ZOONOTIC PATHOGENS IN THE FOOD CHAIN

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Zoonotic Pathogens in the Food Chain

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Preface

Historically the transmission of disease from animals has increased as humans have evolved from a hunter–gatherer existence to the domestication of animals. As animals became domesticated, humans were in closer proximity to animals themselves, their excreta and the pathogens they carried. These pathogens could contaminate food and water, and resulted in human sickness.

During the 1950s and 1960s, there was an explosion in agricultural productivity, a dramatic decline in animal and human infectious disease – largely as a result of the use of antimicrobials, and the emergence of a relatively cheap food supply. During the 1970s and 1980s there was a perception that the 'food production problem' had been solved, but we have seen an increase of not only classical food safety issues but also of concern about how livestock production may affect human health in general.

New problems have arisen in the food supply. Chapter 1 of this book describes the transformation of the food supply from a local activity to a global activity, and the problems with food safety that globalization brings. Chapter 2 gives a reasonably comprehensive list of zoonoses that are, and are likely to be in the future, high on the list of important pathogens. Manure, the subject of Chapter 3, has always been a concern because it is one of the most important sources of disease to humans, but a less well-studied source of zoonoses in the food supply is the actual feed that comes on to or leaves a farm (Chapter 4).

In the past, pasteurization had a critical role in making the milk supply safe for humans, but more recently there has been a trend towards the use of raw (non-pasteurized) milk (Chapter 5), partly because of a public concern with the use of antimicrobials in the food production system and the potential implications of this for human health (Chapter 6). As a result of these new challenges in securing the food supply, a number of on-farm mitigation strategies have been developed (Chapter 7). Organic agriculture has become popular partly because of the concerns raised by antibiotics in food production, and some regard organic production as a mitigation strategy for food safety in

general (Chapter 8). However, food safety is a dynamic area, and mitigation strategies will be updated in the light of new threats to human health such as avian (and swine) influenza (Chapter 9), and Crohn's disease in humans (Chapter 10).

The last chapter (Chapter 11) is a very interesting case study that illustrates what happens when a human disease threat posed by animal agriculture becomes public. This chapter examines the course of events and the policy development surrounding the outbreak of bovine spongiform encephalopathy (BSE) in the UK, with some comparative data from North America also included. While BSE is a threat to human health, its risk to the public is still relatively small compared with other food-safety risks.

Denis Krause
May 2010

1 Globalization of the Food Supply and the Spread of Disease

SUSAN C. CORK AND SYLVIA CHECKLEY

Historical Perspectives and Current Trends

In the globalized political economy of the late 20th century, increasing social, political and economic interdependence occurred as a result of the rapid movement of people, produce and other commodities across national borders (Harlan and Jacobs, 2008). A consequence of increased trade, travel and migration is the growing risk of transmitting biological and other hazards from country to country on a large scale. With greater connectedness, new and emerging diseases have the potential to travel very fast and subsequently trace back, and control is often difficult. Growing international trade in food commodities and the development of transnational cooperations (TNCs) has corresponded with an increase in cross-border reports of foodborne illnesses highlighting the need for international cooperation in animal and human disease reporting and control initiatives (Kaferstein *et al.*, 1997; King *et al.*, 2004; Kobrin, 2008).

The exchange of food and animal products across regions, nations and continents has occurred for centuries. There have been a number of anthropological studies on the cultural impact of trade in specific products such as sugar (Mintz, 1985) and spices (Miller, 1995; Mintz and Du Bois, 2002). More recently, there has been a wide range of literature focused on the development and implementation of a range of regulations and agreements to facilitate the safe exchange of animal- and plant-based primary produce.

The international circulation of food products as commodities, along with the transnational expansion of food-based cooperations, has resulted in the need for global governance of food safety and quality. To a large extent, this has occurred through the World Trade Organization (WTO) and the implementation of standards outlined in the Sanitary and

Phytosanitary (SPS) Agreement[1] and the Codex Alimentarius[2] (Vallat and Mallet, 2006).

Disease and the Food Chain

Global outbreaks of foodborne disease can have socio-economic impacts on consumer food choices and other behaviour (Sockett, 1993; Knowles *et al.*, 2007). Our understanding of the epidemiology of foodborne diseases has evolved in recent decades corresponding, in part, to improvements in pathogen detection and reporting systems. In addition, new pathogens have emerged to correspond with a changing food supply, an increase in the number of people with heightened susceptibility to foodborne diseases and a greater diversity of food preparation practices and food preferences. This has posed a number of challenges for the veterinary profession and public health agencies (Epp, 2008; BeVier, 2008). Alongside these changes, the global economy has facilitated the rapid transport of perishable foods, increasing the potential for new populations to be exposed to foodborne pathogens prevalent in distant parts of the world.

There have been a number of studies developed to examine the impact of trade in food beyond national boundaries, including the effect of the promotion of non-traditional imports to support consumer demand and the implications of evolving free-trade agreements (Barrientos, 2000; Bonanno, 2004; Phillips, 2006). The key drivers for the emergence of globalization are largely economic, with large cooperations seeking more cost-effective ways in which to produce primary produce to include in the compilation of processed animal feeds and foods for human consumption. Other factors include the greater mobility of ethnic groups and the subsequent greater demand for 'exotic' foods not typically grown in the country of residence. The current trend of facilitating free flow of traded commodities between industrialized and less industrialized nations has led to the availability of a greater variety of fresh and preserved produce for citizens. The concurrent rise in modern processing methods and the availability of a wide range of distribution options has also facilitated enhanced potential for international trade in both perishable and non-perishable commodities. However, this increased trade is not without risks. The expansion of food-related TNCs has had some negative consequences. Processed products manufactured from raw ingredients imported from many different sources makes traceability difficult, and comprehensively tracking contaminated foodstuffs may be impossible – as seen in milk and pet food products contaminated with melamine (Brown *et al.*, 2007; Ingelfinger, 2008).

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[1] http://www.wto.org/english/tratop_e/sps_e/sps_e.htm (accessed 17 May 2010).
[2] http://www.codexalimentarius.net/web/index_en.jsp (accessed 17 May 2010).

Foodborne Disease Control: a Transnational Challenge

> Disease knows no boundaries and borders are porous to disease.
>   (Kaferstein *et al.*, 1997)

The world has moved from a situation in which the majority of food was produced locally and sold in local markets to a system in which food is transported great distances and marketed through large chains of supermarkets. Despite the international agreement, which provides a framework for the import health standards required for traded animal- and plant-based products, the expansion of trade in fresh and processed primary produce has resulted in a number of significant biosecurity breaches in recent years. The most recent examples are the 2001 foot-and-mouth disease (FMD) outbreak in the UK, which probably occurred as a result of feeding contaminated animal protein to livestock, and the occurrence of bovine spongiform encephalopathy (BSE) in Europe following the export of BSE-containing feed from the UK. BSE then spread to North America following the export of young animals infected with BSE that were later rendered and entered the North American animal feed chain.[3]

Owing to the growth in international trade, ever-increasing quantities of genetic material and animal and plant products are regularly transported across the world. This has contributed to the spread of some diseases, including those caused by foodborne bacteria, viruses and parasitic agents, to humans (Seimenis, 2008). Although international regulations have been developed by the OIE (World Organisation for Animal Health; see later section of this chapter)[4] and other organizations through the SPS agreement and the Codex Alimentarius to reduce the risk of trading contaminated products, the degree of implementation of relevant regulations, and the extent of inspection and enforcement, varies from country to country, and sometimes within countries (Arambulo, 2008; Pires *et al.*, 2009).

Factors Affecting the Changing Pattern of Foodborne Diseases

Increased surveillance and reporting

In industrialized countries, the systems for reporting foodborne disease outbreaks have become more sophisticated over the past few decades. This, along with better pathogen detection methods and the ability to trace the origin of infections to specific food products, has resulted in more awareness of food safety (Cooke, 1990; Hartung, 2008). Mild disease and sporadic cases of foodborne infection probably still go unreported, but better public education and

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[3] http://www.inspection.gc.ca/english/anima/heasan/disemala/bseesb/ab2003/evale.shtml (accessed 17 May 2010).

[4] http://www.oie.int/eng/OIE/en_about.htm?e1d1 (accessed 17 May 2010).

greater media engagement in communicating potential food hazards and food recalls have contributed to enhanced reporting of many foodborne diseases. Computer-based databases such as FoodNet and PulseNet in the USA, Enter-Net for *Salmonella* spp. and *Escherichia coli* O157:H7 in Europe, and WHO-Global Salm-Surv are now available to assess the trends and changes, and help to predict numbers and types of infection using current and historical data. These and other surveillance networks, as well as the emergence of disease modelling, show potential for the future prediction of and early intervention for better control of foodborne zoonoses (Singer *et al.*, 2007).

Changes in food manufacturing and agricultural processes

Food safety should be examined from the farm setting, where the primary produce is grown, right through to the processing, handling and preparation of food by the consumer (Beuchat and Ryu, 1997). Before food reaches the consumer, contamination can occur at any stage of the food production chain – from production and processing through to packaging. Modern food-processing facilities are typically large and centralized compared with the traditional small family-run units where food was usually sold locally. In many countries there has been a drive, usually due to economic factors, to consolidate the food-producing sector (Howard, 2009). Technological changes in the food-manufacturing industry have enabled producers to maximize output, but although some risks have been minimized with modern processing methods (Galligan and Kanara, 2008), bulk production does also have a downside. For example, owing to the need to preserve food for wider distribution over long distances, appropriate conditions during transport and safe handling of food stuffs have become especially important (Siegford *et al.*, 2008; Burton, 2009). A number of regulations have been enforced by different countries to ensure that good handling practice is maintained by food producers and manufacturers, but compliance can vary from country to country and from company to company. Because of the rise of transnational food corporations and wide-scale distribution between and within countries, international agreements have been developed to ensure that minimum standards are set and enforced. The increase in the trade of fresh produce and the minimal treatment processes required for some 'natural' and organic-based products has also led to an increase in some foodborne diseases, especially in cases where the consumer fails to wash the product well, cross-contaminates foodstuffs during preparation and/or fails to cook food properly before eating it. Raw fruits and vegetables have increasingly been identified as a source of foodborne infections (Beuchat, 2002; Noah, 2009). In order to obtain raw fruit and vegetables out of season, as is now common in many countries, produce is transported many thousands of miles from growing areas to various suppliers. As a result of this trend, outbreaks of foodborne diseases can occur in a number of regions at once. These outbreaks often go unrecognized, but a few key examples involving *Listeria monocytogenes* are provided later in this chapter. Other examples involving protozoal pathogens are discussed in Chapter 2.

Changes in consumer habits

With the rise in the number of middle-class families in many parts of the world, people are eating more meals outside the home and this has, in some cases, been associated with an increase in some foodborne diseases, especially where food-handling practices and hygiene in fast-food outlets and restaurants are not well regulated. The increased consumption of raw, chilled and fresh produce has also resulted in a rise in some foodborne diseases, especially where consumers are not well informed about food-safety risks associated with unwashed fresh produce that may have been imported from parts of the world where unfamiliar diseases are common. For example, many parasitic diseases have the intermediate stages of their life cycles in or on vegetation. The emergence of aquaculture, especially of shellfish, as a key export industry in Asia and other parts of the world (Langan, 2008) also raises concerns about importing produce contaminated by organisms that may not be detected by standard inspection practices. Organic produce and other produce, such as fresh fruit juices, may also harbour potential pathogens and these have been associated with a number of cases of foodborne disease in consumers who are often unaware of the potential risks (Burnett and Beuchat, 2001; Cooper *et al.*, 2007). The growing popularity of organic food and the associated public-health implications are considered in a later section of this chapter.

Increased travel and increased cultural diversity in many large towns has also resulted in the import and consumption of foods not normally consumed and this can lead to improper food preparation on the part of the person not familiar with the food-safety risks associated with that food (Ramos *et al.*, 2008). International vacations have also led to the exposure of travellers to foodborne pathogens to which they have little or no immunity; some of these organisms may inadvertently be carried back to their home country where sick or recovered travellers can still spread the organism, often without knowing it (Johnston *et al.*, 2009).

Increased 'at risk' population (immunocompromised, elderly, etc.)

In some countries, a changing demographic profile with an ageing population has led to an increase in the number of elderly people, many of whom have pre-existing health problems. These people, especially those in residential nursing homes or hospitals, are more susceptible to foodborne diseases. Along with the growth in the elderly population, better medical care has also resulted in improved life expectancy for vulnerable members of the population, such as infants, pregnant women and the immunocompromised. Professional health-care workers need to be well informed about the risks associated with certain food products and to ensure that appropriate food-preparation practices are followed (Mainzer, 2008). In cases where individuals are likely to be more susceptible to foodborne diseases, it is recommended that they avoid higher-risk foodstuffs such as chilled ready-to-eat (RTE) meals, delicatessen meats and salads, soft unripened cheeses, raw meat, raw eggs and raw fish.

Patients with acquired immune deficiency syndrome (AIDS), those undergoing chemotherapy and those on immunosuppressive treatment following organ transplant are especially susceptible to infectious disease, including foodborne disease, so those responsible for the selection and preparation of food for these people must take this into consideration, i.e. ensure that fresh foods are well washed and/or cooked properly.

Improved detection methods and tracking of pathogens

Enhanced pathogen-detection methods, especially molecular techniques, have increased our ability to detect and trace pathogens associated with foodborne diseases. This has led to better reporting and a greater ability to implement food recalls and to raise the alert once a food source associated with a disease outbreak has been identified. Biosensors, immunoassays and PCR techniques are very sensitive and can be used to screen batches of products before sale as part of food-safety monitoring programmes. As new technologies have become available, current and emerging diseases have been more readily detected, thus allowing better reporting and more effective intervention in disease outbreaks. New molecular tools have also facilitated studies towards a better understanding of disease ecology (Chandra *et al.*, 2008; Galligan and Kanara, 2008).

Emerging pathogens with improved survivability

In the past decade, a number of new foodborne pathogens have been identified. Some of these have been discovered as a result of better detection methods and others represent the development of more virulent strains of pathogens such as bacteria, e.g. *E. coli* O157:H7. These new variants may have arisen as a result of selection pressure associated with livestock husbandry practices such as large-scale intensive production systems and the use of antibiotics as growth promoters. However, unequivocal evidence to support this hypothesis has, thus far, been lacking. Although antibiotic resistance has been reported in a wide range of enteric bacteria, including *E. coli*, *Salmonella enterica* and *Campylobacter* spp., it is not known to what extent this is a result of natural selection versus a consequence of antibiotic use in both the human and animal population, and subsequent environmental contamination.

As discussed in Chapter 2, it is clear that many foodborne infections in industrialized nations, especially viral infections such as norovirus, reflect human-to-human transmission, with animals having a minor or insignificant role. However, other foodborne diseases such as listeriosis, *E. coli* O157:H7 and many outbreaks of campylobacteriosis have proven links to livestock production and food preparation practices. Owing to the similarity of the clinical signs associated with a range of food- and waterborne diseases in the human population, the causative agent in many cases remains unconfirmed. In developing nations, emerging diseases such as Nipah virus have the potential to be foodborne and have probably infected humans as a result of pressure on

wildlife habitats, with subsequent spillover of the pathogen from its natural host (e.g. bats) into livestock (e.g. pigs) and the human population (through the consumption of palm sap contaminated with bat faeces, or contact with pigs). Other new and emerging diseases, such as severe acute respiratory syndrome (SARS), may be inadvertently spread across the world as a result of the increasing, and generally illegal, trade in bushmeat and animal-based medicinal compounds, as well as being spread by humans as a result of international air travel. These isolated reports of emerging zoonotic pathogens that may be foodborne may reflect rare events, but they may also act as early indicators of diseases that may be exported around the world where biosecurity measures, and food safety and inspection standards are not enforced.

Ensuring the Safety of the Food Supply

Surveillance of foodborne disease outbreaks

Surveillance plays an important role in the early detection of foodborne diseases and their control. Early identification of the source of a disease outbreak is becoming increasingly important as commodities are traded efficiently in high volumes internationally. Increased mass production means that disease outbreaks have the potential to affect hundreds of people in multiple countries. Examples of large foodborne disease outbreaks include over 168,000 cases of salmonellosis in the UK in 1985, 224,000 cases of salmonellosis in the USA in 1993, >310,000 cases of hepatitis A in China in 1988, >3050 cases of Norwalk-like virus in Australia in 1991 and >6000 cases of *E. coli* O157 infection in Japan in 1996 (Kaferstein *et al.*, 1997). Good surveillance and reporting can help to identify the source of a problem early and so prevent spread; for example, in a 1993 outbreak of listeriosis in France, which was caused by a potted pork product and involved 39 cases, eight miscarriages and one death, the public-health authorities traced its source within a week, recalled the product and reduced further cases (Kaferstein *et al.*, 1997). Early detection of disease outbreaks should also help to minimize the associated costs, both in terms of human disease and public health dollars.

International reporting on foodborne diseases is becoming more important as it facilitates an early and effective response to emerging foodborne disease risks and can prevent disease spread (Berlingieri *et al.*, 2007). Epidemiological data from foodborne disease outbreaks can provide public-health authorities with important information about the types of food implicated in outbreaks, as well as about the populations at risk, practices that lead to food contamination, and the growth and survival of foodborne pathogens; they can also provide an early warning for the development of new and emerging pathogens. In industrialized nations, effective disease reporting has demonstrated the potential role of food handlers in spreading infectious disease. Disease databases indicate a growing role of bacterial pathogens such as *Campylobacter, Salmonella* and *E. coli* O157 versus chemicals and other hazards in food as a primary cause of serious disease outbreaks resulting in high morbidity and mortality.

Surveillance systems are set up to collect, collate, analyse and report surveillance data from animal-health and food systems so that action can be taken based on the findings (Thacker, 2000; Salman, 2003). Some surveillance systems, such as reportable disease systems, rely on timely and accurate reporting by health workers and/or producers (in animal health) when a disease or event occurs. Other surveillance tools mine existing data systems, for example diagnostic laboratories, for changes in health outcomes (Marvin *et al.*, 2009). Some surveillance systems use sentinel cases or syndromic trends for early warning of foreign or emerging disease. In animal-health systems, one objective is to look at animal health/disease or events of public-heath significance preharvest (Davies *et al.*, 2007),[5] potentially allowing more intervention/mitigation options. Other surveillance systems function further along the food production continuum, i.e. at the abattoir or processing plant. Knowledge of the epidemiology of the organisms causing foodborne disease is required to optimize the use of surveillance dollars by targeting the surveillance correctly. Some food-safety surveillance systems are hazard focused and collate reports of non-compliance or problems in food-safety audits (Marvin *et al.*, 2009).

As a complement to better reporting of surveillance data, the growing acceptance of the Hazard Analysis and Critical Control Point (HACCP)[6] system has resulted in a reduction in the number of foodborne disease outbreaks associated with certain manufacturing and preparation processes. Application of HACCP to food preparation permits the identification of practices that may be potentially hazardous and allows manufacturers and food safety inspectors to suggest modifications. In addition, it also allows food technologists and food inspectors to identify which practices within the manufacturing process are critical for ensuring the safety of foods, and therefore to determine the critical control points that require specific monitoring. The first principle of HACCP is to conduct a hazard analysis; this requires epidemiological data on specific foodborne diseases and allows an appraisal of the risk of those pathogens being present at the start of, and during, the food processing and preparation process. The production or persistence of toxins, antibiotics or physical agents in foods is also considered.

Risk assessment and international food standards

Microbiological risk assessment can be used to provide an estimate of the probability of a specific pathogen or hazard being present in a given commodity as well as the likelihood of disease arising from the presence of a given amount of a specific pathogen or hazard in a specific population (Fosse *et al.*, 2008a,b; Hallman, 2008). It can also be used to identify high-risk foods or

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[5] http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/afs10440 (accessed 24 November 2009).
[6] http://www.fao.org/DOCREP/005/Y1579E/y1579e03.htm#TopOfPage (accessed 20 May 2010).

processing methods (Swaminathan and Gerner-Smidt, 2007). However, although risk assessment is a useful tool, the conclusions derived from the risk-assessment process must be viewed in context, as the assessment is usually qualitative. This is because there is often insufficient data available to produce a quantitative measure of the risks associated with a specific commodity. Essentially, risk assessment is a structured and objective process comprising four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. Risk assessment, risk management and risk communication together constitute risk analysis. Risk analysis has a wide range of applications in food safety, ranging from informing national and international food-safety policies to the implementation of specific sanitation measures to achieve pathogen reduction at certain points in the farm-to-fork (table) continuum. The process is well described in a number of publications (Murray, 2002; Mumford and Kihm, 2006; Ross, 2008; Wooldridge, 2008).

In brief, risk assessment is defined as a scientifically based process that has the following steps:

1. Hazard identification (i.e. the identification of biological, chemical and physical agents present in a particular food or group of foods that can cause illness).
2. Hazard characterization (i.e. the qualitative or quantitative evaluation of the nature of the illness associated with biological, chemical and physical agents that may be present in food).
3. Exposure assessment (i.e. the qualitative or quantitative evaluation of the likely intake of the hazard).
4. Risk characterization (i.e. the qualitative or quantitative estimation, including uncertainties, of the probability and severity of known impacts in a given population on the basis of hazard identification, characterization and exposure assessment).

In many, if not most, cases there is not enough data to undertake quantitative risk assessments of biological hazards.

In a broader context, risk analysis that includes risk assessment, risk management and risk communication is used by regulatory authorities to prepare for and manage 'risk'. In 2003, the US Department of Agriculture (USDA), in collaboration with the Food Safety Inspection Service of the USDA and the (US) Centers for Disease Control and Prevention (CDC), released the results of a risk assessment to predict the risk of listeriosis from different food types (Swaminathan and Gerner-Smidt, 2007). As a result of regulations implemented over the past decade to prevent *Listeria* in food commodities for sale to the public, the incidence of listeriosis in the USA has declined, with reported cases reduced by 40% between 1996 and 1998.

International agreements on food standards

Increased trade opportunities following the Uruguay Round of multilateral trade negotiations in 1986–1994, and the increased liberalization of trade,

caused concern within nations over the safety of imported food, and high-lighted the need to develop transparent regulations to ensure that traded raw and processed products would be produced to the same standards as products in the country importing these products. This is relevant to hazards with the potential to affect human health as well as to chemical toxins, genetically mod-ified agents and invasive microorganisms and pests that might have an effect on animal and plant health. The WTO emerged as an entity in 1995 (after the Uruguay Round) along with guidelines for the Application of Sanitary and Phytosanitary (SPS) measures designed to address concerns about the assess-ment and control of hazards in imported products. Along with the SPS mea-sures, food safety issues are specifically addressed by the Codex Alimentarius Commission, which has standards, guidelines and recommendations to ensure food safety in traded products. The purpose of these standards is to facilitate trade among WTO members while ensuring that quality and safety standards are met.

The increased volume of global food trade also highlights the need for consistent and effective surveillance and reporting to facilitate effective risk assessment. In this regard, Article 5 of the SPS agreement explicitly requires WTO members to prepare, or refer to, scientific and consistent risk assess-ments. In addition, the World Health Organization (WHO) has recommended that the application of the HACCP system at every stage of the food chain represents an effective approach for governments to meet the terms outlined in the agreement.

Over 60% of known human infectious diseases have their source in ani-mals (whether domestic or wild), as do 75% of emerging human diseases and 80% of the pathogens that could potentially be used in bioterrorism. At the global level, the OIE has modernized its worldwide information system on animal diseases (including zoonoses) with the creation of World Animal Health Information Service (WAHIS), a mechanism whereby all countries are linked online to a central server that collects all the compulsory notifications sent to the OIE, which covers 100 priority terrestrial and aquatic animal dis-eases. The WHO has adopted the International Health Regulations, placing new obligations on its members. The OIE, WHO and Food and Agriculture Organization (FAO) have created GLEWS, the Global Early Warning System, a platform shared by the three organizations to improve early warning on ani-mal diseases and zoonoses worldwide.

The World Organisation for Animal Health (OIE)

The need to fight animal diseases at global level led to the creation of the Office International des Epizooties (OIE) through an international agree-ment signed on 25 January 1924. In May 2003, the OIE became the World Organisation for Animal Health, but kept its historical acronym, OIE. The OIE is the intergovernmental organization responsible for improving animal health worldwide. It is recognized as a reference organization by the WTO and, as of April 2009, had a total of 174 Member Countries and Territories.

The OIE maintains permanent relations with 36 other international and regional organizations and has regional and subregional offices on every continent.

OIE's claimed mission is to:

- guarantee the transparency of animal disease status worldwide;
- collect, analyse and disseminate veterinary scientific information;
- provide expertise and promote international solidarity for the control of animal disease; and
- guarantee the sanitary safety of world trade by developing sanitary rules for international trade in animals and animal products.

Codex Alimentarius

The Codex Alimentarius Commission was created in 1963 by FAO and WHO to develop food standards, guidelines and related texts such as codes of practice under the Joint FAO/WHO Food Standards Programme. The main purposes of this Programme are protecting health of the consumers, ensuring fair trade practices in the food trade, and promoting the coordination of all food standards work undertaken by international governmental and non-governmental organizations (Dawson, 1995; Droppers, 2006; Slorach, 2006).

The WTO agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) considers that WTO members that apply the Codex Alimentarius Standards meet their obligations under this agreement. Scientifically based risk assessment plays an important role in the setting of Codex standards. Epidemiological data on foodborne diseases is important for the development of these risk assessments. One example is an assessment of the risk of contracting listeriosis following the consumption of products that may contain varying amounts of *L. monocytogenes*. A joint study conducted by the USDA and the US Food and Drug Administration (FDA) identified foods as being of high, medium and low risk with respect to harbouring *L. monocytogenes* (Swaminathan and Gerner-Smidt, 2007). High-risk foods include delicatessen meats, high-fat dairy products, soft unripened cheese and unpasteurized fluid milk. Medium-risk products include pasteurized milk, fresh soft cheeses, RTE meals, salami, fruit and vegetables. To reduce the risk of products harbouring *L. monocytogenes*, good food production standards such as HACCP have also been implemented. The Codex Alimentarius Commission adopts standards, codes of practices and other related texts that are prepared by specialized Codex Committees and ad hoc Task Forces.

Organic food production

There remains a fair bit of controversy about the benefits and risks associated with organic food production, especially in relation to food safety and food quality. A well-managed organic farm can provide many environmental benefits

and has socio-economic value, especially when catering for small-scale niche markets (Hovi *et al.*, 2003; Lund, 2006; Dangour *et al.*, 2009). Some studies have demonstrated a lower prevalence of antimicrobial resistance in bacteria isolated from animals on organic farms compared with those on conventional farms (Schwaiger *et al.*, 2008) although this is not the case in all studies. Large-scale processing of products for international and national distribution has to comply with the same basic food safety standards as conventional produce. Production standards, however, are usually set by national and international certification bodies that outline what husbandry and veterinary intervention practices are required and/or allowed, as well as the production standards for growing, storage, processing, packaging and shipping.

In Europe, the organic sector is quite well developed (Vaarst *et al.*, 2005, 2006; Sundrum *et al.*, 2006, 2007; Sundrum, 2008)with an active research programme underway to examine new approaches to raising livestock with minimal use of chemicals such as anthelmintics (Deane *et al.*, 2002; Keatinge, 2005; Maurer *et al.*, 2007) and antibiotics (Schwaiger *et al.*, 2008). European organic legislation is regularly revised.[7] Guidelines on Organic Standards in the USA are provided by the USDA, with information on sustainable livestock production systems available through the Alternative Farming Systems Information Centre,[8] and the National Organic Program.[9] Owing to the evolving nature of organic regulations hard-copy literature is frequently out of date, so agencies tend to rely on the Internet and news bulletins to update producers. In most countries, organic certification remains the responsibility of government or specialized agencies.

Disease and the Globalized Food Supply: Case Studies

Globalization has changed the face of disease outbreaks and how we respond to them. The globalization of legal and illegal trade in animals, animal parts and food has led to large-scale disease outbreaks that have thwarted historical approaches to disease control. These may also have significant public-health effects. The large centralized food-processing facilities that have emerged in recent years have been associated with a number of significant foodborne disease outbreaks, some of which have had impact on a number of different countries owing to the widespread trade of processed foods and animal feed.

Foot-and-mouth disease in the UK: a new era in disease control

In 2001, the UK was besieged by an outbreak of foot-and-mouth disease (FMD), which resulted in extremely high disease control costs, required extensive efforts for the reopening of trade markets, and had a number of

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[7] http://ec.europa.eu/agriculture/organic/splash_en (accessed 17 May 2010).
[8] http://www.sare.org/publications/organic/resource.htm (accessed 17 May 2010).
[9] http://www.ams.usda.gov/AMSv1.0/nop (accessed 17 May 2010).

direct and indirect impacts on human and animal health and welfare. In previous outbreaks involving exotic diseases such as FMD, the implementation of response policies that advocated containment and cullings had proved effective in the UK. In this case, though, it proved difficult to control the outbreak owing to the widespread undetected dissemination of FMD virus throughout the country before the disease was recognized and/or reported (Scudamore and Harris, 2002). Although the source of the outbreak is not known for sure, it is likely to have been introduced into the UK via FMD-infected meat or other animal products. As a result of the outbreak, large numbers of animals were culled amid significant public outcry.

FMD is considered to be a minor zoonosis because human infection is rare and usually mild. However, the psychosocial and economic effects of the outbreak caused significant stress and distress in the agricultural community and beyond (Mort *et al.*, 2005). Fear of a new disaster and loss of trust in the government authorities figured prominently (Mort *et al.*, 2005). Since 2001, there has been a considerable amount of research done, both within the UK and at an international level, to address questions raised during and after the UK outbreak. This has facilitated better preparedness planning for future outbreaks using a range of different strategies, including the judicious use of vaccination, improved vaccine options, enhanced reporting and surveillance systems, and changes to the veterinary infrastructure (Cottam *et al.*, 2006; Green *et al.*, 2006; McLaws *et al.*, 2006; Bessell *et al.*, 2008; Schley *et al.*, 2009; Tildesley *et al.*, 2009).

Listeriosis – control, risk assessment and regulations

Systemic listeriosis is a serious, but usually sporadic, invasive disease that primarily afflicts pregnant women, neonates and immunocompromised individuals. The causative agent is *L. monocytogenes*, which is primarily transmitted to humans through contaminated foods. Outbreaks of listeriosis are usually spread by the faecal–oral route, resulting in self-limiting gastroenteritis in healthy humans, and have been reported in North America, Europe and Australasia. Soft cheeses made from raw milk and RTE chilled delicatessen meats are high-risk foods for susceptible individuals. The infectious dose of *L. monocytogenes* is not known (Swaminathan and Gerner-Smidt, 2007). Efforts by food processors and food regulatory agencies to control *L. monocytogenes* in food products have been successful in many countries, but sporadic outbreaks, often with severe consequences, continue to occur.

Although only a small percentage of the listeriosis cases reported are traced to a common source, public-health officials place a high priority on investigating the outbreaks for the following reasons:

1. Listeriosis is a serious disease and has a relatively high mortality rate in some cases, especially in individuals with compromised immune systems (e.g. immunodeficiency disorders, the elderly, neonates and pregnant women).
2. Morbidity and mortality can be reduced by prompt action by public-health agencies, i.e. trace back and recall implicated food, etc.

Table 1.1. Gastrointestinal disease outbreaks caused by listeriosis (1993–2001) (adapted from Swaminathan and Gerner-Smidt, 2007).

Location	Year	Food involved	Serotype	No. cases reported
Italy (northern)	1993	Rice salad	1/2b	18
USA (Illinois)	1994	Chocolate milk	1/2b	44
Italy (northern)	1997	Cold maize and tuna salad	4b	1566
Finland	1998	Cold smoked fish	1/2a	–
New Zealand	2000	Ready-to-eat (RTE) meats	1/2	32
USA (California)	2001	RTE meat – turkey	1/2a	16
Sweden	2001	Raw milk/cheese	1/2a	48
Japan	2001	Cheese	1/2b	38

3. Outbreak investigation can help to identify key risk factors and prevent future cases.

During the past decade there have been several significant outbreaks of listeriosis reported worldwide (see Table 1.1).

The availability and use of molecular typing plays an important role in the early recognition of listeriosis and in tracing the source of contamination. It is especially useful to group cases that may occur in geographically distinct regions, or where cases occur in several states or countries. This is discussed further in Chapter 2.

Our knowledge of listeriosis has increased significantly over the past decade. This has been achieved largely by applying risk assessment to food processing and implementing the required sanitation at key intervention points. However, *L. monocytogenes*, cannot be entirely eliminated as it occurs naturally in the environment. Education of consumers and international cooperation in ensuring that food standards are met is important to control both this and other foodborne diseases.

Salmonellae and antimicrobial resistance

Salmonellae have been associated with a number of foodborne diseases linked to traded animal products. Aside from the immediate food-safety issues, there is also significant concern about the growing presence of antibiotic resistance in *S. enterica* isolates (Oloya *et al.*, 2009). Determination and characterization of *Salmonella* spp. isolated from domestic animals and humans in North Dakota, USA was performed to assess their potential role in transferring antimicrobial resistance (AMR) to humans. The National Antimicrobial Resistance Monitoring Systems panel was used to compare AMR profiles of animal and human isolates to assess a possible role of domestic animals in the transfer of AMR to humans. The panel found that *Salmonella enterica* serovar Typhimurium was the predominant serotype in both humans (13.4%) and

domestic animals (34.3%), followed by *S. enterica* serovar Newport in animals (2.6%) and humans (3.9%). *Salmonella arizona* (0.7%), *S. enterica* serovar Give (0.9%) and *S. enterica* serovar Muenster (3.5%) were isolated from sick or dead animals; the AMR levels were generally higher in isolates from animals than humans. However, the genes involved in AMR in animal isolates may not reflect those found in human isolates; therefore, there is a need to assess genotype and phenotype before source attribution can be confirmed (Hopkins *et al.*, 2007). This topic is discussed further with respect to trade in Chapter 2.

Conclusion

Many of the diseases that have grown in importance in human medicine in recent decades are transmitted via the food and water supply. As the world has moved from a situation in which the majority of food was produced locally and sold in local markets to a system in which food is transported great distances and marketed through large chains of supermarkets, the pathogen profile to which humans are exposed has expanded. Despite the international SPS agreement that provides a framework for the import health standards required for traded animal- and plant-based products (Domenech *et al.*, 2006), the expansion of trade in fresh, chilled and processed produce has resulted in a number of significant biosecurity breaches in recent years. Current SPS requirements emphasize the importance of science-based risk assessment and hazard control programmes for the continued reduction of pathogens at relevant points of the 'farm-to-fork' food production chain. This approach, along with good consumer education about food preparation and handling practices is likely to be the best approach for reducing risks to human health in the modern globalized society.

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2 Epidemiology of Pathogens in the Food Supply

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Introduction

The World Health Organization (WHO) estimates that 1.8 million people in the developing world die each year from complications associated with diarrhoea (WHO, 2010).[1] Many of these deaths are caused by pathogens transmitted to humans in food and water supplies. Common sources of food-borne infections are directly contaminated food products and foods contaminated by environmental sources, including water (Gajadhar *et al.*, 2006). Although many of these infections are typically caused by pathogens transmitted from human to human, others also occur in animals and potentially have a zoonotic origin (see Table 2.1 and associated references). To develop appropriate interventions it is important to understand the ecology of these diseases and to be able to attribute significant disease outbreaks to specific sources. This not only requires a sound grasp of epidemiology and the surveillance tools used to map infections, but also a good knowledge of pathogen biology and the behaviour and susceptibility of the populations at risk (Pires *et al.*, 2009). As urban areas expand and previously protected habitats are developed, new and re-emerging diseases, many of which are zoonotic in origin, continue to be reported. This has brought about a recognition that human and animal health agencies need to work together to develop robust and timely disease detection, reporting and prevention policies (King *et al.*, 2004; Gray and Kayali, 2009).

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[1] http://www.who.int/water_sanitation_health/publications/facts2004/en/ (accessed 23 February 2010).

Patterns and Trends in Enteric Infections

The burden of foodborne disease, even in industrialized countries, remains substantial. It is estimated that 76 million cases of foodborne disease occur each year in the USA, with 325,000 hospitalizations and 5000 deaths linked to foodborne and waterborne diseases each year (CDC, 2010).[2] Among the known zoonotic foodborne pathogens, a few that are reported (i.e. *Escherichia coli* O157, *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*) tend to dominate the food safety literature. This trend may reflect the investment provided to develop reliable detection methods and surveillance systems for these pathogens, thereby ensuring timely reporting and intervention. Other agents that cause enteric disease have probably remained under-detected, e.g. emerging protozoal diseases such as caused by *Cyclospora* spp. (Mead *et al.*, 1999).

In addition to the emergence or recognition of new enteric pathogens, the globalization of the food supply, along with modern processing methods, the availability of imported foodstuffs to a wide range of consumers and a growing preference for fresh and ready-to-eat produce, has resulted in widely dispersed outbreaks of some foodborne diseases (e.g. listeriosis, salmonellosis, some protozoal infections) (Kaferstein *et al.*, 1997; Kobrin, 2008; Oloya *et al.*, 2009). Other trends that have been noted include an increase in the detection and reporting of antimicrobial resistance (Hopkins *et al.*, 2007) and enhanced identification of pathogens that are highly opportunistic (i.e. affecting only the most high-risk subpopulations). New pathogens, or new variants of well-known organisms, can emerge as a public-health problem as a result of natural selection pressure or changes in consumer preferences and food production technologies (i.e. increased availability of fresh chilled food products) that connect a potential pathogen with the food chain (e.g. *L. monocytogenes*) (Little *et al.*, 2003; Bhunia, 2008a).

A selection of some pathogens reported to cause enteric disease in humans is provided in Table 2.1. Although this list is not comprehensive, it provides an overview of the wide variety of agents that can be associated with outbreaks of foodborne diseases in humans. Some of these will be considered in more detail later in this chapter.

The impact of specific pathogens on human health will depend on the virulence of the pathogen and the susceptibility of the host, as well as on the level of exposure, presence of co-infections and host immune status. The epidemiological picture depends on a wide range of other factors, including the predominant method of disease transmission, the requirement, or not, for disease vectors or intermediate hosts, the role of reservoir hosts, the demographics of the human population that is exposed and whether or not the pathogen is an opportunistic or an obligate pathogen.

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[2] http://www.cdc.gov/ncidod/dbmd/diseaseinfo/foodborneinfections_g.htm (accessed 23 February 2010).

Table 2.1. Pathogens transmitted by food and water supplies that may have an animal source.

Agent	Food/water source most commonly implicated	Common symptoms in humans
Viruses		
Enteroviruses	Contaminated water	Gastroenteritis and systemic signs
Adenoviruses[a]	Contaminated water	Gastroenteritis, may have respiratory signs
Hepatitis A[b]	Contaminated water and food, including shellfish	Hepatitis with or without jaundice
Hepatitis E	Contaminated food, including pork and venison	Hepatitis, often without jaundice (Banks *et al.*, 2007; Chandra *et al.*, 2008; Khuroo and Khuroo, 2008)
Astroviruses[c]	Contaminated water and food, including shellfish	Gastroenteritis with watery diarrhoea, vomiting and anorexia
Rotaviruses	Contaminated water and food	Gastroenteritis with vomiting and diarrhoea
Norwalk-like viruses (noroviruses)[b]	Contaminated water and food, including shellfish	Gastroenteritis with vomiting, diarrhoea and abdominal pain
Bacteria		
Escherichia coli (many variants)[b]	Contaminated food, including meats (e.g. beef) and vegetables washed in contaminated water	Gastroenteritis, may have significant systemic signs (Oporto *et al.*, 2008)
Salmonellae[d]	Contaminated food, including meats (e.g. chicken) and vegetables washed in contaminated water	Gastroenteritis, may have fever and other systemic signs (Hopkins *et al.*, 2007; Oloya *et al.*, 2009)
Shigellae[b]	Contaminated food, including salads; primates and humans are the key hosts	Gastroenteritis, often with blood in faeces
Vibrio spp.[b]	Contaminated water and food, including shellfish and salads; primates and humans are the key hosts	Gastroenteritis with vomiting and fever
Campylobacter spp.	Contaminated food, including meats (e.g. chicken)	Gastroenteritis, may have fever and other systemic signs
Listeria monocytogenes	Contaminated food, including shellfish, chilled meats and salads	Gastroenteritis, may have other systemic signs (Walse *et al.*, 2003)
Yersinia enterocolitica	Contaminated food, including meats	Gastroenteritis, may have other systemic signs
Yersinia pseudotuberculosis	Contaminated meat and vegetation	Gastroenteritis, may mimic appendicitis and also have other systemic signs
Mycobacterium avium paratuberculosis	Contaminated milk?	May be associated with irritable bowel disease and Crohn's disease (Cirone *et al.*, 2007; Scanu *et al.*, 2007)

Continued

Table 2.1. *Continued*

Agent	Food/water source most commonly implicated	Common symptoms in humans
Mycobacterium bovis	Contaminated milk	Associated with lymph gland enlargement and localized or systemic signs (Thoen and LoBue, 2007; de Kantor *et al.*, 2008)
Staphylococcus aureus[b]	Food contaminated with toxin	Gastroenteritis, may have other systemic signs
Clostridium perfringens	Insufficiently cooked meat or reheated food	Gastroenteritis, may have other systemic signs
Clostridium difficile	Contaminated ground beef, pork or turkey	Mild gastrointestinal signs associated with toxin (Rupnik, 2007)
Bacillus cereus	Reheated food, especially rice	Gastroenteritis, may have other systemic signs
Coxiella burnetii	Contaminated milk products	Fever, systemic signs such as nausea, headache, myalgia
Brucella spp.	Milk and milk products from infected ruminants	Variable, undulant fever, aches, headache, may become chronic (Mantur and Amarnath, 2008)
Leptospira interogans – various serovars	Humans usually infected via direct contact with urine from infected animals or via a contaminated food or water supply	Variable, fever, jaundice, myalgia, vomiting, abdominal pain, diarrhoea (Monahan *et al.*, 2009)
Protozoa		
Giardia lamblia	Contaminated water	Gastroenteritis, abdominal gas and discomfort (Appelbee *et al.*, 2003; Smith *et al.*, 2007)
Sarcocystis spp.	Raw or undercooked beef	Gastroenteritis, may have other systemic signs (Vercruysse *et al.*, 1989; Pathmanathan and Kan, 1992)
Toxoplasma gondii	Raw or undercooked meat (pork, sheep, goat)	Gastroenteritis (Dubey *et al.*, 2002a; Lindsay *et al.*, 2002; Bhopale, 2003)
Entamoeba histolytica[b]	Contaminated food	Gastroenteritis, may develop abscesses and other complications (Stanley, 2003)
Cryptosporidium parvum[b]	Contaminated water and raw fresh produce	Gastroenteritis, diarrhoea (Millar *et al.*, 2002; Graczyk *et al.*, 2003; Smith *et al.*, 2007; Jagai *et al.*, 2009)
Cyclospora	Contaminated water and fresh produce; human host	Gastroenteritis
Nematodes		
Trichinella spp.	Consumption of raw or undercooked meat from infected animals (wild boar, bears, walruses, pigs, horses, etc.)	Variable, hypersensitivity, can be fatal (Pozio *et al.*, 1996; Cui and Wang, 2005; Kaewpitoon *et al.*, 2008; Gottstein *et al.*, 2009)

Continued

Table 2.1. *Continued*

Agent	Food/water source most commonly implicated	Common symptoms in humans
Angiostrongylus spp.	Consumption of raw or undercooked crabs, crayfish, snails, also contaminated fresh produce, etc.	Depends on species of parasite; acute abdominal or cerebral involvement (Lin *et al.*, 2005)
Anisakis spp.	Consumption of raw or undercooked saltwater fish, squid	Epigastric pains, vomiting
Capillaria spp.	Consumption of raw or undercooked fish from fresh or brackish water	Gastrointestinal signs
Trematodes		
Fasciola hepatica	Consumption of plant material contaminated with metacercariae	Variable (Hammami *et al.*, 2007)
Fasciolopsis buski	Consumption of water chestnuts contaminated with metacercariae	Epigastric and hypogastric pain, gastrointestinal signs
Clonorchis sinensis	Consumption of raw or undercooked fish	Variable, cholangitis
Paragonimus	Consumption of freshwater crabs and crayfish	Chest pain, cough and fever (Liu *et al.*, 2008)
Cestodes		
Taenia saginata	Consumption of undercooked beef	Often no signs
Taenia solium	Consumption of undercooked pork	May be no signs or serious systemic involvement with CNS signs (Kyvsgaard *et al.*, 2007)
Echinococcus spp.	Consumption of vegetation washed with water contaminated with dog faeces	Often no signs until cysts are large, serious if cysts rupture (Jenkins *et al.*, 2005)
Diphyllobothrium spp.	Consumption of raw or undercooked freshwater fish	Often no signs (Margono *et al.*, 2007)

[a] Adenoviruses are commonly found in human sewage and have been isolated from both human and animal sources. Disease outbreaks in humans have been reported after consumption of contaminated shellfish (Myrmel *et al.*, 2004). Most adenovirus infections in humans probably result from human-to-human transmission although a zoonotic source cannot be ruled out.

[b] Although these pathogens are predominantly human pathogens that can be transmitted by food and water sources to other humans, new data suggest that some variants may have a potential animal source, especially where non-human primates and other wildlife species are present (Cunningham, 2005; Ekanayake *et al.*, 2006).

[c] Astroviruses are frequently associated with mild cases of gastroenteritis in children and are occasionally isolated from adults. Infections in adults are often associated with the consumption of contaminated shellfish (Le Guyader *et al.*, 2000).

[d] Salmonellae typically cause mild-to-severe gastroenteritis in humans but are also responsible for human typhoid fever (*Salmonella enterica* serovar Typhi) and paratyphoid (*S. enterica* serovar Paratyphi), which are considered to be an important cause of human foodborne infections worldwide. On a global scale it is thought that there are over 16 million cases of typhoid each year, with 1.3 billion cases of enteritis and 3 million deaths attributed to *S. enterica*. Although the majority of these cases are likely to have been transmitted from human to human via contaminated water or food, rodents, other wildlife and domestic animals can also be a source of infection. Specific source attribution of *Salmonella* infections in non-industrialized nations is often difficult owing to the lack of molecular tools and surveillance systems (Gassama-Sow *et al.*, 2006).

Bacterial Foodborne Diseases

A large number of the bacteria that cause enteric disease in humans and animals, as well as those that comprise the normal gut flora, are members of the family Enterobacteriaceae. These are commonly transmitted via contaminated food and water from human sources, or to humans and animals from material contaminated with faeces from other animals (Bhunia, 2008b). The epidemiology of these foodborne pathogens depends on locality, food preparation practices, food preferences, hygiene, access to clean water, level of community education and availability of public health services, as well as on the development and enforcement of food and water safety regulations.

Enteric bacteria commonly transmitted from human to human through food and water sources include *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Vibrio* spp., *Shigella* spp. and *Yersinia* spp. (Cooke, 1990; Bhunia, 2008a). Human infection with other enteric bacteria such as *Campylobacter* is most often associated with specific foodstuffs such as chicken meat, but may also be transmitted to humans from wildlife and other domestic animals. Other agents, such as *L. monocytogenes*, are ubiquitous in the environment but can be present in animals and can contaminate vegetation and meats as well as shellfish. Many of the enteric diseases transmitted to humans via shellfish are not typically considered to be zoonotic as the associated pathogens are often common in the human population and are likely to originate from a human source. However, as we learn more about the complex ecosystems associated with some aquaculture practices this assumption may need to be revised (Graczyk and Schwab, 2000).

A wide range of other bacteria can cause disease in humans, many of these, such as mycobacteria, *Brucella* spp., *Coxiella burnetii* and leptospirosis, cause occasional sporadic disease in developing countries and are now less commonly reported in the northern hemisphere. In many countries this is the result of targeted disease control and food safety practices, but these organisms may still pose a risk to travellers visiting regions where the diseases associated with these organisms remain endemic.

The bacterial diseases outlined in the following sections have been selected to illustrate the epidemiology of some specific bacterial pathogens in more detail.

Listeria monocytogenes

Listeria is a Gram-positive facultative pathogen that was first isolated from rabbits in 1926. It was initially considered to be primarily an animal pathogen causing 'circling disease' in ruminants, pigs and dogs and cats (Cossart, 2007). In the 1970s, *Listeria* was identified as a foodborne pathogen causing numerous outbreaks of gastroenteritis in North America, with up to 2500 cases recorded a year. In humans, the incubation period in susceptible adults is 3–70 days, with the median incubation period estimated to be 3 weeks.

Listeriosis can be a serious problem in pregnant women, newborns, the elderly, and immunocompromised or debilitated hosts. Pregnant women may experience either a mild, flu-like syndrome with fever, chills, headache, slight dizziness or gastrointestinal signs. This may be followed in a few days to weeks by abortion, stillbirth, premature birth or septicaemia in the newborn. Newborns may be infected either *in utero* or from bacteria found in the vagina during delivery. Infected infants can develop septicaemia, disseminated granulomatosis, respiratory disease or meningitis; symptoms may be present at birth or develop within a few days to several weeks. In elderly, immunocompromised or debilitated persons, *L. monocytogenes* can cause meningitis, meningoencephalitis or, less frequently, septicaemia (López *et al.*, 2006). The organism survives well in chilled food products, including salads, shellfish and soft cheeses kept in the fridge. The disease attracts the attention of both the public and regulatory authorities because there is often a higher mortality rate associated with listeriosis than with most other foodborne bacteria (Rocourt *et al.*, 2003). In the USA, a zero-tolerance policy was introduced to ensure that *L. monocytogenes* was not present in ready-to-eat foods. Internationally, and in the USA, there have been a number of widespread multi-state outbreaks reported. Most of these have been followed up by food recalls. Tainted turkey meat and other delicatessen meats were implicated with one case in 1998–1999 that involved 22 states in the USA, 101 illnesses, 15 deaths and six miscarriages (Swaminathan and Gerner-Smidt, 2007). In 2000–2001 a Mexican type soft cheese made from unpasteurized milk was identified as the source of infection in North Carolina and caused five miscarriages, with 12 other people reported to be sick. In 2008, in Canada, there was a listeriosis outbreak linked to ready-to-eat meats produced at a Maple Leaf food plant in Ontario. Although there are thought to be between 100 and 140 cases of listeriosis reported in Canada each year, there are usually very few deaths attributed to *Listeria*. The 2008 outbreak resulted in 20 deaths across five provinces. The authorities worked effectively with industry partners to control the outbreak, but its extent generated a lot of media interest and caused significant public concern as outlined in the 2008 Public Health Agency of Canada report on the outbreak.[3]

In an attempt to assess the risk of *Listeria* in food, a joint study was conducted by the US Department of Agriculture (USDA) and the US Food and Drug Administration (FDA) which categorized foods as being high, medium and low risk with respect to harbouring *L. monocytogenes*. High-risk foods included delicatessen meats, high-fat dairy products, soft unripened cheese and unpasteurized fluid milk. Medium-risk products included pasteurized milk, fresh soft cheeses, ready-to-eat meals, salami, fruit and vegetables. Low-risk products included cultured milk products, hard cheeses and frozen products. To maximize disease prevention, good food production standards have been implemented by the major food manufacturers to minimize the risk

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[3] http://www.phac-aspc.gc.ca/fs-sa/listeria/2008-lessons-lecons-eng.php (accessed 24 February 2010).

of contamination with pathogens such as *L. monocytogenes* (Ivanek *et al.*, 2004; Swaminathan and Gerner-Smidt, 2007).

Increased awareness of listeriosis in both the food-processing industries and among consumers has helped to reduce some of the risks associated with *L. monocytogenes* in certain food sources, but there remains a need to be proactive in this area. Owing to the ubiquitous nature of the organism and its presence in the environment, it is unlikely that this pathogen will be totally eliminated, but continued consumer education about appropriate food selection and preparation remains an important mechanism by which listeriosis and other foodborne pathogens can be controlled.

Escherichia coli

The majority of *E. coli* isolates from humans and animals are non-pathogenic and exist harmlessly in the intestinal tract. Because of the wide range of non-pathogenic strains of *E. coli* that are present in the environment it is important to be able to distinguish these from potential pathogens. Pathogenic *E. coli* is subdivided into different pathotypes which can then be further classified into virotypes, based on the virulence genes that they possess. A virotype reflects a particular combination of virulence genes. Important virulence factors encoded by these genes include fimbrial adhesins, enterotoxins, cytotoxins, capsule and lipopolysaccharides (LPS). Pathogenic *E. coli* may also be differentiated by serotype based on antigenic differences in the O antigen of the LPS, in the flagellar or H antigens, and in the fimbrial or F antigens. The pathogenic *E. coli* virotypes are usually referred to as follows: enterotoxigenic (ETEC), enteropathogenic (EPEC), verocytotoxigenic (VTEC) (this includes enterohaemorrhagic (EHEC) strains), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adhering (DAEC). Some VTEC strains produce a Shiga-like toxin that kills vero cells *in vitro*; hence, the group is also referred to as STEC (Bell and Kyriakides, 1998).

Pathogenic *E. coli* can cause a variety of diseases in humans, including gastroenteritis, dysentery, haemolytic uraemic syndrome (HUS), urinary tract infections (UTI), septicaemia, pneumonia and meningitis. Several of the verotoxic *E. coli* (VTEC) have been isolated from cases of bloody diarrhoea and haemolytic uraemia in humans (Karmali *et al.*, 2003), and a number of disease outbreaks have recently been recorded in developed countries. The VTEC group includes *E. coli* O157:H7, which has also been isolated from the faeces of healthy cattle, as well as from goats, chickens, sheep, pigs, dogs, cats and seagulls. Foodborne disease outbreaks in humans have been associated with the consumption of contaminated ground beef in the USA, Canada and Europe. Outbreaks have also been traced back to contaminated lettuce and spinach, raw milk, mayonnaise, apple cider, fresh fruit, salami and sprouts, and the general assumption is that the *E. coli* responsible has originated from infected animal faeces. However, although a number of studies have been conducted (Cooley *et al.*, 2007) conclusive evidence for this has not always been provided, as is illustrated in a number of examples discussed in detail by Bell and Kyriakides (1998).

Salmonellae

Salmonellae are Gram-negative, non-spore forming bacilli commonly found in the intestinal tract of birds, reptiles, farm animals, wildlife and humans. Isolates of *Salmonella enterica* can be classified in a number of different ways and are usually represented as *S. enterica* followed by the specific serovar, i.e. *S. enterica* serovar Choleraesuis (Dubansky, 2008). In Europe, foodborne disease outbreaks associated with *Salmonella* spp. have often been attributed to the consumption of poultry and poultry products. *Salmonella enterica* serovar Enteritidis is often implicated although this organism does not generally cause disease in infected poultry. *S.* Enteritidis has the ability to colonize the ovary of the laying hen and can be isolated from the contents of freshly laid eggs (Keller *et al.*, 1995). Eggs stored at room temperature may contain up to 10^9 colony forming units (cfu), which may reflect the level of faecal contamination after laying (Lublin and Sela, 2008). In Europe, there are currently a number of regulations in place to control the level of *S.* Enteritidis in poultry on the farm, i.e. the use of vaccination as well as strict regulations over the processing and packaging of eggs and poultry products to reduce contamination (Korsgaard *et al.*, 2009).

Other Salmonellae of interest include *Salmonella dublin*, which can cause gastroenteritis in humans although it is more typically associated with disease in cattle. *S.* Choleraesuis is associated with disease in pigs, and *Salmonella arizonae* has been isolated from healthy and sick reptiles. As with other bacterial pathogens, when attributing sources for foodborne salmonellosis it is necessary to compare isolates using molecular methods to assess whether or not the isolates from animals or infected humans match with those in the suspect food source. In the past this was not always possible, and there was reliance on classical classification techniques which may not be sufficiently specific to prove a direct causal relationship in an outbreak. In many diagnostic facilities, salmonellae are still classified according to the somatic/outer cell wall (O) and flagella (H) antigens and the capsular (Vi) antigenic patterns. Currently, there are thought to be over 2443 *S. enterica* serotypes with six subspecies: type I (*enterica*), II, IIIa, IIIb, IV and VI (Bhunia, 2008b).

In industrialized countries, human cases of salmonellosis are frequently traced back to the consumption of contaminated meat, milk, poultry and eggs. However, dairy products, including cheese and ice cream, as well as fruit and vegetables contaminated with infected faecal material, or fresh foodstuff that has been washed in contaminated water, have also been implicated. The widespread distribution of foodstuffs from central facilities and the blending of products for sale in supermarkets has possibly led to rapid and widespread distribution of potentially contaminated produce. For example *S. enterica* serovar Typhimurium DT104, which is an emerging pathogen in the USA, Canada and Europe, causes high mortality in cattle and can be transmitted to humans in contaminated beef, especially ground beef. In 2005, there were four cases of multi-drug resistant *S.* Typhimurium DT104 isolated in beef imported into Norway from Poland. This was reported to the Norwegian Institute of Public Health reference laboratory, where the source was traced back to Polish

imported beef. The isolates from the disease outbreak had identical multi-locus VNTR (variable number of tandem repeats) analysis profiles and antibiotic-resistance patterns. This highlights the value of molecular techniques for the characterization of isolates (Lindstedt *et al.*, 2003) for trace back and re-enforces the need to monitor imported and blended products.

Campylobacter spp.

Campylobacter is a member of the Campylobacteriaceae and was first identified as a cause of abortion in sheep in 1913 (Bhunia, 2008c). Since then it has been isolated from a range of animals and is considered to be a significant cause of foodborne disease in humans (Clements, 2009). Examination of reported cases of foodborne disease in humans in the USA indicates that 14.2% have been associated with *Campylobacter* (Vasickova *et al.*, 2005). Between 1998 and 2002, the Centers for Disease Control and Prevention (CDC)[4] reported 61 outbreaks of campylobacteriosis in the USA, with 1440 cases of *Campylobacter*-related illness in humans. The higher number of cases reported in recent times may reflect better isolation methods and enhanced reporting as the microaerophilic nature of the organism meant that it was not routinely detected in samples unless its isolation was specifically requested (Humphrey *et al.*, 2007). This and other factors, such as improvements in awareness, have influenced the prevalence of reported cases of campylobacteriosis in Europe and elsewhere (Gillespie *et al.*, 2009).

Animals are considered to be the main reservoir for *Campylobacter* spp., with isolates obtained from rabbits, birds, sheep, horses, cows, pigs, poultry and domestic pets. Isolates have also been cultured from vegetables and shellfish. Risk assessments have been used to determine the source of infections in humans, and contaminated chicken meat is considered to be the main cause of foodborne *Campylobacter jejuni* infection in humans (Calistri and Giovannini, 2008). *C. jejuni* and *Campylobacter coli* can colonize the caecae of poultry, and contamination of meat often occurs during processing. Currently, campylobacteriosis is more commonly reported in developed rather than developing countries, but this may reflect the priorities and focus of the surveillance systems in place.

Campylobacteriosis has been widely studied in New Zealand, where the high prevalence of infection in humans has been associated with consumption of contaminated poultry meat that has not been properly cooked (Eberhart-Phillips *et al.*, 1997). The New Zealand Food Standards Agency, along with the poultry industry, and university and government research facilities, have done risk assessment and case-controlled studies, and identified key risk mitigation measures. The outcome of this work has been a significant reduction in the number of human cases of campylobacteriosis over the last few years (Lake *et al.*, 2007).

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[4] http://www.cdc.gov/foodnet/factsandfigures.htm (accessed 20 May 2010).

Yersinia enterocolitica

Yersinia spp. are members of the Enterobacteriaceae. The genus has 11 species including *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*. The majority of the others are non-pathogenic except in immunocompromised individuals. All three of these organisms are facultative intracellular pathogens and possess a range of virulence factors. *Y. pseudotuberculosis* and *Y. enterocolitica* have both been implicated as causes of foodborne disease whereas *Y. pestis* is more typically transmitted through direct contact with sick rodents, or via flea bites as in the historic outbreaks of plague also known as the Black Death. Both pneumonic (respiratory form) and bubonic forms of plague still occur, but outbreaks are usually self-limiting or involve only sporadic cases[5] (Bhunia, 2008d).

Yersiniae are common in the environment and can be found in the intestinal tract of healthy and sick birds and mammals (Cork *et al.*, 1995). The CDC indicates that *Y. enterocolitica* is responsible for up to 87,000 cases of gastroenteritis annually, and 90% of these are thought to be foodborne. Between 1988 and 2002 there were eight outbreaks of yersiniosis reported as attributable to *Y. enterocolitica*, involving 87 cases. In humans, *Y. pseudotuberculosis* is more frequently associated with sporadic cases of mesenteric adenitis, and occasionally septicaemia (Cork *et al.*, 1998). The latter is more likely with patients being treated for iron overload and related conditions. Pseudotuberculosis in animals has been reported following consumption of vegetation contaminated by bird faeces (Cork, 1994). *Y. pseudotuberculosis* is less often associated with foodborne disease than *Y. enterocolitica*, although both can be transmitted via contaminated vegetation. Disease transmission for both is often more common in the winter owing to the psychrophilic tendency of Yersiniae.

Y. enterocolitica is classified into five biogroups: 1 (1A and 1B), 2, 3, 4 and 5. This grouping is based on pathogenicity and ecological and geographical distribution. There are about 60 serotypes recognized, with several within each biogroup, e.g. 1A (O:5; O:6,30; O:7,8, etc.). Those predominantly causing disease worldwide are serotypes O:3, O:8, O:9 and O:5,27. Potentially pathogenic strains of *Y. enterocolitica* are found in sewage, environmental sources and a wide range of animal faeces (ruminants, dogs, cats, birds, etc.), but human disease has most frequently been linked to pigs. Although *Y. enterocolitica* is a common commensal present in the pig intestinal tract it can cause disease in pigs, and is emerging as a significant zoonotic pathogen in pigs (De Boer *et al.*, 2008; Truszczynski, 2009). In one European study, it was found that pathogenic strains of *Y. entercolitica* were isolated from 13 (9.3%) of 140 samples of porcine tonsils and from five (3.3%) samples of pig faeces examined (De Boer *et al.*, 2008). Another source of foodborne yersiniosis has been chocolate milk, and also other dairy products, which have been associated with cases of enteritis reported in children. The disease is usually self-limiting, but in a few cases septicaemia and death have been reported. Prevention of

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[5] http://www.who.int/mediacentre/factsheets/fs267/en/ (accessed 26 February 2010).

disease requires pasteurization of dairy products and proper cooking of meat, especially pork. There have been reports of pasteurized products, especially milk, containing *Y. enterocolitica*, but although there are some heat-tolerant strains, most cases have been traced back to contamination of the product at or after packaging (Walker and Gilmour, 1986; Schiemann, 1987; Ackers *et al.*, 2000). Ionizing radiation and other food-preservation methods can also be used to control *Yersinia*.

Q fever: *Coxiella burnetii*

Q fever, or 'query fever', is a zoonotic disease caused by *Coxiella burnetii*, a species of bacteria that is distributed globally although it has not been reported in New Zealand (Frazer and Rooney, 2009). In 1999, Q fever became a notifiable disease in the USA, but reporting is not required in many other countries. Because the disease is under-reported, it is not possible to reliably assess how many cases of Q fever have occurred worldwide (Maurin and Raoult, 1999). Although there are few reliable statistics on the prevalence and incidence of Q fever worldwide, it is estimated that there are 50–60 cases of Q fever reported in the USA each year, and that the average annual reported incidence is 0.28 cases per million persons.[6] Similar statistics are reported by Karakousis *et al.* (2006). Cattle, sheep, and goats are thought to be the primary reservoirs of *C. burnetii*, although infection has also been noted in a wide variety of other animals, including other species of livestock, birds and domesticated pets. *C. burnetii* does not usually cause clinical disease in these animals, although abortion in goats and sheep has been linked to infection with the bacterium. The organisms are excreted in the milk, urine and faeces of infected animals and, during parturition, infected dams may pass high numbers of organisms in the amniotic fluids and the placenta. *C. burnetii* has also been isolated from poultry eggs, although foodborne transmission via this and other routes is considered very rare (Maurin and Raoult, 1999). Organisms of *C. burnetii* are resistant to heat, drying and many common disinfectants, thus enabling the bacteria to survive for long periods in the environment.

The most common route of infection in humans is probably via the inhalation of aerosols containing dried placental material, birth fluids and excreta of infected herd animals. Other potential modes of transmission include ingestion of infected unpasteurized milk and tick bites.[7] However, these routes of infection are uncommon, and human Q fever is primarily an occupational disease of farmers, abattoir workers, veterinarians and laboratory workers (Hartzell *et al.*, 2008).

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[6] http://www.bt.cdc.gov/agent/qfever/clinicians/epidemiology.asp (accessed 3 March 2010).

[7] *C. burnetii* can be maintained in a tick–vertebrate cycle, although the ticks are not necessary for the organism to persist. Some ticks may act as a vector between wild and domestic animals.

Although reported cases are uncommon, humans are considered to be very susceptible to the disease, and very few organisms are required to cause infection. In the majority of cases, the disease is a non-specific flu-like illness with a 1–3 week incubation period, often remaining undiagnosed. In a minority of cases there will be a clinical atypical pneumonia or hepatitis. Should the course of disease become chronic, endocarditis and chronic hepatitis can develop. Chronic Q fever is often fatal, and may be more likely to develop in immunocompromised individuals and pregnant women (Maurin and Raoult, 1999).

Brucellosis: *Brucella melitensis*, etc.

Brucellosis is an infectious disease caused by bacteria in the genus *Brucella*, which include *Brucella melitensis*, *Brucella abortus* and *Brucella suis*. These bacteria are primarily passed among animals and cause clinical disease in a range of vertebrates, including sheep, goats, cattle, deer, elk, pigs, dogs, hares and several other species, including wild animals. Although brucellosis is not commonly reported in developed countries (for example, there are fewer than 100 cases reported a year in the USA, and fewer than 50 in Australia), it can be common in countries where animal disease control programmes have not reduced its prevalence in ruminants.

In humans, brucellosis is a multi-systemic disease with a broad spectrum of clinical presentations. Clinical signs may appear insidiously or abruptly. Typically, brucellosis begins as an acute febrile illness with non-specific flu-like signs such as fever, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats may occur, particularly at night. Splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen. Gastrointestinal signs, including anorexia, nausea, vomiting, diarrhoea and constipation occur frequently in adults, but less often in children. In many patients, the symptoms last for 2–4 weeks and are followed by spontaneous recovery. Other patients develop an intermittent fever and other persistent symptoms that typically undulate at 2–14 day intervals. Most people with this undulant form recover completely in 3–12 months. A few patients become chronically ill. Relapses can occur months after the initial symptoms, even in successfully treated cases (Corbel, 1997; Sauret and Vilissova, 2002).

B. melitensis is considered a food safety concern in Mediterranean regions because it may be present in local cheeses and other dairy products if they have been made from the milk of infected sheep and goats. *B. abortus* is more frequently associated with bovids, and is passed in high numbers in placental material and fluids when associated with abortion. *B. suis* is typically found in pigs as a cause of infertility and abortion. Other *Brucella* spp. have been found to cause disease in humans and animals, but are less commonly reported. Humans typically become infected with *Brucella* spp. by coming into contact with animals or animal products that are contaminated with these bacteria (Ramos *et al.*, 2008; Swai and Schoonman, 2009).

Although brucellosis can be found worldwide, it is more common in countries that do not have standardized and effective public health and domestic animal health programmes. Areas currently listed as high risk are the Mediterranean Basin (Portugal, Spain, Southern France, Italy, Greece, Turkey, North Africa), South and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East. Unpasteurized cheeses, sometimes called 'village cheeses', from these areas may represent a particular risk for tourists, so brucellosis should be considered in the differential diagnoses of returned travellers presenting with a fever (Johnston *et al.*, 2009).

Viral Foodborne Diseases

Viruses cause a wide range of diseases in humans and animals, and may be transmitted by a variety of routes. Although viruses are strict intracellular parasites requiring host cells in which to multiply, some can survive for significant periods of time in the environment, leading to transmission to new susceptible hosts. Many common human viruses are transmitted via water, through either the consumption of contaminated drinking water, the consumption of contaminated shellfish or the ingestion of fresh fruit and vegetables washed in contaminated water (Appleton, 2000). Other viruses, many of which are thought to be zoonotic, are transmitted via infected or contaminated meat (e.g. hepatitis E, some influenza A viruses). The latter can occur if the animal host is slaughtered for human consumption when it is still viraemic, or as a result of contamination of the carcass after slaughter. Examination of reported cases of foodborne disease in humans indicates that 66.6% of food-related diseases in the USA are associated with viruses, compared with 9.7% and 14.2% for *Salmonella* and *Campylobacter*, respectively (Vasickova *et al.*, 2005). The majority of cases are likely to be associated with direct human-to-human transmission of enteric viral agents (i.e. most noroviruses, enteroviruses, hepatitis A, astroviruses, some adenoviruses and rotaviruses). However, there is a growing recognition that animals may also serve as the reservoir for some emerging foodborne viral diseases. For example, some strains of rotavirus, hantavirus, foot-and-mouth disease apthoviruses (López-Sánchez *et al.*, 2003), some flaviviruses, Nipah virus (Looi and Chua, 2007) and others have been detected in food sources, and have the potential to sporadically infect humans via the oral route.

Up until quite recently it was assumed that most viruses were highly host specific, but there are numerous examples of new viral pathogens identified in humans that may have an animal source – for example, the coronavirus responsible for SARS (severe acute respiratory syndrome), hepatitis E, some strains of rotavirus A and some strains of norovirus. All of these viruses have been detected in the faeces of infected animals so it is possible that they may be transmitted to humans via the food chain (WHO, 2008). RNA viruses such as SARS coronavirus, hepatitis E, rotavirus A and noroviruses are known to have a high mutation rate, and many human strains appear to be closely related to those found in mammals, although they may not be identical. The

epidemiology of many of these agents is not fully understood. There is also limited information on the environmental persistence of many of these emerging agents, and more work is required to determine whether current water-treatment methods are able to inactivate all of the potential viral pathogens that might be present (Gannon *et al.*, 2004).

Norovirus

Noroviruses cause the majority of human cases of acute viral gastroenteritis worldwide (Hutson *et al.*, 2004). During 1983–1987 in the USA, noroviruses were responsible for one-fifth of the foodborne disease outbreaks (Cliver, 1997a). They were previously called Norwalk-like viruses and are members of the family Caliciviridae. Noroviruses were first recognized in 1968 as the cause of an outbreak of acute gastroenteritis in an elementary school in Ohio (Adler and Zickl, 1969). Many cases are mild, but many people can be involved, for example, the outbreak of norovirus infection in Queensland, Australia in August 1996 associated with the consumption of oysters (Stafford *et al.*, 1997).

Although most of the reported cases are considered to reflect human-to-human transmission via contaminated food and water sources there have been some recent studies to indicate that some noroviruses have been identified in animals (Hutson *et al.*, 2004). In New Zealand, Wolf *et al.* (2009) studied faecal specimens from sheep and pigs and compared norovirus isolates with those isolated from humans. Samples from animals on New Zealand farms were examined using a multiplex real-time RT-PCR (reverse transcriptase PCR) and norovirus was found in 9% (2/23) of porcine samples and 24% (8/33) of ovine samples. Of the porcine samples, all were genogroup II, and those from ovine samples were genogroup III. Whether or not these cases reflect animal or human-to-animal transmission versus a true animal reservoir for some strains of norovirus is not yet determined.

Enterovirus

Another common cause of foodborne gastroenteritis in humans includes viruses of the genus *Enterovirus*. These are members of the family Picornaviridae and include five major groups: polio virus, group A and B coxsackie viruses, echoviruses and some newer enteroviruses. These are ubiquitous enterically transmitted viruses responsible for a wide spectrum of illnesses in infants and children (Cliver, 2000). The enteroviruses multiply in the intestinal tract and are fairly resistant to environmental factors so may persist on fomites and are readily transmitted via fruit and vegetables that might have been contaminated several days earlier (Koopmans and Duizer, 2004; Cook and Rzezutka, 2006). Shellfish can also be a source of enterovirus (Beuret *et al.*, 2003).

The hepatitis A virus, another picornavirus, is classified as a hepatovirus. It is the only member of this genus and is the cause of significant morbidity

in humans, especially in developing countries; it is a pathogen that can be transmitted by food and water supplies. There are four recognized human genotypes of this virus, with three genotypes naturally infecting non-human primates (Cliver, 1997b; Fiore, 2004). The role of non-human primates in transmission to humans is not clear, although in some parts of the world this may have contributed to the evolution of the virus. The virus has a marked tropism for the liver and causes hepatitis with associated fever, jaundice and malaise. Shellfish and the consumption of food contaminated by food handlers have also been implicated as the cause of several outbreaks in humans (Coelho *et al.*, 2003).

Rotavirus

Rotaviruses are members of the family Reoviridae and demonstrate a large degree of genetic variability. Most clinical cases in humans are caused by group A rotaviruses, but infection with group B and C rotaviruses have also been reported. Within group A there are 3 subtypes and 11 serotypes. Other groups (D–G) have also been reported and some have an animal source. Some cases of rotavirus gastroenteritis in humans have been associated with the consumption of undercooked meat or linked to fresh or cooked food that has been contaminated with infected material (Richards, 2001; Cook *et al.*, 2004). Animal rotaviruses have been detected in drinking water, and researchers have speculated about the role of water in the spread of animal strains to humans and the potential for the emergence of new reassorted strains. Bovine–human reassortment strains have been detected in infants in Bangladesh (Ward *et al.*, 1996) and may possibly have been transmitted from humans to cows or vice versa via faecal contamination of food and/or water.

Hepatitis E

Hepatitis E virus (HEV) has emerged as a significant cause of clinical hepatitis in the developing world (Nicand *et al.*, 2009). HEV was initially classified as a member of the Caliciviridae but has now been reclassified as a hepevirus. It is generally transmitted by the faecal–oral route, with outbreaks reported in tropical and subtropical countries (Yazaki *et al.*, 2003; Tamada *et al.*, 2004). Cases in humans have been associated with the consumption of pork (Banks *et al.*, 2007; Leblanc *et al.*, 2007; Nicand *et al.*, 2009) and deer meat (Vasickova *et al.*, 2005). Both foodborne and direct routes of transmission have been identified. Genetic analysis indicates that hepatitis E strains isolated from humans are closely related to swine hepatitis E virus. In one study, anti-HEV antibodies were detected in 20% of people exposed to infected pig herds. In some countries the growing prevalence of hepatitis E in the human population has prompted the public-health authorities to consider the development of a vaccine (Khuroo and Khuroo, 2008).

Other viruses

There is a wide range of other viruses that can infect humans, many of which are zoonotic. Most of these are primarily transmitted by non-enteric routes, but some have the potential to be transmitted via the faecal–oral route in some cases, e.g. Lassa fever and lymphocytic choriomeningitis. These are both arenaviruses with bat and rodent reservoirs. A potential link between human cases and the consumption of contaminated food has been reported (Acha and Szyfres, 2003). Hantaviruses, members of the family Bunyaviridae, are found in the urine and droppings of infected deer mice, and there have been occasional reports of human infection after eating faecally contaminated food/water (Acha and Szyfres, 2003; Heyman *et al.*, 2009).

The apthovirus that causes foot-and-mouth disease has occasionally caused clinical disease in humans. Several human cases have been reported following the consumption of raw milk and dairy products from infected ruminants (López-Sánchez *et al.*, 2003). There are many other animal viruses that have the potential to cause disease in humans, e.g. tick-borne encephalitis (Kohl *et al.*, 1996; Appleton, 2000), avian influenza viruses, paramyxoviruses (Alexander and Brown, 2000; Alexander, 2006) although other routes of transmission are more common.

Prions

Prion diseases such as bovine spongiform encephalitis (BSE) and chronic wasting disease (CWD) in deer are caused by a transmissible protein and have generated a lot of interest over the past few decades. This topic is discussed in detail in Chapter 11.

Parasitic Foodborne Diseases

It has been estimated that humans harbour about 300 species of parasitic worms and over 70 species of protozoa. Many have coexisted with humans for thousands of years, as evidenced by archaeological records. Many of these parasites are transmitted by food and water sources, but many are not (Doyle, 2003). Parasitic infections are often asymptomatic in humans, but some can cause significant morbidity and can persist, causing chronic ill health (Anantaphruti, 2001).

The epidemiology of parasitic diseases can be complex, with the environmental route of transmission being very important for many protozoan and helminth parasites. The availability of suitable environmental conditions, such as suitable temperature, humidity, food and water sources, and favourable soil and vegetation, are particularly significant. The parasites' biological potential for producing large numbers of infective stages, as well as their environmental robustness (they can survive in moist microclimates for prolonged periods of time), can pose a significant challenge for human- and animal-health authorities.

Increased demands on natural resources, as well as the growing trade in non-traditional fresh food products, has the potential to increase the likelihood of humans encountering environments and fresh produce contaminated with the infective stages of a variety of parasites. Robust, efficient detection, viability assessments and typing methods are required to assess risks and to enhance our understanding of the epidemiology of these emerging parasitic diseases (Anantaphruti, 2001).

There is a wide variety of food products that may be contaminated with one or more species of parasite. The prevalence of specific parasites in food supplies varies between countries and regions. One of the major factors influencing the prevalence of parasitic infections in the human population is the preference for, and traditional popularity of, consuming raw or inadequately cooked foods. The parasites that may be acquired by eating these foods include nematodes, trematodes, cestodes and protozoa (Pozio, 2008). A number of significant zoonoses are associated with the consumption of muscle tissues from infected meat (*Toxoplasma gondii, Sarcocystis hominis, Sarcocystis suishominis, Diphyllobothrium latum, Taenia solium, Taenia saginata, Opisthorchis felineus, Anisakis* spp.) and contaminated food and water supplies (i.e. *Giardia duodenalis, Cryptosporidium* spp., *T. gondii, Echinococcus granulosus sensu latu, Echinococcus multilocularis, T. solium, Taenia multiceps*). The effective control and prevention of the majority of these agents requires a focus on the education of both producers and consumers.

Although many parasitic diseases have traditionally been considered to be confined to tropical countries, and therefore of little concern to industrialized nations, recent outbreaks of parasitic diseases in North America have demonstrated that this is incorrect. One example is the outbreak of gastroenteritis caused by *Cryptosporidium* in Milwaukee (USA), which was transmitted through the public water supply (MacKenzie *et al.*, 1994). Consumers are also more frequently exposed to parasites that originate in the tropics during international travel to exotic locations, e.g. *Angiostrongylus* sp. in tourists returning from Jamaica, and also as a result of the availability of a wide variety of imported fresh produce in the local marketplace through the globalization of trade, e.g. *Cyclospora* sp. on raspberries from Guatemala (Herwaldt, 2000). Selected protozoan and helminth parasites will be considered in more detail in the following sections.

Protozoan parasites

Protozoan parasites are frequently found in freshwater sources that have been contaminated by human or animal faeces. Fruits and vegetables washed with contaminated water may also be a source of infection. Some protozoa (e.g. *Sarcocystis* sp.) can be transmitted directly through the handling or consumption of fresh meat. Most species of protozoan parasite have environmental phases in their life cycles and form resistant resting stages (cysts or oocysts) that can withstand drying and disinfectants. This can make them hard to control. Although many protozoal organisms are transmitted from human to

human with little or no animal involvement, many (i.e. some strains of *Giardia*, *Cryptosporidium* and other protozoa) have been found in both humans and animals, indicating that zoonotic transmission may occur. Clinical disease associated with protozoal diseases in humans, and in other vertebrates, depends on a wide range of factors, including level of exposure, virulence of the parasite, host immunity and the presence of concurrent bacterial, viral and other protozoal infections. The latter situation is not uncommon, especially in the young, immunocompromised or travellers visiting areas where they are exposed to organisms against which they have no immunity.

Toxoplasmosis (Toxoplasma gondii)

The life cycle and epidemiology of *T. gondii* have been well categorized (Lindsay *et al.*, 2001; Dubey *et al.*, 2002 a,b,c; Kniel *et al.*, 2002; Hill *et al.*, 2007). However, in some parts of the world there are data to suggest that the transmission pathways can be more complex than previously thought, for example, the emerging role of many non-felid species as sources of infection for humans and other mammalian species. As a zoonotic disease, the clinical picture varies widely with a large proportion of the human population, even in urban populations, exposed to *Toxoplasma* oocysts, but only a small percentage showing clinical signs of infection.

    Toxoplasma spp. are widespread worldwide. In a survey of Central and South America, up to 40% of the human population in developed regions, and 80% in underdeveloped regions, were found to be infected (Hill and Dubey, 2002). The prevalence is also high in France, where raw meat is regularly consumed, with 84% of pregnant women in some regions demonstrating antibodies to *Toxoplasma* spp. compared with 32% in New York City. In adults, the infection tends to be asymptomatic, but significant prenatal damage can occur when pregnant women not previously exposed to *Toxoplasma* are infected during pregnancy (Ajzenberg *et al.*, 2002). The organism crosses the placenta and can cause a range of birth defects, from blindness to hydrocephalus. Toxoplasmosis is responsible for the deaths of 10–30% of AIDS patients in Europe and the USA, and has been linked to encephalitis in immunocompromised patients (Bowie *et al.*, 1997; Choi *et al.*, 1997). The clinical picture in these patients is quite varied (Bhopale, 2003). There does not seem to be much genetic diversity among strains of *Toxoplasma*, with only three lineages recognized (Ajzenburg *et al.*, 2002). *T. gondii* infects all warm-blooded animals, but only domestic cats and other felids commonly act as the definitive host that provides a source of infection for other animals. Up to 8000 million infective oocysts can be excreted in the faeces of felids (wild and domestic) to contaminate pasture, water supplies and vegetation. In animals other than cats, *Toxoplasma* cells migrate out of the intestine and encyst in muscle tissue where they remain for the life of the animal. Cysts in birds and rodents are consumed by felids, and the life cycle resumes. Sheep, pigs and other livestock, including poultry, may also become infected with cysts and these can be a source of infection for humans. Consumption of undercooked mutton or pork has been thought to be the source of a number of human infections (Lundén and Uggla, 1992; Lundén *et al.*, 2002).

Giardiasis

Giardia lamblia (*intestinalis*) is the most commonly reported intestinal parasite in humans. The CDC estimates that there are over 2 million cases of giardiasis a year in the USA. *Giardia* sp. cysts may be excreted for prolonged periods in the faeces of infected people (Nichols and Smith, 2002) and can occasionally cause severe damage to the gastrointestinal tract, with chronic malabsorption and other complications if untreated. Severe cases can occur especially in immunocompromised individuals. Most cases of foodborne giardiasis occur following the consumption of fruit and vegetables irrigated with faecally contaminated water. In one study in the USA and Central America, it was found that of 25 samples of water used to irrigate food crops that were eaten raw, 60% contained *Giardia* (Thurston-Enriquez *et al.*, 2002). Food handlers are also thought to be responsible for transmitting the infection in food; for example, in 1996 an outbreak in the USA was traced back to the consumption of contaminated ice cream (Olsen *et al.*, 2000). Other human cases have been traced to pre-prepared food such as sandwiches, salads, etc. (Hancock *et al.*, 1998; Rose and Slifco, 1999; Nichols, 2000). *Giardia* cysts have been isolated from surface water in areas remote from towns, and are thought to have come from the faeces of wild animals, including beavers and coyotes (Thompson *et al.*, 2009). Backpackers have been found to be infected after drinking from freshwater streams, and it is thought that wildlife carry the organism and shed it in their faeces into watercourses. Domestic livestock such as cattle (Appelbee *et al.*, 2003; Dixon, 2009) also shed this organism, with up to 5800 cysts g^{-1} detected in the faeces of domestic cattle (Heitman *et al.*, 2002). Some strains of *Giardia* sp. found in other species do not often infect humans (Appelbee *et al.*, 2003; Snel *et al.*, 2009).

Cryptosporidiosis

There is a wide range of species in the genus *Cryptosporidium* (i.e. *Cryptosporidium bovis, Cryptosporidium felis, Cryptosporidium hominis, Cryptosporidium suis, Cryptosporidium galli*), most of which can be readily transmitted between hosts. Because of this transmission between host species, and the wide range of potential vertebrate hosts infected, it was suggested that the *Cryptosporidium* spp. should be grouped together under the species name *Cryptosporidium parvum* (Xiao and Cama, 2006).

Over the past decade there have been several large outbreaks of gastroenteritis in humans associated with *C. parvum* (Millar *et al.*, 2002). In 1993, over 400,000 people in Milwaukee were affected, with 69 fatalities involving immunocompromised individuals (Naumova *et al.*, 2003). The economic cost was estimated to have been about US$96.2 million (Corso *et al.*, 2003). In most cases, the organism causes a mild and self-limiting disease, but this depends on the infective dose, the immunity of the host, and the virulence of the strain involved (DuPont *et al.*, 1995). Foodborne infections are often associated with the consumption of raw produce contaminated by infected food handlers or water. *C. parvum* has been found in cider, unpasteurized milk and in the faeces of cattle and other livestock (Deng and Cliver, 1999, 2001; Dixon, 2009). Water infected with sewage is often thought to be the source of contamination

involving fresh fruits and vegetables and beverages (Friedman *et al.*, 1997). The oocysts of *C. parvum* can survive in fresh, brackish and saltwater for a number of months (Gomez-Couso *et al.*, 2003) and have been isolated from clams, oysters and other shellfish (Fayer *et al.*, 1998). However, human infection associated with eating shellfish does not seem to be common, possibly because cooking destroys the parasite, although raw shellfish do pose a potential risk (Graczyk *et al.*, 2003).

Cyclospora

Cyclospora cayetensis has been the cause of numerous foodborne disease outbreaks in recent decades. In the 1990s, there was a multi-state outbreak of cyclosporosis in the USA following the consumption of imported raspberries. Other outbreaks have been traced back to the consumption of salad greens, basil and a range of berries, all of which had been contaminated with water containing *Cyclospora* spp. during irrigation (Rose and Slifco, 1999). Only sporulated oocysts are infectious to humans, so food handlers probably have little role in the transmission of this organism. The epidemiology of *Cyclospora* is not fully known, but it is endemic in many developing countries and is spread by the faecal–oral route. At the present time, it is thought that humans are the main host for *C. cayetensis*, but evidence for the absence of the organism in animal hosts is lacking. The organism causes diarrhoea and debility in infants and the immunocompromised (Bern *et al.*, 1999). Complications of infection include Guillain–Barré syndrome (Richardson *et al.*, 1998) and reactive arthritis or Reiter's syndrome (Connor *et al.*, 2001).

Sarcocystis

Human infections may occur as a result of infection with *S. hominis* and *S. suihominis* involving a two-host cycle and consumption of contaminated undercooked meat, especially beef and pork. There have been only a limited number of human cases of *Sarcocystis* infection reported, and most of these are from Asia. In Malaysia, *Sarcocystis* has been reported to be common in domestic and wild animals, including rats, bandicoots, slow loris, buffaloes and monkeys. The overall seroprevalence in humans tested in the same region was 19.8% (Kan and Pathmanathan, 1991). Although many human cases present with gastroenteritis, other clinical signs can occur, such as myositis and also malignancy (Pathmanathan and Kan, 1992). In New Zealand, muscle tissue from the oesophagus and diaphragm of 500 beef cattle was examined, and it was found that all the cattle were infected with *Sarcocystis*; of these, 98% had *Sarcocystis cruzi* and 79.8% had *Sarcocystis hirsuta/S. hominis* (Böttner *et al.*, 1987). A similar study in Belgium found that 97% cattle were positive for *Sarcocystis*. Thick-walled cysts were recovered from 56% of the animals, but these could not be specifically identified as *S. hirsuta* or *S. hominis* on morphological grounds (Vercruysse *et al.*, 1989). A study in India, which examined muscle samples from 890 slaughtered pigs, determined a prevalence rate of 67%, with *Sarcocystis meischeriana* identified in over 40% of cases and 47% of cases with *S. suihominis* (Saleque and Bhatia, 1991). In Canada, it has been found that 53% of some caribou herds can be infected (Khan and Fong, 1991). Other studies

have shown similar results in a range of livestock, including poultry. *Sarcocystis* is killed by proper cooking of meats and this is especially recommended for game meats and pork (Cama, 2006).

Entamoeba

Entamoeba histolytica is an important cause of diarrhoea in people in tropical and subtropical countries (Barwick *et al.*, 2002). Although it is not traditionally considered to be zoonotic, it is mentioned here because it has been found in non-human primates and other wildlife species (Stedman *et al.*, 2003; Verweij *et al.*, 2003), and is a significant human pathogen. Morphologically it resembles *Entamoeba dispar*, which is non-pathogenic, so it is important to distinguish between the two in diagnostic samples. In the USA, *Entamoeba* is not uncommon in immigrants and travellers returning from areas in which it is endemic. It is also common in people living along the US border with Mexico. It should be noted that, in humans, *E. histolytica* is the second leading parasitic cause of death (after malaria). It has been estimated that 50 million people worldwide are infected, of whom 40,000–100,000 die each year from associated complications of the infection, such gut invasion and liver infection, as well as secondary bacterial and other diseases (Stanley, 2003). In most healthy adults, the infection is often asymptomatic as a degree of resistance develops over time. The usual source of infection is sewage-contaminated water that has been used to wash fresh produce. Food handlers can also transmit the disease (Barwick *et al.*, 1998; Leber, 1999).

Helminths

Nematoda

The nematodes, or roundworms, include a number of important human pathogens, such as *Trichinella*, *Ascaris*, *Anisakis*, *Angiostrongylus* and *Gnathostoma*. Some of these parasites have complex life cycles involving an intermediate host, for example *Trichinella*, *Anisakis* and *Gnathostoma*, which exist as cysts in the muscles of mammals or fish and develop into adults in humans that consume the infected flesh. Proper cooking of meats and fish should prevent human infection (Hayunga, 2007). Other nematode parasites, such as *Ascaris*, have a more simple life cycle and are typically transmitted from human to human, so are not considered to be zoonotic.

TRICHINELLOSIS (*TRICHINELLA SPIRALIS*)   Trichinellosis, also called trichinosis, is caused by eating raw or undercooked meat of animals infected with the larvae of the nematode *Trichinella spiralis* and related species (Khumjui *et al.*, 2008). Infection occurs commonly in certain wild carnivorous animals, but may also occur in domestic pigs. Humans have become infected after the consumption of walrus, horse, bear, pig, cougar and seal meats.

When a human or animal eats meat that contains infective *Trichinella* cysts, the acid in the stomach dissolves the hard covering of the cyst and releases the larval worms, which pass into the small intestine and, in 1–2 days, become

mature. After mating, adult females lay eggs. Eggs develop into immature worms, travel through the arteries, and are transported to muscles. Within the muscles, the worms curl into a ball and encyst (become enclosed in a capsule). In humans, clinical signs occur 1–2 days after infection and can include nausea, diarrhoea, vomiting, fatigue, fever and abdominal discomfort. Additional symptoms are varied and can develop within days, weeks or months after infection; these include fever, joint and muscle pain, difficulty in breathing, itchy skin, etc. If the infection is heavy, patients may experience difficulty coordinating movements, and have heart and breathing problems. In severe cases, death can occur. For mild-to-moderate infections, most symptoms subside within a few months, although fatigue, weakness and diarrhoea may last for months.

In emerging economies, such as parts of Eastern Europe, the prevalence of trichinellosis in rural communities can be quite high (Takumi *et al.*, 2009). Cases are usually related to the consumption of *T. spiralis* in pig meat originating from small backyard farms. In contrast, in most parts of Europe, infections are limited because control measures have been implemented and so the pathogen is rarely found in commercial pig units. Trichinellosis can be avoided by cooking meat thoroughly – at 60°C or higher for at least 1 min to kill the infective stage of the parasite – or by freezing meat at –15°C or colder for at least 20 days. Ordinary curing and salting, smoking or microwaving of pork products will not kill the juvenile worms. Human trichinellosis remains a major foodborne zoonosis in Eastern Europe and Asia (Barennes *et al.*, 2008) with a high health, social and economic impact. Infection can also occur in travellers as a result of the consumption of infected wild boar, bear and other game that has not been well cooked (Sterling, 2006; Kurdova *et al.*, 2008).

Cestoda

Meat and fish may contain larval tapeworms that can develop into adults in the human intestine. Clinically important cestodes pathogenic to humans are the *Taenia* species *T. solium* (pork tapeworm), *T. saginata* (beef tapeworm), the *Diphyllobothrium* species *D. latum* (fish or broad tapeworm), *Hymenolepis nana* (dwarf tapeworm) and the *Echinococcus* species *E. granulosus* and *E. multilocularis* (hydatids) (Murrell *et al.*, 2005).

TAENIASIS (*TAENIA SOLIUM*, *TAENIA SAGINATA*)   The *Taenia* cestodes have a worldwide distribution, but the incidence of human infection is higher in developing countries. In North America, the infection rate is thought to be as low as 1:1000, but it can be as high as 10% in some developing countries. Humans can be a definitive host for both the beef tapeworm (*T. saginata* and the closely related *Taenia asiatica*) and the pork tapeworm (*T. solium*), with the latter more commonly reported (Bowman *et al.*, 2006). These tapeworms are parasites that have coevolved with humans and domestic livestock, and remain common in many parts of the world.

Adult *Taenia* tapeworms can be up to 4–6 m long with a long, flat body comprising several hundred segments (proglottids). There are some

morphological differences between adult worms, but the eggs of *T. solium* and *T. saginata* are indistinguishable. In most cases, human infection occurs when a tapeworm larval cyst (cysticercus) is ingested with poorly cooked infected meat. Once ingested, the larvae escape from the cyst and pass to the small intestine where they attach to the intestinal mucosa by the scolex suckers. The proglottids develop as the worm matures in 3–4 months. The adult cestode may live in the small intestine for as long as 25 years and pass gravid proglottids with the faeces. Eggs extruded from the proglottids contaminate, and persist on, vegetation for several days, so in areas where sanitation is poor and latrines are scarce, these proglottids can be consumed by cattle or pigs in which they hatch and form cysticerci.

Light infections remain asymptomatic, but heavier infections may produce clinical signs, such as abdominal discomfort, epigastric pain, vomiting and diarrhoea. In the case of the pork tapeworm, eggs can also infect humans and cause cysticercosis (larval cysts in the lungs, liver, eyes and brain) resulting in blindness and neurological disorders. In some parts of the world, the incidence of cerebral cysticercosis can be as high 1:1000 in the population and may account for a significant proportion of reported neurological disease in some countries (e.g. Mexico, Bhutan); cysticercosis ocular involvement occurs in about 2.5% of patients, and muscular involvement is as high as 10% (India). Prevention of human infection requires good education along with a thorough inspection of beef and pork (and game such as wild boar), with condemnation of meat containing cysts (Gonzales *et al.*, 2003; Bowman *et al.*, 2006). Improving sanitation and providing latrines to prevent ruminants and pigs gaining access to human faecal material is also important. Adequate cooking or freezing of meat are also effective precautions because cysticerci do not survive temperatures below –10°C or above 50°C. Prevention of the disease in humans requires good cooperation with communities and is based on an understanding of the epidemiology of the parasites (Bowman *et al.*, 2006). Apart from the human-health risk associated with taeniasis, cysticercosis renders beef unmarketable and is globally responsible for over US$2 billion in yearly losses (Hoberg, 2002).

DIPHYLLOBOTHRIASIS (FISH TAPEWORM INFECTION)   Diphyllobothriasis in humans is a benign tapeworm infection of the small intestine caused by eating raw fish, a quite common practice in many parts of the world (e.g. in the Baltic countries, Finland and Canada/Alaska). The causative agents are the *Diphyllobothrium* species *D. latum* and *D. pacificum*. *D. latum* is common in northern temperate regions where fish are eaten raw, whereas *D. pacificum* is common in coastal South America, especially Peru. The definitive hosts of *D. latum* include humans, dogs and cats. The natural reservoirs for *D. pacificum* are seals. The two main intermediate hosts include a crustacean and a freshwater fish. Gravid proglottids pass in the faeces of the definitive host and the eggs hatch in lakes and waterways where they infect crustaceans. Freshwater fish consume these and the larvae encyst in the musculature. These intermediate hosts can be eaten by larger fish which can still transmit the infection. Humans acquire the parasite by eating raw infected fish, so although the disease is usually

asymptomatic in humans or other definitive hosts (i.e. dogs, cats, foxes, bears, pigs), there are food-safety implications for the aquaculture industry. The prevalence of *D. latum* in the USA is estimated to be less than 0.5%, although outbreaks have been recently associated with the increased availability of fresh salmon and sushi (Bowman *et al.*, 2006).

ECHINOCOCCOSIS (HYDATIDS)   Hydatid tapeworms (*Echinococcus* spp.) are parasites of canids that can infect humans and animals who accidentally ingest the eggs of the tapeworm after they have been passed out in the faeces of the host. In humans, the two main cestodes associated with 'hydatid' disease are *E. granulosus*, which causes 'cystic' disease, and *E. multilocularis*, which causes 'alveolar' disease.

E. granulosus has worldwide distribution and is the most common form of hydatid in humans. There are typically two forms of the disease recognized: the European form, which is globally distributed in domestic animals; and the Northern form, which is restricted to the tundra and taiga of North America and Eurasia. Sylvatic cycles of transmission in wild animals may also result in the accidental infection of humans, especially when the level of environmental contamination with parasite eggs is high (Jenkins *et al.*, 2005).

The definitive hosts for *E. granulosus* are predominantly members of the family Canidae, which harbour the adult tapeworm in the small intestine. Most infections in canids result in no clinical signs. Most human infections with *E. granulosus* occur in places where hygiene is poor and dogs are used to herd grazing animals, particularly sheep and yaks, which act as intermediate hosts (Yang *et al.*, 2009). In sheep and other ruminants, hydatid cysts can cause considerable condemnation of meat and loss of production. Humans are considered 'dead end' hosts for the parasite, with infection frequently the result of ingesting vegetation contaminated with infected dog faeces. The disease is common throughout southern South America, the Mediterranean and the Middle East, Central Asia and East Africa.

Implementation of proper meat-inspection practices and preventing carnivores from consuming the infective cysts in meat has reduced the incidence of hydatids in many countries. This, along with improving hygiene, carefully washing vegetables such as salads before eating them and regularly treating dogs with suitable anthelmintics, has minimized the risk to humans in many parts of the world, but isolated cases still occur in Eastern Europe, Russia, Australasia, India and parts of the Mediterranean (Jenkins, 2006; Garippa and Manfredi, 2009). In North America, endemic foci have been reported from the western USA, the lower Mississippi Valley, Alaska and northwestern Canada (Somily *et al.*, 2005).

The life cycle for *E. multilocularis* involves foxes as a definitive host and rodents such as voles and meadow mice as intermediate hosts (Vuitton *et al.*, 2008). Domestic dogs and cats can also become infected with the adult tapeworm when they eat infected wild rodents. Human disease associated with *E. multilocularis* has been reported in parts of central Europe, much of Siberia, northwestern Canada, and western Alaska (McManus *et al.*, 2003; Somily *et al.*, 2005).

Trematoda

Most trematodes, or flukes, have a life cycle that requires one or two interme-
diate hosts. Juvenile flukes may be present on aquatic vegetables or foods
washed in contaminated water (e.g. *Fasciola* and *Fasciolopsis*), while others
encyst in fish (*Clonorchis*) or crabs and wild boar (*Paragonimus*). Although
trematodes have not received a lot of attention from food-safety authorities,
foodborne trematodiasis is an emerging public-health problem, particularly
in South-east Asia and the Western Pacific region. It is estimated that millions
of people are currently at risk of infection with fluke parasites such as *Clonor-
chis sinensis, Paragonimus* spp., *Fasciola* spp., and *Opisthorchis* spp. In Asia, where
aquaculture is of growing importance, rural and some urban residents living
near the freshwater habitats for these parasites have a 2.15-fold higher risk
(95% confidence interval 1.38–3.36) for infections than residents living fur-
ther from the water. The exponential growth of aquaculture may be the most
important risk factor for the emergence of foodborne trematodiasis (Keiser
and Utzinger, 2005; Robinson and Dalton, 2009).

FASCIOLIASIS   Human and animal fascioliasis is a matter of growing concern in
Egypt (Soliman, 2008), but although the parasite occurs worldwide clinical
disease in humans is rarely reported in Western countries. However, there has
been a recent increase in human cases reported from Europe, the Americas,
Oceania, Africa and Asia. Human fascioliasis is considered to be an important
emerging disease, with most cases associated with consumption of infective
stages in vegetation such as watercress, containing intermediate hosts such as
snails (Soliman, 2008).

Other foodborne zoonotic trematodes (FZT) pose risks to human health,
many of these are transmitted in raw or inadequately cooked fish raised in fish
farms. A number of studies in South-east Asia have found that FZT are a
significant problem in hatcheries supplying stock to fish farms. Farmed fish
in Asia include perch (*Anabas* sp.), carp (*Cyprinus* sp., *Ctenopharyngodon* sp.),
gouramy (*Osphronemus* sp., *Helostoma* sp.), tilapia (*Oreochromis* sp.) and other
species that comprise an important source of protein for consumers (Thien
et al., 2009). Recent concerns associated with the presence of trematode meta-
cercariae in fish flesh have been responsible for substantial economic losses
in the aquaculture industry owing to restrictions on exports and reduced con-
sumer demand because of food safety concerns. Current food inspection pro-
cedures may not detect the presence of all parasites in imported fish products,
so there can be a risk of human infection if the fish or shellfish are consumed
raw or lightly cooked. International travel and the increasing availability of,
and interest in, ethnic foods also contribute to the risk of infection in non-
endemic areas (Soliman, 2008).

Conclusions

A wide range of zoonotic pathogens are capable of causing significant morbid-
ity and mortality in the human population. Outbreaks of zoonotic enteric

disease in urban populations of the industrialized world have typically been attributed to bacterial pathogens (e.g. *E. coli* O157, *Salmonella* spp., *L. monocytogenes, Campylobacter* spp.) present in contaminated dairy products, eggs, meat and processed foods. Other outbreaks have been traced back to the presence of bacteria (e.g. *E. coli*) or protozoa (e.g. *Cryptosporidium* spp.) in contaminated water supplies used to wash fresh produce. Several recent outbreaks of food-borne disease have been linked to hygiene failures in modern food production plants, with further risk associated with the ready availability of fresh and chilled food products, which are often transported large distances both nationally and internationally. In an attempt to reduce the risk to consumers, regional and international regulatory authorities have been working together to regulate the food-production industry at all levels – from the producer through to the consumer. In most countries, there are international agreements in place to ensure that biosecurity and food-safety standards are applied to all traded food commodities. This applies to both exports and food for sale in local markets and in food outlets. This topic is discussed further in Chapter 1.

In less industrialized countries, and in more rural settings, the range and extent of diseases that are transmitted from animals to humans via the food chain is more varied. The water supplies and food-production methods are often less closely regulated and there is more potential for disease transmission between humans, wildlife and livestock. The range of pathogens to which humans are exposed is also greater now that people have the opportunity to travel away from their home environment and have access to a range of food options that were previously not available (e.g. game meats, fresh raw fish, farmed shellfish, and novel fruits and vegetables that may not have been well washed or cooked). The epidemiology of many of the predominantly food-borne and waterborne diseases outlined in this chapter is complex as it is dependent on the interaction between the pathogen, the environment and the host, as well as on the interplay between other potential hosts present in an area. Food preferences, food availability, water quality and food-preparation methods will also have an effect on the nature and extent of foodborne diseases present in a particular area.

A greater understanding of the interplay between humans, the food supply and the environment has started to emerge with the development of interdisciplinary studies on the epidemiology of zoonotic and emerging diseases. These studies involve microbiologists, molecular scientists, ecologists, risk-assessment experts, geographers, veterinarians, wildlife ecologists, medical specialists, food technologists and others (Daszak *et al.*, 2000, 2007; Meslin *et al.*, 2000; Croft *et al.*, 2003; Bengis *et al.*, 2004; Dagendorf, 2004; de La Rocque *et al.*, 2008; Tee *et al.*, 2008; Gould and Higgs, 2009; Gray *et al.*, 2009). Broad-based surveillance efforts are required to inform veterinary and public health authorities about current and emerging disease risks associated with the food and water supply. The successes of the 20th century and the new challenges we face mean that public-health vigilance, careful investigation of new problems, responsible attention to food safety from farm to table, and partnerships to bring about new foodborne disease control measures will be needed for the foreseeable future.

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3 Manure as a Source of Zoonotic Pathogens

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Introduction

In addition to providing nutrients, the gastrointestinal system plays an integral role in the physiological, immunological and protective functions of the host (Zoetendal *et al.*, 2004). The microbiome of the gastrointestinal system, and its extensive and diverse populations of gut microorganisms, is increasingly recognized to have a major role in numerous aspects of the host's health, including stimulation of the immune response, protection from pathogens, production and metabolism of toxins and gene expression in host epithelial tissue (Daly and Shirazi-Beechey, 2003). The gastrointestinal microbiome is metabolically adaptable and rapidly renewable (Zoetendal *et al.*, 2004). However, it may also harbour a wide variety of pathogenic organisms of both veterinary and human significance.

A review of the scientific literature in 2001 identified 1415 species of infectious pathogens reported to cause disease in humans; 61% (868 species) of these pathogens were identified as known zoonoses (Taylor *et al.*, 2001). These 868 species were determined to be derived from 313 different genera and included, overall, viruses and prions (19%), bacteria or rickettsiae (31%), fungi (13%), protozoa (5%) and helminths (32%). Further highlighting the importance of zoonoses in global public health was the finding in the study of Taylor *et al.* (2001) that 75% (132) of emerging pathogens are zoonoses. These finding are supported by a study of emerging infectious diseases from 1940 to 2004, which found 60.3% of emerging infectious diseases to be caused by zoonotic pathogens (Jones *et al.*, 2008). Both the study of Jones *et al.* (2008) and that of Taylor *et al.* (2001) identified two groups as the main source of emerging infectious diseases: viruses and prions (25.4% and 44% for the respective studies) and bacteria and rickettsiae (54.3% and 30% for the

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respective studies); these discrepancies were attributable to the classification by Jones *et al.* (2008) of each drug-resistant microbial strain as a separate pathogen. The Jones *et al.* (2008) study determined that the majority of emerging infectious disease originated from wildlife (71.8%), rather than from companion or production animals. This chapter will focus on the most significant of these zoonotic pathogens that are derived from manure, the modes by which humans become exposed to these organisms and the significance of the diseases caused.

Manure, Zoonoses and Modes of Infection

Manure is generally regarded as livestock excreta (urine and faeces) mixed with bedding materials, such as straw, that is traditionally disseminated into the environment as a source of fertilizer for crops and pastures. It is important to recognize that, in addition to excreta and bedding material, manure may also contain water (drinking and cleaning) and excretions from the nose, throat, vagina, mammary glands and skin, as well as blood, and typically contains 10^{10} bacterial cells g^{-1} (Pell, 1997). In 1997, manure production by housed livestock in England and Wales was estimated at around 70 million tonnes, the majority of which was produced by the beef and dairy industries (77% combined), followed by the pig (15%), poultry (6%) and sheep industries (2%) (Hutchison *et al.*, 2000). In the USA, estimates indicate that in excess of 1.2 billion tons of cattle manure are produced yearly (Sanchez *et al.*, 2002a). Traditionally, manure was seen as an agricultural waste product that necessitated disposal, but this view has changed and manure is now viewed as an industry by-product with a number of valuable uses both within the agricultural setting and outside these industries (Bicudo and Goyal, 2003). The primary use of manure is as a fertilizer source. Manure is recycled back into agricultural land and provides an economical means of reintroducing organic material and nutrients to soil, and maintaining or improving quality and fertility (Hutchison *et al.*, 2000). While the vast majority of manure is utilized as fertilizer, other common uses for manure include use as a source of solid fuel for cooking and heating (Venkataraman *et al.*, 2005) and for biogas production (Amon *et al.*, 2007). The handling and use of manure for these applications has the potential to expose humans, either directly or indirectly, to potential zoonoses, and constitutes a significant public health issue.

Infection may occur either via direct exposure to contaminated faecal matter, such as through the handling or processing of manure, or through a number of indirect routes, such as contaminated meat, milk, other produce or water. A study by Adak *et al.* (2005) reported that foodborne disease resulted in over 1.7 million infections and 687 deaths in England and Wales between 1996 and 2000, with animal-based food products (including meat, milk/dairy products and seafood) accounting for 65% and 68% of cases and deaths, respectively. Meat products can become contaminated with manure and resident pathogenic bacteria during slaughtering, butchering or processing, and contamination may originate either directly from the gastrointestinal tract

or from the hide of the animal. The same study of foodborne disease in England and Wales reported that fruits and vegetables account for only 3% and 2% of cases and deaths, respectively (Adak *et al.*, 2005) – yet despite this relatively low incidence of foodborne diseases from fruits and vegetables, these are becoming increasingly recognized as potential sources of infection, particularly with the increase in popularity of organic farming practices (Leifert *et al.*, 2008; Sofos, 2008). Milk contamination may occur through poor udder hygiene (Ramirez *et al.*, 2004) and, combined with dairy products, was reported to account for 7% and 5% of cases of foodborne illness and death, respectively (Adak *et al.*, 2005). The exact proportion of foodborne infections that can be directly attributed to manure contamination is, though, not well established (Cliver, 2009).

Water contaminated with pathogenic microorganisms is a major source of human morbidity. A large number of pathogens transmissible via contaminated water can be found in livestock manure, but as humans and a wide variety of wildlife species can also be a source of these organisms, documented outbreaks are often unable to be definitively attributed to a source (Bicudo and Goyal, 2003). Environmental contamination with manure containing zoonotic agents may, via a number of hydrological pathways, result in contamination of water sources accessed by humans (Williams *et al.*, 2008). Leaching may result in the contamination of groundwater, while runoff has the potential to contaminate watercourses and stored water, especially during heavy rainfall events (Williams *et al.*, 2008). Thawing snow may facilitate the entry of zoonotic microorganisms into watercourses or catchments in areas with winter snow cover (Unc and Goss, 2006). It should also be noted that contamination of ground, irrigation or drinking water supplies provides not just a source of infection for humans, but also a means by which these organisms can be spread between animals or herds.

The intensification and industrialization of the animal production industries have resulted in an increase in microbial load in the production environment through the increased presence of animal feed, animals and associated waste products, including manure (Millner, 2009). These factors have necessitated increased handling and management of both animals and animal wastes, and put persons in direct contact with either animals or manure at increased risk of contracting infections associated with manure. Bio-aerosols, defined as a collection of aerosolized biological partials (Cox and Wathes, 1995), may potentially be generated within animal housing facilities from either solid manure or slurries, but also from feed, litter or the animals themselves (Pillai and Ricke, 2002). As a result, within animal housing facilities, workers can become exposed to aerosolized pathogenic microorganisms or endotoxins, leading to increased risk of disease (Clark *et al.*, 1983; Pillai and Ricke, 2002). Mechanical spreading of manure as a fertilizer also results in the production of aerosols, which constitute a potential source of infection if inhaled, or in environmental, water or crop contamination through spray drift (Hutchison *et al.*, 2000). Trials using rain-gun manure sprayers have shown the potential of these systems to contaminate the environment over 100 m from the spray site (Hutchison *et al.*, 2008).

Control measures for preventing zoonotic infections arising from manure can be implemented at multiple steps during animal and crop production, and have been reviewed in detail with particular reference to the contamination of vegetable crops (Leifert *et al.*, 2008) and verocytotoxigenic *Escherichia coli* (VTEC) from cattle (Khanna *et al.*, 2008). Briefly, practices should be implemented that reduce pathogen burden within the host animal (Doyle and Erickson, 2006). Implementation of this policy often makes economic sense, as many zoonotic pathogens cause disease in the host, thus reducing productivity and, as such, profitability (Pell, 1997). Prevention of environmental contamination reduces the risk of herd reinfection and of human disease resulting from pathogen contamination of crops or water sources. Pathogenic organisms may remain viable in manure for extended periods (for example, *E. coli* O157:H7 can survive and remain infectious in faecal matter for up to 21 months; Zhao *et al.*, 2001). Environmental contamination can be reduced through various manure management systems, including the composting of manure, and various physical, chemical or biological treatments before application to land; these methods have been reviewed in detail (Bicudo and Goyal, 2003; Hutchison *et al.*, 2005a,b). Appropriate soil management and irrigation practices can also be beneficial in reducing the risk of contaminating fruit and vegetable crops (Holley *et al.*, 2008; Leifert *et al.*, 2008), and the use of filter strips has been shown to be efficacious in reducing watercourse contamination through runoff (Larsen *et al.*, 1994). Finally, food processing facilities should implement appropriate risk-minimization practices to reduce the chance of contamination of products with manure (Zhao *et al.*, 2001; Stecchini and Del Torre, 2005; Khanna *et al.*, 2008).

Significant Zoonotic Infections Contractible from Manure Exposure

Pathogen types and numbers in manure differ with animal species, geographical location and the physicochemical composition of the manure (Bicudo and Goyal, 2003). Bacterial, viral and protozoan zoonotic organisms are all transmissible to humans through manure, although the majority of pathogens of concern are bacterial (Cliver, 2009). A study of 38,629,641 cases of foodborne diseases of known aetiology in the USA concluded that six pathogens alone accounted for approximately 70% of total cases and 95% of the 2718 reported deaths: *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%) and *E. coli* O157:H7 (3%) (Mead *et al.*, 1999). For all of these organisms, with the exception of Norwalk-like viruses and *Toxoplasma*, manure can be either the direct source of infection or the source of contamination by which these pathogens enter the food chain. Furthermore, a recent publication evaluated and prioritized a list of 51 foodborne and waterborne zoonoses (Cardoen *et al.*, 2009), which corroborated the results of Mead *et al.* (1999); *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and VTEC were ranked as the most significant of the diseases analysed.

Escherichia coli

E. coli is a normal inhabitant of the gastrointestinal system of warm-blooded animals. Commensal *E. coli* colonizes the neonate gastrointestinal system within hours of birth, and plays an intrinsic role in maintaining normal gut physiology in the host (Mackie *et al.*, 1999). Gut commensal *E. coli* is rarely responsible for disease. However, through the acquisition of virulence factors, strains of *E. coli* have developed the capacity to cause either enteric or extra-intestinal diseases, and are commonly associated with disease resulting from the exposure of foodstuffs to manure contamination. The *E. coli* O157:H7 serotype is associated with over 50% of VTEC infections in the EU (European Centre for Disease Prevention and Control, 2009); this, combined with the involvement of the organism in large outbreaks, and the severity of the disease, makes *E. coli* O157:H7 the serotype of most interest. *E. coli* O157:H7 infection within the EU was reported to affect 0.6 persons per 100,000 in 2007 (European Food Safety Authority, 2009).

Of most concern, with regard to the context of this document, are the enterohaemorrhagic *E. coli* (EHEC), a subgroup of VTEC with the capacity to produce toxins (vero, Shiga or Shiga-like) similar to *Shigella dysenteriae* type 1 cytotoxin (O'Brien and Holmes, 1987), but also possessing the locus of enterocyte effacement (LEE) pathogenicity island, which encodes for a type III secretion system (Kaper *et al.*, 2004). Animals are the primary reservoir for EHEC (Khanna *et al.*, 2008), with bovine products linked to around 75% of cases of *E. coli* O157:H7 outbreaks (Callaway *et al.*, 2009). In cattle, *E. coli* O157:H7 colonizes mucosal surfaces of the large intestine, and particularly of the terminal rectum (Naylor *et al.*, 2003), where the organisms may reside and continue to be shed for up to 3 months (Sanchez *et al.*, 2002a). Prevalence rates of *E. coli* O157:H7 range from 0 to 28% for individual cattle, and up to 75% for herds (Sanchez *et al.*, 2002a). Hide contamination with VTEC is typically higher than faecal carriage, and a single animal has the capacity to contaminate, either directly or indirectly through environmental contamination, the hides of many animals (Rhoades *et al.*, 2009). *E. coli* O157:H7 is not invasive, and undercooked meat contaminated with cattle faeces constitutes the leading source of infections (Sanchez *et al.*, 2002a). Studies have shown that as much as 19% of retail uncooked beef may be contaminated with *E. coli*, and 4% with *E. coli* O157:H7 (Zhao *et al.*, 2001); the initial *E. coli* O157:H7 cases were associated with the consumption of undercooked hamburgers (Kaper *et al.*, 2004). It should be noted that other livestock can also act as reservoirs, and *E. coli* O157:H7 has been found in pork (1.5%), lamb (2%) and poultry (1.5%) meat, as well as in various other foods, including unpasteurized milk (Zhao *et al.*, 2001). Contamination of food products may occur at one or more of several steps along the food processing, distribution, retail and preparation line (Zhao *et al.*, 2001), and estimated infectious doses for EHEC are very low (ID_{50} between 100 and 1000 cells) (Teunis *et al.*, 2008). Shedding rates for *E. coli* O157:H7 are typically lower than 100 colony forming units (cfu) g^{-1} faecal matter, though shedding rates of greater than 10^7 cfu g^{-1} faeces have been reported (Rhoades *et al.*, 2009). Water sources, such as lakes or ponds

contaminated with infected cattle-manure runoff, and swimming pools, have been identified as sources of *E. coli* O157:H7 outbreaks, as has contact with animals, particularly calves, at petting zoos or farms (Sanchez *et al.*, 2002a; Callaway *et al.*, 2009). *E. coli* O157:H7 has the capacity to survive in the environment for long periods of time, especially in water, with studies showing *E. coli* O157:H7 to survive and remain infectious in water (farm drinking troughs) for 8 months and in faecal matter for 21 months (Zhao *et al.*, 2001).

Salmonella

Of the zoonotic pathogens associated with manure, *Salmonella* constitutes the broadest risk (Cliver, 2009). *Salmonella* is ubiquitous in nature and has been isolated from a wide variety of vertebrate hosts (Pell, 1997). Salmonellae are commonly found in production animals, their environments and the manure produced by animal industries (Thorns, 2000; Guard-Petter, 2001; Hutchison *et al.*, 2004; Farzan *et al.*, 2009; Rhoades *et al.*, 2009). Furthermore, *Salmonella* was ranked by 35 scientific experts in the fields of animal and public health, food, and clinical microbiology and epidemiology, as the single most important foodborne zoonotic microorganism (Cardoen *et al.*, 2009). Non-typhoidal salmonellosis is reported to account for 9.7% and 6.6% of total foodborne disease in the USA and in England and Wales, respectively (Mead *et al.*, 1999; Adak *et al.*, 2002). Human disease resulting from *Salmonella* infection is typically self-limiting, and characterized by diarrhoea, stomach cramps, vomiting and fever (Rhoades *et al.*, 2009). However, *Salmonella* is reported to account for more deaths from foodborne infections in the USA and in England and Wales than any other organism: 30.6% and 29.2%, respectively (Mead *et al.*, 1999; Adak *et al.*, 2002).

In livestock, salmonellosis results in illness ranging in severity from moderate to severe, but the animals can also be asymptomatic carriers (Van Kessel *et al.*, 2007). Faecal contamination of hides is common, and hide carriage rates of *Salmonella* by feedlot cattle have been reported to be as high as 71.0% (yearly mean), compared with 4.3% by faecal carriage for the same animals (Barkocy-Gallagher *et al.*, 2003). Stored manure is reported to contain as many as 10^6 *Salmonella* cells g^{-1} (Hutchison *et al.*, 2004), and the organisms are more likely to be detected in stored manure than from faecal samples, highlighting the potential significance of manure as a source of environmental contamination (Farzan *et al.*, 2009). Long-term *Salmonella* contamination of farms has been described and appears to be widespread (Winfield and Groisman, 2003).

Manure may constitute a source of human salmonellosis via a number of routes of infection. A wide range of food types, ranging from meat products, milk, eggs, fruit juice, salad and other fresh produce through to peanut butter, has been associated with *Salmonella* outbreaks (Denno *et al.*, 2007). Faecal contamination of meat during processing poses a risk of *Salmonella* entry into the food chain, and a number of studies have been conducted to evaluate the level of *Salmonella* contamination on abattoir carcasses at different stages of processing. In these studies, up to 45.2% of pre-evisceration carcasses were

found to be contaminated (Rhoades *et al.*, 2009). *Salmonella* contamination of chicken, turkey, pork and beef meat from retail stores in the Greater Washington, DC area reported contamination rates ranging from 1.9% to 4.2% (Zhao *et al.*, 2001). Enteric disease has been associated with the consumption of a large range of contaminated fresh produce, and *Salmonella* constitutes the most commonly identified enteropathogen in these cases (Heaton and Jones, 2008). Contamination of these products may occur either postharvest, through mechanisms such as inadequate hygiene, or preharvest, through mechanisms discussed above such as direct environmental contamination by animals or the application of manure or contaminated water (which may have initially been contaminated by manure). *Salmonella* survivability trials in soils amended with pig manure have indicated that the largest decreases in viable *Salmonella* occurred in the initial 2 weeks, and this led the authors to suggest that a 30-day delay between manure application and land use would minimize the risk of *Salmonella* contamination of crops and animals (Holley *et al.*, 2006); in this study, no *Salmonella* survived for longer than 180 days, although the organisms have been shown to survive for periods of up to 300 days (Baloda *et al.*, 2001). Occupational contact with animals and animal waste is reported to constitute a risk for contracting salmonellosis (Sanchez *et al.*, 2002b). Flies may also act as vectors of *Salmonella* (Winfield and Groisman, 2003); muscoid flies on dairy and poultry farms have been demonstrated to have infection rates of 67% and 13%, respectively and total *Salmonella* defecation rates in experimentally infected flies have been shown to be as high as 10^7 (Greenberg and Klowden, 1972).

Listeria

Listeriosis constitutes a serious public-health concern owing to the epidemic potential and high mortality rate characteristic of this disease. Of the six recognized species of *Listeria*, two are known to cause disease in humans: *L. inanovii* and *L. monocytogenes* (Snapir *et al.*, 2006); virtually all human cases can, however, be attributed to the latter. While direct animal-to-human transmission is possible, particularly in persons working with aborted fetuses, ingestion of contaminated food is the primary source of infection, with 99% of listeriosis cases attributed to this (Mead *et al.*, 1999). *L. monocytogenes* has been isolated from 42 mammalian species and 22 avian species, as well as fish, crustaceans and insects (Wiedmann *et al.*, 1996), and a vast array of plant and soil environments, groundwater, sewage and silage (Freitag *et al.*, 2009). It is the ubiquitous nature of this organism that facilitates its epidemic and zoonotic potential, but it is cattle and sheep, through the contamination of meat and milk, which constitute the greatest zoonotic threat as a source of *L. monocytogenes* infections (Czuprynski, 2005). *L. monocytogenes* is resilient, able to grow under a wide range of temperatures (3–42°C) and pH ranges (<5.5–9.0) and high salt concentrations (Pell, 1997), and has the capacity to become established in food-processing facilities if appropriate controls are not introduced (Freitag *et al.*, 2009).

Animal infection most commonly occurs through the ingestion of contaminated feed, and studies showing correlations between feeding poorly prepared silage (pH > 5) and *L. monocytogenes* have been published (Pell, 1997; Czuprynski, 2005). Livestock infection most commonly presents as neurological signs; altered behaviour and depression, developing into facial hypalgesia, paralysis, ataxia and, particularly in sheep, head tilt and permanent circling movement (Wagner *et al.*, 2005). Asymptomatic *L. monocytogenes* carriage and faecal shedding is common (Czuprynski, 2005) and has been well reported for a wide range of livestock and wildlife (Lyautey *et al.*, 2007); *L. monocytogenes* prevalence levels as high as 33% have been reported in healthy cattle (Weber *et al.*, 1995). Under favourable conditions, *L. monocytogenes* has been shown to have the capacity to survive in soil amended with bovine manure for several weeks (Jiang *et al.*, 2004), thus posing a significant risk of transmission through the contamination of fruits or vegetables entering the human food chain (Czuprynski, 2005).

Campylobacter

The *Campylobacter* genus has long been recognized as associated with both animals and human disease. *Campylobacter*-like organisms were first observed in the colon of babies that had died of diarrhoeal disease in 1886 (Escherich, 1886), and in 1913 *Campylobacter*-like organisms were observed to be commonly associated with aborted sheep fetuses (McFadyean and Stockman, 1913). It was, however, not until the development of appropriate isolation and cultivation techniques in the early 1970s that *Campylobacter* infection became recognized as both a common and significant cause of enteric illness in humans (Dekeyser *et al.*, 1972). *Campylobacter* is now recognized worldwide as being both a significant public-health concern and an economic burden, and it is the leading cause of bacterial foodborne illness in the USA, accounting for 14.2% of total infections and 5.5% of mortalities (Mead *et al.*, 1999). In England and Wales, *Campylobacter* was reported to account for around 27% of total foodborne infections (359,466 of 1,338,772) in 2000 (Adak *et al.*, 2002), and these acute infections have been estimated to cost, on average, £1315 per case (Humphrey *et al.*, 2007).

Most human infections are attributable to three species: *Campylobacter jejuni*, *Campylobacter coli* and, particularly in the developing world, *Campylobacter upsaliensis*. Around 90% of confirmed cases of campylobacteriosis are attributed to *C. jejuni* (Altekruse and Tollefson, 2003). Cardoen *et al.* (2009) ranked *Campylobacter* as the second most significant foodborne zoonotic organism after *Salmonella*.

Campylobacter spp. may cause gastrointestinal disease and septic abortions in animals (Cole *et al.*, 1999), although they are generally considered as normal gastrointestinal microflora of many mammals and birds (Crushell *et al.*, 2004). Both *C. jejuni* and *C. coli* are commonly found in the gastrointestinal tract of production animals, and colonization with these organisms is thought to occur largely through contact with a contaminated environment

(Humphrey *et al.*, 2007). Human campylobacteriosis is most commonly sporadic and not linked with environmental contamination (Crushell *et al.*, 2004). Infections from faecally contaminated water, unpasteurized milk and raw vegetables are recorded, though campylobacteriosis is most commonly associated with the consumption of raw or undercooked meat products (Horrocks *et al.*, 2009), particularly poultry meat (Suzuki and Yamamoto, 2009). Direct transmission of *Campylobacter* from animals to humans is also possible, but infections via this route account for only a minority of infections (Crushell *et al.*, 2004). Studies of meat and animal contamination by *Campylobacter* spp. have demonstrated mean rates of contamination for cattle to be 30.0% and 62.1% for dairy and beef cattle, respectively, and 31.1% for sheep and 61% for pigs (Humphrey *et al.*, 2007); chicken, turkey and duck flocks had mean contamination rates of 58.7%, 78.0% and 38.0%, respectively, while 3.2% of raw milk returned positive results for *Campylobacter* contamination. Of most concern are the reported contamination rates for meats at retail: chicken (57.4%), turkey (47.8%), duck (30.2%), pork (2.0%), beef (2.7%) and lamb (6.0%). *C. jejuni* is the most commonly isolated *Campylobacter* species from cattle and poultry species, while *C. coli* is more commonly associated with swine (Cole *et al.*, 1999; Farzan *et al.*, 2009).

Protozoa

The protozoan species of most concern, with regard to manure exposure, are *Cryptosporidium* spp. and *Giardia duodenalis* (Hunter and Thompson, 2005). *Cryptosporidium* spp., along with *Giardia* spp., constitute the most common enteric parasites of domestic animals and livestock (Thompson *et al.*, 2008) and, together, are thought to constitute the most common cause of protozoan diarrhoea in humans (Caccio *et al.*, 2005). It was not, however, until the 1980s, with the establishment of the role of this organism in the death of AIDS patients (Current *et al.*, 1983), that *Cryptosporidium* became recognized as a significant zoonotic pathogen (Ramirez *et al.*, 2004). Infections by both organisms occur via mechanisms commonly associated with manure contamination: the faecal–oral route, predominantly through the ingestion of contaminated food or water, via host-to-host contact or, for *Cryptosporidium*, via aerosol transmission (Leoni *et al.*, 2006; Xiao and Feng, 2008).

Cattle are the major reservoir for *Cryptosporidium parvum* (Hunter and Thompson, 2005), and constitute an important source of zoonotic cryptosporidiosis infections, largely through the contamination of food or water with manure containing *Cryptosporidium* oocysts (Xiao and Feng, 2008). Overall, contaminated water constitutes the leading source of human infection (Ramirez *et al.*, 2004). Disease is usually self-limiting and does not pose long-term health risks for healthy, mature animals (Pell, 1997); infected, immunocompetent animals typically clear the parasite within 3 weeks of infection (Chappell *et al.*, 1999). If diarrhoea develops, cattle infected with *C. parvum* are reported to excrete between 10^5 and 10^7 oocysts ml^{-1} faeces. *C. parvum* is among the most common enteropathogens of calves (Thompson *et al.*, 2008),

and subclinical infections are not uncommon in older animals (Ridley and Olsen, 1991). Excreted oocysts are immediately infectious once released and may remain viable in the environment for months (Thompson *et al.*, 2008); they are resistant to the majority of common disinfectants (Campbell *et al.*, 1982). *C. parvum* infectious doses have been determined in healthy, serologically negative (DuPont *et al.*, 1995) and positive (Chappell *et al.*, 1999) adults and the 50% infectious dose (ID_{50}) was found to be 132 and 1880 oocysts, respectively.

Overall, the exact significance of cryptosporidiosis as a zoonotic disease remains unclear. Reports indicate that *C. parvum* and *Cryptosporidium hominis* account for over 90% of cases of cryptosporidiosis (Xiao and Feng, 2008), and as many as 98% of sporadic cryptosporidiosis cases (Leoni *et al.*, 2006) in industrialized countries. Of these, *C. parvum* is responsible for slightly more cases in the Czech Republic, England, France, New Zealand, Northern Ireland, Portugal, Slovenia, Switzerland and Wales, while *C. hominis* is the dominant species in Australia, Canada, Japan and the USA (Xiao and Feng, 2008). Interestingly, *C. hominis* alone accounts for 70–90% of cases of cryptosporidiosis in developing countries, suggesting that cases of zoonotic origin are of less importance in these countries (Xiao and Feng, 2008). The proportion of infections occurring from *C. parvum* of animal source is unclear, as both humans and animals may be the source of infection (Xiao and Feng, 2008). Furthermore, *Cryptosporidium* is often not considered in the differential diagnosis of gastrointestinal diseases of immunocompetent patients, resulting in the under-reporting of this disease (Ramirez *et al.*, 2004). Persons exposed to livestock are, however, reportedly at increased risk of *Cryptosporidium* infection (Lengerich *et al.*, 1993).

Like cryptosporidiosis, giardiasis presents as an acute, mild-to-severe gastrointestinal illness, characterized by transient diarrhoea and associated gastrointestinal symptoms (Wolfe, 1992). Chronic disease may develop and can persist over a number of years (Pell, 1997). The *Giardia* genus contains a number of different species which are known to affect various species of domestic animals and wildlife, as well as humans. Of these, only assemblages A and B of *G. duodenalis* are pathogens of humans (Hunter and Thompson, 2005). The routes of infection and specific roles of animals in the transmission remain unclear, although the available evidence suggests that livestock only constitute a small public-health risk with regards to transmission of *Giardia* (Caccio *et al.*, 2005).

It should also be noted that *Toxoplasma gondii* (which was reported by Mead *et al.* (1999) to account for 21% of reported deaths) is a zoonotic organism, for which cats (Felidae) are the primary host. Human infection by this organism occurs through either direct or indirect contact with feline faecal matter contaminated with *T. gondii* oocysts, and while it is not spread by exposure to manure, it is a possibility that cats can contract toxoplasmosis from manure (Tenter *et al.*, 2000). Livestock may also constitute a source of infection for both definitive and intermediate hosts, including humans, through the ingestion of tissue cysts (Tenter *et al.*, 2000; Tenter, 2009).

Viruses

A wide variety of viruses are known to affect production animals; however, few of these have been shown to be transmissible to humans through manure (Cliver, 2009). The study of food-related illness and death in the USA by Mead *et al.* (1999) reported Norwalk-like viruses (of the Norovirus genus) to account for 66.6% of foodborne illnesses and 6.9% of deaths. Norovirus has recently been detected in the faeces or gastrointestinal tract of a number of species of production animals (van Der Poel *et al.*, 2000). Furthermore, norovirus resembling a human strain has been detected in swine faeces and retail meat (Mattison *et al.*, 2007; Wolf *et al.*, 2009), and these human Norovirus strains have been demonstrated to cause symptoms under experimental infection (Cheetham *et al.*, 2006). While there is concern of the potential of these organisms to cause zoonotic disease, zoonotic infection has not been detected, and the general consensus is that while zoonotic infection may be possible, outbreaks are more likely to occur from human rather than from animal sources (Farkas *et al.*, 2005; Koopmans, 2008; Cliver, 2009).

Viral zoonotic organisms of more concern with respect to human infection from contact with manure are hepatitis E virus (HEV), avian influenza A (H5N1) virus (Cole *et al.*, 1999) and, more recently, swine influenza A (H1N1) virus (Tomley and Shirley, 2009). Described as 'the last great plague of man' (Kaplan and Webster, 1977), influenza virus is unique in its potential to rapidly infect billions of people worldwide (Greger, 2007). Wild waterfowl constitute the natural reservoir of avian influenza A viruses, and these can be transmitted to domestic terrestrial poultry in which they may mutate from the low-pathogenicity strain acquired into a highly pathogenic form (WHO, 2007). Infected animals shed large quantities of virus, which can become incorporated into manure and has the capacity to contaminate water supplies and the environment via surface runoff, through groundwater or via wind dispersal (WHO, 2007; Halvorson, 2009). The route of transmission of avian influenza H5N1 from poultry to humans has yet to be fully elucidated. Current evidence suggests that infection may occur via direct animal contact or through contact with faecal matter via the respiratory or faecal–oral route (WHO, 2007). Regarding the H1N1 virus, work still needs to be done to establish disease susceptibility in host species, transmission dynamics and virulence of this organism. However, it is proposed that natural transmissions between pigs and humans are a feature of this disease (Tomley and Shirley, 2009), and the role that manure may have in the spread of the disease remains to be elucidated.

HEV is a non-enveloped, single-stranded RNA virus commonly associated with large-scale waterborne acute hepatitis epidemics and sporadic infections, particularly in developing countries (Khuroo, 2008). The transmission of HEV to humans can occur via a number of routes, including parenterally in association with blood transfusion, or vertically, although transmission primarily occurs via the faecal–oral route through contaminated water or the meat of wild or domestic animals (Goens and Perdue, 2004; Khuroo, 2008). In endemic countries, HEV is reported to account for in excess of 50% of cases of hepatitis

(Dalton *et al.*, 2008). While primarily associated with developing countries and with travel within these, autochthonous HEV infections in developed countries are becoming recognized as a more important source of infection than previously recognized (Dalton *et al.*, 2008). The main source for sporadic infection in developed countries remains unclear, though virological evidence of HEV has been found in pigs and antibodies to HEV have been detected in other domestic livestock, including cattle, sheep, goats and horses (Dalton *et al.*, 2008). Studies have shown that 50–90% of pigs are anti-HEV seropositive, and that infected animals have the capacity to shed high levels of infective HEV in their faeces for weeks post-infection (Teo, 2006). The exact mechanisms of transmission of autochthonous HEV infections in developed countries are unknown, but it is probable that zoonotic foodborne transmission via manure, particularly from pigs, is involved (Lewis *et al.*, 2009).

Exposure to a number of viruses associated with cancer development in food animals has been implicated as a potential mediator of cancer development in humans (Johnson *et al.*, 1997; Fritschi, 2000; Johnson *et al.*, 2007). Persons at most risk are those in close contact with animals, such as veterinarians, animal workers and meat workers. Of particular note are viruses associated with poultry: the avian leukosis/sarcoma viruses, reticuloendotheliosis viruses and Marek's disease virus (Netto and Johnson, 2003), all of which may be shed in faeces and therefore incorporated into manure (Weyl and Dougherty, 1977; Cole *et al.*, 1999). The significance of these viruses in human cancer development, however, remains to be conclusively established.

Manure as a Source of Antimicrobial-resistant Organisms/ Resistance Genes

In addition to contributing to zoonotic disease directly or through the introduction of pathogens into the environment, manure can also contribute indirectly to zoonotic disease via the use of antimicrobials in animal agriculture. Varying classes of antimicrobials are used in the livestock industries as either growth promotants or prophylactics, or are used for the treatment of disease, either at the herd level or in individual animals (Prescott, 2008; Silbergeld *et al.*, 2008; Venglovsky *et al.*, 2009). The development of communities of organisms resistant to these compounds is well documented in production animals (Aarestrup *et al.*, 2008; Call *et al.*, 2008; Gyles, 2008).

The establishment of antimicrobial-resistant bacterial populations in production animals poses a risk to humans in two key areas: (i) as a source of antimicrobial-resistant organisms with the potential to infect humans; and (ii) as a reservoir of antimicrobial resistance genes and their precursors, contained in both pathogenic and non-pathogenic bacteria, and termed the 'resistome' (Wright, 2007). The development of such a resistome facilitates the acquisition of antimicrobial resistance by pathogenic bacteria. Horizontal gene transfer has been demonstrated to occur in a number of complex media, including both the gastrointestinal tract and animal faeces (Walsh and Fan-

ning, 2008) and, as such, manure constitutes a potential source of infection or environmental contamination by organisms (or genetic elements) that have acquired antimicrobial resistance genes from these environments. Interestingly, this is not the only mechanism by which manure may be involved in the development of antimicrobial resistance. Manure may also contain significant quantities of antimicrobial residues, and its application to the environment – largely through the use of manure as fertilizer – poses environmental problems through both the toxicity of these residues to soil microorganisms and the potential of this practice to increase antimicrobial resistance in environmental microorganisms (Venglovsky *et al.*, 2009). Furthermore, experiments evaluating the effects of applying manure containing varying levels of sulfamethazine to maize (*Zea mays*), lettuce (*Lactuca sativa*) and potato (*Solanum tuberosum*) crops showed a positive correlation between antimicrobial concentrations in the manure and in the plant tissue (Dolliver *et al.*, 2007), leading the authors to question the human-health implications of this process.

Other Notable Zoonoses Contractible from Manure

Disease associated with organisms contractible from manure occurs, largely, via the faecal–oral route and manifests as gastrointestinal disease. In excess of 100 zoonotic pathogens have been described that affect humans through entry into the food chain (Walton and White, 1981). Despite the large number of zoonotic pathogens that have the potential to cause disease in humans, the vast majority of disease is attributable to only a few organisms (Mead *et al.*, 1999). This section will, very briefly, focus on a small number of other zoonotic pathogens of note, contractible through exposure to manure, which have not been covered in this chapter so far.

Numerous bacterial species constitute potential zoonoses contractible through manure exposure. *Yersinia enterocolitica*, a foodborne pathogen responsible for acute gastroenteritis and mesenteric lymphadenitis, is the third most reported zoonosis in the EU (Laukkanen *et al.*, 2009). Pigs are considered to be a major reservoir of this organism and infection is most frequently associated with undercooked pork (Tauxe, 1997; Farzan *et al.*, 2009; Laukkanen *et al.*, 2009). *Helicobacter* spp. are associated with human gastric disease and have been found in sheep (*Helicobacter pylori*), pigs (*Helicobacter suis*) and cattle (Candidatus *Helicobacter bovis*) (Haesebrouck *et al.*, 2009). *Clostridium difficile* has recently been demonstrated in cattle, pigs and broiler chickens, and in retail meat from all three animals (Indra *et al.*, 2009), while *Clostridium perfringens* inhabits the gastrointestinal system of numerous animal species, including production animals (Uzal and Songer, 2008).

Maternal and neonatal tetanus is reportedly responsible for around 180,000 deaths worldwide annually (Roper *et al.*, 2007), predominantly in developing countries, and the elimination of this disease is an objective of the World Health Organization (WHO, 2006). The organism responsible for tetanus, *Clostridium tetani*, can be found in the faeces of numerous domestic animal species (Edlich *et al.*, 2003). In some developing countries, ghee (clarified

butter) is applied to the umbilical wounds of neonates for its perceived healing and strengthening powers. When the ghee is heated with cow dung fuel, this practice has been shown to be significantly associated with the development of neonatal tetanus (Bennett *et al.*, 1999).

Brucella melitensis, a re-emerging zoonotic organism and the leading cause of brucellosis, a febrile disease, has been reported to be associated with occupational contact with manure, particularly from goats, but also from sheep and cattle (Corbel, 1997; Wallach *et al.*, 1997). Similarly, contact with manure has been associated with the development of leptospirosis (Diesch, 1971; Levett, 2001) and Q fever (*Coxiella burnetii*) (Salmon *et al.*, 1982; Jorm *et al.*, 1990; Berri *et al.*, 2003), while *Rhodococcus equi* has been found in the manure of a wide variety of herbivores (Prescott, 1991; Votava *et al.*, 1997). *Erysipelothrix rhusiopathiae*, although most commonly associated with pigs and turkeys, is shed in the faeces of a number of species, including cattle, sheep and horses (Pell, 1997); clinical presentation of *E. rhusiopathiae* infection is, most commonly, a localized, non-pyogenic cellulitis, typically on the hands; however, other more serious syndromes may also be associated the infection (Brooke and Riley, 1999). *Streptococcus suis* type 2 is a communal bacterium of swine tonsils and may also be found in the gastrointestinal tract and faeces of swine (Devriese *et al.*, 1994); meningitis resulting from *S. suis* infection has been associated with exposure to pigs (Pell, 1997). *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) also warrants mention for its putative, albeit contentious, role in Crohn's disease (Mendoza *et al.*, 2009; Pierce, 2009). *M. paratuberculosis* is the aetiological agent of Johne's disease, which constitutes a major economic and veterinary issue of the agricultural industries, particularly the cattle industry (Harris and Barletta, 2001). Cattle are not the only animals affected by *M. paratuberculosis*; Johne's disease has been reported in other ruminants as well as in pigs and rabbits (Harris and Barletta, 2001). Infection typically occurs via the faecal–oral route, either vertically or through exposure to manure-contaminated environments, and studies have shown that *M. paratuberculosis* remains viable in faeces, water and cattle slurry for up to 250 days (Harris and Barletta, 2001; Begg and Whittington, 2008).

Poultry constitute a potential source of a number of other zoonotic microorganisms. In birds, *Bacillus anthracis* infections are usually asymptomatic. Chickens appear to be particularly resistant and pose a risk of both infection and environmental contamination through the shedding of spores in faeces (Senanayake and Baker, 2007). *Cryptococcus neoformans* is associated with the faeces of birds, including chickens, but particularly pigeons (Emmons, 1955; Levitz, 1991; Bovers *et al.*, 2008). Clinical cases of cryptococcosis are relatively rare, and studies indicate that most people mount a sufficient immune response to suppress disease upon exposure to the organism (Levitz, 1991); studies of pigeon breeders indicated that, while exposure to *C. neoformans* is common, there was not an increased incidence of cryptococcosis (Newberry *et al.*, 1967). Psittacosis is a potentially fatal disease resulting from infection with *Chlamydophila psittaci*. Clinical symptoms are highly variable, and infection typically presents as respiratory infection, but can progress to affect other organs (Beeckman and Vanrompay, 2009). *C. psittaci* infections are reported

to occur in over 465 bird species, including chickens, turkeys, ducks and geese (Kaleta and Taday, 2003); disease is predominantly caused through the inhalation of aerosolized urine, respiratory or eye secretions, or from dried faeces (Beeckman and Vanrompay, 2009).

Treating Manure to Reduce the Risk

The development of zoonotic disease through exposure, either direct or indirect, to livestock manure constitutes a real and significant risk of public-health concern. Manure may potentially harbour numerous species of zoonotic pathogens and, for many zoonotic microorganisms, constitutes a significant reservoir. The public-health risk posed by manure has increased with both the intensification of the livestock industries and the use of manure outside these industries (Martens and Bohm, 2009). This risk has driven the development of numerous methods to mitigate the potential of manure to harbour pathogenic microorganisms, and these are discussed in great detail elsewhere (Bicudo and Goyal, 2003; Burton and Turner, 2003). Manure-treatment regimes can be classified as chemical, physical or biological in process, and are typically applied as either as a general prophylactic hygiene measure or as a means of controlling or eradicating a specific organism or group of organisms, such as an organism responsible for a notifiable disease (Martens and Bohm, 2009). It should be noted that reduction in pathogen numbers is not necessarily the primary objective in manure management regimes; treatment is often applied for other purposes, such as ammonia stripping or phosphate precipitation (Martens and Bohm, 2009). Despite this, these practices may have a positive effect through reduction of pathogen viability.

A number of compounds have been identified that can be used as a means of chemical treatment of both solid manure and slurries. These chemicals include: limewash, caustic soda, formalin, peracetic acid and calcium cyanamide (Burton and Turner, 2003). Chemical treatment of manure is, however, most commonly employed as a means of control during an epidemic, rather than as a routine means of controlling manure-associated pathogens. Furthermore, the non-microbial residues left from chemical treatment processes may constitute a greater environmental risk than the microbial pathogens themselves (Cliver, 2009). Physical manure-treatment regimes, such as thermal treatment or irradiation, can also be employed as a means of disinfecting manure. This process, with the exception of the drying of poultry manure and exposure of spread manure to sunlight, is not commonly used for routine manure management, although it is, once again, employed by some countries for the control of certain disease outbreaks, such as foot-and-mouth disease (Martens and Bohm, 2009). Biological systems employed for the control of pathogenic microorganisms in manure include both aerobic and anaerobic biotechnological treatments; the function of these systems is described in greater detail elsewhere (Bicudo and Goyal, 2003).

Biological systems achieve a reduction in viable pathogen numbers through a number of factors, including: antibiosis, pH alterations, redox-potential

adjustments, antagonism, nutrient deficiencies and exothermic metabolism, but the most effective factor has been demonstrated to be through elevation in temperature (Martens and Bohm, 2009). Manure is commonly treated in either batches or under a semi-batch manner (a cycle consisting of a short feeding period followed by a long stabilization period) and can be processed either in the mesophilic or the thermophilic range (Martens and Bohm, 2009). The composting of manure has also been shown to be effective in reducing pathogen numbers (Bernal *et al.*, 2009). The simple act of storage alone has been demonstrated to reduce both viable pathogen levels in manure and the presence of virulence genes (Duriez *et al.*, 2008). The act of storing manure before use can, essentially, be classified as a biotechnological treatment. Survival rates, however, vary markedly depending on the microorganism monitored, the source and physical properties of the manure and the climatic conditions. Furthermore, storage alone is unlikely to achieve safe and efficient decontamination of all pathogens (Bicudo and Goyal, 2003).

Appropriate treatment has the capacity to significantly reduce pathogen levels contained in manure, and, consequently, reduce the risk associated with the use of this material. As such, manure management policies and legislation have been developed within numerous countries aimed at limiting the negative impacts of manure usage (Burton, 2009). A number of factors need to be considered in determining the most appropriate system for controlling potentially pathogenic microorganisms within manure. Considerations to be taken into account include, but are not limited to: the physical properties of the manure to be treated (solid manure or slurry); the pathogens that are potentially associated within the material to be treated; the eventual application of the treated manure (some processes can adversely alter the physiochemical properties of manure; Martens and Bohm, 2009); the risk posed by these microorganisms through its intended use of the manure; and the economics of treating the manure. The best results for ameliorating the risk posed by zoonotic organisms associated with manure are achieved through the adoption of integrated systemic approaches: employing management strategies to eliminate these organisms from their animal reservoirs (Cliver, 2009); selecting and implementing an appropriate manure management and treatment system (Bicudo and Goyal, 2003; Burton and Turner, 2003); identifying and employing low-risk manure uses, such as utilizing as much manure as possible in low-risk cropping systems (Burton, 2009); and identifying areas of particular risk and minimizing these through appropriate management practices (Zhao *et al.*, 2001; Stecchini and Del Torre, 2005).

Summary

Manure is a combination of livestock excreta (urine and faeces) mixed with bedding materials, traditionally viewed as an agricultural waste product, and disposed of through its dissemination into the environment. Large amounts

of manure are produced, utilized for a number of purposes, and eventually reintroduced into the environment; annual manure production by housed livestock alone, in England and Wales, is estimated at 70 million tonnes (Hutchison *et al.*, 2000), while the US cattle industries are estimated to produce 1.2 billion tons annually. The view of manure as an agricultural by-product necessitating disposal is, however, shifting and its value is becoming increasingly recognized, despite the fact that it contains high numbers of microorganisms, including, potentially, a large variety of organisms capable of causing disease in humans. These organisms comprise a large number of bacterial species, such as *E. coli* and *Salmonella*, protozoa and viruses, which are responsible, in particular, for many of the reported foodborne disease outbreaks. Furthermore, manure can contribute to zoonotic disease through the introduction of antimicrobial resistance genes, and even antibiotics themselves, into the environment, where they can be horizontally transferred, or selected for antimicrobial-resistant pathogens, respectively. While manure is a valuable agricultural commodity, this must be weighed against the risk of spreading zoonotic disease.

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4 Animal Feed as a Source of Zoonotic Pathogens

RICHARD A. HOLLEY

Introduction

Steps to effectively control bacterial pathogens in food responsible for causing illness in humans will be possible once it is understood where vulnerabilities lie in the food production, manufacture, distribution and preparation continuum. Recent foodborne illness outbreaks worldwide, where produce was contaminated by *Salmonella enterica* serovars or verocytotoxigenic *Escherichia coli* (VTEC) (most often *E. coli* O157:H7), point to a variety of causes, largely represented by lapses in maintenance of accepted sanitary practice (Beuchat, 2006). Yet where there has been adoption of these practices, there appears to have been little recent progress towards reducing overall frequencies of foodborne illness caused by the major bacterial pathogens (CDC, 2009). This suggests that a fresh approach is needed.

Unlike the rest of Europe (Larsson, 2007) and North America (Sapkota *et al.*, 2007), the Scandinavian countries of Finland (Maijala *et al.*, 2005) and Norway (Vestby *et al.*, 2009) have, for more than 17 years, adopted a policy of zero tolerance for all serovars of *Salmonella* in the food and animal feed supply. This concentration of effort to control *Salmonella* has yielded reduced rates of human gastroenteritis caused by these organisms (Mäkelä, 2007), but perhaps at the expense of illnesses caused by *Campylobacter* and VTEC. *Salmonella* control in feed and food in Denmark (Hald *et al.*, 2006; Hermansson, 2007) and Sweden (Wierup, 2006) is less aggressive than in Norway and Finland, but is more stringent than in the remaining EU countries.

The link between the frequency of animal carcass contamination by *Campylobacter*, *Salmonella* or VTEC and human illness caused by the consumption of contaminated food of animal origin is well established (Doyle and Erickson, 2004; Callaway *et al.*, 2008). Evidence indicating that environmental contamination by manure and waste products from food production can yield

contaminated produce is also strong (Rogers and Haines, 2005; Beuchat, 2006). Our inability to decontaminate produce has highlighted the importance of the prevention of preharvest contamination of fruits and vegetables to control their contamination at consumption (Beuchat, 2006; Doane *et al.*, 2007).

Infection of livestock and poultry by *Campylobacter, Salmonella* and *E. coli* O157:H7 is followed by variable periods of pathogen shedding (sometimes at high numbers), but clinical symptoms are rarely seen in the infected animals. This is also true of *Yersinia* in hogs (Nesbakken, 2006), but, in contrast, *Listeria monocytogenes* causes mortality in small ruminants (sheep and goats) although it is carried asymptomatically and is shed by cattle (Jemmi and Stephan, 2006). Further, while *L. monocytogenes* is ubiquitous in the animal environment, the other major foodborne illness agents (*Salmonella*, VTEC and *Yersinia*) can be widely distributed but are not truly ubiquitous as animals are commercially raised in their absence (Forshell and Wierup, 2006). The situation with *Campylobacter* is poorly understood. Cattle appear to be a major reservoir for *E. coli* O157:H7, hogs are a reservoir for *Yersinia enterocolitica* (Laukkanen *et al.*, 2009), and poultry are more commonly contaminated by *Salmonella* and *Campylobacter*. Sources of these zoonotic pathogens include other animals, the animal environment, and feed and water (Doyle and Erickson, 2004). Intensified animal production facilitates horizontal transfer of these organisms among flocks and herds when individual contaminated animals are present. Animal passage amplifies the numbers of these zoonotic pathogens and accelerates their spread on the farm.

Studies in the UK and the USA show that VTEC is present on most cattle farms, at least occasionally, with frequencies of positive faecal samples ranging from 0.5% to 36%, with a mean of 15.7% (Collins and Wall, 2004). In the USA, 27–31% of dairy, 25–48% of swine herds and 19% of poultry flocks were found to have animals shedding *Salmonella* (Callaway *et al.*, 2008), and the latter summary included the observation that 60% of swine farms in Alberta, Canada, were *Salmonella* positive. Rogers and Haines (2005) noted that, in the USA, 18% of whole chickens and 45% of turkeys were *Salmonella* positive, but 95% of chickens and 2% of turkeys were *Campylobacter* positive. In the EU, *Salmonella* contamination of broiler flocks ranged from 0.2% to 66%, and 0–55% of swine herds and 0–7% of cattle herds were *Salmonella* positive. The largest variation in *Salmonella* frequencies occurred at the country level, with values being higher in southern Europe (Mäkelä, 2007).

Scandinavian countries reported values of animal and food contamination by *Salmonella* of <1% (Maijala *et al.*, 2005; Wierup, 2006). The rare occurrence of salmonellosis in Finland and Norway (80% of cases are travel related, according to Lunestad *et al.*, 2007), and the lower frequency in Sweden than in most of the other countries for which data are available (Table 4.1), suggest that a solution to the problem of salmonellosis in the rest of the EU and North America might be evident by examining country-specific policies regarding animal husbandry. Data from Denmark are less likely to provide a solution because salmonellosis frequency is higher than in the USA or Canada. Several years ago, upper limits or National Health Objectives were set in the USA for illnesses caused by *Salmonella*, VTEC, *Listeria* and *Campylobacter*.

Table 4.1.   Frequencies of human illnesses per 100,000 persons caused by zoonotic bacteria.

Country/region	Salmonella	Campylobacter	Escherichia coli (verocytotoxigenic)	Listeria monocytogenes	Yersinia
USA[a]	16.2	12.7	1.1	0.29	0.36
EU[b]	35	46	1.5	<1	2.0
Denmark[c]	26	_[f]	_[f]	_[f]	_[f]
Sweden[d]	5.6	_[f]	_[f]	_[f]	_[f]
Canada[e]	18	29.7	3.1	_[f]	1.8

[a] CDC (2009) (data from 2008). *Salmonella* frequency in the state of Georgia was 30.2 per 100,000 persons.
[b] Mäkelä (2007) European Food Safety Agency (data from 2006, applies to 27 EU countries).
[c] Wegener (2009) Danish Zoonosis Centre (data from 2007), DTU National Food Institute.
[d] Forshell (2006).
[e] PHAC (2007) Public Health Agency of Canada (data from 2006).
[f] Data not available.

Targets were to be achieved by 2010, but in 2009 salmonellosis in the USA was 2.4 times higher than the objective (CDC, 2009).

In the following discussion, the available data will be evaluated to determine the importance of feed contamination as a vector for zoonotic pathogen contamination of livestock and to examine the link between this contamination and foodborne illness in humans caused by the pathogens.

Feed as a Potential Disease Vector

The animal feed industry is international, and ingredients are frequently accessed from developing countries in South America, South Asia and Africa. The industry plays a vitally important role by formulating livestock and poultry diets to satisfy species-specific nutritional needs. In addition to forages, grain, seed meals (canola or rapeseed, cotton, soybean, groundnut, safflower), fats, oils, minerals and vitamins, feed will contain by-products of the agri-food industry and waste streams from the poultry, livestock (rendered, inedible protein, carcasses) and marine food (fishmeal) industries (Sapkota *et al.*, 2007). Although this recycling of nutrients contributes significantly to the sustainability of agriculture, it may also serve to concentrate, amplify and recycle zoonotic pathogens as well as redistribute heavy metals and other toxic substances.

Realization of the risk to human health from an unprotected animal feed supply has come from the UK and North American experience with mad cow disease (bovine spongiform encephalopathy, BSE), and dioxin contamination of poultry feed in Belgium in 1999 (Sapkota *et al.*, 2007) and of hog (pig/swine) feed in Ireland in 2008 (Wall, 2009).

The animal feed industry since the early 1970s has become large and complex to serve the needs of an integrated and intense animal production industry

in developed countries. The feed industry in the USA produced 120 million tons of primary feed in 2004 (Sapkota *et al.*, 2007) and in the EU feed production was estimated to be 420 million tonnes yearly (EFSA, 2008). To address issues associated with contamination by biological and chemical agents, the EU officially recognized that animal feed was part of the human food chain by passing the Feed Hygiene Regulation (EC183/2005). This regulation does not specify zero tolerance for specific zoonotic bacterial pathogens in animal feed but does require that they be 'controlled' (EC, 2005). In practice, in the EU, *Salmonella* control in the feed industry ranges from the very strict control in place in most of the Scandinavian countries to essentially no strategy at all in others (Larsson, 2007).

Zoonotic Pathogens in Feed

Work conducted since the early 1990s to evaluate the extent of *Salmonella* contamination of animal feed in North America has yielded widely variable results. Feed ingredient and mixed (compound) feed contained *Salmonella* in 25–50% of samples (Whyte *et al.*, 2003; Maciorowski *et al.*, 2006, 2007; Sapkota *et al.*, 2007). Rates of <8% were not uncommon (Jones and Richardson, 2004; Rodriguez *et al.*, 2006), but these usually climbed once there was uncontrolled access to the feed (Kidd *et al.*, 2002). Pelleted feed was usually less frequently contaminated (4–9%) (Jones and Richardson 2004; Bucher *et al.*, 2007). Similarly, in the EU, results from *Salmonella* analyses of feed varied widely among countries: from 0.3% in Norwegian fish feed (Lunestad *et al.*, 2007) to <8% in feed imported to Sweden (Lofstrom *et al.*, 2004), and 3.4% in pig feed before final heat treatment in Switzerland (Sauli *et al.*, 2005). In the EU in general, *Salmonella* was present in compound feed at rates of 0–9.5% and in feed ingredients at rates of <2.5% (Mäkelä, 2007). Younus *et al.* (2000) reported that 21% of poultry feed samples analysed in Pakistan were *Salmonella* positive. In most EU countries, poultry feed is given a lethal heat treatment, often after the addition of an organic acid (1–2% formic acid) to kill *Salmonella* (75°C for >30 s). This is a mandatory requirement in Sweden (Häggblom, 2006) and Ireland, but 60 s is used in Ireland (Whyte *et al.*, 2003). Factors affecting the lethality of these treatments and combinations of formic and propionic acid have been discussed (Maciorowski *et al.*, 2006).

Less work has been done to examine animal feed for contamination by *E. coli* O157:H7, and work cited here is from the USA. Hancock *et al.* (2001) and Davis *et al.* (2003) found *E. coli* O157:H7 present in <0.8% of feed samples. However, Doane *et al.* (2007) found the organism present in >17% of fresh poultry and swine feed in a five-farm survey conducted over a 2-year period. Levels of feed contamination by both *Salmonella* and *E. coli* O157:H7 are significant when it is considered that cattle consume about 35 kg feed day^{-1}. Numbers of *Salmonella* present in feed have been reported to range from 1 cell g^{-1} to 3 log cfu (colony forming units) g^{-1} (Lofstrum *et al.*, 2004; Franco, 2005). The frequencies of feed contamination reported here do not include data where animals had contact with the feed and have not considered the

potential growth of both of these organisms in moistened feed. *Campylobacter* does not appear to survive long enough in animal feed to be detected (Whyte *et al.*, 2003; Hansson *et al.*, 2007; Rasschaert *et al.*, 2007). When tests have been done, *Y. enterocolitica* has not been found in animal (hog) feed (Gurtler *et al.*, 2005). In contrast, poor-quality or improperly fermented silage is a vehicle for the transmission of *L. monocytogenes* to cattle (Nightingale *et al.*, 2004).

Risk of Feed Ingredient Contamination

Salmonella is regarded in many countries as the most important bacterial pathogen in feed because it is a major cause of human illnesses worldwide (Forshell and Wierup, 2006). In the opinion of many, *Salmonella*-contaminated feed is of concern because its consumption increases periods of asymptomatic animal shedding, increases carcass contamination and influences the occurrence of human illnesses (Davies, 2006; Sapkota *et al.*, 2007). Of significant importance in some countries is the presence of VTEC O157:H7 in feed (Doyle and Erickson 2004; Doane *et al.*, 2007) because of its potential, as with *Salmonella*, to contaminate produce that is not usually cooked before consumption (Rogers and Haines 2005; Beuchat, 2006).

Although many ingredients may find their way into feed (Sapkota *et al.*, 2007) the risk that some may be contaminated by *Salmonella* is greater than other risks, and this is likely to be true for *E.coli* O157:H7 as well, although little information on the latter organism is currently available. Maciorowski *et al.* (2007) and Hancock *et al.* (2001) provided evidence that *E. coli* O157:H7 is transmitted to cattle by contaminated feed. Animal feed ingredients considered by regulators in most countries to be at high risk of contamination by *Salmonella* are rendered animal protein meals and products from the vegetable oil seed-crushing industry, including meals from soybean seeds, rapeseed (canola), palm kernels, sunflower seeds, cotton-seed and safflower seeds (Salomonsson *et al.*, 2005). In addition, fishmeal and eggshells also represent considerable risk of *Salmonella* contamination when used as feed ingredients (Larsson, 2007). Most often, cereals and cereal products are considered low-risk materials, but some suppliers (poor performers) can change the level of risk associated with these ingredients (Hermansson, 2007). In reiterating the high-risk ingredients for *Salmonella* contamination of feed noted above, EFSA (2008) noted that non-processed soybeans were often found to be *Salmonella* positive.

With the withdrawal of bonemeal as a feed ingredient in 1991 – during the BSE crisis – oilseed meals increasingly have become a popular protein and energy substitute (Morita *et al.*, 2006). Their more widespread use as feed ingredients will have a greater influence on the final contamination of compound feeds by *Salmonella*.

Salmonella spp. are uniquely adapted to survive in the dry environments found in oilseed, fishmeal, animal-rendering plants and feed compounding mills, as well as in poultry-rearing environments, and can survive in niches there for years (Pedersen *et al.*, 2008). Thermal treatments used in animal-protein

rendering and oilseed extraction are sufficient to eliminate the organism; however, final meals become contaminated after the heating step. *Salmonella* can survive on oily equipment surfaces and floors, be aerosolized in dust in the plant and recontaminate the meal at cooling. *Salmonella* spp. are able to grow in regions of the meal where condensation from the cooling of equipment has contacted the meal (Morita *et al.*, 2006).

Both *E. coli* O157:H7 (Davis *et al.*, 2003) and *Salmonella* have been frequently found in compounding feed mills (Whyte *et al.*, 2003; Maciorowski *et al.*, 2006; Lunestad *et al.*, 2007). Although a persistent strain of *S. enterica* serovar Senftenberg (*S.* Senftenberg) was not found to have enhanced resistance to desiccation (Pedersen *et al.*, 2008), strains of *Salmonella* more capable of forming biofilms in feed mills were most likely to contaminate the plant and feed (Vestby *et al.*, 2009).

As with oilseed crushing mills, in compound feed mills at the pellet forming operation, condensation at the pellet cooler and contaminated dust at the cooler air intake have been implicated as sites where *Salmonella* contamination of feed occurs (Jones and Richardson, 2004; Maciorowski *et al.*, 2006). Even though thermal treatment of pelleted feed reduced *Salmonella* contamination, Lo Fo Wong *et al.* (2004) found during hog feeding studies that hogs fed pelleted feed were more likely to be *Salmonella* positive than those fed mash feed and whey. Particle size may have been a factor that influenced *Salmonella* survival in the animals. None the less, the recently revised and released code of practice for voluntary control of *Salmonella* in animal feeds by the UK (DEFRA, 2009) should prove to be of value in setting standards for minimizing *Salmonella* contamination during feed-mill operation.

Animal Feed-derived Human Health Effects

Although it is clear that animals are infected by *Salmonella* or *E. coli* O157:H7 in contaminated feed, it is less clearly established whether these infectious agents in feed are responsible for clinical illness in humans. Proof for such a relationship is only possible from a continuous line of evidence from surveillance of veterinary and human health programmes that monitor agents in feed, health effects in animals (detection of infection), presence of the contaminant in animal- (or plant-) based foods, and illnesses in humans. Systems capable of monitoring sequential movement of a pathogen through this chain of locations do not exist worldwide. Various reports from many countries have only characterized either the first or last two steps of the chain of transmission (Crump *et al.*, 2002; Sapkota *et al.*, 2007).

Perhaps the most complete line of evidence for concluding that feed can be the source of a zoonotic pathogen causing human foodborne illness comes from the report of an outbreak of illness in the USA caused by *S. enterica* serovar Agona (*S.* Agona) contamination of imported Peruvian fishmeal formulated into poultry feed (Clark *et al.*, 1973). Chickens contaminated by the feed were consumed and caused illness, initially in Arkansas, but later in several other US states. In parallel, the Peruvian fish meal was shipped to the UK,

Israel and the Netherlands, and similarly caused foodborne illness there. This introduction of *S.* Agona was visible because at that time (1968) this serovar was unusual in humans. During the next few years, *S.* Agona became established and caused over 1 million human cases of salmonellosis in the USA alone (Crump *et al.*, 2002), and in 2005 it was one of the top ten serovars of *Salmonella* isolated from most livestock and poultry species in the USA (Foley and Lynne, 2008). Currently, the two serovars causing most cases of salmonellosis in the USA are *S. enterica* serovar Typhimurium (*S.* Typhimurium) and *S. enterica* serovar Enteritidis (*S.* Enteritidis). Serovars of human-health importance change over time. In the 1970s, *S.* Agona was important; in the 1980s *S.* Enteritidis was most important; and in the 1990s *S.* Typhimurium DT 104 dominated in the USA (Maciorowski *et al.*, 2007). Franco (2003) questioned the reliability of evidence in the Crump *et al.* (2002) report linking feed contamination to human illness, and noted that the majority of *S. enterica* serovars isolated from feed were rarely found in humans. Other studies support this observation (Franco, 2005; Häggblom, 2006). Crump *et al.* (2003) pointed out that although *Salmonella* serovars differ in virulence, data indicate that all serovars have the potential to cause human illness. Hald *et al.* (2006) essentially agreed, and the Danish government regulatory authority (Plant Directorate) used their data in adopting the position that any *Salmonella* serovar was dangerous in feed.

Salmonella serovar distribution

Variable results regarding the similarity of *Salmonella* serovars in feed and human disease are found in the literature. Although Younus *et al.* (2009) found *S.* Enteritidis or *S.* Typhimurium in 10–21% of feed in Pakistan, these serovars were rarely isolated from feed in Sweden (Häggblom, 2006) or Norway (Lunestad *et al.*, 2007). Of 194 *S. enterica* isolates serotyped following their isolation from rendered animal protein meal, Franco (2005) found that, when combined, 7.5% were *S.* Typhimurium, *S.* Enteritidis, *S.* Agona and *S. enterica* serovar Infantis (*S.* Infantis). Maciorowski *et al.* (2006) noted earlier studies in which isolates of *S. enterica* serovar Hadar (*S.* Hadar), *S. enterica* serovar Heidelberg (*S.* Heidelberg), *S. enterica* serovar Virchow (*S.* Virchow) and *S.* Agona from human illness and present in chickens and cattle were traced back to contaminated bone and fish meal. Lunestad *et al.* (2007) found that the most common serovars in Norwegian fish meal were *S.* Senftenberg and *S. enterica* serovar Montevideo (*S.* Montevideo), which had established themselves as house strains in feed factories. They also noted from epidemiological studies that common *Salmonella* serovars in feed ingredients, fish feed and feed-factory environments accounted for about 2% of the *Salmonella* isolates from domestically acquired human salmonellosis cases in Norway. Similarly, Hald *et al.* (2006) estimated that 1.7–2.1% of human salmonellosis cases in Denmark were the result of contaminated feed. Angulo (2004) in the USA estimated that 10% of foodborne salmonellosis cases were caused by contaminated feed.

Underestimating *Salmonella* serovar importance

An approach to understanding the frequent disparity in serovar identity between isolates from feed and those from animals (Hald *et al.*, 2006) was offered by Davies (2006). It was suggested that where differences have been observed, a source other than feed was likely to be predominant. However, this conclusion was not considered to have ruled out the possibility that feed may have been the original source of infection at some earlier point in time. Complicating understanding of this issue is the different ability of *Salmonella* serovars to be dominant in the inhospitable feed environment or the more hospitable animal intestinal environment. Further, in animals the dominant serovars change as shedding occurs. Infection may be briefly transient or sustained for months in animal reservoirs, and require intervention. In addition, the detection of one serovar may obscure the simultaneous presence of another. A concern here is that an 'exotic' or rare strain in imported feed may become established in new regions (Clark *et al.*, 1973). While there appears to be doubt about the public-health significance of feed-related *Salmonella* serovars, there is little doubt that feed (especially imported products) represents significant risk as a vehicle for the establishment of future epidemic strains in the food chain (Davies, 2006).

Weight of evidence

At an expert meeting in Rome convened by FAO/WHO (2008), it was concluded that there was insufficient scientific evidence to define the importance of feed in disease transmission and food safety. There appeared to be little correlation between contaminated feed and the infection of livestock by the same strain of *Salmonella* implicated in the contamination of meat, milk or eggs produced from these animals. Clearly there is a need for further study of the transmission of zoonotic pathogens, particularly *Salmonella*, along the feed–animal–food chain to humans. But even more basic studies are needed to better characterize the dynamics of *Salmonella* serovar dominance in the chain. Another neglected area is the contribution that on-farm feed formulation and mixing of feed may make to the overall burden of animal contamination.

Feed Decontamination

Some study has been directed toward feed decontamination, but there is need for further work in this area (EFSA, 2008). In the Nordic countries – Sweden, Norway, Finland and Iceland – all feed must be analysed and found *Salmonella* negative before it is delivered to farms (Herland, 2006). Feed for poultry farms in most EU countries is heat treated at ≥75°C for 30 s to 2 min (Hermansson, 2007; Larsson, 2007; Hendricksen *et al.*, 2008). When found *Salmonella* positive, feed is treated first with an organic acid (formic acid) and then heated at pelleting (Häggblom, 2006). However, Aspar (2007) offered an industry (EU

Grain and Oilseed Trader's Association) view that when large volumes of bulk feed are imported, heating becomes expensive and it is difficult to apply organic acid treatments. Although Hazard Analysis Critical Control Point (HACCP) systems are strongly recommended in the EU countries, it is not a requirement on farm at mixing. It was suggested that *Salmonella* should be a point of attention (POA) rather than a critical control point (CCP) in feed, and that the presence of *Salmonella* serovars other than those mentioned in EU Regulation 1003/2005 should not trigger the requirement for heat treatment or a report to the EFSA rapid alert system for food and feed (RASFF). Clearly, the feed industry is unconvinced that *Salmonella* in feed represents an important risk to human health, even in Europe. It is also significant that Danish *Salmonella* control over increasingly larger quantities of imported feed is not as stringent as it was when smaller quantities were imported (Hald *et al.*, 2006).

Use of Antibiotics in Feed

The challenge of proving that the use of antibiotics as growth promoters in feed yields antibiotic-resistant pathogens that cause human foodborne illness is as complex as proving that zoonotic pathogens in animal feed cause food-borne illness in humans. None the less, the EU has again taken the lead and has banned antibiotic use at sub-therapeutic levels in animal feed. Presently, a restricted list is permitted for the latter use in North America, which includes some antibiotics used in human clinical medicine (Sapkota *et al.*, 2007). Concern has been repeatedly expressed that use of sub-therapeutic levels of antibiotics in feed to promote livestock growth elicits the development of antibiotic resistance (sometimes multiple resistances) in bacterial populations (Kidd *et al.*, 2002; Velge *et al.*, 2005; Forshell and Wierup 2006). Examples of resistance development in response to antibiotic use, and the reverse where reductions in isolations of antibiotic-resistant bacteria occur following the removal of antibiotics from the animal diet have been reported (Sapkota *et al.*, 2007). Antibiotic use in animals can have undesirable effects, sometimes triggering the spread of *Salmonella* through a group of closely confined animals (Forshell and Wierup, 2006). In a recent study in the USA, multi-drug resistance was found in 60% of *Salmonella* isolates from poultry raised conventionally (fed feed-grade antimicrobials), but these were found in only 11% of isolates from poultry raised on pasture without access to antibiotics (Siemon *et al.*, 2007). In animals raised intensively, roughly 10–30% of *Salmonella* isolates were antibiotic resistant, but this rose to 60–90% when antibiotics were used (Forshell and Wierup, 2006). Continued use of antibiotics in North American animal feeds is motivated by the economic advantages associated with greater animal production efficiency. In addition, there is the suspicion in North America that withdrawal of antibiotic use as growth promoters will result in a greater total use of antibiotics for animal therapy in response to the development of more frequent clinical conditions in livestock and poultry requiring intervention. However, it is likely in the longer term that antibiotics will not be permitted for use as growth promoters in North America.

Conclusion

Evidence linking the use of feed contaminated by *Salmonella* or *E. coli* O157:H7 to animal infection is strong, and if the expectation is that by reducing the shedding of these zoonotic pathogens we can reduce human illnesses (Doyle and Erickson, 2004), then continuing to use feed contaminated by these organisms on farms is not completely logical. If evidence directly linking animal-feed contamination by these organisms to human illness were stronger, motivation might exist in developed countries to halt this practice. Our current inability to control fresh produce contamination by *Salmonella* and VTEC should serve as a warning sign that a new approach is needed to reduce our vulnerability to foodborne illness. That approach must include the prevention of feed contamination by these organisms. Adoption of zero tolerance in feed, at the beginning of the food continuum, will afford on-farm HACCP plans a critical control point, and thereby make them useful.

In animal environments where *Salmonella* contamination is almost ubiquitous, and cycles of animal reinfection are continuous, the use of *Salmonella*-contaminated feed probably makes little contribution to the level or frequency of animal contamination (Davies, 2006). However, use of contaminated feed ensures maintenance of the positive *Salmonella* status of livestock and poultry (Sapkota *et al.*, 2007), and lengthens the period and extent of animal shedding. Contamination of poultry and hogs by *E. coli* O157:H7 from contaminated feed is a recent development; previously, this organism was limited to cattle (Doane *et al.*, 2007). On farms where *Salmonella* in the environment and animals is controlled, the use of *Salmonella*-contaminated feed can almost instantly change the *Salmonella* status of the animals.

As it is accepted that the frequencies of *Salmonella* and *E. coli* O157:H7 shedding by animals are directly related to foodborne illness (the link is strongest with *Salmonella* and poultry), effort to reduce zoonotic pathogen shedding in animals should be productive in reducing foodborne illness. Recent progress has been achieved in the EU (EFSA, 2008) but the pattern of foodborne illness in the USA during the last 3 years has not responded to the preharvest interventions adopted there (CDC, 2009). The issue of zoonotic pathogen contamination of feed has largely been ignored in North America, but not in Europe. But even in Europe, FAO/WHO (2008) felt there was a need for more scientific evidence to directly link feed contamination to human foodborne illness. This is likely to be partly the result of the Finnish and Norwegian experience, where *Salmonella* serovars in feed are substantially different from those causing illnesses in humans (Maijala *et al.*, 2005; Lunestad *et al.*, 2007). However, the infrequent occurrence of salmonellosis in humans in those countries makes it difficult to draw such conclusions legitimately.

Reluctance to tackle the issue of feed contamination by zoonotic pathogens is understandable given the international character, size and complexity of the feed industry. The existence of reservoirs of *Salmonella* and *E. coli* O157:H7 on the farm serving to reinfect animals also fosters acceptance of the present situation. As neither *Salmonella* nor VTEC is yet ubiquitous on farms,

initiatives to halt the initial introduction of zoonotic pathogens to the farm in the short term will, with other measures such as HACCP, have an immense payback in reducing foodborne illness in humans in the longer term.

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5 Milk and Raw Milk Consumption as a Vector for Human Disease

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Introduction

In many parts of the world, especially in underdeveloped and developing countries, the sale of raw milk is commonplace and a large segment of society consumes raw milk and/or products made from raw milk. An increasing number of people are consuming raw milk in developed countries as well, even if the sale of raw milk is discouraged or prohibited by law. Raw milk is milk from cows, sheep, goats and other animals that has not been pasteurized. Those that advocate consumption of raw milk cite enhanced nutritional qualities, better taste, demand for natural, unprocessed foods and freedom of choice as reasons for increased interest in raw milk consumption. On the one hand, there is a perception by some that raw milk consumption confers health benefits. On the other hand, raw milk has long been recognized as an important source of pathogens that can cause disease in humans. Consequently, many public health and regulatory agencies in various countries throughout the world recommend that milk be pasteurized and oppose the consumption of raw milk because of the potential risks of foodborne pathogen contamination. Pasteurization is a process in which raw milk is heated for a short time to destroy pathogens that may be present. Raw milk advocates claim that pasteurization of milk results in several undesirable effects, which, for the most part, have not been substantiated. The controversy surrounding the consumption of raw or pasteurized milk has been around for decades. However, the increased demand for raw milk has intensified the raw milk debate – and so the debate continues! This chapter re-examines some of these issues, and discusses the potential threats that raw milk consumption poses to consumers.

Raw Milk Consumption

The sale of raw, unpasteurized milk is allowed in many parts of the world, including underdeveloped, developing and developed countries, while in other countries, such as Canada, the sale of raw milk is prohibited. Over 75% of milk marketed in many developing countries is sold raw through informal channels (Staal and Kaguongo, 2003). For example, in East Africa, most milk is produced by smallholders, and the bulk of the milk (86% in Kenya and 92% in Uganda) is traded through informal channels as unpasteurized milk or milk products (Grace *et al.*, 2008). These informal milk markets thrive because they provide social and economic benefits to smallholder producers, small market agents and consumers in terms of higher farm-gate prices, and they also create employment and competitive consumer prices (Kang'ethe *et al.*, 2007). In the USA, it is a violation of federal law to sell raw milk packaged for consumer use across state lines, although intra-state sale of raw milk is legal in many states. According to a recent survey by the US National Associa-tion of State Departments of Agriculture (NASDA, 2008a,b), 29 states allow the sale of raw milk by some means. In places where the purchase of raw milk is prohibited and/or illegal, cow-share or leasing programmes, and the sale of raw milk as pet food, have been used as means for consumers to obtain raw milk.

Estimates of raw milk consumption in the USA are difficult to obtain. The consumption of raw milk has always been common among farm families and farm employees, ranging from 35% to 60% (Rohrbach *et al.*, 1992; Jayarao and Henning, 2001; Jayarao *et al.*, 2006), probably because it is a traditional practice and it is less expensive to take milk from the bulk tank than to buy pasteurized retail milk (Hegarty *et al.*, 2002). Consumption of raw milk by the urban community is more difficult to estimate. Headrick *et al.* (1998), in a study on the epidemiology of raw milk-associated foodborne disease outbreaks in the USA from 1973 to 1992, indicated that raw milk accounted for <1% of the total milk sold in states that permit the sale of raw milk. Headrick *et al.* (1997) conducted another study to determine the prevalence of raw milk consumption in California which, at the time of the study, was the largest pro-ducer of certified raw milk in the USA. Among 3999 survey respondents, 3.2% reported drinking raw milk in the previous year. The demographic and behav-ioural characteristics of raw milk consumers were as follows: raw milk drinkers were more likely than non-drinkers to be younger than age 40, male, Hispanic and to have less than a high school education (Headrick *et al.*, 1997). A more recent estimate reported that 3.5% of people who participated in a survey conducted in 2002 consumed unpasteurized milk within a 7-day period before the survey was taken (CDC, 2004). If the results of this survey and the report by Headrick *et al.* (1997) are representative of the US population, this would imply that over 10.5 million people are consuming raw milk regularly, perhaps daily. This estimate may be too low based on information from The Weston A. Price Foundation (2007), a non-profit education foundation that promotes the consumption of clean raw milk from healthy grass-fed cows, which indi-cated that the demand for raw milk is growing rapidly – by some estimates at

40% per year. The concept of 'produce, sell and buy local' and the demand for natural and unprocessed foods are growing consumer trends that are likely to have resulted in an increased interest in raw milk consumption.

Proposed Benefits of Raw Milk Consumption

A variety of reasons appear to influence the consumption of raw milk by consumers. These include: the perception of a better quality product; enhanced nutritional qualities; health benefits; superior taste; a demand for natural, unprocessed food; consumers who are interested in sustainable agriculture and support producers who use methods that are environmentally friendly; and freedom of choice. An important tenet of raw milk advocates is that milk to be pasteurized is inferior in quality to raw milk that is to be consumed directly. The number of somatic cells in milk, referred to as the somatic cell count or SCC, is used throughout the world as an indicator of milk quality. Poor-quality milk has a high number of somatic cells, while excellent-quality milk has a very low number of somatic cells. The current regulatory limit for somatic cells in milk in the USA, as defined in the Pasteurized Milk Ordinance, is 750,000 ml^{-1} (US FDA, 2007). There is continuing pressure in the USA from a variety of groups and organizations to reduce the regulatory limit for somatic cells in milk from the current 750,000 ml^{-1} to 400,000 ml^{-1} or less in order to be more competitive with the EU and other countries that have a lower SCC limit. A recent report published by the US Department of Agriculture (USDA) Animal Improvement Program Laboratory (Norman *et al.*, 2009) summarized SCC data from all herds in the USA enrolled in the Dairy Herd Improvement (DHI) testing programme for 2008. The good news is that the national SCC average for 2008 was 262,000 cells ml^{-1} of milk, which is 14,000 cells ml^{-1}, or 3%, lower than it was in 2007 (Miller *et al.*, 2008). The bad news, however, was that 3.4% of herds in the USA had SCCs > 750,000 ml^{-1}, and 22.4% of the national dairy herd had SCCs > 400,000 ml^{-1}. State-average SCCs were often lower than the national average in the north-east and the far west, and higher in the south-east, mid-Atlantic and central states, a finding that is consistent with previous reports. What is not known from this report is which herds are producing milk that is pasteurized and which are producing milk that is consumed directly without pasteurization – but to assume that milk to be consumed directly without pasteurization is a better quality product than milk to be pasteurized is clearly without merit. Regardless of whether milk is pasteurized, or consumed directly without pasteurization, some herds produce maximum quantities of very high-quality milk, other herds produce average quantities of average-quality milk, while some herds produce poor-quality milk.

Raw milk advocates feel that the pasteurization of milk is associated with lactose intolerance, increased allergic reactions and reduced nutritional value of milk, and causes pathogens to multiply, destroys antibodies and other protective bioactive factors found in milk, destroys milk proteins and polypeptides, kills beneficial bacteria, and is associated with the development of

arthritis and autism. Most of these claims are anecdotal and/or based on testi-monials with little to no science-based data to support the contentions. The reader is referred to the Marler Blog (http://www.marlerblog.com) for an overview of the pros (Marler, 2008a) and cons (Marler, 2008b) of consuming raw milk, and other interesting reviews on raw milk.

Influence of pasteurization on nutritional qualities of milk

Pasteurization is the most effective known method of enhancing the microbio-logical safety of milk and milk products. Pasteurization is a process that kills harmful bacteria by heating milk to a specified temperature for a set period of time. Pasteurization protocols approved and commonly used in the USA are summarized in Table 5.1. Similar protocols and/or their equivalents are employed internationally.

Over 25 years ago, Potter *et al.* (1984) stated that 'Meaningful differences in nutritional value between pasteurized and unpasteurized milk have not been demonstrated, and other purported benefits of raw milk consumption have not been substantiated'. From a nutritional perspective, the major com-ponents of milk, including lactose, casein and most milk proteins, are not affected significantly following pasteurization (Potter *et al.* 1984; LeJeune and Rajala-Schultz, 2009). Heating milk can result in degradation of lactose to lactulose and epilactose (Lopez-Fandino and Olano, 1999; Teuri *et al.*, 1999), and large amounts of indigestible carbohydrates such as lactulose can cause digestive disturbances in individuals who have difficulties digesting lactose. However, pasteurization generally does not cause detectable levels of lactulose in pasteurized milk. In addition, pasteurization will kill lactase-producing bacteria that might be beneficial to those with lactose intolerance (Lopez-Fandino and Olano, 1999; Teuri *et al.*, 1999). Whey proteins such as lactofer-rin and immunoglobulins retain their biological activity except following ultrahigh temperature pasteurization (Paulsson *et al.*, 1993; Li-Chan *et al.*,

Table 5.1. US FDA's times and temperatures for pasteurization of fluid milk.[a]

Temperature	Time
63°C (145°F)	30 min
72°C (161°F)	15.0 s
89°C (191°F)	1.0 s
90°C (194°F)	0.5 s
94°C (201°F)	0.1 s
96°C (204°F)	0.05 s
100°C (212°F)	0.01 s

[a] Compiled from US FDA (Food and Drug Administration) Center for Food Safety and Applied Nutrition (2007).

1995). Some bovine enzymes in milk are reduced by pasteurization, although most are not used by humans to aid in digestion. Other enzymes found in low concentrations in bovine milk, such as lactoperoxidase (Marks *et al.*, 2001), lysozyme (Fox and Kelly, 2006) and xanthine oxidase (Walstra *et al.*, 1999) are still active following pasteurization. Pasteurization has little effect on vitamins A, D, E, and K, but does result in slight reductions in vitamin C (Fox and Kelly, 2006).

Perceived health benefits of raw milk

Raw milk advocates claim that raw milk has medicinal qualities, including those of allowing lactose-intolerant individuals to consume such milk without digestive problems, reducing allergies and asthma, and reducing digestive problems caused by Crohn's disease. More recently, there have been testimonials that raw milk was associated with the improvement of autistic children. Raw milk advocates feel that pasteurization of milk is associated with lactose intolerance, increases allergic reactions, destroys antibodies and other protective bioactive factors found in milk associated with disease prevention/resistance, and is associated with the development of arthritis and autism. Few studies have been done in the USA or in other countries to prove or disprove the health benefits associated with the consumption of raw milk, but many of the health benefit claims are anecdotal or based on testimonials. Additional studies are certainly needed to determine whether raw milk is indeed associated with health benefits, and the specific factor(s) in raw milk that are protective.

Some published studies, primarily from the EU, have indicated that children from farming environments had fewer allergic conditions (asthma, hay fever and eczema, among others), and that the consumption of raw milk was one of the protective factors associated with these reduced allergies (Kilpelainen *et al.*, 2000; Riedler *et al.*, 2000; Wickens *et al.*, 2002; Waser *et al.*, 2007). Other factors, including barn exposure and contact with animals, were also associated with reduced allergies. Because of the potential health hazards from foodborne pathogens in raw milk, the authors indicated that raw milk could not be recommended to prevent allergies.

Nutritional significance of bovine milk and milk products

According to the (US) National Research Council (1995), good nutrition starts with a balanced diet that provides the necessary levels of essential nutrients and adequate energy. The USDA recommends daily consumption of two to three servings of dairy products, thus the nutritional significance of these products cannot be overstated (US Department of Health and Human Services and USDA, 2005). Milk and milk products, primarily from cattle, water buffaloes, goats, sheep and other species, are important components of the human diet (LeJeune and Rajala-Schultz, 2009). Inclusion of dairy products in the diet aids in the prevention of diseases such as obesity, hypertension and

diabetes, and dairy products are also a source of calcium, which is important for growing bones and the prevention of osteoporosis. Furthermore, dairy products are an important dietary source of protein, vitamins and other minerals. The consumption of milk products has been associated with overall diet quality and adequacy of intake of many nutrients, including calcium, potassium, magnesium, zinc, iron, riboflavin, vitamin A, folate, vitamin D and protein (McCarron and Heaney, 2004; US Department of Health and Human Services and USDA, 2005; Huth *et al.*, 2006).

Prevalence of Foodborne Pathogens in Raw Milk and Milk Products

Prevalence data for raw milk-borne pathogens were obtained from peer-reviewed literature published internationally from 1994 to 2009, although most of the reports were from 2000 to 2009. Prevalence rates for the common zoonotic pathogens that were isolated worldwide from raw milk and products derived from raw milk are summarized in Tables 5.2–5.8. The majority of the reports are from the USA, which has a highly developed capacity for research and surveillance, and where data are easily available via international publications and the Internet. From the literature that was reviewed, the most commonly researched and/or reported bacterial pathogens from raw milk and raw milk products were *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and non-O157:H7 Shiga toxin-producing *E. coli*, in order of decreasing number of prevalence data reports. Oliver *et al.* (2009a) recently reviewed the literature on food-safety hazards associated with the consumption of raw milk in the USA, focusing primarily on the prevalence of foodborne pathogens in raw milk and raw milk-borne disease outbreaks. Other recent reviews on this topic focused on foodborne pathogens in the farm environment (Oliver *et al.*, 2005) and developments in and the future outlook for preharvest food safety (Oliver *et al.*, 2009b).

Isolation rates for *L. monocytogenes* in raw milk (Table 5.2) ranged from 2.7% to 7.0% in North America (Jayarao and Henning, 2001; Muraoka *et al.*, 2003; Van Kessel *et al.*, 2004; Goff and Griffiths, 2006; Jayarao *et al.*, 2006; D'Amico *et al.*, 2008) and were higher (12.6%) in in-line milk filters (Hassan *et al.*, 2000). In Asia (Table 5.3), reported isolation rates of *L. monocytogenes* were 0% in China (Chao *et al.*, 2007) and 1.9% in Malaysia (Chye *et al.*, 2004). In South America (Table 5.4), prevalence rates for *L. monocytogenes* were 0% in two studies in Brazil (Nero *et al.*, 2008; Moraes *et al.*, 2009). *L. monocytogenes* isolation rates were not available from other continents. Overall isolation rates for *L. monocytogenes* reported internationally in raw milk worldwide were 0–7%.

In North America, *Salmonella* isolation rates (Table 5.2) ranged from 0% to 11.8% in normal bulk tank milk (Jayarao and Henning, 2001; Murinda *et al.*, 2002b; Warnick *et al.*, 2003; Van Kessel *et al.*, 2004; Karns *et al.*, 2005; Goff and Griffiths, 2006; Jayarao *et al.*, 2006; D'Amico *et al.*, 2008; Van Kessel *et al.*, 2008), and from 1.5% to 66% in in-line milk filters (Hassan *et al.*, 2000; Warnick *et al.*, 2003; Van Kessel *et al.*, 2008), whereas isolation rates were 15% in

Table 5.2. Prevalence rates for isolation of bacterial pathogens from bulk tank milk in the USA and Canada.

Product	Pathogen	Country	Prevalence rates (%)	Reference
Raw milk	*Listeria monocytogenes*	Canada	2.73	Goff and Griffiths (2006)
Raw milk	*L. monocytogenes*	USA	2.8	Jayarao *et al.* (2006)
Raw milk	*L. monocytogenes*	USA	4.6	Jayarao and Henning (2001)
Raw milk	*L. monocytogenes*	USA	5.6	Van Kessel *et al.* (2004)
Raw milk	*L. monocytogenes*	USA	4.9–7.0	Muraoka *et al.* (2003)
Raw milk	*L. monocytogenes*	USA	4.8	D'Amico *et al.* (2008)
Filters	*L. monocytogenes*	USA	12.6	Hassan *et al.* (2000)
Raw milk	*Salmonella* spp.	Canada	0.17	Goff and Griffiths (2006)
Raw milk	*Salmonella* spp.	USA	6.0	Jayarao *et al.* (2006)
Raw milk	*Salmonella* spp.	USA	2.24	Murinda *et al.* (2002b)
Raw milk	*Salmonella* spp.	USA	6.1	Jayarao and Henning (2001)
Raw milk	*Salmonella* spp.	USA	2.6	Van Kessel *et al.* (2004)
Raw milk	*Salmonella* spp.	USA	2.6/11.8[a]	Karns *et al.* (2005)
Raw milk	*Salmonella* spp.	USA	0	D'Amico *et al.* (2008)
Colostrum	*Salmonella* spp.	USA	15	Houser *et al.* (2008)
Milk and filters	*Salmonella* spp.	USA	1.1/12.6	Warnick *et al.* (2003)
Milk and filters	*Salmonella* spp.	USA	11/66	Van Kessel *et al.* (2008)
Filter	*Salmonella* spp.	USA	1.5[b]	Hassan *et al.* (2000)
Raw milk	*Campylobacter jejuni*	USA	9.2	Jayarao and Henning (2001)
Raw milk	*C. jejuni*	USA	2.0	Jayarao *et al.* (2006)
Raw milk	*Campylobacter* spp.	Canada	0.47	Goff and Griffiths (2006)
Raw milk	*Yersinia enterocolitica*	USA	6.1	Jayarao and Henning (2001)
Raw milk	*Y. enterocolitica*	USA	1.2	Jayarao *et al.* (2006)
Raw milk	O157:H7 STEC[c]	USA	0.23	Karns *et al.* (2007)
Raw milk	O157:H7 STEC	USA	0	Jayarao and Henning (2001)
Raw milk	O157:H7 STEC	USA	0.75	Murinda *et al.* (2002a)
Raw milk	O157:H7 STEC	USA	0	D'Amico *et al.* (2008)
Raw milk	Non-O157:H7 STEC[d]	USA	2.4	Jayarao *et al.* (2006)
Raw milk	Non-O157:H7 STEC	USA	3.8	Jayarao and Henning (2001)
Raw milk	Non-O157:H7 STEC	USA	3.96	Karns *et al.* (2007)
Raw milk	Non-O157:H7 STEC	USA	3.5	Cobbold *et al.* (2008)
Raw milk	Non-O157:H7 STEC	Canada	0.87	Goff and Griffiths (2006)

[a] Conventional versus real-time PCR method.
[b] One of six isolates was *Salmonella enterica* serotype Typhimurium DT104.
[c] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.
[d] Non-O157:H7 STEC, Shiga toxin-producing *E. coli* (non-O157:H7).

colostrum samples (Houser *et al.*, 2008). This implies that feeding colostrum to calves and the consumption of colostrum by humans could be a potential health hazard to both calves and humans. A strain of *Salmonella enterica* serovar Typhimurium DT104 was isolated from one in six *Salmonella*-positive milk filter samples (Hassan *et al.*, 2000). In general, isolation rates of pathogens were higher in in-line milk filter samples than in bulk tank milk. The prevalence

Table 5.3. Prevalence rates for isolation of bacterial pathogens from raw milk and milk products in Asia and the Middle East.

Product	Pathogen	Country	Prevalence rate (%)	Reference
Milk and milk products[a]	O157:H7 STEC[b]	India	1.8	Sehgal *et al.* (2008)
Raw milk	O157:H7 STEC	Malaysia	33.5	Chye *et al.* (2004)
Raw milk	O157:H7 STEC	China	0	Chao *et al.* (2007)
Raw milk	*Listeria monocytogenes*	Malaysia	1.9	Chye *et al.* (2004)
Raw milk	*L. monocytogenes*	China	0	Chao *et al.* (2007)
Raw milk	*Salmonella*	Malaysia	1.4	Chye *et al.* (2004)
Raw milk	*Salmonella*	China	4	Chao *et al.* (2007)
Raw milk	*Vibrio parahemolyticus*	China	0	Chao *et al.* (2007)
Raw milk	*Mycobacterium avium* subsp. *paratuberculosis*	Iran	11 (herd)	Haghkhah *et al.* (2008)
Raw milk	*M. avium* subsp. *paratuberculosis*	Iran	8.6–23 (sample)	Haghkhah *et al.* (2008)

[a] It was assumed they were both unpasteurized.
[b] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.

Table 5.4. Prevalence rates for isolation of bacterial pathogens from milk and dairy products in South America and the Caribbean.

Product	Pathogen	Country	Prevalence rates (%)	Reference
Raw milk	*Salmonella*	Brazil	0	Nero *et al.* (2008)
Raw milk soft cheeses	*Salmonella*	Brazil	0	Moraes *et al.* (2009)
Raw milk	*Listeria monocytogenes*	Brazil	0	Nero *et al.* (2008)
Raw milk soft cheeses	*L. monocytogenes*	Brazil	0	Moraes *et al.* (2009)
Raw milk	O157:H7 STEC[a]	Trinidad	23.6	Adesiyun (1994)

[a] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.

rates of *Salmonella* spp. in Asia (Table 5.3) were 1.4–4% (Chye *et al.*, 2004; Chao *et al.*, 2007). However, in two studies conducted in Brazil (Table 5.4), the pathogen was not isolated (Nero *et al.*, 2008; Moraes *et al.*, 2009).

Isolation rates for *Campylobacter jejuni* were 2.0% (Jayarao *et al.*, 2006) and 9.2% (Jayarao and Henning, 2001) in the USA, whereas, Goff and Griffiths (2006) reported prevalence rates of 0.47% in Canada for *Campylobacter* spp. (Table 5.2).

The prevalence of Shiga toxin-producing *E. coli* (STEC) in bulk tank milk was investigated in the majority of studies. Worldwide prevalence rates for the

Table 5.5. Prevalence rates for isolation of bacterial pathogens from raw milk and milk products in Europe.

Product	Pathogen	Country	Prevalence rates (%)	Reference
Raw milk[a]	O157:H7 STEC[b]	Spain	0.3	Rey *et al.* (2006)
Bulk tank milk[a]	Non-O157:H7 STEC[c]	Spain	10.8	Rey *et al.* (2006)
Fresh cheese curds[a,d]	Non-O157:H7 STEC	Spain	3.9	Rey *et al.* (2006)
Cheese[a,d]	Non-O157:H7 STEC	Spain	5	Rey *et al.* (2006)
Raw milk	*Mycobacterium paratuberculosis*	UK	1.6	Goff and Griffiths (2006)
Raw milk	*M. paratuberculosis*	Switzerland	19.7	Corti and Stephan (2002)
Raw milk	Tick-borne encephalitis	Czech Republic	11	Kríz *et al.* (2009)

[a] Milk and milk products from goats and sheep.
[b] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.
[c] Non-O157:H7 STEC, Shiga toxin-producing *E.coli* (non-O157:H7); the non-O157:H7 STEC serotypes isolated from these products included *E. coli* O27:H18, O45:H38, O76:H19, O91:H28, ONT:H7, ONT:H9 and ONT:H21.
[d] Made from raw milk.

Table 5.6. Prevalence rates for isolation of bacterial pathogens from raw milk and milk products in Africa.

Product	Pathogen	Country	Prevalence rates (%)	Reference
Raw milk	O157:H7 STEC[a]	Kenya	0.7–0.8	Kang'ethe *et al.* (2007)
Raw milk	O157:H7 STEC	Uganda	1.2	Nasinyama and Randolf (2005)
Raw milk	O157:H7 STEC	Egypt	6	Abdul-Raouf *et al.* (1996)
Raw milk	O157:H7 STEC	Morocco	14.3	Benkerroum *et al.* (2004)
Jben[b]	O157:H7 STEC	Morocco	40.2	Benkerroum *et al.* (2004)
Raw milk	*Brucella abortus*	Kenya	0–10[c]	Arimi *et al.* (2005)

[a] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.
[b] Traditional fresh Moroccan cheese.
[c] Prevalence rates increased with increasing intensity of farming operation.

isolation of O157:H7 STEC in raw milk ranged from 0% to 33.5% (Tables 5.2–5.6). Low prevalence rates of 0–0.75% (Table 5.2) were established in the USA (Murinda *et al.*, 2000a; Jayarao and Henning, 2001; Karns *et al.*, 2007; D'Amico *et al.*, 2008). The pathogen was found at the high prevalence rates of 33.5% (Table 5.3) and 23.6% (Table 5.4) in raw milk in Malaysia (Chye *et al.*, 2004) and Trinidad (Adesiyun, 1994), respectively. In general, most researchers have indicated low prevalence rates for non-O157:H7 STEC in bulk tank

Table 5.7. Prevalence rates for isolation of mastitis pathogens in raw milk worldwide.

Pathogen	Country	Prevalence rates (%)	Reference
Staphylococcus aureus	USA	42[a]	Houser *et al.* (2008)
S. aureus	USA	31	Jayarao *et al.* (2004)
S. aureus	USA	37[b]	Khaitsa *et al.* (2000)
S. aureus	USA	27.4	D'Amico *et al.* (2008)
S. aureus	Kenya	61	Ombui *et al.* (1992)
S. aureus	Malaysia	60	Chye *et al.* (2004)
S. aureus	Trinidad	94.3	Adesiyun (1994)
S. aureus	China	34	Chao *et al.* (2007)
S. aureus	Brazil	30.9	Moraes *et al.* (2009)
Coagulase-negative *Staphylococcus* spp.	USA	11.4[c]	Gillespie *et al.* (2009)
Coagulase-negative *Staphylococcus* spp.	USA	>74[a]	Houser *et al.* (2008)
Streptococci	USA	>71[a]	Houser *et al.* (2008)
Streptococcus agalactiae	USA	10	Jayarao *et al.* (2004)
S. agalactiae	USA	0	Khaitsa *et al.* (2000)
Mycoplasma	USA	0	Khaitsa *et al.* (2000)

[a] Colostrum.
[b] Herd prevalence.
[c] Quarter prevalence.

Table 5.8. Prevalence rates for isolation of bacterial pathogens in raw non-bovine milk and milk products worldwide.

Product	Pathogen	Country	Prevalence rates (%)	Reference
Water buffalo milk	O157:H7 STEC[a]	Turkey	1.4	Seker *et al.* (2008)
Water buffalo milk	Non-tuberculosis mycobacteria	Brazil	21.7	Jordao *et al.* (2009)
Ewe milk	O157:H7 STEC	Spain	3.5	Caro *et al.* (2006)
Ewe milk	O157:H7 STEC	Greece	1	Dontorou *et al.* (2003)
Goat milk	O157:H7 STEC	USA	0.75	D'Amico *et al.* (2008)
Goat milk	*Listeria monocytogenes*	USA	17	Abou-Eleinin *et al.* (2000)
Goat milk	Tick-borne encephalitis	Czech Republic	56.3	Kríz *et al.* (2009)
Goat cheese	O157:H7 STEC	Morocco	0	Benkerroum *et al.* (2004)
Camel milk	O157:H7 STEC	Morocco	0	Benkerroum *et al.* (2004)
Ewe milk cheese	Tick-borne encephalitis	Czech Republic	33	Kríz *et al.* (2009)

[a] O157:H7 STEC, Shiga toxin-producing *Escherichia coli* O157:H7.

milk in North America compared with O157:H7 STEC. Non-O157:H7 STEC has also been isolated (Rey *et al.*, 2006) in raw milk products such as fresh cheese curds (3.9%) and cheese (5%) in Spain (Table 5.5).

Prevalence rates of some less frequently targeted zoonotic pathogens from raw milk, such as *Yersinia, Vibrio, Brucella* and *Mycobacterium* spp., have also been reported; rates reported for *Yersinia enterocolitica* in the USA (Table 5.2) were 1.2% (Jayarao *et al.*, 2006) and 6.1% (Jayarao and Henning, 2001). In Kenya (Table 5.6), *Brucella abortus* was isolated in 0–10% of raw milk samples that were tested; the prevalence rates increased with degree of intensity of dairying operations (Arimi *et al.*, 2005). In studies conducted in China (Table 5.3), *Vibrio parahemolyticus* was not isolated from any of the samples that were evaluated for the pathogen (Chao *et al.*, 2007). *Mycobacterium avium* subsp. *paratuberculosis* (MAP) was isolated in 1.6% (Goff and Griffiths, 2006) and 8.6–23% (Haghkhah *et al.*, 2008) of raw milk samples in the UK (Table 5.5) and Iran (Table 5.3), respectively; additionally, herd prevalences of 11% were established in Iran (Table 5.3) (Haghkhah *et al.*, 2008). Corti and Stephan (2002) reported that 19.7% of bulk tank milk samples in Switzerland were positive for MAP (Table 5.5). Bovine tuberculosis due to *Mycobacterium bovis* is a major cause of human gastrointestinal tuberculosis in developing countries where raw milk is consumed; Bonsu *et al.* (2000) reported prevalences for *M. bovis* of 13.8% in cattle, and as high as 50% in some kraals (i.e. cattle pens) in Ghana. Interestingly, studies conducted in the Czech Republic (Table 5.5) indicated that 11% of raw bovine milk samples tested positive for tick-borne encephalitis, a viral disease that is normally transmitted via tick bites (Kríz *et al.*, 2009).

Prevalence of mastitis pathogens in raw bovine milk

The percentage of bulk tank milk samples that were positive for *S. aureus*, the major pathogen associated with contagious mastitis worldwide, ranged from 27.4% to 94.3% (Table 5.7). In the USA, colostrum had higher *S. aureus* prevalence rates (42%) than raw milk (Jayarao *et al.*, 2004; D'Amico *et al.*, 2008; Houser *et al.*, 2008). In other countries, prevalence rates ranged from 30.9% to 94.3%. These rates were higher in countries with hotter climates, namely, Kenya, Malaysia and Trinidad (Table 5.7). Contamination of raw milk and colostrum with coagulase-negative *Staphylococcus* spp. (CNS) (Houser *et al.*, 2008; Gillespie *et al.*, 2009) and *Streptococcus* species (Houser *et al.*, 2008), including *Streptococcus agalactiae* (Jayarao *et al.*, 2004), has also been reported. In the USA, Jayarao *et al.* (2004) reported an increase in the frequency of isolation of *Staphylococcus aureus* and *Streptococcus agalactiae*, which were significantly associated with increased bulk tank milk somatic cell counts. *Staphylococcus aureus* isolated from the milk of cows with mastitis has been demonstrated to harbour enterotoxin genes at high frequencies (Srinivasan *et al.*, 2005). This is a concern of epidemiological significance to raw milk consumers because *S. aureus* is a common zoonotic foodborne pathogen. The CNS isolated by Gillespie *et al.* (2009) from milk samples were further characterized by Sawant *et al.* (2009), who demonstrated that some of the *Staphylococcus epidermidis*

samples isolated from milk were antibiotic resistant; these could pose a public-health hazard to those who consume raw milk. Khaitsa *et al.* (2000) did not isolate *Mycoplasma* or *Streptococcus agalactiae* from the raw milk samples they evaluated.

Bovine colostrum is used traditionally for feeding dairy calves and providing passive immunity to calves that are born hypogammaglobulonaemic. More recently, colostrum has gained popularity as a human food because it has been advocated as an excellent source of bioactive proteins, which have been claimed to inhibit viral and bacterial pathogens, improve gastrointestinal health and enhance body condition (Houser *et al.*, 2008). In a study that was conducted to determine bacteriological quality and the occurrence of *Staphylococcus aureus, Streptococcus agalactiae*, CNS, other streptococci and other parameters, bovine colostrum was associated with high contamination rates with milk-borne pathogens, with *Staphylococcus aureus* (42%), CNS (>74%) and *Streptococcus* spp. (>71%) predominating (Houser *et al.*, 2008). Thus, consumption of unpasteurized colostrum could pose a potential health risk to those consuming this product. Results from these studies also emphasize that pathogens are frequently found in colostrum even though it contains high concentrations of bioactive compounds and antibacterial factors such as antibodies and lactoferrin (Oliver and Sordillo, 1989).

Contamination of non-bovine milk samples by foodborne pathogens

Although worldwide milk and milk products come primarily from dairy cattle, in some countries water buffaloes, goats, sheep and other species are important milk-producing animals. With regard to carriage of zoonotic foodborne pathogens, these alternative sources of raw milk for consumers are not any safer than bovine milk (Table 5.8). In the USA, Abou-Eleinin *et al.* (2000) isolated *L. monocytogenes* from 17% of raw goat bulk milk samples that were tested. D'Amico *et al.* (2008) isolated *E. coli* O157:H7 from one goat milk sample (prevalence rate, 0.75%; *n* = 49), whereas milk samples from cows (*n* = 62) and sheep (*n* = 22) were found to be negative for the pathogen.

A total of 1% of raw ewe's milk samples that were tested in Greece were positive for *E. coli* O157:H7 (Dontorou *et al.*, 2003), whereas in Turkey 1.4% of raw milk samples from water buffaloes tested positive for this pathogen (Seker *et al.*, 2008). *Escherichia coli* O157:H7 was also isolated from 3.5% of ewe's milk samples in Spain (Caro *et al.*, 2006). Benkerroum *et al.* (2004) did not isolate this pathogen from the goat's cheese and camel milk they analysed. Unpasteurized goat milk (56.3%) and sheep milk cheeses (33%) made from unpasteurized milk were associated with tick-borne encephalitis (a disease that is commonly associated with tick-bite transmission) in the Czech Republic (Kríz *et al.*, 2009); correspondingly lower prevalence rates, i.e. 11% of dairy cow milk samples, were associated with this disease. Raw milk from water buffalo is popularly used for the manufacture of mozzarella cheese in Brazil; five of 23 (21.7%) water buffalo milk samples that were examined tested positive for non-tuberculosis mycobacteria (NTM), which are considered to be emerging

milk-borne pathogens in Brazil (Jordao *et al.*, 2009). The isolation of opportunistic NTM pathogens, such as *Mycobacterium kansaii, Mycobacterium simiae* and *Mycobacterium lentiflavum,* represents a risk to consumers of mozzarella cheese made from raw buffalo milk.

Potential Threats that Raw Milk Consumption Poses to Consumers

Milk can harbour a variety of microorganisms and can be an important source of foodborne pathogens. The presence of foodborne pathogens in milk can be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Raw milk and raw milk products have long been regarded as an important source of zoonotic bacterial pathogens that cause disease in humans. The symptoms caused by the foodborne pathogens found in raw milk range from nausea, vomiting, chills and diarrhoea to, in some cases, death (Table 5.9). For example, *L. monocytogenes* is associated with flu-like symptoms, diarrhoea and meningitis, and can also cause abortions. O157:H7 STEC is associated with several different conditions including haemorrhagic colitis, haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (i.e. HUS + fever), kidney failure, fever and death.

People continue to consume raw milk even though numerous epidemiological studies have shown clearly that it can be contaminated by a variety of pathogens, some of which are associated with human illness and disease.

Table 5.9. Diseases caused by zoonotic pathogens commonly isolated from milk and milk products.[a]

Zoonotic pathogen	Disease symptoms
Salmonella spp.	Nausea, vomiting, abdominal pain, headache, chills, diarrhoea, fever
Listeria monocytogenes	Flu-like illness, diarrhoea, meningitis, abortions
Yersinia enterocolitica	Diarrhoea, fever, vomiting, abdominal pain
Vibrio parahemolyticus	Acute gastroenteritis, nausea, vomiting, abdominal cramps, fever, chills, diarrhoea
Staphylococcus aureus	Nausea, vomiting, diarrhoea
Shiga toxin-producing *Escherichia coli* O157:H7	Haemorrhagic colitis, haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (HUS + fever), kidney failure, death, fever
Campylobacter jejuni	Profuse diarrhoea, sometimes bloody diarrhoea, stomach cramps, nausea, dizziness, fever
Viruses	Gastroenteritis, fever, vomiting, diarrhoea

[a] Compiled from Mortimore and Wallace (1994).

Several documented milk-borne disease outbreaks occurred from 2000 to 2008 and were traced back to the consumption of raw unpasteurized milk (Oliver *et al.*, 2009a). Numerous people were diagnosed with infections, some were hospitalized, and a few died. In the majority of these outbreaks, the organism associated with the milk-borne outbreak was isolated from the implicated product(s) or from subsequent products made at the suspected dairy or source. In contrast, fewer milk-borne disease outbreaks were associated with consumption of pasteurized milk during this same time period. For a comprehensive review of the hazards associated with the consumption of raw milk see Oliver *et al.* (2009a).

Summary

Safety and quality of dairy products start at the farm and continue throughout the processing continuum. One thing is certain – it is impossible to transform a low-quality raw milk product into a high-quality finished dairy product! To meet increased raw milk quality standards, dairy producers must adopt production practices that reduce mastitis and reduce the bacterial contamination of bulk tank milk. Use of effective management strategies to minimize the contamination of raw milk and proven mastitis control strategies will help dairy producers achieve these important goals. However, it is important to recognize that use of these methods *will not* eliminate the risk of pathogen contamination of raw milk and the potential for milk-borne diseases.

An increasing number of people are consuming raw unpasteurized milk. Enhanced nutritional qualities, superior taste, health benefits, demand for natural, unprocessed foods and freedom of choice, among others, have all been advocated as reasons for increased interest in raw milk consumption, although science-based data to substantiate these claims are limited or lacking. Although milk and milk products are important components of a healthy diet, if consumed unpasteurized, they can present a health hazard resulting from possible contamination with pathogenic bacteria. People continue to consume raw milk even though numerous studies have shown clearly that it can be contaminated by a variety of pathogens, some of which are associated with human illness and disease.

Where raw milk is offered for sale, strategies to reduce the risks associated with raw milk and products made from raw milk are needed. The development of uniform regulations, including microbial standards for raw milk to be sold for human consumption, the labelling of raw milk, improving sanitation during milking, and enhancing and targeting educational efforts directed towards producers and consumers are potential approaches to this issue. Development of on-farm preharvest and postharvest control measures to effectively reduce bacterial contamination is critical to the control of pathogens in raw milk. The International Dairy Foods Association and the US National Milk Producers Federation have called for legislation requiring all facilities producing raw or unpasteurized milk products for direct human consumption to register with the US Food and Drug Administration (FDA) and

adhere to food-safety requirements that are followed by all other facilities producing milk products.

Liability is another very important aspect that should be considered by both producers and consumers of raw milk. Dairy producers supplying raw milk must be well informed of the risks and liabilities associated with the milk they sell to consumers. In addition, consumers purchasing raw milk need to recognize the inherent risks of the product and that they may not have protection if they become ill from consuming the contaminated product.

Advocates of raw milk are long on the proposed benefits of consuming raw milk but short on data to support most of these claims. Given the current body of knowledge on this topic, it is clear that the disadvantages of raw milk consumption far outweigh the proposed benefits. Even in studies that have shown a benefit of raw milk consumption for allergic conditions, authors of many of these studies do not recommend this practice because of the potential health hazards from contamination by foodborne pathogens. Additional science-based studies are needed to evaluate raw milk quality, determine the potential benefits of raw milk consumption and delineate the factors in milk that are beneficial. However, until those studies are done, one sure way to prevent raw milk-associated foodborne illness is for consumers to refrain from drinking raw milk and from consuming dairy products manufactured using raw milk.

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6

The Contribution of Antibiotic Residues and Antibiotic Resistance Genes from Livestock Operations to Antibiotic Resistance in the Environment and Food Chain

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Introduction

Antibiotic-resistant bacteria and their antibiotic-resistance genes (ARGs) have become emerging contaminants of concern in the 21st century. One of the main reasons that this issue is gaining public interest is the adverse health impacts that are associated with these contaminants. According to the World Health Organization (WHO), deaths arising from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria and tuberculosis account for more than 85% of the mortality from infection worldwide. Further compounding the problem is that the pathogens causing these diseases are gaining resistance to first-line drugs for treatment of disease, and, in some instances, resistance to second- and third-line drug agents. An additional complication is that immunocompromised groups are at a heightened risk of developing adverse health implications from these infections (WHO, 2001).

Many factors can possibly account for the emergence of antibiotic resistance. The rampant use of antibiotics in clinical settings, coupled with a large pool of

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immunocompromised patients, has resulted in selective pressure that favours the rapid emergence of antibiotic-resistant bacterial strains (McDonald *et al.*, 1997). For example, one of the most notorious antibiotic-resistant bacterial strains to have emerged from clinical settings includes methicillin-resistant *Staphylococcus aureus* (MRSA). In the USA in 2005, MRSA has been associated to 18,650 deaths (Klevens *et al.*, 2007), and MRSA-associated infections have also been widely reported globally (Diekema *et al.*, 2001). To curb the dissemination of antibiotic-resistant bacteria from clinical settings, initiatives that promote the prudent use of antibiotics and consumer education are currently in place to reduce unnecessary antibiotic prescriptions (CDC, 2009).

Despite these efforts, it is widely recognized that the emergence of antibiotic resistance still remains as a serious threat, in part because of the use of antibiotics in livestock-production operations. Antibiotics are routinely used in livestock production for therapeutic treatment of disease, at sub-therapeutic concentrations to prevent disease (prophylaxis) and for growth promotion. The classes of antibiotics so used include tetracyclines, macrolides, lincosamides, polypeptides, streptogramins, cephalosporins, penicillins, sulfonamides, aminoglycosides and fluoroquinolones (Schmidt, 2002; Chee-Sanford *et al.*, 2009), all of which include drug members that were originally intended for disease treatment in humans but are also being used for livestock-production purposes.

Because the use of antibiotics in livestock production may lead to increased emergence and spread of antibiotic-resistant bacteria, it is a cause for concern and requires deeper understanding in order to control and prevent the dissemination of resistance. This chapter aims to provide a summary of the global use of antibiotics in the livestock industry, and of the possible dissemination routes of antibiotic resistance from livestock-production operations into the environment. Scientific evidence will be provided to support claims that antibiotic use in livestock production has adversely affected the environment. Lastly, we provide an outlook on the potential health impact and the current knowledge gaps that need to be addressed to tackle this global problem.

Global Use of Antibiotics in the Livestock Industry

The use of antibiotics in livestock production seems to be a ubiquitous practice. Swine, poultry and cattle production, as well as aquaculture, have reported the use of antibiotics for the treatment (i.e. therapeutic use) of and prevention (i.e. prophylactic use) of animal diseases, and for growth promotion. An estimate of the quantity of antibiotics used in animal husbandry is difficult to obtain and is often not reliable. For example, the Animal Health Institute (AHI) and Union of Concerned Scientists (UCS) have reported two different estimates of antibiotic usage in animal production in the USA. AHI reported that approximately 9.3 million kg of antibiotics were sold for animal use in 1999, while UCS reported that more than 11.2 million kg of antibiotics were used for non-therapeutic purposes alone (Chee-Sanford *et al.*, 2009). In the USA, no regulation is currently enacted to ban the use of antibiotics for livestock production that were originally intended for use in human medicine.

Livestock production companies can, however, voluntarily withdraw the use of antibiotics in their production systems, or consumers can purchase meat produced without the use of antibiotics (Schmidt, 2002), usually at increased cost.

In the EU, antibiotics that are used in human medicine have been progressively phased out from being added to animal feed as growth promoters. The animal growth promoter avoparcin, a glycopeptide antibiotic related to vancomycin, was one of the first such antibiotics to be banned in 1997, followed by tylosin, spiramycin, bacitracin and virginiamycin in 1999. Subsequently, a ban on monensin sodium, salinomycin sodium, avilamycin and flavomycin for growth promotion entered into effect from 2006 (Casewell *et al.*, 2003; Europa, 2005). However, it is noted that antibiotics are still allowed and critical for therapeutic and prophylactic purposes. Also, a ban on the use of antibiotics as growth promoters has not necessarily equated to an end of the resistance problem. Casewell *et al.* (2003) argued that banned antibiotics that were previously used as growth promoters also showed prophylactic effects. A ban on their use has led to an increase in the number of diseased animals and an associated increase in the usage of therapeutic antibiotics in animal feed. This may explain why the amount of antibiotics used in the EU, although lower than in the USA, has remained high even after the ban. For example, the European Federation of Animal Health reported in 1998 that approximately 5 million kg of antibiotics were used for veterinary applications and for growth promotion, a mere twofold lower consumption than in the USA (Barton, 2000).

In China, regulatory and surveillance frameworks to encourage the responsible use of antibiotics in animal husbandry are generally not in place. Public awareness of the potential health impact remains low, and data on the quantity of antibiotics given to livestock are not easily available to the public or government agencies. WHO estimates that quinolone consumption in animals approximates 0.5 million kg year^{-1} in China (WHO, 1998). In a report published by WHO, it was noted that in practice other antibiotics such as tetracyclines and the mycelial by-products from the production of antibiotics are also added into animal feeds (Jin, 1997). As developing countries typically rely on the agricultural industry to contribute to their gross domestic product, concentrated animal farming operations (CAFOs) are becoming the norm, and the use of antibiotics in the feed formulations of these operations is likely to increase even further in the near future (Sarmah *et al.*, 2006).

In addition to swine, poultry and beef livestock production, aquaculture is a rapidly growing industry in both developing and developed countries, and it is estimated that more than twofold growth in industrial aquaculture was achieved worldwide over the past 15 years (Naylor *et al.*, 2000; Cabello, 2006). Antibiotics such as oxytetracycline, erythromycin and sulfonamides are typically administered in the feed or medication that is added to ponds and holding tanks in aquaculture (Sorum, 2006). In many instances, fish do not effectively metabolize antibiotics and will excrete largely unused and untransformed antibiotic residues back into the environment. Coupled with the good mobility and, therefore, the better dissemination capabilities of water, it is anticipated that

the use of antibiotics in aquaculture will have an environmental and health impact that is equal to or more adverse than that of swine, poultry and beef livestock production (Cabello, 2006).

Despite the lack of statistically reliable data on the global usage of antibiotics in livestock production, it is clear that the use of antibiotics that were originally intended for use in human medicine is now routine in livestock production (Chee-Sanford *et al.*, 2009). As such, animal production and animal feed have often been implicated in the spread of antibiotic resistance, with adverse impacts on human medicine.

Sources of Antibiotic Residues and Antibiotic Resistance Genes and their Dissemination Routes

With the increase in the global demand for meat production, the livestock industry responded by implementing the widespread use of CAFOs to meet production demands. Livestock housed in CAFOs are concentrated in a single location at high densities, typically ranging from a few hundred to a few thousand animals at one location. To achieve rapid weight gains and to keep animals healthy under such conditions, most CAFOs resort to administering antibiotic growth promoters to the animals through their feeding (Aarestrup and Jenser, 2007). The US Food and Drug Administration (FDA) defines a subtherapeutic concentration of antibiotics as an amount of <0.2 g kg^{-1} of feed. In practice, the actual amount of antibiotics added to the feed varies according to the class and treatment effectiveness of the drug. On a daily basis, some amount of these antibiotics may be atmospherically dispersed into the environment through the ventilation fans equipped in a CAFO and through the dispersion of dust generated during feed mixing and the filling of feed troughs.

Given the dense population of livestock within a single confined area, a large quantity of manure is also produced. It is estimated that each pig typically produces approximately 1500 kg of manure during a 5–6 month production cycle, and that each gram of animal manure in turn contains approximately 10^{11}–10^{15} bacteria (Dowd *et al.*, 2008). Besides the high bacterial load emitted on a daily basis, animal faeces and urine also contain antibiotic residues and their breakdown by-products. For example, approximately 50–90% of the erythromycin parent compound is excreted via faeces, and approximately 10% is discharged in the urine. The remaining percentage is metabolized by demethylation in the body to generate erythromycin-H$_2$O, and is discharged from the body as waste products (Schlüsener *et al.*, 2006; Yang *et al.*, 2006). Owing to the large amount of waste products generated on a daily basis, routine cleaning of the housing areas is necessary to maintain a basic level of hygiene within the confined area. Typical management practices include scraping and flushing waste products in the housing areas to a trench. As illustrated in Fig. 6.1, accidental spillage and atmospheric dispersion of antibiotics into the environment can happen at this point by releases via construction flaws (e.g. broken liners or cracks in trenches).

Concentrated animal farming operation

Atmospheric dispersion of antibiotic contaminants

Waste lagoon

Leaching through flawed liner

Accidental spillage while transporting manure to lagoon

Daily cleaning and accidental release through engineering flaws

Land application

Affected ecosystems

Soil

Surface water

Ground water

Vertical seepage and surface runoff

Fig. 6.1. Possible dissemination routes for antibiotic contaminants in concentrated livestock production farms.

In most CAFOs, the waste products are next transported to an aerated lagoon for removal of biochemical and chemical oxygen demand, as well as volatilization of the organic nitrogen content. Solid retention time (SRT) in a manure lagoon is typically 5–30 days depending on the extent of treatment desired (Burton and Turner, 2003). However, this treatment process is not sufficient to remove all antibiotics present in the manure. For instance, given a 10-day SRT, Kim *et al.* (2005) reported that the removal of tetracycline in an activated sludge process was approximately 85%, and was primarily achieved by physical sorption of antibiotics to the sludge flocs. Therefore, the removal efficiency of an antibiotic is highly dependent on the sorption capabilities exhibited by that particular antibiotic. Although lagoon treatment achieves a certain percentage removal of antibiotics from the manure, a major limitation of such lagoons is that they are susceptible to deterioration with time. Leachate contaminated with antibiotics of low sorption capabilities can diffuse through flawed liner or sealing systems and contaminate groundwater sources (Fig. 6.1). Furthermore, manure lagoons require periodic removal of the accumulated solids that are contaminated with antibiotics of high sorption capabilities. Thus antibiotic contaminants are merely transported from the manure lagoon to another location during the desludging process.

Subsequently, the treated manure, which may still contain antibiotic contaminants, is disposed of by land application (Fig. 6.1), which is a common practice in most CAFOs, as faecal material contains a high content of nitrogen and/or phosphorus, and it can be used to enrich soil for agriculture (Chee-Sanford *et al.*, 2009). Land application is also one of the most economical ways to dispose of waste products. However, this practice can facilitate the dissemination of antibiotic contaminants into the soil and water environment. For instance, antibiotics that were previously adsorbed on to the manure particulates may leach out to contaminate groundwater and nearby surface waters during seasons of heavy rainfall, and under extreme irrigation conditions (Blackwell *et al.*, 2009).

Transfer of Antibiotic-resistance Genes at the Microbial Cell Level

Upon entry into the environment, different antibiotics have different half-lives and fates, and these may include physical sorption on to solid particulates, chemical degradation and biodegradation (Chee-Sanford *et al.*, 2009). Some antibiotic contaminants can persist for up to 100 days in the manure (Boxall *et al.*, 2006; Chee-Sanford *et al.*, 2009), and coupled with regular dosing and application of the contaminated manure on to fields, the build-up of antibiotic residues will favour the selection of antibiotic-resistant bacteria in the environment. Together with the antibiotic-resistant bacteria that originate from the manure, these bacteria can promiscuously disseminate a vast amount of ARGs via mobile genetic elements.

Plasmids, transposons and integron gene cassettes are three different types of mobile genetic elements that can disseminate ARGs among microorganisms (Fig. 6.2). A plasmid is defined as a collection of functional genetic modules that are organized into a stable, self-replicating entity, and usually confers non-core genetic functions (e.g. antibiotic resistance) on to the microbial recipient (Frost *et al.*, 2005). The first resistance plasmid (R plasmid) was discovered in Japan in strains of enteric bacteria, and was found to carry more than one ARG. For example, plasmid R100 carries genes encoding resistance to sulfonamides, streptomycin, spectinomycin, chloramphenicol and tetracycline. Furthermore, it can be transferred promiscuously between various enteric bacteria, therefore making it a serious threat to human health (Mathur and Singh, 2005; Madigan, 2009). Transposons are genetic elements that can relocate or transpose themselves to different positions within the chromosomal DNA or plasmid. A key distinctive feature of transposons is that they carry transposase, which is an enzyme necessary for transposons to move around the chromosomal DNA (Madigan, 2009). An integron gene cassette is a combination of an integron, which is a chromosomal insertion site for genes, and one or more modular gene cassettes that encode the functional genes. An integron gene cassette has several distinctive traits: it comprises the integrase gene (*int*), an adjacent recombination site (*attI*), and one or more gene cassettes that can be expressed based on the promoter in the integron (P_{ANT}) (Hall and
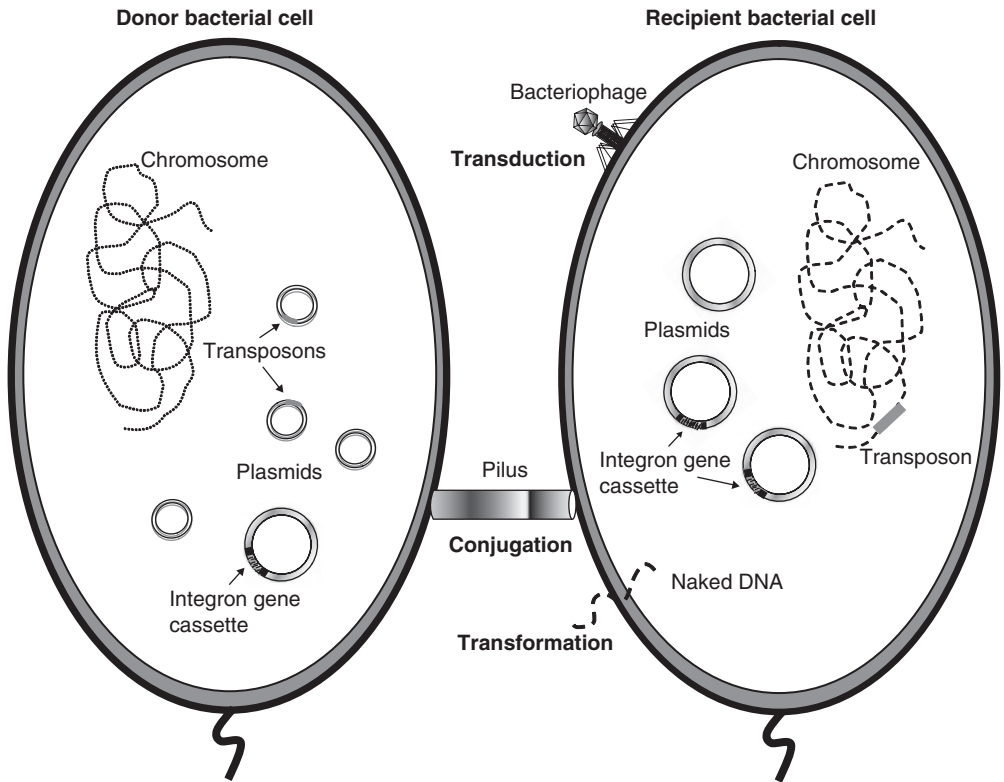
Fig. 6.2. Mobile genetic elements and different mechanisms for horizontal gene transfer (courtesy Frost *et al.*, 2005).

Collis, 1995). Unlike transposons, the insertion of integrons is highly site specific. A survey of over 600 full or partially sequenced bacterial genomes revealed that 9% of the sequenced genomes contained integrons that can be categorized into classes 1, 2 or 3. Among these three classes, class 1 integrons have been frequently identified as the central player in the worldwide dissemination of ARGs (Boucher *et al.*, 2007; Gillings *et al.*, 2008).

A recent PCR-based survey of 17 European habitats, including farm soils, waste water, and cattle, chicken, and pig manure, was able to detect the presence of markers for several mobile genetic elements, including broad-host-range plasmids, conjugative transposons and integron gene cassettes (Smalla *et al.*, 2000). These mobile genetic elements encode genes that confer resistance to the antibiotics gentamycin (Heuer *et al.*, 2002), sulfadiazine (Heuer and Smalla, 2007), and amoxicillin (Binh *et al.*, 2007). These surveys provided an outlook on the ubiquity, persistence and stability of a vast pool of mobile genetic elements in antibiotic-affected hot spots or sites where gene transfer and recombination are higher than in non-affected locations.

Under favourable conditions, the pool of mobile genetic elements can be transferred promiscuously among different bacteria by three mechanisms:

(i) conjugation, a process of genetic transfer that involves cell-to-cell contact; (ii) transformation, a process by which free DNA is incorporated into a competent cell and brings about genetic change in the recipient; and (iii) transduction, a process by which DNA is transferred by bacteriophage (Fig. 6.2). Conjugation was originally thought to play a more important role in disseminating ARGs across microorganisms of different genera and species (Courvalin, 1994) because the ubiquity of biofilm formation or high cell densities and close cell contact found in gut ecosystems would favour the dissemination of ARGs via conjugation (Molin and Tolker-Nielsen, 2003). However, with the advent of molecular tools, it is now recognized that various ecosystems, such as activated sludge and marine waters, harbour a large diversity and abundance of bacteriophages along with the bacterial population. The high numbers of bacteriophages suggest that transduction may also be a major force for horizontal gene transfer in ecosystems such as those in close proximity to aquaculture farms (Cabello, 2006). Likewise, the stability of naked DNA in the soil environment suggests the possibility for bacteria to acquire genetic elements through transformation. Therefore, although the relative contribution of each mechanism remains unknown, it is likely that all three may play significant roles in disseminating ARGs among different microorganisms.

The Spread of Antibiotic Resistance

In this section, we provide evidence to show that the use of antibiotics in animal husbandry can contribute to the spread of ARGs and antibiotic-resistant bacteria to the environment by presenting two case studies. The first involves data that we have gathered over a 3-year monitoring study conducted in three Illinois swine-production facilities. This case study documents the presence of ARGs in waste lagoons and nearby groundwater-monitoring wells and soil ecosystems. The second case study, which is based on several reports from published literature, documents the presence of ARGs and antibiotic-resistant bacteria in food products sold to consumers. This provides indirect evidence that antibiotic use in livestock production can possibly affect human health.

Case study 1: swine production farms

Two swine production farms in Illinois (designated as Sites A and C) were monitored extensively over a 3-year period. As illustrated in Fig. 6.3a and b, each site was fitted out with a network of groundwater sampling wells (designated A1–A16 and C1–C7, respectively) for the monitoring of antibiotic contaminants and ARGs. These wells were distributed around the open waste-treatment lagoons, allowing monitoring of groundwater both 'upstream' and 'downstream' of the waste lagoons. The 'upstream' wells served as on-site controls, allowing us to distinguish ARGs originating in waste lagoons from those derived from other sources in the landscape (e.g. the indigenous environmental microbiota). Additionally, we surveyed agricultural soils at a third site (Site E, Fig. 6.3c), where manure from a deep pit treatment system was applied as fertilizer.
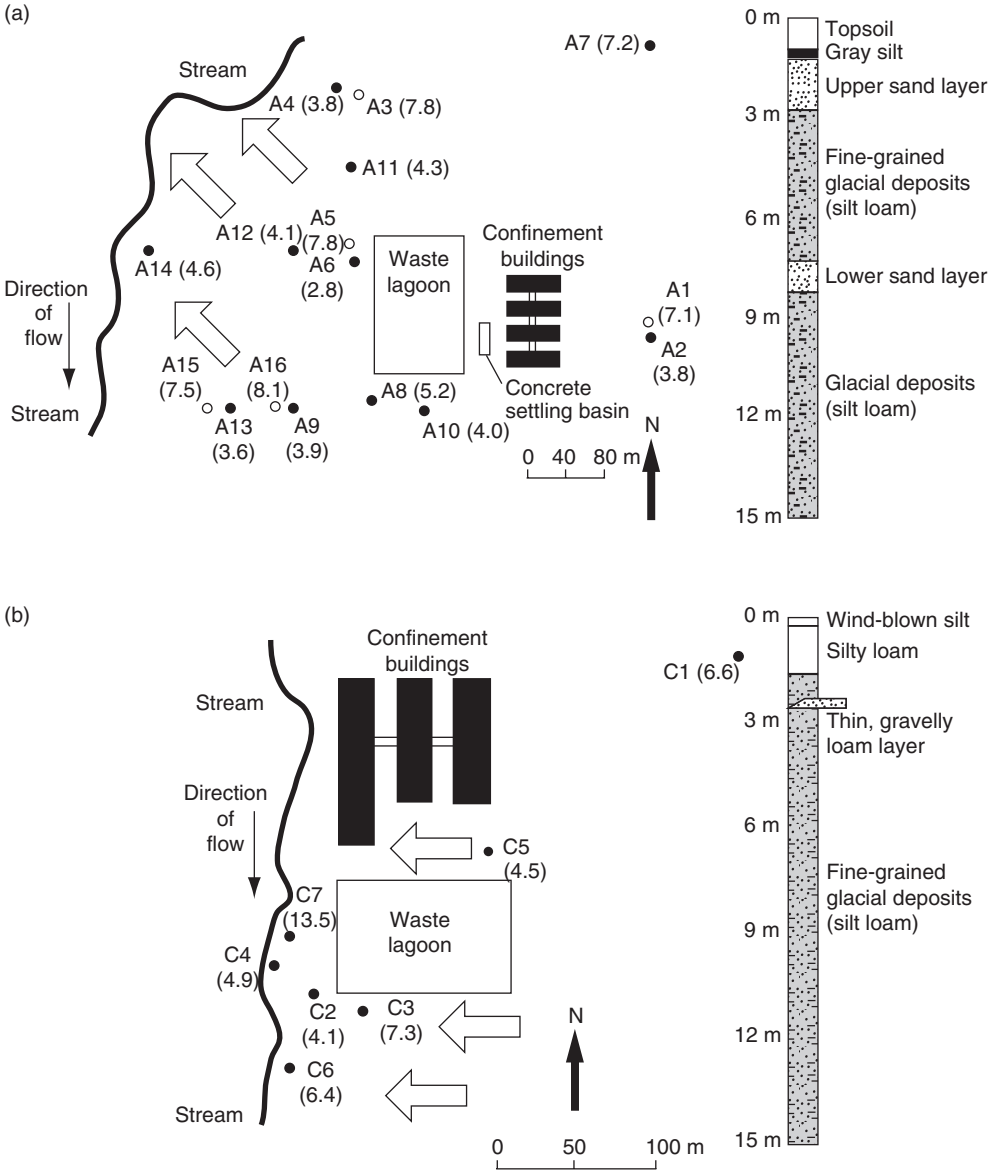
Fig. 6.3. Site maps and corresponding stratigraphic columns indicating the characteristics of sand layers at different vertical depths at three experimental sites. (a) Site A: wells A1 to A16 are groundwater monitoring wells; A7 is an upstream background monitoring well. (b) Site C: wells C1 to C7 are groundwater monitoring wells; C1 is an upstream background monitoring well.

Continued

(c)
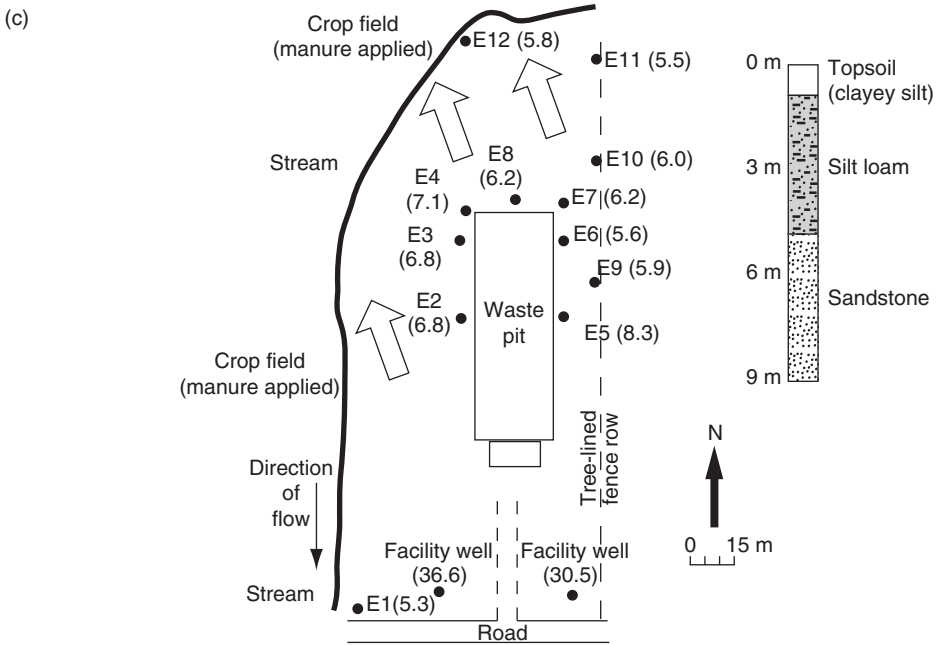
Fig. 6.3. *Continued.* (c) Site E: wells E1 to E12 are groundwater monitoring wells; there are two wells providing water to the facility (facility wells); well E1 is an upstream background monitoring well. Agricultural wells were surveyed at Site E.

Preliminary surveys of the study sites suggested that genes conferring resistance to tetracycline and tylosin (a macrolide similar to erythromycin) were of particular interest. Tetracycline and tylosin are both commonly used for prophylaxis and growth promotion at Sites C and E. Site A had used both antibiotics before our study but had reduced its antibiotic usage considerably in the later stages by maintaining a high-health status herd. Erythromycin, which is strictly reserved for human use, was not used at these sites. However, erythromycin and tylosin are both macrolide antibiotics, and *erm* genes have been shown to confer resistance to both these antibiotics.

Detection and quantification of ARGs were first achieved by designing primer sets that targeted four major groups of antibiotic resistance genes, namely: (i) four classes of genes (*tet*(M), *tet*(O), *tet*(Q), *tet*(W)) that confer resistance to tetracycline by means of ribosomal protection proteins; (ii) three classes of genes (*tet*(C), *tet*(H), *tet*(Z)) that confer resistance to tetracycline by means of efflux pump proteins; (iii) two classes of genes (*tlr*(B), *tlr*(D)) that confer resistance to tylosin; and (iv) six classes of methylase genes (*erm*(A), *erm*(B), *erm*(C), *erm*(F), *erm*(G), *erm*(Q)) that confer resistance to erythromycin (Aminov *et al.*, 2001, 2002; Mackie *et al.*, 2006; Koike *et al.*, 2007, 2010).

Our findings showed that manure-treatment lagoons and storage pits at these sites always contained every *tet* gene for which we surveyed (Koike *et al.*, 2007), and, likewise, five out of six *erm* genes found at these sites were detected in nearly every lagoon sample (Koike *et al.*, 2010). A subset of groundwater

wells contained both *tet* and *erm* genes with much higher frequencies than other wells, and the detection frequencies of most *tet* and *erm* genes for these wells were close to 100%. The wells concerned were all located in close proximity to the source lagoon, and most of them were situated in a relatively porous aquifer that bisected the lagoon.

Cloning and sequencing of the *tet*(W) genes revealed phylogenetic patterns that correlated well with gene detection frequency patterns of affected versus non-affected wells. There was a distinct sub-cluster consisting of *tet*(W) genes that were found only in 'upstream' background control wells, while *tet*(W) genes from 'downstream' affected wells were often of similar or identical phylogeny to those from lagoons or swine manure (Koike *et al.*, 2007). Thus, at least a portion of the *tet*(W) gene pool of the native groundwater microbiota was readily distinguishable (99.8% gene similarity) from the pool of genes associated with swine-production activities and affected wells. In addition, the phylogenetic distribution of *tet*(W) sequences from lagoons was broader than that of sequences recovered from background wells, which suggests that the *tet*(W) gene pool of swine waste was more diverse than that of the native groundwater microbiota. The detection of *erm* genes at these sites suggested selection due to tylosin usage, as no erythromycin was used at all three farms.

Besides detecting ARGs in the groundwater wells, our surveys at Site E further showed that soils were also affected by the addition of manure. Before manure injection, we did not detect the presence of *tet*(C), *tet*(H) and *tet*(Z) in our grab samples. Approximately 3 days after manure injection, it was possible to detect these three tetracycline-resistant genes, along with *tet*(M), *tet*(O), *tet*(Q) and *tet*(W) in most of the soil samples surveyed. Over time, the detection frequency of *tet*(C), *tet*(H), *tet*(M) and *tet*(Z) returned to near-zero, while others, such as *tet*(O), *tet*(Q) and *tet*(W) persisted for up to 7 months (A. Yannarell, unpublished data).

Interestingly, the temporal and spatial patterns of ARGs in soils and water did not seem to depend on direct selection pressure resulting from antibiotic persistence in the environment, as levels of antibiotic residues were generally found to be low or below the detection limit in soil and water samples. These results support previous observations that the problem of antibiotic-resistant bacteria is not necessarily linked to the persistence of antibiotic residues in the environment (McEwen, 2006). Instead, the spatial and temporal patterns of antibiotic resistance genes at these three sites suggest that exposure to hog waste is the most important factor.

Case study 2: contaminated food produce

While the spread of ARGs to soil and water represents an environmental concern, the presence of ARGs in food produce provides a direct pathway for these contaminants to enter into the human food chain, and thus these contaminants may impose adverse health impacts when consumed. To evaluate this concern, Kumar *et al.* (2005) first demonstrated that antibiotics are bioaccumulated in crops that were grown on land to which manure had been applied.

Garofalo *et al.* (2007) went on to collect faecal samples from chickens and pigs, as well as specimens of raw chicken and pork meat from production factories that were located in central Italy. Samples were subsequently extracted for bacterial DNA, and a PCR-based approach was utilized to detect 11 types of genes encoding resistance against tetracycline, erythromycin, vancomycin, aminoglycosidase, methicillin and β-lactams. Genes conferring resistance against tetracycline and erythromycin, namely *tet*(K) and *erm*(B), were prevalent in the meat samples, and Garofalo *et al.* (2007) concluded that contamination is likely to be due to the breeding process rather than to processing techniques in the production farms.

Similarly, Donabedian *et al.* (2003) isolated a total of 360 enterococci from food purchased in grocery stores, and from faeces of healthy chickens, turkeys, cattle and pigs reared on farms. The isolates were evaluated for genes that confer resistance against gentamicin, which is an antibiotic that is commonly used in swine farming and widely used in chicken and turkey rearing in the USA. Donabedian *et al.* (2003) found that when gentamicin resistance genes are highly prevalent in the faeces of food-producing animals, there was an equally high prevalence of those resistance genes in the food specimens. These findings reiterated those reported by Garofalo *et al.* (2007), and provided evidence of the spread of gentamicin-resistant enterococci from livestock production to food.

Independent studies have also detected the presence of ARGs in fermented dairy products and probiotic food. For example, Huys *et al.* (2004) isolated 187 enterococci from European cheeses and found that 24% of these isolates exhibited phenotypic resistance to tetracycline. Furthermore, 4% of the isolates exhibited multiple resistance to tetracycline, erythromycin and chloramphenicol. It was further demonstrated that some of the isolates were able to transfer ARGs to recipient strains through conjugative transposons (Huys *et al.*, 2004). In a separate study, Hummel *et al.* (2007) examined the antibiotic resistance of 45 strains of lactic acid bacteria. Using a PCR-based approach, genes that confer resistance to β-lactams, chloramphenicol, tetracycline and erythromycin were found in approximately 7% of the isolates. However, in some of the strains that possessed the chloramphenicol-resistant genes, the genes were found to be silent after performing a reverse transcription PCR and phenotypic trait testing. The Hummel *et al.* (2007) study demonstrated that genotypic detection of ARGs alone is not sufficient to distinguish between silent and active resistance genes, and that phenotypic testing of ARGs should also be performed. However, it should be noted that phenotypic testing typically relies on the isolation of bacteria, and that currently, cultured bacteria are estimated to constitute only less than 0.001–15% of the total cell counts in environmental samples (Amann *et al.*, 1995; Tamaki *et al.*, 2005). Therefore, phenotypic testing often underestimates the total antibiotic resistance gene pool compared with the genotypic approach (D'Costa *et al.*, 2006; Sommer *et al.*, 2009). It is important, therefore, to use genotypic and phenotypic testing as complementary approaches to examine both the total antibiotic resistance gene pool and the expressed ARGs.

The Spread of Antibiotic Resistance and Why Do We Care?

The detection of ARGs in soil and water ecosystems, as well as in food produce, suggests that antibiotic-resistant bacteria can be transmitted to humans through various routes, such as skin contact and oral ingestion. To evaluate how the use of antibiotics in livestock production has affected the human population, we typically rely on epidemiology studies to compare the percentage occurrence of antibiotic-resistant bacterial infections in the human population before and after an antibiotic ban was imposed. An example of such a study is a comparative evaluation of the occurrence of vancomycin-resistant enterococci (VRE) in Europe before and after the related antibiotic avoparcin was banned as an animal growth promoter in 1997.

During the late 1990s, after approximately 20 years of approval of the use of avoparcin as an animal growth promoter in Europe, the community prevalence of VRE was estimated at 2–12%. Interestingly, the infected population included people with no prior history of hospitalization, and suggested that some percentage of the infections within the community reservoir was due to the use of avoparcin in livestock production (McDonald *et al.*, 1997; Smith *et al.*, 2005). Since avoparcin was banned in 1997, there has been a marked reduction in the prevalence of VRE within the European Community (EC) (Klare *et al.*, 1999). This observation further reiterates the possible impact on the community due to the use of antibiotics in livestock production.

Besides potential adverse impacts on human health, the dissemination of antibiotic contaminants from animal husbandry can also create a microbial 'perfect storm' in which unpredictable and unforeseen factors converge and result in the emergence of novel microbial threats. For example, a 2003 report from the Institute of Medicine of the National Academies (of the USA) identified 13 broad categories of socio-economic, ecological, environmental and biological factors that play a role in the emergence of novel microbial threats (Smolinski *et al.*, 2003). Among these are factors relating to: (i) land use and agricultural practices, particularly the promiscuous use of antibiotics in food production; (ii) changes in human susceptibility to microbial risk, including the acquisition of antibiotic resistance by pathogens; and (iii) microbial adaptation, which is enhanced when microorganisms are introduced to novel environments, interact with each other in species-rich environments, and participate in horizontal gene exchange.

As Smolinski *et al.* (2003) point out, the rampant use of antibiotics in livestock production can create situations where the combined gene pools of animal-associated enteric bacteria, antibiotic-resistant bacteria and environmental microbiota are conducive to the generation of novel and possibly undesirable and even dangerous microbial threats. For example, environmental bacteria may acquire 'enteric' genes that enable them to spread to human or animal hosts. Conversely, enteric pathogens may acquire genes that enable them to persist in new soil or water environments, which then serve as reservoirs for the spread of these microorganisms. In addition, enteric bacteria may acquire from environmental microorganisms traits that serve one purpose in

soil or water but that also contribute to virulence in a mammalian host (Casadevall, 2006). For example, capsules that help organisms to survive predation in the soil may help pathogens to evade mammalian immune systems (Steenbergen *et al.*, 2001), and molecules that serve nutritional functions in the environment (e.g. proteases or phospholipase) can cause damage to host cells in a mammalian context (Casadevall and Pirofski, 2003; Casadevall, 2006).

The confluence of several of these factors can generate favourable conditions in which novel microbial threats emerge with elevated frequency and impose a compounded health risk (Fig. 6.4). These threats include new human and animal pathogens, zoonotic pathogens that can pass between human and animal hosts, and previously seen pathogens that have acquired new traits, such as antibiotic resistance, that increase their potential to cause harm. Novel zoonotic pathogens can also emerge from genetic rearrangements that allow otherwise harmless organisms to acquire new traits associated with human or animal virulence, including the ability to: (i) infect and survive in new hosts; (ii) persist in environments that increase their contact with human or animal hosts; (iii) evade host immune response; (iv) resist medical/veterinary treatment (e.g. antibiotics); or (v) damage host cells.

In early 2009, we witnessed the emergence of a novel influenza A (H1N1) viral strain of swine origin (S-OIV). This new strain is a reassortment of two
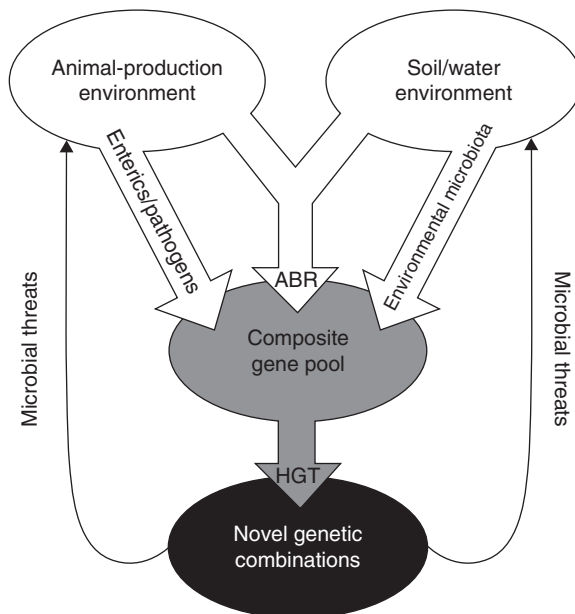
Fig. 6.4. Conceptual model for a microbial 'perfect storm'. Enteric microorganisms, antibiotic-resistant bacteria (ABR), and environmental microbiota can interact in soil and water systems affected by animal-production activities. Their combined gene pools drive microbial adaptation via horizontal gene transfer (HGT). Emergent microbial threats can pose a risk to human and animal health, or they may persist in the environment, thus increasing the risk of exposure and driving further adaptation.

previously circulating strains: a 'triple-reassortment' swine influenza that has been circulating in North America since 1998, and an H1N1 strain that has been circulating for decades in swine populations in Europe and Asia (Smith *et al.*, 2009a). The lack of systematic surveillance of influenza in swine has favoured the mixing of genetic elements, and ultimately led to the emergence of viruses with pandemic potential (Smith *et al.*, 2009b). Summing up, we need to be cautious and consider the possibility that new antibiotic-resistant microbial threats can emerge as a consequence of the unchecked use of antibiotics in livestock production.

Knowledge Gaps

Much of what is known about resistance mechanisms and horizontal gene transfer have come from the study of clinical isolates. In contrast, the total ARG pool in the agriculture-affected environment remains elusive (D'Costa *et al.*, 2006). Current genotypic and phenotypic approaches for examining ARGs rely on prior knowledge and detect only those genes that were already known. Such approaches do not provide new insights into unknown antibiotic resistance mechanisms (Sundsfjord *et al.*, 2004).

Recent advances in genome sequencing technologies have provided a cost-effective way to determine the ARG pool. For example, through the study of various opportunistic pathogens and clinical isolates, it was revealed that the genes encoding efflux proteins are commonly found in most sequenced bacterial genomes (Wright, 2007). The vast collection of efflux pumps presumably provides robustness and flexibility to the microorganism so that it can survive in diverse environments (Stover *et al.*, 2000; Poole and Srikumar, 2001; Piddock, 2006a). Opportunistic pathogens of environmental origins can also utilize such traits to promote pathogenicity (Piddock, 2006b). In the near future, more sequencing effort should be undertaken to provide information on pan-microbial genomes and possibly discover new ARGs and antibiotic-resistant mechanisms (Wright, 2007).

Besides the need to elucidate the ARG pool, there is also a need to increase our understanding of the potential for gene exchange among environmental, animal-associated and antibiotic-resistant microorganisms in agriculture-affected environments (Smalla and Sobecky, 2002; van Elsas and Bailey, 2002). Because horizontal gene transfer can effectively expand the gene pool available to a potential pathogen, it is important to understand what happens when human activities bring together microorganisms with a variety of threat-related genes, such as virulence and ARGs. However, this remains a daunting task to perform, primarily because of the complexities involved in an environmental setting (Nielsen and Townsend, 2004). For example, soil typically contains approximately 10^7 bacterial cells g^{-1} (Gans *et al.*, 2005), and acquiring virulence genes together with ARGs in an endemic soil microorganism may be a rare event that occurs at low transformation rates. Therefore, conventional PCR-based and cultivation methods would not be able to detect the occurrence of this rare event easily unless an extensive sampling and surveillance effort had been carried out (Nielsen and Townsend, 2004).

Concluding Remarks

In their natural habitat, some microorganisms produce antibiotics that are selective against their enemies. In response to antibiotics, the targeted micro-organisms gradually evolve antibiotic-resistance mechanisms and genes in a bid to outcompete. When human beings eventually discovered these amazing resources, we ingeniously harnessed them and hailed new milestones in human medicine. However, what was once useful to bring humans back to good health has now become a potential menace as a result of inappropriate use in clinical settings and livestock production. Such practices have indirectly caused human pathogens to evolve and rapidly acquire multiple drug resistance that renders our existing antibiotics ineffective.

As of now, many published studies have demonstrated the presence of ARGs in the environment and in food produce, but they have not provided extensive epidemiological evidence to conclusively prove the link between ARGs released by animal husbandry and the emergence of new diseases. However, one should always handle environmental concerns and human-health related issues with prudence. It has been suggested that if we react fast enough to remediate the current situation, we could perhaps delay the adverse effects brought about by rapid dissemination of antibiotic contaminants (Johnsen *et al.*, 2009). As the race to develop new antibiotics is speeding up, more research and surveillance studies need to be performed to ensure responsible use of antibiotics in both clinical settings and livestock production.

Finally, the American Academy for Microbiology has recently published a report entitled *Antibiotic Resistance: An Ecological Perspective on an Old Problem* (AAM, 2009). The scope of the problem is much larger than that which has been considered in this chapter. However, several of the conclusions of the report are pertinent and worth reiterating. The first is that antibiotic resistance is a pandemic that compromises treatment of all infectious diseases, and that is at present uncontrollable. The reasons controlling the establishment and spread of antibiotic resistance are complex, mostly multifactorial and largely unknown. The second conclusion is that responsibility is due partly to medical, veterinary and industrial practice, but also to economics and politics, as well as to antibiotics themselves. Thus, the use of growth-promoting antibiotics in livestock production is only a part of problem and should not be a scapegoat. Third and last, ARGs are not new entities; they should be considered as fundamental components of microbial diversity and life, as well as components that represent evolutionary phenomena.

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7 On-farm Mitigation of Enteric Pathogens to Prevent Human Disease

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Introduction

The importance of controlling foodborne pathogenic bacteria postharvest, starting with abattoir processing, is well recognized. Indeed, a recent review of the beef production chain showed significant reduction in the prevalence of *Escherichia coli* O157, *Salmonella enterica* and *Listeria monocytogenes* throughout abattoir processing (Rhoades *et al.*, 2009). However, despite efforts to mitigate pathogens, contaminated meat products still enter the food chain and pathogenic bacteria are frequently detected in food products. For example, *Salmonella* spp. (Delhalle *et al.*, 2009), *E. coli* O157:H7 (Naugle *et al.*, 2005) and *Campylobacter* spp. (Moran *et al.*, 2009) have all been isolated from retail meats.

It is evident that pathogenic contaminants from animals can enter the food chain during processing (Arthur *et al.*, 2007). Using pulsed-field gel electrophoresis (PFGE), *E. coli* O157:H7 on carcasses (Barkocy-Gallagher *et al.*, 2001) and antimicrobial-resistant *E. coli* in ground beef (Alexander *et al.*, 2010) have been source tracked to cattle at slaughter. Additionally, *Salmonella* spp. (Vieira-Pinto *et al.*, 2006) and *Yersinia enterocolitica* (Laukkanen *et al.*, 2009) isolated from pig carcasses had similar PFGE genotypes to isolates originating from digesta and tonsil samples. While both the animal and bacterial species influence dissemination, pathogen load also affects the likelihood of carcass contamination (Brichta-Harhay *et al.*, 2008), with higher carriage levels making it more difficult to avoid the adulteration of meat products.

The prevalence of foodborne pathogens in cattle (Gansheroff and O'Brien, 2000), pigs (Letellier *et al.*, 2009) and poultry (Arsenault *et al.*, 2007) preharvest has been shown to be positively correlated with carcass

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contamination. Reducing colonization and the shedding of pathogens can, therefore, play an integral role to mitigating foodborne disease postharvest. Additionally, contamination of produce with enteric pathogens, such as occurred during the 2006 *E. coli* O157:H7 outbreak in the USA (Jay *et al.*, 2007), has prompted greater assessment of the role of animal waste as a vehicle for transmission. To address these issues, an increasing number of studies are investigating on-farm mitigation strategies. Preharvest control points are being analysed that include altering microbial populations of the digestive tract or direct targeting of the pathogen at the animal level. Equally as important are strategies to manage waste in order to prevent environmental contamination and the transfer of pathogens among animals. This chapter will review the on-farm methods for controlling enteric pathogenic bacteria. As an example, a schematic of how these control points can be used to mitigate the spread of *E. coli* O157:H7 in a feedlot production system is illustrated in Fig. 7.1.

Reducing Pathogens in Animal Feed and Water

Feeds from poultry, swine and cattle production systems have been identified as vectors of foodborne pathogens to livestock (Doyle and Erickson, 2006). For example, isolates of *S. enterica* serovar Typhimurium (*S.* Typhimurium) or *E. coli* O157:H7 that were cultured from stored feedstuffs at feedlots were genetically related to isolates collected from faeces of cattle at the same feedlot (Davis *et al.*, 2003). For poultry and swine, feed is often pelleted or extruded, and exposed to temperatures as high as 90°C (Doyle and Erickson, 2006). Exposure to heat can denature enzymes that confer anti-nutritional properties, and pelleted feed flows more easily in handling systems; during processing, exposure to heat has also been recognized as an efficient method of mitigating feed-borne pathogens. Mashed poultry feed has been reported to be more frequently contaminated than pelleted feed (21% versus 1.4%; Veldman *et al.*, 1995). Himathongkham *et al.* (1996) showed that heat treatment of poultry feeds for 90 s at 93°C will cause a 10,000-fold reduction in contaminating *Salmonella*. The addition of propionic acid to the feed can increase the sensitivity of pathogens such as *Salmonella* to heat. For example, heating feed for 80 s at 71°C in the presence of 0.2% propionic acid resulted in a 4 log (unit) reduction in *Salmonella* (Matlho *et al.*, 1997). However, the effectiveness of heating is dependent on the bacterial species as heating feed at 71°C for 120 s only reduced *E. coli* O157:H7 in the feed by 2.2 log units (Hutchison *et al.*, 2007). Additionally, the reduction of pathogen loads in feeds by heat treatment does not preclude the possibility of subsequent contamination of the feed during downstream handling, either at the feed mill or on the farm. Furthermore, many livestock operations do not heat treat feed before providing it to livestock (Davies *et al.*, 2004). Also, rodents, birds and wildlife can contaminate stored feed with pathogens (Davies *et al.*, 2004), necessitating the need for proper hygiene practices and storage of feed throughout the production continuum.
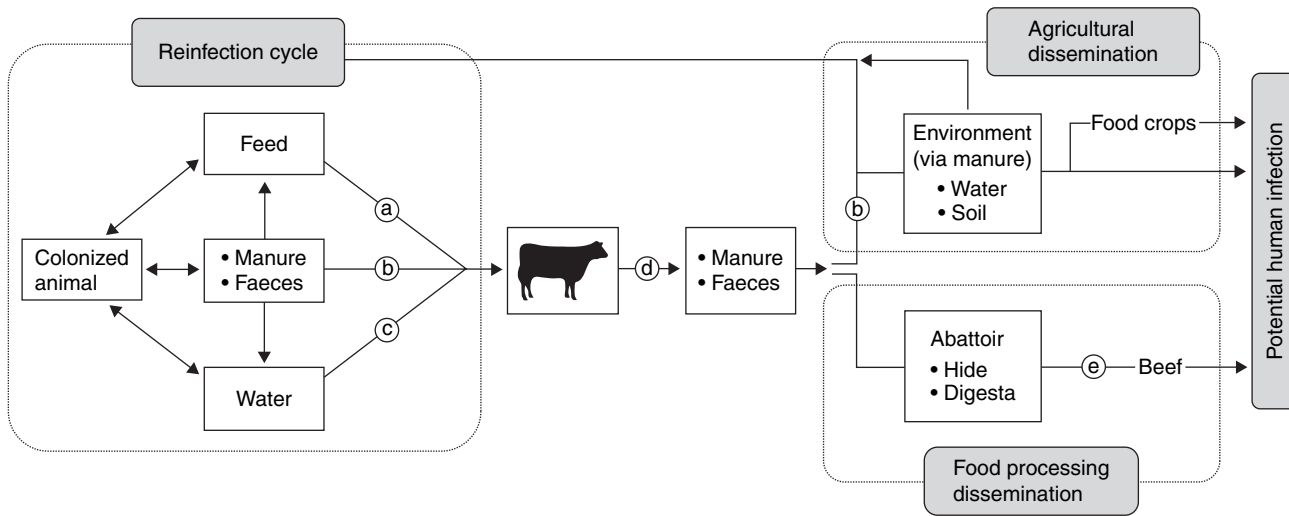
Fig. 7.1. Dissemination of *Escherichia coli* O157:H7 within a beef production system and to food and environmental sources. Letters a–d within the arrows indicate possible on-farm mitigation control points for the bacterium: a, feed hygiene; b, feedlot hygiene, waste management; c, water treatment; d, use of antimicrobials, bacteriophages, probiotics, vaccination, immunotherapy. Letter e, postharvest mitigation.

Water can also serve as a vector of pathogens to livestock. For example, several studies have shown that water troughs in feedlots can harbour large numbers of *E. coli* O157:H7. In one study, 12% of water troughs at feedlots tested positive for *E. coli* O157:H7 (Van Donkersgoed *et al.*, 2001), and it appears that long-term survival of the bacterium in this environment (up to 245 days) is possible (LeJeune *et al.*, 2001). LeJeune *et al.* (2004) observed no differences in the prevalence of *E. coli* O157:H7 in feedlot water troughs that contained chlorinated or non-chlorinated water. Similarly, Zhao *et al.* (2006) reported that chlorine had little effect on *E. coli* O157:H7 in water that contained rumen contents. Contaminating faeces are often observed in feedlot water troughs, and the resulting elevated organic matter can negate the efficacy of chlorine as an antimicrobial treatment for water (Doyle and Erickson, 2006). In the study by Zhao *et al.* (2006), four treatments containing a combination of chemicals that included lactic acid, acidic calcium sulfate, caprylic acid, sodium benzoate or chlorine dioxide reduced numbers of enterohemorrhagic *E. coli* by up to 5 log units, even in the presence of rumen or faecal contents. Unfortunately, these treatments reduced water consumption in a manner that could adversely affect the health of cattle; the authors suggested that periodic treatment of the water may overcome this problem, but such a strategy lacks practicality at the commercial level. Other methods shown to reduce *E. coli* O157:H7 in cattle drinking water include the use of electrolysed oxidizing water (Stevenson *et al.*, 2004), and the addition of sodium caprylate (Amalaradjou *et al.*, 2006), or the plant essential oil *trans*-cinnamaldehyde (Charles *et al.*, 2008). However, many of these strategies are expensive, and their effects on water consumption have not yet been examined.

Antimicrobial Feed Additives

Several types of antimicrobial feed additives have been investigated for targeting specific or general groups of pathogenic bacteria. The application of neomycin sulfate has been reported to reduce *E. coli* O157:H7 to undetectable levels in naturally colonized cattle (Elder *et al.*, 2002) and to lower its prevalence in feedlot cattle (Woerner *et al.*, 2006). While these preliminary results are impressive, adoption of the mass medication of cattle with an antimicrobial of the same class of antibiotics as those commonly used in human medicine is unlikely. This concern arises over promotion of the emergence of antimicrobial-resistant bacteria in livestock, which can also enter the food chain through the contamination of meat in the abattoir (Alexander *et al.*, 2010). Most research on novel antimicrobials has therefore focused on agents unrelated to traditional antibiotics.

Some chemicals have been used to target specific metabolic pathways of pathogens. *Salmonella* and *E. coli* encode nitrate reductase, which reduces nitrate to nitrite under anaerobic conditions (Oliver *et al.*, 2009). The enzyme does not differentiate between nitrate and chlorate, and when the latter is present, it is reduced to bactericidal chlorite. Evaluation of sodium chlorate has shown its potential to reduce *Salmonella* and *E. coli in vivo*. Sodium chlorate

supplementation resulted in a 2 and 3 log reduction of *E. coli* O157:H7 in the rumen and faeces, respectively, of cattle challenged with this pathogen (Callaway *et al.*, 2002), with similar results in sheep (Callaway *et al.*, 2003). This approach has also been shown to be effective against *Salmonella*. In broilers challenged with *S.* Typhimurium, birds administered a chlorate product had reduced prevalence (2% versus 36.7%) and numbers of this bacterium in their caeca (0.96 versus 2.52 log colony forming units (cfu) g^{-1}) than birds not fed the product (Byrd *et al.*, 2003). Reduced concentrations of *S.* Typhimurium in the crops of turkeys have also been reported in birds administered chlorate (Moore *et al.*, 2006).

Campylobacter utilizes amino acids as a principal energy substrate and, as a result, inhibiting amino acid catabolism can potentially reduce their competitiveness in the gut (Horrocks *et al.*, 2009). For example, the inclusion of the deaminase inhibitors diphenyliodonium chloride and thymol in mixed faecal cultures from pigs reduced the number of *Campylobacter* by more than 3 log units (Anderson *et al.*, 2009). Caprylic acid, a medium-chain fatty acid with eight carbons, has also been shown to mitigate *Campylobacter* (Solis de los Santos *et al.*, 2008); in chickens challenged with *Campylobacter*, doses of 0.35–0.87% caprylic acid in dietary dry matter decreased caecal concentrations of this bacterium by 2.0–5.0 log units. Caprylic acid has also been shown to reduce the numbers of *S. enterica* serovar Enteritidis (*S.* Enteridis) colonizing intestinal tissues in experimentally inoculated chickens (Johny *et al.*, 2009). Similarly, the short-chain fatty acid butyric acid has been reported to reduce colonization and shedding of *Salmonella* in chickens (Van Immerseel *et al.*, 2005). The exact mechanisms by which these acids reduce colonization remain unknown.

The bacteriostatic and bactericidal activities of plant extracts termed essential oils have been well described (Ojha and Kostrzynska, 2007) and, more recently, their antimicrobial effects on pathogenic organisms in livestock have been examined. Tasco-14™ is a proprietary product (from Acadian Seaplants Ltd, Dartmouth, Nova Scotia) obtained from the brown seaweed *Ascophyllum nodosum*, which grows along the North Atlantic coastline. This plant product enhances immune function, improves carcass characteristics and extends the retail display shelf life of beef (Allen *et al.*, 2001; Montgomery *et al.*, 2001; Braden *et al.*, 2007). The product has also been investigated for reducing the shedding of *E. coli* O157:H7. The inclusion of Tasco-14™ at a level of 2% in the diet of cattle for a period of 2 weeks before slaughter reduced the prevalence of *E. coli* O157:H7 in faecal samples (11%) and hide swabs (36%) compared with the levels 1 day before being fed the additive (Braden *et al.*, 2004). Bach *et al.* (2008) tested the same product on feedlot cattle experimentally inoculated with *E. coli* O157:H7. In that study, steers were fed Tasco-14™ in the diet at a level of 1% for 14 days, 2% for 7 days, or 2% for 14 days after inoculation; the dietary treatments commenced 7 days after inoculation. Throughout the sampling period, detection and concentration of the pathogen in faecal samples was less frequent when the additive was fed at 1% for 14 days or 2% for 7 days, compared with samples from animals fed Tasco-14™ at 2% for 14 days after inoculation and control animals

receiving no additive. Wang *et al.* (2009) reported that phlorotannins extracted from *A. nodosum* were both bactericidal and bacteriostatic to *E. coli* O157:H7. Other studies have indicated that plant tannins (Min *et al.*, 2007) and essential oils (Callaway *et al.*, 2008b) exhibit bactericidal activity against *E. coli* O157:H7, although the extent of this antimicrobial activity varies among plant sources (Min *et al.*, 2007).

Probiotics

Probiotics are any of a number of live microorganisms, including yeast, *Lactobacillus* or other bacterial strains, extracts and enzyme preparations (Elam *et al.*, 2003) that are known to be safe and produce beneficial results when fed individually or as mixtures to livestock. Probiotics have been used in the cattle industry for over 20 years, primarily to improve growth performance, milk production or feed conversion efficiency (LeJeune and Wetzel 2007). Recently, a new generation of probiotics has started to be developed which, along with growth performance benefits, also exhibits activity against pathogens in livestock (Callaway *et al.*, 2009).

In the poultry industry, a direct-fed microbial (DFM) containing a mixture of *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium* and *Bifidobacterium bifidium* (Talebi *et al.*, 2008) and marketed under the trade name of PrimaLac has been well characterized. This DFM has been shown to improve poultry gut health and bird performance after oral challenge with *S.* Typhimurium, *S. enterica* serovar Heidelberg (*S.* Heidelberg) and *S. enterica* serovar Kentucky (*S.* Kentucky) (Rahimi *et al.*, 2009). PrimaLac has also been shown to reduce the prevalence of *Campylobacter jejuni* in broiler chicks (Willis and Reid, 2008) and reduce shedding of sporulated ooycsts of *Eimeria acervulina*, thereby acting as an alternative to the anti-protozoal products that are presently used to control coccidiosis (Dalloul *et al.*, 2003).

In the swine industry, a DFM containing *Bacillus subtilis* served as an alternative to the use of sub-therapeutic antibiotics as it reduced scours in piglets that were challenged with *E. coli* K88 (Bhandari *et al.*, 2008). A DFM containing *Bifidobacterium lactis* Bb12 and *Lactobacillus rhamnosus* LGG reduced adhesion of *Salmonella, Clostridium* and *E. coli* to swine intestinal tissues *in vitro* (Collado *et al.*, 2007), and similar results were observed *in vivo* (Konstantinov *et al.*, 2008). These researchers demonstrated that a DFM containing *Lactobacillus sobrius* DSM 16698 was able to reduce the colonization of enterotoxigenic *E. coli* in piglets immediately after weaning. Inclusion of *E. faecium* in sow diets has also been shown to reduce the rate of shedding of Chlamydiaceae and the transmission of this pathogen to piglets (Pollman *et al.*, 2005).

In contrast to poultry and swine studies, DFMs for cattle have focused primarily on the control of pathogenic strains of *E. coli* (Elam *et al.*, 2003; Zhao *et al.*, 2003; Callaway *et al.*, 2004; LeJeune and Wetzel, 2007). The most extensively studied DFM for cattle contains *L. acidophilus* strain NP51 and this has been found to reduce shedding of *E. coli* O157:H7 by 48–80% when fed at

10^9 cfu (Brashears *et al.*, 2003; Younts-Dahl *et al.*, 2004, 2005; Stephens *et al.*, 2007a,b). Tabe *et al.* (2008) found that *L. acidophilus* strain BT1386 in the diet reduced faecal shedding of *E. coli* O157 in feedlot steers, but had no impact on the shedding of *Salmonella*. Reduced faecal shedding of *Salmonella* was observed when a combination of 10^9 cfu *L. acidophilus* NP51 and 10^9 cfu *Propionibacterium freudenreichii* NP24 was fed to steers (Stephens *et al.*, 2007b). The efficacy of DFMs may also depend on the dosage, a relationship that has been shown to be apply for *L. acidophilus* NP51 when it is administered to cattle to reduce the shedding of *E. coli* O157 (Younts-Dahl *et al.*, 2005).

Although the ability of DFMs to control pathogens in various livestock species has been reported, the mechanisms to which this response is attributed are not well characterized (Callaway *et al.*, 2008c). The efficacy of a DFM may be compromised if the pathogen(s) fully colonizes the gastrointestinal tract (GIT) before introduction of the DFM. Once pathogens form biofilms, changes in gene expression can stabilize the population, making it resistant to a wide variety of antimicrobial agents (Ito *et al.*, 2009). This may explain why positive responses to DFMs are generally more often observed in younger as opposed to older animals. For example, administration of a DFM containing mixtures of lactobacilli to preterm piglets precluded the colonization of the intestinal tract by *Clostridium perfringens* (Siggers *et al.*, 2008).

Prebiotics

Prebiotics have been defined as non-digestible dietary ingredients that stimulate growth or activity of native microbial populations in the digestive tract, ultimately benefiting the health of the host (Collins and Gibson, 1999). Examples of prebiotics include fructo-oligosaccharides (FOS; e.g. oligofructose), galacto-oligosaccharides, inulin and lactulose (Collins and Gibson, 1999). Prebiotics have been used to enhance health in humans, but their potential to provide a competitive advantage to select resident bacteria has led to their possible use for preharvest mitigation of pathogens (Callaway *et al.*, 2008c).

Different types of oligosaccharides have been tested in poultry for their ability to improve animal health and growth performance. Meta-analyses have shown that mannan-oligosaccharides (MOS) improve body weight and reduce mortalities in poultry (Hooge, 2004; Rosen, 2007), but the mechanisms responsible for these effects are not clear. Despite reported differences in the capacity of prebiotics to alter bacterial populations in poultry, most reports have consistently documented a decline in intestinal colonization by *Salmonella* when the products are administered. For example, the addition of lactose to the diets of broiler chickens inhibited colonization (Corrier *et al.*, 1990) and numerically decreased *Salmonella* in caecal contents compared with animals fed no lactose (Ziprin *et al.*, 1990). The response was attributed to the increased production of bacteriostatic volatile fatty acid concentrations in the caeca, and the associated lower pH as a result of the inclusion of lactose in the diet. Furthermore, the impact of MOS (Spring

et al., 2000) or FOS (Fukata *et al.*, 1999) may be specific to different species of microbial pathogens as they have been shown to reduce numbers of *Salmonella*, but have no effect on the numbers of potentially beneficial *Bifidobacterium* and *Lactobacillus* spp in caecal contents. Conversely, in some instances, MOS may even promote an increase in the numbers of *Bifidobacterium* and *Lactobacillus* within the intestinal tract of poultry (Fernandez *et al.*, 2002). At times, numbers of *Bifidobacterium* in the caecal contents of broilers fed MOS have increased, while populations of *E. coli* and *Campylobacter* have decreased (Baurhoo *et al.*, 2009). Inclusion of MOS in diet has also been shown to alter intestinal morphology, including increasing villus height and the number of goblet cells associated with each villus. Goblet cells secrete mucins which may further inhibit the establishment of pathogens within the caeca of poultry.

Several types of prebiotics have been shown to increase the concentrations of *Lactobacillus reuteri* and *Lactobacillus amylovorus* as well as the overall genetic diversity of bacteria in colonic samples from pigs (Konstantinov *et al.*, 2004). A chito-oligosaccharide also increased *Labctobacillus* counts in the faeces of weaning pigs and favourably altered intestinal morphology (Liu *et al.*, 2008); additionally, in this study *E. coli* counts and the incidence of diarrhoea decreased in animals fed the prebiotic. Similarly, FOS improved intestinal function and protected pigs against *S.* Typhimurium, reducing the incidence of diarrhoea (Correa-Matos *et al.*, 2003). Prebiotics have also been shown to reduce the adhesion of pathogens that utilize cell-surface oligosaccharide-binding proteins as adhesion receptors through competitive binding (Rhoades *et al.*, 2006). A galacto-oligosaccharide (GOS) mixture increased *Bifidobacterium* concentrations in the caecal contents of pigs and also inhibited attachment of enteropathogenic *E. coli* and *S.* Typhimurium to HT29 cells *in vitro* (Tzortzis *et al.*, 2005). The feeding of a synbiotic (probiotic plus prebiotic) containing *Lactobacillus plantarum* and FOS has also been reported to reduce adhesion of *E. coli* O8:K88 to the intestinal mucosa of the jejunum and colon in pigs (Nemcová *et al.*, 2007).

Few studies have investigated prebiotic use in ruminants, mainly because of their prohibitive costs as additives and the complex microbial ecosystem of the rumen (Callaway *et al.*, 2008c). Rumen microbes produce an array of polysaccharidases with the capacity to degrade many of prebiotics, limiting their ability to exert effects within the lower digestive tract of ruminants. Purified GOS has been shown to inhibit the adherence of enteropathogenic *E. coli* to HEp-2 and Caco-2 cell lines *in vitro* (Shoaf *et al.*, 2006), but expression of this activity in the lower intestinal tract of cattle seems unlikely. When sorbitol was added to rumen cultures, inoculated *E. coli* O157:H7 was only displaced after 72 h (de Vaux *et al.*, 2002), leaving ample time for this pathogen to pass to the lower tract with the fluid fraction of digesta. However, the effects of prebiotics on *E. coli* O157:H7 *in vivo* have not been examined. As with probiotics, prebiotics may have a greater efficacy in pre-ruminant calves. Recently, lactulose fed in combination with *E. faecium* was shown to improve the immune status of pre-ruminant calves, but the implications of this response for colonization by pathogens was not explored (Fleige *et al.*, 2009).

Immunization

Vaccines have been effectively used to manage disease in livestock. Typically, vaccine development has been targeted towards pathogens that adversely affect animal health and production (Oliver *et al.*, 2009). Efforts to elicit immune responses to foodborne pathogens as a means of potentially reducing infection in humans have recently been undertaken. In some instances (e.g. *Salmonella*), the organism can be pathogenic to both the livestock animal and humans, raising the possibility that a vaccine could have benefits for livestock production as well as the general public. In contrast, the foodborne pathogens may be asymptomatic in the animal (e.g. *Campylobacter, E. coli* O157:H7). Under these circumstances, the economic incentive for the producer to utilize the vaccine is not immediately apparent, a factor that may pose a barrier to vaccine development (LeJeune and Wetzel, 2007).

Infection of poultry with *Salmonella* induces a serological immune response (Skov *et al.*, 2000) which can reduce the duration of infection as well as reinfection (Gast, 2007). Vaccination against *Salmonella* produces similar responses and may also afford long-term protection (Mastroeni *et al.*, 2001). While live *Salmonella* vaccines have been shown to enhance cell-mediated immunity in comparison to killed vaccines (Babu *et al.*, 2003), multiple commercial vaccines of both types are utilized to some extent in the poultry industry. Broiler breeder hens challenged with *Salmonella* and vaccinated with a commercial bacterin or live vaccine exhibited reduced excretion of the pathogen and a reduction in the extent to which it colonized the spleen, liver and caecum (Penha Filho *et al.*, 2009). Vaccination with a live *Salmonella* vaccine at 1 day, 6 weeks and 16 weeks of age has also been shown to reduce the incidence of oviduct and internal egg contamination in layer hens intravenously challenged with *S.* Enteritidis (Gantois *et al.*, 2006). Recently, an analysis of layer-hen flocks across the EU found that vaccination of hens against *Salmonella* reduced the prevalence of *Salmonella,* with the exception of *S.* Typhimurium (EFSA, 2007). While current vaccines do not eliminate *Salmonella* from poultry, their use may reduce the transmission of this pathogen, as fewer human-associated infections of *S.* Enteritidis were reported in both the UK (Cogan and Humphrey, 2003) and Belgium (Collard *et al.*, 2008) with the introduction of layer-flock vaccination programmes.

Campylobacter is prevalent in poultry production systems and colonizes the caeca, large intestine and cloaca. Although colonized birds display no signs of pathology, the bacterium can trigger a systemic and mucosal immune response (de Zoete *et al.*, 2007). After oral infection of chickens, serum and mucosal *Campylobacter*-specific antibodies increase (Cawthraw *et al.*, 1994), and this increase has been shown to coincide with a reduction in colonization (Lin, 2009). Additionally, high titres of maternal anti-*Campylobacter* antibodies are present in chicks for up to 7 days after hatching, at which point they decline (Sahin *et al.*, 2001). The decrease in titre also coincides with the point at which *Campylobacter* is more commonly isolated from the digestive tract. Combined, these data suggest that there may be some potential to vaccinate poultry against *Campylobacter*, although efforts to develop vaccines against *Campylobacter* have largely been unsuccessful. This lack of success may be attributable

to the genomic and phenotypic instability and diversity of *Campylobacter*, making it difficult to use attenuated variants of the bacterium or to identify antigen targets with sufficiently broad specificity (de Zoete *et al.*, 2007). In one study, oral vaccination of chickens with an avirulent *Salmonella* strain expressing the *C. jejuni* CjaA antigen resulted in a sixfold reduction of colonization upon challenge with a wild type *C. jejuni* strain (Wyszyńska *et al.*, 2004). The study did not include a control group of birds receiving *Salmonella* that did not express CjaA, and only a few birds were included in the experiment. Consequently, it was not possible to determine whether the immune response resulted from *Salmonella* or from the presence of the CjaA antigen. However, a recent study in which chickens were also vaccinated with attenuated *Salmonella* expressing CjaA confirmed the induction of CjaA-specific serum antibodies (Buckley *et al.*, 2010). In this latter study, only a 1.4 log reduction of *Campylobacter* per gram of caecal contents was observed.

As for poultry, commercial vaccines against *Salmonella* are available for use in swine. Only a few clinical trials have been conducted, but a recent review has shown that vaccination of swine is associated with reduced *Salmonella* prevalence just before and at harvest (Denagamage *et al.*, 2007). Roesler *et al.* (2004) vaccinated 4-week-old piglets with a live *S.* Typhimurium vaccine and then subjected them to a challenge 3 weeks later. Of the vaccinated pigs, 90% did not show any clinical signs of salmonellosis, whereas all control animals showed moderate-to-severe clinical symptoms. Additionally, colonization of internal organs was reduced in the vaccinated group compared with the control (42.5% versus 87.5%). Vaccination of sows also appears to confer protection against *S.* Typhimurium in piglets. From birth to 142 days old, *S.* Typhimurium was not detected in faecal samples of piglets originating from vaccinated sows, whereas a prevalence rate of 47.7% occurred in piglets from unvaccinated sows (Roesler *et al.*, 2006).

Vaccine development against *E. coli* O157:H7 is complicated because the bacterium acts as a commensal in cattle and sheep (LeJeune and Wetzel, 2007). Only recently have vaccines targeting *E. coli* O157:H7 been tested in cattle. Most vaccines against *E. coli* O157:H7 have attempted to capitalize on proteins excreted by the type III-secretory system as antigens. First-generation vaccines grew *E. coli* O157:H7 under conditions that promoted the excretion of these proteins, whereas second-generation recombinant vaccines will be likely to focus on specific proteins within this system. Vaccination with a first-generation vaccine against *E. coli* O157:H7 was shown to reduce both the level and duration of shedding of this bacterium in challenged cattle (Potter *et al.*, 2004). However, use of this vaccine in a commercial feedlot found no difference in prevalence of *E. coli* O157:H7 between pens of vaccinated and non-vaccinated cattle, a result that was attributed to the adjuvant used in the formulation or the need for more than two immunizations (van Donkersgoed *et al.*, 2005). Subsequent studies using an altered formula of the vaccine and three vaccinations found that vaccinated cattle were 98% less likely to be colonized by *E. coli* O157:H7 at the terminal rectum mucosa (Peterson *et al.*, 2007). Under commercial conditions, vaccination three times over the feeding period is impractical, and as a result two-dose vaccine strategies have generally

been used. In a large-scale trial using 20,556 cattle, administering a two-dose vaccine to cattle resulted in a 12% reduction in the likelihood of pens of cattle shedding *E. coli* O157:H7 (Smith *et al.*, 2008). From that same study, a subset of animals was tested for colonization of the terminal rectum (Smith *et al.*, 2009a). Compared with unvaccinated cattle (17.0%), there was a lower incidence of colonization in vaccinated cattle (2.9%). This vaccine has also been shown to reduce faecal shedding and hide contamination, but these effects were negated when unvaccinated cattle were mixed with those that were vaccinated, demonstrating the likely need to vaccinate all cattle in order for vaccination to be an effective mitigation strategy (Smith *et al.*, 2009b). Vaccination of feedlot cattle against *E. coli* O157:H7 with a vaccine based on a siderophore receptor and porin proteins reduced the prevalence and level of shedding of *E. coli* O157:H7 in feedlot cattle (Thomson *et al.*, 2009). An interesting follow-up to this study involved vaccination of cattle that had previously been identified to be shedding *E. coli* O157:H7 (Fox *et al.*, 2009). Vaccination of these animals reduced the prevalence of *E. coli* O157:H7 (17.7%) compared with unvaccinated controls (33.7%) and lowered the number of individuals that were shedding high levels of this pathogen.

Passive immunization using egg-yolk antibodies has also been investigated to control pathogens in swine and ruminants. Antibodies produced by hens immunized with specific antigens or whole cells are transferred to the egg yolk, and these antibodies can be administered to the animal without activating the mammalian complement (Cook *et al.*, 2005). In swine, the use of egg-yolk antibodies has been tested for the control of *E. coli* K88+, but with mixed results. One study showed that challenged piglets treated with egg yolk containing anti-*E. coli* K88+ antibodies were protected, whereas a control group experienced a 62% mortality rate (Marquardt *et al.*, 1999). In contrast, Chernysheva *et al.* (2003) reported no differences in rates of diarrhoea or mortality between pigs treated with anti-*E. coli* K88+ egg yolk and control animals. In calves, a high dose of *Salmonella*-specific egg-yolk antibodies resulted in protection from a challenge strain, whereas calves in low-dose or control groups experienced high mortality rates (Yokoyama *et al.*, 1998). No studies with *Salmonella* have been conducted in a commercial setting. Anti-*E. coli* O157:H7 chicken egg-yolk antibodies have been tested in sheep challenged with *E. coli* O157:H7 (Cook *et al.*, 2005). Throughout 85 days post-challenge, the number of *E. coli* O157:H7 shed and the duration of shedding was shorter for lambs given a high and medium dose of egg yolk antibodies, compared with control animals. A subsequent *in vitro* study by Cook *et al.* (2007) showed that polyclonal antibodies targeting adherence-associated factors were effective in preventing adhesion and colonization by *E. coli* O157:H7 to cultured HeLa cells.

Bacteriophages

Bacteriophages are highly specific viruses that target bacteria. They naturally inhabit a diversity of ecosystems, including the mammalian GIT (Dabrowska *et al.*, 2005), and significantly influence microbial communities

and function (Rohwer and Thurber, 2009). Their use to mitigate bacterial pathogens is not novel, but there has been renewed interest in bacteriophages because of the development of antimicrobial resistance in bacteria. As a pre-harvest strategy to reduce enteric pathogens, bacteriophages are attractive because they are highly specific, non-toxic, self-amplifying, and can overcome multiple-drug resistant bacteria (Ojha and Kostrzynska, 2007). Additionally, there is evidence that bacteriophages may be transferred between animals, which may result in spread of a protective agent among herd cohorts (Rozema *et al.*, 2009).

The administration of two broad-range phages to broiler chickens challenged with *S.* Enteritidis or *S.* Typhimurium resulted in 4.2 and 2.19 log reductions, respectively, of these bacteria in caecal contents within 24 h of treatment (Atterbury *et al.*, 2007). However, it was also observed in this study that some of the *Salmonella* isolates collected were phage-resistant. Similarly, Sklar and Joerger (2001) isolated phage-resistant *S.* Enteritidis from chickens that were challenged with the bacterium and subjected to phage therapy; they noted a reduction of 0.3–1.3 log reduction in the population of *S.* Enteritidis in caecal contents, but the results were not consistent across five experiments. Two other studies reported that phage therapy was successful in short- but not long-term elimination of *Salmonella* from the intestinal tracts of chickens. Administering two phages separately, or in combination, reduced the numbers of *S.* Enteritidis after 24 h, but after 48 h populations were similar to those in the control treatment (Andreatti Filho *et al.*, 2007). Likewise, counts of *S.* Enteritidis in digestive contents were reduced 12 h after inoculation with phages, but this effect was less apparent after 24 and 48 h (Berchieri *et al.*, 1991). In the latter study, bacteriophage treatment was most effective when numbers of *Salmonella* exceeded 10^6 cfu ml^{-1}.

An epidemiological study in the UK found that the number of *C. jejuni* present in the caeca of broiler chickens was noticeably lower if *Campylobacter*-specific bacteriophages were also detected within intestinal contents (Atterbury *et al.*, 2005). Subsequent experiments have shown that bacteriophage therapy can reduce *Campylobacter* in poultry. A thorough study by Loc Carrillo *et al.* (2005) reported a 0.5–5 log reduction in caecal counts of *C. jejuni* when challenged birds were administered *Campylobacter*-specifc bacteriophage. The results were dependent on several factors, including host specificity, dosage and the time that samples were collected after phage administration. Wagenaar *et al.* (2005) reported therapeutic and preventive applications of bacterio-phages in broilers. The time of administration of bacteriophage relative to the time of infection may be a particular important factor in determining the effi-cacy of bacteriophage therapy. Broiler chickens administered bacteriophage before challenge with *C. jejuni* exhibited delayed colonization, whereas those that received bacteriophage post-challenge showed a threefold decline in *C. jejuni* caecal contents (Wagenaar *et al.*, 2005), although these effects were short term, with populations of *C. jejuni* returning to near pretreatment levels over time.

Less work with bacteriophages has been undertaken in swine. Wall *et al.* (2010) showed that administering an anti-*Salmonella* phage cocktail to pigs

inoculated with *S.* Typhimurium reduced colonization by 99%, and gave a 2–3 log reduction of the pathogen population. Under commercial conditions, pigs inoculated with *S.* Typhimurium were co-mingled with cohorts treated with either a phage cocktail or a placebo. Pigs that received the phage cocktail had lower numbers of *S.* Typhimurium in their caecal contents.

A recent study of feedlot cattle in a commercial setting reported that a higher prevalence of *E. coli* O157:H7-specific bacteriophage in faecal pats or water trough samples was associated with reduced prevalence of *E. coli* O157:H7 in rectal faecal samples (Niu *et al.*, 2009a). This suggests that the use of bacteriophages may have applications in mitigating infection by this bacterium, although the complexity of the ruminant digestive tract may pose challenges. Bach *et al.* (2003) showed that administration of phages could accelerate the elimination of *E. coli* O157:H7 from an artificial rumen system, but in sheep challenged with *E. coli* O157:H7, inoculation with bacteriophages had no effect. Similarly, phages that displayed *in vitro* activity against *E. coli* O157:H7, and eliminated *E. coli* O157:H7 in infected mice, did not elicit the same response in challenged cattle (Sheng *et al.*, 2006); however, in this study, concentrations of *E. coli* O157:H7 shed in faeces were reduced for up to 10 days by bacteriophage therapy. Similarly, a short-term reduction in *E. coli* O157:H7 in challenged sheep showed that bacteriophage-treated animals had reduced levels of the pathogen in intestinal contents and faeces 24 h (Callaway *et al.*, 2008a) and 2 days (Raya *et al.*, 2006) after treatment.

Mitigating bacterial pathogens with bacteriophages has had variable results, but this can be expected given their diversity. Additionally, the highly specific nature of phages will limit the susceptibility of bacteria, which has been shown to change according to bacterial genotype and geographical origin of the bacterial isolates (Niu *et al.*, 2009b). A bacteriophage cocktail would, therefore, be the most efficacious method of using phages to mitigate pathogens on the farm. Also, the use of phages may be most beneficial directly before animals are sent to slaughter, given the potential for the development of bacterial resistance and the transient protection against pathogens observed in some studies.

Waste Management

Proper management of animal waste is important in preventing the spread of pathogens from livestock to the greater environment. For example, in the poultry industry, litter can harbour pathogens, and its prolonged retention in barns can promote the spread of bacteria (Vicente *et al.*, 2007). Additionally, compromised vaccine performance has been related to poor hygiene in laying houses (Gast, 2007). Throughout feedlots, rapid spread of *E. coli* can occur within and between animal pens (Stevenson *et al.*, 2003). *E. coli* O157:H7 can be present in environmental samples even when cattle do not harbour this pathogen (Davis *et al.*, 2005), and faeces on pen floors have been implicated as a significant source of infection (Bach *et al.*, 2005). Removal of waste from animal housing is therefore necessary to reduce the degree of horizontal
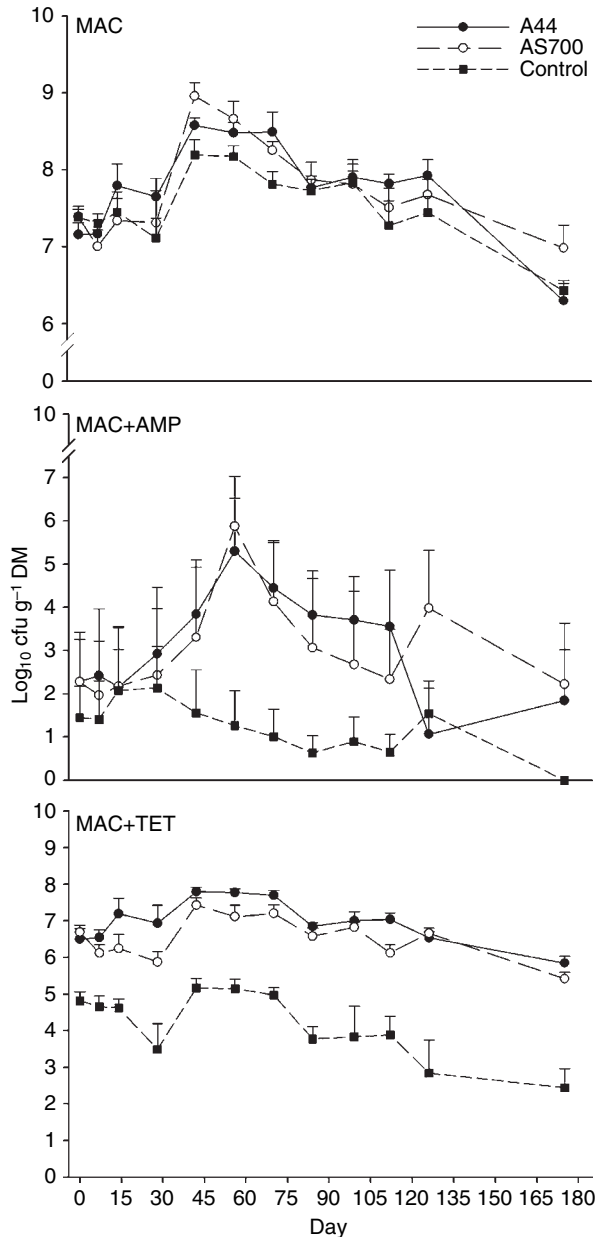
Fig. 7.2. Mean counts (*n* = 3, plus SE) of total, ampicillin-resistant and tetracycline-resistant *Escherichia coli* isolated from faecal deposits using MacConkey agar (MAC) or MAC amended with ampicillin (MAC+AMP, 32 μg ml^{-1}) or tetracycline (MAC+TET, 16 μg ml^{-1}), respectively. The treatments were as follows: control, no antimicrobial agents added to the diets of steers from which faecal deposits originated; A44, chlortetracycline (44 ppm); and AS700, chlortetracycline and sulfamethazine (each at 44 ppm). Faecal deposits were left under ambient field conditions for 175 days. DM, dry matter; cfu, colony forming units. (From Alexander *et al.,* 2009.)

transmission among individuals. Bacteria from faecal material can also migrate to surface water, thereby potentially contaminating water sources used by humans (Schuster *et al.*, 2005). Containment of waste within and outside housing structures is critical to preventing environmental contamination.

　　Typically, livestock waste is applied to land as a fertilizer. Enteric pathogens survive well in manure and can therefore also be transmitted to fields and waterways. Antimicrobial-resistant *E. coli* has been shown to grow in cattle faeces and survive in this environment for more than 175 days (Alexander *et al.*, 2009; Fig. 7.2). Long-term survival of *Salmonella* in faeces (Sinton *et al.*, 2007) and soil (Holley *et al.*, 2006) has also been reported. Therefore,
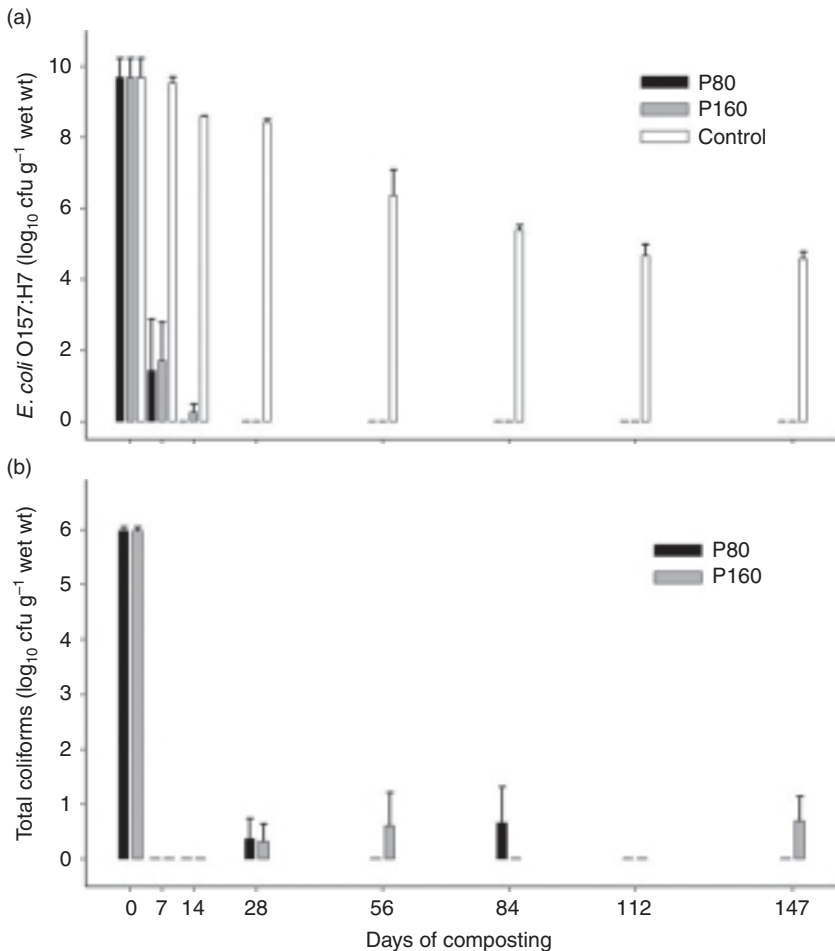
Fig. 7.3.   Survival of *Escherichia coli* O157:H7 and total coliforms in compost during 147 days of composting (mean ± SE, *n* = 4). (a) Effect of heat on *E. coli* O157:H7 inactivation. Autoclaved manure was inoculated with *E. coli* O157:H7, sealed in polypropylene vials, and embedded at depths of 80 and 160 cm (P80 and P160) in compost. Control samples were retained on the laboratory bench (20°C). (b) Enumeration of total coliforms in compost at P80 and P160. (From Xu *et al.*, 2009.)

treatment of waste before its application to land is recognized as an important step in reducing the transmission of pathogens to other hosts and/or environments (Leifert *et al.*, 2008).

Composting has been established as an effective technology to reduce pathogens (Wilkinson, 2007). Within a compost pile of spent broiler litter, reductions in *E. coli*, faecal coliforms and enterococci ranged from 5.96 to 8.18 log units over a 110-day period (Mohee *et al.*, 2008). Shepherd *et al.* (2010) measured the prevalence of *E. coli* and *Salmonella* in compost piles on five poultry farms that employ a variety of composting practices. *E. coli* was detected in 63% of samples collected from the pile surface and in 9.8% of samples collected from the inside of the pile during the primary composting phase. Prevalence at these locations declined to 16.7% and 0%, respectively, during the second composting phase. With further turning of the pile, *E. coli* was no longer detected, suggesting that that heat-sensitive pathogens can be virtually eliminated from composted poultry waste. Similar to these results, *E. coli* O157:H7 was found to survive for up to 4 months on the surfaces of compost piles composed of dairy cattle manure (Shepherd *et al.*, 2007). In this study, most locations within the compost pile reached temperatures of 50°C, although temperatures were not uniform throughout the compost pile. At locations where temperatures clearly reached 50°C or higher, such as the centre and bottom of the pile, *E. coli* O157:H7 was not detected after 14 days. A biosecure static composting system containing bovine mortalities and manure also showed temperature stratification (Xu *et al.*, 2009). At depths of 80 cm, temperatures of 55–65°C were maintained for more than 30 days, whereas at a depth of 160 cm, temperatures failed to exceed 55°C. Regardless of location, *E. coli* O157:H7 was rendered undetectable after 7 days, suggesting that factors other than heat alone contribute to the ability of the composting process to reduce the viability of pathogens (Fig. 7.3). DNA isolated from *Campylobacter* was amplifiable for 84 days at 80 cm and 160 days at 160 cm but, overall, there was a greater than 6 log reduction of this bacterium. The above studies indicate that, when executed properly, thermophilic composting can substantially reduce the likelihood of viable pathogens entering the environment when compost is applied to land as a fertilizer.

Conclusion

Despite postharvest efforts to control enteric pathogens entering the food chain, contamination of food can occur. Reduction of both the prevalence and concentration of pathogens in livestock would ease the burden on existing control measures within abattoirs. Preharvest mitigation strategies have therefore been investigated and implemented to control on-farm pathogens. Additionally, methods to mitigate the environmental spread of pathogens, such as composting animal waste, are important. While none of the methods results in complete eradication of pathogens, reducing pathogen prevalence by a single method or a combination of methods reduces the risk of food and environmental dissemination.

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8 Organic Agriculture and its Contribution to Zoonotic Pathogens

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Introduction

Organic agriculture is defined as a form of agriculture that relies on crop rotation, green manure, compost, biological pest control and mechanical cultivation to maintain soil productivity and control pests, excluding or strictly limiting the use of synthetic fertilizers and synthetic pesticides, plant growth regulators, livestock feed additives and genetically modified organisms (GMOs).

The organic movement started in the 1930s as a criticism of mainstream conventional farming with its increasing industrialization and use of pesticides and chemical fertilizers. A British biologist, Sir Albert Howard, travelled to India in order to teach the Indians about conventional agriculture. Instead, he was amazed by traditional Indian farming practices and was specifically interested in the connection between a healthy soil and the healthy population, livestock and crops. From then on, he started to promote Indian farming practices. In Howard's tradition, biodynamic agriculture was then founded by the Austrian philosopher Rudolf Steiner, who emphasized the interrelationships of animals, plants and soil, and saw farms as unified organisms. Steiner's ideas contributed significantly to the development of modern organic farming.

Organic agriculture has many advantages above conventional agriculture, at least at a local scale. In the case of organic animal production, animals have a lower stocking density, obligatory straw bedding and outdoor access, and are fed with organic feed and/or roughage. The weaning periods of pigs are longer, while tail, teeth and beak clipping is prohibited. In poultry production, broiler breeds that grow more slowly are used in order to lower the number of broilers that cannot keep up and therefore die.

According to consumer perception, organically raised animals are thus reared under higher welfare conditions. Moreover, the products from these

animals are thought to contain fewer residues (pesticides and veterinary drugs) than products from conventional animal production systems, as the use of chemical pesticides or artificial fertilizers is not permitted. Although consumers and producers sometimes claim that organic produce is healthier food than conventional produce (Edwards, 2005; Vaarst *et al.*, 2005), the current scientific evidence does not support this contention (Trewavas, 2004).

Nowadays, the intentions of organic livestock production have been formulated by the International Federation of Organic Agriculture Movements (IFOAM). However, each region implements its own version of these intentions and rules may differ significantly. In the EU, there are strict regulations on the use of antibiotics (longer waiting times after medical treatment before products are delivered to the market) and GMOs in feed and application of growth promoters are not allowed. The EU has regulated organic animal husbandry via EU regulation 2092/91 in the year 2000 (Council of the European Union, 2007). In the USA, the application of antibiotics is forbidden in organic livestock production by the USDA Organic Foods Production Act (OFPA) (Jacob *et al.*, 2008). If an animal receives medical antibiotic treatment, that animal loses its organic status and should be sold as a regular conventional product.

In organic plant production, use of pesticides is prohibited. Moreover, seedlings should come from organic sources, and the use of artificial fertilizer is not allowed. Farmers should use manure from organic farms instead or use 'green manure' (nitrogen-fixing plants) in order to keep their land fertile. As a result of the reduction of inputs, food production in organic systems is less efficient than in conventional farming systems, and some therefore question the sustainability of organic production at a global scale. Moreover, the consumption of organic products is in many countries still marginal: only in Switzerland and Austria does the consumption of organic products exceed 5%.

The Importance of Safe Food

Despite their relative low market share, it is important that products of organic origin fulfil the highest standards in the field of food safety (Colles *et al.*, 2008). Generally, consumers believe that organically grown produce will pose fewer risks than conventionally grown produce (Williams and Hammitt, 2001). In a specific consumer study, over 90% of the respondents estimated lower pesticide-related mortality risks associated with the consumption and production of organically grown produce compared with conventionally grown produce, while about 45% estimated lower natural toxin and microbial pathogen risks (Williams and Hammitt, 2001). However, this perception is not based on evidence. Contrary to the popular perception that chemical residues form the major source of food contamination, most foodborne disease outbreaks have demonstrated that microbial hazards are much more important for food safety (Cliver, 1999). Risks due to pesticide residues and food additives are relatively minor compared with both the acute and chronic effects caused by microbiological and other naturally occurring toxicants (Cliver, 1999; Magkos *et al.*, 2003).

Organic production systems might experience problems concerning food safety resulting from: (i) their relatively open character; and (ii) the fact that organic systems are primarily based on biological cycles.

Concerning the relatively open character of organic production systems, this is of particular importance in organic animal husbandry. Animals have the opportunity to go outdoors and may more easily come into contact with pathogens or vectors that might spread pathogens. Wild fauna (e.g. rodents, flies, etc.) are often mentioned as a potential source of pathogens for organic livestock (Meerburg *et al.*, 2006, 2007; Meerburg and Kijlstra, 2007; Kijlstra *et al.*, 2008), but the role of domestic animals such as cats should also not be forgotten (Kijlstra *et al.*, 2004a). This is specifically important as these animals are often used to counter rodent presence, as farmers perceive this method as more 'natural' than the application of rodenticides. Although it is doubtful whether pathogen transmission is always problematic for the animals themselves (as it is often assumed that animals have a better immune response towards prevailing pathogens under organic conditions than under conventional conditions (Kijlstra and Eijck, 2006)), pathogen transmission might eventually result in the contamination of products in the food chain.

With regard to the principle of biological cycles, which includes the use of organic manure within the farm, the risk of recirculation of infectious pathogens emerges (Tauxe *et al.*, 1997). Manure from farm animals that is used as fertilizer for the crops that are used as animal feed, or are for human consumption, may contain enteric pathogenic microorganisms, and its use as fertilizer for organic crops can lead to pathogen entry into the food chain (Pell, 1997). Plants can become infected via the roots or by water splashing on to the leaf surfaces (Rembiałkowska, 2007). In pot experiments in which contaminated manure was mixed with soil, it was demonstrated that *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) declined steadily but could still be traced after 56 days, while the survival of *Escherichia coli* O157:H7 varied from 2 to 56 days (Franz *et al.*, 2005). No pathogens were detected in the edible parts of lettuce grown in the pots 2 weeks after mixing with manure, and only one plant showed the presence of any pathogen (*E. coli* O157:H7 on root samples). Even though the composting and drying of manure on the field may decrease the number of viable pathogens (Pell, 1997; Semenov *et al.*, 2009), some authors suggest that traditional composting practices are not sufficient to render animal manure safe for use on vegetables with the advent of pathogens such as *E. coli* O157:H7 (Tauxe *et al.*, 1997). Moreover, drying manure on the field will have negative environmental consequences from the formation of NH_3, N_2O and other greenhouse gases (Huijsmans *et al.*, 2008).

Although manure is used as a source of crop fertilizer in both organic and conventional agriculture, the importance of manure as an alternative source of plant nutrients is greater in organic production systems. Conventional farmers have a variety of synthetic fertilizers at their disposal, while organic farmers are not allowed to use these. Therefore, the relative risks for food contamination will most likely be higher in organic production systems (Albihn, 2001). However, the risk may be determined by the type of plant to which the manure is applied and the pathogen species. In a recent study

in which crisphead lettuce was grown in ground that was contaminated with *E. coli* O157:H7, these bacteria persisted in the ground for a substantial time, but the lettuce itself did not contain them in its edible parts (Johannessen *et al.*, 2005). In contrast, the same pathogen is able to persist on organic onions and carrots for several months (Islam *et al.*, 2005). Moreover, when another pathogen, *S.* Typhimurium, was added to the ground, it was detected for up to 63 days on lettuce and 231 days on parsley (Islam *et al.*, 2004a), while it was found for 84 days on radishes and 203 days on carrots (Islam *et al.*, 2004b).

Risks of Organic Plant Products

Several food incidents have been reported as resulting from the consumption of organic plant products. In 1992, a 2-year-old child died in the USA after consuming manured vegetables that were inadequately washed. The cause of infection was identified as *E. coli* O157:H7 (Cieslak *et al.*, 1993). In 1995, a summer outbreak of gastroenteritis followed by haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura and death was reported in a nursery school (Tschäpe *et al.*, 1995). The butter that was used to prepare sandwiches contained organic parsley and this was thought to be the vehicle of infection. Clonally identical verocytotoxigenic *Citrobacter freundii* were found as the causative agents of HUS and gastroenteritis, and were also detected on the parsley (Tschäpe *et al.*, 1995).

In the USA, both organic and conventional lucerne sprouts were tested for the presence of *Salmonella* spp. (Doyle, 2000); *Salmonella* was detected in 7.7% (3 of 39 samples) of organic sprouts, but not in 39 samples of conventional sprouts. During the same trial, lettuce was tested for *E. coli*. The bacterium was found in 16.7% (8 of 48 samples) of organic spring mix (lettuce) at an average count of 10^6 cfu *E. coli* g^{-1}, whereas it was detected in 8.3% (4 of 48 samples) of conventional spring mix at an average count of 10^4 cfu *E. coli* g^{-1} (Doyle, 2000). In a study from Minnesota (Mukherjee *et al.*, 2004), microbiological analyses of both organically and conventionally produced fresh fruits and vegetables (tomatoes, leafy greens, lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples) were conducted to determine the coliform count and the prevalence of *E. coli*, *Salmonella* and *E. coli* O157:H7. A total of 476 and 129 produce samples were collected from 32 organic (of which eight were certified) and eight conventional farms, respectively. The proportion of *E. coli*-positive samples in conventional and organic produce were 1.6% and 9.7%, respectively. However, the *E. coli* prevalence in certified organic produce was 4.3%, which is not statistically different from other samples (Mukherjee *et al.*, 2004). Organic lettuce had the largest prevalence of *E. coli* (22.4%) compared with other produce types. Organic samples from farms that used manure or compost aged less than 12 months had a prevalence of *E. coli* 19 times greater than that of farms that used older materials. Serotype O157:H7 was not detected in any produce samples, but *Salmonella* was isolated from one organic lettuce and one organic green pepper (Mukherjee *et al.*, 2004).

In 2001, a microbiological study was performed on uncooked ready-to-eat organic vegetables in the UK to determine the presence of a number of pathogens (*Listeria monocytogenes, Salmonella, Campylobacter* and *E. coli* O157). In this study (Sagoo *et al.*, 2001) it was found that the majority of the samples (3185 of 3200; 99.5%) were of satisfactory/acceptable quality, while only 15 (0.5%) were of unsatisfactory quality. The latter was primarily the result of levels of *E. coli* and *Listeria* spp. (other than *L. monocytogenes*) in excess of 10^2 cfu g^{-1}. The authors indicated that, overall, agricultural, hygiene, harvesting and production practices for organic vegetables were good (Sagoo *et al.*, 2001). In Northern Ireland, commercially available organic vegetables (*n* = 86) were examined for the presence of *Salmonella, Campylobacter, E. coli* O157, *L. monocytogenes* and *Aeromonas* spp. (McMahon and Wilson, 2001); *Aeromonas* spp. were isolated from 34% of the total number of organic vegetables examined, while no other enteric pathogens were found. In a recent study in the UK on edible dried seeds from the retail stores, no difference was found between seeds labelled as organic and those that were conventional as regards *Salmonella* contamination, but a significantly higher proportion of organically produced seed samples had unsatisfactory levels of *E. coli* ($\geq 10^2$ cfu g^{-1}) (2.4%) compared to those that were not (1.2%) (Willis *et al.*, 2009).

In Norway, a study was performed that aimed to investigate bacteriological quality in organically grown leaf lettuce (Loncarevic *et al.*, 2005). In total, 179 organic samples were collected from 12 producers. *E. coli* was isolated from 16 of the lettuce samples, but in 12 of these contamination was sufficiently low (<100 cfu g^{-1}) that they would be considered to be of acceptable bacteriological quality. *E. coli* O157 and *Salmonella* were not detected in any of the samples. *L. monocytogenes* serogroups 1 and 4 were isolated from two samples (Loncarevic *et al.*, 2005).

In a recent study in the Netherlands, organic lettuce was compared with conventional lettuce (Hoogenboom *et al.*, 2008). The organic lettuce was sampled at three distribution centres for supermarkets. Conventional lettuce was obtained from local stores. None of the samples in this study tested positive for *Salmonella* or *E. coli* O157. The authors concluded that introduction through the use of animal manure should, therefore, not be considered as a common problem, but also that the presence of pathogenic microorganisms cannot be excluded in a small product fraction. To minimize potential contamination risks, several countries (including Canada and the USA) do not allow the use of non-composted manure (Hoogenboom *et al.*, 2008).

So, although bacterial contaminations are found and the amount of contamination is sometimes higher in organic produce, this is not always the case. Moreover, the impact of consumption of organic plant products on human health remains unknown. The incidents that were linked to contamination of food with *E. coli* (O157:H7) have stimulated the debate on whether the use of animal manures in certified organic food production systems might confer any extra health risk for consumers (Bourn and Prescott, 2002). According to recent literature however, farmers can take measures to reduce the probability of infection of their products, e.g. by choosing a clean water source and minimizing

the chances of faecal material splashing on to the crop (Leifert *et al.*, 2008). Such a risk approach might contribute to a reduction of pathogen presence on organic plant products, thus decreasing the risks for consumers.

Risks of Organic Products of Animal Origin

In veterinary medicine, the prevention of the transfer of infectious diseases, such as bacterial diseases (e.g. those caused by *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp. and *Mycobacterium paratuberculosis*) or parasitic diseases (e.g. those caused by *Toxoplasma gondii* and *Ascaris suum*) is based on the breaking of cycles. In organic livestock systems, organisms causing disease are essentially the same as those in conventional production systems. However, breaking of their life cycles could be more difficult owing to less hygienic circumstances (the provision of straw and roughage in some production systems) and outdoor access for the animals (Höglund *et al.*, 2001; Hovi *et al.*, 2003). This can lead to more infections with enteric pathogens of zoonotic potential compared with conventional systems (Honikel, 1998; Thamsborg *et al.*, 1999; Hermansen, 2003; Eijck and Borgsteede, 2005). There are some helminth and protozoan infections with zoonotic potential that can enter humans via food or the environment. Examples of foodborne zoonoses are *T. gondii*, *Taenia saginata* (cattle), *Taenia solium* (pig), *Trichinella* spp. (wild boar, domestic pigs, horses), *Sarcocystis bovihominis* (cattle) and *Sarcocystis suihominis* (pigs). Of these, *T. gondii* is by far the most important and frequent. However, in tropical regions, *T. solium* can be a dangerous parasite, not as an adult worm, but as the larval stage, which can cause neurocysticercosis. Among the zoonotic pathogens that can enter humans via the environment, there are the helminths *Fasciola hepatica* and *A. suum*, and the protozoans *Giardia duodenalis* and *Cryptosporidium parvum*. Infections with these helminths are rare, while the incidence of infections with the protozoans is rather high, but the origin of the infection is not always clear. Thus, it is unlikely that organic farming contributes much to infections with these pathogens. However, a recent human hookworm infection (probably *Ancylostoma duodenale*) in Japan was associated with consumption of imported organic produce (Kajiya *et al.*, 2006), indicating that individual cases do occur. Bacteria can also cause trouble. In Denmark, an outbreak of Shiga toxin-producing *E. coli* O26:H11 infection in 20 patients (median age 2 years) was recently described (Ethelberg *et al.*, 2009); the source of infection was identified as an organic fermented beef sausage that was sold in a supermarket.

In this section, we will discuss the three most relevant pathogens among the bacteria, protozoa and helminths – *T. gondii*, *Salmonella* and *Campylobacter* – and see whether differences can be found in their occurrence between regular and organic produce.

Toxoplasma gondii

Toxoplasmosis, caused by *T. gondii*, is the most prevalent parasitic zoonotic disease throughout the world (Tenter *et al.*, 2000). It is an important cause of

abortion in humans and livestock (sheep) and was recently shown to be the third most frequent cause of death following foodborne illnesses (Mead *et al.*, 1999). Recently, the estimated disease burden of congenital toxoplasmosis in the Netherlands was estimated to be similar to that for salmonellosis in terms of disability adjusted life years per year (Havelaar *et al.*, 2007).

In humans the parasite is known to cause encephalitis, mental retardation and blindness. Treatment is sometimes difficult, especially the treatment of ocular toxoplasmosis (Stanford *et al.*, 2003). So at present, the prevention of infection by *T. gondii* is the best strategy. Cats are definitive hosts for the parasite and can excrete millions of eggs – once over a short period in their lifetime – that are spread throughout the environment (Kijlstra *et al.*, 2008). Favourable climatic conditions may contribute to the survival of the pathogen (Meerburg and Kijlstra, 2009). When the excreted eggs or infected vectors (e.g. rodents) are consumed by farm animals, tissue cysts may develop in the meat of the livestock. If improperly cooked (at a temperature <67°C) humans may acquire infection.

In the Netherlands, during 1995–1996, a population-based seroprevalence study was conducted in pregnant women; the results were compared with those from a study conducted during 1987–1988 in order to estimate the change in seroprevalence (Kortbeek *et al.*, 2004). In total, 7521 sera were tested and the national seroprevalence was 40.5%. The seroprevalence among women aged 15–49 years was 10% lower in the study of 1995–1996 compared with that of 1987–1988 (45.8%) (Kortbeek *et al.*, 2004), but the steepest rise in seroprevalence still occurred among the subjects aged 25–44 years. One of the reasons for this could be the consumption of undercooked pork meat, which is considered a major risk factor for contracting toxoplasmosis in humans. In a previous study, it was found that conventionally (indoors) raised pigs are free from *T. gondii* infection, while animal-friendly raised pigs (with outdoor access) were contaminated in 2.9% of the cases (Kijlstra *et al.*, 2004b). However, *T. gondii* infection can also be contracted by consumption of the meat of many other animals, including poultry (Kijlstra and Jongert, 2008). Therefore, it is important that all meat is frozen or properly cooked before consumption.

Salmonella

In a recent study (Hoogenboom *et al.*, 2008), the presence of *Salmonella* in pigs at regular and organic pig farms was compared. No large differences were observed, but the authors got the impression that *Salmonella* incidence at farms that had transformed from regular farming to organic farming some years earlier was lower. In Denmark, Zheng *et al.* (2007) found a lower *Salmonella* prevalence in pigs with outdoor access (both in conventional and organic systems) compared with pigs that were reared indoors. Because these researchers did not find a difference in the numbers of antibodies between the groups, they came to the conclusion that pigs with outdoor access must have a better defence mechanism against *Salmonella*. However, in earlier Danish and Dutch studies, *Salmonella* seroprevalence has been shown to be higher in free-range

finishing pigs than in those produced in conventional intensive systems (Wingstrand *et al.*, 1999; Van der Wolf, 2000). These contradicting results are a complicating factor. Another problem is the absence of clinical signs of salmonellosis in the majority of pigs, which may result in the entrance of undetected carriers into the food production chain.

In a Belgian study in which the health status of organic broiler chickens and the contamination rates with *Salmonella* and *Campylobacter* were compared at slaughter, no difference could be found in prevalence of *Salmonella* between organic and conventional broilers (Van Overbeke *et al.*, 2006). In the USA (Bailey and Cosby, 2005), it was found that *Salmonella* was more prevalent in free-range (31%) and all-natural (25%) chickens than in chickens from the US commercial poultry industry surveyed for the USDA Food Safety Inspection Service reports in 2000–2003 (9.1–12.8% prevalence); it should be noted, though, that ten of the 22 lots of free-range chickens and all of the natural chickens under study had no detectable *Salmonella* (Bailey and Cosby, 2005).

In an older prospective case-control study of sporadic *Salmonella enterica* servovar Enteridis infection in Denmark (1997–1999), the consumption of eggs was identified as the key source of infection, but these authors were unable to demonstrate an association with the consumption of organic eggs (Molbak and Neimann, 2002).

Campylobacter

Campylobacter in broilers has proved to be higher in organic systems than in conventional flocks (Heuer *et al.*, 2001; Rodenburg *et al.*, 2004; Van Overbeke *et al.*, 2006). Heuer *et al.* (2001) isolated *Campylobacter* spp. from 100% of organic broiler flocks, 49.2% of extensive indoor broiler flocks and 36.7% of conventional broiler flocks. Schwaiger *et al.* (2008) also found a higher prevalence in organic flocks, but claim that this is the result of differences in the testing procedures. Most likely, organic broilers become infected with *Campylobacter* between weeks 7 and 10 (Van Overbeke *et al.*, 2006).

In a Danish study in pigs, it was found that the increased exposure of outdoor pigs to *Campylobacter jejuni* from the environment may cause a shift from a normal dominance of *Campylobacter coli* to more *C. jejuni*, which may imply a concern of reduced food safety (Jensen *et al.*, 2006). Apparently, in the case of *Campylobacter*, the contact of farm animals with outdoor wildlife, such as birds and rodents, is vital (Jensen *et al.*, 2006; Meerburg *et al.*, 2006).

Conclusions

Food safety hazards are currently an inherent but probably major risk in organic production systems. It is difficult to drive out this risk, as the whole farming system is generally more open, which might facilitate the introduction or preservation of hazards. Moreover, organic production is

not a guarantee that the environmental performance of the farming system will improve (Cederberg and Mattsson, 2000; Rigby and Cáceres, 2001; Trewavas, 2001; De Boer, 2003), although sometimes it will (Reganold *et al.*, 2001; Kumm, 2002; Pacini *et al.*, 2003).

Nevertheless, there are also advantages to organic production systems. In organic animal husbandry, the use of antibiotics is not allowed. Consequently, at organic pig and broiler farms a lower resistance of a number of selected microorganisms to antibiotics has been demonstrated (Hoogenboom *et al.*, 2008). Furthermore, organic farmers often claim that animal welfare in organic animal husbandry is generally better, as farm animals are allowed to lead a more natural life (Lund, 2006), although it is rather questionable what effects raising the housing standards will have on the total well-being of the animals (Sundrum, 2001). Other authors even claim, on the basis of practical experience, that organic livestock production is not a guarantee of good animal health and welfare (Hovi *et al.*, 2003; von Borell and Sørensen, 2004).

Some studies have compared nutrient compositions of organically and conventionally produced crops and animal products (meat, milk and dairy products). Very few compositional differences have been reported (Brandt and Mølgaard, 2001; Caris-Veyrat *et al.*, 2004), although there are reasonably consistent findings for higher nitrate and lower vitamin C contents of conventionally produced vegetables, particularly leafy vegetables (Williams, 2002). Additionally, in a recent study where the organic food consumption by infants was associated with developing atopic manifestations in the first 2 years of life, it was found that the consumption of organic dairy products led to a lower incidence of eczema (Kummeling *et al.*, 2008). More of these studies need to be performed in order to substantiate these results.

When considering the above, one might speak about a true dilemma. The consumer has to decide which factor(s) he or she thinks is most important. Consumers who choose organic foodstuffs should be aware that there is a potential risk of food contamination – potential, as there are currently gaps in scientific knowledge that make it difficult to weigh the exact risks of organic production. The attribution of human illness has been recently recognized as an important tool to better inform food safety decisions. Analysis of outbreak data sets is necessary for that purpose, but so far only a limited number of such studies has been performed. Often, it proves difficult to directly link the agent and food vehicle. In a recent Canadian study for example, foodborne outbreak data sets covering 30 years were investigated to estimate food attribution in cases of gastrointestinal illness (Ravel *et al.*, 2009); although overall 6908 foodborne outbreaks were described, the agent and food vehicle were only identified in 2107 of these cases, and in most of these studies organic production was not incorporated as a specific factor.

To consumers, it should be made clear that 'organic' products are not by definition 'safe'. Currently, many consumers perceive organic food as healthier than conventional food products (Schifferstein and Oude Ophuis, 1998; Trewavas, 2001; Zanoli and Naspetti, 2002; Magnusson *et al.*, 2003). In a study in the USA, over 90% of survey respondents perceived a reduction in pesticide residue risk associated with substituting organically grown

produce for conventionally grown produce, and nearly 50% perceived a reduction in risk due to natural toxins and microbial pathogens (Williams and Hammitt, 2001). In reality, organic products can be expected to contain fewer agrochemical residues and lower levels of nitrate than conventional products, but might contain more food hazards of other origin, such as microbial pathogens and mycotoxins (Lu *et al.*, 2006; Magkos *et al.*, 2006).

Consequently, there is friction between the consumer perception of the food safety of organic produce and the true safety risks. Thus, more attention should be paid to consumer education about possible food safety hazards and the communication of risk in order to maintain public trust (Kijlstra *et al.*, 2009). Beside this, producers should adopt proper agricultural practices in order to limit the risks associated with organic production as much as possible (e.g. by usage of parasite-safe pastures), and processors of organic products should do their utmost to prevent food contaminations, e.g. by decontamination.

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9 Zoonotic Implications of Avian and Swine Influenza

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Avian Influenza

Avian influenza (AI) is an infectious viral disease of birds caused by type A viruses of the family Orthomyxoviridae (Swayne and Halvorson, 2003). Although the first official description of AI was documented in Italy in 1878 (Lupiani and Reddy, 2009), it is believed to have existed since ancient times (Hirsch, 1883). In the 20th century, there have been three influenza pandemics, which were caused by H1N1, H2N2 and H3N2 subtypes of the influenza virus in the years of 1918, 1957 and 1968, respectively (Morens and Fauci, 2007). Of these, the H1N1 pandemic of 1918, the so-called 'Spanish Influenza', was the deadliest, and resulted in more than 50 million deaths worldwide (Erkoreka, 2009). The emergence of the H2N2 and H3N2 subtypes caused relatively mild pandemics (Scholtissek *et al.*, 1978). Furthermore, AI causes worldwide morbidity and mortality in domestic and wild birds. The emergence of new highly pathogenic avian influenza (HPAI) strains of type A viruses has led to devastating consequences for the poultry industry, resulting in the culling of hundreds of millions of birds (Swayne and Suarez, 2000).

The greatest threat posed by the AI virus lies in its zoonotic potential and ability to infect and cause significant morbidity and mortality in humans (Yen and Webster, 2009). The frequent outbreaks of AI in poultry and the transmission of AI viruses to humans reflect the potential for pandemic spread of these viruses. In 1997, an AI outbreak in Hong Kong attracted attention worldwide when a highly pathogenic strain of AI virus, H5N1, crossed the species barrier and infected humans, resulting in six fatalities out of 18 infected humans (Li *et al.*, 2004). The culling of all poultry in Hong Kong contained further viral dissemination, but the H5N1 viruses continued to circulate among wild aquatic birds in coastal regions of China (Chen *et al.*, 2004). Since 1997, more than 500 human cases of AI infections have been reported, with a case fatality rate of about 60%. Interestingly, HPAI viruses are exhibiting an unprecedented

geographical distribution and increased host range (Alexander, 2007), which substantiates the belief that an influenza pandemic is imminent. AI has, therefore, become one of the greatest concerns for public health in recent times.

Avian influenza viruses

The influenza viruses have been found to infect a variety of hosts, ranging from birds to mammals, but only viruses of the influenza A genus are known to infect birds. The virus has a negative-sense single-stranded RNA genome. The genome has eight segments with a total length of about 14 kb. Influenza A viruses may be classified into subtypes on the basis of the antigenic properties of their surface glycoproteins – haemagglutinin (HA) and neuraminidase (NA). Sixteen subtypes of HA (H1–H16) and nine subtypes of NA (N1–N9) have been reported with almost all subtype combinations (Palese and Shaw, 2006). HA helps in virus attachment to the eukaryotic cell receptors and the fusion of viral and cellular membranes, while NA cleaves the sialic acid receptors from the host cell membrane, thereby facilitating the release of the virus from the host cell. Furthermore, the genes encoding the internal virus proteins are highly conserved between influenza A viruses. The matrix 1 (M1) protein is found between the core and the membrane, and plays an important role in the assembly of the nucleocapsids (the genome plus its protein coat, or capsid) and membrane-bound proteins. The matrix 2 (M2) protein, an integral membrane protein, operates as a hydrogen ion channel regulating the pH environment of the virus. The multi-segmented viral genome is coated with the nucleocapsid protein (NP). The NP and the RNA polymerase proteins PB1 and PB2 (polymerase basic proteins 1 and 2) and PA (polymerase acidic protein) are potential targets of the cell-mediated immune system (Wright, 2007).

Pathogenesis of avian influenza

On the basis of clinical manifestations in poultry, the influenza viruses can be divided into two groups: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). HPAI viruses cause severe disease in poultry, leading to almost 100% mortality. In contrast to this, the LPAI viruses cause a much milder form of the disease, causing less morbidity and almost no mortality in infected birds (Webster *et al.*, 1992). The HPAI and LPAI viruses in domestic poultry are said to be transmitted from wild birds. Moreover, the LPAI H5 and H7 viruses circulating into poultry from wild birds may mutate and transform into HPAI viruses. The LPAI and HPAI viruses show structural differences at the so-called cleavage site of the precursor of the viral HA, which must be cleaved into HA1 and HA2 for the virus to become infectious. For all influenza A viruses, the HA glycoprotein is produced as a precursor, HA0, which requires post-translational cleavage by host proteases before it is functional and virus particles become infectious (Rott, 1992). The LPAI viruses

have only one or a few basic amino acids at this site, and cleavage occurs exclusively by trypsin-like host proteases. Virus replication is, therefore, restricted to sites in the host where such enzymes are found, i.e. the epithelia of the respiratory and intestinal tracts. HPAI viruses, in contrast, possess multiple basic amino acids at their HA cleavage sites and can be cleaved by a broad range of cellular proteases (Chen *et al.*, 1998; Pantin-Lockwood and Swayne, 2009). The transition from an LPAI phenotype to the HPAI phenotype is achieved by the introduction of basic amino acids into the HA cleavage site, which facilitates systemic virus replication, causing an acute generalized disease in poultry in which mortality may be as high as 100% (Webster and Rott, 1987). However, studies of AI viruses in chickens and ducks show that the NA, NS (non-structural), PA, PB1, PB2 and NP proteins can also play an important role in pathogenesis of the disease (Pantin-Lockwood and Swayne, 2009).

Emergence of new avian influenza virus strains

The influenza viruses continuously modify their HA and NA antigenic profiles via antigenic drift and antigenic shift. Antigenic drift is caused by point mutations leading to minor antigenic changes in the HA or NA proteins; this results from a lack of proofreading ability by the RNA polymerases during viral replication (Holland *et al.*, 1982). In contrast, antigenic shift refers to major antigenic changes resulting in new HA or NA proteins; because the proteins are distinct from the previously circulating strains, populations will have no immunity to the new subtype. Antigenic shift may occur as a result of genetic reassortment, which occurs when a host cell is infected by two influenza A viruses at the same time. The segmented nature of the genome facilitates this reassortment by allowing the mixing of genome segments from distinct AI viruses inside the infected cell (Hayashida *et al.*, 1985) (Fig. 9.1). Pandemic influenza occurs when a new virus to which the human population has no or little immunity emerges (Wright, 2007). A pandemic AI virus possesses certain characteristics, e.g. entry and replication in the human body, causing the disease, being shed in the excreta and effectively spreading among humans (Brankston, 2007).

Avian influenza ecology and outbreaks in poultry

Aquatic birds are the natural reservoir of influenza A viruses (Webster *et al.*, 1992). AI viruses have a broad range of avian hosts, e.g. ducks, geese, swans, gulls, terns, waders, etc. However, turkeys and domestic poultry are most susceptible to AI viruses (Alexander, 2000; Olsen *et al.*, 2006). The AI viruses replicate in the respiratory and intestinal tracts of aquatic birds, which show no clinical symptoms of the disease (Kida *et al.*, 1980). The mechanisms by which influenza viruses pass from one bird to another and bring about infection are poorly understood. In the past, some attempts were made to
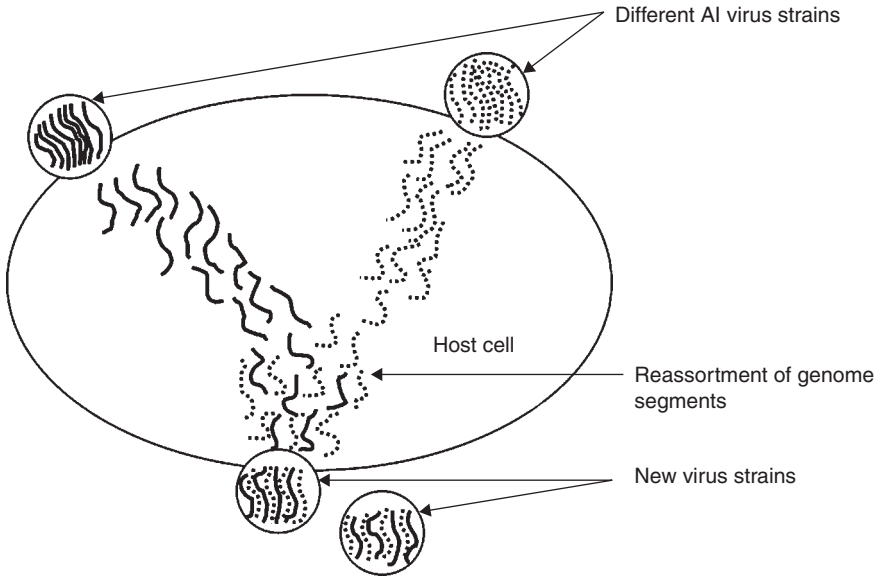
Fig. 9.1. Schematic of the process of genetic shift during avian influenza virus replication.

assess the transmissibility of LPAI and HPAI viruses in domestic poultry experimentally. The results suggested that bird-to-bird transmission is extremely complex and depends on strain of virus, the species of bird and environmental factors. The warm winter in South-east Asia attracts migratory birds from northern climates that spend the winter in this region. The high density of human populations and prevalence of backyard poultry and pigs provide the opportunity for close interactions between these reservoirs of influenza virus. Pigs possess the receptors for AI viruses (sialic acids with a 2,3-galactose linkage) as well for as human influenza viruses (sialic acids with a 2,6-galactose linkage), and have been considered as 'mixing vessels' for generating reassortant viruses (Scholtissek, 1995). Furthermore, the live poultry market system provides optimal conditions for influenza virus evolution, with transmission between avian species and possible infection of humans (Peiris *et al.*, 2007). In both natural and experimental infections, virulent viruses have tended to show much poorer transmission from infected to susceptible chickens and turkeys than viruses of low pathogenicity. The ability of the virus to spread easily should be related to the amount of virus shed through the respiratory or intestinal route. The highly pathogenic viruses cause extremely rapid deaths in these birds and it is possible that relatively little virus is excreted during the course of such infections. Nevertheless, this contaminates lake or pond water to the extent that virus may be isolated from lake water where large numbers of waterfowl are found.

The prognosis of influenza infection for wild birds is different from that for domestic birds (Alexander, 2000). In wild birds, the infections do not usually show clinical symptoms, and most influenza viruses isolated from wild birds are low pathogenic (LP) for poultry. This supports the hypothesis that HP (highly pathogenic) H5 or H7 viruses emerged after the introduction of an LPAI virus from wild ducks to poultry. Interestingly, viruses that are HP for poultry usually replicate poorly or to a limited degree in wild birds. However, the 1961 H5N3 HPAI outbreak in terns in South Africa and the HP H5N1 virus have caused significant mortality and morbidity among wild birds. The H5N1 virus has caused disease and death among migratory geese populations in western China. The increased virulence of some of the recent H5N1 isolates for some duck species has also been confirmed in experimental studies (Sturm-Ramirez *et al.*, 2005). Also, some recent H5N1 subtypes may still cause subclinical disease in aquatic wild birds. The target tissue for LPAI viruses is mainly the intestine, where they replicate and are subsequently shed in faeces. The AI H5N1 strain prefers the respiratory tissues of wild birds (Brown *et al.*, 2006). These HPAI viruses include the H5 or H7 subtypes of the AI viruses, but all H5 or H7 subtypes are not highly virulent. The influenza A viruses of subtype H5 and H7 may become highly pathogenic after introduction into poultry, and can cause outbreaks of HPAI (Table 9.1). Taking account of the severity of the disease, HPAI is classified as a list A disease by the World Organisation for Animal Health (OIE, originally Office Internationale des Epizooties) (Alexander, 2000). The OIE defines highly pathogenic notifiable AI as any AI virus with an intravenous pathogenicity index in 6-week-old chickens greater than 1.2 or that causes at least 75% mortality in 4- to 8-week-old chickens infected intravenously (Swayne and Halvorson, 2003).

Avian influenza zoonoses

A few subtypes of AI viruses are a public health threat as they have the potential to cause serious illness and death in humans, but only six strains – H5N1, H7N2, H7N3, H7N7, H9N2 and H10N7 – have been found to infect humans (Table 9.2). Of these, H5N1 is highly virulent and considered to have the potential to cause the next pandemic in human populations.

The sudden outbreak of Hong Kong HPAI H5N1 virus in 1997 caused a devastating effect on the poultry industry, leading to the culling of millions of poultry (Li *et al.*, 2004). The virus strain from which the H5N1 emerged is still circulating in aquatic birds in south China, without showing any clinical symptoms in these birds. The H5N1 infection underwent further expansion in many South-east Asian countries, including South Korea, Vietnam, Japan, Cambodia, Indonesia, Thailand, China and Laos. The viruses were contracted by the wild bird population from domestic poultry, resulting in huge mortality of thousands of migratory birds at the Qinghai Lake Nature Reserve in China (Olsen *et al.*, 2006). Since the 1997 outbreak, the H5N1 viruses are showing an increased geographical expansion, and have reached Europe

Table 9.1. Highly pathogenic avian influenza (HPAI) outbreaks in poultry since 1959 (adapted from Alexander, 2007).

HPAI virus	Subtype	Approximate numbers of poultry involved
1 A/chicken/Scotland/59	H5N1	1 small farm
2 A/turkey/England/63	H7N3	29,000
3 A/turkey/Ontario/7732/66	H5N9	8,000
4 A/chicken/Victoria/76	H7N7	58,000
5 A/chicken/Germany/79	H7N7	1 chicken farm, 1 goose farm
6 A/turkey/England/199/79	H7N7	9,000
7 A/chicken/Pennsylvania/1370/83	H5N2	17,000,000
8 A/turkey/Ireland/1378/83	H5N8	307,000, mostly ducks[a]
9 A/chicken/Victoria/85	H7N7	240,000
10 A/turkey/England/50–92/91	H5N1	8,000
11 A/chicken/Victoria/1/92	H7N3	18,000
12 A/chicken/Queensland/667-6/94	H7N3	22,000
13 A/chicken/Mexico/8623-607/94	H5N2	Unknown (? millions)
14 A/chicken/Pakistan/447/94	H7N3	>6,000,000
15 A/chicken/NSW/97	H7N4	160,000
16 A/chicken/Hong Kong/97b	H5N1	3,000,000
17 A/chicken/Italy/330/97	H5N2	8,000
18 A/turkey/Italy/99	H7N1	14,000,000
19 A/chicken/Chile/2002	H7N3	700,000
20 A/chicken/Netherlands/2003	H7N7	>25,000,000
21 A/chicken/Eurasia and Africa/ 2003–2006	H5N1	Unknown (100s of millions)
22 A/chicken/Texas/2004	H5N2	6,600
23 A/chicken/British Columbia/2004	H7N3	16,000,000
24 A/ostrich/South Africa/2004	H5N2	30,000
25 A/chicken/North Korea/05	H7N7	219,000
26 A/turkey/England/07	H5N1	160,000
27 A/chicken/Canada/2007	H7N3	540
28 A/chicken/England/2008	H7N7	15,000
29 A/chicken/Spain/2009	H7N7	30,000

[a] A very closely related virus was isolated from ducks in the area, and the epidemiological data suggested that the virus had been maintained in ducks as a subclinical infection before transmission to turkeys.

and Africa. HPAI H5N1 virus is notorious for its zoonotic potential, and causes high mortality and morbidity in humans. The recent spread of HPAI H5N1 across Asia, Europe and Africa raises the concern of a possible new pandemic, in the case that the virus attains the ability to become transmissible from person to person. The evolution of H5N1 into a pandemic threat could occur through a single reassortment of its segmented genome or through the slower process of genetic drift (Fauci, 2006). Furthermore, the cases of human infection with H5N1 viruses in 1997 in Hong Kong

Table 9.2. Cases of human infection with avian influenza viruses from 1997 (source: WHO, 2009).

Virus strains	Year	Country	Clinical symptoms	Number of cases (deaths)
H5N1	1997	Hong Kong	Conjunctivitis, pneumonia, influenza-like symptoms	18 (6)
	2003–2009	Azerbaijan, Bangladesh, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Laos, Myanmar, Nigeria, Pakistan, Thailand, Turkey, Vietnam	Pneumonia, influenza-like symptoms	444 (262)
H9N2	1999	Hong Kong	Influenza-like symptoms	2
	1999	China		5
	2003	Hong Kong		1
	2007	Hong Kong		1
H7N7	2003	Netherlands	Conjunctivitis, influenza-like symptoms, pneumonia	89 (1)
H7N3	2004	Canada	Conjunctivitis	2
	2006	UK		1
H7N2	2002	USA	Influenza-like symptoms	11
	2003	USA	Upper and lower respiratory infection	1
	2007	UK	Conjunctivitis	1
H10N7	2004	Egypt	Cough and fever	2

established the fact that AI viruses could be transmitted directly from poultry to humans (Claas *et al.*, 1998). However, the transmission of the H5N1 viruses from birds to humans has been uncommon compared with the transmission of the virus among birds.

The clinical symptoms of H5N1 infection in humans vary from severe pneumonia to mild upper respiratory tract infection without pneumonia. Gastrointestinal symptoms are also reported in patients suffering from certain clades of H5N1 viruses (Abdel-Ghafar *et al.*, 2008). Although the mechanism behind avian-to-human infection is poorly understood, close contact with infected birds and the consumption of undercooked poultry meat are important causes of human infection (Beigel *et al.*, 2005; Abdel-Ghafar *et al.*, 2008). Also, the limited human-to-human infections that have occurred have been observed in family members taking care of H5N1-infected patients (Ungchusak *et al.*, 2005). In addition, the intrauterine transmission of H5N1 from mother to fetus has been reported (Gu *et al.*, 2007).

In February 2003, there was an HPAI H7N7 outbreak in poultry in the Netherlands. The outbreak spread further to the neighbouring countries of

Belgium and Germany. To contain the outbreak, more than 30 million poultry were culled. This outbreak of H7N7 infected 89 humans with a single mortality. Infected people did not show the typical influenza-like clinical symptoms, but rather conjunctivitis (Koopmans *et al.*, 2004). In 2004, an HPAI H7N3 outbreak was reported in Canada (British Columbia), leading to the culling of more than 17 million domestic poultry (Berhane and Hooper-McGrevy, 2009). Two humans were infected and also presented with conjunctivitis (Hirst *et al.*, 2004).

Swine Influenza

Swine influenza is an economically important respiratory disease of pigs throughout the world. It was first recognized as a disease of pigs in the Midwestern USA in 1918, coinciding with the 'Spanish Influenza' pandemic in the human population (Koen, 1919; Webster, 2002). Swine influenza is manifested as an acute respiratory disease characterized by fever, lethargy, decreased food intake, respiratory distress, coughing, sneezing, rhinitis, nasal discharge and conjunctivitis (Alexander and Brown, 2000; Richt *et al.*, 2003). Swine influenza also contributes to the porcine respiratory disease complex in combination with the porcine respiratory and reproductive syndrome virus, *Mycoplasma hyopneumoniae* and other bacterial pathogens. Human infections with swine influenza viruses are well documented, and humans occupationally exposed to pigs are at increased risk of infection with swine influenza viruses and developing influenza-like illness (Olsen *et al.*, 2002; Gray *et al.*, 2007; Myers *et al.*, 2007). Thus, swine influenza also poses a threat to public health.

Swine influenza viruses

Influenza viruses were first isolated from pigs in 1930 (Shope, 1931) and were the initial examples of the classical H1N1 lineage of swine influenza A viruses. Since then, H1N1 viruses have circulated in swine populations in North America, South America, Europe and Asia. However, the predominant virus in Europe is an avian H1N1 virus introduced by wild ducks into the swine population in 1979 (Van Reeth, 2007). Although there are genetic, antigenic and pathogenic similarities between early swine and the 1918 human H1N1 viruses, it is not clear whether a progenitor virus was transmitted from pigs to humans or from humans to pigs (Memoli *et al.*, 2009). For nearly 70 years, classical H1N1 remained the predominant swine influenza virus subtype in North America without much genetic and antigenic change (Sheerar *et al.*, 1989; Luoh *et al.*, 1992; Noble *et al.*, 1993).

In 1998, a new 'triple reassortant' H3N2 swine influenza virus emerged in the USA which caused severe influenza-like illness in pigs. This triple reassortant H3N2 swine influenza virus contained HA, NA and PB1 genes of human influenza virus origin, NP, M and NS genes of classical swine

H1N1 virus origin, and PB2 and PA genes of North American avian virus origin. Swine influenza viruses antigenically and genetically related to the triple reassortant H3N2 viruses were subsequently established in the US swine population (Webby *et al.*, 2000). Further evolution of H3N2 viruses through genetic mutation and reassortment with the classical H1N1 viruses led to the emergence of a number of reassortant swine influenza viruses, including new H3N2 viruses (Webby *et al.*, 2000, 2004; Richt *et al.*, 2003), H1N2 viruses (Choi *et al.*, 2002; Karasin *et al.*, 2002) reassortant H1N1 viruses (Webby *et al.*, 2004) and H3N1 viruses (Lekcharoensuk *et al.*, 2006; Ma *et al.*, 2006). Currently, the H3N2, reassortant H1N1 and H1N2 viruses co-circulate in swine populations in most regions of the USA and Canada. Although the origin and time of introduction are different, reassortant H3N2 and H1N2 viruses are circulating in Europe as well (Van Reeth, 2007). Emergence of reassortant swine influenza viruses with mixtures of genes from human, swine and avian viruses support the notion that pigs may serve as 'mixing vessels' for genetic reassortment between human and avian viruses. Thus, pigs may play an important role in the generation of influenza viruses with pandemic potential.

In addition to the H1N1 and H3N2 reassortant viruses, a number of novel swine influenza virus subtypes were isolated from pigs in different parts of the world. An H1N7 virus containing gene segments from human and equine influenza viruses was isolated from a pig farm in the UK in 1992 (Brown *et al.*, 1994). An avian influenza H4N6 virus of North American lineage was isolated from pigs with pneumonia on a commercial swine farm in Canada in October 1999. This avian virus was probably introduced by waterfowl from a nearby lake (Karasin *et al.*, 2000). In 2003, transmission of human H1N2 to pigs was reported in Ontario. This H1N2 virus was genetically and antigenically distinct from the classical swine H1 virus (Karasin *et al.*, 2006). Researchers in the USA identified a new H2N3 subtype of swine influenza virus from two groups of infected pigs at separate production facilities in 2006. The new H2N3 swine influenza virus belonged to the group of H2 influenza viruses that caused the influenza pandemic in 1957 and continued to circulate among the human population until 1968. The affected swine production facilities apparently used water from ponds frequented by waterfowl, which are natural hosts for all subtypes of influenza A viruses. Use of this pond water might have introduced avian influenza viruses into the swine population, although the exact source of this virus remains unclear. Laboratory studies also revealed that the new H2N3 virus has undergone adaptations that enabled it to efficiently infect and replicate in mammals, including pigs, mice and ferrets (Ma *et al.*, 2007). Many of the newly emerged viruses are not able to establish themselves in the swine population. However, the frequent transmission of human and avian viruses to the swine population increases the possibility of the emergence of new influenza virus subtypes by reassortment which could have an impact on public health. Introduction of new viruses and constant changes in the genetic make-up of circulating swine influenza viruses also pose a challenge to disease control programmes.

Swine influenza zoonoses

Human infections with swine influenza viruses have been reported on several occasions in the USA, Canada, Europe and Asia. In a recent study (Myers *et al.*, 2007), 50 cases of zoonotic swine influenza infections were identified and described. This number may not reflect the actual number of cases of human infection with swine influenza viruses, as there are no unique clinical features to discriminate between infections caused by swine and human influenza viruses. Most of the cases resulted from direct exposure to swine or human-to-human transmission within a family cluster. Swine influenza H1N1 virus subtype caused more infections in humans than the H3N2 virus subtype (Myers *et al.*, 2007). A notable case is the swine influenza virus infection in humans caused by an H1N1 virus at Fort Dix, New Jersey, in 1976. This outbreak resulted in one death, and respiratory disease in 12 soldiers, along with serological evidence of infection in more than 200 soldiers (Gaydos *et al.*, 1977). No evidence of exposure to swine was found in these cases and the virus had disappeared from the human population within a short period of time (Gaydos *et al.*, 1977).

On 21 April 2009, the Centers for Disease Control and Prevention (CDC) in the USA announced the identification of a new strain of H1N1 influenza virus, the pandemic H1N1 2009 virus (pH1N1) in humans. This virus consists of genomic RNA segments PB2 and PA of North American avian virus origin, the PB1 gene of human H3N2 virus origin, HA, NP and NS genes of classical swine H1N1 virus origin, and NA and M genes of Eurasian avian-like swine origin. The pH1N1 virus is believed to have originated from the reassortment of recent North American H1N1 and H3N2 triple reassortant swine viruses with Eurasian avian-like swine viruses (Brockwell-Staats *et al.*, 2009). Although the exact nature of origin of this new virus is not known, it spread in humans so quickly that by 11 June 2009 WHO declared a phase six pandemic (Brockwell-Staats *et al.*, 2009; Neumann *et al.*, 2009). The virus was also isolated from a swine herd in Canada on 2 May 2009, and pH1N1 has now become an emerging swine influenza virus in several countries. Experimental and field infections of pH1N1 virus in swine appear to be similar in nature to the circulating swine influenza virus infections (Lange *et al.*, 2009). The risk of transmission of this virus from pigs to humans is yet to be determined, and it is still uncertain whether the pH1N1 virus will become established in swine populations in any part of the world.

Recent studies have shown that persons who work with swine, including farmers, meat-processing workers, veterinarians and their spouses are at increased risk of zoonotic influenza virus infections (Olsen *et al.*, 2002; Gray *et al.*, 2007; Myers *et al.*, 2007). These zoonotic infections could increase the possibility of the mixing of swine and human influenza viruses in swine workers, leading to the generation of new influenza viruses with pandemic potential. People who are exposed to swine could accelerate the transmission of new pandemic influenza viruses to their communities if such viruses become enzootic in the swine population (Saenz *et al.*, 2006). However, the frequency of transmission of swine influenza virus to occupationally exposed people

is low or negligible compared with the number of people exposed to pigs (Van Reeth, 2007).

Conclusion

Since the first documented case of bird-to-human infection with the HPAI H5N1 virus in 1997, there have been several reports of human infections with a variety of HPAI viral strains (Table 9.2), although all these viruses were devoid of the ability to spread from human to human, which is a prerequisite for them to become established in the human population. The question still remains as to whether AI viruses will acquire the capacity for consistent human-to-human transmission. HPAI is constantly evolving over the course of time by antigenic drift and shift. If the AI virus attains the capability of human-to-human transmission, a global influenza pandemic will be inevitable. It is also accepted that AI viruses can have a major role in the initiation of an influenza pandemic in the human population (Capua and Alexander, 2004). Furthermore, cases of human infections with swine influenza have been reported, especially among people occupationally exposed to pigs, which have been claimed to work as a 'mixing vessel' for the genetic reassortment of human and avian influenza viruses (Scholtissek, 1995). However, the significance of zoonotic swine influenza infection is still not very clear owing to the lack of consistent screening and reporting programmes. Nevertheless, the zoonotic potential of the avian and swine influenza viruses raises serious public health concerns as the cause of a future pandemic in the human population, which underscores the need for a comprehensive influenza pandemic preparedness strategy.

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10 Crohn's Disease in Humans and Johne's Disease in Cattle – Linked Diseases?

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Introduction

With advancing world trade, and as new agricultural regions develop, guaranteed quality and cost-effective production are important to maintain both local demand and the export position of the dairy and beef sector in Canada. The consumer's perception of the cattle sector is important, and is affected by both the quality of the end product and, increasingly, the way this product is produced. The concerns of the consumer go beyond the actual risk of consuming this product; the perception of risk is also important and can undoubtedly affect the consumer's behaviour. Animal health is a cornerstone to providing the consumer with the desired assurances on food safety. One example of perceived consumer risk is *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which is known to cause Johne's disease (JD) in cattle and may confer a risk of developing Crohn's disease (CD) in humans. The cause of CD has been linked to infection ever since it has been described, and of the putative infectious agents, MAP has been argued to fulfil Koch's postulates (Hansen *et al.*, 2010). Further evidence linking CD to MAP, as well as disproving the link, continue to appear, which may be partly attributed to the heterogenous phenotype of CD.

Johne's Disease

Johne's disease in cattle is a chronic granulomatous inflammation of the gut and other organs caused by infection with MAP. MAP is a major health problem in ruminants, resulting in intermittent diarrhoea, loss of body condition and lower productivity. In the terminal phase, which most cows will not reach, animals die with a very poor body condition. Infected cattle shed MAP in

manure and milk in increasing quantities as the disease progresses. The disease is widespread in cattle populations in almost all countries with a cattle industry, and causes great economic losses because of lower productivity, but even more by loss of future income due to early culling (McKenna *et al.*, 2006). In Canada, the economic damage caused by JD is unknown; however, the percentage of cattle infected is similar to that in the USA, where the disease is estimated to cost US$100 per animal on an infected farm, or US$250 million a year to the cattle industry (Ott *et al.*, 1999). JD is likely to spread further if control measures are not implemented, because as herds increase in size farmers are more likely to purchase animals, often from herds with unknown JD status.

Testing for MAP infection in cattle is difficult as many infected animals do not have detectable organisms in their faeces or antibodies in their blood until shortly before clinical disease develops. In Canada, using antibody tests such as enzyme-linked immunosorbent assay (ELISA), 1.3% (Prince Edward Island) to 9.1% (Alberta) of the dairy cows were found to be infected, while 10% (Ontario) to 60% (Alberta) of the herds had at least one infected cow (VanLeeuwen *et al.*, 2001; Scott *et al.*, 2006). Furthermore, a recent slaughterhouse study performed in Atlantic Canada using bacteriological culture of lymph nodes and intestines revealed that 16% of dairy cattle were infected (McKenna *et al.*, 2004). When using only an ELISA as the method of testing, 10–25% of infected cattle are identified – meaning that the true percentage of infected animals and herds is highly underestimated. Most infected animals do not manifest symptoms of the disease, and thus act as silent carriers that can infect other cattle. Many MAP-infected herds will therefore never show any symptoms of the disease as these animals are often removed for reasons other than clinical JD, i.e. low milk production, mastitis, etc.

Beef cattle in North America are more extensively managed than dairy cattle, resulting in lower ELISA prevalence estimates at the cow and herd levels: 1% and 7%, respectively (Dargatz *et al.*, 2001; Waldner *et al.*, 2002). However, when MAP is present on a beef farm, the within-herd prevalence can become quite high. As opposed to dairy production, beef cows and calves are commingled from birth until weaning, thus allowing ample opportunity for transmission.

Other ruminants, such as sheep and goats, also become infected with MAP and may present with JD. The hallmark sign of JD in small ruminants is weight loss, as signs of malabsorptive diarrhoea are often absent. Sheep are predominantly infected with a different strain from that of cattle and goats, although sheep can still be infected with cattle strains, and transmission between these species is possible (Motiwala *et al.*, 2006). Small ruminant production is much more common in Australia, New Zealand and the UK. Given the relatively low economic value of the individual animal, JD prevention has focused more on the flock and, in particular, on the use of vaccination. As new infections are established very early in life, vaccination is quite limited in its ability to directly prevent infection, but its ability to reduce faecal shedding minimizes environmental contamination and thus helps

to control JD. Vaccination is not used widely for cattle in North America because of its impact on future JD serological testing and tuberculosis surveillance.

Complicating the epidemiology of JD are the wild ungulates (deer, elk, moose, etc.) that can serve as sylvatic reservoirs. The role that these species play as a nidus of infection for livestock has not been well established, but they are more likely to be innocent victims of infected cattle, sheep and goats. MAP survives well in the environment, even in the presence of light (sun and UV), heat, cold and desiccation. Recently, concerns are also being raised as to MAP as a waterborne pathogen.

Inflammatory Bowel Disease

The inflammatory bowel diseases (IBDs) of humans consist of CD and ulcerative colitis (UC), and are chronic, relapsing inflammatory conditions of the gastrointestinal tract. Despite their significant impact on patients and society, there is no cure and their cause remains unknown. The onset of IBD is greatest in early adulthood, with peak incidence among people aged 18–35 years, and it affects quality of life, employment and psychosocial functioning. About 10–15% of IBD occurs in childhood or adolescence, when the disease may be particularly aggressive. For unknown reasons, Canada has among the highest incidence rates of IBD in the world (Bernstein *et al.*, 2006). IBD is most predominant among industrialized nations, which points, at least in part, to environmental and microbial influences. The disease has shown a prominent recent increase in incidence in the Far East, Middle East and Eastern Europe, suggesting environmental influence.

The prevailing theory describing the aetiology of IBD is that the inflammation results from dysregulation of the gut's immune system in genetically predisposed individuals who are exposed to unknown environmental triggers (Podolsky, 2002). Recent advances have identified a multitude of IBD-specific genes and environmental risk factors. However, none uniformly explain the processes that drive the pathogenesis of IBD and none can be shown to be a contributing factor in more than a minority of cases. Overall, many of these associated genetic variants impair the clearance of intracellular bacteria. The difficulty in understanding the aetiology of IBD is in part because of the complex interactions between genes, intestinal microbes and the environment. While the pathogenesis of this chronic disorder is poorly understood, the burden to the patient in quality of life, morbidity and hospitalizations, as well as to the health-care system in direct and indirect costs, is staggering (Yu *et al.*, 2008).

Crohn's Disease

As already stated, CD and UC are the two most important manifestations of IBD in humans. Although they are seen as different diseases, they overlap,

and both diseases can be classified into several phenotypes that may have different pathogenetic pathways. CD is a chronic relapsing inflammatory disease that may affect virtually any part of the intestinal tract. Symptoms are chronic urgent diarrhoea, abdominal pain, nausea, vomiting, fever, intestinal bleeding, malnutrition and fatigue. A cure does not exist for CD; consequently, most patients are on chronic immune-suppressing medications and often require at least one IBD-related intestinal operation. Currently, 50,000–100,000 people have been estimated to be living with CD in Canada, and over a half a million people are believed to have the disease in the USA. The percentage of people affected with CD is increasing in industrialized parts of the world (Loftus, 2004). The pathogenesis of CD is unclear, and there are different theories on the development of the disease. The predominant theory of pathogenesis is that interactions between intestinal microflora and exposure to environmental factors (e.g. smoking and diet) in genetically susceptible individuals results in dysregulated inflammation and chronic gastrointestinal injury. Secondarily, investigators have proposed that chronic inflammation is provoked following a gastrointestinal infection (e.g. with MAP or strains of *Escherichia coli*). A characteristic histology is non-caseating granuloma, which may be present in up to 50% of patients. In the small intestine, the disease often starts as aphthous ulcers overlying Peyer's patches, which provide a portal to bacterial entry and sampling.

Genetic susceptibility

Genetic susceptibility to IBD is well recognized (Cho and Weaver, 2007; Franke *et al.*, 2008). Approximately 20% of IBD patients report a family history of IBD and twin studies have demonstrated higher concordance rates among monozygotic twins (Halfvarson *et al.*, 2003).

The first susceptibility gene identified for CD was the *NOD2* gene. Caucasians who are heterozygote carriers of a *NOD2* gene variant are three times more likely to be diagnosed with CD, while those who are homozygous carriers have 23 times increased risk (Cuthbert *et al.*, 2002). *NOD2* has also been shown to predispose to CD occurring in the terminal ileum and manifesting with fibrostenotic disease. The prevalence of *NOD2* variants is different across populations (Economou *et al.*, 2004). For example, Japanese people have not been shown to carry mutations of the *NOD2* gene (Yamazaki *et al.*, 2002). Recently, a meta-analysis of genome-wide association studies confirmed 11 CD genetic loci and identified 21 new genetic loci (Mathew, 2008). The genes identified to date have provided insight into the pathogenesis of CD by implicating defects in innate and adaptive immunity, epithelial barrier function and mucosal defence (Yamamoto-Furusho, 2007; Hussey *et al.*, 2008; Mathew, 2008). The specific linkage of three genes to CD is of particular interest when considering the role of bacteria in CD pathogenesis, as these genes are involved in host sensing (*NOD2*) and the elimination through autophagy (*ATG16L1* and *IRGM*) of intracellular bacteria.

Likewise, genetic susceptibility is important in UC. The major histocompatability complex region on chromosome 6p (IBD3) has been shown to be associated with UC (van Heel *et al.*, 2004). Additionally, the *IL1RA*, *MDR1* and *PTPRS* genes have been associated with an increased risk of UC (Yamamoto-Furusho, 2007). Finally, family clusters of IBD have reported mixed inheritance patterns. These findings suggest that genetic overlap may lead to CD in one family member and UC in another (Cho and Weaver, 2007). Alternatively, environmental influences may shift phenotypes in genetically predisposed family members. Thus, the prevailing view is that IBD is a spectrum of clinical disorders with the eventual phenotype determined by a combination of both genetic and environmental factors. However, gene–environment interactions in IBD have not been well studied and so the causal relationships between IBD genes and environmental risk factors are not known. Specifically, several of the genetic defects identified in CD may increase susceptibility to MAP. *NOD2/CARD15* has been shown to play a role in the susceptibility of cattle to MAP (Pinedo *et al.*, 2009), and *NOD2* also plays a critical role in the host type I interferon response to *Mycobacterium tuberculosis* (Pandey *et al.*, 2009). A genome-wide association study of leprosy showed that variants of the NOD2 signalling pathway are associated with susceptibility to *Mycobacterium leprae* (Zhang *et al.*, 2009), raising the intriguing possibility of a common genetic fingerprint in a known mycobacterial disease and CD (Schurr and Gros, 2009). MAP is recognized by NOD2 and TLR2/TLR4 receptors (Ferwerda *et al.*, 2007). The autophagy gene variants *ATG16L1* and *IRGM* impair the ability to clear intracellular *Mycobacteria*, which provides further links between the known genetic associations of CD and possible MAP infection (Glasser and Darfeuille-Michaud, 2008).

Environment

While IBD genes have provided insight into disease pathogenesis, genetics has not completely explained the aetiology of IBD. The majority of patients with IBD have neither a family history nor a known genetic defect (Loftus, 2004). Moreover, IBD has emerged predominantly in industrialized nations in the last century; as developing nations have more recently become industrialized, the incidence of IBD in these countries has risen (Loftus, 2004). In many countries, the incidence rate is rising faster than can be explained by genetic factors alone, which suggests that the aetiology of CD is also likely to be to caused by result of environmental conditions (Loftus, 2004). Environmental factors that are specific to modernization play an important role in the development of IBD, although despite numerous studies that have evaluated environmental risk factors, only a handful of disease determinants have been reproducibly confirmed and many others remain controversial. A paradoxical relationship between smoking and IBD has been consistently demonstrated. A meta-analysis concluded that active smokers were less likely to develop UC, while more likely to develop CD (Calkins,

1989). Additionally, the contraceptive pill was implicated in CD and UC (Godet *et al.*, 1995), whereas appendectomy was shown to be protective in UC (Koutroubakis *et al.*, 2002). Also, IBD occurs more commonly in urban centres (Ekbom *et al.*, 1991). Diet has been extensively studied in relation to IBD (Wild *et al.*, 2007), but has yielded variable results. An analysis of the association between CD onset and refined sugars gave inconsistent findings (Riordan *et al.*, 1998), but a study in Japan showed that high fat diets may increase the risk of CD (Shoda *et al.*, 1996), whereas breastfeeding may protect against CD (Klement *et al.*, 2004). Finally, helminth infections, which are more common in developing countries, may protect against CD in particular (Weinstock *et al.*, 2004). However, a recurrent environmental factor implicated in CD has been microorganisms such as MAP or *E. coli* derived from food-chain contamination. The preceding risk factors have not completely explained the occurrences of IBD, and risk factors associated with industrialization and urban predominance have been incompletely investigated.

Microbiota

Genetic and environmental studies implicate commensal gut microorganisms in the induction and perpetuation of IBD, as well as many of its clinical complications (e.g. abscesses, phlegmon, fistulae) (Sartor, 2008). Genes associated with IBD affect innate and adaptive immunity, as well as epithelial barrier function, which are key determinants of the composition of the commensal flora and its interactions with the host (Yamamoto-Furusho and Podolsky, 2007; Gibson *et al.*, 2008; Lundin *et al.*, 2008). A variety of genetically engineered rodents spontaneously develop chronic intestinal inflammation that mimics IBD in the presence of commensal bacteria, whereas germ-free animals remain disease free (Rath *et al.*, 2001). Surgical diversion of the ileum from the faecal stream prevents postoperative recurrence of CD, whereas reinserting gut content from the ileostoma into the diverted intestine results in rapid-onset inflammation (Harper *et al.*, 1985). Additionally, acute gastroenteritis and antibiotic exposure are associated with increased risk of developing IBD (Garcia Rodriguez *et al.*, 2006). Furthermore, probiotic bacteria have been used to treat active IBD (Fedorak and Madsen, 2004). Finally, candidate organisms in the pathogenesis of CD include adherent-invasive strains of *E. coli* and MAP.

Despite several lines of evidence implicating the importance of gut microbiota, the mechanism by which these microbiota influence IBD remains elusive. We hypothesize that environmental factors affect the commensal intestinal microbiota in genetically predisposed individuals, resulting in the initiation and perpetuation of IBD. Alternatively, specific bacterial species may play a more direct role in triggering, maintaining or modulating the disease, as has been proposed for candidate organisms in the pathogenesis of CD (MAP and adherent-invasive strains of *E. coli*).

Infection with MAP

CD was described by Dalziel (1913), who wrote: 'Tissue characteristics from Johne's and Crohn's patients are so similar as to justify a proposition that the diseases may be the same'. Since 1952, researchers have tried to grow *Mycobacteria* from surgically removed CD tissue. Chiodini *et al.* (1984) cultured MAP from the gut wall of children with CD. Further corroboration of such findings in early-onset CD has been provided recently by an Australian group (Kirkwood *et al.*, 2009). Additionally, MAP and *E. coli* strains have been demonstrated to be present in the blood and intestinal tissue of CD patients (Selby, 2004; Kotlowski *et al.*, 2007). However, as already mentioned, despite several lines of evidence implicating the importance of gut microbiota, the mechanism by which they influence IBD remains elusive. The challenge with defining a causative relationship between MAP (and other pathogens) and CD is temporal: does the presence of MAP in a subset of CD patients provide evidence that MAP infection results in CD, or does the development of CD predispose to a benign colonization of MAP in CD patients? This is reminiscent of the debate surrounding *Helicobacter pylori* and peptic ulcers in the 1980s.

Does MAP Cause Crohn's Disease?

Recent studies have shown that a high percentage of people with CD are infected with MAP compared with a low percentage of people who do not have the disease (e.g. Sanderson *et al.*, 1992; Sechi *et al.*, 2001; Kirkwood *et al.*, 2009). This seems to be a finding specific to CD, as MAP is not significantly associated with UC. Furthermore, it seems to be an association specific to MAP, as other environmental *Mycobacteria* are found as often in people with CD as in patients with other inflammations of the bowel. In a study of 28 CD patients, MAP was cultured from blood samples in 50%, while this was the case in only 22% of patients with UC, and none from people who did not have either disease (Naser *et al.*, 2004). CD has been consistently demonstrated to be more predominant in urban centres. In contrast, rural regions and farmers have greater exposure to MAP, but increased rates of CD have not been observed in these populations. However, not all studies have documented such urban–rural differences (Armitage *et al.*, 2004).

Case–control studies exploring an association between MAP and CD have yielded heterogeneous results. While several studies have demonstrated a relationship between MAP and CD, a population-based matched case–control study from Canada demonstrated no differences in rates of serology for MAP between CD, UC, randomly sampled controls and unaffected siblings; in all four groups, one-third of participants were seropositive (Bernstein *et al.*, 2004). Overall, three recently published meta-analyses and systematic reviews summarized the results of relevant studies on the association between MAP (as assessed by ELISA or PCR) and CD, and concluded that an association is evident (Feller *et al.*, 2007; Abubakar *et al.*, 2008; Waddell *et al.*, 2008). These

studies, however, stated that the evidence of an association between MAP and CD does not prove that MAP causes CD. The higher prevalence of MAP in CD may be secondary to the damage inflicted to the intestinal wall, which places these patients at higher risk for infection or colonization with MAP after the disease had developed. This issue can only be resolved by therapy to eradicate MAP in patients with CD associated with presence of MAP.

If MAP infection causes CD, as is known for JD, then treating MAP should intuitively treat CD. Many open-label studies evaluating different regimens of antimicrobial agents targeting MAP have been published, with varying results. The inconsistency in the data motivated the development of a randomized controlled trial whereby 213 active CD patients were randomized to 2 years of maintenance treatment with anti-MAP antibiotics or placebo, in combination with a 16-week course of prednisolone for induction of remission at the onset of the study (Selby *et al.*, 2007). The trial used a combination of three anti-mycobacterial agents – clarithromycin, rifabutin and clofazimine – justified on the basis of proven efficacy against the *Mycobacterium avium* complex (MAC), intracellular penetration, tolerability, safety and redundancy of effect to avoid resistance. More patients achieved remission in the prednisolone and antibiotics group at 16 weeks than with prednisolone and placebo (66% versus 50%). During the maintenance phase of the trial, the proportion of antibiotic-treated patients that relapsed tended to be less than in the placebo group: at 52 weeks 39% in the antibiotics group versus 56% in the placebo group; and at 104 weeks 26% in the antibiotics group versus 43% in the placebo group. In the 12 months after treatment ceased, there was no significant difference in the proportion of patients that relapsed (59% in the antibiotics group versus 50% in the placebo group). In summary, the only statistically significant benefit of anti-MAP antibiotics in this trial was the 16% absolute benefit seen in achieving steroid-induced remission at 16 weeks. While it remains possible that this early benefit is attributable to specific effects on MAP, this should have been more readily apparent in the maintenance phase, as effective treatment of atypical *Mycobacteria* generally requires prolonged antibiotics. Rather, this finding is likely to be a non-specific effect of the antibiotics, or even a consequence of the direct anti-inflammatory or immunomodulatory effects that have been described for macrolide antibiotics in particular. Considering that the study did not specifically enrol CD patients shown by molecular detection to have 'MAP-associated' disease, it may not have been adequately powered to discern a benefit in a subset of patients that presumably harbour MAP. Publication of the Selby trial (Selby *et al.*, 2007) led to a vigorous debate and correspondence, including reanalysis of the data, which provided support for a favourable influence of combination antibiotics on CD. A replication of this study in MAP-positive CD patients is required.

Ultimately, the predominant treatment strategy for CD is suppression of the immune system with drugs such as corticosteroids, azathioprine and monoclonal antibodies against tumour necrosis factor (TNF) (e.g. infliximab). If human infection with MAP was the dominant pathogenesis for the development of CD, then suppression of the immune system should result in widespread infection of MAP. Take for example infliximab, which has been

clearly demonstrated to reactivate tuberculosis in CD patients with latent infections. While MAP has been shown to cause disease in HIV patients, similar reports of MAP-related disease have not been observed in CD patients receiving long-standing anti-TNF therapy, which argues against a clinically relevant role for MAP in CD. It is possible that immunosuppression favourably affects the inflammation associated with microorganisms, but the persistence of microorganisms relates to inevitable relapse following discontinuation of immunosuppression in the majority of patients. In addition, several drugs used in the treatment of CD, such as azathioprine, 6-mercaptopurine (Shin and Collins, 2008), 5-aminosalicylic acid (Greenstein *et al.*, 2007), cyclosporin, rapamycin and tacrolimus (Greenstein *et al.*, 2008) and thalidomide (Greenstein and Brown, 2009) have all been shown to inhibit MAP growth, raising the possibility that immunosuppressive drugs used in CD may have anti-MAP efficacy. In addition, antibiotic studies in CD not specifically directed against MAP had variable results in often uncontrolled trials.

Overall, numerous studies have demonstrated an association between MAP and CD, although there are no conclusive data yet to support the idea that human infection with MAP causes CD. An alternative concept is that MAP may contribute to the development of CD in a subset of patients, though the effects of MAP may not be due to direct infection. Increased understanding of the genetics of CD suggests that defects in innate immunity and autophagy predispose to the development of CD. Among genetically susceptible individuals with defects in immune response, exposure to MAP may incite a dysregulation of inflammation. Additionally, intestinal inflammation may be a secondary or terminal manifestation of systemic MAP infection, and difficulties in discerning a causal role could reflect that fact that most studies of MAP have focused on the intestinal mucosa rather than on distant sites (e.g. circulating macrophages, regional lymph nodes, mesenteric fat) (Behr, 2010). Future studies exploring gene–MAP interactions will be necessary to elucidate the mechanisms by which MAP exposure influences the development of CD.

MAP strains

The broad host range and zoonotic potential of MAP have been suggested numerous times. This is based on the isolation of MAP from many different host species, including humans. The isolates from CD patients have commonly been reported to be of restricted genetic heterogeneity. However, the genotypes of only a limited number of such human isolates have been studied (Francois *et al.*, 1997; Pillai *et al.*, 2001; Bull *et al.*, 2003; Ghadiali *et al.*, 2004; Overduin *et al.*, 2004). Therefore, it is currently impossible to conclude whether the association of MAP with CD is restricted to a limited number of specific MAP genotypes. The main reason for the small numbers of human isolates in these studies and in public collections is that MAP strains are very difficult to isolate from humans, and may require several months to years to produce colonies. Future comprehensive comparative studies will require larger

numbers of human isolates and typing techniques than can be applied to poorly growing isolates. In most comparative studies, the genotypes attributed to the human isolates are the same as for cattle isolates (Chiodini *et al.*, 1990; Pavlik *et al.*, 1995; Whittington *et al.*, 2000). This is in contrast with only one study in which the seven human MAP isolates analysed were unique and did not cluster with either the bovine or ovine strains.

Evidence of strain sharing between cattle and humans is of special interest because it would imply the existence of a potential animal reservoir for CD. Recently, new genotyping techniques have been developed for MAP that have a high enough discriminatory index to further investigate the transmission between animals and humans. Currently, a combination of a fingerprinting and PCR-based techniques is necessary to achieve a high enough discriminatory index (Möbius *et al.*, 2008; Sevilla *et al.*, 2008). Future studies using techniques with high resolving power might uncover interesting associations between animals and human sources. This has already been demonstrated in a recent study, when two MAP strains that shared both a rare combination of short repetitive sequences and fingerprinting patterns were isolated from humans and cattle from the same geographical origin, raising the question of a common source (Thibault *et al.*, 2007). Although this suggests a close association of the human and animal strains, it does not provide direct evidence for zoonotic transmission, nor a causal role of MAP in CD.

In summary, solid evidence of host specificity of MAP isolates is lacking. Thus, the true degree of host adaptation or preference of MAP isolates remains unknown.

Possible Transmission Pathways for Crohn's Disease: Prevalence in Milk, Beef and Water

The incidence of CD has been correlated with increased intake of meat and milk protein (Shoda *et al.*, 1996). Live MAP bacteria have been found in a small proportion of retail milk samples in different countries (Grant *et al.*, 2002; Ayele *et al.*, 2005; Ellingson *et al.*, 2005), while in all studies a significant percentage of those samples contained genetic material (DNA) of MAP (e.g. Gao *et al.*, 2002). Although in some studies a small number of retail milk samples contained live MAP, this does not explain the high number of CD patients in these countries. Furthermore, Sweden does not have JD (Lewerin *et al.*, 2007), but has a fair number of CD patients (Lapidus, 2006). It must be noted, however, that Sweden imports milk from other countries (Wahlström, 2002). As regards beef, slaughterhouse prevalence studies have proven that infected cattle are processed for human consumption. A recent study from Canada suggests that some MAP will survive cooking of meat to a medium-rare condition (63°C), but that their numbers will be greatly reduced, while cooking to a well-done condition (71°C) can be expected to render meat free from viable MAP (Mutharia *et al.*, 2010).

If people are infected with MAP coming from cows, then it is likely that pathways other than the consumption of contaminated milk or meat are also

involved. CD is more often found in people living in cities than in rural populations, and farmers do not have an increased risk of CD (Jones *et al.*, 2006), despite a relatively high proportion of farming families that consume unpasteurized milk (e.g. Hegarty *et al.*, 2002). However, in Europe, robust evidence for transmission of MAP between wildlife and domestic ruminants has been described, which provides support for food-chain contamination (Stevenson *et al.*, 2009). People may become infected while swimming in infected water (children often swallow water when swimming) or drinking infected water (Pierce, 2009). Additionally, MAP is not the only organism that has been mentioned as a possible causal factor of CD – e.g. invasive *E. coli* has also been described (Packey and Sartor, 2009); for these bacteria, other transmission pathways need to be examined.

Conclusions

The suggestion that MAP plays a role in CD is nearly 100 years old. MAP may play a role in the pathogenesis of CD; however, this relationship is not proven. The pathogenesis of CD is likely to be multifactorial in light of the different susceptibility genes and phenotypes of the disease. Consequently, MAP may only influence a subset of CD patients. Transmission of MAP to people through milk and meat is possible, but the impact of water is not well studied and may play a larger role than infection by means of animal products. While a final consensus has not been reached regarding the association of MAP with CD, the evidence to support this association is increasing. Future studies are needed to determine whether MAP is an innocent bystander, an infectious cause in a subset of CD, or an influence on the dysregulation of immune response through gene–MAP interactions. Further combination antibiotic therapy in CD patients shown to have MAP requires well designed and adequately powered trials. Understanding the exact nature of the involvement of MAP in human disease is important because of its potential consequences on public health. Finally, irrespective of the relationship of MAP with CD, it causes JD, which is a serious threat to the cattle industry. Consequently, studying MAP in CD and controlling outbreaks of JD should be a top priority.

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11 Transmissible Spongiform Encephalopathies as a Case Study in Policy Development for Zoonoses

MICHAEL TREVAN

Introduction

When, on 20 March 1996, the UK government announced the discovery of ten recent cases of a variant form of Creutzfeldt–Jakob disease (vCJD) in young people that may have been caused by eating bovine spongiform encephalopathy (BSE)-contaminated beef products, the public felt betrayed (Phillips, 2000) and the media were outraged. Several weeks later, supermarkets in the UK heavily discounted the retail price of beef, and the inexorably growing mountain of unsold beef was cleared from the shelves in less than 48 hours! It would appear that the public's capricious response to risk could be encapsulated by the attitude that we have been eating this risky beef for years anyway, and that piece of prime sirloin is a real bargain.

Transmissible Spongiform Encephalopathies

BSE is one of a group of diseases known as transmissible spongiform encephalopathies (TSEs). These are diseases that, over time, produce debilitating and eventually mortal destruction of normal brain tissue in an individual. The best known TSE is a disease of sheep and goats called scrapie, first identified in the UK in 1732. The symptoms of scrapie include nervousness, itching and motor dysfunction. The disease rarely occurs in sheep of less than 2 years of age, and in its 280-year history has never been shown, or suspected, to be transmissible to any other species through either the food chain or direct contact. Other known TSEs have been discovered more recently: chronic wasting disease (CWD) of deer and elk in the USA in 1967; kuru, a disease prevalent among the ritually cannibalistic Fore people of the mountains of New Guinea; Creutzfeldt–Jakob disease (CJD), a form of dementia seen in humans over the

age of 40; and vCJD, first recorded in young people in the UK in 1995. Several other mammalian species, e.g. cats and mink, also exhibit TSE diseases.

The generally accepted cause of TSE diseases, first proposed by Prusiner in 1982, is that proteins normally present in nervous tissue are stimulated to refold into an abnormal conformation as a result of the introduction of abnormally folded versions of the same protein. This disruption to the molecular architecture of the neural tissue presents a histological pattern of sponge-like holes in the brain, sometimes associated with the appearance of amyloid plaques. Prusiner coined the term 'prion protein' for the proteinaceous infective particle. TSE diseases are different from all other infectious diseases in that the putative infective agent, a small protein, contains no DNA or RNA and, significantly, induces no immune response.

It is well known that any single protein molecule can fold into a number of different 3-D shapes, depending upon the environment in which the molecule is placed (Poltorak *et al.*, 1999a,b). The normal prion protein (PrP^c) is a relatively small, globular, heat-labile protein molecule containing significant amounts of α-helix in its secondary structure, whereas the abnormal prion (PrP^{sc} or PrP^{res}), despite having an identical sequence of amino acids, contains a high proportion of β-sheet secondary structure and, consequently, is extremely heat stable and highly resistant to proteolysis. PrP^{res} is stable for up to 15 min in dry heat at 600°C; this is significant because it means that the protein will not be denatured during normal rendering, cooking or canning processes. Prions are glycoproteins that have bound to them a variable percentage of monosaccharides and disaccharides. This variation in sugar content seems to give rise to distinct 'strains' of prion even within the same animal species; these different strains can be characterized by Western blotting techniques. For example, prions extracted from the brain tissue of classical sporadic CJD, or iatrogenic CJD, can be identified as prion types 1 to 3 by Western blotting, whereas vCJD prions from brain tissue are identified as type 4, and the prions isolated from tonsils of vCJD sufferers (type 4t) show a similar, but not identical, Western blot pattern. The glycosylation pattern of each of these five types is different. Thus, types 1 to 3 have twice the amount of monoglycosylation to diglycosylation, type 4 has slightly more disaccharides than monosaccharides, and type 4t is about 60% diglycosylated and only 27% monoglycosylated (Wadsworth *et al.*, 2003). Within the same species, these different 'strains' of prion create different clinical and histopathological symptoms.

Symptomatic differences occur between the various forms of TSE, even within a species. For example, the three best characterized TSEs of people, sporadic CJD, vCJD and kuru, differ significantly in the outward display of symptoms. Sporadic CJD patients are usually between the ages of 45 and 75 (the clinical definition of CJD was in part that the patient would be at least 40, which made the diagnosis of any form of CJD in those less than 40 years old a significant challenge), and the principal symptom is dementia, which increases rapidly, with death occurring within a few months. In vCJD, however, the first symptoms are usually pain in the limbs, slurred speech, tingling sensations, involuntary movements and memory loss. Death often does not occur until after 12 months following the first onset of clinical signs, and dementia may

occur as a final symptom. The first symptoms of kuru are an unsteady gait, slurred speech and tremor, while dementia is rarely present; mood changes are often seen in the latter stages of disease. These differences in symptoms are probably a result of which area of the brain is most infected. In kuru, it is known that the cerebellum, which controls motor function, is the principal site of infection (NINDS, 2010).

For the most part, TSEs are transmitted within a specific host species either horizontally to other members of the population or vertically from dam to progeny, e.g. scrapie. It is well established that some TSEs are acquired when an individual eats material contaminated with abnormal prions. For example, CWD appears to be spread horizontally, most likely through healthy individuals eating plants contaminated with urine, faeces or saliva from diseased individuals. Kuru is known to have spread in the Fore peoples of New Guinea through the ritual practice of eating tissue, including brain tissue, of deceased relatives. It reached epidemic levels in the 1950s and 1960s, and following government campaigns to discourage the practice, the incidence of kuru (which means 'shiver' in the Fore language) has declined substantially.

BSE – the UK Experience

The advent and course of BSE and its relationship to vCJD in the UK have been extensively described in a report to the UK government that was compiled by Lord Phillips (2000). The full report runs to several thousand pages; the first chapter alone is 308 pages. Much of the detail that follows is taken from that report. In 1985, the first case of BSE in the UK was reported, but was not diagnosed as a TSE. It was not until the end of 1986 that the Pathology Laboratory of the Central Veterinary Laboratory (CVL) in the UK reported that the first two cases of a new TSE in cattle had been diagnosed. However, an embargo on immediate publication led to a 6-month delay in this diagnosis being communicated. By the end of 1987, John Wilesmith, the Head of Epidemiology at CVL, had concluded that this new disease had not been transmitted by contact between individuals. He reached this conclusion because of the enigma that the first 200 index cases of BSE had all occurred in unconnected, and mostly closed, herds across the country (an index case is the first diagnosed case in any one herd). Wilesmith perceptively and rapidly realized that the only possible connection between these index cases, which had occurred almost exclusively in dairy cows, must be their feed. At that time, beef cattle were mostly fed on a regime of grass and grain supplements, whereas dairy cattle required an additional protein supplement, and Wilesmith correctly identified the practice of feeding rendered meat and bone meal (MBM), largely from cattle, as the probable cause. The ban on the feeding of MBM produced from ruminants to ruminants followed quickly (1988), and was the most decisive measure introduced that eventually brought the burgeoning epidemic under control.

With the appearance of BSE, and the knowledge that it had probably spread within the cattle population through the consumption of contaminated

feed, the question that most concerned those responsible for advising government was: how likely was it that this new TSE could cross a species barrier? More explicitly, could eating BSE-contaminated beef cause a new TSE in people? When these first cases of BSE were recognized in 1987 in the UK, the CVL concluded that although there was a possible risk of transmission to people, the risk was extremely low because BSE was thought to have entered the bovine herd through the consumption of scrapie-contaminated feed, and scrapie had never been transmitted to people. John Wilesmith assumed that the first cases of BSE reported were index cases, and he incorrectly hypothesized that the infective agent was probably the scrapie prion from sheep, and that it was changes in the rendering process for animal carcasses introduced into the UK in the early 1980s that had permitted the survival of the scrapie prion in the feed chain. Consequently, this new disease was rightly viewed as a major hazard to bovine health, but it was not seen as a problem for human health, because scrapie had not been shown to be transmitted to humans in its over 200-year history, and if BSE had been caused by scrapie it was unreasonable to suppose that it would be transmitted to people.

It is now clear that the cause of the 1986 cases of BSE was bovine prion-contaminated feed, and that rendering processes had never been capable of disabling prion proteins. It is still unclear where the disease first originated. The exact relationship between the (abnormal) prion protein and the route of infection has been the subject of much controversy. The only certainty is that for clinical disease to develop the prion protein must be expressed. Mice devoid of the *prnp* gene do not develop clinical symptoms even when inoculated with abnormal prions. The key works are summarized in Baron and Biacabe (2007), but see also Bueler *et al.* (1993), Prusiner (1998), Chesebro (1999), Lasmézas *et al.* (1997), Nonno *et al.* (2003) and Lezmi *et al.* (2004).

By the end of 1987, government officials in the UK Ministry of Agriculture, Fisheries and Food (MAFF) had become concerned that BSE might be transmissible to humans through eating contaminated beef. However, they did not report this to the Ministry of Health until March 1988. When they did, the Chief Medical Officer promptly set up a working party, chaired by Sir Richard Southwood, to investigate the matter. By June 1988, Southwood's committee recommended banning BSE-infected cattle from human feed. They did this on the basis of no evidence that there was potential for transmission between species, but on the precautionary principle that if there was a possibility of transmission, the consequences could be catastrophic.

In August 1988, compulsory slaughter for herds where BSE had been diagnosed in an individual was introduced and compensation was paid to farmers. In January 1989, the Southwood Committee reported to government knowing that their report would be made available to the public. It subsequently appeared that the wording of the report was carefully considered so that it would not cause an alarmist response by the public. Thus, they stated that 'it was most unlikely that BSE would have any implications for human health'. They based this statement on the following assumptions: BSE was derived from scrapie and would behave like scrapie; scrapie was known not to affect humans; and any specific medical or occupational risks that might occur

could be dealt with by the relevant authorities. But they did not make clear in their report this basis for their conclusions. When, subsequently, the assumptions they had made turned out to be invalid, their conclusions still held weight because they had not reported their assumptions and therefore there was nothing to challenge. Nevertheless, precautionary measures were put in place and in 1989 a ban on the inclusion of specified bovine offal (SBO) in human foods was introduced.

In May 1990, a cat in the UK was diagnosed with 'scrapie-like symptoms'. The fact that a carnivore could be susceptible to a TSE appeared to cause surprise, despite the fact that a TSE of farmed mink had been recognized in North America since the 1930s. It also concerned the public, whose fears about eating British beef had been growing since the initial announcement of BSE, and because of the suggestion of a possible link to CJD. Twenty Local Education Authorities had already banned beef from school dinners. The government launched a campaign of reassurance that, infamously, showed the then Minister of Agriculture, John Gummer, feeding a beefburger to his 4-year-old daughter at a boat show in his constituency on 16 May 1990 (BBC, 1990), just 6 days after the news of TSE in a cat had broken and 6 months after the government's own ban on bovine offal. John Gummer was later publicly ridiculed for this act (see Fig. 11.1). Over the next 6 years, the principal government policy appears to have been to contain public fears with reassurances that beef was safe to eat. In March 1993, the new Chief Medical Officer, Kenneth Calman, repeated his predecessor's assurances. By July of that year, the 100,000th BSE case was recorded, although the rate of infection of cattle had started to decline. The government campaign culminated in a statement by Prime Minister John Major in December 1995 that 'there is no scientific

Fig. 11.1.   Cartoon of John Gummer, then UK Minister of Agriculture, and the then UK Prime Minister John Major (wearing underpants) (copyright Steve Bell, 2000, originally published in *The Guardian* newspaper).

evidence that BSE can be transmitted to humans or that eating beef causes it in humans'.

In 1990, the UK government established the CJD Surveillance Unit (CJDSU) as a precautionary measure to actively look for any new spongiform encephalopathy disease within the human population that might, epidemiologically, be linked to BSE. The CJDSU identified the first possible cases in farmers in 1992, but these turned out to be cases of classic CJD. The UK government had also established a Spongiform Encephalopathy Advisory Committee (SEAC). In 1994, SEAC reported that, because of the precautionary regulations that had already been taken, the risk to humans from BSE was very low.

Appearance of Variant Creutzfeldt–Jakob Disease in the UK

The political time bomb was primed in 1995 when the CJDSU reported the first two cases of CJD in young people, at a time when only four other cases of CJD in young people had been reported worldwide. The diagnosis of CJD had, hitherto, been almost exclusively confined to individuals over the age of 40. By March 1996, ten cases of this new variant form of CJD (nvCJD or vCJD) had been found in the UK population. BSE was thought to be the cause because exposure through food to the BSE agent would have been greatest in the mid-1980s, and the sudden emergence of cases of CJD in young people was consistent with a 5–10-year incubation period. This, of course, was a somewhat circular argument, because if BSE had appeared only 10 years previously, the incubation period for transmission to people could only be a maximum of 10 years. It did not explain why there had not been a sudden appearance of this vCJD across the whole population, and only in those under 40 years of age. The suggested, and entirely subjective, reason was that it was the consumers under 40 who ate most beefburgers! On 8 March 1996, the CJDSU informed SEAC of these ten cases. SEAC duly notified the Secretary of Health, Stephen Dorrell, that it believed that these ten cases of vCJD had been caused through BSE-infected beef entering the human food chain. Their advice made it clear that there was no scientific evidence for this view, but there was a lack of any other credible explanation for the appearance of this new vCJD. The Royal Society repeated this in its statement made on 23 July 1996:

> Is the new variant form of CJD caused by BSE transmission to humans? No explanation other than the ingestion of 'BSE-prion' contaminated food has come to light, and this explanation must still be considered the most likely cause at the present time. There is insufficient evidence on the use of bovine offal … to know with any precision where it might have entered the human food chain. It is believed however that mechanically recovered meat may have contaminated some of the relevant bovine offal.

The UK government's policy response to the BSE crisis was hampered by miscommunication and limited knowledge. At the time, it was usual for abattoirs to remove the last traces of meat from a carcass mechanically, using high pressure to produce what was known as mechanically recovered meat (MRM),

Table 11.1. Abbreviated chronology of events in the UK related to the occurrence and control of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD) (source: Hueston and Bryant, 2005).

1988	Restricted ruminant protein from ruminant feed
1989	EU banned export of UK cattle born before feed ban (July 1988)
1989	Banned specified bovine offal (SBO) from human food
1990	Ban SBO from animal food and export of SBO to EU
1991	Banned export of SBO to rest of world
1994	Restricted all mammalian protein from ruminant feed
1995	Banned mechanically recovered meat (MRM) from the vertebral column
1996	Banned use of cattle >30 months old (except for leather)
1996	Restricted all MBM from animal feed/fertilizer
1997	Sale of beef on the bone banned in UK, but is lifted later
1999	Destroyed all offspring of BSE cattle born after 1 August 1996
2001	Banned all MRM from sheep, cattle and goats
2001	Brain examination of all slaughtered cattle >30 months old

a mince-like slurry that was used principally in the cheaper brands of processed meat products, e.g. burgers, frozen mince and meat pies. At its peak, the UK produced 5000 t of beef MRM a year. MAFF first questioned the use of MRM in human food in 1989, because some of the MRM would have come from the spinal column of the carcass, and raised the issue with SEAC in 1990. However, MAFF believed, erroneously, that SEAC's view was that a small amount of spinal cord contamination in MRM was of no concern; SEAC believed, incorrectly, that all spinal cord material could be excluded from MRM. This miscommunication was compounded because in 1990 there was no evidence that spinal cord tissue contained infective particles. Consequently, it was not until December 1995 that the use of MRM in human food products was banned. Despite the best efforts of various agencies, even by 2002 it was not clear how much MRM or infective spinal cord had entered the UK human food supply between 1980 and 1995 (UK Food Standards Agency, 2002). The Phillips report also noted that the measures government had introduced were reasonable in guarding cattle from a known hazard, and people from an unknown hazard, but some officials lacked rigour in turning policy into action. This was, in part, because of the belief that persisted that BSE was not a threat to human health, and that bureaucratic processes lead to unacceptable delays. Consequently, these 'sensible measures' were delayed, inadequately implemented and not appropriately enforced.

Table 11.1 summarizes the main measures implemented in the UK concerning the control of BSE and vCJD.

Not Just in Food

Similar confusion to that documented above occurred between government departments in the UK when it came to the issues around non-food products

made from cattle, or the use of bovine parts. At the heart of the matter was the subdivision of responsibility between government departments. For example, MAFF first discussed the practice of dissection of bovine eyeballs in schools in September 1989, shortly after the ban on SBO was enacted. The close relation-ship between eye and brain was thought to pose a possible risk of transmission of the BSE prion to pupils and teachers. In February 1990, the theoretical risk was raised with the Medical Adviser to the Department of Education and Science (DES). In June of the same year, SEAC issued its advice that only eyeballs taken from cattle less than 6 months old should be used in school biology classes, and this advice was relayed to the DES Medical Adviser. A rec-ommendation to the Minister responsible for the DES that dissection of bovine eyeballs should be discontinued in schools was first drafted in August 1990. By February 1992, the submission to the Minister in the DES was still in its third and final draft form, but the DES, which is not usually responsible for pupil/teacher safety in schools (that remit belonged to the independent Health and Safety Executive), apparently felt that it must consult widely across other departments, particularly with the Department of Health (DH) and MAFF. By October 1992, in response to pressure from the DH, the DES finally agreed to make the submission to its Minister recommending the banning of bovine eyeball dissection in schools. By the beginning of 1993, three and a half years after the issue was first raised by MAFF, guidance to schools in England and Wales was finally issued! Scotland, which, through partial devolution of gov-ernment in the UK had independent control of its school system, had issued similar guidance to its schools in February 1990. The cause of this delay appears to have been the result of the interaction of a number of factors. First, the DES was not primarily responsible for pupil/teacher safety and it felt 'distant' from the BSE crisis, but was receptive to the repeated advice from government that beef was 'safe'. Second, other issues at the time seemed more important to the major responsibilities of key civil servants in the DES and the DES Medical Adviser, having agitated for the guidance to be issued, appears to have concluded in May 1992 that such guidance was no longer timely. Third, the longer the delay in issuing guidance, the less attractive the proposition, presumably for fear of attracting adverse comment on the delay itself. In Lord Phillips' words, 'Here as in other areas, excessively reassuring language about the risk from BSE sedated those who needed to act'.

Cosmetic goods were another type of product through which people could be exposed to 'hazardous' bovine material. Cosmetics are usually employed by application to the skin, lips and eyelids, and bovine products occur in many types of cosmetic preparations. The assessment of the potential hazard was informed by the source of bovine material and the extent of processing required to obtain the final preparation. For most regular cosmetics, both the type of bovine material and the heavy processing required suggested that there was minimal risk. However, some premium brands of anti-ageing and anti-wrinkle creams contained only lightly processed brain, placenta, spleen and thymus materials. The safety of cosmetics was governed by the 1987 EU Cosmetics Directive and Regulations (part of the Consumer Protection Act), but the implementation of these regulations was essentially a voluntary

undertaking by industry, as there was no requirement for cosmetics to be licensed. In the UK, the cosmetics industry was the responsibility of the Department of Trade and Industry (DTI). Responsibility for enforcement lay with Trading Standards Officers who worked for local government bodies, who could only take action following a complaint of harm caused by a cosmetic product.

The Tyrrell Committee, chaired by Dr David Tyrrell, Director of the MRC Common Cold Unit, was established in 1988 to advise MAFF and the DH on research priorities related to spongiform encephalopathies. In June 1989 it reported that it had identified a potential hazard of introducing BSE prions to people through the use of cosmetics, but neither the DH nor MAFF proactively informed the DTI of this potential hazard. It was not until January 1990 that the DTI sought advice about the matter from the DH. On receiving advice that there was a potential hazard, the DTI promptly informed the Cosmetic, Toiletry and Perfumery Association (CTPA), which in turn informed its members, advising them either to reformulate the products to avoid bovine material, or else to source that material from outside the UK. The question remains: why did neither the DH nor MAFF think to inform the DTI of the potential hazard? It appears that those responsible in MAFF considered this to be a human health issue and therefore the responsibility of the DH. For their part, the officials in the DH appear to have believed that the risk of transmission through cosmetic products was so small that there was no point in alerting the DTI to the possibility. Later, the DTI recognized a flaw in their system: that despite having been prompt in delivering the warning on bovine products to the CTPA, who were equally prompt in advising their members, not all cosmetic producers were members of the CTPA. In addition, no organization seems to have had the knowledge of what bovine products were incorporated into which cosmetics, and no one was clear as to which body should be taking the lead. CPTA was asked to collect information from its members, but its attempts to do so produced little result. In 1993, the EU Working Party on Cosmetics became involved, and progress towards European guidance became ensnared by bureaucratic procedures, infrequent meetings of the relevant parties and national differences of position. Thus it took until March 1994 for the EU Health Council to proclaim that existing measures in respect of cosmetics were sufficient to protect public health. Subsequent to the emergence of vCJD in 1996, the EU Cosmetics Directive was amended. Meanwhile, the CTPA, in consultation with the French cosmetics industry, issued guidance to its members in March 1994 that was essentially the same as that issued by the World Health Organization (WHO) in 1991.

The EU Perspective

On 25 March 1996, 5 days after the UK government's announcement that BSE might be the cause of a new human vCJD, the EU Commissioner for Agriculture and Rural Development, Franz Fischler (Fischler, 1996), announced a ban on the export of all beef from the UK. By the beginning of April, the UK government had introduced a ban on any cattle over the age of 30 months entering

the food chain. The reasoning and timing of this were mostly to do with the EU ban, and with public reassurance. It was also based on the principle that BSE took 4–5 years to incubate in cattle, therefore there was a high likelihood that cattle under 30 months of age would be disease free.

Over the next few months, the UK worked hard to get the EU ban relaxed: it signalled that it could remove its opposition to ratification of the Europol police cooperation convention if there was movement on the restrictions on British beef; it threatened to disrupt EU business; it refused to give assent to new EU bankruptcy rules; it instituted a policy of non-cooperation with the rest of the EU; and it blocked a trade pact with Mexico. By 5 June 1996, the UK had vetoed 40 measures as part of its non-cooperation protest against the beef ban, and Prime Minister John Major issued a statement that the policy of non-cooperation would continue unless and until the EU produced a timetable to lead to the eventual lifting of the ban on British beef. By July, accusations surfaced that the EU had attempted to cover up the BSE crisis by asking the UK not to publish the results of its research into BSE, and that the European Court of Justice had upheld the EU's worldwide ban on British beef exports at the same time that the EU beef management committee had approved an €850 million package of special aid to beef farmers. The accusations and acrimony rumbled on until December 1996, by which time the Dutch had banned the import of beef from Switzerland, and the French had requested a review of EU aid for veal producers in the wake of an incident in which French cattle breeders hijacked Dutch meat trucks and burned the contents in protest against EU policies and actions. The EU ban on UK beef exports, which had cost the industry over £670 million, was eased in 1999 to allow de-boned beef and beef products to be exported, and was eventually lifted on 8 March 2006, when the number of new cases of BSE had fallen to below 200 per million live cattle. The easing of the ban on de-boned beef and beef products in 1999 was not uniformly or immediately implemented. France continued to ban British beef imports, citing concerns over human health, despite an EU court ruling that their ban was illegal. In October 2002, the French government announced that it was lifting the ban, on advice from its experts that British beef was (now) safe, just a few days before it faced fines of £100,000 per day if it persisted.

The Epidemic Under Control

Although the epidemic of BSE in the UK peaked in around 1992, the number of new cases being reported was still high. This was to be expected because of the long and variable incubation period, which shortens as the age at infection decreases and as the infective dose increases. The number of new cases of BSE did not fall as rapidly as might have been expected after the peak in 1992, suggesting that the MBM ban of 1988 had not been completely effective, given an average incubation period of 4 years. Had the 1988 ban been totally effective, it would be reasonable to expect that the number of new cases of BSE would have fallen to a few hundred by 1996, whereas some 10,000 cases were reported that year (see Fig. 11.2, next section). Leakage of farm feed not intended for

ruminants into cattle feed was suspected, and in 1996 a reinforced feed ban was imposed prohibiting all MBM from being used in any animal feed, or for fertilizer. As Stevenson *et al.* (2005) reported, there was cross-contamination of feed. Before the reinforced feed ban of 1996, the regional density of cases of BSE correlated better with the number of pigs in the region than it did with cattle density. However, it became apparent the cattle born after the 1996 reinforced feed ban were still contracting BSE (called BARB cases – for Born After the Reinforced Ban). The number of such cases increased from 3 in 2000/1 to almost 100 by June 2004 (Table 11.2). A number of cases were probably missed before the introduction of active surveillance in 2001.

In response to these continuing cases, the UK Department for Environment, Food and Rural Affairs (DEFRA) commissioned a report from Professor William Hill (2005) of Edinburgh University, with the remit to investigate the likely causes of these BARB cases of BSE and to identify whether or not this might be a new and different strain of BSE, or whether any significant new risk factors might have arisen. Hill concluded that this was not a new strain of BSE, nor was its occurrence caused by differences in genetic susceptibility of cattle. He found no evidence of vertical or horizontal transmission, nor that any but a few cases could have arisen spontaneously, and was drawn to the inevitable conclusion that the most likely explanation was either deliberate or accidental failure to remove all traces of contamination from the feed bins on farms. Accidental importation of contaminated feed seemed an unlikely cause because of the random distribution of the BARB cases across the country. At the same time, authorities elsewhere in Europe were reporting the appearance of a variant form of BSE, one in which amyloid plaques were found in the brain on autopsy, and where the glycosylation patterns of the PrP^{res} were different. This new bovine TSE disease, termed bovine amyloidotic spongiform encephalopathy (BASE), had a lot of the characteristics of classic CJD in humans, and might well have been the result of sporadic mutations. Hill concluded his report:

> Conclusions: Elimination of feed borne sources is now, as before, the key to elimination of BSE. The incidence of the disease can be greatly reduced but not readily eliminated in any country by adequate imposition of controls, particularly on animal feed. As the level of incidence falls both in the UK and internationally, the risks of contamination through feed, or indeed through any other source, fall whether or not controls in the UK and abroad are further tightened. With the current expertise in DEFRA and the VLA [Veterinary Laboratories Agency], GB is well placed to keep on top of and promote developments.

> Recommendations: It is essential that appropriate, risk based, controls and monitoring should be maintained on animals and feed until no cases of BSE are found, and controls tightened up where feasible, both in the UK and elsewhere that the UK can influence. In view of the very long incubation period of BSE in some animals, long-continued vigilance is necessary. It is not evident, however, that specific new measures are needed. Basically it is necessary to 'keep taking the medicine'. Nevertheless, in view of new discoveries on the nature of the disease and the possibilities of new or changed TSEs arising, relevant research capacity in GB should be maintained.

Table 11.2. Numbers of cattle that went on to develop bovine spongiform encephalopathy (BSE), by year of birth; and cases of BARB (BSE cases in cattle born after the reinforced feed ban of 1996), both by year of birth (as for BSE) and by year of diagnosis (source: Hill, 2005).

Year from July	1992/3	1993/4	1994/5	1995/6	1996/7	1997/8	1998/9	1999/2000	2000/1	2001/2	2002/3	2003/4	2004/5	Total
BSE by year of birth	3493	2960	2128	1059	62									9702
BARB by year of birth					42	30	15	5	0	1				93
BARB by year of diagnosis									3	14	35	28	13	93

Did BSE Cause vCJD?

Did BSE cause this new variant of CJD? Until at least 1997, the evidence was at best highly circumstantial. Here was a potentially new hazard to human health with a risk of unknown proportion. Epidemiologists were soon to predict a devastating epidemic of vCJD on the basis of almost no numerical information. They estimated that the number of new cases would rise to hundreds of thousands a year in the UK alone – a disease with a mortality rate of 100% and no known cure. This was not the universal opinion, but it was the mood that prevailed and influenced policy. More than 6 years of reassurance by government ministers and senior scientists that British beef was safe to eat now appeared to be, at best, plain wrong, and, at worst, a conspiracy to hide the truth. Witness the headline of the period in the national *Daily Mirror* newspaper, 'We've already eaten 100,000 mad cows'. The consequences of this loss of confidence in the government's mechanisms for securing food safety were severe. It was perhaps the major cause of public scepticism over the introduction of genetically modified (GM) crops into the UK. The continual and repeated support of the new Labour government of Prime Minister Tony Blair (elected in 1997) for GM crops in all probability hardened the negative attitude of the British public towards these crops. The public had lost all confidence in MAFF and in agricultural practices, and shortly after the general election in 2001, the re-elected Labour Government disbanded MAFF and vested most of its responsibilities in the new DEFRA. Responsibility for food safety was vested in a new arms-length non-governmental organization, the Food Standards Agency (FSA), under the chairmanship of Sir John Krebs. This agency, through an agenda of promiscuous transparency, did much to restore the public's faith in its food supply.

In 1997, the first laboratory reports of BSE transmission to mice were presented as evidence that BSE had indeed caused vCJD. The conclusion of the authors, Bruce *et al.* (1997), was that the RIII strain of experimental mice, when inoculated with brain tissue from humans who had died of vCJD, showed a histopathology similar to that when the mice were inoculated with brain tissue either from BSE-infected cattle or cats with feline spongiform encephalopathy. The same mice, when inoculated with brain tissue from humans who had died of classic CJD, or with brain tissue from sheep with scrapie, showed a different pathology. Bruce *et al.* (1997) concluded that their observations 'provide compelling evidence of a link between BSE and vCJD'. The other conclusion to be drawn, however, is that, if BSE and vCJD are linked through the pattern of disease that they cause in this strain of mice but scrapie causes a different pattern, then scrapie is unlikely to have been the cause of BSE. Wadsworth *et al.* (2003) demonstrated that prion proteins from patients who had died of classic CJD and vCJD appeared to exhibit different strains of prion protein. Prions from cases of classic CJD were classified as types 1–3, and characteristically had a ratio of monoglycosylation to diglycosylation > 1, while prions from vCJD patients of types 4 and 4t (from tonsils) had a ratio of < 1. Assante *et al.* (2003) reported the effect of inoculation of transgenic mice with prions from BSE or vCJD. The transgenic mice had their own PrP^c gene

removed and replaced with a human PrP^c gene, homozygous for either methionine or valine at amino acid residue 129. Three strains of mice were studied: Tg35, Tg45 and Tg152. Tg35 mice expressed human PrP^c homozygous for methionine at twice the level found in human brain; Tg45 mice expressed the same prion at four times the level found in human brain; Tg 152 mice expressed human PrP^c that was homozygous for valine at residue 129. Both vCJD and BSE prions caused symptoms similar to vCJD in a small minority of the Tg 35 and 45 mice, but some mice that at death had the PrP^{res} prions in their brain tissue had shown no clinical symptoms of disease. Tg152 mice that died of old age had no PrP^{res} in their brain tissue, suggesting that the homozygous 129 valine prion may be protective against BSE prions. Both the BSE and vCJD inocula produced 'florid' plaques in mouse Tg35 brains, but inoculation with classic CJD did not produce florid plaques. This was cited as additional evidence that BSE and vCJD were related, and different from classic CJD, and therefore that BSE could be the cause of vCJD. Inconveniently, it was also observed that the same mice inoculated with scrapie prions exhibited the same form of florid plaques. This might have suggested that scrapie had been the cause of BSE, had not the earlier work of Bruce *et al.* (1997) suggested the exact opposite. It was these key scientific papers that convinced governments that BSE had indeed caused vCJD. Yet still the questions remained: why only in young people? And did the fact that prions from BSE-infected cattle and vCJD-infected humans cause similar symptoms when inoculated into mice actually prove that BSE had been transmitted to humans through the food chain to cause vCJD? It is, of course, possible that any patient presenting with CJD-like symptoms, but who was over the age of 40, could be diagnosed with classic CJD even if suffering from vCJD. The evidence was at best still circumstantial – correlation does not prove cause and effect; but that did not prevent authority from proclaiming it as a fact – and this inconvenient truth was all that various jurisdictions needed to justify trade barriers to British beef.

By 2000, when the Phillips report had declared that 'BSE has caused a harrowing fatal disease for humans', this universal truth was embedded in the belief structure of a nation. But from 2001, the number of annual cases of vCJD started to decline, leading the epidemiologists to revise their doom-laden predictions. For the first 2 or 3 years of the new millennium, it appeared that reports of new cases of vCJD had plateaued. This led many commentators to speculate that vCJD had simply been a disease that had always existed, but had not been diagnosed until the scientific community set out to search for it. An existing rare, but previously unrecognized, disease would show an appearance of cases very similar to that seen for vCJD between 1995 and 2000 – an initial rapid rise followed by a steady state. Latterly, though, the statistics have shown something different. The first question must be: why had this 'new' disease only appeared in significant numbers in the UK? Despite active surveillance, vCJD has not appeared in substantial numbers in any other country, in particular in those countries where BSE had become established, which does not fit well with the idea that vCJD was an existing, but hitherto undiscovered, disease. Equally, if BSE had become established in a country, the lack of vCJD cases could indicate that BSE did not cause vCJD. However, where BSE
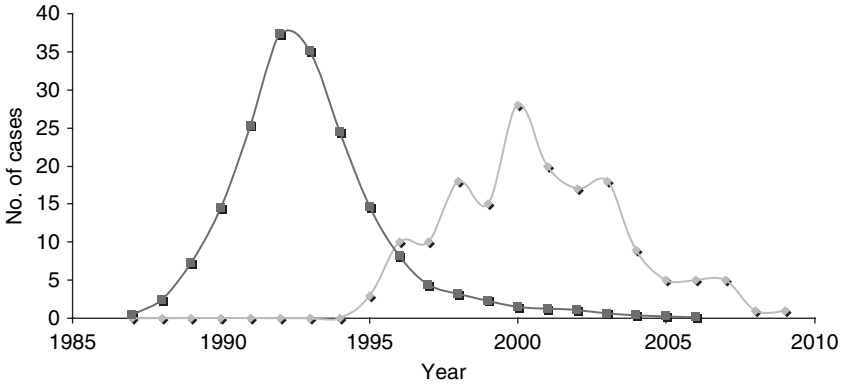
Fig. 11.2.   UK cases of bovine spongiform encephalopathy (BSE) (1000s) (■) and deaths from variant Creutzfeldt–Jakob disease (vCJD) (♦) from the late 1980s to 2009 (sources: The National Creutzfeldt–Jakob Disease Surveillance Unit (NCJDSU), 2010; World Organisation for Animal Health (OIE), 2010).

subsequently appeared in other countries, particularly in the EU, all of these countries had adopted the UK's precautionary measures that were predicated to prevent the transmission of BSE across the species barrier to humans.

Figure 11.2 shows the UK figures for the annual cases of both BSE and vCJD. If one assumes an incubation period for vCJD of 8 years (not that different from the official figure of 10–12 years assumed by SEAC), then the curves for the appearance of BSE and vCJD are almost identical and suggest a low infectivity rate of one human case of vCJD per 1000 cases of BSE in cattle. But the simple correlation between these figures for BSE and vCJD takes no account of the UK government's precautionary measures that were instigated to prevent the possibility that BSE might cause a similar disease in humans. In other words, the similarity of shape of the BSE and vCJD curves is misleading because, assuming that vCJD is caused by the consumption of BSE-infected beef products, cases of vCJD should not mirror cases of BSE, but instead represent the presence of BSE infectious material in the human food chain. In addition, two potentially confounding events occurred: the banning of SBO from human food in 1989, and the ban on the use of MRM in food products in late 1995. The effect of these events on the apparently simple relationship between BSE in UK cattle and vCJD in UK people should depend upon which of these two measures (if either) was the sole, or major, action that kept BSE-infected material out of human food. It is difficult to say with any certainty which, if either, of these two measures had the greatest effect, although it is likely that the SBO ban was the most significant because it removed from human food items known to contain prion-infected materials. MRM was a product that could, by chance, pick up prions from associated spinal cord material. One would expect that all the time the number of cattle infected with BSE was rising exponentially and infected material was entering the human food chain (assuming that the virulence of the infective agent, or its

concentration in those foods, did not change over the period) that the number of subsequently detected cases of vCJD would rise exponentially, which is what we observe. Then again, the sudden removal of the major carrier of infection, SBO, from human food would be expected to result in a precipitous decline in observed new cases of vCJD, which did not happen.

Experimental models have shown that the length of the incubation period for an acquired TSE is inversely proportional to the infective dose acquired. Thus, the declining tail of new cases of vCJD shown in Fig. 11.2 could be due to variation in incubation period among the individual cases. All this is, of course, highly speculative and, given the small total numbers of vCJD cases, impossible to verify. Despite that, it remains very important, because the peak of 28 vCJD cases in 2000 (see Fig. 11.2) ought to relate to 1989 when SBO was removed from human food. This would put the incubation time for vCJD to an average of 10 years, and the ratio of cases of vCJD to BSE at about 28:7228 or approximately 1:260, rather than the 1:1200 suggested by a simple analysis of Fig. 11.2, implying a 4.5× higher rate of transmission. Is this difference significant? Probably not, because we are considering a population of 60 million, of whom at least 40 million could have been regular consumers of beef products. But what if those responsible for advising government on policy options had taken the view that BSE could not be transmitted to humans, and, as a result, SBO and MRM had remained part of the food supply? It is certain that the actions taken to eradicate BSE from the cattle herd would have been implemented irrespective of the possible risk to people, because BSE was causing dairy and cattle producers significant economic losses. If nothing else had changed, then we might have expected to see a peak of 135 cases of vCJD occurring in about the year 2000, and a total number of UK cases of vCJD close to 1000. However, the optics that figures such as these place upon the influencers of government policy are out of all proportion to real risk: 135 deaths represents less than 0.02 deaths per 10,000 of the UK population. It is common, though, for the perception of a hazard to be significantly heightened by its novelty. It should be noted that the only other country in which there have been significant numbers of cases of vCJD identified is France, where 25 cases have been reported. Finally, we must consider the possibility that the decline in reported vCJD cases in the UK from the year 2000 might be the result of decreased diagnosis because it was assumed that the BSE epidemic was well under control and, therefore, no longer a risk to human health. To quote a more ancient authority, it could be a case of you find what you are looking for: 'Ask, and it shall be given you; seek, and ye shall find; knock, and it shall be opened unto you' (King James Bible (1611), Matthew 7:7).

North America

The first case of BSE in North America occurred in 1993 in Canada in a cow that had been imported from the UK. This isolated case was dealt with swiftly: the entire herd and any traced outsourced animals were eradicated (Kellar and Lees, 2003). Over the next decade, both Canada and the USA enacted

measures modelled on the UK's experience and designed to prevent the ingress and spread of BSE in North America. Table 11.3 shows some of the comparable actions taken in the EU, Canada and the USA.

Up until 2003, both Canada and the USA had regarded the risk of BSE becoming an issue in their cattle herds as being practically zero, and not without cause. Many of the measures introduced to prevent the importation of animal diseases, e.g. foot-and-mouth disease, would also have been effective against the importation of BSE. A ban on beef products from other EU countries not considered free of BSE was implemented in 1991. Canada banned the importation of UK cattle in 1990 in response to the growing BSE epidemic in the UK, and officially monitored all 182 animals imported from the UK since 1982. Of these, 14 were still in quarantine at the time and were not released, 68 had died or been slaughtered, and, of the remaining 100, one developed BSE in 1993. Ten of the 68 animals no longer alive in 1990 were later traced back to farms in the UK where BSE had subsequently occurred, and it is likely that it was through (some of) these cattle being incorporated into the feed chain that BSE entered Canada (Health Canada, 2005). Similar circumstances pertained in the USA.

It must be assumed that BSE-infected feed entered the complex animal feed chain in North America. However, it is now known that the susceptibility of cattle to infection with BSE increases with decreasing age – younger cattle

Table 11.3. Actions taken to prevent spread and possible effects of bovine spongiform encephalopathy (BSE) (source: Hueston and Bryant, 2005).

Action taken	EU	Canada	USA
Banned import of live ruminants (and products) from UK	1989	1990	1989
Started active surveillance for BSE	–	–	1989
Banned mammalian rendered meat and bone meal (MBM) from ruminant feed	1994	1997	1997
Banned export of UK cattle and milk	1996	n/a	n/a
Banned import of live ruminants and ruminant products from EU	n/a	–	1997
Banned use of specified risk material (SRM) from sheep and cattle	2000	–	–
Banned import of all rendered animal proteins	2000	2000	–
Banned all use of mammalian protein in feed for livestock	2001	–	–
Introduced routine testing of advanced meat recovery (AMR) products for spinal cord	–	–	2002
Banned 'downer' cattle SRMs from over 30-month-old animals from human food	–	2004	2004
Banned MSM (mechanically separated meat) from human food	–	–	2004
Introduce enhanced surveillance programme	–	2004	2004
Banned SRM/MSM products from cosmetics and diet supplements	–	–	2004

are more susceptible. The North American practice of slaughtering cattle at a younger age, when, even if infected with BSE, they would not have had time to develop high levels of infectivity, would also have prevented BSE from becoming an established endemic disease. However, from 2004, 17 cases of BSE have been recorded in Canadian cattle (the latest in March 2010). While hardly an epidemic, this does raise the question of where the latter of these cases have come from, given that most were born well after the 1997 feed ban was imposed. Given that it is still uncertain how BSE arose in UK cattle in the first place, and that the original hypothesis that it came from scrapie-infected meal has been discredited by the science that shows distinct differences in patterns of infectivity in 'humanized' mice when inoculated with scrapie or BSE prions, then the hypothesis that BSE is a disease akin to classic CJD in humans needs to be re-examined. Could BSE have been a sporadic TSE of elderly cattle that was amplified through the rendering of cattle remains to cattle feed? If so, then given current surveillance levels for BSE, it would be surprising if, in a national herd of 10–20 million cattle, the very occasional case of BSE was not detected.

The first US case of BSE was announced on 23 December 2003 in a dairy cow in Washington state. Almost immediately, 53 countries, including the USA's major markets of Canada, Mexico, Japan and South Korea, banned the import of US beef products and cattle. This reaction was not surprising, given that the beef economies of the countries involved suffered from US competition, but it was against the recommendations of the World Organisation for Animal Health (OIE). The USA had already banned the import of cattle from Canada following the first recognized case of endogenous BSE in Canada in May 2003, and had not itself endorsed or operated the OIE recommendations. However, after significant political pressure, the US Department of Agriculture (USDA) put in place regulations (effective March 2005), to allow importation of cattle under 30 months of age from 'minimal risk' countries such as Canada. That, however, did not stop the US industry group Ranchers-Cattlemen Action Legal Fund (R-CALF) from successfully gaining an injunction in 2004 from a court in Montana (presided over by the aptly named Judge Cebull) blocking the federal measure (R-CALF, 2004). R-CALF's argument was that it was concerned with the implications of BSE for human health, despite the fact that most authorities had by then recognized that this was at best highly tenuous, if not illusory. The economic issue was the potential losses that might be sustained through competition from Canadian cattle producers. Marsh *et al.* (2005) had estimated that Canadian imports would reduce the price of US feeder cattle by US$4.75 per cwt. The costs of subsequent export restrictions on US beef, following their own first case of BSE, probably lie in the range of US$3–5 billion. This, of course, does not include the negative effect on processors. US beef processors relied on both US and Canadian beef supply to make their activities economically sustainable. The sudden reduction in supply of cattle because of the import ban on Canadian animals had a significant negative effect on their businesses.

Testing for BSE has been another source of controversy. Before the first case of BSE in the USA, testing for BSE had been below international

standards. For example, in 2003 the USDA tested 20,000 cattle compared with the EU's 8 million (Fox and Peterson, 2004). As a result of the discovery of the Washington case of BSE, the USDA announced an enhanced surveillance programme, targeted at high-risk cattle populations. The USDA's calculation was that by testing 268,000 cattle on a selective basis, they could predict with 99% confidence the occurrence rate of a 1:1,000,000 incidence of BSE. Obviously, the lower the detection rate, the less the negative effect on US beef prices. Mitchell (2004) criticized the choice of test used by the USDA as being one that reported a high level of inconclusive results, thus minimizing positive results and insulating the US market from the over-reporting of BSE cases. Some producers argued for more extensive voluntary testing. Coffey *et al.* (2005) suggested a net premium between US$27.50 and US$48.50 per head in sales of beef to the Japanese and South Korean markets if voluntary testing were instituted. However, in 2004 the USDA rejected a request from a Kansas-based company, Creekstone Farms, to test all slaughter cattle for BSE in an attempt to regain the Japanese export market. R-CALF continued to lobby the USDA to restrict imports of Canadian-bred beef, latterly on the basis of inadequacies in the Canadian BSE testing regime compared with that described in the EU Report on the monitoring and testing of ruminants for the presence of transmissible spongiform encephalopathies (TSEs) in the EU in 2007 (EU, 2009). In their letter of July 2009 to the USDA, R-CALF states:

> The 2007 EU Report provides valuable statistical data concerning the status of the EU's monitoring and testing program for bovine spongiform encephalopathy ("BSE") and other transmissible spongiform encephalopathies ("TSEs"). When these EU data and their results are compared to and contrasted with the monitoring and testing program (hereafter "testing program") that Canada practices for BSE, it is abundantly clear that Canada's BSE testing program is woefully inadequate to: 1) reliably determine the prevalence and evolution of BSE in Canada; and, 2) protect the food supply, both in Canada and the U.S., from contamination by beef from BSE-infected animals.

Concluding Remarks

What these preceding accounts show is how much local and national food safety issues are as much governed by politics and economics as they are informed by science, and that the outcome is as much determined by a government's coordinated response to risk management as it is by the quality of scientific advice.

As a final comment, it would be well to remember that the formulation of policy is the remit and responsibility of politicians. Science can provide information to guide the derivation of policy, it can never supplant the process. The corollary is that it ill-behoves politicians (and scientists or media reporters) to pretend that their pronouncements are supported by scientific evidence when they are not. To do so inevitably renders science an impotent force in society, which in the end benefits nobody.

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