed foods*. The prime control is efficient refrigeration, the lowest recorded temperature for growth of *St. aureus* being 7 °C and for toxigenesis 10 °C. In foods, such as cream, and some meat products, refrigeration is the sole growth-controlling factor. *St. aureus*, however, is generally resistant to preservatives and gains a selective advantage in cooked cured products, owing to its tolerance of NaCl and nitrite. Sensitivity to other preservatives varies, but any effect is likely to be secondary to refrigeration. *Staphylococcus aureus* is able to grow in vacuum and modified atmosphere packs, although SE synthesis may be inhibited. It is not possible, however, to depend on this factor as a means of preventing staphylococcal enterointoxication.
- *Fermented foods*. Manufacture of many fermented foods involves a stage at which growth of *St. aureus* is, potentially, very rapid. Safety is dependent on rapid acidification, either by active starter cultures, or by addition of acidulants. Other practices, including 'slopping back' in salami manufacture and cheese making by 'natural inoculation', are fundamentally unsafe with respect to *St. aureus* and other foodborne pathogens.
- *Dried and intermediate moisture foods*. Problems result from a stage in manufacture in which *St. aureus* is selectively favoured by reduced a_w at a

temperature suitable for rapid growth. Control lies in design of the process to avoid such a combination of conditions.

14.4 Future trends

The general trend for staphylococcal enterointoxication has been downwards for some years. This has been attributed to increased refrigeration efficiency, but probably also reflects improved general hygiene. There is increased potential risk in the growth of the market for convenience foods, such as centrally pre-prepared sandwiches and mixed salads, which require considerable handling during manufacture.

A possible enhanced risk of staphylococcal enterointoxication exists in connection with carcass decontamination procedures, especially steam decontamination proposed for control of verocytotoxin-producing *Escherichia coli*. Such procedures are effectively 'pasteurisation' and remove a significant proportion of the spoilage microflora. In the absence of precautions against recontamination, difficult to apply in an abattoir, the risk of development of *St. aureus* exists if refrigeration is inadequate. It may be argued that introduction of carcass decontamination on a wide scale would require significant amendments to current operating systems to ensure safety with respect to *St. aureus*.

14.5 Further information

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14.6 Characteristics of the genus *Yersinia*

14.6.1 Introduction

The genus *Yersinia* comprises Gram-negative, rod-shaped bacteria, which are capable of both oxidative and fermentative metabolism of carbohydrates. The genus is a member of the family Enterobacteriaceae, although it has a number of distinctive characteristics, including small colony size and, in many circumstances, coccoid morphology of cells. Yersinias are also considered as 'low-temperature' pathogens and, unlike most other members of the Enterobacteriaceae, are able to grow at 4 °C. Incubation temperature has a profound effect on growth characteristics between 28–30 °C and 37 °C (Sheridan *et al*., 1998). Biochemical characteristics and suppression of expression of cellular components are involved. The genus comprises environmental organisms, which may be oppor-

tunistic pathogens, and three specific pathogens, *Yersinia enterocolitica*, the cause of foodborne yersiniosis, *Y. pseudotuberculosis*, which may be foodborne under some circumstances, and *Y. pestis*, the causative organism of plague. In *Y. enterocolitica* pathogenicity is largely restricted to certain bio-serotypes, others being considered non-pathogenic. Bio-serotype O:3/4 is prevalent in human infections in Europe and some parts of the USA.

National surveillance suggests that the importance of *Y. enterocolitica* varies geographically. It is of high prevalence in Belgium and the third most common bacterial infection in Sweden. In the UK, 42 cases of foodborne yersiniosis were reported to the Public Health Laboratory Service in 2000. Numbers of reported cases have fallen from a peak of 726 in 1989.

14.6.2 Nature of foodborne *Yersinia* infections

Yersinia enterocolitica

Infection by *Y. enterocolitica* can involve many symptoms, which vary according to host and bacterium-associated factors (Table 14.1). The most common are gastrointestinal, appearing within 2–3 days and characterised by abdominal pain and diarrhoea. The temperature is usually raised and vomiting may be a secondary symptom. Severity of symptoms can vary considerably and pain can be very severe, resembling that of appendicitis. This results from acute terminal ileitis and inflammation of the mesenteric lymph nodes. In adolescents, pain may be confined to the right quadrant of the trunk (right fossa iliaca), leading to possible confusion with appendicitis. *Yersinia enterocolitica* is also associated with extraintestinal symptoms, which may not be preceded by gastroenteritis. Systemic spread may occur, especially with the invasive serotype O8, predominant in North America. Symptoms include septicaemia, most common amongst the elderly and immunocompromised, soft tissue infections, conjunctivitis and exudative pharyngitis. There may also be long-term sequelae, especially arthritis.

Yersinia enterocolitica is an infectious pathogen, virulence of which is mediated partially by a 48 MDa plasmid, pVYe. This plasmid, which is similar in size and function to the virulence plasmids of other pathogenic yersinias, mediates a number of temperature-regulated phenotypes associated with pathogenicity (Table 14.2). Initial stages of infection, adherence and penetration, however, appear to be primarily under chromosomal control. Spread of most serotypes beyond the epithelial cells is limited, except in susceptible persons, and Peyer's patches are the most heavily colonised tissues. A systemic infection can result from *Y. enterocolitica* draining into the mesenteric lymph nodes (Bottone, 1997). Serotype O8 is of greater invasive capability than others, possibly because of its lower requirement for iron, yersinias being unusual Enterobacteriaceae in not producing siderophores. *Yersinia enterocolitica* produces a heat-stable enterotoxin, Y-ST, which has a similar biological activity to *E. coli* ST_A toxin. The role of the toxin in pathogenicity, however, is uncertain.

Table 14.1 Symptoms of *Yersinia enterocolitica* infections in relation to host- and becterium-related factors

Symptoms	Predisposing factors	
	Host-related	Bacterium-related
Gastroenteritis: abdominal pain, diarrhoea	All	All
Erythema nodosum	Adults	Serotype O:3
Rheumatoid arthritis	Adults	Serotype O:3
Sjøgren's syndrome	Females: middle-aged	All
Autoimmune disease	Adults	Serotype O:3
Septicaemia	Elderly, immunocompromised	Serotype O:8
Osteomyelitis	All	Serotype O:8
Pharyngitis	All	Serotypes O:3, 13a, 13b

Table 14.2 Temperature-regulated phenotypes of *Yersinia enterocolitica*

Phenotype	Expressed at	
	25 °C	37 °C
Motility	+	−
Voges–Proskauer reaction	+	−
O-Nitrophenyl galactoside (ONPG) hydrolysis	+	−
Ornithine decarboxylase	+	−
HeLa cells		
Adherence	+	±
Invasion	+	±
Serum resistance	−	+

Other species of Yersinia

Mild gastroenteritis has been associated with 'environmental' species, especially *Y. fredericksenii*, although a causal role may sometimes be doubted. Symptoms are those of gastroenteritis. *Yersinia pseudotuberculosis* is recognised as a cause of a severe, typhoid-like septicaemia, but the organism also causes less severe gastrointestinal symptoms.

14.7 Risk factors, detection methods and control procedures

14.7.1 Risk factors

Age is an important factor in determining risk of *Y. enterocolitica* infection. Persons at extremes of age are most susceptible, infants aged less than 1 year

being at greatest risk. Susceptibility to *Yersinia* infection remains high, however, until about 14 years of age. Although there is a general susceptibility to *Y. enterocolitica* among the immunocompromised, the most important predisposing conditions involve cirrhosis, or other liver disorder, and iron overload. Liver disorders and iron overload also predispose to more serious systemic infections. Hospitalised patients, in general, are at enhanced risk of *Yersinia* infection. Bio-serotypes not usually considered pathogenic have been involved on some occasions. Hospitalised patients, especially children, may also be at risk from *Y. frederickensii* and other species not usually considered pathogenic.

Most cases of yersiniosis are sporadic. The organism has been isolated from a wide range of foods, but has been associated only with outbreaks involving milk, pork, tofu and water. The carriage rate of *Y. enterocolitica* is high in pigs, the tonsil and surrounding tissue usually being involved, although the incidence can be particularly high when diarrhoeal symptoms are present in the animals. Carriage of *Y. enterocolitica* in pigs usually involves bio-serotype O:3/4, most commonly involved in human infections, although typing by pulsed-field gel electrophoresis suggests strain differences (Asplund *et al*., 1998). Contamination of meat inevitably occurs during butchery, although a direct causal link between pork and yersiniosis has been established in only a small number of cases. A strong epidemiological link has been established between consumption of raw pork mince and yersiniosis in Belgium, one of the few countries where raw pork is widely eaten (Tauxe *et al*., 1988).

The highly virulent serotypes O8 and O21 are not associated with pigs and it is unlikely that pork is a vehicle of infection. It has been suggested that wild rodents form a reservoir for these serotypes and that transmission to humans largely involves fleas, although contamination of water is also a possibility. Fleaborne transmission implies poor living conditions as a risk factor for *Y. enterocolitica* infections, although there is likely to be occupational risk for workers in agriculture, sewer maintenance and pest control.

14.7.2 Approaches to detection and enumeration

It is not usual to examine for *Y. enterocolitica* on a routine basis. Where examination is made, cultural methods are most commonly used. Most media will not differentiate between species of *Yersinia* and identification is required as part of the detection and enumeration procedure. The situation is further complicated by the fact that only certain bio-serotypes are recognised pathogens and that further testing may be required. The level to which putative isolates of *Y. enterocolitica* are identified depends on circumstances and the purpose of the work. In surveys, for example, identification to species level is usually sufficient and, in cooked foods, the presence of other *Yersinia* species may well be considered cause for concern.

Detection procedures, based on cultural methodology, have been developed specifically for *Y. enterocolitica*. Enrichment is necessary and may involve either incubation at low temperatures (4–15 °C) in non-selective broth (cold

enrichment), or selective enrichment at 25 °C. Selective enrichment is now preferred, bile–oxalate–sorbitol (BOS) usually being the medium of choice. Alkali treatments may be applied in conjunction with enrichment where the number of competing microorganisms is high, but its value in many circumstances is doubtful (Varnam and Evans, 1996). Cephaloridin–irgasan–novobiocin medium (CIN) is widely used and is generally effective, although serotype O3 may be inhibited. If necessary, a second medium should be used to recover this serotype, a modification of *Salmonella–Shigella* agar being recommended.

Confirmation of identity of *Y. enterocolitica* may be made using commercial identification kits (incubated at 30 °C). Relatively simple cultural methods also exist for determination of virulence, although interpretation can be difficult (Varnam and Evans, 1996). Rapid genetic methods for detection of *Y. enterocolitica* have been developed. A number of workers have developed polymerase chain reaction (PCR) methods, the most effective selecting primers directed both at chromosomal genes and the plasmid-borne virulence, *virF*, gene. Advantages have been claimed in terms of selectivity and specificity (Thisted-Lambertz *et al*., 1996), but use in the food industry is limited and commercial kits are not currently available.

14.7.3 Control procedures

Control procedures for foodborne *Y. enterocolitica* are similar to those for other zoonotic pathogens; reduction of levels of contamination in the pre-process food chain, destruction by thermal processing and adequate domestic cooking and prevention of recontamination. *Y. enterocolitica*, however, is generally considered to be much less important a cause of human morbidity than *Campylobacter* or *Salmonella* and relatively little effort has been put into specific precautions against the organism.

Reduction of contamination in the pre-process food chain

A high incidence of *Y. enterocolitica* carriage in pigs has been associated with particular agricultural practices, especially ‘buying in’ of pigs to the farm. This system is dictated by economic circumstances and is likely to continue. Some control of carriage rates can be exerted by good hygiene and it is common practice to quarantine incoming pigs before entry into a flock. Carriage of *Y. enterocolitica*, however, is long term and, usually, asymptomatic and the scope for control of infection at this stage is limited. Control by vaccination is a possibility, but introduction seems unlikely at present. Incidence of carriage can be modulated by a number of factors, including feeding of growth-promoting antimicrobials (Asplund *et al*., 1998).

Contamination of meat with zoonotic pathogens at a high level is often associated with poor slaughterhouse practice and improvements in technology may reduce carriage (Andersen, 1988). Concern over *E. coli* O157:H7 has enhanced efforts to reduce carcass contamination by decontamination procedures. Many decontamination treatments have been developed, ‘steam-vacuuming’ being con-

sidered particularly effective (Corry *et al*., 1995). Although some decontamination procedures are effective in reducing the incidence of pathogens, the possibility of enhanced growth if recontamination occurs is a general concern (Jay, 1996) and has been discussed above with respect to *St. aureus*. Work with *Y. enterocolitica* on pork suggests little difference in growth rate on decontaminated meat (Nissen *et al*., 2001), although longer shelf-life, resulting from reduction in the spoilage microflora could, potentially, permit a significant increase in numbers.

Although porcine carriage of *Y. enterocolitica* may involve colonisation of the gastrointestinal tract, the tonsils are more commonly involved. Control by improving standards of butchery has been proposed, with particular emphasis being placed on preventing contamination of pork mince. Although obviously desirable, the extent to which this can be achieved in industrial-scale butchery is questionable.

Elimination by processing

Yersinia enterocolitica is not unusually heat resistant and will be eliminated by adequate thermal processing, including high-temperature short-time pasteurisation of milk and generally applied processes for cooking of meat. Although there has been relatively little work on alternative processing methods in relation to the bacterium, it is unlikely that response will be significantly different from that of other members of the Enterobacteriaceae. Equally generic measures taken to prevent recontamination after processing are likely to be effective against *Y. enterocolitica*. Contamination with *Y. enterocolitica*, however, is potentially serious owing to its ability to grow at 4 °C. Vacuum-packing offers no protection and sensitivity to preservatives is similar to that of other Enterobacteriaceae, although sodium lactate, in combination with mild heat processing, has been found to be effective in control of *Y. enterocolitica* in *sous vide* foods (McMahon *et al*., 1999). Carbon dioxide is also effective in limiting growth of *Y. enterocolitica* in modified atmosphere packed fish (Davies & Slade, 1995).

With respect to risk of contamination from food handlers, human carriage is known, although there is disagreement over the extent and significance. Figures suggesting a higher carriage rate than non-typhoid salmonellas have been quoted, but it is not clear if this is true, or convalescent, carriage. Precautions against contamination by human carriers are the same as those devised for *Salmonella* and include exclusion from work during acute illness, but return to be permitted after recovery, providing stools are well formed and personal hygiene good. Convalescent persons should not, however, be allowed to handle foods to be consumed without cooking until three consecutive stool samples have tested negative for *Y. enterocolitica*.

14.8 Future trends

The relative lack of information concerning causal relationships between yersioniosis and specific foods means that predicting future trends is difficult. It

is probable that the number of cases will follow general trends, although these are likely to be biased by consumption of pork. Consumption of raw meat has gained some popularity in the USA as a supposed means of ensuring general well-being and also as 'therapy' for conditions including AIDS. Protagonists of raw meat consumption can only be described as irresponsible in the extreme and the practice can only lead to increased morbidity due to zoonotic pathogens, including *Y. enterocolitica*. There is also evidence of culinary fashion involving consumption of raw, or lightly cooked, meat in marinades. *Yersinia enterocolitica* is relatively sensitive to low pH, which reduces risk. In pork-eating countries, however, knowledge of parasitic infections associated with the meat mean that there is considerable resistance to consumption of meat which is less than fully cooked.

14.9 Further information

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14.10 Characteristics of the genus *Shigella*

14.10.1 Introduction

The genus *Shigella* is a member of the Enterobacteriaceae and conforms to the general characteristics of that group. The genus is non-motile and is relatively inert in tests for carbohydrate fermentation and other biochemical properties. *Shigella* has been thought of as a biochemically inactive variant of *E. coli* and shares some common antigens. A close relationship has been demonstrated by DNA homology, but the genera are distinct (Brenner, 1984). The genus contains four main subgroups differentiated by a combination of biochemical and serological characteristics, A (*Sh. dysenteriae*), B (*Sh. flexneri*), C (*Sh. boydii*) and D (*Sh. sonnei*).

Shigella is highly host adapted and usually infects only humans and some other primates, although a few infections of dogs have been reported. People are usually the main reservoir of infection and person-to-person transmission is the most common route of infection. The organism is also transmitted by contaminated water and food. There is a common conception that water is the more important vehicle and that food is of relatively little significance. Public health data suggest, however, that food is more important than water (ICMSF, 1996). The infectious dose of the bacterium is low, ranging from 1 to 10^4 cells (Morris, 1986).

Shigellosis is common in countries where hygiene standards are low. The infection is less common elsewhere, although there may be importation by

travellers. In the UK, 966 cases were reported in 2000, falling from a peak of 18 069 in 1992.

14.10.2 Nature of *Shigella* infections

Classic *Shigella* (bacillary) dysentery is characterised by frequent passing of liquid stools, which contain blood, mucus and inflammatory cells. The incubation period is usually 12–50 hours; onset is rapid and accompanied by fever and severe abdominal pain. Symptoms usually last 3 to 4 days, but can persist for 14 days, or longer. In healthy adults, death is rare, but *Shigella* dysentery is a major cause of death among infants in countries where hygiene is poor. Bacteraemia is an unusual symptom, being most common amongst persons aged less than 16 years, but also occurring in compromised persons, the death rate approaching 50%.

Infections with *Sh. dysenteriae* almost always develop full and severe symptoms of dysentery. Similar symptoms, although often less severe, can also be associated with *Sh. boydii* and *Sh. flexneri*. Most adult infections by these species, however, and virtually all by *Sh. sonnei* do not progress beyond relatively mild, non-bloody diarrhoea. Symptoms may differ in young children and be of greater severity, possibly involving extraintestinal symptoms, including convulsions, headaches and delirium.

Shigella is primarily an invasive pathogen, the primary site of invasion being the colon. Cells multiply in the lumen and penetrate the colonic epithelium by receptor-mediated endocytosis. Lesions form in the gastric mucosa, covered by a pseudomembrane composed of polymorphonuclear leukocytes, bacteria and cell debris in a fibrin network. Entry into the lamina propria probably results in formation of ulcerative lesions and the appearance of bloody, mucosal stools. The mechanism of ulceration is cell death caused by accumulation of metabolic products and release of endotoxin.

Invasive strains of *Sh. dysenteriae* Type 1 produce high levels of a heat-sensitive cytotoxin, commonly designated Shiga toxin. The toxin has a molecular weight of *ca* 70 kDa and consists of two polypeptide subunits A and B, which are combined in the ratio 1 : 5.7. The B subunit mediates binding of the toxin to surface receptors on target cells. The A subunit enters cells by endocytic transport, binding to 60S ribosomes and inhibiting protein and DNA synthesis, leading to cell death (O'Brien and Holmes, 1987). There is evidence for enterotoxic and neurotoxic activity as well as cytotoxic, but the mechanism is not fully understood.

Although only invasive strains of *Sh. dysenteriae* 1 produce large quantities of Shiga toxin, low levels of a Shiga-like toxin are produced by some strains of *Sh. flexneri* and *Sh. sonnei*.

Full expression of virulence by *Shigella* requires at least three genetic determinants, but a large (120–140 kDa) plasmid, present in all virulent shigellas and enteroinvasive strains of *E. coli*, determines invasive ability. A second small

(6 kDa) plasmid is also carried by virulent shigellas and codes for O antigen production, but not invasion (Watanabe and Timmis, 1984), while plasmid genes are also involved in early blockage of respiration in infected cells (Scotland, 1988). Chromosomal genes are involved in stability of the small plasmid and Shiga toxin production is probably chromosomally mediated (Pal *et al*., 1989).

14.11 Risk factors, detection methods and control procedures

14.11.1 Risk factors (including foods commonly involved)

Individual susceptibility to *Shigella* infection varies according to age and the presence of predisposing conditions. Infection is rare, however, in neonates and very young children owing to lack of receptor sites on colon epithelial cells. Immunocompromised persons are of particular susceptibility and there is a greater likelihood of bacteraemia, with consequent high mortality.

Shigella is not an environmental organism and the overwhelming source of contamination of foods is humans. The organism is readily destroyed by processing and foods involved are invariably consumed without cooking, or processing, after contamination. Any food may, in principle, be contaminated and a wide range has been implicated as vehicles of shigellosis (Table 14.3).

In practice, foods that receive significant handling during preparation are of greatest risk where contamination is direct from handlers. However, the bacterium can survive for extended periods on foods and contamination of salads, etc. can occur before harvest from soil, water, etc., containing infected faecal material. In either case, risk is greatest where hygiene standards are poor and the incidence of *Shigella* infection is high among the general population. In developed countries, shigellosis can be associated with localised failure of hygiene, leading to sporadic outbreaks in schools, military establishments, summer camps, religious communities, etc. A very large outbreak in the USA involved more than 1300 culture-confirmed cases of *Sh. sonnei* infection during 1986–87, most of the affected being tradition-observant Jews. In developed countries, shigellosis is also

Table 14.3 Foods implicated as vehicles for *Shigella* infection

Salads, various	Spaghetti
Tuna salads	Shrimp cocktail
Lettuce	Potato salad
Milk	Mashed potato
Soft cheese	Chocolate pudding
Cooked rice	Stewed apples

a disease of poverty and in the USA and Australia, the urban poor, migrant workers and native peoples are at a continuing high risk (Varnam & Evans, 1996).

14.11.2 Approaches to detection and enumeration

It is not general practice to examine foods for *Shigella* in non-outbreak situations. Examination for the organism on a regular basis is unlikely to be of any benefit in safety assurance and is a diversion of resources from management of critical control points.

Isolation is difficult owing to relatively poor growth on commonly used media, especially when other members of the Enterobacteriaceae are present. A number of media have, however, been re-formulated to improve recovery of shigellas, although use is primarily with clinical samples. Direct plating of food is unlikely to be successful, but little attention has been given to enrichment media. Selenite broth and Gram-negative (GN) broth have been used, in combination with selective plating. *Salmonella–shigella* agar, deoxycholate–citrate agar, xylose lysine–deoxycholate agar, various MacConkey agars and eosin–methylene blue agar have all been used. Increased interest in foodborne shigellosis in the 1980s led to attempts to develop new media. The most promising approach has been supplementation of various media with novobiocin. Despite this, the scope for further improvement of traditional, cultural methods appears limited.

Both serological and genetic approaches have been used in attempts to develop alternative methods for detection of *Shigella* in foods. The major concern has been detection of shigellas in stool samples and, while possibly useful, none is fully satisfactory with foods. Some effort has been made with the fluorescent antibody technique, but non-specific reactions limit use. A more promising method, the Bactigen® slide agglutination test, separately detects *Salmonella* and *Shigella*, but has not been fully validated for foods. A further approach is use of enzyme-linked immunosorbent assay (ELISA) to detect the virulence marker antigen of virulent shigellas and enteroinvasive *E. coli*, or Shiga toxin itself, but use in foods has been limited. DNA hybridisation methods using invasion-essential gene segments as probes are successful with virulent strains, but problems occur due to loss of the plasmid and selective deletion of invasion associated genes during cultivation and storage of isolates. A preferred approach is detection of the *ipaH* gene sequence which is present on both plasmid and chromosome (Venkatesan *et al*., 1989). Invasive strains of *Shigella* may also be detected in foods using PCR-based assays (Lampel *et al*., 1990; Vantarakis *et al*., 2000). None is available commercially.

14.11.3 Control procedures

The epidemiology of foodborne *Shigella* infections is such that control procedures must be directed against contamination from human sources. Shigellosis in communities is difficult to control, owing to a high incidence of person-to-person infection, multiple exposure to the bacterium and significant secondary spread.

Recognition that a problem exists is often considered the first step in control (ICMSF, 1996), accompanied by hygiene education and measures such as supervised hand washing by children.

With respect to shigellosis associated with contamination of particular foods, the Critical Control Points are prevention of contamination of salads and other crops eaten without cooking and control of contamination during handling, especially after heating or other lethal processing has been applied. In contrast to previous opinion that shigellas are delicate and have only limited survival capability in foods, the organism can survive refrigerated and frozen storage on a variety of foods. Illness may therefore occur at a time remote from the point of contamination. At the level of primary agriculture, risk has been associated with use of nightsoil as manure, or irrigation with polluted water. Leafy salad vegetables, such as lettuce, are most commonly involved and washing is unreliable even where extensive use is made of disinfectants.

Contamination during handling almost invariably involves direct human contact, although there has been one, apparently authentic, case of *Sh. flexneri* infection caused by a monkey touching a child's ice cream (Rothwell, 1981). Control involves minimising hands-on procedures, exclusion of potential excretors of *Shigella* from handling food and ensuring good standards of personal hygiene. Several outbreaks of shigellosis attributable to contamination by food handlers have been described by Smith (1987). These included an outbreak of *Sh. boydii* infection, which affected 176 persons following a pasta meal. The situation can be complicated by atypical symptoms making shigellosis difficult to recognise. The greatest risk is from convalescent excretors and return to work must be carefully monitored, three consecutive negative stool samples usually being required. Healthy excretors are considered to be of limited importance.

14.12 Future trends

In countries where *Shigella* infection is endemic, only improvement in standards of hygiene and provision of clean water supplies will alleviate the problem. It is possible that climate change leading to reduction in water supplies will exacerbate problems and that areas of endemic infection will increase. This is likely to be a general pattern involving other enteric pathogens, such as *Vibrio* spp., as well as *Shigella*. There are implications for areas outside those that may be directly affected by climate change. These include a higher incidence of imported shigellosis amongst returning travellers and contacts. There may also be a general food-associated increase, if hygiene standards fall in countries supplying salad crops and fruit.

14.13 Further information

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14.14 Characteristics of the genus *Vibrio*

14.14.1 Introduction

The genus *Vibrio* comprises Gram-negative, oxidase-positive, facultatively anaerobic rods. Differentiation from *Aeromonas* may be made on the basis of the sensitivity of most vibrios to the vibriostat O/129. Cells are often curved, or comma-shaped, and motile by a characteristic sheathed, polar flagellum. With the exception of *V. costicola*, the genus is of estuarine, or marine origin, many species having an obligate requirement for the Na^+ ion. Some species, including *V. cholerae*, may be more prevalent in fresh water than has previously been thought. Enteropathogenic *Vibrio* spp. are generally found in greater numbers in warm waters and may show a marked seasonal variation in occurrence, which correlates with temperature (Varnam and Evans, 2000). The genus is among those able to enter the putative viable non-recoverable (VNR) state (Oliver *et al*., 1995), which can complicate the epidemiology of infections.

The genus *Vibrio* contains a number of species, of which 11 have a proven association with intestinal or extraintestinal disease. The main species of importance with respect to foods are *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Seafood is the most common vehicle of foodborne infection, although water is historically associated with *V. cholerae* infection. *Vibrio vulnificus* is also an important cause of wound infections.

Foodborne infections with *Vibrio* spp. are most common in Asia. Infections are rare in Europe and usually associated with imported foods, or travellers returning from affected areas. Not all US states report *Vibrio* infections, but 30 to 40 cases of *V. parahaemolyticus* infection occur each year in Gulf Coast states, such as Texas and Alabama. *Vibrio vulnificus* infection is less common, *ca* 300 cases being reported between 1988 and 1995.

14.14.2 Enteropathogenic species (*V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*): infection and epidemiology

The three main enteropathogenic species are distinguishable on the basis of phenotypic properties, have a distinct ecology within the aquatic environment and cause illness of different types and severity.

Vibrio cholerae

The cause of serious human disease since antiquity, *V. cholera* is the causative agent of Asiatic cholera. The current cholera pandemic is the eighth and the disease is present in many parts of the world. Cholera is classically a waterborne disease, but food is also an important vehicle, especially in countries with a sea-coast. Classic Asiatic cholera is caused only by strains of *V. cholerae*

serogroup O1 and O139, which also produce cholera toxin. Serogroup O1 has two biotypes, classic and El Tor, and three serotypes, Inaba, Ogawa and Hikojima. The incubation period of cholera varies from 6 hours to as long as 3 days. Although initial symptoms may be mild, the infection rapidly progresses to copious diarrhoea ('rice water stools'), with rapid loss of body fluids and mineral salts, especially potassium. Fluid loss is up to 1 litre/hour, resulting in dehydration, hypertension and salt imbalance. Where treatment is unavailable, death may be rapid, but recovery in 1 to 6 days is normal where rehydration and salt replacement are possible.

Other illness is caused by *V. cholerae* other than O1 and O139, or by strains of these serotypes which lack the ability to produce cholera toxin. Symptoms are usually milder, but severity varies and can approach that of cholera. Gastroenteritis produced by *V. cholerae* other than O1 and O139 is characterised by diarrhoea, which is bloody in *ca* 25% of cases, abdominal cramps and fever (*ca* 70%). Nausea and vomiting accompany diarrhoea in *ca* 20% of cases. Illness caused by non-toxigenic strains of O1 and O139 usually involves diarrhoea only. Mild diarrhoea is also caused by a separate species within the *V. cholerae* group; *V. mimicus*.

V. cholerae adheres to the surface of the small intestine, little being known of the mechanism of adhesion, although there is an important role for cell-associated haemagglutinins. Cells grow, producing cholera toxin, a true enterotoxin, which is an oligomeric protein (MW 84 000), composed of two subunits, A1 (MW 21 000) and A2 (MW 7000), which is linked by covalent bonds to five B subunits (MW 10 000). The B subunits bind to a receptor ganglioside in mucosal cell membrane, while the A1 subunit enters the cells, irreversibly activating adenylate cyclase, leading to increased intracellular levels of cyclic AMP, and a biochemical reaction cascade, resulting in hypersecretion of water and salts. The role of the A2 subunit is probably in mediating entry into the cell.

Other toxins are produced by *V. cholerae*, although their significance is uncertain. These include heat-stable (ST) and heat-labile (LT) enterotoxins, a Shiga-like cytotoxin, a haemolysin similar to that of *V. parahaemolyticus* and an endotoxin. This is produced in significantly greater quantities by non-toxigenic strains. At least some of the pathogenic mechanisms of *V. cholerae* are shared by *V. mimicus*.

Vibrio parahaemolyticus

V. parahaemolyticus causes a predominantly diarrhoeal syndrome. Typical symptoms are diarrhoea, abdominal cramps and nausea. Vomiting occurs in *ca* 50% of cases and there may be a headache. Mild fever and chills occur in *ca* 25% of cases. Onset is usually after 4–24 hours, although longer and shorter periods have been reported. Symptoms usually abate within 2–3 days and death is very rare. A second, more severe, dysenteric form has been described (Twedt, 1989), which is characterised by bloody, or mucoid, stools. Human pathogenicity of *V. parahaemolyticus* usually correlates with presence of direct, thermostable haemolysin activity (Kanagawa reaction). Kanagawa-negative strains can be a cause of diarrhoea, however, and are important outside tropical countries.

Vibrio parahaemolyticus differs from *V. cholerae* in being invasive and in not producing a classic enterotoxin. Much attention has been paid to the role of the direct, thermostable haemolysin (Vp-TDH), but relatively little is known of other aspects of pathogenicity. Both flagella and cell-associated haemagglutinins are involved in adhesion to the epithelial cells before entry and penetration to the lamina propria. The direct, thermostable haemolysin promotes *in vitro* vascular permeability and has enterotoxin properties, but its precise role in induction of diarrhoea is unknown. Other toxins may be produced, including a Shiga-like cytotoxin, which may be involved in the less common dysenteric form of infection. Pathogenicity in these strains is associated with production of a second haemolysin, Vp-TDH related haemolysin.

Vibrio vulnificus

Foodborne *V. vulnificus* infections are almost invariably associated with underlying medical conditions (see below). Although similar to *V. parahaemolyticus*, *V. vulnificus* differs from this, and other enteropathogenic vibrios, in having a septicaemic rather than an enteric human pathology. In healthy persons, infection may involve no more than mild and self-limiting diarrhoea, but most cases involve primary septicaemia with fever, chills and, in many cases, nausea. Hypotension occurs in *ca* 33% of cases and *ca* 66% of patients develop skin lesions on extremities and trunk. The normal incubation period of *V. vulnificus* infection is 16–48 hours, septicaemia progressing rapidly and being difficult to treat. Mortality is 40–60%.

Invasion occurs rapidly after adhesion and is accompanied by extensive tissue damage. The organism has a number of virulence factors which protect against host defences. These include cell-associated factors protective against normal serum and complement-induced lysis and a surface component (capsule) which is antiphagocytic and anticomplementary. *Vibrio vulnificus* also produces active siderophores, which facilitate iron acquisition and which are associated with enhanced virulence when blood iron levels are high.

A number of products may be responsible for tissue damage. At least two haemolysins, which possess cytotoxic activity, are produced but, while contributing to virulence, these are not essential determinants. Specific proteinases, collagenase and phospholipases are also produced, which affect vascular permeability and cause tissue destruction.

14.15 Risk factors, detection methods and control procedures

14.15.1 Risk factors (including foods commonly involved)

Vibrio cholerae

Cholera is a disease of poverty and the malnourished and individuals at extremes of age are at particularly high risk. Genetic factors are also involved and persons

of blood group O are more highly susceptible than those of other groups. Low stomach acidity, due to over-medication with antacids, or to clinical factors, also increases susceptibility to *V. cholerae* infection.

In countries where cholera is endemic, contaminated water, used for drinking or food preparation is the most common vehicle of infection. *Vibrio cholerae* O1 can become established in the environment, which was probably the origin in an outbreak involving crabmeat in Louisiana (Blake *et al*., 1980). Most outbreaks, however, have been attributed to direct sewage pollution. Many have involved molluscs and, to a lesser extent, other shellfish and crustaceans, but outbreaks have been caused by other foods, including lettuce. An outbreak of cholera involving bottled mineral water in Portugal was attributed to contamination of the source with sewage-contaminated river water, which had percolated through subsurface strata. Despite these examples, shellfish can be a high-risk food, especially when consumed raw.

Vibrio parahaemolyticus

Little is known of individual variations in susceptibility to *V. parahaemolyticus*, with the exception of a higher risk of infection when stomach acidity is low.

Isolation of *V. parahaemolyticus* is made only occasionally from foods other than those of marine origin. Isolation can be made at any time in warm climates, but is normally a problem only in summer in temperate countries. In Europe and the USA, there is an enhanced risk of infection associated with consumption of raw molluscs (oysters, clams). In warmer areas, such as India, Japan and Africa, a similar association exists with raw fin fish. *Vibrio parahaemolyticus* infection is also associated with cooked seafood, especially crab and shrimp. This is not a consequence of inherent risk, but of poor hygiene, including the practice of cooling cooked fish with untreated sea water (Varnam and Evans, 1996).

Vibrio vulnificus

There is a strong association between invasive *V. vulnificus* infections and underlying medical conditions. Underlying predisposing conditions include chronic cirrhosis, hepatitis, thalassemia major and haemachromatosis and there is often a history of alcohol abuse. Less commonly, *V. vulnificus* infections occur where there are underlying malignancies, gastric disease, including inflammatory bowel disease and achlorhydria, steroid dependency and immunodeficiency. Males are markedly more susceptible than females and account for over 80% of infections (Oliver, 1989).

Vibrio vulnificus infections are almost uniquely associated with consumption of raw oysters. The incidence of infection follows a seasonal distribution and numbers of the bacterium may be high in oysters when water temperatures are >21 °C. Isolation, either from water or fish, is rare when water temperature is below 10–15 °C. Geographical factors are also involved, however, and while, during summer months, *V. vulnificus* is readily isolated in significant numbers in California, for example, the incidence is low in the Spanish Mediterranean sea, probably because of the high salinity value (Arias *et al*., 1999).

14.15.2 Approaches to detection and enumeration

Laboratory testing for enteropathogenic *Vibrio* plays an important part in control of foodborne infection. In many situations determination of numbers in the water of the growing beds is of greatest importance. Under most conditions, numbers are small ($<10^3$/ml), although significantly higher numbers can be present if water temperatures are very high.

Conventional cultural methods are well established for enteropathogenic vibrios. Enrichment is usually necessary and the relatively non-selective alkaline peptone water is widely used. This medium is not always effective, especially in isolation of *V. vulnificus*, if numbers of competing bacteria are high and supplementation with polymixin B can improve performance (O'Neill *et al*., 1992). Several, more selective, enrichment media have been developed, including the widely used glucose–salt–teepol broth (Beuchat, 1977), have been developed, although the value of these is not universally accepted.

Thiosulphate–citrate–bile salts–sucrose (TCBS) agar is the most widely used selective plating medium for the common enteropathogenic species. *Vibrio cholerae* (sucrose fermenting) may be distinguished from *V. parahaemolyticus* and *V. vulnificus* (sucrose non-fermenting), but other bacteria may be able to grow. Confirmatory testing is, therefore, required. Commercial identification kits primarily intended for the Enterobacteriaceae (e.g. API 20E) have been used with success, although Dalsgaard *et al*. (1996) found the API 20E to be unsatisfactory in identification of *V. vulnificus* from the environment.

A particular problem with *V. parahaemolyticus* is that most isolates from water and foods (*ca* 98%) are non-pathogenic. Where specific information is required, the Kanagawa test may be required, involving determination of β-haemolysis on Wagatsuma agar (Twedt, 1989).

Rapid genetic methods, mostly based on PCR and colony hybridisation techniques, have been developed for each of the main enteropathogenic species of *Vibrio*, including toxigenic *V. cholerae* O1 (Shangkuan *et al*., 1996). None of these has been applied on a large scale and none is commercially available.

14.15.3 Control procedures

Vibrio cholerae

In most circumstances, *V. cholerae* is not present in the food production and processing environment and no specific measures are required. As a general precaution applying to all enteropathogenic vibrios, however, harvesting of shellfish from inshore waters should cease when water temperatures exceed 25 °C, unless full processing is applied.

Where cholera is endemic, it is necessary to break the cycle of infection by protecting food from sewage contamination. This means that foods that are to be eaten without full processing should be harvested only where absence of contamination can be guaranteed. In the case of cooked foods, precautions against recontamination must be taken. Cooked crabmeat, which is picked by hand, is a

particular risk and a secondary pasteurisation to 80 °C internally is recommended. The bacterium is also highly radiation-sensitive and irradiation may be preferred where heat-induced changes are undesirable, or for secondary pasteurisation.

In areas of endemic cholera, risk of contamination is low, but constant. Regular bacteriological testing of waters of shellfish beds and less frequent checks on shellfish offer significant, but not absolute, protection. Bacteriological testing is also required for water used in food processing and for bottled water. Bacteriological testing should be used in conjunction with general precautions, including control of shellfish growing beds, application of HACCP in food processing operations and education of food handlers. It is likely to be necessary to apply regulatory control to prevent unsafe food entering the food supply system. Control of unofficial food supply can be difficult where shortages exist.

Vibrio parahaemolyticus

Control of *V. parahaemolyticus* at source can have some effect on incidence of infection and harvesting from obviously polluted beds should be prevented. Depuration or relaying in clean water is used for reducing levels of enteric pathogens in oysters and other shellfish. Properly controlled, this procedure removes loosely attached sewage-derived bacteria, but is of less efficiency with *V. parahaemolyticus*, which is a normal inhabitant of the intestinal tract of some shellfish. Under most circumstances, however, *V. parahaemolyticus* is unlikely to be present in sufficient numbers to cause illness, unless growth in the food occurs. Refrigeration to below 5 °C offers a significant level of control and such temperatures should be maintained at all stages of handling of shellfish, such as oysters and clams, and dishes, such as sushi, which are eaten raw. *Vibrio parahaemolyticus* is readily destroyed by heating, death occurring at temperatures as low as 47 °C. Recontamination must be prevented by good practice. Growth on cooked fish is very rapid at temperatures above 20 °C and efficient cooling to below 5 °C is necessary if the product is not to be consumed within 2 hours.

Vibrio vulnificus

Infection is a significant problem only in the restricted circumstances of raw oyster consumption by susceptible persons. A high level of complete control could, therefore, be achieved by ensuring that such individuals do not eat raw oysters. This requires extensive and continuing public education, including warnings on packaging, and is unlikely to be entirely successful. Risk can be reduced by suspending harvesting when water temperatures are above 25 °C, rapid chilling to less than 15 °C and maintaining strict temperature control during storage at below 5 °C. Further measures, including fishing only at cool times of day, harvesting from deeper, colder waters and protecting catch from sunlight have been described by Brenton *et al*. (2001). *Vibrio vulnificus* is heat sensitive, but cooking is not acceptable to those who insist on consuming oysters raw. Post-harvest irradiation at 1.0–1.5 kGy, which would kill *V. vulnificus*, but not the oyster, has been proposed, but is unlikely to be applied.

14.16 Future trends

Within the enteropathogenic vibrios as a whole, an increase in the incidence of infection may be predicted as a consequence of climate change. Higher water temperatures are likely to mean an increase in numbers of vibrios, especially *V. parahaemolyticus* and *V. vulnificus*, and a higher rate of infection in areas where a significant problem already exists. At the same time, warming of seas in temperate regions is likely to introduce the problem to areas where non-imported infections are rare. Problems are likely to be compounded by increasing salinity in some waters and by a general shortage of clean water for washing, etc. In the specific case of *V. cholerae*, there is a well-recognised relationship between drought and epidemic cholera (Fleurat, 1986). It is, therefore, possible that the disease will spread to, and become established in, regions where it is currently rare. These include southern European countries, such as Spain and Portugal.

The strong association of illness caused by *V. parahaemolyticus* and *V. vulnificus* with consumption of raw shellfish means that dietary fashion may directly affect the incidence of infection with these organisms. A factor in the rise of *V. vulnificus* infections in California and elsewhere in the USA was the increased popularity of the 'raw bar' (Blake, 1983). Shellfish bars are becoming increasingly popular in the UK, although the risk of *Vibrio* infection is low, owing to the very low incidence in fish caught in UK waters.

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14.18 Characteristics of the genera *Aeromonas* and *Plesiomonas*

14.18.1 Introduction

The genus *Aeromonas* comprises Gram-negative, oxidase-positive, facultatively anaerobic rods. These are insensitive to the vibriostat O/129, have no requirement for Na^+ and further differ from vibrios in producing gas during sugar fermentation (most strains). There is considerable phenotypic variation among *Aeromonas*. Gas production is variable and may be temperature-dependent, while

there can also be marked differences in cellular morphology. Two distinct groups of *Aeromonas* exist, the psychrophilic group represented by *A. salmonicida*, a fish pathogen, rarely isolated in a free-living state and the mesophilic (more correctly psychrotrophic), genera, which are usually motile. The genus has undergone taxonomic reassessment in recent years and now comprises 10 phenotypic species, of which 8 have been associated with human enteric disease (Table 14.4).

The taxonomy of the genus has been further refined by DNA–DNA hybridisation techniques and comprises at least 14 genospecies (DNA hybridisation groups: HGs). Some species of *Aeromonas* are pathogens of fish and reptiles, while others are associated with human disease (Altwegg *et al*., 1991; Deodhar *et al*., 1991). Although epidemiological links are relatively easily established, these have not been confirmed with feeding trials and direct causal links exist in only a few cases. Despite a probable involvement in foodborne disease, the status of *Aeromonas* remains as a putative enteropathogen.

Plesiomonas, which comprises a single species *P. shigelloides*, is a member of the family Vibrionaceae and shares characteristics with both *Vibrio* and *Aeromonas*, although it contains the Enterobacteriaceae common antigen. Like *Aeromonas*, it has been regarded as a putative enteropathogen, although a direct causal relationship has been established following outbreaks of illness associated with water and oysters (Wadström and Ljungh, 1991).

The putative nature of *Aeromonas* as a pathogen means that data concerning incidence are not readily available. In the UK, 261 isolations were made from faecal samples during 2000, of which 144 were identified with *A. hydrophila* and 75 with unidentified species. In the same year 31 isolations of *Plesiomonas* from faeces were made.

14.18.2 *Aeromonas* and *Plesiomonas* as human pathogens

Aeromonas has been associated with both gastroenteritis and extraintestinal disease, usually in immunocompromised hosts. Traditionally, *Aeromonas hydrophila* has been most commonly involved, although descriptions of outbreaks have not always distinguished between this species, *A. caviae*, *A. sobria* and *A. veronii*. Most human pathogenic strains are now recognised as grouping in three

Table 14.4 Phenotypic species of *Aeromonas*

A. caviae	*A. eucrenophila*[a]
A. hydrophila	*A. jandei*
A. media	*A. salmonicida*[a]
A. schubertii	*A. sobria*
A. trota	*A. veronii*

[a] Fish pathogens.

genomic species, *A. hydrophila* HG 1, *A. caviae* HG 4 and *A. veronii* biovar sobria HG 8. As the role as an enteropathogen is not definitely established, descriptions of symptoms must be treated with care. Strains vary in pathogenicity, Kirov *et al*. (1994) postulating that this results from differences in the degree to which virulence factors can be expressed. Gastroenteritis associated with *Aeromonas* varies in severity from mild diarrhoea to a life-threatening, cholera-like illness (ICMSF, 1996). Two syndromes have been described. The first, which accounts for *ca* 75% of cases is characterised by watery diarrhoea with mild, or absent, fever, possibly accompanied by abdominal pain and nausea. The second form is dysentery-like and characterised by bloody, mucoid stools. The incubation period is not known. Mild infections are self-limiting and recovery is complete within 1 to 7 days. In severe cases, symptoms are prolonged and may persist for a month or more, often requiring antibiotic therapy.

Aeromonas is associated with a range of extraintestinal disease, of which septicaemia and meningitis are most common. Other symptoms include endocarditis, eye infections and osteomyelitis. Portal of entry may be the intestinal tract and mortality can exceed 60%.

Aeromonas is able to colonise the intestine and invade epithelial cells. Significant systemic spread, however, usually occurs only in the immunocompromised. The bacterium produces a number of putative toxins, although their relationship with illness in man is not fully resolved. Both a cytotonic enterotoxin, with genetic relatedness to cholera toxin, and a cytotoxic enterotoxin, with β-haemolysin activity, have been described (Varnam and Evans, 1996). The relative importance of the two enterotoxins is disputed, but it has been suggested that the toxins of *Aeromonas* act by facilitating colonisation of the intestine and/or invasion rather than directly inducing enteritis (Todd *et al*., 1989).

Symptoms of *Pl. shigelloides* infection appear within 24 to 48 hours and are predominantly diarrhoeal (94%). Diarrhoea is accompanied by abdominal pain (74%), nausea (72%), chills (49%), fever (37%), headache (34%) and vomiting (33%). Recovery is usually within 1 to 9 days, but may be prolonged. Three types of diarrhoeal symptoms have been described, secretory, shigella-like and cholera-like. Secretory diarrhoea is most common and can vary greatly in severity. The shigella-like type is also potentially severe, with symptoms of prolonged duration, while the cholera-type illness is very rare.

Plesiomonas shigelloides is also a relatively uncommon cause of extraintestinal infection, usually where predisposing conditions are present. The intestine is the portal of entry in at least some cases and there may be prodromal enteritis. Meningitis is most common and has a mortality rate of *ca* 80%.

Although *Pl. shigelloides* has a number of putative virulence factors, including ability to adhere to epithelial cells, invasive ability (possibly only in certain strains) and toxin production, direct relationships with pathogenicity have been difficult to demonstrate. Two distinct enterotoxins are produced and there is also evidence for endotoxin, protease, elastase and haemolysin production.

14.19 Risk factors detection methods and control procedures

14.19.1 Risk factors (including foods commonly involved)

Greatest risk of *Aeromonas* infection involves persons with predisposing conditions, especially leukaemia and a deficient immune system. Persons with liver disease are also at higher risk. Males are generally more susceptible and account for *ca* 80% of cases involving persons with underlying cancer. Among healthy persons, young children are at greatest risk. Most cases involve children aged 6 months to 2 years and there is a markedly lower incidence beyond 5 years. It has been suggested that, owing to reduced intestinal pH, breast-fed children are at lower risk than formula-fed with respect to *A. caviae* infection.

Aeromonas can be isolated from a wide range of foods and is capable of growth during storage at low temperature. Isolation has been made from plant and animal foods and some strains are probably part of the normal spoilage microflora of meat, poultry and fish. Neyts *et al*. (2000), for example, recovered *Aeromonas* from 26% of vegetable samples, 70% of meat and poultry and 72% of fish and shrimps at levels of 10^2–10^5 colony-forming units (cfu)/g. Despite variation between surveys, these results are probably typical. The significance in foods, in relation to human disease, is not known and many isolates may be non-toxigenic. The potentially pathogenic genospecies HG4 (*A. caviae* complex) was, however, isolated by Neyts *et al*. (2000), especially from vegetables. *Aeromonas* has been isolated from piped water supplies, as well as bored well water and bottled mineral water (Krovacek *et al*., 1989; Slade *et al*., 1986). The bacterium can be present in high numbers in river water and there is a risk in any water abstracted from such sources for human use (Schubert, 1991), although survival rates have been reported to be highest in mineral water (Brandi *et al*., 1999). *Aeromonas* does not have enhanced resistance to chlorine and its presence in chlorinated water close to point of treatment is indicative of high organic loading, resulting in inadequate chlorination (Schubert, 1991).

There has been considerable discussion concerning the relative importance of water and food in *Aeromonas* infections, but the two sources are probably interrelated. The bacterium does appear to have an association with water and it is probable that risk of exposure is greatest through consumption of contaminated water, or food processed with contaminated water.

Plesiomonas shigelloides potentially infects all ages, but while there is disagreement over the age group at highest risk, it is generally considered that the very young, the elderly and the infirm are most susceptible. Susceptibility to infection is strongly influenced by a number of underlying conditions. Malignancies, especially leukaemia, are of importance, while low gastric acidity is a general risk factor, with a number of possible causes. These include malnutrition, gastric surgery, malignancy and, possibly, over-use of antacid preparations.

Plesiomonas shigelloides is widely distributed in the wild and is a common contaminant of natural, non-saline waters, especially in warmer months. Although the bacterium is also associated with a number of animals, risk of exposure is

most likely to involve drinking contaminated water, or consumption of raw foods, such as oysters which have had contact with contaminated water.

14.19.2 Approaches to detection and enumeration

Several authors have stated the need for monitoring for *Aeromonas* as a parameter of water quality. Although the organism can readily be isolated from foods, it is not generally examined for on a routine basis. Detection and enumeration are based on conventional cultural methods. Media were originally devised for use with water, but are suitable for foods, starch–ampicillin medium being considered most effective (ICMSF, 1996). Ampicillin–bile salts–inositol–xylose agar (*Aeromonas* medium), however, is more effective for recovery of *Aeromonas* from water when other bacteria are in high numbers (Villari *et al*., 1999) and may also be superior with unprocessed foods, especially if *A. sobria* is present (Pin *et al*., 1994). Enrichment before plating is not normally used, but tryptone–soy broth plus ampicillin is effective when other organisms are present in large numbers. Biochemical confirmation of identity is likely to be required, commercial kits are suitable, but may differ in performance (Ogden *et al*., 1994). Rapid methods have not been developed.

Foods are not usually examined for *Pl. shigelloides* and while selective media exist, these have been developed for clinical and environmental samples. Media designed for *Salmonella* are still widely used, but are over-selective and not recommended. Two media have been specifically devised for *Pl. shigelloides*, *Plesiomonas* medium and inositol–brilliant green–bile salts medium (IBB). Use of both media is likely to be most satisfactory, but IBB is recommended for single use. If enrichment is considered necessary, alkaline peptone water, or tetrathionate broth have been used, although neither is considered particularly effective (Varnam and Evans, 1996). Rapid methods have not been developed.

14.19.3 Control procedures

Basic control procedures for both *Aeromonas* spp. and *Pl. shigelloides* are those used for all Gram-negative pathogens; prevention of contamination, thermal or other, processing for safety and prevention of recontamination. In the case of salads and vegetables eaten raw, water used for washing should be chlorinated or otherwise disinfected and care should be taken to ensure that water distribution systems are not colonised by *Aeromonas*. Studies in a remote area of Italy, however, showed that while *Aeromonas* was frequently present in water sources, there was no evidence that it grew in the water distribution system after chlorination (Legnani *et al*., 1998). Presence of *Aeromonas* is independent of anthropic pollution and these authors, and others, consider that monitoring of the organism should be used as a further measure of water quality.

Water is also a potential source of contamination of processed foods and similar precautions must be taken to prevent contamination of distribution systems. Water and soil may be considered as part of an ecological continuum and

laboratory survival experiments suggest that soil may play an important role in the epidemiology of human *Aeromonas* infection (Brandi *et al*., 1996).

Human carriage of *Aeromonas* spp. or *Pl. shigelloides* is not a significant hazard and special precautions are unnecessary in food handling. *Aeromonas*, but probably not *Plesiomonas*, is capable of growth at 4 °C or below and growth cannot be totally prevented by refrigeration. There is doubt, however, that strains capable of growth at low temperatures are enteropathogenic. Little is known of the effect of preservatives, although *Aeromonas* is sensitive to NaCl and pH below 6.0 and has been shown to be inhibited by CO_2 in modified atmosphere packaging of fish (Davies and Slade, 1995).

Risk of *Aeromonas* and *Pl. shigelloides* infections is significantly greater among the immunocompromised and may be reduced by avoidance of suspect foods. This is difficult, however, in the absence of information concerning the relationship between infection and specific foods.

14.20 Future trends

Uncertainties over the role of *Aeromonas* and *Plesiomonas* mean that future trends are difficult to predict. There do not, however, appear to be any factors specific to these organisms. Numbers in the environment may increase with higher overall temperatures, while some strains of *Aeromonas* are able to grow at low temperatures and there is a possibility of a higher incidence of infection associated with further dependence on refrigeration for controlling microbial growth on foods.

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15

Characteristics of spore-forming bacteria

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15.1 Introduction

The spore-forming bacteria belong to the prokaryotic family Bacillaceae, a heterogeneous group of rod-shaped, Gram-positive bacteria. Bacterial spores are essentially 'resting stages' in the life-cycle of the bacteria, their formation generally resulting from a depletion of some essential nutrient in the growth milieu. Spores are dormant structures, with a central 'core', where the genetic element(s) of the cell resides, together with the enzymes necessary for the germination and initial outgrowth of the germinated spore. The core is highly protected from the external environment by a thick protein coat and a cortex. The protein coat is highly resistant to chemical and radiation damage, whereas it is thought that the cortex provides a very low a_W surrounding for the core, resulting in a high resistance to heat. Spores are very resistant to many environments lethal to vegetative cells, e.g. high temperatures, drying, UV and other ionising irradiation, disinfectants and chemical sterilants. These properties make for difficulties in the safe processing of foods, especially canning.

The two main genera, *Bacillus* and *Clostridium*, contain several serious pathogens of humans, animals and insects, e.g. anthrax, botulism, tetanus, several different types of 'black disease' of cattle, enterotoxaemias and gas gangrene. Many species from the genera in this family produce powerful extra-cellular enzymes responsible for degradation of carbohydrates, proteins and fats (some of these are used in 'biological detergents'). Members of the genus *Bacillus* are aerobic or facultative, whereas members of the genus *Clostridium* are anaerobic to aerotolerant. *Bacillus* species can only sporulate under aerobic conditions and *Clostridium* species only under anaerobic conditions.

15.2 *Clostridium botulinum*: general characteristics

Botulism as a neuroparalytic intoxication was recognised at least a century ago as often being derived from eating sausage (Latin, *botulus*). The producing organism is a strictly anaerobic, Gram-positive, spore-forming bacillus and is widely distributed in soils and, fresh water and marine sediments. The organism is particularly prevalent in marine and freshwater sediments where there is considerable land drainage from rivers and little water movement, e.g. the Baltic and coasts of Scandinavia, and some areas of the Great Lakes in North America. Although all strains of clostridia capable of producing the neurotoxin were originally called *Cl. botulinum*, it is now recognised that certain strains of other clostridia may be capable of producing the toxin, e.g. *Cl. butyricum*, *Cl. barati*. There are four distinct phenotypic groups recognised within this taxon (designated groups I–IV) (see Table 15.1), plus two other groups, *Cl. butyricum* and *Cl. baratii*. For food microbiologists only the group I and II strains are of major concern, producing toxin types A, B, E and rarely type F. The group III strains rarely cause illness in humans and there are only a very few reports describing organisms belonging to the other 3 groups. The organism producing type G toxin differs phenotypically from other *Cl. botulinum* types and is now designated as a distinct species, *Cl. argentinese*.

Ingestion of botulinal toxins results in muscle paralysis by blockade of the release of acetylcholine from the nerve cell across the neuromuscular synapse. Occasionally in infants, botulinal spores can germinate and colonise the intestine, releasing toxin and resulting in 'floppy baby' syndrome. This syndrome is not unknown in adults with major changes in gastrointestinal flora. The initial symptoms of ingestion of toxic food can occur between 12 and 36 hours but may be delayed up to 10 days or longer; the earlier symptoms occur, the more serious is the intoxication. The early symptoms are generally nausea, and/or vomiting,

Table 15.1 Characteristics of groups of botulinum toxin-producing clostridia[1]

Characteristic	Group			
	I	II	III	IV
Neurotoxin type	A,B,F	B,E,F	C,D	G
P/NP[a]	P	NP	NP	P
Min. growth temp. (°C)	10	3.3	15	ND[c]
Opt. growth temp. (°C)	35–40	18–25	40	37
Min. pH for growth	4.7	5.0	ND	ND
Min. a_W for growth (%NaCl)[b]	0.94 [10]	0.97 [5]	ND	ND
$D_{100\,°C}$ of spores (min)	25	<0.1	0.1–0.9	0.8–1.1
$D_{121\,°C}$ of spores (min)	0.1–0.2	<0.001	ND	ND

[a] P, proteolytic, NP, non-proteolytic.
[b] % NaCl-on-water.
[c] ND, not determined.
Source: adapted from Dodds & Austin[1].

closely followed by neurological symptoms such as double vision and fixed dilated pupils, speech impediment, difficulty in swallowing, etc. These symptoms then develop into more general lack of muscular coordination and weakness, fatigue and respiratory distress. The intoxication has often been misdiagnosed as Guillain–Barré syndrome, but whereas this begins in the peripheral systems and ascends towards the head, botulism affects the cranial nerves first. The toxic dose for humans is unknown but for mice the dose is less than 0.1 ng/kg, among the most toxic natural poisons.

Treatment of the intoxication is by rapid administration of antitoxin, although once the toxin has entered the nerve cell at neuromuscular junctions, it is unavailable for neutralisation by the antitoxin. Other medical treatment is restricted to supportive interventions to alleviate the symptoms, particularly artificial ventilation. In infant botulism the use of antitoxin is contra-indicated and supportive ventilation is needed in a minority of cases (*ca* 25%). Since the availability of antitoxin, the case fatality rate has fallen considerably. In China before antitoxin was available, *ca* 50% of all cases were fatal but more recently with antitoxin treatment this has fallen to *ca* 8%.[1]

15.2.1 Incidence of botulism

Because botulism is a serious illness, the statistics of occurrence probably more accurately reflect the true incidence of this form of food-poisoning than others, at least in well-developed countries. Misdiagnosis is still a problem in some outbreaks as many medical practitioners will not have seen botulism.[2] Commercial products generally have a good record with respect to botulism since the canning industry adopted safe heat processes and good manufacturing practices. There have been some specific exceptions that arose from not understanding the ubiquitous nature and characteristics of the organism and changing preservation mechanisms without due consideration. Thus a well-researched case in the UK involved substitution of high levels of sugar by an intense sweetener in the preparation of a hazelnut flavouring for yoghurt without consideration of the resulting high a_W and altering the pasteurisation process to a full 12-D canning process (i.e. a 12 log reduction of *Cl. botulinum* spores). Similarly, inclusion of fresh herbs and spices in oils, such as garlic in oil, is risky as shown by cases in the USA and Canada. Most cases arise from home-prepared foods such as home-canned vegetables (e.g. in USA), improperly salted or lightly cooked fish (e.g. in Scandinavia, USA, Egypt) or improper time and temperature of holding after cooking (see Dodds and Austin[1]). In Northern colder countries and generally food from marine or freshwater sources, the main causative group is Group II (psychrotrophic, non-proteolytic) strains, toxin types E and occasionally B. However, from meat and vegetables (generally previously cooked or cured), the most common type of toxin detected in outbreaks is B and occasionally A (Group I strains).

The statistics of outbreaks, cases and deaths from botulism in different countries and the foods involved indicate that in European countries one to five out-

breaks per year occur on average. Germany tends to record about 20 outbreaks per year and Poland recorded over 400 outbreaks per year over the three-year period between 1984 and 1987. The number of cases per outbreak is generally low, averaging one to five cases although some outbreaks involved more than ten persons, and the most common food involved is meats.

As the consumer demand builds for ever-decreasing processing of foods for freshness of texture and taste, and lower levels of preservatives – lower salt, nitrite, sugar etc., – combined with requirements for longer shelf-life and ready-to-eat products, there are distinct possibilities for an increase in botulism cases. Food processors would be well advised to increase their vigilance regarding the potential for the presence, survival and growth of *Cl. botulinum* in re-formulated products. The use of computer models for predicting the survival or growth of botulinum is to be recommended (e.g. FoodMicroModel).

15.2.2 Conditions for growth

Most foods contain sufficient nutrients for the growth of *Cl. botulinum*. The limiting physico-chemical parameters for growth under otherwise ideal conditions for the two main groups are summarised in Table 15.1. The solute used for lowering of a_W is salt (NaCl) but different solutes have differing effects on the growth of cells at any given a_W, and should be investigated carefully before committing changes in formulations of a food. Combinations of less than limiting conditions can be efficacious in preventing growth. A good example is that of pasteurised cured meats, in which salt (NaCl), nitrite, pH below neutral, perhaps with added polyphosphates and ascorbates, mild heat treatment and cool storage temperatures, can be combined to produce a safe stable product. If other organisms can grow in a product initially safe against botulinum, conditions may be sufficiently altered to permit toxin production; examples are pickled vegetables in which equilibration of internal pH has been too slow, or yeast or moulds have grown, raising the local pH value to permissive values. New combinations of preservation conditions should either be confirmed as safe by computer predictions or challenge studies. Reliance on a single preservative characteristic, particularly low temperatures, is unwise as consumers or retailers can abuse these conditions resulting in growth and toxin production. An example is that of the outbreak of botulism from mascarpone cheese in which the intrinsic properties (pH, a_W and absence of lactic acid bacteria) are not growth limiting, and storage at 4 °C is the only preservative factor.[3,4] Under certain special conditions, the absolute lower limits for pH and of temperature have been decreased marginally, but growth is very slow. Incorporation of oxygen in packs of foods will not prevent the growth of the obligately anaerobic clostridia, as fresh foods generally have active enzyme systems and active microbial populations that reduce the oxidation/reduction potential (E_h) to values permitting clostridial growth which may be as high as +150 mV,[5] and cooked foods have a low E_h intrinsically.

The low heat resistance of Group II botulinum spores enables the use of comparatively low heat treatments for products to be stored under refrigeration (less

than 10 °C). However, recent research has shown that lysozyme and certain other similar enzymes stimulate the germination of heat-damaged Group II spores, and can persist through mild heat.[6,7] Currently used heat processes may need re-evaluation, although there are no known cases of botulism resulting from this cause.[8]

In order to eliminate the most heat-resistant spores of *Cl. botulinum*, the canning industry adopted the concept of a 12 log reduction in spores as the benchmark of thermal processes. This reduction is achieved by the 'Botulinum Cook', based on a $D_{121\,°C}$ value of 0.2 minutes (i.e. 1 log reduction is achieved by 0.2 minutes at 121 °C). Addition of a further safety margin (12 log reduction at 121 °C = 2.4 minutes) results in a thermal process of 3 minutes at 121 °C at the slowest heating point in a product. Adopting a z value (increase or decrease in temperature required to change the D value by 1 log) of 10 °C allows the calculation of any equivalent process at alternative temperatures and integration of effective heat processes.

15.3 *Clostridium perfringens*: general characteristics

This organism was formerly known as *Cl. welchii*, and the various toxin types (A–E) are responsible for a number of serious diseases in human and animals, such as gas gangrene and enterotoxaemias. The organism is a Gram-positive, short rod-shaped cell, typically with blunt or square ends. Spores although formed, are not commonly found in media and special techniques or additives must be used to encourage sporulation. The organism, like all clostridia, is a strict anaerobe, but is rather more aerotolerant than many species for short times. The organism, although not a thermophile, has quite a high optimum temperature for growth, 43–45 °C and will continue to grow up to *ca* 50 °C. Growth slows significantly below 15 °C and ceases at 6 °C. *Cl. perfringens* is not particularly tolerant of low a_W or low pH values (Table 15.2). Under optimum conditions

Table 15.2 Growth characteristics of *Clostridium perfringens*

Growth characteristic	
Min. growth temp. (°C)	12
Opt. growth temp. (°C)	43–45
Max. growth temp. (°C)	50
Min. pH for growth	5.5
Min. a_W for growth [NaCl][a]	0.95 [8%]
$D_{95\,°C}$ of spores heat sensitive	1–3 min
$D_{95\,°C}$ of spores heat resistant	18–64 min
E_h range for growth[b]	−125 to +350 mV

[a] Different a_W limits may apply with other solutes.
[b] Growth will not occur in the presence of air, e.g. on surfaces of agar media.

for growth the organism exhibits probably the fastest growth rate of all foodborne bacteria, doubling in number every 8–10 minutes.

Any one strain of the organism can be identified by the production of a limited number of the 4 (of 13) major toxins. Although the enterotoxaemias are serious diseases, they are relatively rare compared with the food poisoning caused by some of the type A strains. The alpha-toxin produced by these strains is not that responsible for type A food poisoning as there is now known to be a specific enterotoxin related to sporulation in the GI tract.

The symptoms of *Cl. perfringens* type A food-poisoning occur between 8 and 22 hours, usually 12–18 hours, after eating contaminated foods. The symptoms consist of severe abdominal pain, flatulence and diarrhoea; nausea, vomiting and fever are rare. Recovery is generally complete within a further 24 hours and the attack is relatively mild; in a few cases in elderly persons rapid dehydration can occur, but death is rare. The infective dose of vegetative cells is typically 10^6 to 10^8 cells of a food-poisoning strain. There is no specific treatment necessary other than to replace lost fluid and salts, as the disease is self-limiting.

15.3.1 Incidence of *Cl. perfringens* food poisoning

As the disease is of short duration and the symptoms are relatively mild, there is significant under-reporting of cases. Only when there are large numbers of persons involved simultaneously, such as in a large catering operation, is there likely to be public health involvement and investigation. Thus Todd[9] has estimated that reporting in Canada and the USA is of the order of only 0.15% of actual cases. In the UK reporting may be slightly better, perhaps 1–5% of cases. There is a downward trend in the number of outbreaks and cases in both the UK and North America, by *ca* 50% in the UK since 1990 and a rather greater fall in the USA. This fall may be related to better awareness of the importance of prompt chilling and refrigeration of cooked foods and also perhaps to a decline in mass catering in factories. Although type A food-poisoning can occur at any time of the year following temperature abuse, cases are slightly more common in summer months, probably as a result of warmer temperatures and difficulties in keeping cooked foods cool.

The main vehicles of *Cl. perfringens* type A food-poisoning are cooked meats, particularly beef but also including poultry. In the USA Mexican foods that include cooked meats are emerging as a vehicle for *Cl. perfringens* food-poisoning. The major factor contributing to this type of food poisoning is temperature abuse after cooking, but incomplete cooking can stimulate the rapid germination of *Cl. perfringens* spores on cooling.

The possible future trend of food poisoning by type A *Cl. perfringens* seems likely to be downward if the pattern since about 1995 continues. However, the increasing availability of ready-to-eat meat dishes, e.g. ethnic foods, and the potential for temperature abuse, particularly in the summer months, may reverse this trend. It also seems unlikely that the rate of reporting the symptoms will increase since they are so mild. As the population of the elderly increases, there

may be opportunities for more outbreaks in residential homes where preparation of food in bulk may occur for reasons of economy. Investigations of such outbreaks will, in future, probably concentrate more on identification of *Cl. perfringens* enterotoxin (CPE)-producing strains and their origin, rather than just *Cl. perfringens*. Kits are already available for detecting CPE in faecal samples, and may become available for detecting the *cpe* gene, as induction of sporulation and production of CPE in laboratory media is difficult to achieve routinely.

15.3.2 Conditions for growth of *Cl. perfringens*

The organism although not a thermophile, has quite a high optimum temperature for growth, 43–45 °C, and will continue to grow up to *ca* 50 °C. Growth slows significantly below 15 °C and ceases at 12 °C. Vegetative cells of *Cl. perfringens* are not particularly tolerant of low temperatures, refrigeration or freezing resulting in death. Spores are more resistant to low temperatures and may survive in chilled or frozen cooked foods, only to germinate and begin rapid growth on reheating the food. Cultures to be stored should not be chilled but stored at ambient temperatures (spores are rarely formed in laboratory media), or freeze-dried. *Cl. perfringens* is not particularly tolerant of low a_W or low pH values (Table 15.2). For complete inhibition of growth, 6–8% salt is required, and up to 400 ppm of nitrite. Thus commercially cured products of acceptable preservative levels may not completely inhibit the growth of this organism. However, the observation that commercially cured meats are relatively rarely involved in type A food poisoning indicates that curing salts are effective in significantly reducing growth of *Cl. perfringens*.[10]

As indicated previously, the organism is very tolerant of E_h conditions that would inhibit many other clostridia (Table 15.2). Under optimum conditions for growth the organism exhibits probably the fastest growth rate of all foodborne bacteria, doubling in number every 8–10 minutes.

15.4 Bacillus spp.: general characteristics

15.4.1 *Bacillus cereus*

Bacillus cereus is a large (greater than 0.9 µm wide), motile, Gram-positive, facultative aerobic, rod-shaped bacillus, sporulating readily only in the presence of oxygen, and is widely distributed in the environment. There are several *Bacillus* spp. closely related physiologically to *B. cereus*, e.g. *B anthracis* (causative agent of anthrax), *B. thuringiensis* (a crystal-forming organism responsible for a lethal infection/intoxication of several insect larvae) and *B. mycoides* (producing a fungal-like colony). The resistance of the spores to adverse environmental conditions, and the ability to produce a range of food-degrading enzymes, e.g. protease, amylase, lecithinase, enable *B. cereus* to survive and grow well in many different conditions. It is typically responsible for the defect in temperature-

abused pasteurised milk called 'bitty cream' that results from degradation of lecithin, the cream-stabilising emulsifier in milk, during growth of the organism from spores surviving pasteurisation.

Only in the early 1950s was *Bacillus cereus* recognised as a food-poisoning bacterium.[11] It is now known that a restricted number of strains of this organism can produce one or more of a number of toxins responsible for symptoms of food poisoning. The emetic toxin produced in foods, causes nausea and vomiting and occasionally diarrhoea, *ca* 1–5 hours after consumption of a contaminated meal, and closely resembles symptoms of food poisoning caused by *Staph. aureus*. *Bacillus cereus* can produce at least three different enterotoxins, two of these are tripartite toxins and both of these are associated with foodborne illness. The most studied is HBL and this is thought to be the primary virulence factor in *B. cereus* diarrhoea. The diarrhoeagenic toxin produced in the GI tract, causes abdominal pain and diarrhoea, but rarely nausea and vomiting, *ca* 8–16 hours after a contaminated meal, and closely resembles the symptoms caused by *Cl. perfringens* enterotoxin. The effective dose for the emetic syndrome is 10^5–10^8 cells/g of food where the toxin is pre-formed in the food, and 10^5–10^7 total cells to infect the small intestine for the diarrhoeagenic syndrome (see Table 15.3). For either intoxication, symptoms resolve after 6–24 hours, and while some patients may be sufficiently severely affected to be hospitalised, reports of mortality are extremely rare. Recovery is usually complete in 24–48 hours, and no specific treatment other than replacement of fluid and salts loss is indicated. Some other species of the genus *Bacillus* have occasionally been shown to cause food poisoning of either type (see later).

The emetic toxin has only recently been isolated and characterised, as the only test methods were either induction of emesis in primates or vacuolation of Hep-2 cells (or more recently Vero, CHO or McCoy cells) in tissue culture. This toxin, now named cereulide, is a ring structure of four amino acids repeated three times, hydrophobic in nature, resistant to heat, low pH and proteolysis and is non-immunogenic.[12,13]

Table 15.3 Characteristics of *Bacillus cereus* resistance and growth

Character	Values
Spore heat resistance D_{100}	1.2–8 min
z	6–9 °C
Germination temperatures – limits	5–50 °C
optimum	30–35 °C
Growth limits, pH	4.3–9.3
Temperature[a]	5/15–35/50 °C
A_W, broth	0.95
boiled rice	*ca* 0.91
Generation times, broth culture 35 °C	*ca* 20 min
boiled rice 30 °C	*ca* 30 min

[a] 5/35 °C limits for psychrotrophic strains; 15/50 °C for mesophilic strains. Spore germination may occur up to about 60 °C.

Table 15.4 Characteristics of *Bacillus cereus* food-poisoning syndromes

Characteristic	Emetic syndrome	Diarrhoeal syndrome
'Infective' dose	10^5–10^8/g food	10^5–10^7 total cells consumed
Production site of toxin	In food	In small intestine
Symptoms	Nausea and vomiting; possibly also diarrhoea	Abdominal pain, diarrhoea; occasionally nausea
Incubation time	1–5 hours	8–16 hours
Duration of symptoms	6–24 hours	12–24 hours
Toxin characteristics	Small peptide; heat resistant; acid stable; hydrophobic; protease stable; non-immunogenic	At least three protein enterotoxins recognised; heat and protease sensitive; immunogenic
Most common food vehicles	Re-heated rice, farinaceous products	Meat products, stews, soups, sauces, puddings, milk products

15.4.2 Incidence of *B. cereus* food poisoning

The incidence of either form of this disease is seriously under-reported as the symptoms in most cases, are relatively mild and of short duration. Only when an outbreak involves several cases and arises from a single source, is the disease likely to be reported and investigated. The emetic syndrome is most commonly associated with consumption of rice and some other farinaceous foods in which the spores have survived cooking, germinated and allowed sufficient time at moderate temperatures for growth and toxin production to occur. The diarrhoeagenic syndrome is more often associated with meat-containing dishes such as stews and soups, although vegetables, sauces and milk products may also be vehicles (Table 15.4).

Differences in the reported incidences of *B. cereus* seem to reflect differences in eating habits but also the frequencies of other food-poisoning incidents in various countries. Thus in Japan, North America and Europe (in general), the reported outbreaks are between 1% and 22% of all outbreaks. However, the frequency of the emetic and diarrhoeal syndromes differ; in Japan the emetic syndrome is ten times more frequently reported than the diarrhoeal syndrome, whereas in Europe and North America the latter is more frequently reported. In the UK the most common source of the emetic syndrome used to be fried or re-heated rice dishes, until the importance of rapid cooling and chill storage in prevention was realised and advice given.[14] In Norway, however, whereas the country is almost free of *Salmonella* and *Campylobacter* food-poisoning, *B. cereus* is the most commonly reported food-poisoning syndrome.

15.4.3 Conditions for growth

Bacillus cereus produces a wide range of extracellular enzymes capable of hydrolysing proteins, fats and starch, as well as a range of other carbohydrates. The organism can therefore utilise a wide range of food materials to support growth, but starch-containing foods seem to be optimal for growth. Even though the diarrhoeal syndrome is mainly associated with meat-containing dishes, many of these will have been thickened with starch or contain spices that are often heavily contaminated with *B. cereus* spores.

B. cereus is a mesophilic organism with an optimum temperature for growth of 30–35 °C, although some strains are psychrotrophic, growing at temperatures down to *ca* 5 °C. These latter strains are mostly associated with milk and dairy products. The upper temperature limit for germination of spores (i.e. loss of the specific spore characteristics of heat resistance, refractility under phase contrast microscopy) is rather higher than for vegetative growth of cells. Thus germination will occur during cooling of boiled rice (at 55–60 °C) with rapid generation of vegetative cells on further cooling. Although *B. cereus* is not generally regarded as tolerant of low pH and a_W values, it will grow in boiled rice with a_W values of *ca* 0.91. The main characteristics of the organism are summarised in Table 15.3.

15.4.4 Other *Bacillus* species, *B. subtilis*, *B. licheniformis*, *B. thuringiensis*

These first two species have occasionally been shown to be responsible for incidents of food-poisoning resembling one or other of the syndromes typical of *B. cereus*.[14–17] Thus *B. subtilis* food-poisoning is of rapid onset (*ca* 2–3 hours) and consists of acute vomiting often followed by diarrhoea, thereby resembling the emetic syndrome of *B. cereus*. *B. licheniformis*, on the other hand, produces mainly diarrhoea about 8 hours after consuming contaminated food, although vomiting is also quite common. This latter syndrome resembles food poisoning caused by *Cl. perfringens* or the enterotoxin of *B. cereus*. There is also an 'intermediate' species, *B. pumilus*, having many of the characteristics of both *B. subtilis* and *B. licheniformis* group, which has also been recorded in a very few cases of vomiting and diarrhoea in the UK. The numbers of organisms recovered from incriminated foods indicates that in excess of 10^6 colony-forming units (cfu)/g are present, generally as a result of temperature abuse. Faecal samples from patients also often yield an almost pure culture of the organism.

There is currently some concern being expressed regarding the potential production of one of the toxins of *B. cereus* by some strains of *B. thuringiensis*, an organism being used as an insecticide for certain crops. During an investigation of an outbreak of gastroenteritis in Canada,[18] it was shown that all the clinical isolates were cytotoxic (to McCoy cells) and identified as *B. thuringiensis* by virtue of crystal formation on sporulation. Damgaard *et al.*[19] isolated several strains of *B. thuringiensis* (lethal for cabbage white caterpillar) from foods almost all of which were toxic to Vero cells. In a similar study with an improved cyto-

toxicity test, of seven strains of *B. thuringiensis* tested on McCoy cells, all were cytotoxic; two strains tested were also positive in immunological assay kit tests.[20] Three of six strains of *B. mycoides* and three of seven strains of *B. subtilis* also tested on McCoy cells, were also cytotoxic and some were positive in immunological assays. Hsieh *et al.*[21] conducted a thorough investigation of several strains of the *B. cereus* group (including *B. thuringiensis*, *B. anthracis*, *B. mycoides*) for possession of enterotoxigenic genes and cytotoxicity. All *B. thuringiensis* strains exhibited more than one of the enterotoxin genes and all were cytotoxic to CHO cells. Similarly, all strains of *B. mycoides* tested were cytotoxic. These results call into question whether many cases of '*B. cereus* food-poisoning' are in fact due to *B. thuringiensis*, *B. mycoides* or other closely related strains of the *B. cereus* group. The increasing use of *B. thuringiensis* insecticides may therefore be of concern, unless manufacturers have checked that their strain(s) does not contain the enterotoxigenic genes. Generally the identification of '*B. cereus* food-poisoning' does not extend to more detailed investigation of the exact subspecies responsible, e.g. para-sporal crystal formation for *B. thuringiensis*.

15.5 Methods of detection: *Clostridium botulinum*

There are no sufficiently selective/indicator media for the detection of the organism. There have been immunofluorescence methods developed for detection of the main groups of *Cl. botulinum*.[22,23] However, reliance is generally placed on growth of the organism in a food enrichment culture at 25–30 °C for 5–10 days and detection of toxin. In most cases, vacuum-packaging the food sample, perhaps with adjustment of pH and addition of *ca* 10%w/w of freshly steamed and cooled cooked meat broth for additional nutrients, is sufficient. Botulinum toxin is detected by intra-peritoneal injection of mice with filter-sterilised cultures or food extracts and observation of typical neuroparalytic symptoms over a period of up to 3 days. Toxin can be neutralised by boiling for 10 minutes, the usual negative control for toxin tests, or with specific antiserum. Several authors have devised immunologically based assays, e.g. radio-immunoassays (RIA), enzyme-linked immunosorbent assays (ELISA; see, e.g., Notermans and Kozaki,[24] Shone *et al.*,[25] Gibson *et al.*[26]), but these do not measure toxicity and may suffer from interference by toxoid or other proteins, and a general lack of sensitivity compared with the animal model. Similarly, detection of the genes responsible for coding for the toxins (using specific probes and polymerase chain reaction, PCR), also does not detect production of toxin, but can be valuable in detecting the presence of the organism in samples (see, e.g., Aranda *et al.*,[27] Franciosa *et al.*[28]). The specific mechanism of action of botulinum toxins is the cleavage of one of the complex of peptides responsible for conducting the neurotransmitter acetylcholine from the containing vesicle within the motor nerve cell into the synaptic space, and thus paralysing the nerve–muscle junction. The action of the botulinal toxins on these peptides is a result of their very specific peptidase activity.[29,30] An assay based upon this peptidase activity is now being

developed using recombinant or synthetic peptides[31,32] and may eventually replace *in vivo* tests.

15.6 Methods of detection: *Clostridium perfringens*

Early methods relied on the rapid formation of a 'stormy clot' reaction in milk, but this is not necessarily definitive and is easily mis-read. Agar or liquid media containing sulphite and iron salts are commonly used to detect clostridia by formation of black precipitates, but are not sufficiently specific for *Cl. perfringens*. Liquid iron-sulphite containing media may be used in Most Probable Number (MPN) techniques. Since the organism does not readily form spores in laboratory media, heating presumptive positive (black) cultures in liquid media and subculturing into fresh medium and examining for growth, is rarely successful.[33] However, streaking of the presumptive positive culture on to a diagnostic medium often yields characteristic colonies of *Cl. perfringens*.[33] Selective agar media are now generally employed for the isolation and enumeration of *Cl. perfringens*, one of the most commonly used being egg yolk-free tryptose–sulphite–cycloserine agar incubated at 37 °C.[34] Confirmation of the identity of the organism is by relatively few physiological tests, reduction of nitrate to nitrite, fermentation of lactose, lack of motility and indole production, fermentation of lactose and gelatin digestion (but not casein) being some of the more important tests. A very useful diagnostic agar medium for the common clostridia in foods was devised by combining lactose fermentation, egg yolk reaction (Nagler; lecithinase activity), lipase and and proteolysis of milk (Lactose Egg Yolk Milk (LEYM) agar).[35]

However, the simple detection of *Cl. perfringens* type A does not necessarily indicate a food-poisoning hazard exists. Only a small proportion of strains are capable of producing CPE, and Saito's[36] survey of a range of foods and faecal samples from food handlers using very sensitive CPE serological assays indicated that only shellfish and faecal samples yielded CPE-positive cultures. Although no CPE-positive cultures were detected in poultry, pigs or cattle in Saito's survey, in a later survey using *cpe* gene technology, positive cells were recovered from a variety of animals including food animals.[37] However, it also appears that the gene controlling CPE production can be either plasmid- or chromosomally-borne, giving rise to speculation that there may be two distinct populations of food-poisoning strains of the organism.[38] There is also the possibility that CPE-negative strains may acquire a plasmid-borne *cpe*-gene under certain conditions. However, for most practical purposes in a food microbiology laboratory, the demonstration of large numbers in an incriminated food or in faecal samples from patients with typical symptoms, preferably backed up by CPE-positive results, is sufficient evidence for *Cl. perfringens* type A food poisoning. However, tracing the definitive food source of the organism requires the use of specific agglutinating antisera, of which 75 were described by Stringer *et al.*[39] In general these are only available in public health laboratories.

CPE has now been isolated and characterised and consists of a single polypep-

tide of *ca* 35 kDa, with an isoelectric point of pH 4.3. The amino acid structure appears to be highly conserved between the CPEs produced by several strains.[38]

15.7 Methods of detection: *Bacillus* spp.

Almost all techniques for the detection of *B. cereus* are based on plating media formulated to demonstrate one or more of the physiological properties of the growing cells. Thus many indicator agar media incorporate egg yolk to demonstrate lecithinase activity (a white precipitate around colonies), or haemolytic activity (horse, rabbit, sheep or human red cells may be used). Various egg yolk-containing agar media have been described, but the most commonly used is that described by Holbrook and Anderson[40] (PEMBA) or a derivative of the formulation (e.g. PEMPA).[41] These contain a low level of peptone to stimulate sporulation, pyruvate to reduce the size of colonies, polymixin as a selective agent, egg yolk to demonstrate lecithinase production, mannitol and a pH indicator (*B. cereus* does not ferment mannitol unlike many other *Bacillus* spp.). PEMPA is claimed to result in typical colonial appearance in 24 hours rather than 48 hours for PEMBA, with formation of spores that may be observed by phase contrast microscopy.

If determination of a spore count is required, then a pasteurisation treatment (e.g. 70 °C for 10 minutes) may be applied to samples before plating. However, spores may germinate once hydrated from dry foods, giving a low spore count.

The occurrence of high numbers of *Bacillus* spp. from a suspect food should always be regarded with suspicion, whether or not the typical characteristics of *B. cereus* are present. The species of the genus *Bacillus* are often difficult to differentiate as the metabolic and physiological properties of the genus seem to be a continuum of small differences. *B. subtilis* can be differentiated from *B. cereus* and *B. licheniformis* by its inability to grow anaerobically, although even the smallest amount of oxygen will permit limited growth. *B. cereus* is the only one of these three species that produces lecithinase, but does not ferment mannitol, xylose or arabinose. Thus *B. cereus* can readily be distinguished from the other two species on PEMBA or PEMPA.

15.8 Control issues: *Clostridium botulinum*

Since the organisms responsible for botulism are widely distributed in the environment, it is essential that effective measures be taken to control growth and toxin production. However, the spore load in the environment differs considerably in different parts of the world. Food from different regions therefore, will be contaminated to a greater or lesser extent.[1] Modification of products, whether changes in the packing of cans, e.g. affecting the fluidity of the product (i.e. changing convection heating to conduction heating), changing one acid for another, e.g. in pickling, or the level or type of solute (e.g. NaCl, glucose,

glycerol, sucrose) or degree of cooking or baking resulting in adjustment of a_w, must be considered carefully and tested. Thus the reduction in salt in certain preserved products may reach values that cannot prevent the growth of the organism, e.g. in cold-smoked fish levels of less than 3.5% salt on water have been found.[42] In general the tendency towards more lightly preserved, lower thermal processing of foods could readily lead to more outbreaks of botulism as more reliance is placed on low temperatures (less than 3 °C) alone to prevent or limit growth. Temperature abuse, in the distribution chain or in the domestic refrigerator, can occur readily.

15.9 Control issues: *Clostridium perfringens*

Since the organism is found in many natural environments, it must be assumed that many foods, especially animal-derived foods, will contain at least low numbers of the organism. Prevention of food poisoning must minimise cross-contamination from soil and faecal material. Careful attention to hygienic practices in the abattoir is the first line of defence. As the organism is generally present in soil and faecal matter, the initial control point is presentation of clean animals from the farm. To minimise distribution of the organism via the blood stream, cleaning of the hide prior to 'sticking' and a clean stick knife, are also control points. Careful removal of the hide (to minimise contamination of the skinned carcase by dust or faecal matter), and of the GI tract, and minimising cross-contamination by abattoir line workers are all important practices. However, while these practices can minimise, they cannot be relied upon to prevent contamination. As the infective dose of the organism is high, prevention of growth assumes prime importance. This is readily achieved in practice by control of the chilling regime and chill storage of raw meats, proper cooking schedules, prompt cooling and refrigeration of cooked meats, and proper re-heating and holding temperatures, i.e. heating to above 70 °C and holding above 60 °C. Type A food-poisoning from CPE pre-formed in foods has only occasionally been suspected, and CPE is quite heat sensitive. Therefore, destruction of vegetative cells by proper heating and prevention of their growth is an important control point in food service operations. Cross-contamination of cooked from raw meats in food service is also important. In preparation and storage of cured meats, the correct combination of curing salts (more than 3.5%w/w salt and more than 100 ppm nitrite) and cool temperatures will minimise or prevent the growth of *Cl. perfringens*.

15.10 Control issues: *Bacillus* spp.

These organisms are frequently found in dried foods, e.g. spices, farinaceous ingredients, milk powders, as heat-resistant spores. On rehydration of such foods, spores will germinate and begin to grow under suitable conditions. Germinated

spores can be readily killed by pasteurisation, but it is known that not all spores will germinate at the same time, as the specific germinating agent L-alanine is racemised by the germinating spores to D-alanine, which is a potent inhibitor of germination. Control consists of limiting germination of spores and more importantly limitation of opportunities for growth, by temperature, low a_W or pH values or combinations of these. If it is necessary to store cooked foods, then rapid cooling and chill storage will minimise growth. For example, rice should be cooked in small quantities, cooled quickly, preferably by cold water either poured through the rice or submersion of the cooking container in cold water, and storage in a refrigerator once cool.[15] Although psychrotrophic strains may continue to grow at temperatures down to 5–8 °C, the growth rate is very slow (doubling time *ca* 15 hours at 5 °C).

15.11 Sources of further information and advice

15.11.1 *Clostridia*

Several sources of advice are available to the food industry. Leatherhead Food Research Association has operated a Botulinum Laboratory for many years and offers member companies advice and challenge-testing facilities for assessing the botulinal safety of food products. Leatherhead Food RA also markets Food MicroModel, a computer-based set of predictive models for the growth and death of all the major foodborne pathogens, including *Cl. botulinum* and *Cl. perfringens*. LFRA Library has several books on *Cl. botulinum* and other food-poisoning organisms, and the Information Group at Leatherhead Food RA can also access the scientific literature on all aspects of food poisoning, and provide information on the current situation or conduct literature searches on specific items. Campden and Chorleywood Food Research Association (CCFRA) also has similar facilities for work with clostridia.

The Central Public Health Laboratory (CPHL), Colindale, London, has facilities for examining foods for botulinal toxin, *Cl. perfringens* and *B. cereus*, but generally is active only in outbreak situations. Much of the recent research work on alternative methods for detecting the botulinal toxin, has been in conjunction with the Centre for Applied Microbiological Research (CAMR), part of the Microbiological Research Authority within the Department of Health.

Much of the recent research on heat resistance of the spores of psychrotrophic strain of *Cl. botulinum* and lysozyme activation of germination, has been done at the Institute for Food Research, Norwich, together with determination of the limitation of growth by temperature and salt.

Useful texts on botulism and the organism include the following:

DOYLE M P, BEUCHAT L R and MONTVILLE T J, (1997) *Food Microbiology, Fundamentals and Frontiers*, ASM Press, Washington, DC, Section III, ch. 15, K L Dodds and J W Austin.

HAUSCHILD A H W and DODDS K L, (1993) Clostridium botulinum: *Ecology and Control in Foods*, Marcel Dekker, Inc., New York.

HUI Y H, GORHAM J R, MURRELL K D and CLIVER D O, (1993) *Foodborne Pathogens*, Marcel Dekker, Inc., New York, K L Dodds, pp. 97–131.
DOYLE M P, (1989) *Foodborne Bacterial Pathogens*, Marcel Dekker, Inc., New York, A H W Hauschild, pp. 111–89.

15.11.2 *Clostridium perfringens*

Useful texts on *Cl. perfringens* include the following:

WRIGLEY D M, (1994) *Clostridium perfringens*, in Y H Hui, J R Gorham, K D Murrell and D O Cliver (Eds) *Foodborne Diseases Handbook, vol. 1 Diseases Caused by Bacteria*, Marcel Dekker Inc., New York, pp. 133–67.
LABBE R, (1989) *Clostridium perfringens*, in M P Doyle (Ed.) *Foodborne Bacterial Pathogens*, Marcel Dekker Inc., New York, pp. 192–227.

15.11.3 *Bacillus* spp.

Food MicroModel has models for the growth of mesophilic and psychrotrophic strains of *B. cereus* allowing estimation of growth rates at different temperatures, pH value and salt content (a_W). Current work in Leatherhead Food RA is modelling the growth of *B. cereus* during cooling of a model food using growth rates predicted by Food MicroModel combined with the Ratkowsky Square Root Model to produce a dynamic model. The predictions are being validated in a cooling rice milieu. Advice, modelling and challenge testing can also be carried out by LFRA. Testing for the diarrhoeagenic toxin by ELISA or latex agglutination techniques, and for the emetic toxin by vacuolation of Hep-2 cells, can also be performed.

The Central Public Health Laboratory, Colindale, London has antisera for serotyping strains isolated during food-poisoning outbreaks, which can help in identifying the food source.

Useful texts on *B. cereus* include:

BOUWER-HERTZBERGER S A and MOSSEL D A A, (1982) Quantitative isolation and identification of *Bacillus cereus*, in J E L Corry, D Roberts and F A Skinner (Eds) *Isolation and Identification Methods for Food Poisoning Organisms*, Academic Press, London, pp. 255–9.
KRAMER J M, TURNBULL P C B, MUNSHI G and GILBERT R J, (1982) Identification and characterization of *Bacillus creus* and other *Bacillus* species associated with foods and food poisoning, in J E L Corry, D Roberts and F A Skinner (Eds) *Isolation and Identification Methods for Food Poisoning Organisms*, Academic Press, London, pp. 261–86.
GRANUM P E, (1997) *Bacillus cereus*, in M P Doyle, L R Beuchat and T J Montville (Eds) *Food Microbiology, Fundamentals and Frontiers*, ASM Press, Washington, DC, pp. 327–36.
SCHULTZ F J and SMITH J L, (1994) *Bacillus*: recent advances in *Bacillus cereus* food poisoning research, in Y H Hui, J R Gorham, K D Murrell and D O Cliver (Eds) *Foodborne Disease Handbook, Volume 1: Diseases caused by Bacteria*, Marcel Dekker, New York, pp. 29–62.

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Part III

Non-bacterial and emerging foodborne pathogens

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16

Viruses

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16.1 Introduction

Viruses are increasingly recognised as causes of foodborne and waterborne illness in humans (Motarjemi *et al*., 1995). They get into the food chain by contamination with faecal material from infected persons. Numerous viruses replicate in the human gut, but only a few of these are commonly recognised as important foodborne pathogens. The enteric viruses can be categorised into three main groups, according to the type of illness they produce: viruses that cause gastroenteritis, the enterically transmitted hepatitis viruses, and a third group of viruses that replicate in the human intestine, but cause illness after they migrate to other organs such as the central nervous system (Table 16.1) (Codex Alimentarius, 1999). Foodborne illness has been documented for most of these viruses, but recent studies suggest that the Norwalk-like caliciviruses (NLV) and hepatitis A virus (HAV) are the most common cause of illness by this mode of transmission (Mead *et al*., 1999). Incidentally, large foodborne outbreaks have occurred with group B and C rotaviruses, and waterborne outbreaks with hepatitis E virus and group B rotavirus (Hung *et al*., 1984; Su *et al*., 1986; Matsumoto *et al*., 1989; Oishi *et al*., 1993). Food or water may be contaminated by release of faecal material from infected persons. In addition, virus transmission via vomiting and the subsequent generation of aerosols has been suggested for some of the gastroenteritis viruses (Chadwick and McCann, 1994; Chadwick *et al*., 1994; Patterson *et al*., 1997).

16.1.1 Description of organism

The NLV (also known as small-round-structured viruses or SRSV) and the Sapporo-like viruses (SLV) belong to the family *Caliciviridae*, hepatitis A,

Table 16.1 Viruses that replicate in the gut, according to type of illness associated with infection and likelihood of food- or waterborne transmission

Likelihood of food- or waterborne transmission	Illness		
	Gastroenteritis	Hepatitis	Other
Common	Norwalk-like calicivirus	Hepatitis A virus	
Occasionally	Enteric adenovirus (types 40/41) Rotavirus (group A–C) Sapporo-like calicivirus Astrovirus Aichivirus	Hepatitis E virus (waterborne)	Enterovirus
No			HIV cytomegaloviras (CMV)

enteroviruses, and Aichivirus to the family *Picornaviridae*, rotavirus to the family of *Reoviridae*, adenoviruses to the family *Adenoviridae*, and astrovirus to the family of *Astroviridae* (Green *et al*., 2000a; Melnick, 1992; Yamashita *et al*., 1998). The hepatitis E viruses have not yet been grouped in one of the existing virus families (Berke and Matson, 2000). Most are among the smallest viruses (22–38 nm), and have a quite simple structure: the RNA genome is folded inside a spherical capsid (Table 16.2). The adenoviruses are larger and have a double-stranded DNA genome, whereas rotavirus measure approximately 70 nm and have a segmented double-stranded RNA genome (Russell *et al*., 1991; Estes, 1991). Neither of these viruses is surrounded by a lipid envelope, which renders them insensitive to the effect of lipid solvents and chloroform.

16.1.2 Symptoms caused in humans

People infected with NLV, SLV, astrovirus and rotavirus develop symptoms after a short incubation period, typically 24–48 hours, whereas incubation times for adenoviruses and enterovirus are somewhat longer (average 7–14 days; Table 16.2). The longest incubation times are found for hepatitis A virus infection, ranging up to 50 days, and hepatitis E infection (up to 10 weeks; Mast and Alter, 1993). Most illness associated with astrovirus, rotavirus, adenovirus and enterovirus occurs in (young) children (Koopmans *et al*., 2000; de Wit *et al*., 2001a, b). For NLV, in addition to this, clinical symptoms commonly occur in adults, often seen as explosive outbreaks in institutions such as nursing homes

Table 16.2 Characteristics of (infections with) enteric viruses

Virus	Calicivirus	Hepatitis A virus	Rotavirus	Adenovirus	Astrovirus	Enterovirus	Hepatitis E virus
Size (nm)	30–38	22–30	70	80–110	28–30	22–30	30–38
Genome	ssRNA	ssRNA	dsRNA	dsDNA	ssRNA	ssRNA	ssRNA
Average incubation period	24–48 h	up to 50 days	24–48 h	7–14 days	24–48 h	1–2 weeks	Up to 10 weeks
Symptoms	Vomiting and diarrhoea	Hepatitis	Diarrhoea, vomiting, fever	Diarrhoea	Diarrhoea	Diarrhoea, meningitis, encephalitis, paralytic illness, hand, foot and mouth disease, rash illnesses	Hepatitis
Affected age groups	All	Depending on level of endemicity	Children <5 year	Children <5 year	Children <10 year	Children <15 year	All
Duration of illness	days	weeks	days	Days–weeks	days	Days–weeks sometimes lifelong	Weeks
Severity of illness	Mild	Severity increasing with age of first infection	Major cause of death in developing countries	Mild	Mild	Can be severe, but majority of infections subclinical	Generally mild, except for pregnant women
Special risk groups	Institution-alised hospitalised	Fulminant course with underlying hepatitis					Pregnant women

and hospitals (Vinjé and Koopmans, 1996; Vinjé *et al*., 1997). The onset of illness may be abrupt, with vomiting observed in a majority of those infected (NLV), or watery diarrhoea (NLV, astrovirus, rotavirus, adenovirus). In outbreaks of illness with NLV, the attack rate is high (30–100%) and secondary transmission is quite common. Recovery usually is rapid, although deaths associated with NLV infection have been reported (Djuretic *et al*., 1996). Hepatitis A virus infects people of all ages, but results in more serious illness with increasing age of first contact with the virus (Hadler and McFarland, 1986). The infection will result in non-specific symptoms such as fever, headache, fatigue, nausea and vomiting, followed by signs of hepatitis 1–2 weeks later. Virus shedding typically continues until 1 week after onset of jaundice in adults and 1–2 weeks in children, although prolonged intermittent shedding may occur for up to 90 days in patients with clinical relapses (Bower *et al*., 2000). Relapses have been reported in 1.5–18.5% of persons. Children with symptoms were found to excrete low levels of HAV RNA for up to 10 weeks after the onset of symptoms (Robertson *et al*., 2000). In a recent study, HAV RNA was detected an average of 17 days before the onset of jaundice (and the concomitant increase in the levels of alanine aminotransferase), and viraemia persisted for an average of 79 days after the liver enzyme peak. The average duration of viraemia was 95 days (range, 36–391 days; Bower *et al*., 2000).

Since contaminated foods may contain multiple agents, mixtures of symptoms may occur.

16.1.3 Treatment

In healthy, immunocompetent hosts, most enteric viral infections are self-limiting. Occasionally, diarrhoea and vomiting may lead to dehydration, necessitating parenteral fluid therapy and hospitalisation. Deaths associated with NLV outbreaks have been reported, but probably are rare. In young children, rotavirus infection is among the most common causes of hospitalisation, and may lead to dehydration (de Wit *et al*., 2001c). For HAV infection, treatment is aimed at maintaining comfort and an adequate nutritional balance (Hollinger and Ticehurst, 1996). There is no specific antiviral treatment for any of the viruses described.

16.2 Current level of incidence

16.2.1 Community incidence

Gastroenteritis viruses

Recent studies in The Netherlands and the UK have shown a quite high community incidence of gastro-enteritis (283 cases per 1000 persons per year for The Netherlands, 190 per 1000 for the UK) (Wheeler *et al*., 1999; de Wit *et al*., 2001b). In The Netherlands, viruses accounted for a total of 33.5% of cases, with

NLV in 16.1% of all cases, SLV in 6.3%, rotavirus in 7.3%, astrovirus in 2%, and adenovirus types 40/41 in 3.8% (de Wit *et al*., 2001b). Of these, 8 per 1000 persons per year seek medical help in The Netherlands, with rotaviruses found in 5.3%, adenoviruses in 2.2%, astrovirus in 1.5%, NLV in 5.1% and SLV in 2.4%. From these studies, the NLV emerged as the most common cause of illness in people of all age groups (Koopmans *et al*., 2000). The estimates for NLV were somewhat lower in the UK, possibly resulting from the difference in methods used to screen stool samples for viruses (electron microscopy versus reverse transcriptase, polymerase chainreaction, RT-PCR) (Tompkins *et al*., 1999). From molecular typing of the NLV positive stool samples from The Netherlands, it was shown that numerous lineages or genotypes of NLV circulate in the community (Koopmans *et al*., 2000).

It is difficult to estimate the proportion of cases that could be due to foodborne infection. Mead *et al*. (1999) estimated that this is the mode of transmission for 40% of all NLV infections, but there are insufficient data at present to support this estimate.

Hepatitis A virus

For hepatitis A virus, the community incidence varies significantly, depending on geographical region. This variability reflects an important aspect in HAV epidemiology: with increasing levels of hygiene, the incidence of HAV infections has gone down to an estimated 10 cases or less per 100 000 persons per year in low endemic regions (Mast and Alter, 1993). This is reflected by the decreasing seroprevalence of HAV antibodies, observed in most countries with high standards of living, with a concomitant increase in the proportion of non-immune, hence susceptible, individuals and a shift of infections to a higher age group in which the rate of subclinical infections is much lower. Figure 16.1 shows an example of the differences in age-specific seroprevalence for some countries across Europe (Stroffolini *et al*., 1991; Dubois *et al*., 1992; Beutels *et al*., 1998; de Melker *et al*., 1998; Beran *et al*., 1999; Dal-Ré *et al*., 2000). The net result is that after an initial decline in the number of HAV cases their incidence and the potential for (larger) outbreaks of HAV infection have increased. In countries with lower standards of hygiene, HAV infections occur typically during childhood, and often go unnoticed since a large proportion of HAV infections in children are asymptomatic.

16.2.2 Data from outbreaks

Several studies of outbreaks of gastroenteritis have now clearly shown that a large proportion of these is caused by NLV, including foodborne outbreaks, and waterborne outbreaks (Vinjé and Koopmans, 1996; Vinjé *et al*., 1997; Fankhauser *et al*., 1998; Brugha *et al*., 1999; Kukkula *et al*., 1999; Noel *et al*., 1999; McCarthy *et al*., 2000; Iritani *et al*., 2000; Koopmans *et al*., 2000; Schreier *et al*., 2000). The contamination often occurs by infected food handlers, and it is important to realise that this may be in any step of the production chain

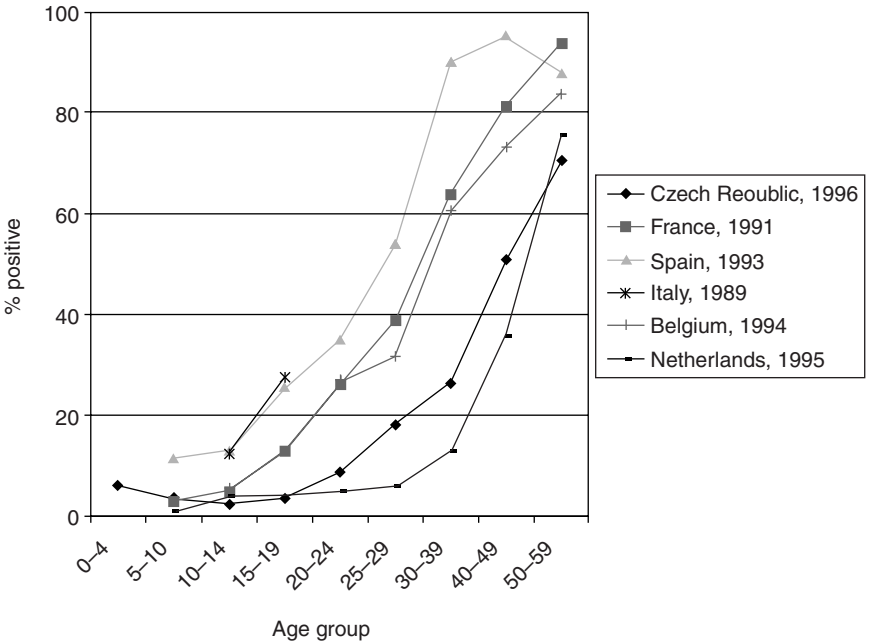

Fig. 16.1 Seroprevalence of HAV antibodies in some countries in Europe (from: Czech Republic: Beran *et al*., 1999; France: Dubois *et al*., 1992; Spain: Dal-Ré *et al*., 2000; Italy, Stroffolini *et al*., 1991; Belgium, Beutels *et al*., 1998; The Netherlands, de Melker *et al*., 1995).

(e.g. seasonal workers picking berries). As a result, outbreaks have occurred from the consumption of foods that had been grown and harvested in a different region, but was contaminated early in the chain of production (Pönkä *et al*., 1999; Gaulin *et al*., 1999; Berg *et al*., 2000). Outbreaks have been linked to infected food handlers who fell ill after preparing a meal, or who had recovered from intestinal illness (Lo *et al*., 1994; Parashar *et al*., 1998). In this context, it is important to note that the duration of shedding of NLV may be at least up to 10 days post-infection. Aerosol transmission may occur through the generation of aerosols during vomiting (Chadwick *et al*., 1994). These aerosols may result in widespread environmental contamination (Cheesbrough *et al*., 2000). Outbreaks of HAV are detected less commonly. In countries with high levels of hygiene they are often linked to import of infection via a traveller that contracted the virus in an area of high endemicity (Termorshuizen and van de Laar, 1998). Occasionally, outbreaks of HAV via contaminated food or water have been reported (Halliday *et al*., 1991; Skala *et al*., 1993; Malfait *et al*., 1996; CDC, 1997; Leoni *et al*., 1998; De Serres *et al*., 1999; Hutin *et al*., 1999; Massoudi *et al*., 1999; Bosch, 2000; Hemmer and Hansen-Koenig, 2000). An inverse correlation between dose and incubation period has been observed (Istre and Hopkins, 1985).

16.3 Conditions of growth and survival

Viruses, unlike bacteria, are strict intracellular parasites and cannot replicate in food or water. Therefore, viral contamination of food will not increase during processing, and may actually decrease. Most enteric viruses grow only under special conditions *in vitro*, but the NLV have not been adapted successfully to cell or organ culture despite numerous attempts. Some HAV strains have successfully been adapted to cell culture, but isolation of HAV from patient specimens remains difficult.

Most food- or waterborne viruses are relatively resistant to heat, disinfection and pH (>pH 3). It is no coincidence that most virus groups implicated in outbreaks are small, non-enveloped particles, rather than large, fragile, enveloped viruses. Problems in the detection of viral contamination of food or water are that – generally – the contaminated products will look, smell and taste normal, and that (molecular) diagnostic methods are not routinely available in food microbiology laboratories. Abad *et al*. (1994) have shown that viruses persisted for extended periods on several types of materials commonly found in institutions and domestic environments. Overall, HAV and human rotavirus were more resistant to inactivation than enteric adenovirus and poliovirus. NLV were not tested in this study (Abad *et al*., 1994). For HAV, it was demonstrated that survival was inversely proportional to the level of relative humidity and temperature, and the half-lives of the virus ranged from greater than 7 days at low relative humidity (25%) and 5 °C to about 2 h at high relative humidity (95%) and 35 °C (Mbithi *et al*., 1991). Similar findings were reported for rotavirus (Sattar *et al*., 1986). However, the opposite was found for enteroviruses such as poliovirus and enterovirus 70 (Mbithi *et al*., 1991; Sattar *et al*., 1988). Survival was longer on non-porous surfaces and variable on porous surfaces (Sattar *et al*., 1986; Nauheim *et al*., 1990). Adenoviruses were found to survive in desiccated circumstances for up to 35 days on a plastic surface (Nauheim *et al*., 1990). It should be noted that numerous strains or genotypes exist for all foodborne viruses, which may differ in their behaviour. In dried faeces HAV remained viable for 30 days when stored at 25 °C and 42% relative humidity (McCaustland *et al*., 1982). Heating at 60 °C for 10 minutes was sufficient to kill the relatively resistant HAV in solution, but not after addition of $MgCl_2$ or of organic matter such as oystermeat, suggesting that inactivation profiles need to be assessed for each individual critical step in a production process (Croci *et al*., 1999).

Aerosols – generated while vomiting – are thought to play a role in the transmission of foodborne viruses (Caul, 1994), and rotaviruses were found to survive best in aerosols at medium relative humidity (50% ± 5%), where its half-life was nearly 40 h. Three per cent of the infectious virus was detectable in the air after 9 days of aerosol age at 20 °C. Rotaviruses could, therefore, survive in air for prolonged periods, thus making air a possible vehicle for their dissemination (Sattar *et al*., 1984). Again, enteroviruses behave quite differently, with most favourable conditions for survival at high relative humidity (Ijaz *et al*., 1985). These findings stress the need for independent assessment of behaviour for

different viruses. This poses a problem for the NLV, which cannot be grown in cell culture and therefore are not readily tested under the experimental conditions described above. It remains to be seen if other viruses that can be grown in tissue culture may serve as a model for the NLV as has been suggested for feline caliciviruses (Slomka and Appleton, 1998).

Finally, in water, viruses may survive for prolonged periods of time, with over 1 year survival of poliovirus and rotavirus in mineral water at 4 °C (Biziagos *et al*., 1988). This is particularly relevant given the finding of Beuret *et al*. (2000). By testing 69 mineral waters from 29 brands, they found that 33% contained NLV RNA. Clearly, it is not known if the presence of PCR-detectable RNA indicates the presence of infectious virus.

16.4 Detection methods

Infection with gastroenteritis viruses is typically diagnosed by the detection of the pathogen in stool samples from sick people, rather than by measuring the antibody response in serum. Commercial ELISA-based assays are available for detection of group A rotaviruses, adenoviruses and astroviruses, and for some lineages of NLV (Vipond *et al*., 2000; Kobayashi *et al*., 2000). For non-group A rotaviruses, SLV and the remaining NLV, the diagnosis can be made by detection of viral nucleic acid by RT-PCR assays (Gouvea *et al*., 1991; De Leon *et al*., 1992; Vinjé *et al*., 2000b). A problem with NLV is the great variability of the viral genome, making it difficult to develop a single generic detection assay (Green *et al*., 2000b; Vinjé *et al*., 2000a). For the hepatitis viruses, detection of specific IgM antibodies is diagnostic of recent infection. In addition, virus can be detected in stools and in serum by RT-PCR. For most enteric viruses, strains can be subtyped by sequence analysis of genome fragments (Gouvea *et al*., 1990; Gentsch *et al*., 1992; Robertson *et al*., 1992; Belliot *et al*., 1997; Vinjé *et al*., 2000b). By doing so, common source outbreaks have been diagnosed, even though a link between different outbreaks had not been suspected on the basis of the epidemiological investigation (De Serres *et al*., 1999; Kukkula *et al*., 1999; Robertson *et al*., 2000).

The advances in genome characterisation also led to the development of molecular detection assays adapted for food. However, a major problem remains, as molecular tests do not provide information on viability of the pathogen, thereby rendering the assays of little use for food manufacturers and processors at present. In addition, while several methods have been developed, very few have been used under field conditions with the inherent problem of heterogeneity of samples, and uneven distribution of contaminants in faecal-soiled foodstuffs.

16.5 Control issues

It is clear that most problems with foodborne viruses occur from contamination of food products during manual handling in combination with minimal process-

ing of foods afterwards. Given the high incidence of NLV infections, which peaks in winter in temperate climates, it is probably wise to assume that the introduction of viruses into the food chain is a probable event that needs to be prevented by stringent hygienic control. Food handlers with young children are at special risk of introducing viruses, given the high incidence of infections in this age group. Increasing the awareness of all food handlers about transmission of enteric viruses is needed (including the spread of viruses by vomiting), with special emphasis on the risk of 'silent' transmission by asymptomatically infected persons and those continuing to shed virus following resolution of symptoms. At present, insufficient data are available to determine which steps are going to be critical for all foods in a Hazard Analysis Critical Control Point (HACCP) system. Water used in combination with the culturing or preparation of food should be of high quality, although there is not currently enough information available on the methods of choice for enumeration of the presence of viruses in waters. Guidelines specifically aimed at reduction of viral contamination are needed, as it has become clear that the current indicators for water and shellfish quality are insufficient as predictors of viral contamination. Based on a review of outbreaks of gastroenteritis in hospitals in the UK, the Public Health Laboratory Service has recommended special precautions for contamination control in food preparation areas. This includes disinfection with freshly prepared hypochlorite-based cleaner containing 1000 ppm available chlorine (Chadwick *et al*., 2000).

Documented outbreaks of foodborne infections could be reported faster using, for example, the 'rapid alert system for food' of the European Union or the US Foodnet and would be much more informative if typing information of virus strains would be included.

A vaccine is available for hepatitis A, and contacts can be treated with the administration of immunoglobulin up to two weeks after exposure. The Advisory Committee on Immunisation Practices (ACIP, 1996), USA, suggests that HAV vaccination should be considered for food handlers, although the risk assessment will be different for each country given the great differences in seroprevalence of HAV.

16.6 Sources of further information and advice

Special issue of the *Journal of Infectious Diseases* (2000), **181** Suppl. 2, on Caliciviruses.

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17

Parasites: *Cryptosporidium*, *Giardia* and *Cyclospora* as foodborne pathogens

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17.1 Introduction

Giardia, *Cryptosporidium* and *Cyclospora* are intestinal protozoan parasites that parasitise both human and non-human hosts. Increasing evidence since 1970 has implicated these organisms as significant contaminants of food. Their life cycles consist of reproductive stages, which infect the intestine, and transmissive stages (cysts of *Giardia* and oocysts of *Cryptosporidium* and *Cyclospora* [(oo)cysts]) which are excreted in the faeces of infected hosts. Of great importance is that (oo)cysts are environmentally robust, being capable of prolonged survival in moist dark environments. Whereas cysts of *Giardia* and oocysts of *Cryptosporidium* are infectious to susceptible hosts immediately following excretion, oocysts of *Cyclospora* are not infectious when excreted and require a period of maturation in the environment before they become infective to other hosts. Of the various species of *Giardia*, *Cryptosporidium* and *Cyclospora*, *Giardia duodenalis*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* are significant pathogens of humans.

17.2 Description of the organisms

17.2.1 Life cycles

Giardia

The genus *Giardia* consists of five species: *G. agilis*, infecting amphibians, *G. muris*, infecting rodents, *G. duodenalis*, infecting mammals, *G. psittaci*,

infecting budgerigars and parakeets, and *G. ardeae*, infecting great blue herons. The parasites that infect humans are also known as *G. intestinalis* (= *lamblia*) and are ascribed to the *duodenalis* species. *G. intestinalis* is regarded by some authorities as a race of *G. duodenalis*. *Giardia* parasites infecting humans can also infect non-human hosts. In this chapter we use the species name *duodenalis* to describe those *duodenalis* 'type' parasites which infect both human and non-human hosts. Exposure to the acidity of the stomach and the alkalinity of the jejunum induces the cyst to excyst, producing two pyriform (pear-shaped) *G. duodenalis* trophozoites which attach onto the apical surfaces of enterocytes and divide by binary fission. Detachment from enterocytes, together with exposure to increased concentration of bile salts and elevated pH during passage through the lumen of the small intestine cause trophozoites to encyst into ovoid cysts which are excreted in faeces. The life cycle of *Giardia* is presented in Fig. 17.1.

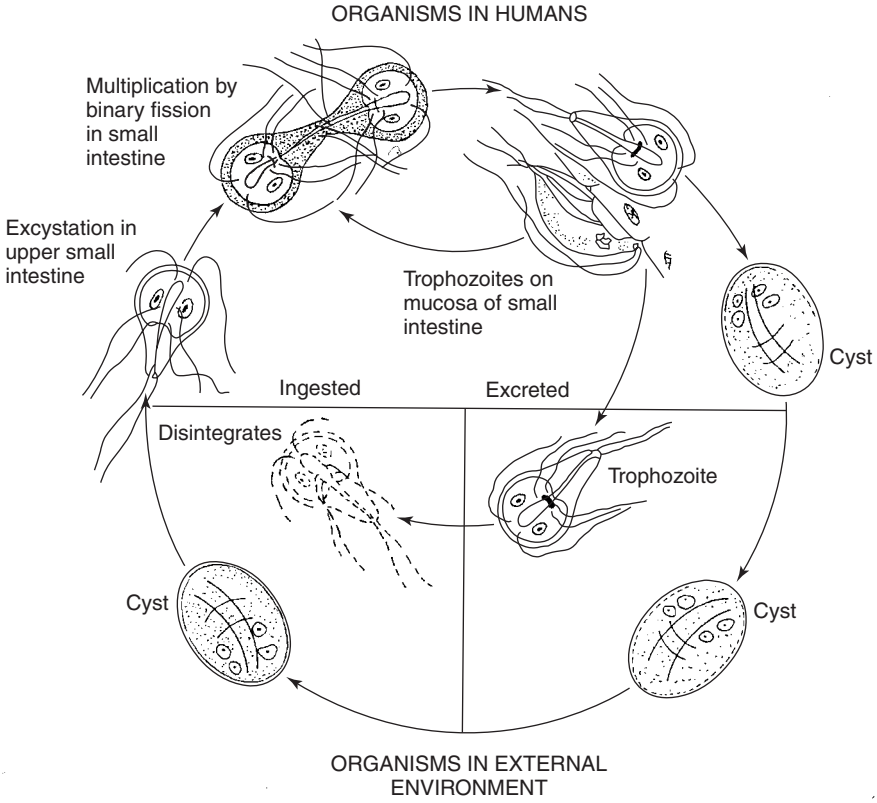

Fig 17.1 Life cycle of *Giardia*. The life cycle is direct, requiring no intermediate host, and the parasite exists in two distinct morphological forms, namely the cyst and trophozoite. Redrawn from Meyer and Jarroll (1980).

Cryptosporidium

Originally described by Tyzzer (1910, 1912), *Cryptosporidium* has emerged as an important pathogen of human beings in the last 25 years. Although more than 20 'species' of this coccidian parasite have been described on the basis of the animal hosts from which they were isolated, host specificity as a criterion for speciation appears to be ill founded as some 'species' lack such specificity. Currently, there are ten 'valid' species: *C. parvum*, *C. andersoni* and *C. muris* which infect mammals; *C. baileyi* and *C. meleagridis* which infect birds; *C. serpentis* and *C. nasorum* which infect reptiles and fish respectively; *C. wrairi* has been described in guinea pigs; *C. felis* in cats and *C. saurophilum* in lizards. *Cryptosporidium felis* has also been identified as a cause of infection in humans, in a small number of cases. The discovery of DNA sequence-based differences within the ribosomal RNA (rRNA) gene repeat unit between individual isolates within a 'valid' species means that the taxonomy of the genus remains under revision. Recently, *C. meleagridis* has been described from 6 immunocompetent (out of 1735 specimens) human patients. Purified oocysts from the patient's faecal material were indistinguishable from *C. parvum* by conventional methods, but showed genetical identity to *C. meleagridis* determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of the *COWP* gene and sequencing of the COWP, TRAP-C1 and 18S rRNA PCR gene fragments (Pedraza-Diaz *et al*., 2000).

The life cycle of *C. parvum* is complex (Fig. 17.2), comprising asexual, sexual and transmissive stages in a single host (monoxenous). The spherical oocyst measures 4.5–5.5 μm in diameter and contains four naked (not within a sporocyst) crescentic sporozoites (Table 17.1; Fig. 17.2). Fayer *et al*. (1990) provide a good account of the biology of *Cryptosporidium*.

Two genotypes of *C. parvum* have been identified: genotype 1, found primarily in humans, and genotype 2 with a much broader host range, including humans, and other mammals. As yet, no recombinant of these two genotypes has been identified, suggesting that they maintain separate reproductive strategies.

Cyclospora

Recently identified as a coccidian parasite, *Cyclospora* organisms have been implicated in human enteritis since 1977. Prior to 1992, their classification remained in doubt, being referred to, among others, as 'cyanobacterium-like bodies' and 'coccidia-like bodies'. The species that infects humans, *Cyclospora cayetanensis* (Ortega *et al*., 1993), is closely related to the genus *Eimeria* (Relman *et al*., 1996). Eleven species of *Cyclospora* have been described from moles, rodents, insectivores, snakes and humans. Recently, three new species of *Cyclospora* isolated from monkeys and baboons from western Ethiopia have been proposed: *C. cercopitheci* from green monkeys, *C. colobi* from colobus monkeys and *C. papionis* from baboons (Eberhard *et al*., 1999). *Cyclospora* oocysts are spherical, measuring 8–10 μm in diameter, and are excreted unsporulated.

The life cycle of *Cyclospora* is not fully understood, but involves both sexual and asexual stages of development in a single host. As for *Giardia* and

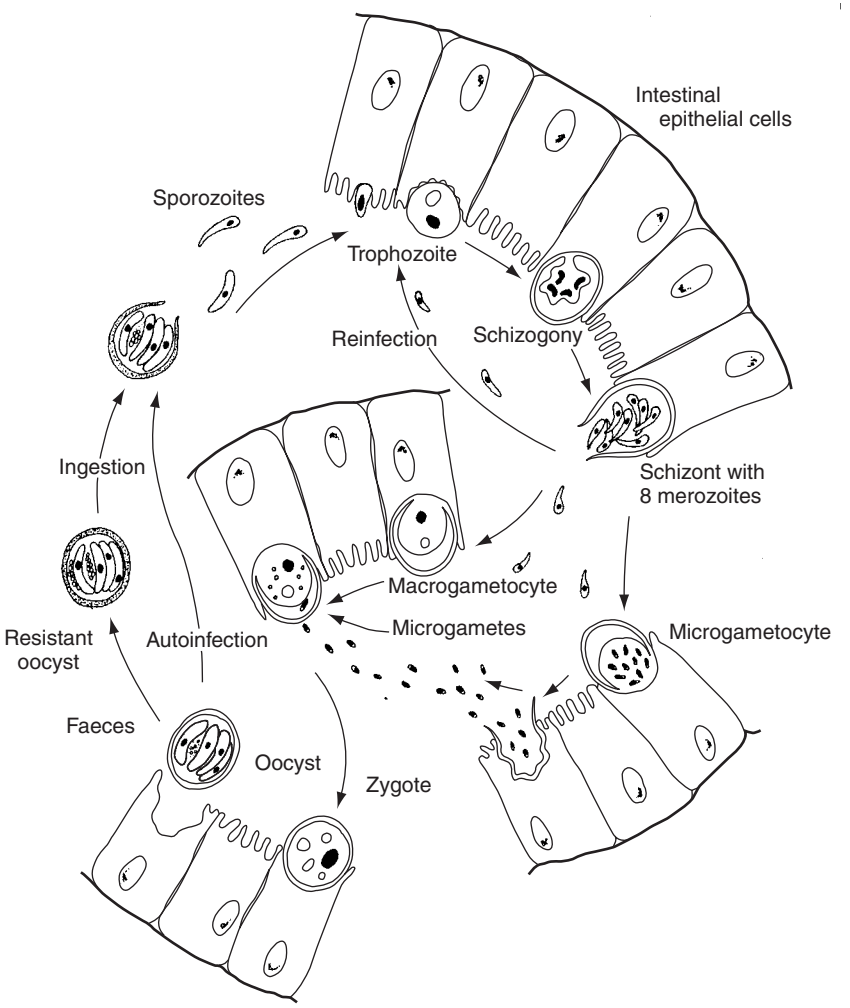

Fig. 17.2 Life cycle of *Cryptosporidium*. Reproduced with permission from Smith and Rose (1980).

Cryptosporidium, exposure to the acidity of the stomach and the alkalinity of the jejunum causes the sporozoites contained within sporocysts to excyst. Two types of meronts and sexual stages were observed in the jejunal enterocytes of biopsy material from oocyst excreting humans (Ortega *et al*., 1997a). Under laboratory conditions, 40% of oocysts exposed to temperatures of 25–30 °C sporulated after 1–2 weeks, each oocyst containing two sporocysts, with two sporozoites within each sporocyst (Ortega *et al.*, 1993; Smith *et al*., 1997).

Table 17.1 Characteristic features of *G. duodenalis* cysts and *C. parvum* and *C. cayetanensis* oocysts by epifluorescence microscopy and Nomarski differential interference contrast (DIC) microscopy

Appearance of *G. duodenalis* cysts and *C. parvum* oocysts: under the FITC (fluorescein isothiocyanate) filters of an epifluorescence microscope

The putative organism must conform to the following fluorescent criteria: uniform apple green fluorescence, often with an increased intensity of fluorescence on the outer perimeter of an object of the appropriate size and shape (see below).

Appearance of *C. cayetanensis* oocysts: under the UV filters of an epifluorescence microscope

The putative organism must conform to the following fluorescent criteria: uniform sky blue autofluorescence on the outer perimeter of an object of the appropriate size and shape (see below).

Appearance under Nomarski differential interference contrast (DIC) microscopy

Giardia duodenalis cysts	*Cryptosporidium parvum* oocysts	*Cyclospora cayetanensis* oocysts
• Ellipsoid to oval, smooth walled, colourless and refractile	• Spherical or slightly ovoid, smooth, thick walled, colourless and refractile	• Spherical, smooth, thin walled, colourless and refractile
• 8–12 × 7–10 µm (length × width)	• 4.5–5.5 µm	• 8–10 µm
• Mature cysts contain four nuclei displaced to one pole of the organism	• Sporulated oocysts contain four nuclei	• Unsporulated oocysts contain developing sporocysts
• Axostyle (flagellar axonemes) lying diagonally across the long axis of the cyst	• Four elongated, naked (i.e. not within a sporocyst(s)) sporozoites and a cytoplasmic residual body within the oocyst	• Sporulated oocysts contain two ovoid sporocysts, each containing two sporozoites
• Two 'claw-hammer'-shaped bodies lying transversely in the mid-portion of the organism		

17.3 Symptoms caused in humans

17.3.1 Giardiasis

Giardiasis is self-limiting in most people. The short-lived acute phase is characterised by flatulence with sometimes sulphurous belching and abdominal distension with cramps. Diarrhoea is initially frequent and watery but later becomes bulky, sometimes frothy, greasy and offensive. Stools may float on water. Blood

and mucus are usually absent and pus cells are not a feature on microscopy. In chronic giardiasis, malaise, weight loss and other features of malabsorption may become prominent. Stools are usually pale or yellow and are frequent and of small volume and, occasionally, episodes of constipation intervene with nausea and diarrhoea precipitated by the ingestion of food. Malabsorption of vitamins A and B_{12} and D-xylose can occur. Disaccharidase deficiencies (most commonly lactase) are frequently detected in chronic cases. In young children, 'failure to thrive' is frequently due to giardiasis, and all infants being investigated for causes of malabsorption should have a diagnosis of giardiasis excluded (Smith *et al*., 1995a; Girdwood and Smith, 1999a).

Cyst excretion can approach 10^7/g faeces (Danciger and Lopez, 1975). The prepatent period (time from infection to the initial detection of parasites in stools) is on average 9.1 days (Rendtorff, 1979). The incubation period is usually 1–2 weeks. As the prepatent period can exceed the incubation period, initially a patient can have symptoms in the absence of cysts in the faeces.

17.3.2 Cryptosporidiosis

In immunocompetent patients

Cryptosporidium is a common cause of acute self-limiting gastroenteritis, symptoms commencing on average 3–14 days post-infection. Symptoms include a 'flu-like illness, diarrhoea, malaise, abdominal pain, anorexia, nausea, flatulence, malabsorption, vomiting, mild fever and weight loss (Fayer and Ungar, 1986). From 2 to more than 20 bowel motions a day have been noted, with stools being described as watery, light-coloured, malodorous and containing mucus (Casemore, 1987). Severe, cramping (colicky) abdominal pain is experienced by about two-thirds of patients and vomiting, anorexia, abdominal distension, flatulence and significant weight loss occur in fewer than 50% of patients. Gastrointestinal symptoms usually last about 7–14 days, unusually 5–6 weeks, while persistent weakness, lethargy, mild abdominal pain and bowel looseness may persist for a month (Casemore, 1987). In young malnourished children, symptoms may be severe enough to cause dehydration, malabsorption and even death. Histopathology of infected intestinal tissue reveals loss of villus height, villus oedema and an inflammatory reaction. Mechanisms of severe diarrhoea are primarily consequences of malabsorption, possibly due to a reduction of lactase activity. The ratio of symptomatic to asymptomatic cases is not known.

Illness and oocyst excretion patterns may vary owing to factors such as immune status, infective dose, host age and possible variations in the virulence of the organism; however, oocyst shedding can be intermittent and can continue for up to 50 days after the cessation of symptoms (mean: 7 days). In humans, the prepatent period is between 7 and 28 days. The mean incubation period (time from infection to the manifestation of symptoms) is 7.2 days (range 1–12) with a mean duration of illness of 12.2 days (range 2–26) (Jokipii and Jokipii, 1986). As the prepatent period can exceed the incubation period, initially a patient can have symptoms in the absence of oocysts in the faeces.

Oocyst excretion by either human or non-human hosts can be up to 10^7/g during the acute phase of infection. Infected calves and lambs excrete up to 10^9 oocysts daily for up to 14 days (Blewett, 1989).

In immunocompromised patients
In patients with Acquired Immune Deficiency Syndrome (AIDS), other acquired abnormalities of T lymphocytes, congenital hypogammaglobulinaemia, severe combined immunodeficiency syndrome, those receiving immunosuppressive drugs and those with severe malnutrition, symptoms include very frequent episodes of watery diarrhoea (between 6 and 25 bowel motions daily, passing between 1 and 20 litres of stool daily). Associated symptoms include cramping, upper abdominal pain, often associated with meals, profound weight loss, weakness, malaise, anorexia and low-grade fever (Whiteside *et al*., 1984). Infection can involve the pharynx, oesophagus, stomach, duodenum, jejunum, ileum, appendix, colon, rectum, gall bladder, bile duct, pancreatic duct and the bronchial tree (Soave and Armstrong, 1986; Cook, 1987). Except in those individuals in whom suppression of the immune system can be relieved by discontinuing immunosuppressive therapies, symptoms can persist unabated until the patient dies (Soave and Armstrong, 1986). Cryptosporidiosis in the immunocompromised can be a common and life-threatening condition in developing countries, causing profuse intractable diarrhoea with severe dehydration, malabsorption and wasting. AIDS triple therapies can reduce the severity of the clinical consequences of cryptosporidiosis. Oocyst excretion can continue for 2–3 weeks after the disappearance of symptoms (Soave and Armstrong, 1986).

17.3.3 Cyclosporiasis

Cyclosporiasis is a 'flu-like illness, and diarrhoea with weight loss, low-grade fever, fatigue, anorexia, nausea, vomiting, dyspepsia, abdominal pain and bloating have been described as symptoms (Ortega *et al*., 1993; Huang *et al*., 1995; Fleming *et al*., 1998). The incubation period is between 2 and 11 days (Soave, 1996) with moderate numbers of unsporulated oocysts being excreted for up to 60 days or more. In immunocompetent individuals the symptoms are self-limiting and oocyst excretion is associated with clinical illness, whereas in immunocompromised individuals diarrhoea may be prolonged. The self-limiting watery diarrhoea can be explosive, but leukocytes and erythrocytes are usually absent. Often, diarrhoea can last longer than 6 weeks in immunocompetent individuals. The diarrhoeal syndrome may be characterised by remittent periods of constipation or normal bowel movements (Ortega *et al*., 1993). Malabsorption with abnormal D-xylose levels has also been reported (Connor *et al*., 1993).

17.4 Infectious dose and treatment

The infectious dose to human beings is between 25 and 100 cysts for *G. intestinalis* (Rendtorff, 1954, 1979), although a volunteer study demonstrated that a

human-source isolate can vary in its ability to colonise other humans (Nash *et al.*, 1987), suggesting that certain isolates may be less infectious to some humans than others. For *Cryptosporidium*, human volunteer studies indicate that the infectious dose varies from isolate to isolate, being between 30 and 132 oocysts for the Iowa (bovine, genotype 2, originally isolated by Dr H Moon, University of Iowa, from a calf and passaged in calves at the Sterling Parasitology Laboratory, University of Arizona) isolate of *C. parvum* (DuPont *et al*., 1995), 1042 oocysts for the UCP (UCP = Ungar *C. parvum*; bovine, genotype 2 received from Dr Beth Ungar in 1989, originally from Dr R. Fayer at the United States Department of Agriculture and passaged in calves by ImmuCell Corp., Maine) isolate, and nine oocysts for the TAMU (Texas A & M University; equine, genotype 2, isolated from a human exposed to an infected foal and passaged in calves) *C. parvum* isolate (Okhuysen *et al*., 1999). An infective dose between ten and 100 has been suggested for *C. cayetanensis* (Adams *et al*., 1999).

While effective chemotherapy is available for giardiasis (nitroimidazole compounds, quinacrine, furazolidone, albendazole and mebendazole), cyclosporiasis (trimethoprim-sulfamethoxazole, excluding those who are intolerant to sulpha drugs), no effective chemotherapy is available for cryptosporidiosis.

17.5 Current levels of incidence

Contamination of fresh produce, especially fruit, vegetables, salads and other foods consumed raw or lightly cooked, with viable (oo)cysts has been responsible for several outbreaks of giardiasis, cryptosporidiosis and cyclosporiasis (Tables 17.2–17.4). Other food types known to have been contaminated or epidemiologically associated with outbreaks include Christmas pudding, home-canned salmon, chicken salad, sandwiches, fruit salad, ice, raw sliced vegetables, cold pressed (non-alcoholic) apple cider, raspberries, noodle salad, basil pesto pasta salad and mesclun lettuce (Tables 17.2–17.4). Our knowledge of incidence is scarce owing to the lack of a reproducible, sensitive detection method (see Table 17.5). Infectious (oo)cysts can be transmitted to a susceptible host via any faecally contaminated matrix, including water, aerosol, food and transport hosts. Food products can became contaminated with (oo)cysts in a variety of ways, and it is likely that more than one route may be involved in transmission, particularly in endemic areas.

- *Person to person (anthroponotic) transmission.* Anthroponotic transmission has been documented particularly for foods that are intended to be consumed raw, or for those that are handled after being cooked. Direct contamination, by symptomatic or asymptomatic (oo)cyst excretors, during food preparation, or following food handler contact with (oo)cyst excretors are frequently reported routes for foodborne giardiasis and cryptosporidiosis (Tables 17.2 and 17.3), and are due to poor personal hygiene standards of that food handler. The hygienic practice of washing hands before preparing food can minimise (oo)cyst contamination and transmission. Guidelines exist for food handlers

Table 17.2 Some documented foodborne outbreaks of giardiasis

No. of persons affected	Suspected food-stuff	Probable/possible source of infection	Reference
3	Christmas pudding	Rodent faeces	Conroy (1960)
29	Home-canned salmon	Food handler	Osterholm *et al.* (1981)
13	Noodle salad	Food handler	Petersen *et al*. (1988)
88	Sandwiches	–	White *et al.* (1989)
10	Fruit salad	Food handler	Porter *et al*. (1990)
–	Tripe soup	Infected sheep	Karabiber and Aktas (1991)
27	Ice	Food handler	Quick *et al*. (1992)
26	Raw sliced vegetables	Food handler	Mintz *et al*. (1993)

Table 17.3 Some documented foodborne outbreaks of cryptosporidiosis

No. of persons affected	Suspected food-stuff	Probable/possible source of infection	Reference
160	Cold pressed (non-alcoholic) apple cider	Contamination of fallen apples from infected calf	Millard *et al*. (1994)
25	Cold pressed (non-alcoholic) apple cider	? Contaminated water used to wash apples	Anon. (1997a)
15	Chicken salad	Food handler	Anon. (1996)
54	Not identified	Common food ingredient	Anon. (1998a)
152	Eating in one of two university campus cafeterias	Food handler	Quiroz *et al*. (2000)

suffering diarrhoea, or those with recent symptoms. The most recently documented foodborne outbreak of cryptosporidiosis, involving 88 cases, originated from a food handler who continued to work in spite of having gastroenteritis (Quiroz *et al*., 2000). Washing uncooked fruit and vegetables before consumption is also recommended; however, one study indicates that washing is not sufficient to remove all *C. parvum* oocysts seeded onto lettuce surfaces (Ortega *et al.*, 1997b).

- *Animal to person (zoonotic) transmission.* There are no recorded outbreaks of zoonotic foodborne transmission of *Giardia* or *Cyclospora*. Direct contact of food with bovine faeces was the suggested cause of the largest foodborne outbreak of cryptosporidiosis, which occurred in Maine, USA. In this outbreak, apples collected from an orchard in which a *Cryptosporidium*-infected calf grazed were made into non-alcoholic cider (Millard *et al.*, 1994) (Table 17.3).

Table 17.4 Some documented foodborne outbreaks of cyclosporiasis

No. of persons affected	Suspected food-stuff	Probable/possible source of infection	Reference
1465	Guatemalan raspberries	? Aerosolisation of oocysts during application of insecticides or fungicides	Herwaldt e*t al.* (1997)
1450	Guatemalan raspberries	? Aerosolisation of oocysts during application of insecticides or fungicides	Anon. (1998b)
48	Basil pesto pasta salad	Unknown	Anon. (1997b)
Unknown	Mesclun lettuce	Unknown	Anon. (1997c)

17.5.1 Foodborne giardiasis

Foodborne transmission was suggested in the 1920s (Musgrave, 1922; Lyon and Swalm, 1925) when water, vegetables and other foods were found to be contaminated with cysts. Since then, cysts have been detected on vegetables including lettuce (Mastrandrea and Micarelli, 1968; Barnard and Jackson, 1980) and soft fruit (e.g. strawberries, Kasprzak *et al*., 1981; Barnard and Jackson, 1980). One report identifies the possibility of offal (tripe) being intrinsically infected (Karabiber and Aktas, 1991). The remaining seven documented outbreaks presented in Table 17.2 occurred from 1977 onwards.

17.5.2 Foodborne cryptosporidiosis

Five outbreaks of foodborne transmission have been documented, all of which occurred in the USA (Table 17.3). Two occurred following the consumption of non-alcoholic, pressed apple cider, in 1993 and 1996 affecting a total of 185 individuals. In the first outbreak, apples were collected from an orchard in which an infected calf grazed. Some apples had fallen onto the ground (windfalls) and had probably been contaminated with infectious oocysts then (Millard *et al.*, 1994). The source of oocysts in the second outbreak is less clear as windfalls were not used and waterborne as well as other routes of contamination were suggested (Anon., 1997a). A foodborne outbreak, which affected 15 individuals, occurred in 1995 with chicken salad, contaminated by a food handler, being the probable vehicle of transmission (Anon., 1996). In 1997, an outbreak was documented in Spokane, Washington. Among 62 attendees of a banquet dinner, 54 (87%) became ill. Eight of 10 stool specimens obtained from ill banquet attendees were positive for *Cryptosporidium*. Epidemiological investigation suggested that foodborne transmission occurred through a contaminated ingredient in multiple menu items (Anon., 1998a). All *Cryptosporidium* faecal samples from this outbreak were of genotype 1 (Quiroz *et al*., 2000).

During September and October 1998, a cryptosporidiosis outbreak, affecting

Table 17.5 Some reported recovery rates for *Cryptosporidium*, *Giardia* and *Cyclospora* (oo)cysts from food produce

Food matrices	Extraction and concentration methods	ID methods	Recovery rates	References
200 g seeded with *Cryptosporidium* spp. oocysts (1/g) • cabbage and lettuce leaves	FDA method: sonication in 1% SDS and 0.1% Tween 80, centrifugation	IF	• 1%	Bier (1990)
• Milk • Orange juice • White wine	Seed 10 to 1000 *C. parvum* oocysts in 70–200 ml; filtration	IF on filters	• 4–9.5% • <10% • >40%	Bankes (1995) (experimental recoveries)
• Cilantro leaves • Cilantro roots • Lettuce	Rinse according to Speck (1984); centrifugation	*Cryptosporidium* oocysts by Koster stain	• 5.0% (4 samples) • 8.7% (7 samples) • 2.5% (2 samples)	Monge and Chinchilla (1996) (Costa Rica)
Raw milk	Bacto-Trypsin & Triton X-100 treatment of seeded samples (20 ml); centrifugation	PCR and probe hybridisation	10 seeded *C. parvum* oocysts	Laberge *et al.* (1996a) (experimental recoveries)
Seeded lettuce leaves • *Cyclospora* (50) • *C. parvum* (100)	Rinse in tap water; centrifugation	Acid-fast stain, IF and direct wet mount	• *C. parvum* 25–36% • *Cyclospora* 13–15%	Ortega *et al.* (1997a, b) (experimental recoveries)
• 110 vegetables and herbs from 13 markets • 62 vegetables and herbs from 15 markets	Rinse in tap water; centrifugation	Acid-fast stain, IF and direct wet mount	• *Cryptosporidium* • (i) 14.5%; (ii) 19.35% • *Cyclospora* • (i) 1.8%; (ii) 1.6%	Ortega *et al.* (1997a, b) (Peru)

Table 17.5 Continued

Food matrices	Extraction and concentration methods	ID methods	Recovery rates	References
Homogenised milk	100 ml samples; centrifugation followed by IMS	Direct PCR	10 *C. parvum* oocysts	Deng *et al.* (2000) (experimental recoveries)
Apple juice	• Sucrose gradient, IMS • Flotation or flotation and IMS • Flotation	• IF • direct PCR • acid-fast stain	• 10–30 *C. parvum* oocysts • 30–100 oocysts • 3 000–10 000 oocysts	Deng and Cliver (2000) (experimental recoveries)
• Four leafy vegetables and strawberries • Bean sprouts	Rotation and sonication in elution buffer; centrifugation and IMS	IF	• *Giardia* 67%; *C. parvum* 42% • *Giardia* 4–42%; *C. parvum* 22–35%	Robertson and Gjerde (2000) (experimental recoveries)
Lettuce leaves	100 *C. parvum* oocysts seeded, elution buffer (pH 5.5) • Pulsifying • Stomaching Centrifugation and IMS	IF	 • 40% • up to 85%	Wilkinson *et al.* (2000) (experimental recoveries)

FDA US Food and Drug Administration; SDS sodium dodecyl sulphate; IF immunofluorescence; PCR polymerase chain reaction; IMS immunomagnetisable separation.

152 individuals, occurred on a university campus in Washington, DC. A case control study with 88 case patients and 67 control subjects revealed that eating in one of the two cafeterias was associated with illness. One food handler, positive for *Cryptosporidium*, had prepared raw produce on 20–22 September. All samples analysed by molecular typing (25 cases, including the food-handler) were of genotype 1 (Quiroz *et al*., 2000).

17.5.3 Foodborne cyclosporiasis

The first report of foodborne transmission of *Cyclospora* may have occurred in 1995, when an airline pilot presented with diarrhoeal illness after eating food prepared in a kitchen in Haiti that was then brought on board the aeroplane (Connor and Shlim, 1995). In 1996, outbreaks of cyclosporiasis, affecting more than 1400 individuals, occurred in the USA and Canada, with imported raspberries being implicated (Herwaldt *et al*., 1997). At that time, no method existed for detecting *Cyclospora* oocysts on foods and their presence on the foods implicated could not be confirmed. In 1997, outbreaks in the USA were also associated with imported raspberries, and later that year, with contaminated basil and lettuce (Anon., 1997b,c; Table 17.4). In 1998, clusters of cases, again associated with fresh berries from Guatemala, were recorded in Ontario, Canada (Anon., 1998b). Most cases occurred during spring and summer. The four, better documented, outbreaks are presented in Table 17.4.

17.6 Conditions for growth

Giardia, *Cryptosporidium* and *Cyclospora* are obligate parasites, and require a host to reproduce. (Oo)cysts can be transmitted via water, food or transport hosts (e.g. seagulls, waterfowl, flies, bivalves); however, the parasites cannot replicate in these matrices.

17.6.1 Infectivity and viability

In vitro methods for assessing the viability of (oo)cysts (reviewed by Smith, 1998 and O'Grady and Smith, 2002) have been developed as surrogates for *in vivo* models since the latter are expensive, require closely regulated home office licences and specialised animal housing facilities.

Giardia

Assessment of *G. duodenalis* cyst infectivity can be undertaken *in vivo* in neonatal mice or adult gerbils (Faubert and Belosevic, 1990), while *Giardia* cyst viability (reviewed by Smith, 1998 and O'Grady and Smith, 2002) can be undertaken *in vitro* by (a) excystation (Bhatia and Warhurst, 1981; Smith and Smith, 1989), (b) fluorogenic vital dyes (Schupp and Erlandsen, 1987a,b; Schupp *et al*., 1988; Smith and Smith 1989; Sauch *et al*., 1991; Taghi-Kilani *et al*., 1996, Smith, 1998),

(c) propidium iodide vital dye staining and morphological assessment of cysts observed under Nomarski DIC optics (Smith, 1996) or (d) RT-PCR to amplify a sequence of the mRNA of *Giardia* heat shock protein 70 (*hsp 70*) (Abbaszadegan *et al*., 1997; Kaucner & Stinear, 1998).

Cryptosporidium
Determination of *Cryptosporidium* oocyst viability (reviewed by Smith, 1998 and O'Grady and Smith 2002) can be undertaken *in vitro* by (a) excystation (Blewett, 1989; Robertson *et al*., 1993), (b) fluorogenic vital dyes (Campbell *et al*., 1992; Belosevic *et al.*, 1997), (c) *in vitro* infectivity (Upton *et al.*, 1994; Slifco *et al*., 1997; Rochelle *et al*., 1997), (d) *in vitro* excystation followed by PCR (Wagner-Wiening and Kimmig, 1995; Deng *et al*., 1997), (e) RT-PCR of *C. parvum hsp 70* (Stinear *et al*., 1996; Kaucner and Stinear, 1998), (f) fluorescence *in situ* hybridisation (Vesey *et al*., 1998) or (g) electrorotation (Goater and Pethig, 1998).

Genotype 2 *C. parvum* (as well as *C. muris* and *C. meleagridis*) oocysts can establish infection in neonatal or immunosuppressed mice (reviewed in O'Grady and Smith, 2002). An *in vitro* cell culture infectivity method, developed for genotype 2 *C. parvum* oocysts (Upton *et al*., 1994; Slifco *et al*., 1997) has been used to determine the infectivity of oocysts subjected to (a) drug treatment, (b) disinfection sensitivity and (c) sensitivity to treatments in food processing, as well as pathophysiological changes in cell permeability due to *C. parvum* infection and the secretion of interleukin-8. Genotype 1 *C. parvum* oocysts do not infect neonatal mice nor do they initiate infection as readily as genotype 2 isolates; in currently established cell culture systems, however, successful propagation of genotype 1 oocysts was recently demonstrated in gnotobiotic piglets (Widmer *et al*., 2000).

Cyclospora
No animal model or *in vitro* viability assay has been identified for *C. cayetanensis*. Observation of *in vitro* sporulation is the only criterion available for assessing oocyst viability. *Cyclospora* sp. oocysts sporulate maximally at 22 °C and 30 °C (Ortega *et al*., 1993; Smith *et al*., 1997), however, storage at either 4 °C or 37 °C for 14 days retards sporulation. Up to 12% of human- and baboon-associated oocysts previously stored at 4 °C for 1–2 months sporulate when stored for 6–7 days at 30 °C (Smith *et al*., 1997).

17.6.2 Resistance to physical and chemical treatments

(Oo)cysts are resistant to a variety of environmental pressures and chemical disinfectants normally used in water treatment. *Giardia* and *Cryptosporidium* (oo)cysts survive better at lower than at higher temperatures. While less is known of *C. cayetanensis* oocyst survival in the environment, current evidence indicates that sporulation is delayed (presumably increasing survival) at lower temperatures (Smith *et al*., 1997). The details of experimental procedures and data analy-

ses for both *Giardia* and *Cryptosporidium* have critical effects on both the interpretation and comparability of results, often making comparisons between studies difficult. In addition, for *Giardia*, the more resistant *Giardia muris* cysts have often been used as a surrogate for those of the human parasite, *Giardia duodenalis*. As published data are numerous and varied, we summarise some of the more important trends and findings in Table 17.6.

17.7 Detection methods

Methods developed for detecting protozoa as surface contaminants on foods are modifications of those used for water (e.g. Anon., 1994, 1998c, 1999a,b; Smith, 1995, 1998; Smith and Hayes, 1996) and, as such, they must be capable of detecting small numbers of (oo)cysts (1–100). Methods for detecting (oo)cysts on foods can be subdivided as follows: (a) desorption of organisms from the matrix, (b) concentration and (c) identification.

Desorption can be accomplished by mechanical agitation, stomaching, pulsifying and sonication of leafy vegetables or fruit suspended in a liquid that encourages desorption of (oo)cysts from the food. Detergents including Tween 20, Tween 80, sodium lauryl sulphate and Laureth 12 have been used to encourage desorption. Lowering or elevating pH affects surface charge and can also increase desorption. Depending on the turbidity and pH of the eluate, (oo)cysts eluted into non-turbid, neutral pH eluates can be concentrated by filtration through a 1 µm flat bed cellulose acetate membrane (Girdwood and Smith, 1999b). (Oo)cysts eluted into turbid eluates can be concentrated by immunomagnetisable separation (Campbell and Smith, 1997; Table 17.5). (Oo)cysts are identified by epifluorescence and Nomarski differential interference contrast microscopy (Tables 17.1 and 17.5), although recently, the polymerase chain reaction (PCR) has been used to determine the presence of parasite DNA in eluates (reviewed in Smith, 1988; Girdwood and Smith, 1999a,b; Table 17.5).

Modifications to the methods described will undoubtedly occur, in order to provide optimised recovery from specific matrices. The most important stage for maximising the detection of (oo)cysts is their efficient extraction from the food matrix in question. At present, most publications report on seeding experiments (Table 17.5), although *Cryptosporidium* and *Cyclospora* oocysts have been detected on vegetables for sale in market places in Peru (Ortega *et al*., 1997b) and Costa Rica (Monge and Chinchilla, 1996) (Table 17.5).

17.8 Control issues

Analyses of foodborne outbreaks identify two major sources of contamination: water and food handlers. Surface contamination of produce accounts for ten recorded outbreaks, and foods that receive minimal treatment pose the greatest risks. Surface contamination of such produce at source can be direct or indirect

Table 17.6 Some conditions for inactivation and survival of *Cryptosporidium* and *Giardia* (oo)cysts

Parasite	Physical inactivation	Chemical inactivation
C. parvum (genotype 2)	• 71.7 °C for 15 s (pasteurisation) for bovine oocysts in either water or milk completely abrogates infectivity to neonatal mice (Harp *et al*., 1996). • 64.2 °C for 2 min or 72.4 °C for 1 min for bovine oocysts: no infection established in BALB/c mice (Fayer, 1994). • Air-drying (18–20 °C; cervine-ovine oocysts, isolate MD) for 2 h: 97% death; for >2 h: 100% death (Robertson *et al*., 1992). • Bovine oocysts (AUCP-1 isolate) at −70 °C for 1 h; −20 °C up to 168 h; −15 °C for 168 h: no developmental stages observed in BALB/c mice; −20 °C up to 5 h; −15°C up to 24 h and at −10 °C for 168 h: developmental stages observed in mice (Fayer and Nerad, 1996). • Snap-freezing (MD isolate) in liquid nitrogen: 100% death (Robertson *et al*., 1992). • Infectious to mice after storage at 4 °C (isolate GCH1) for 39 weeks or after 20 weeks at 20 °C (Widmer *et al*., 1999). • Storage at −20 °C for 775 h, 168 h and 24 h reduces viability to 1.8%, 7.9% and 33% respectively (isolate MD) (Robertson *et al*., 1992).	• Free chlorine: ineffective. Up to 16 000 mg L^{-1} for 24 h at 5 or 20 °C pH 6, 7 and 8 or 8 000 mg L^{-1} for 24 h at 5 °C pH 6 and 7 or at 20 °C pH 8 required for total inactivation of human or bovine oocyst isolates (Smith *et al*., 1989). • Chlorine dioxide: 78 mg l^{-1} min^{-1} required for 90% inactivation (Korich *et al*., 1990). • Ozone: at 20 and 25 °C up to 3–8 mg l^{-1} min^{-1} required for up to 4 $\log_{10}$ inactivation. Ozone requirements increase with decrease in temperature (Smith *et al*., 1995b). • UV irradiation: a dose of 8 748 mW s cm^{-2} during two 5 min periods, produces >2 $\log_{10}$ reduction in oocyst viability (Campbell *et al*., 1995). • 4% iodophore; 5% cresylic acid; 3% NaOCl; 5% benzylkonium chloride and 0.02 M NaOH for 18 h: viable oocysts observed. 5% ammonia or 10% formol saline for 18 h: completely destroy oocyst viability (Campbell *et al*., 1982).
Giardia duodenalis; G. muris	• Freezing (–13°C) for 14 days and thawing (<1% viable cysts) (Bingham *et al*., 1979). • *G. duodenalis* cysts at 8 °C for 77 days (<5% excystation) (Bingham *et al*., 1979). • *G. muris* cysts 3 months in cold raw water sources (DeRegnier *et al*., 1989).	• Free chlorine: up to 142 mg l^{-1} min^{-1} required for 99% *G. duodenalis* inactivation (Hoff, 1986). At 5 °C, pH 8, 2 mg ml^{-1} for 30 min produces <30% *G. duodenalis* cyst inactivation. At 25 °C, pH 8, 1.5 mg ml^{-1} for 10 min produces >99% cyst inactivation (Jarroll *et al*., 1981). • Chlorine dioxide: 11.2 mg l^{-1} min^{-1} required for 99% inactivation (Hoff, 1986). • Ozone: up to 2.57 mg l^{-1} min^{-1} required for up to 4 $\log_{10}$ *G. duodenalis* inactivation (Finch *et al*., 1993).

(Table 17.7) and effective methods for the isolation and identification of these parasites from foods should be developed and validated.

Mains potable water or treated borehole-derived water used by the food industry for its manufacturing and ancillary processes and all water used for direct food contact and food contact surfaces must be at least of potable quality and should be free of pathogens. Water used by industry include: direct incorporation into foods as an ingredient, washing of food containers (e.g. cans prior to passing in to high-risk processing areas), washing raw vegetables, raw fruits, animal carcasses, etc. Water used for cleaning, which has the potential to become contaminated with pathogens washed from produce, is increasingly being reconditioned, especially for the preparation and processing of food as long as the microbiological safety and quality of each food can be assessed.

Standardised methods for detecting *Giardia* and *Cryptosporidium* in mains potable water are available (Anon., 1994, 1998b, 1999a,b) and should be applicable to treated borehole-derived water. Where undertaken, results of routine water testing for *Cryptosporidium* (and possibly *Giardia*) will be held by water companies. In the UK, *Cryptosporidium* non-compliances are reported to the Drinking Water Inspectorate. The presence of parasites in reconditioned water can be tested using the above methods, although less data are available regarding their recovery efficiencies.

While some data on the survival of (oo)cysts in food industry processes and product are available, most are generic or extrapolatory, with few pertaining to actual processes undertaken by specific manufacturers at full scale. Because of the robustness of these parasites, issues regarding the survival of (oo)cysts in treatment and production processes, on surfaces, in disinfectants used in the food industry and in/on product must be addressed, otherwise effective Hazard Analysis Critical Control Point (HACCP) strategies cannot be implemented.

Table 17.7 Possible sources of food contamination

- Use of cyst and oocyst contaminated faeces (night soil), farmyard manure and slurry as fertiliser for crop cultivation.
- Pasturing infected livestock near crops.
- Defaecation of infected feral hosts onto crops.
- Direct contamination of foods following contact with cyst and oocyst contaminated faeces transmitted by coprophagous transport hosts (e.g. birds and insects).
- Use of contaminated wastewater for irrigation.
- Aerosolisation of contaminated water used for insecticide and fungicide sprays and mists.
- Aerosols from slurry spraying and muck spreading.
- Poor personal hygiene of food handlers.
- Washing 'salad' vegetables, or those consumed raw, in contaminated water.
- Use of contaminated water for making ice and frozen/chilled foods.
- Use of contaminated water for making products which receive minimum heat or preservative treatment.

As poor personal hygiene is a major contributor to protozoan parasite contamination incidents, guidelines currently in use for individuals working in the preparation of food in restaurants and industry with infectious, diarrhoeal disease should be extended to incorporate giardiasis, cryptosporidiosis and cyclosporiasis. Measures include obligatory, paid abstinence from work during gastrointestinal illness and strict procedures on hand washing and the use of clean facilities in the workplace.

Globalisation of food production and new food trends can contribute to increasing opportunities for protozoan parasite contamination. Sourcing ingredients from various countries or regions, which are then incorporated into a final food product, can complicate the tracing of a particular contaminated constituent. Knowledge of parasite biology and survival as well as local agricultural practices can assist risk assessment. Effective risk assessment requires confidence in recovery and identification methods and survival data. For *Giardia*, *Cryptosporidium* and *Cyclospora* contamination of food, surveillance and control are measures that are still being developed.

More focused quality issues have followed the adoption of HACCP-based control systems. This approach requires substantial information on the significance of transmission routes and (oo)cyst contamination of, and survival in, matrices commonly encountered by the food industry. While such criteria are being striven for *Giardia* and *Cryptosporidium*, many remain unknown for newer emerging foodborne parasites such as *Cyclospora* and the microsporidia.

17.9 The regulatory framework

17.9.1 Public health

While not reportable in England and Wales, giardiasis and cryptosporidiosis were made laboratory reportable diseases in Scotland in 1989. Cyclosporiasis is not reportable in the UK. Following numerous outbreaks in the eastern USA in 1996 and 1997 and Canada, the Centers for Disease Control and Prevention established cyclosporiasis as a reportable disease. *C. cayetanensis* is also included as an emerging pathogen in the Food Safety initiative which focuses on monitoring outbreaks, research into the selected pathogens, and enforcement of regulations.

17.9.2 Water

The European Union 'drinking water' directive requires that 'water intended for human consumption should not contain pathogenic organisms' and 'nor should such water contain: parasites, algas, other organisms such as animalcules'. In recognising the impracticality of the current zero standard, the proposed revision to this Directive will make it a general requirement 'that water intended for human consumption does not contain pathogenic micro-organisms and parasites in numbers which constitute a potential danger to health'. No numerical standard for *Giardia* or *Cryptosporidium* is proposed.

In the UK, *Cryptosporidium* is regulated in drinking water. The regulation sets a treatment standard at water treatment sites determined to be of significant risk following risk assessment. Daily continuous sampling of at least 1000 litres over at least 22 h period from each point at which water leaves the water treatment works is required and the goal is to achieve less than an average density of 1 oocyst per 10 litres of water (UK Statutory Instruments 1999 No. 1524).

The US Environmental Protection Agency has issued several rules to address the control of *Cryptosporidium* and *Giardia*. One of the goals of the Surface Water Treatment Rule (SWTR), formulated to address the control of viruses, *Giardia* and *Legionella*, was to minimise waterborne disease transmission to levels below an annual risk of 10^{-4}. In order to accomplish this goal, treatment, through filtration and disinfection requirements were set to reduce *Giardia* cysts and viruses by 99.9% and 99.99%, respectively. The Surface Water Treatment Rule (SWTR) also lowered the acceptable limit for turbidity in finished drinking water from a monthly average of 1.0 nephelometric turbidity unit (NTU) to a level not to exceed 0.5 NTU in 95% of 4 hour measurements. The Enhanced Surface Water Treatment Rule (ESWTR) includes regulation of *Cryptosporidium* but in order to implement this rule, a national database on the occurrence of oocysts in surface and treated waters was required to be collected under the Information Collection Rule (ICR). Water authorities serving more than 10 000 individuals began an 18 month monitoring programme for *Cryptosporidium* under the ICR, which was issued in 1996 and is now completed.

17.9.3 Food

The UK Food Safety Act (1990) requires that food, not only for resale but throughout the food chain, must not have been rendered injurious to health; be unfit; or be so contaminated – whether by extraneous matter or otherwise – that it would be unreasonable for it to be eaten. A set of horizontal and vertical regulations (which are provisions in food law) covering both foods in general and specific foodstuffs also ensure that food has not been rendered injurious to health. Other than being potential microbiological contaminants, *Giardia*, *Cryptosporidium* and *Cyclospora* are not identified in these regulations.

The US Food and Drug Administration is responsible for enforcing the regulations detailed in the Federal Food, Drug and Cosmetic Act. Regulations do not address these protozoan parasites specifically, but contaminated products are covered by sections of the Act depending on whether the foods are domestic (either produced within the USA or already imported and in the domestic market [section 402(a)(l)],) or imported (at the port of entry [section 801(a)(1)]). Analysis of regulatory samples by the FDA follows the procedures contained within the *Bacteriological Analytical Manual* (1998).

17.9.4 Agricultural practices and wastes

Apart from regulations governing agricultural wastes, current regulations in the UK and USA do not require risk-based standards or guidance based on protection

from microbial contaminants such as *Giardia* and *Cryptosporidium*. Good management practices (GMPs) are also suggested for farms and agricultural wastes. The EC Agri-Environment Regulation, under the Common Agricultural Policy, promotes schemes which encourage farmers to undertake positive measures to conserve and enhance the rural environment in Europe.

In the USA, guidance on GMPs and good manufacturing practices for fruits and vegetables is available on FDA's web page (http://www.fda.gov). The guide consists of recommendations to growers, packers, transporters and distributors of produce to minimise the risks of foodborne diseases. Its purpose is the prevention of microbial contamination, by applying basic principles to the use of water and organic fertilisers, employee hygiene, field and facility sanitation, and transportation. Advice is given on the establishment of a system for accountability to monitor personnel and procedures from the producer to the distributor.

17.10 Sources of further information and advice

Key texts are: Adams *et al*. (1999); Casemore (1990); Girdwood and Smith (1999a, b); Jaykus (1997); Laberge *et al*. (1996b); Nichols (1999); Smith (1993); Smith and Nichols (2001); Tauxe (1997).

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18

Toxigenic fungi

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18.1 Introduction

The terrestrial filamentous fungi include species that produce macroscopic fruiting bodies, such as mushrooms and toadstools, as well as many species that produce microscopic sporing structures and are generally referred to as moulds. Some of the macrofungi, such as the cultivated mushroom *Agaricus bisporus*, are valuable foods but some, such as the deathcap *Amanita phalloides*, produce virulent toxins and the consumption of a single fruit body may lead to death through liver and kidney failure. Several mould species are also widely used in the production of foods, such as the mould-ripened cheeses, tempeh, miso and soya sauce, but there are also species that can produce toxic metabolites and when these contaminate foods, or animal feeds, they are referred to as mycotoxins (Moss, 1998). These can certainly be considered as foodborne hazards and will be dealt with in this chapter.

Many toxic mould metabolites are known but only a few are consistently associated with foodborne illness to the extent that individual countries may set maximum tolerated limits for their presence in foods (Boutrif and Canet, 1998). Mycotoxins are produced primarily in foods of plant origin, the most important of which are cereals (especially maize), legumes (especially groundnuts), pulses, oilseeds and treenuts. However, they can also pass through the food chain into foods of animal origin such as meat and milk. It has been estimated that as much as 25% of the world's food crops may be contaminated with mycotoxins (Boutrif and Canet, 1998) and such materials may be either destroyed, diverted to non-food uses, or be consumed in those countries that do not have legislation regulating their use as food or animal feed.

Most mycotoxins are acutely poisonous but they are not as virulent as some of the toxins of cyanobacteria, dinoflagellates or food poisoning bacteria such as

Table 18.1 Acute toxicities of some mycotoxins and other microbial toxins

Compound	Producing microorganism[a]	LD_{50} (mg kg^{-1})
Aflatoxin B_1	*Aspergillus flavus*	0.5 (dog)
Sterigmatocystin	*Aspergillus versicolor*	166 (rat)
Patulin	*Penicillium expansum*	35 (mouse)
Ochratoxin A	*Penicillium verrucosum*	20 (rat)
Citrinin	*Penicillium citrinum*	67 (rat)
T-2 toxin	*Fusarium sporotrichioides*	5 (mouse)
Deoxynivalenol	*Fusarium graminearum*	70 (mouse)
Fumonisin B_1	*Fusarium moniliforme*	1–4 (horse)[b]
Aeruginosin	*Microcystis aeruginosa*	0.05
Saxitoxin	*Alexandrium tamarense*	0.03
Botulinum toxin	*Clostridium botulinum*	10^{-6}

[a] Many of these toxins are produced by several species.
[b] 1–4 mg/kg body mass/day over 29–33 days will induce equine leucoencephalomalacia in horses (Kellerman *et al*., 1990).

Clostridium botulinum (see Table 18.1). The justification for concern about their presence in food is not their acute toxicity but their chronic toxicity which may include carcinogenicity, chronic organ damage and immunosuppressive activity.

18.2 Aflatoxins: occurrence and significance

The aflatoxins are produced by a small number of species of *Aspergillus* which currently includes *A. flavus*, *A. parasiticus*, *A. nomius* and a species originally isolated from Ivory Coast soil, *A. ochraceoroseus* (Bartoli and Maggi, 1978; Klich *et al.*, 2000). A strain of *A. tamarii* has also been shown to produce aflatoxin (Klich *et al.*, 2000), but this may be a brown spored form of *A. flavus* and is certainly very closely related. It should be emphasised that not all strains within a species are toxigenic. On a worldwide basis the majority of strains of *A. parasiticus* are aflatoxigenic but only about 35% of strains of *A. flavus* produce aflatoxins. Indeed some strains of *A. flavus* have been used to produce koji and the more widely used species *A. oryzae* is probably a domesticated form of this species (Cruikshank and Pitt, 1990). At the level of acute toxicity the aflatoxins cause liver damage and, in animals such as cattle, the farmer will initially see reduced feed consumption, depressed milk production, weight loss and eventually death due to severe liver damage. They were discovered following an outbreak of acute toxicosis affecting turkey poults and game birds in which large numbers of birds died.

The aflatoxins are a family of compounds the most toxic of which is aflatoxin B_1 (Fig. 18.1). In humans it is both acutely toxic and probably carcinogenic. It was responsible for a major outbreak of toxicosis in India, initial reports of which found that 397 people were ill of whom 106 died, following consumption of afla-

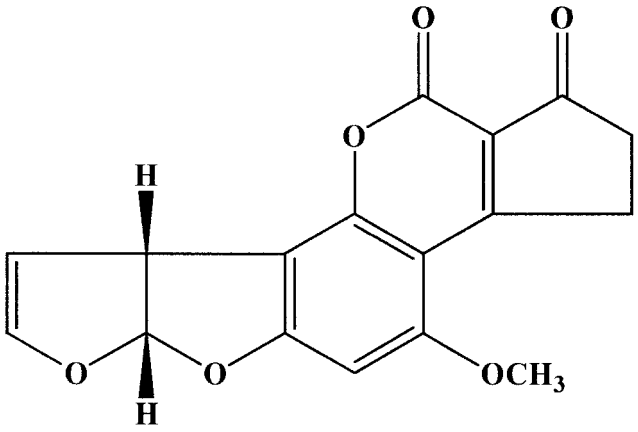

Fig. 18.1 Aflatoxin B_1.

toxin contaminated maize (Krishnamachari *et al*., 1975). This outbreak affected people and dogs in several villages and the most overt symptom was jaundice preceded by vomiting and anorexia.

A fascinating aspect of the toxicology of aflatoxin B_1 is the variation in response among different species and even between the sexes within a species. Indeed, for some animal species, such as the rat, it is among the most carcinogenic compounds known and yet in others, such as the guinea pig, it may not be carcinogenic. Although it is difficult to disentangle the interactions of aflatoxin with other carcinogenic agents, such as hepatitis B virus, it is clearly prudent to assume that aflatoxin B_1 is a human carcinogen and a review of the risk assessment leading to this assumption is presented by Castegnaro and McGregor (1998). The reason for the diversity of response to aflatoxin B_1 is because it has to be metabolised first and the balance of metabolism varies from one species to another. This aspect of the toxicology is reviewed by Moss (1998).

The moulds producing aflatoxins are ubiquitous in their ocurrence but they are only normally active and competitive in the warmer parts of the world, the optimum temperature for growth being 35–37 °C, and the optimum temperature for aflatoxin production 30 °C. The commodities most usually contaminated are maize and groundnuts but a wide range of other plant products may also contain aflatoxin B_1. These include Brazil nuts, almonds, figs and spices for human consumption as well as cottonseed meal and copra meal for animal feeds. Levels can range from a few $\mu g\,kg^{-1}$ to several $mg\,kg^{-1}$ (Pittet, 1998). The moulds producing aflatoxins grow particularly well on appropriate substrates when these are stored at high moisture content and elevated temperatures. Under these conditions levels of contamination can be high enough to cause acute toxicity as occurred in India in 1974 when it was estimated that as much as 2–6 mg of aflatoxin could be consumed daily over a period of several weeks (Krishnamachari *et al*., 1975).

However, these same species can infect crops such as maize and groundnuts in the field, before harvest, by establishing an endophytic relationship with the plant. When such an infected plant is stressed by, for example, a period of drought, aflatoxin may be produced which will then be present in the crop at harvest even though it may look perfectly sound. Under these conditions only relatively low concentrations are formed but they may be higher than the maximum tolerated levels set by individual countries. Incidents of field contamination of crops by aflatoxin B_1 have been well documented for the southern states of the USA (Hagler, 1990). In 1980, for example, 65.7% of samples of maize from the harvest in North Carolina contained more than $20\,\mu g\,kg^{-1}$ of aflatoxin with an estimated cost to producers and handlers of $30 816 000 (Nichols, 1983). These losses arose because $20\,\mu g\,kg^{-1}$ is the maximum tolerated level in commodities for human consumption in the USA. Attempts to harmonise national legislation at the international level have so far failed and maximum permitted levels for aflatoxin B_1 in foods may be as low as $2\,\mu g\,kg^{-1}$ (European Union) or as high as $30\,\mu g\,kg^{-1}$ (India).

Although aflatoxin M_1, which is secreted in milk after consumption and metabolism of aflatoxin B_1, is less toxic than its precursor, most countries set much more stringent levels ($0.05\,\mu g\,kg^{-1}$ in the EU) because milk may be consumed by very young children who may be at high risk because of an intrinsically higher sensitivity and light body weight.

18.3 Control measures

Because aflatoxins can be produced in the field, in stored commodities, or as a result of passage through the food chain to contaminate foods that had not been mouldy, their control requires an integrated management programme (Lopez-Garcia *et al*., 1999). Such a programme must include the control of formation in the field by preventing insect damage, alleviating drought stress, and ensuring that potential inoculum does not build up in plant residues after harvest. Following harvest it is essential that crops be dried to reduce their water activity and stored in cool, dry conditions. When mould and aflatoxin contamination occur they are usually very heterogeneous and the recognition and removal of hot spots of contamination can be effective. In the case of relatively large particulate commodities such as groundnuts and pistachio nuts it may be possible to physically remove highly contaminated nuts. In a particular batch of pistachio nuts it has been shown that 90% of the aflatoxin was present in 4.6% of the product and, if this could be removed, it would be possible to reduce the average aflatoxin contamination of this commodity from 1.2 to $0.12\,\mu g\,kg^{-1}$ for product going to the market for human consumption (Schatzki, 1995; Schatzki and Pan, 1996). A similar study of aflatoxin contamination in a consignment of figs also demonstrated a very skewed distribution (Sharman *et al*., 1991). There have been many studies of the possible inactivation of aflatoxins by physical, chemical or biological processes but only the use of ammonia at either high pressure/high

temperature or atmospheric pressure/ambient temperature has proved efficacious (Lopez-Garcia *et al*., 1999).

18.4 Ochratoxin A: occurrence and significance

Ochratoxin A (Fig. 18.2) is produced by *Penicillium verrucosum* in temperate climates and by a number of species of *Aspergillus* in the warmer parts of the world, the most important of which is *A. ochraceus*. In temperate climates it is most commonly associated with barley and other cereals but in tropical and sub-tropical regions, a much wider range of foodstuffs may be contaminated, including coffee, cocoa, vine fruits and spices (Pittet, 1998). During the last few years levels of contamination in foods for human consumption sampled in Europe range from as little as 0.01–7.0 $\mu g\,kg^{-1}$ in wine to 0.05–121 $\mu g\,kg^{-1}$ in rye (Pittet, 1998). Ochratoxin is primarily a nephrotoxin and has been associated with Balkan endemic nephropathy. This condition in turn is linked to the occurrence of urinary tract tumours and current discussions in the EU to set maximum tolerated levels in human foods are based on the assumption that ochratoxin A should be considered carcinogenic to humans. A few countries already have maximum tolerated levels in place which vary from 5 to 50 $\mu g\,kg^{-1}$ (Boutrif and Canet, 1998). It has a relatively long residence time in the animal body and so can be found in the flesh of animals, such as pigs, after slaughter for human consumption. There is not yet any evidence that ochratoxin is produced in plants in the field but this may reflect our current lack of knowledge of the detailed ecology of the moulds known to produce ochratoxins. The possibility of uptake from the soil has been suggested and supported by studies with radiolabelled ochratoxin A (Mantle, 2000).

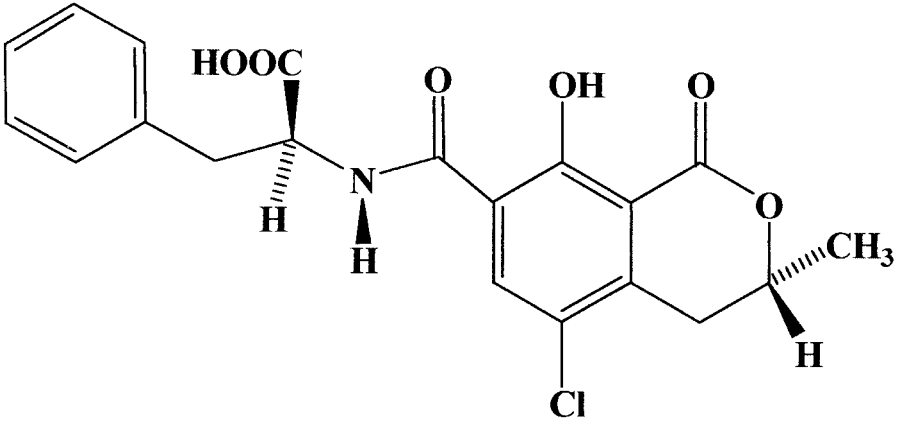

Fig. 18.2 Ochratoxin A.

18.5 Control measures

Ochratoxin is a relatively stable metabolite as demonstrated by the survival of significant amounts in roasted coffee and in wine after fermentation. Its control must thus depend on the prevention of mould growth at every stage in the production of foods for human consumption. In the case of cereals it is clear that care in harvesting and subsequent drying and adequate storage is required. However, some commodities such as coffee involve complex processes, some of which are inevitably at a high water activity, and there is a need to understand the relationship between the ecology of ochratoxin-producing fungi and the stages in these processes. Frank (1999) has demonstrated the importance of the drying process in the production of ochratoxin-free coffee beans and has attempted to apply the Hazard Analysis Critical Control Point (HACCP) approach to the study of coffee production.

18.6 Patulin: occurrence and significance

Patulin (Fig. 18.3) is produced by a number of species of *Penicillium*, *Aspergillus* and *Byssochlamys* but in the context of human foods the most important is *Penicillium expansum*. This species is responsible for a soft rot of apples and a number of other fruits. It would not normally be a problem in fresh fruits because the rot is usually obvious and the fruit discarded. There are a few cultivars of apple, such as the Bramley, with an open core structure in which infection and mould growth can occur without any external evidence of a problem. Again, in the case of fresh fruit, the moulding would be apparent on cutting up the apple and it would most probably be discarded. The major source of patulin in the human diet is from fresh apple juice (and other fruit juices) because a perfectly palatable juice can be expressed from fruit in which some fruits are mouldy. Levels of contamination of fruit juices (mainly apple juices) over the last few years have ranged from 5 to 1130 $\mu g\,l^{-1}$ and the incidence of positive samples has ranged from 21 to 100% (Pittet, 1998). The UK has an advisory level of 50 $\mu g\,l^{-1}$ and those few countries

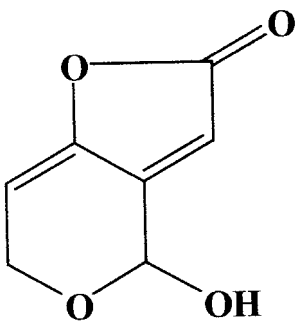

Fig. 18.3 Patulin.

that have set maximum tolerated levels also use this figure although, in the Czech Republic, the level is reduced to $30\,\mu g\,l^{-1}$ for children and $20\,\mu g\,l^{-1}$ for infants (Boutrif and Canet, 1998). Although patulin has a significant acute toxicity, early suggestions that it is carcinogenic have not been confirmed for oral administration. It is excreted very rapidly from the body (Dailey *et al*., 1977) but clearly, with a vehicle such as fresh apple juice which is increasingly popular, and may be consumed on a daily basis, it is sensible to ensure that exposure is as low as practically possible.

18.7 Control measures

Patulin is not particularly stable in an aqueous environment except at reduced pH when it will even survive elevated temperatures (Lovett and Peeler, 1973), hence its occurrence in pasteurised apple juice. Fresh apple juice is the commodity of most concern and the removal of mouldy apples, or even the overtly mouldy part of individual apples, is an effective control measure. As indicated above there is the possibility of mould infection, and patulin contamination, in some apple cultivars that do not appear to be overtly mouldy and further study is required to understand the circumstances in which they become infected. Patulin is destroyed by the active fermentation of apple juice to cider by *Saccharomyces cerevisiae*. It can also be removed with activated charcoal and by treatment with sulphur dioxide.

18.8 Fumonisins: occurrence and significance

The fumonisins, of which the best studied is fumonisin B_1 (Fig. 18.4), are produced by *Fusarium moniliforme* and a few closely related species of *Fusarium* as well as *Alternaria alternata* f. sp. *lycopersici*. By far the most important substrate is maize in which it can be found in concentrations ranging from $6\,\mu g\,kg^{-1}$ to $>37\,mg\,kg^{-1}$ with incidences from 20 to 100%. The fumonisins are clearly widespread in maize and maize products and may occur at relatively high concentrations and yet it is unclear what their role may be in human health. Fumonisin B_1 is certainly responsible for a number of serious animal illnesses, such as encephalomalacia in horses (Kellerman *et al*., 1990), pulmonary oedema in pigs and hepatocarcinoma in rats. The presence of *Fusarium moniliforme* in maize had been linked to human oesophageal carcinoma as long ago as 1981 (Marasas *et al*., 1981) and it is possible that, of the several toxic metabolites produced by this species, the fumonisins are implicated in this particular form of human cancer.

18.9 Control measures

Fusarium moniliforme (= *F. verticillioides*) can be a seedborne endophyte in maize and its elimination is difficult. When maize is growing well there may be

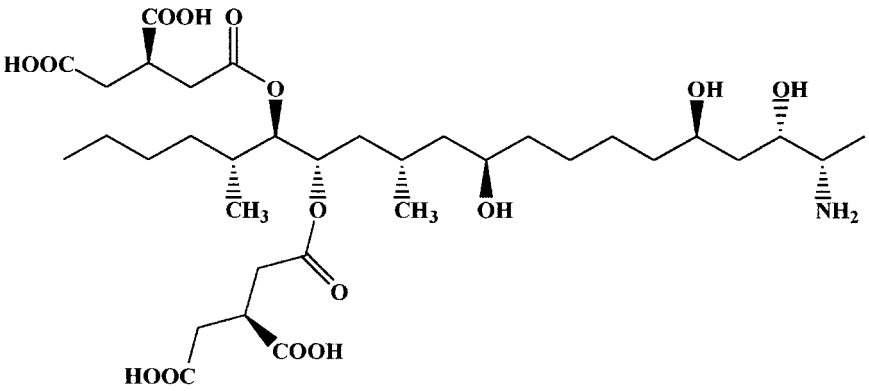

Fig. 18.4 Fumonisin B_1.

no overt symptoms but warm dry weather early in the growing season, followed by wet weather during the development of the cob, has been associated with ear rot disease of maize which in turn is associated with high levels of fumonisin. Insect damage is also associated with high levels of infection and fumonisin contamination and the breeding of cultivars resistant to such damage is a possible control strategy. Although fumonisin levels can increase during poor storage it is clearly important to control disease and infection in the field. The possibility of using biological control with biocompetitive bacteria and non-toxigenic strains of *Fusarium moniliforme* are being actively investigated. This range of possible control strategies have been reviewed by Riley and Norred (1999).

18.10 Other mycotoxins

Although the aflatoxins, ochratoxins, patulin and the fumonisins are presently perceived to be the most important mycotoxins in the context of human health, there are many more that undoubtedly affect farm animals, have been implicated in human health in the past, and may become important in the future. The trichothecenes are produced by several species of *Fusarium* in cereals such as barley, wheat and maize. As a group they are all immunosuppressive and this may be a matter of concern following long-term exposure at low concentrations. The acute toxicity varies considerably from one member of the family to another. T-2 toxin is very toxic and was implicated in the serious outbreak of alimentary toxic aleukia in Russia at the beginning of the last century but is relatively uncommon. Deoxynivalenol, also known as vomitoxin, is much less acutely toxic but much more common in cereals grown throughout the world. Another *Fusarium* toxin is zearalenone, often produced at the same time as deoxynivalenol, which, although without acute toxicity, is oestrogenic and it may be undesirable to expose the human population to a known oestrogen through the food chain.

Other *Penicillium* toxins include citrinin which, like ochratoxin, is nephrotoxic but it is much less stable than ochratoxin and there is no evidence of it being a kidney carcinogen. A number of species of *Aspergillus* produce sterigmatocystin which is a precursor in the biosynthetic pathway to the aflatoxins as well as a metabolite in its own right. It is much less commonly found in human foods than aflatoxin and is very much less acutely toxic and carcinogenic. One species that secretes sterigmatocystin is *A. versicolor* which can grow at relatively low temperatures and can form part of the mould flora on the surfaces of cheese stored for long periods at low temperatures. Sterigmatocystin has been found in such cheeses but it does not penetrate more than a few millimetres below the surface. Toxic metabolites of moulds continue to be discovered although their significance in human health may not be apparent and the risks associated with their presence in foods may be low. It is important to maintain an awareness of the potential for moulds to produce toxic metabolites in foods without detracting from the long-standing and invaluable use of moulds in the production of a wide range of foods.

18.11 Sources of further information and advice

The following journals are especially useful for details of mycotoxin studies:

- *Applied and Environmental Microbiology*
- *Food Additives and Contaminants*
- *Journal of the Association of Official Analytical Chemists*
- *Mycotoxin Research*

Reports of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the International Agency for Research on Cancer (IARC) and the following monographs are also invaluable sources of information:

ICMSF (1996) *Microorganisms in Foods 5. Microbiological Specifications of Food Pathogens*. Blackie Academic & Professional, London.

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19

Mycobacterium paratuberculosis

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19.1 Introduction

The organism that we now know as *Mycobacterium avium* subsp. *paratuberculosis* was first isolated by Johne and Frothingham in 1895 during an investigation of the cause of chronic diarrhoea in cattle. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a member of the family *Mycobacteriaceae* (Wayne and Kubica, 1986). These bacteria are characterised by their slow growth rate and resistance to acid and alcohol. This resistance is due to a strong cell wall containing a high lipid concentration. They are Gram-positive, strictly aerobic, non-motile, acid-fast, rod-shaped bacteria with fastidious growth requirements. MAP requires the presence of mycobactins, iron-binding hydroxamate compounds, for growth. The organism will grow in the temperature range 25–45 °C with an optimum of 39 °C. It will grow at salt concentrations below 5% and at a pH of 5.5 or greater. The characteristics of MAP have been reviewed by Collins *et al*. (2001).

MAP is the causative agent of Johne's disease, an incurable, chronic, infectious enteritis of ruminants. The disease is characterised by diarrhoea, weight loss and, ultimately, death. It is recognised as being one of the most common causes of bacterial infection in domesticated animals worldwide. It is most common in cattle where asymptomatic carriage may occur. There is some evidence of host specificity because of the reported evidence of different varieties of MAP that cause paratuberculosis in cattle and in small ruminants (Juste *et al*., 1991). Results of a national survey for Johne's disease conducted in the USA in the 1980s showed that the infection was present in 1.6% of all cattle and 2.9% of cull cows (Merkal *et al.*, 1987). A survey of veterinary practices and farms in southwest England concluded that 1% of farms had cattle with Johne's disease and 2% of

the animals in these herds were infected (Çetinkaya *et al.*, 1996). Similar prevalence rates have been found in other European countries (Scientific Committee on Animal Health and Animal Welfare, 2000).

19.2 *Mycobacterium paratuberculosis* and Crohn's disease

Because the pathological changes that occur in the small intestines of people with Crohn's disease are similar to those observed in cattle suffering from Johne's disease, it has been postulated that MAP is the aetiological agent responsible for Crohn's disease (Chiodini and Rossiter, 1996; Hermon-Taylor *et al.*, 2000). Crohn's disease is a chronic inflammatory disease of humans that most commonly affects the distal ileum and colon. However, it can occur in any part of the gastrointestinal tract. There is no known cure for Crohn's disease and it is a life-long debilitating illness, the symptoms of which generally first appear in people aged 15 to 24. The illness is often cyclical, with patients undergoing intermittent remission followed by recurrence. Surgical intervention is necessary in a large proportion of sufferers. Management of the disease has become easier with the advent of drugs such as aminoslaicylates, budesonide and immunosuppressive drugs (Achkar and Hanauer, 2000; Prantera *et al.*, 1994). Revolutionary treatments involving genetically engineered anti-TNF α antibody, infliximab, may provide ways to radically alter the course of severe Crohn's disease by targeting a specific inflammatory mediator (Bell and Kamm, 2000).

Evidence linking MAP and Crohn's disease is far from conclusive. Studies have shown that the organism can be cultured from about 7.5% of patients with Crohn's disease but from only 1% of healthy individuals (Chiodini and Rossiter, 1996). The results of several surveys are summarised in Table 19.1. There is about a two-fold increase in the prevalence of the organism in Crohn's patients compared with control groups. However, the biggest differences were noticed when a polymerase chain reaction (PCR) method was used to detect the organism (Scientific Committee on Animal Health and Animal Welfare, 2000). Concerns have been raised because this method does not differentiate between viable and dead cells. In addition, many other bacteria have been isolated from biopsies taken from Crohn's patients, including *Helicobacter* spp., *Listeria monocytogenes* and *Escherichia coli* (Tiveljung *et al.*, 1999).

Further evidence implicating MAP as the aetiological agent in Crohn's disease was produced by El-Zaatari and colleagues (El-Zaatari *et al.*, 1994, 1997, 1999; Naser *et al.*, 1999). They identified two MAP proteins, p35 and p36, and then screened sera of Crohn's patients for the presence of antibodies to these two proteins. Eighty-six per cent of patients were seropositive for the p36 antibody and 74% of patients were seropositive when challenged with both proteins. The corresponding values for sera from controls was 11% and 0%, respectively. However, 100% of BCG vaccinated subjects and 89% of subjects with tuberculosis or leprosy were also seropositive for the p36 antibody. Other research has failed to find a link between seroprevalence of MAP antibodies and Crohn's

Table 19.1 Comparison of isolation rates of *Mycobacterium paratuberculosis* from patients with Crohn's disease and healthy controls

Method of isolation	No. positive for MAP/Total no. examined (% +ve)	
	Crohn's	Control
Direct culture	18/221 (8.1)	7/117 (6)
PCR on culture	12/35 (34.3)	1/24 (4.2)
PCR on tissue samples	120/507 (23.7)	69/666 (10.4)
All methods	150/763 (19.7)	77/807 (9.5)

Source: data from Scientific Committee on Animal Health and Animal Welfare (2000).

disease (Tanaka *et al.*, 1991; Walmsley *et al.*, 1996). The interpretation of data concerning seroprevalence is made difficult because many Crohn's sufferers take immunosuppressive drugs which can interfere with immunological assays. The picture is even further complicated by the fact that Crohn's disease results in a 'leaky' intestine and so patients with the disease readily form antibodies to intestinal and food microorganisms (Blaser *et al.*, 1984).

There have also been reports of the efficacy of antimycobacterial drugs in treatment of Crohn's patients, but the antibiotics used are active against many other bacteria (Prantera *et al.*, 1994, 1996). The remission in symptoms achieved with these drugs is generally short-lived and similar results can be obtained with antibiotics not known to be effective against mycobacteria (Prantera *et al.*, 1996).

There is conclusive evidence that hereditary and environmental factors play an important role in the aetiology of Crohn's disease (Kornbluth *et al.*, 1993). This, together with the conflicting results of studies aimed at confirming a link between MAP and Crohn's disease, suggest that even if MAP is involved in development of Crohn's disease it is not the sole cause. Also, if bacteria are involved in Crohn's disease then their action may be the result of a dysfunctional immune response and not due to the virulence of the organism *per se*.

Recently, Naser *et al.* (2000) reported finding MAP in the breast milk of lactating Crohn's disease patients. They sampled seven breast milk samples; two from Crohn's disease patients and five from healthy controls. The controls exhibited no evidence of MAP, but the two samples from Crohn's disease patients did clearly show the presence of MAP.

19.3 *Mycobacterium paratuberculosis* in foods

Because of the widespread occurrence of Johne's disease it seems likely that MAP would be present in raw meats from ruminants, raw vegetables and water. However, no data appear to exist in the literature on the prevalence of this organism from these sources.

Several studies have shown that MAP can be cultured from the milk of cows clinically infected with paratuberculosis (Doyle 1954; Smith, 1960; Taylor *et al.*, 1981). *Mycobacterium paratuberculosis* has been cultured from the faeces of 28.6% of cows in a single herd with high prevalence of infection comprising 126 clinically normal animals. Of the 36 faecal culture-positive cows, MAP was isolated from the colostrum of 8 (22.2%) and from the milk of 3 (8.3%). Cows that were heavy faecal shedders were more likely to shed the organism in the colostrum than were light faecal shedders (Sweeney *et al.*, 1992). Levels of the organism in nine culture-positive raw milks from clinically normal, faecal culture-positive cows were between 2 and 8 colony-forming units (cfu)/ml (Sweeney *et al.*, 1992). However, Nauta and van der Giessen (1998) suggested that faecal contamination was the most important contributor to contamination of milk by MAP and levels as high as 10^4 cfu/ml could be attained.

Cream, whey and pellet fractions of centrifuged whole cow milk were examined by IS900 PCR for MAP by Millar *et al.* (1996). The PCR assay gave the expected results for spiked milk and for native milk samples obtained directly from MAP-free, subclinically and clinically infected cows. Individual cartons and bottles of whole pasteurised cow milk obtained from retail outlets throughout Central and Southern England and South Wales from September 1991 to March 1993 were analysed and peaks in the percentage of positive samples were found in winter and autumn. Overall, 22 of 312 (7.1%) samples tested positive for MAP. Fifty per cent of the PCR-positive milk samples and 16.7% of the PCR-negative milk samples tested positive for MAP after 13–40 months of culture, despite overgrowth by other organisms.

During the period March 1999 to August 2000, a survey was undertaken to determine the prevalence of MAP in raw and pasteurised milks in the UK (Advisory Committee on the Microbiological Safety of Food, 2000). The organism was detected in milk using an initial rapid screening procedure involving immunomagnetic separation coupled to PCR. Conventional culture was used to confirm viability of the isolates and the organism was determined to be MAP if it met the following criteria: (i) acid fast, (ii) slow growth and typical colony morphology on Herrold's egg yolk medium, (iii) presence of IS*900* insertion element confirmed by PCR, and (iv) dependent on mycobactin J for growth. To date results are available for 679 milk samples (Table 19.2), and viable MAP has been found in 1.9% of raw milk samples and 2.1% of pasteurised milk samples. The presence of MAP in pasteurised milk has generated speculation that the organism can survive high-temperature, short-time (HTST) pasteurisation.

Very little work has been published on the ability of MAP to survive in foods. To determine the ability of MAP to survive in cheese, Sung and Collins (2000) investigated the effect of pH, salt and heat treatment on viability of the organism. They showed faster rates of inactivation of MAP at lower pH. The time for a 1 log cycle decrease in count (D value) was 10 ± 2.5, 19 ± 3.9, and 33.3 ± 4.4 days at pH 4, 5 and 6, respectively. It was also concluded that NaCl concentrations between 2 and 6% had little effect on the ability of the organism to survive regardless of pH. However, the inactivation rates were higher in acetate buffer

Table 19.2 Prevalence of *Mycobacterium paratuberculosis* in milk in the UK

Sample	No. of samples tested	No. of samples +ve for MAP (%)	No. of samples –ve for MAP (%)
Raw milk	201	4 (2.0)	197 (98.0)
Pasteurised milk	476	10 (2.1)	466 (97.9)
Whole	191	3 (1.6)	188 (98.4)
Semi-skimmed	145	5 (3.4)	140 (96.6)
Skimmed	140	2 (1.4)	138 (98.6)
UHT	2	0 (0)	2 (100)
All milks	679	14 (2.1)	665 (97.9)

Source: data from Advisory Committee on the Microbiological Safety of Food (2000).

(pH 6, 2% NaCl) than in Queso Fresco cheese (pH 6.06, 2% NaCl). The *D* values for heat-treated (62 °C for 240 s) MAP in buffer and cheese were 3.1 and 36.5 days, respectively, and the corresponding values for non-heat-treated cells were 38.2 and 59.9 days. It was concluded that heat treatment of milk together with a 60 day curing period will reduce numbers of MAP in cheese by about 10^3 cfu/g.

Since the revelation that MAP can be isolated from pasteurised milk, there have been numerous studies to ascertain its heat stability in milk. Using a holder method, Chiodini and Hermon-Taylor (1993) concluded that heat treatments simulating a batch pasteurisation (63 °C for 30 min) and an HTST treatment (72 °C for 15 s) resulted in over 91% and 95% destruction of MAP, respectively. When MAP was heated in milk in a sealed vial, *D* values of 229, 48, 22 and 12 s were reported at 62, 65, 68 and 71 °C, respectively (Collins *et al.*, 2001; Sung and Collins, 1998). Data from studies where the *D* values of MAP were calculated are presented graphically in Fig. 19.1.

Grant *et al.* (1996) used a holder method (63.5 °C for up to 40 min) and a laboratory-scale pasteuriser (72 °C for 15 s) to study the effect of heat on MAP. A rapid decline in numbers was observed during heating but approximately 1% of the initial population remained after heating. This was attributed this to clumping of the bacterial cells (Klijn *et al.*, 2001 and Rowe *et al.*, 2000), and they concluded that laboratory heat treatments simulating pasteurisation did not effectively eliminate MAP from the milk unless initial numbers were below 10 cfu/ml (Grant *et al.*, 1998a) or the holding time at 72 °C was extended to 25 s (Grant *et al.*, 1999). A laboratory method, in which milk inoculated with MAP was heated in sealed tubes at temperatures ranging from 65 to 72 °C for up to 30 min, was compared to results obtained using a small-scale pasteuriser designed to simulate HTST units used in processing plants (Stabel *et al.*, 1997). The holder method showed that about 10 cfu/ml of MAP (from an original population of about 1×10^6 cfu/ml) survived even after heating for 30 min at 72 °C. However, results obtained using the laboratory-scale pasteuriser showed that there were no detectable cells of MAP after heating for 15 s at 65, 70 or 75 °C. It was concluded that results obtained

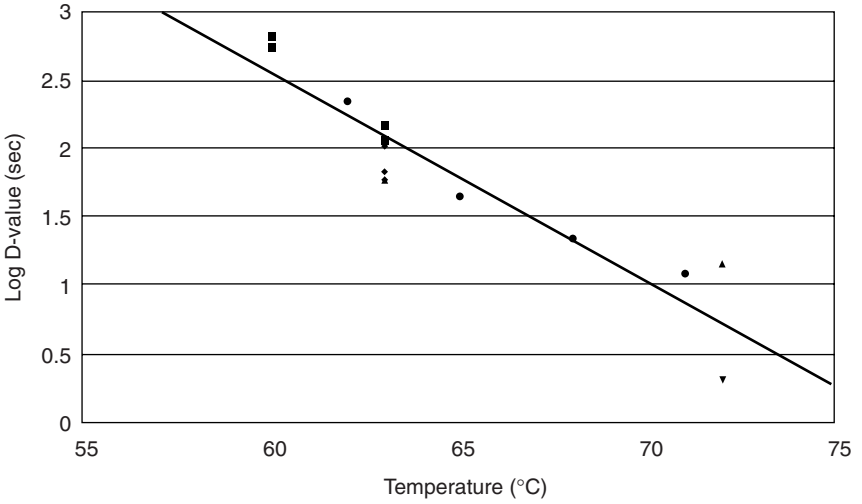

Fig. 19.1. Heat resistance of *Mycobacterium paratuberculosis* in milk. Data of: ■; Keswani and Frank (1998); ●; Sung and Collins (1998); ▲; Stabel *et al.* (1997); ◆; Grant (cited in Stabel *et al*., 2001); ▼; Pearce *et al.* (cited in Stabel *et al.,* 2001). The regression equation is: $y = 11.7 - 0.15x$; and the z value is 6.5 °C.

using the holder method could not be extrapolated to a commercial HTST unit, and that continuous flow is essential for effective killing of MAP in milk.

Further studies using a continuous flow system were described by Hope *et al.* (1997). However, the unit used in this study did not simulate exactly the conditions that would exist in a commercial pasteuriser because a linear holding tube was used. This failed to generate the turbulent flow expected in a plate heat exchanger. Seventeen batches of raw milk were loaded with 10^2–10^5 cfu/ml of MAP and pasteurised at temperatures ranging from 72 to 90 °C for 15–35 s. *M. paratuberculosis* was not isolated from 96% (275/286) of pasteurised milk samples, representing at least a 4 log-cycle reduction in count. Viable mycobacteria were not recovered from the heat-treated milk when raw whole milk was loaded with less than 10^4 mycobacteria per ml, and were not cultured in any of five batches of milk pasteurised at 72–73 °C for 25–35 s, which are the minimum conditions applied when this machine is used commercially to correct for laminar flow in the holding tube. Adequate holding time appeared to be more effective in killing *M. paratuberculosis* than higher temperatures in the small number of batches treated, and this is similar to results reported by Grant *et al.* (1999). Hope *et al.* (1997) cautioned that the survival of *M. paratuberculosis* in experimentally inoculated batches of milk in the small-scale commercial unit cannot be directly extrapolated to commercial pasteurisation of naturally infected milk in dairy factories because of artificially high mycobacterial loads used in these experiments, possible differences between the thermoresistance of laboratory cultured mycobacteria, and features of the small-scale unit. However, they did conclude

that pasteurisation in the continuous flow small-scale unit used in these experiments was more efficient at killing mycobacteria than batch experiments performed in the laboratory. The inaccuracies in heat resistance data produced by laboratory-scale experiments were pointed out by Cerf and Griffiths (2000) and Hasting *et al.* (2001). They also pointed out that it was thermodynamically unfeasible to expect that extending the holding time would have a greater effect on survival of MAP than increasing the heating temperature. A pilot-scale pasteurizer operating under validated turbulent flow was used to study the heat sensitivity of MAP added to raw milk by Pearce *et al.* (2001). Five strains were heated in raw whole milk for 15 s at 63, 66, 69, and 72°C in duplicate trials. No strains survived at 72 °C for 15 s; and only one strain survived at 69 °C. D values (decimal reduction times) at 63, 66, and 72 °C were 15.0 ± 2.8 s, 5.9 ± 0.7 s, and <2.03 s, respectively. This was equivalent to a >7 $\log_{10}$ kill at 72 °C for 15 s. The mean Z value (degrees required for the decimal reduction time to traverse one log cycle) was 8.6 °C. These five strains showed similar survival whether recovery was on Herrold's egg yolk medium containing mycobactin or by a radiometric culture method (BACTEC). The authors concluded that properly maintained and operated equipment should ensure the absence of viable MAP in retail milk and other pasteurized dairy products.

A series of studies are underway worldwide to establish the efficacy of commercial pasteurisation procedures against MAP (Stabel *et al.*, 2001). These results are producing conflicting results. Results from New Zealand and Australia suggest that the current minimum pasteurisation procedures (72 °C for 15 s) used in the dairy industry are effective for controlling MAP. However, studies conducted in Germany using intracellularly located MAP showed that the organism survived in 80 out of 234 trials, albeit at levels less than 1 cfu/ml. Thus, the definitive study to determine whether MAP can survive pasteurisation is still awaited.

19.4 Detection methods

Diagnostic tests for MAP have recently been reviewed (Grant and Rowe, 2001; Nielsen *et al.*, 2001). Culture methods are laborious and time-consuming, taking 8–16 weeks to obtain results. Because of this long incubation period, contamination is often a problem and samples have to be treated with selective agents to reduce numbers of non-mycobacterial organisms. The low numbers present also necessitate a concentration step, such as centrifugation, filtration or, more recently, immunomagnetic separation (Grant *et al.*, 1998b). Usually an enrichment step in liquid medium, such as Dubos broth, is used prior to plating onto a solid medium. Of these, one of the most commonly used is Herrold's egg yolk medium. The identity of the isolate is confirmed if it meets all the following criteria: (i) it must be acid-fast; (ii) it must exhibit slow growth with a typical colony morphology (i.e. colonies are 1–2 mm in diameter, entire and white); (iii) it must be IS900 PCR-positive; (iv) it must require mycobactin J for growth.

A modification of the culture method has been used successfully in which

radioisotope-labelled substrates are incorporated in the growth medium. The assimilation of these substrates can be detected using the BACTEC system. Radiometric culture is faster and more sensitive than traditional culture techniques (Nielsen *et al.*, 2001). The radiometric method has also been combined with PCR to rapidly confirm the MAP status of the sample.

Because of its slow growth there has been considerable interest in the development of more rapid detection tests. These have mainly focused on PCR of the IS900 insertion element which is unique to MAP and is present in multiple copies in the genome of the bacterium. The limitations of this assay have been discussed by Nielsen *et al.* (2001), but the main problem seems to be the high number of false-negative reactions generated by PCR as compared to conventional culture. There is also growing interest in the development of real-time PCR methods, such as the Taqman assay. However, PCR methods, when used alone, cannot distinguish between viable and non-viable states of the organism. To overcome this problem, the use of reverse transcriptase PCR (RT-PCR) and nucleic acids sequence-based amplification (NASBA) are being investigated (Grant and Rowe, 2001).

Immunoassays are widely used for screening cattle for Johne's disease and the use of enzyme-linked immunosorbent assay (ELISA) has been attempted for the screening of bulk tank milks (Nielsen *et al.*, 2000) with little success. Commercial ELISA and immunodiagnostic kits are available from a number of companies.

19.5 Control measures

Arguably the most effective way of controlling MAP is by adopting strategies that will eliminate Johne's disease from cattle on the farm. However, this is easier said than done because of the ability of the organism to survive in the environment, the long incubation period of the disease and the lack of sensitive diagnostic tests to identify infected animals during this time (Kennedy *et al.*, 2001). The risk management strategies used to contain Johne's disease include prevention of infection of the herd through maintaining a closed herd whenever possible and when new animals have to be introduced they should be from herds free from the disease. Cattle should also be kept from pastures and other environments that may be heavily contaminated through prior exposure to animals with the disease. It has been demonstrated that MAP can survive in faeces kept outdoors for up to 246 days, depending on the conditions (Collins *et al.*, 2001). Environmental contamination can also be reduced by good management of water and effluent flows from neighbouring high-risk farms and by good manure management.

Exposure of individual calves to paratuberculosis should be minimised by rearing in clean environments free from adult cattle, coupled with the adoption of good hygienic practices by farm personnel. The animals should be fed milk and water that are free from contamination, and ideally the calf should be

provided with an adequate colostrum intake from a paratuberculosis-negative cow.

The most important source of MAP within the herd is the cow with advanced infection, as the rate of excretion of bacteria in the faeces of these animals increases as they approach clinical disease. Thus, these animals should be identified as early as possible through testing and applying knowledge of the history of disease in the herd. This is best achieved by culling calves of infected cows, early culling of suspect cows and test reactors along with animals that have been in contact with these cattle.

Several countries have adopted national programmes to control paratuberculosis in dairy herds and/or to accredit MAP-negative herds as low-risk sources of replacement cows.

To reduce the level of MAP in milk the UK has recommended increasing the holding time at the minimum pasteurisation temperature of 72 °C from 15 to 25 s.

19.6 Sources of further information and advice

19.6.1 Organisations

International Association for Paratuberculosis: www.paratuberculosis.org
International Dairy Federation: 41 Square Vergote, 1030 Brussels, Belgium (www.fil-idf.org).

19.6.2 Key texts

Bulletin of the International Dairy Federation 362/2001, IDF, Brussels.
Institute of Food Science and Technology: Current Hot Topics. *Mycobacterium paratuberculosis* and milk. www.ifst.org/hottop23.htm
Report of the Scientific Committee on Animal Health and Welfare (2000) *Possible Links Between Crohn's Disease and Paratuberculosis*. European Commission SANCO/B3/R16/2000.

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20

Chronic sequelae of foodborne infections

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20.1 Introduction

Foodborne pathogens cause various health problems. The acute health effects such as diarrhoea nausea, vomiting, abdominal pain, cramps, fever and jaundice are well known by health professionals and the general population. In most cases immunocompetent persons recover within a few days or few weeks. Therefore, foodborne infections are often considered as self-limiting diseases.

During the years, awareness has been raised on the more severe and chronic health consequences of foodborne diseases, and foodborne pathogens have been associated with a number of chronic health problems such as cardiovascular, renal, joint and articular, neural neuramuscular disorders, auto-immune thyroid disease, and affection of respiratory or immune systems (WHO, 1992). The role of a number of foodborne bacteria such as *Mycobacterium paratuberculosis*, *Streptoccocus fecalis* and *Escherichia coli* in causing chronic infections such as the inflammatory bowel disease (Crohn's disease, ulcerative colitis) is still being investigated. Nanobacteria are also being studied for their role in formation of kidney stones.

Archer and Kvenberg (1985) estimated that in the USA chronic health sequelae might occur in 2–3% of foodborne infections. In a survey of 32 448 cases of foodborne diseases in the Russian Federation, chronic health effects occurred in over 11% of patients, with hypertension and cholelithiasis (presence of concretion in the gall bladder) being the most frequent. A number of patients also developed myocardial infarction (Brodov, 1993).

As the incidence of foodborne illness increases, it may be expected that the occurrence of these chronic problems may also increase. However, unless in-depth epidemiological investigation is carried out, chronic complications of

foodborne pathogens will be relatively infrequently associated with foodborne agents and reported as such (Bunning *et al.*, 1997). Further difficulty in determining the sequelae of foodborne infection lies in the fact that chronic health effects may appear weeks, months or even years after the initial infection.

The occurrence of chronic health effects depends on many factors. Some of the determining factors are the virulence and ingested dose of the organism, factors related to the host including the predisposition of patients (e.g. genetic factors, age, immunity, health and nutritional status), as well as timely and adequate treatment. While not in the scope of this chapter, it is important to mention that when foodborne infections persist, they may cause severe malnutrition, which in turn may lead to other infections. A vicious spiral of infection and malnutrition sets in, which in many cases leads to death. In the developing regions of the world where hygiene and food safety practices are poor, such problems are a frequent cause of death in infants and young children (Motarjemi *et al.*, 1993).

The vulnerable populations of the elderly, pregnant women, infants and young children and immunocompromised individuals is more seriously affected by foodborne pathogens. The elderly are both more susceptible to some foodborne infections and may also suffer more severely from the consequences. The increased susceptibility of the elderly has been explained by the age-induced decrease in (a) stomach acid, allowing the survival of pathogens in the intestinal tract, (b) immunity, (c) peristalsis and (d) decrease in food consumption resulting in poor nutritional status (Smith, 1998).

Pregnant women and their unborn children are particularly susceptible to some foodborne pathogens. In a literature survey of foodborne diseases, Smith (1999) reported that transplacental transmission, with subsequent infection of the foetus or the new-born has been observed with hepatitis E virus, *Brucella melitensis, Campylobacter jejuni*, *Coxiella burnetii*, *Listeria monocytogenes*, *Salmonella typhi* and *Toxoplasma gondii*. Consequences are often abortion, stillbirth or preterm delivery (Smith, 1999).

The mechanism of appearance of chronic sequelae is not always well understood. According to Bunning *et al.* (1997) chronic infection may in the case of some pathogens arise without an overt illness, or alternatively it may be unrelated to the acute illness and occur despite the fact the immune system successfully eliminates the primary infection. In other words, the activation of the immune system can by itself initiate the chronic condition due to an autoimmune response (Archer and Young, 1988; Foegeding *et al.*, 1994; Bunning *et al.*, 1997).

This chapter reviews the chronic health complications caused by some major and emerging pathogens. It should however be noted that a broad range of foodborne diseases or contaminants may cause chronic sequelea (Archer and Young, 1988, Lindsay 1997, McDowell and McElvaine, 1997). The focus of this chapter is foodborne infections of microbial origin. A number of foodborne helminthiasis, other than trematode infections mentioned here may also cause chronic diseases. Mention should also be made of chemical contaminants (e.g. heavy metals) or naturally occurring toxins including mycotoxins, and plant and marine biotoxins (WHO, 1990, Käferstein *et al*. In press). The global incidence of the diseases addressed in this chapter, is reviewed in Motarjemi and Käferstein (1997).

20.2 *Aeromonas*

Although evidence on the pathogenicity of this organism is scare, *Aeromonas hydrophila* is viewed as an emerging foodborne pathogen associated with seafood, snails and drinking water. *Aeromonas* is an opportunistic pathogen, causing infections particularly in children, the elderly and the immunocompromised. Acute intestinal symptoms include watery stools, stomach cramps, mild fever and vomiting. Recent case reports have associated chronic colitis with *Aeromonas hydrophila* infection (Ibrahim *et al.*, 1996). Extraintestinal infections may also occur and cause health problems such as bronchopneumonia and cholecystitis (inflammation of gallbladder). The organism is commonly found in aquatic environments and can be transmitted through consumption of seafoods, snails and drinking water. The control measures include treatment and disinfection of water supply and thorough cooking of food. As the organism can grow at low temperatures (for instance at a temperature of 4 °C), long-term refrigeration of ready-to-eat food is a risk factor.

20.3 *Brucella* spp.

Among *Brucella* species, four strains cause human brucellosis*: B. abortus*, *B. melitensis*, *B. suis* and *B. canis*. Infections caused by *B. melitensis* and *B. suis* have a prolonged course of disease and can cause serious complications. Acute symptoms include malaise, chills, sweats, fatigue, and weakness, myalgia (muscular pain) arthralgia (joint pain) and weight loss. Symptoms may appear up to 2 months after exposure to the disease agent. Chronic health problems can be developed particularly when the treatment is inadequate. In 20–60% of patients, the infection can lead to osteoarticular complications, sacroliitis (inflammation of sacroliac joint), genitourinary complications, cardiovascular and neurological conditions, insomnia and depression.

The reservoirs for *Brucella* are cows *(B. abortus*), sheep and goats (*B. melitensis*) and pigs (*B.suis*). Brucellosis is contracted principally from close association with infected animals and therefore is often an occupational disease of farmers, herdsman, veterinarians and slaughterhouse workers. It can also be contracted through consumption of milk (usually goat's or sheep's milk), and products made from non-pasteurised milk, e.g. fresh goat cheese. Control measures include vaccination of animals and eradication of diseased animals. Foodborne transmission can be prevented through pasteurisation or sterilisation of milk for cheese production, ageing of cheese for at least 90 days. Consumers should also be advised not to consume raw milk and cheese made with raw milk.

20.4 *Campylobacter* spp.

Campylobacter species, in particular *C. jejuni* and *C. coli*, cause infections, which vary in symptoms, ranging from asymptomatic to severe chronic illness. Acute health consequences are abdominal pain, fever, nausea and diarrhoea, which

can vary from slight to profuse watery diarrhoea sometimes containing mucus or blood. Chronic health consequences may occur in 2–10% of cases of campylobacteriosis. These include reactive arthritis, meningitis, cholecystitis, endocarditis, erythemea nodosum, abortion and neonatal sepsis. *C. jejuni* is increasingly recognised as a risk factor for Guillain–Barré Syndrome (GBS, the presence of pathogenic organisms, or their toxins, in the blood or tissues), a common cause of neuromuscular dysfunctioning (Bunning *et al.*, 1997). Studies by McCarthy and Giesecke (2001) have shown that the risk of developing Guillain–Barré syndrome during the 2 months following a symptomatic episode of *C. jejuni* infection is approximately 100 times higher than the risk in the general population. Nevertheless, it remains a rare complication and its incidence is estimated as some 30 cases per 100 000 cases of *C. jejuni* infection (McCarthy and Giesecke, 2001).

The reservoir for *Campylobacter* is domestic animals (cats and dogs), livestock (pigs, cattle, sheep), birds (including poultry) and polluted water. Campyloabacteriosis is principally transmitted through ingestion of contaminated food and water. The main food sources are raw milk and undercooked poultry. The bacteria can be transmitted to other foods by cross-contamination, or contamination with untreated water or through contact with animals (pets and farm animals) and birds. The foodborne route of infection can be successfully prevented through pasteurisation/sterilisation of milk, irradiation of poultry and disinfection of water. At the food handler and kitchen level, hygienic practices (including prevention of cross-contamination, thorough heat treatment of milk and poultry, hand hygiene in food preparation and keeping pets away from food-handling areas) are essential control measures.

20.5 Enterohaemorrhagic *Escherichia coli*

Among *E.coli* strains, four strain types are particularly pathogenic and cause gastroenteritis. These are: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (EHEC). Acute symptoms of *E. coli* infections include diarrhoea, abdominal cramp and vomiting. In the developing countries, infections caused by *E. coli* (EPEC, ETEC) are particularly prevalent and are a major cause of undernutrition. In the industrialised countries, it is EHEC which presents the greatest concerns as EHEC infections can have life-threatening health consequences, such as haemolytic ureamic syndrome (HUS). This may occur in up to 10% of patients, particularly young children and the elderly. HUS is characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. EHEC infections can lead to long term or permanent kidney damage. Other sequelae include erythema and thrombotic thrombocytopenia purpura. On average 2–7% of patients with HUS die but in some outbreaks among the elderly, the mortality rate has been as high as 50% (WHO, 1997).

EHEC infections are transmitted through consumption of foods of animal

origin such as raw or undercooked ground meat products and raw milk. Faecal contamination of water or foods which have been contaminated with manure during production. Examples of foods implicated in outbreaks of EHEC infection include: hamburgers, roast beef, raw milk, fresh pressed apple juice, yoghurt, cheese, fermented sausage, cooked maize, lettuce and seed sprouts. Some strains have shown patterns of acid resistance and have survived in fermented foods implicated in outbreaks. As the pathogen has a low infective dose, low levels of contamination may be dangerous and transmission can take place from person to person.

In general, prevention of EHEC infection relies on good hygienic practices all along the food chain from farm to the final preparation to consumption. These include good animal husbandry practices to minimise the spread of contaminated material to animals or on ground used for crops; protection of fruits and vegetables from manure, treatment of potentially contaminated foods, e.g. pasteurisation of milk, irradiation or heat treatment of meat and meat products; thorough hand-washing before preparation of food. Waterborne transmission is also prevented through effective treatment of drinking water and sewage disposal.

20.6 *Enterobacter sakazakii*

Enterobacter sakazakii has been implicated a few times in severe forms of neonatal meningitis and necrotising enterocolitis. Patients surviving *E. sakazakii* meningitis are known to suffer from severe neurological sequelae such as hydrocephalus, quadriplegia and retarded neural development (Nazarowec-White *et al*., in press). In many cases neonates die within days of birth. The case fatality rate may be as high as 75% (Muytjens *et al.*, 1983). Much is unknown about the epidemiology of this organism. In a few outbreaks involving premature babies, the organism was associated with dry infant formula (Willis and Robinson, 1998). Therefore, based on the current knowledge on the epidemiology, to prevent *E. sakazakii* strict hygienic conditions should be observed during the manufacture of infant formula, in particular prevention of recontamination after processing through the environment. Adherence to hygienic rules is equally important during the preparation of infant formula. In particular, infant formula should not be stored for long periods at room temperature or in bottle warmers as these conditions may lead to the growth of the pathogen and increase the risk of infection (Biering *et al.*, 1989).

20.7 *Helicobacter pylori*

Helicobacter pylori is an emerging pathogen found all over the world but with higher prevalence in the developing countries. It colonises gastric epithelial tissue and the mucus layer. It has been associated with gastritis, and has been recognised as a major cause of duodenal and gastric ulcers (NIH, 1994). There is also

epidemiological evidence that *H. pylori* infection is associated with gastric cancer. The mode of transmission remains poorly understood. Different pathways, including waterborne, zoonotic and faecal oral routes, have been suggested (IARC, 1994; Goodman and Correra, 1995).

20.8 *Listeria monocytogenes*

Listeria monocytogenes may cause influenza-like symptoms such as fever, headache and occasionally gastrointestinal symptoms. More severe health consequences may occur in foetuses, neonates, the elderly and immuncompromised individuals. About one-third of clinical cases have been reported in the new-born. In adults infection occurs mainly in those aged 40 or over. In pregnant women, bacteraemia may affect the amniotic sac leading to premature birth, abortion or stillbirth. In elderly and immunocompromised adults (e.g. persons with transplanted organs, lymphomas or AIDS), the infection may lead to meningitis, encephalitis and/or septicaemia. The infection of the central nervous system leads to severe diseases often with a high case fatality rate or with neurological sequelae in survivors (Rocourt and Bille, 1997). The case fatality rate is 30%, but in patients who have not received adequate treatment it may be as high as 70%.

Foodborne transmission of listeriosis has been reported through various types of foods: raw milk, soft cheese, frankfurters, jellied pork tongue, raw vegetables and coleslaw. *Listeria monocytogenes* is psychrotrophic and can grow at refrigeration temperatures; therefore long-term storage of food in refrigerator is a risk factor.

The main ways to prevent listeriosis are thorough heat treatment of foods of animal origin and prevention of opportunities of post-process recontamination. Consumers should be educated to refrigerate perishable foods and consume them within a short period of time. Pregnant women and other vulnerable individuals, e.g the elderly and immunocompromised, should avoid eating raw foods of animal origin, e.g. raw meat, raw milk and foods that support the growth of *Listeria* such as smoked or raw seafood, pâté and soft cheese.

20.9 *Mycobacterium paratuberculosis*

Mycobacterium paratuberculosis, a subspecies of Mycobacterium avium (MAP), is the cause of chronic enteritis in cattle known as John's disease. In North America and Europe, the organism is highly prevalent in a subclinical form in dairy herds and domestic livestock such as sheep and goat. It can also infect a range of wild animals such as rabbits and deer (Kennedy *et al.*, 2001).

The organism has been of concern due to its impact on milk and meat production and subsequent economic losses (EC, 2000). In recent years, there is an increasing concern about potential public health consequences of this organism as some scientists suspect the organism to be associated with the human Crohn's disease (Hermon-Taylor *et al.* 2000; EC, 2000; FSA, 2001).

Crohn's disease (CD) is a highly debilitating chronic inflammation of gastrointestinal tract in humans but most commonly affecting the distal ileum and colon. It is a lifelong disease with no cure. It usually affects young people with the highest incidence rate in the age group 15–24. Genetic and immunologic factors seem to play an important role in the occurrence of the disease. A multicentre European study has reported an incidence rate of 5.6 per 100 000 individuals per year (Shivananda *et al.* 1996).

The aetiology of the disease is not well known. The association of the disease with different infectious agents has been studied without conclusive results. The similarities between John's disease and Crohn's disease has focused the attention on MAP and the possibility that this organism may be involved in causation of Crohn's disease. The putative association of Crohn's disease with MAP has also been strengthened following some studies showing presence of MAP in a significant proportion of CD patients findings (Acheson, 2001; Hermon-Taylor, 2001).

Infected dairy cows secrete MAP in the milk as well as in the pasture contaminating the environment and the water resources. Therefore, milk and water are considered as potential sources of exposure. Other dairy products e.g. cheese as well as meat is also considered a potential vehicle for this organism. Due to its relatively high heat resistance, the organism shows the ability to survive the current pasteurisation conditions of 72 °C and 15 seconds. Surveys carried out in the UK have shown the presence of the organism in a small proportion of raw as well as commercially available pasteurised milk samples (FSA 2001). Another feature of this organism, which makes its control difficult, is its ability to survive in the environment as well as methods of culturing partly due to its slow growth.

20.10 Nanobacteria

Nanobacteria are the smallest cell-walled bacteria (0.2–0.5 μm), recently discovered in human and cow blood. The bacteria have been found in marine limestone, freshwater streams, springs, water pipes and caves (Folk, 1999). During their growth, nanobacteria form carbonate apatite on their cell envelope. Under the scanning electron microscopy, nanobacteria resemble the smallest apatite units in the kidney stone. Researchers now believe that nanobacteria can be the cause of kidney stones. Apatite produced by nanobacteria may facilitate deposit around which crystalline compounds are collected (Bradbury, 1998). In a study of some 72 cases of kidney stones in Finnish patients, Ciftcioglu and co-workers (1999) found that in 97.25% of cases, stones were positive for nanobacteria indicating that the organism may possibly be the cause of kidney stones. Studies of Hjelle *et al.* (2000) also demonstrated the presence of nanobacteria in kidney stone patients. However, the work of other scientists casts doubts on this theory and provides an alternative explanation for kidney stone formation (Cisar *et al.*, 2000). Further studies are needed to prove this hypothesis and to determine the factors that may contribute to kidney stones (species/strain of nanobacteria, vulnerability of individuals) and the ecology and route of transmission of this organism.

20.11 Non-Typhi *Salmonella*

More than 2000 serotypes of non-Typhi salmonellae have been described, of which more than 200 are well-known as human pathogens. The principal symptoms of salmonellosis are fever, headache, nausea, abdominal pain and diarrhoea. However, a broad range of chronic sequelae such as reactive arthritis, Reiter disease, rheumatoid syndromes, pancreatitis, osteomyelitis, myocarditis, colitis, choleocystitis and meningitis have been reported as a consequence of salmonella infection. Reactive arthritis has been observed in 2–16% of patients suffering salmonellosis. In a great proportion of cases the symptoms persisted for over a year (Locht *et al.*, 1993; Smith, 1994). Genetic factors seem to be a significant factor in triggering reactive arthritis (Smith *et al.*, 1993).

Foodborne salmonellosis is transmitted by contaminated foods, particularly foods derived from infected animals, e.g. milk, meat, poultry, eggs. Foods can also be contaminated by infected food handlers who have not observed hand hygiene, pets, pests or by cross-contamination during food preparation. Contamination of food and water may also occur from the faeces of an infected animal or person. Time–temperature abuse are important risk factors in transmission of salmonellosis as it may lead to the increase of the organism to disease causing levels.

Prevention of salmonellosis requires effective bactericidal treatment of foods of animal origin, e.g. pasteurisation of milk, heat treatment or irradiation of meat and poultry, pasteurisation or proper cooking of eggs and egg-based products. Food handlers should strictly observe rules of hygienic preparation including prevention of cross-contamination, thorough cooking and reheating of foods, boiling of milk. The vulnerable group of the population should avoid eating raw and under-cooked eggs and foods containing raw eggs.

20.12 *Vibrio vulnificus*

Vibrio vulnificus may cause profuse diarrhoea in persons with chronic liver disease, chronic alcoholism, haemochromatosis or who are immunodepressed. The organism may also lead to septicaemia. Over 50% of patients with primary septicaemia may die, and the fatality rate may increase to 90% in hypersensitive individuals.

All known cases of *Vibrio vulnificus* infection are associated with seafoods, particularly raw oysters. To prevent infections caused by *Vibrio vulnificus*, consumers and, in particular, vulnerable groups such as the elderly, those with underlying liver disease, the immunodepressed and alcohol-dependent persons should be advised not to eat raw seafood.

20.13 *Yersinia enterocolitica*

Infections caused by *Yersinia enterocolitica* manifests in the form of abdominal pain, diarrhoea accompanied by mild fever, and sometimes vomiting. Symptoms

may last 3 weeks. In 2 to 3% of cases sequelae are observed. These include Grave disease (autoimmune thyroid disease), Reiter syndrome (association of urethritis, mucocutaneous lesion, and arthritis), eye complaints and rash, cholangitis, erythema nodosum, septicemia, hepatic and splenic abscesses, lymphadenitis, pneumonia and spondylitis.

Although a variety of animals harbour the organism, it is most frequently isolated with pigs. The route of transmission is primarily pork products e.g. tongue, gut, but also raw milk and milk products. The organism is psychotrophic and can grow at temperatures as low as 0 °C. The illness can be effectively prevented through proper heat treatment of pork products and milk and prevention of cross contamination during food preparation.

20.14 *Toxoplasma gondii*

Infections due to *Toxoplasma gondii* are often asymptomatic. In case of acute disease, the protozoa may cause lymphadenopathy, and lymphocytosis persisting for a few days or weeks. *T. gondii* cysts can remain in infected tissues (muscle, brain) for long periods and reactivate if the immune system becomes compromised, e.g. by cytotoxic or immunosuppressive therapy or in patients with AIDS in which case the infection may be fulminant or fatal. Transplacental infection may also occur during pregnancy. This may cause stillbirth or perinatal death. The baby may also suffer from congenital toxoplasmosis involving the ocular, auditory and/or central nervous system. Symptoms may appear months or years after birth. A proportion of infected babies may show symptoms of hydrocephalus, intracranial calcification, chorioretinitis causing mental retardation and blindness (Smith, 1999). In immunocompromised individuals it may also cause cerebritis, chorioretinitis, pneumonia, myocarditis, rash and death.

Cats and other felines are the reservoir for *Toxoplasma gondii*. Oocysts shed by these animals become infective 1–5 days later and may remain infective in water or soil for a year. Humans become infected upon ingesting the oocysts. For instance, children may become infected by playing in sand polluted with cat excreta. Infection may also occur through eating raw or undercooked meat containing the oocysts (cattle, birds, sheep, goats and pigs are intermediate hosts and may carry an infective stage of *T. gondii*) or food and water contaminated with feline faeces.

To prevent toxoplasmosis, consumers and in particular pregnant women should be advised to avoid eating raw or undercooked meat, to wash vegetables carefully and wash hands after contact with cats. Irradiation of meat is also effective for killing *T. gondii*.

20.15 Trematodes

Foodborne trematodes refer to a group of flatworms which are transmitted through contaminated aquaculture products such as raw or underprocessed fresh-

water fish (*Clonorchis sinensis*, *Opisthorchis viverrini* and *Opisthorchis felineus*), crustaceans (*Paragonimus westermani*), watercress and salad plants such as dandelions (*Fasciola hepatica* and *Fasciola gigantica*). These helminths can cause acute health problems as well as chronic infections. Acute symptoms are broad and depending on the worm may include fever, fatigue, diarrhoea, anorexia, abdominal pain and discomfort, dizziness, urticaria, jaundice, oedema, etc.

Chronic health consequences of clonorchiasis include affection of the liver, spleen and pancreas, recurrent cholangitis and cholangiocarcinoma. In a case of heavy infection, dwarfism and retardation of sexual development have been associated with this infection.

Fascioliasis caused by *Fasciola hepatica* and *Fasciola gigantica* lead also to chronic health conditions such as necrotic lesions, inflammatory and adenomatus changes in the bilary duct, atrophy of the liver, cholecystitis and cholelithiasis. Opisthorchiasis lead also to cholecystitis, cholangitis, liver abscess, gallstones and cholangiocarcinoma. Paragonimiasis causes pulmonary lesions, leading to chronic coughing, thoracic pain and other symptoms. Extrapulmonary lesions may also occur when the worm migrates to other organs. Migration of the worm to the brain may cause cerebral haemorrhage, oedema or meningitis. Severe headache, mental confusion, seizure, hemiparesis, hypaesthesia, blurred vision, diplopia and meningismus may also occur (WHO, 1995).

Prevention of foodborne trematode infections requires safe disposal of excreta and sewage/wastewater; treatment of wastewater used for aquaculture or watercress beds. For fasciolosis, livestock should be treated against *Fasciola hepatica* and should be prevented access to commercial watercress beds. Freshwater fish could also receive parasiticidal treatments such as irradiation, freezing or heat treatment. Consumers should also be advised to avoid consumption of raw or undercooked freshwater fish and watercress.

20.16 *Taenia solium*

Depending on the animal infected and route of transmission, *Taenia* can be divided into *Taenia saginata* (cattle) and *Taenia solium* (pigs). Unlike *T. saginata*, *T. solium* causes both intestinal infection with adult worms as well as somatic infection with the eggs in human host. The latter type is referred to as 'cysticercosis' and is a serious infection of the central nervous system. When eggs or proglottides of *T. solium* are swallowed, the eggs hatch in the small intestines and the larvae migrate to subcutaneous tissue, striated muscles and other tissues and cysts. When the vital organs such as the central nervous system, the eye or heart are affected the health consequences can be serious. These include epileptiform seizures, signs of intracranial hypertension or psychiatric disturbance. The infection may also be fatal. Symptoms of cysticercosis may appear from a few days to over 10 years.

Infection due to *Taenia solium* (taeniasis) is caused by consumption of raw or

undercooked pork. Cysticercosis is caused by ingestion of *T. solium* eggs by the faecal-oral route, person-to-person, autoinfection (unwashed hands) or consumption of contaminated foods, e.g. vegetables.

Cysticercosis may be prevented through safe disposal of sewage and prevention of faecal contamination of soil, water, human and animal food; use of untreated sewage water for irrigation of crops and vegetables should also be avoided.

20.17 *Trichinella spiralis*

Trichinella spiralis may cause symptoms ranging from apparent infection to fulminating and fatal disease depending on the number of larvae ingested. During the initial phase, corresponding to the development of the larvae into adults in the epithelium the infection manifests itself with nausea, vomiting, diarrhoea and fever. The female worm produces larvae, which migrate in the body through the lymphatic and blood system. The larvae encapsulate in the skeletal muscle. At this stage, other symptoms such as rheumatic conditions, muscle soreness and pain, together with oedema of the upper eyelids, followed by retinal haemorrhages, pain and photophobia may appear. Thirst, profuse sweating, chills, weakness and prostration follow the ocular symptoms. Cardiac and neurological complications appear 3–6 weeks later. In most severe cases death may result from myocardial failure.

T. spiralis is transmitted through consumption of raw or undercooked meat (pork, horse, game) containing the encysted larvae. Transmission of *T. spiralis* can be prevented through irradiation, heat treatment or curing of meat and meat products. Hunters should be advised to cook all game meat thoroughly.

20.18 Viral hepatitis A virus

Infections caused by hepatitis A virus are most severe in adults. In children it is often asymptomatic and confers immunity. Symptoms appear 2 to 6 weeks after the initial infection and they consist of loss of appetite, fever, malaise, abdominal discomfort, nausea and vomiting. These are followed by signs of liver damage such as passage of dark urine, pale stools and jaundice. In older persons, the infection may cause long lasting liver disorder.

Hepatitis A virus is spread through faecal-oral route, primarily person to person. It can also be transmitted through food (e.g. shellfish) and water as a result of sewage contamination or infected food handlers. Measures of prevention include safe disposal of sewage and treatment of water supply, good personal hygiene, in particular prior to handling food, and thorough cooking of shellfish. A vaccine is also available and food handlers as well as travellers are advised to be vaccinated.

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