Ecosystem Health and Sustainable Agriculture

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Food Safety and Public Health

Introduction

Balanced nutrition, housing, clothing, health, a safe environment and personal integrity are some of fundamental needs of people. Public health is a concept of preventive measures provided by society to ensure and support these needs. Each society, following its international commitments, is obliged to focus on the relevant public health problems for its own population. Veterinary medicine is a discipline responsible for the health and welfare of domesticated animals, especially food production animals, in order to provide healthy and wholesome food for consumers. Veterinary surgeons are also important in the control and prevention of zoonotic diseases because their expertise covers the whole food animal production chain starting from primary production, continuing through slaughter and meat processing to retail, food catering and the consumer’s plate. The management of food safety requires a multidisciplinary approach that employs the expertise of researchers at universities and trained experts in the field monitoring food safety. The ‘farm to fork’ approach includes all stages in food production and includes monitoring, surveillance and all aspects of risk identification, analysis, management and communication at each stage of the production chain. A simplified diagram of the food production chain is shown in Figure 34.1.

For many years, the community of food safety professionals has been trying to draw the attention of consumers and society to the importance of food safety for human health and the economics of food production. The importance of public health and maintaining high standards are fundamental objectives of European Union (EU) food laws as laid down in European Commission (EC) regulation No 178/2002. Throughout the EU, the aware-
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ness of consumers has been increasing and consumers require the food industry to provide them with safe, nutritious and healthy food which also has high-grade sensory quality and prolonged shelf-life. To meet the demand for healthier food with high sensory quality, the use of food additives and preservatives has been reduced or eliminated and minimal processing techniques have been introduced. To increase food safety and quality, considerable amounts of time, effort and money have been spent on food safety control and management systems (ISO 22000:2005), including better packaging methods and improved and novel methods for the detection of pathogenic microbes. Nevertheless, there are still few signs in the official statistics of a significant reduction in the incidence of food-borne illnesses in the EU countries.

Todd (1997) reported that in the beginning of 1990s, 73-100% of all European outbreaks with known aetiology were caused by bacteria. Particular concerns were species such as Listeria monocytogenes, Campylobacter jejuni, Salmonella and Escherichia coli O157:H7. Biofilm formation and other problems in the production environment have been in focus lately. Wirtanen et al. (2003) reported that pathogens such as L. monocytogenes, Salmonella Typhimurium and Yersinia enterocolitica can readily produce biofilms, causing severe disinfection and cleaning problems on surfaces in the food industry. There is also a growing problem of human infections that are difficult to treat because of antimicrobial resistance, which is emerging partly because of the increased use of antimicrobials in human medicine and in animal production. Diseases caused by resistant strains can be significantly more severe than diseases caused by susceptible strains (INFOSAN, 2005). Problems related to the occurrence of simultaneous resistance to several antimicrobials within one strain (multiresistance) seem to be increasing and appear to be linked to the use of these antimicrobials in animal production (WHO, 2004).

The objective of this subchapter is to provide a summary of the most important bacterial food-borne pathogens, their contamination sources and routes in the food production. In addition, emerging antimicrobial resistance and current food legislation matters are discussed.

Emerging Zoonotic Food-borne Pathogens

Food-borne disease is any illness that results from ingestion of food. Food can contain microbiological pathogens that cause infections or intoxications, or chemical agents that cause acute or chronic intoxications. Biological and chemical contamination of the environment carries an important risk of food-borne disease, especially where it interfaces with food producing, processing or preparation and consumption. Drinking water supplies and waste disposal systems may be inadequate, particularly in developing countries, thus markedly increasing the risk of spreading of food-borne pathogens. Among the emerging and re-emerging pathogens, food-borne pathogens are prominently represented. They include Campylobacter jejuni and C. coli., enterohaemorrhagic Escherichia coli, Listeria monocytogenes, Yersinia enterocolitica, Cryptosporidium, multidrug resistant Salmonella Typhimurium DT 104, etc. As the etiological agent remains unknown in a considerable proportion of cases of food-borne diseases, many more pathogens need still to be recognised (Käferstein, 2004). Obtaining information on the epidemiology, costs and risks of food-borne pathogens is the key to controlling food-borne diseases (Snowdon et al., 2002). Examples of food-borne disease outbreaks, associated foods and etiological agents are shown in Table 34.1.

Campylobacter spp.
The genus Campylobacter spp. consists of 17 species and 6 subspecies (Euzeby, 2006). These bacteria are microaerophilic (85% N₂, 10% CO₂ and 5% O₂), but some can also grow aerobically or anaerobically. The most important species of Campylobacter are the thermophilic species: C. jejuni subsp. jejuni, C. coli and C. lari. Other species which are known gastrointestinal pathogens include C. sputorum, C. upsaliensis, C. hyointestinalis, C. mucosalis, C. fetus subsp. fetus and C. curvus (EFSA, 2004; Abbott et al., 2005; Euzeby, 2006). Campylobacter jejuni and C. coli are Gram-negative, spirally shaped microaerophilic bacteria. Campylobacter cells are mostly slender, spirally curved rods, 0.2-0.5 μm wide and 0.5-5 μm long. The rods may have one or more helical turns and can be as long as 8 μm. They also appear S-shaped and gull-wing-
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shaped when two cells form short chains (Holt et al., 1994). Cells of some species are predominantly curved or straight rods. Cells in old cultures may form coccoid bodies which are considered degenerative forms rather than a dormant stage of the organism (Hazeleger et al., 1994). The cells of most species are motile, with a characteristic corkscrew-like motion by means of a single, unsheathed, polar flagellum at one or both ends of the cells. Cells of some species such as *C. hominis* and *C. gracilis* are non motile, while other species such as *C. showae* have multiple flagella. *Campylobacter* spp. are relatively inactive biochemically, obtaining their energy from amino acids or tricarboxylic acid cycle intermediates rather than carbohydrates. Carbohydrates are neither fermented nor oxidised. This makes them difficult to speciate by use of classical biochemical tests (On, 1996), so they are often identified to species level by use of DNA-based methods (Bolton et al., 2002; On and Jordan 2003).

Some species are pathogenic for humans and animals. *Campylobacter jejuni* and *C. coli* are the major pathogenic species of interest. They are found in the intestinal tract and oral cavity of man and animals. The predominant species in human infection can be readily grown under a microaerobic atmosphere on selective media without the necessity to use hydrogen. Campylobacters grow optimally in an atmosphere containing 5% oxygen and have an optimum growth temperature between approximately 30 and 45°C. They survive during storage at refrigerated temperatures better than at room temperature. The cells are sensitive to freezing, drying and salt concentrations above 1% sodium chloride. Campylobacters are also sensitive to standard concentrations of common disinfectants (Anonymous, 1993). *Campylobacter* spp. are relatively sensitive to heat and irradiation, and they can readily be inactivated during cooking (ICMSF, 1996). *C. jejuni* and *C. coli* are the main cause of *Campylobacter* enteritis in humans (Skirrow and Blaser, 2000; Hänninen et al., 2003). *C. jejuni* is responsible for 80-90% of campylobacteriosis. It causes more common enteric diseases than *Shigella* spp. and *Salmonella* spp. combined. In the industrialised countries, including Western Europe, USA, Canada, Australia and New Zealand, the rate of human *Campylobacter* infections has been increasing steadily since the mid-1990s. On 12 April 2000, the Scientific Committee on Veterinary Measures relating to Public Health (SCVMPH) issued an opinion on foodborne zoonoses (SCVMPH, 2000). In this opinion the Committee identified *Campylobacter* spp. as one of the

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Food item</th>
<th>Pathogen</th>
<th>No. of illnesses (deaths)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>Chocolate</td>
<td>S. enterica serotype Eastbourne</td>
<td>119</td>
<td>CDC, 2004</td>
</tr>
<tr>
<td>Italy</td>
<td>Salami</td>
<td>S. enterica serotype Typhimurium</td>
<td>23</td>
<td>Connor et al., 2001</td>
</tr>
<tr>
<td>Brazil</td>
<td>Mango</td>
<td>S. enterica serotype Newport</td>
<td>78 (2)</td>
<td>Dubos, 1998</td>
</tr>
<tr>
<td>Israel</td>
<td>Drinking water</td>
<td>E. coli (ETEC)</td>
<td>229</td>
<td>Fratamico, 2002</td>
</tr>
<tr>
<td>France</td>
<td>Brie cheese</td>
<td>E. coli O27:H20 (ETEC)</td>
<td>45</td>
<td>Buzby et al., 1997</td>
</tr>
<tr>
<td>USA</td>
<td>Minced beef</td>
<td>E. coli O157:H7</td>
<td>3</td>
<td>De Jong et al., 2006</td>
</tr>
<tr>
<td>USA</td>
<td>Turkey meat</td>
<td>Listeria monocytenes</td>
<td>29 (7)</td>
<td>Harris, 2002</td>
</tr>
<tr>
<td>USA</td>
<td>Frankfurters</td>
<td>Listeria monocytenes</td>
<td>101 (21)</td>
<td>Harris, 2002</td>
</tr>
<tr>
<td>Sweden</td>
<td>Cold-smoked and gravad rainbow trout</td>
<td>Listeria monocytenes</td>
<td>9 (2)</td>
<td>Colomba et al., 2006</td>
</tr>
<tr>
<td>Hungary</td>
<td>Raw milk</td>
<td>Campylobacter spp.</td>
<td>52</td>
<td>Erkmen, 1996</td>
</tr>
<tr>
<td>USA</td>
<td>Undercooked, barbecued chicken meat</td>
<td>Campylobacter jejuni</td>
<td>11</td>
<td>Eggertson, 2005</td>
</tr>
<tr>
<td>Germany</td>
<td>Chocolate drink made from raw milk</td>
<td>Campylobacter jejuni</td>
<td>24</td>
<td>Farmer et al., 1980</td>
</tr>
</tbody>
</table>

Table 34.1. Food-borne disease outbreaks and associated foods.
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public health priorities among the food-borne zoonotic pathogens. Campylobacteriosis represents an important public health problem with considerable socio-economic impact in the EU. In 2004, a total of 183,961 human cases of campylobacteriosis were reported from the 25 member states of the European Union. The EU incidence was 47.6 cases per 100,000 population. In 2005, 2631 confirmed campylobacteriosis cases were reported in Norway, 4002 cases in Finland, 3677 cases in Denmark, 5969 cases in Sweden and 124 cases in Estonia. The overall incidence of campylobacteriosis in the EU in that year was 51.6 per 100,000 population, ranging from <0.1 to 302.7 cases per 100,000 population (EFSA, 2006). In 2007, a total of 200,507 human cases of campylobacteriosis were reported in EU member states, an increase of almost 25,000 cases compared with 2006, and the EU incidence was 45.2 cases per 100,000 population (EFSA, 2009a). In 2000, 78 campylobacteriosis cases were recorded in Denmark but the estimated incidence of Campylobacter infections may have been 600-8300 cases per 100,000 population (Rosenquist et al., 2003). An estimated 2.5 million cases of Campylobacter infection occur each year in the United States, and 80% of these cases have been found to be the consequence of a food-borne transmission (Bhaduri and Cottrell, 2004). The high Campylobacter contamination of raw poultry products observed by Praakle-Amin et al. (2007) in retail outlets in Estonia may indicate that the prevalence of human campylobacteriosis in Estonia is greater than the 154 cases (11.5 cases per 100,000 inhabitants) reported by the Estonian Health Protection Inspectorate in 2009. Other Baltic countries report low numbers as well. In Lithuania, a total of 564 cases (notification rate 16.4 per 100,000 inhabitants) of human campylobacteriosis were reported in 2007, while no cases were reported in Latvia (EFSA, 2009a).

Associated Foods and Environment

Campylobacter spp. are widespread in nature, not only in wildlife but also among food animals, such as cattle, sheep, swine and avian species, as commensal organisms (Friedman et al., 2000). The avian species are the most common hosts for Campylobacter, probably because of their higher body temperature (Skirrow, 1977), as well their breeding in large dense animal populations which promote transmission of campylobacteria within a flock. Figure 34.2 illustrates possible routes of Campylobacter jejuni transmission. Monitoring studies indicate that chicken flocks are commonly colonised with C. jejuni. There are large differences in the annual prevalence of positive flocks between different countries in the EU (EFSA 2009a). Studies in Europe indicate flock prevalence ranging from 18 to over 90%, with northern countries showing a lower proportion of positive flocks (Barrios et al., 2006). Intestinal colonisation usually leads to contamination of the final product, which cannot be prevented in the processing plant (Hänninen, 2009). Studies carried out in slaughterhouses have shown that the main source of the spread of C. jejuni on poultry carcasses is their intestinal contents (Stern et al., 2003). Campylobacter have also been isolated from food items such as raw milk, pork, beef, lamb and seafood (Duffy et al., 2001).

The presence of Campylobacter spp. in raw food materials and products of animal origin may represent a source of infection, but a real health hazard exists only when meat consumed is raw or undercooked (Domingues et al., 2002). The other major hazard may be a result of improper hygiene habits and disregard of Good Manufacturing Practice (GMP) principles. This is related to the transfer of bacteria from raw meat to other foodstuffs (cross-contamination). An effective quality control programme in a large-scale poultry processing plant in Estonia accounted for the lower contamination levels of fresh chicken meat compared with the contamination level for the same type.
of products in a small-scale plant (Roasto et al., 2005). Altogether, 279 samples of Estonian raw chicken meat (breasts, carcasses, legs, minced meat, thighs and wings) were analysed during 2000 and 2002 (Roasto et al., 2005). Of these, 90 were collected directly from the end of the slaughter line of a small-scale poultry meat plant and 189 from traditional market halls of Tartu town, Estonia. All chicken meat samples from market halls were sold fresh and unpacked. Of the raw chicken products of Estonian origin, 15.8% tested positive for *Campylobacter*. The prevalence of *Campylobacter* in the products (breasts, carcasses, thighs and wings) of the small-scale poultry meat plant (35.6%) was significantly higher than in those originating from the large-scale company (6.3%) (P<0.001). The occurrence of *Campylobacter* spp. in broiler chicken production in Estonia in the period 2002-2007 was analysed by Meremäe et al. (2010). *Campylobacter* spp. were isolated in 163 (12.3%) of 1320 chicken meat samples tested in 2002-2007 and in 115 (6.3%) of 1819 caecal samples tested in 2005-2007. All 1254 faecal samples collected in 2005 and 2006 from two farms with a total of 60 flocks, each containing 20,000 birds, were negative. *C. jejuni* was the most commonly isolated species in Estonian analyses (98.2%), followed by *C. coli* (1.4%) and *C. lari* (0.4%). The seasonal peak of *Campylobacter* contamination was from July to September.

More data about the prevalence of *Campylobacter* spp. on fresh poultry meat world-wide are presented in Table 34.2.

**Listeria Monocytogenes**

*Listeria monocytogenes* is transmitted via three main routes: direct contact with animals, cross-infection of new-born babies in hospital and food-borne infection. The latter two sources result in the majority of listeriosis cases in humans. Listeriosis is an uncommon but a serious food-borne disease that can be life-threatening to the elderly, people with a weakened immune system and pregnant women (Frye et al., 2002). In EU member states, 1558 human cases of listeriosis were reported in 2007 (EFSA, 2009b). *Listeria monocytogenes* accounts for about 2500 cases, 2289 hospitalisations and 449 deaths each year in the United States. The mortality rate of *L. monocytogenes* (~28%) remains the highest of all food-borne pathogens (Wesley, 2009). In humans, severe illness mainly occurs in unborn children, infants, the elderly and those with a compromised immune system. Symptoms vary, ranging from mild flu-like symptoms and diarrhoea to life-threatening infections characterised by septicaemia and meningoencephalitis. In pregnant women the infection can spread to the foetus, which may either be born severely ill or die in the uterus and result in abortion.

*L. monocytogenes* is a Gram-positive and motile bacterium that is commonly present in the environment and occurs in almost all raw food materials sporadically. According to current knowledge the genus *Listeria* contains six clearly distinguishable species. The most commonly occurring species in food are *L. monocytogenes* and *L. innocua*, although *L. monocytogenes* is the only

<table>
<thead>
<tr>
<th>Product</th>
<th>Country of origin</th>
<th>No. of positive samples (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken carcass</td>
<td>Finland</td>
<td>28 (14)</td>
<td>Aho and Hirn, 1988</td>
</tr>
<tr>
<td>Goose carcass</td>
<td>Poland</td>
<td>76 (38)</td>
<td>Kwiatek et al., 1990</td>
</tr>
<tr>
<td>Chicken wings</td>
<td>Northern Ireland</td>
<td>99 (65)</td>
<td>Flynn et al., 1994</td>
</tr>
<tr>
<td>Poultry meat</td>
<td>Chile</td>
<td>117 (93)</td>
<td>Fernandez and Pison, 1996</td>
</tr>
<tr>
<td>Retail poultry meat</td>
<td>The Netherlands</td>
<td>431 (37)</td>
<td>de Boer et al., 2000</td>
</tr>
<tr>
<td>Turkey meat</td>
<td>Denmark</td>
<td>78 (25)</td>
<td>Hald et al., 1998</td>
</tr>
<tr>
<td>Chicken carcass</td>
<td>Japan</td>
<td>13 (59)</td>
<td>Ono and Yamamoto, 1999</td>
</tr>
<tr>
<td>Retail chicken meat</td>
<td>Spain</td>
<td>98 (50)</td>
<td>Dominguez et al., 2002</td>
</tr>
<tr>
<td>Retail poultry meat</td>
<td>South Africa</td>
<td>1 (7)</td>
<td>van Nierop et al., 2005</td>
</tr>
<tr>
<td>Retail poultry meat</td>
<td>Estonia</td>
<td>163 (12)</td>
<td>Meremäe et al., 2010</td>
</tr>
<tr>
<td>Retail poultry meat</td>
<td>Latvia</td>
<td>125 (10)</td>
<td>EFSA, 2006</td>
</tr>
</tbody>
</table>

Table 34.2. *Campylobacter* spp. isolated from fresh poultry meat.
important human pathogen of the genus (Catteau, 1995). Some studies suggest that 1-10% of humans may be intestinal carriers of L. monocytogenes. It has been isolated from at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. Healthy birds may asymptomatically shed L. monocytogenes in faecal material (Skovgaard and Morgen, 1988). Poultry meat is contaminated during slaughtering and processing (Rørvik et al., 2003). Listeria can be isolated from water, soil, silage and other environmental sources. L. monocytogenes is quite hardy and resists the detrimental effects of freezing, drying and heat remarkably well for a non spore-forming bacterium (Johansson, 1999).

Associated Foods
L. monocytogenes has been associated with food sources such as raw milk, improperly pasteurised milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types) and raw and smoked fish (Farber and Peterkin, 1991). Listeria is able to grow at temperatures as low as 3°C and this permits its multiplication in refrigerated foods. It can survive or even grow at pH values as low as 4.4 and at salt concentrations of up to 14% (Berziņš et al., 2007). In a study by Praakle-Amin et al. (2006), a total of 240 raw broiler legs (120 of Estonian origin and 120 of foreign origin) from 12 retail stores in the two biggest cities (Tallinn and Tartu) in Estonia were investigated from January to December 2002. Of the raw broiler legs, 70% tested positive for L. monocytogenes. The prevalence of L. monocytogenes in broiler legs of Estonian origin (88%) was significantly higher than in broiler legs of foreign origin (53%) (P<0.001). Praakle-Amin et al. (2006) concluded that the high prevalence of L. monocytogenes showing various PFGE types in the broiler legs could be caused by cross-contamination at retail level. Ready-to-eat meat products with a long shelf-life are associated with a risk of transmission of L. monocytogenes (Farber and Peterkin, 1991).

The prevalence of L. monocytogenes in cold smoked, sliced, vacuum-packaged pork products during 15-month period from 2003 to 2004 was studied by Berziņš et al. (2007). Samples originated from 8 Latvian and 7 Lithuanian manufacturers. The prevalence of L. monocytogenes in cold-smoked pork varied from 0-67% in Latvian products and 10-73% in Lithuanian products. In order to identify the main risk factors associated with L. monocytogenes contamination, all production steps were studied separately in each meat processing plant. Berziņš et al. (2007) suggested that brining by injection was a significant (P<0.05) risk factor in contamination. Moreover, long cold-smoking times (12 h) had a significant (P<0.014) predictive value for a sample to be positive for L. monocytogenes. Cold-smoking temperatures between 24 and 30°C can have an inhibitory effect on the presence of L. monocytogenes. Low numbers of L. monocytogenes at the end of shelf-life (<100cfu/g) can be explained by the use of starter cultures during processing, which have an antilisterial effect and inhibit the multiplication of L. monocytogenes in pork products. It is recognised that the presence of L. monocytogenes in raw foods cannot be completely eliminated, but through the application of effective hygiene measures, it is possible to reduce its occurrence and level in food products. In order to ensure the safety of food products, growing, harvesting, handling, storage, processing and food supply systems must be managed by food handlers in such a way that they are able to reliably control the growth of L. monocytogenes and to prevent its multiplication to the potentially harmful level of >100/g (Roasto, 2009).

Salmonella
Salmonella is a member of the family Enterobacteriaceae, which comprises a large and diverse group of Gram-negative rods. Members of the genus Salmonella are zoonotic and can be pathogenic in man and animals. Salmonellae are facultatively anaerobic, Gram-negative, straight, small rods, which are usually motile with peritrichous flagella. They are non lactose-fermenting and non spore-forming. There are currently well over 2400 serotypes. Epidemiological classification of Salmonella is based on host preference. One group includes serotypes that infect only humans, for example, S. typhi and S. paratyphi.

Salmonellosis is one of the most common and widely occurring food-borne diseases and constitutes a major public health burden and represents a massive cost to society in many countries. Millions of cases are reported world-wide every year, resulting in thousands of deaths. A Salmonella control programme in food
animal production has been in place for several years in Denmark and the annual estimated cost of this control programme is 10.8 million euros. It is estimated that this programme saves 19.6 million euros for the Danish economy annually. Some countries have managed to limit and even reverse salmonellosis but the spread of two strains of *Salmonella*, namely *Salmonella* Enteritidis and *Salmonella* Typhimurium, is causing increased concern (European Commission, 2004). Multiresistant strains of *Salmonella* are now encountered frequently. The occurrence of multiresistance has increased considerably in recent years owing to the global spread of multiresistant *Salmonella* Typhimurium DT104. While the spread of DT104 may have been facilitated by the use of antimicrobials, international and national trade in infected animals is thought to play a major role in its dissemination (INFOSAN, 2005).

**Associated Foods**

A wide variety of foods have been identified in outbreaks caused by several serotypes of *Salmonella*: raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatine, peanut butter, cocoa, chocolate and even dried chilli (Figure 34.3). Various *Salmonella* serotypes have long been isolated from the outer surface of egg shells. The current status of *S*. *enteritidis* is complicated by the presence of the organism inside the egg, in the yolk. This and other information strongly suggest vertical transmission, i.e. deposition of the organism in the yolk by an infected layer hen prior to shell deposition. Foods other than eggs have also caused outbreaks of *S. enteritidis* disease. *Salmonella* is still the most frequently recorded pathogen in the production chain of food of animal origin. At present the predominant serotypes are *S. enteritidis* and *S*. *Typhimurium*, particularly in terms of the most important meats from pig and poultry. In areas such as Scandinavia, measures against this pathogen have traditionally been more thoroughly implemented, ultimately resulting in a lower prevalence of *Salmonella* in these countries compared with Central Europe (Roasto et al., 2006). Whatever the *Salmonella* serotype, effective controls for minimising/eliminating the hazard of *Salmonella* from foods involve control of the following steps: raw materials, personal and environmental hygiene, process conditions, post-process contamination, retail and catering practices and consumer handling (Roasto et al., 2006).

**Escherichia Coli**

*E. coli* is a short, typically motile, facultatively anaerobic, non spore-forming, Gram-negative, rod-shaped bacterium (1.1-1.5 μm and 2.0-6.0 μm) within the family *Enterobacteriaceae*. The optimum growth temperature is 37°C. The combination of O and H antigens defines the *E. coli* serotype, and serotyping of isolates is useful for characterisation of commensal and pathogenic serotypes and as a tool for epidemiological investigations. *E. coli* forms a part of the natural gastrointestinal microbiota of man and warm-blooded animals. Normally, *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesising appreciable amounts of vitamins. Although most *E. coli* strains are harmless commensal organisms, there are many pathogenic strains capable of causing a variety of illnesses in humans.

![Figure 34.3. Possible route of contamination of chillies with *Salmonella* during drying. Photo: Gul Chotrani, Lombok, Indonesia, 2005.](image)
There are six recognised groups of pathogenic *E. coli* (EAEC, EPEC, ETEC, EIEC, EHEC, VTEC). Each group has different virulence features and mechanisms of pathogenicity (Fratamico et al., 2002; Duffy, 2006). The enteroaggregative *E. coli* (EAEC) are associated with persistent diarrhoea in young children, especially in developing countries. These strains produce three toxins which stimulate intestinal secretion. The enteroaggregative *E. coli* (EPEC) cause severe diarrhoea in infants. Certain EPEC strains produce one or more cytotoxins. The enteroaggregative *E. coli* (ETEC) cause also diarrhoea in humans, both in infants and adults, and in the latter the world-wide illness known as traveller’s diarrhoea. ETEC strains produce enterotoxins. The enteroaggregative *E. coli* (EIEC) produce a cytotoxin and often induce rather severe illness such as colitis and a form of dysentery, accompanied by fever and bloody stools. The enteroaggregative *E. coli* (EHEC) produce cytotoxins which give more severe symptoms. These toxins (verotoxin 1 and verotoxin 2) are closely related or identical to the toxin produced by *Shigella dysenteriae*. They have the same biological activity but can be distinguished immunologically. The toxins are lethal to Vero cells and hence are known as Verocytotoxin producing *Escherichia coli* or VTEC. The toxins destroy the intestinal cells of the human colon, causing haemorrhagic colitis (HC) which is characterised by severe abdominal pain and diarrhoea. About 15% of HC cases, notably in children, develop haemolytic uraemic syndrome (HUS). This is a form of renal failure and haemolytic anaemia and may result in permanent kidney damage. *E. coli* serotype O157:H7 is the most well-known EHEC strain. Due to current detection procedures *E. coli* O157:H7 is the only serotype routinely identified, but other verotoxigenic *E. coli* serotypes such as *E. coli* O26:H11 are known (Forsythe and Hayes, 1998; Fratamico et al., 2002).

**Antimicrobial Resistance**

Many food-borne pathogens can have natural habitats in food animals. They can enter meat and milk production at slaughter and milking, or contaminate raw vegetables when the soil is fertilised with animal manure. The molecular analysis of antibiotic resistance genes, plasmids and transposons has demonstrated that identical elements are found in animals and humans. The use of antibiotics in veterinary medicine, either for preventive or prophylactic purposes or in therapy, selects resistant pathogenic, opportunistic and commensal bacteria. These bacteria are released into the environment. Specific food items, water and direct contact can spread these bacteria from animal microflora to human microflora (Teuber, 2004).

The development of antimicrobial resistance in pathogenic bacteria is a matter of increasing concern. There is growing scientific evidence that the use of antimicrobials in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Van Loooveren et al., 2001). These human food safety concerns have been influential in prompting the European Union to ban the use of antimicrobials as growth promoters in food production and to increase their surveillance for antimicrobial resistance of food-borne pathogens and indicator organisms (Smith et al., 2007). In farm environments, commensal and environmental bacteria may be a reservoir for the transfer of antimicrobial resistance genes to pathogenic bacteria. Bacteria may acquire resistance genes through horizontal trans-
Conjugative genetic elements such as plasmids and transposons are common vectors for the dissemination of antimicrobial resistance genes to diverse microorganisms (Smith et al., 2007). Many scientists and public health specialists expect this resistance problem to worsen unless we act decisively.

Estonian antimicrobial susceptibility studies (Roasto et al., 2007) of *Campylobacter* strains revealed high resistance patterns for several antimicrobials (Table 34.3). A high proportion (27.5% of isolates) of multidrug resistance to three or more unrelated antimicrobials was found. All these isolates were resistant to enrofloxacin and all except one were resistant to nalidixic acid. Hakanen et al. (2003) noted that 20% of the Finnish human *Campylobacter* isolates associated with travel were resistant to three or more antimicrobials. Multiresistant isolates in the study by Roasto et al. (2007) consisted of a combination of all tested antimicrobials. The results showed that multiresistance was significantly associated with enrofloxacin and nalidixic acid resistance (correlation coefficient 0.372 and 0.310, P<0.01). These findings suggest that the use of fluoroquinolones may select multiresistant strains, since resistance to erythromycin, gentamicin or oxytetracycline was exceptional without simultaneous resistance to fluoroquinolones. A recent study on antimicrobial resistance of *Escherichia coli* at a farm where no antimicrobial treatment of the birds was performed during one year before the sampling showed that the resistance to tetracycline, gentamicin and streptomycin persisted but all isolates were susceptible to enrofloxacin (Smith et al., 2007). Thus multiresistant strains may reflect the past history of antimicrobial usage during a longer period. This phenomenon may also partly explain the rather high number of multiresistant strains in the Estonian study (Roasto et al., 2007). Antimicrobial susceptibility patterns of *C. jejuni* isolates from broiler chickens in Estonia in 2005-2006 are shown in Table 34.3

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Antimicrobial concentration range (µg/ml)</th>
<th>Breakpoint (µg/ml)</th>
<th>No. of resistant strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am</td>
<td>0.5-64</td>
<td>32</td>
<td>10 (7.6)</td>
</tr>
<tr>
<td>Ef</td>
<td>0.03-4</td>
<td>1</td>
<td>96 (73.3)</td>
</tr>
<tr>
<td>Em</td>
<td>0.12-16</td>
<td>16</td>
<td>26 (19.8)</td>
</tr>
<tr>
<td>Gm</td>
<td>0.25-8</td>
<td>8</td>
<td>25 (19.1)</td>
</tr>
<tr>
<td>Nal</td>
<td>1-128</td>
<td>32</td>
<td>99 (75.6)</td>
</tr>
<tr>
<td>Tc</td>
<td>0.25-32</td>
<td>4</td>
<td>42 (32.1)</td>
</tr>
</tbody>
</table>

*Antimicrobial agents: Am, Ampicillin; Ef, Enrofloxacin; Em, Erythromycin; Gm, Gentamicin; Nal, Nalidixic acid; Tc, Oxytetracycline.*

The costs of treating antimicrobial-resistant human infections place a significant burden on society. For example, it has been estimated that the in-hospital cost of hospital-acquired infections caused by just six common kinds of resistant bacteria was at least 1.3 billion USD (1 billion euros) in 1992 (http://www.hhs.gov/news/press/2001pres/20010118b.html; accessed 24 May 2007). Multiresistance is a major public health problem because it limits chemotherapeutic options. The concept of Critically Important Antimicrobials for humans and animals should be used by EU member states for setting priorities for improved management of antimicrobials in animal production. Figure 34.4 and 36.5 illustrate the susceptible and resistant *C. jejuni* strains identified using minimal inhibitory concentration testing by E-test™.

**Legislation**

The aim of EU legislation is to be clear about the responsibilities of the various stakeholders in the food production chain. Legislation aims to clarify that responsibility for the safety of food on the market rests on the food operators; that the relevant authorities in individual member states are in charge of monitoring and enforcing this responsibility through national surveillance and control systems; and finally that the Commission must concentrate on evaluating the ability of the relevant authorities to carry out these tasks through audits and inspections. The Commission should continue to reinforce its farm to table policy, covering all sectors of the food chain, including feed production, production on the farm, food processing, storage, transport and retail sale (Daelman, 2002).

**General Food Law – Principles**

The food law aims at ensuring a high level of protection of human life and health, as well taking into account the
protection of animal health and welfare, plant health and the environment. This integrated ‘farm to fork’ approach is now considered a general principle for EU food safety policy. Food law, both at national and EU level, establishes the rights of consumers to safe food and to accurate and honest information. The EU food law aims to harmonise existing national requirements in order to ensure the free movement of food and feed in the EU. The food law recognises the European Union commitment to its international obligations and will be developed and adapted taking international standards into consideration, except where this might undermine the high level of consumer protection pursued by the EU.

Risk Analysis
EU regulation EC 178/2002 establishes the principles of risk analysis in relation to food and establishes the structures and mechanisms for the scientific and technical evaluations which are undertaken by the European Food Safety Authority (EFSA).

Depending on the nature of the measure, food law, and in particular measures relating to food safety, must be underpinned by strong science. The EU has been at the forefront of the development of the risk analysis principles and their subsequent international acceptance. Regulation EC 178/2002 establishes in EU law that the three interrelated components of risk analysis (risk assessment, risk management and risk communication) provide the basis for food law as appropriate to the measure under consideration. Clearly not all food law has a scientific basis, e.g. food law relating to consumer information or the prevention of misleading practices does not need a scientific foundation.

Scientific assessment of risk must be undertaken in an independent, objective and transparent manner based on the best available science. Risk management is the process of weighing policy alternatives in the light of results of a risk assessment and, if required, selecting the appropriate actions necessary to prevent, reduce or eliminate the risk to ensure the high level of health protection determined as appropriate in the EU.

In the risk management phase, the decision-makers need to consider a range of information in addition to the scientific risk assessment. These include, for example, the feasibility of controlling a risk, the most effective risk reduction actions depending on the part of the food supply chain where the problem occurs, the practical arrangements needed, the socio-economic effects and the environmental impact. Regulation EC/178/2002 establishes the principle that risk management actions are not just based on a scientific assessment of risk but also take into consideration a wide range of other factors legitimate to the matter under consideration.
Transparency

Food safety and the protection of consumer interests are of increasing concern to the general public, non-government organisations, professional associations, international trading partners and trade organisations. Therefore, the EU Regulation establishes a framework for the greater involvement of stakeholders at all stages in the development of food law and establishes the mechanisms necessary to increase consumer confidence in food law.

This consumer confidence is an essential outcome of a successful food policy and is therefore a primary goal of EU action related to food. Transparency of legislation and effective public consultation are essential elements of building this greater confidence. Better communication about food safety and the evaluation and explanation of potential risks, including full transparency of scientific opinions, are of key importance.

EU Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs constitutes that foodstuffs should not contain microorganisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. EU Regulation (EC) No 178/2002 lays down general food safety requirements, according to which food must not be placed on the market if it is unsafe. The use of microbiological criteria should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. According to Article 4 of EU Regulation (EC) No 852/2004, food business operators are to comply with microbiological criteria. This should include testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective actions, in accordance with food law and the instructions given by the competent authority. Article 5 of EU Regulation (EC) No 2073/2005 lays down specific rules for testing and sampling, according to which the ISO standard 18593 must be used as a reference method. Food business operators manufacturing ready-to-eat foods, which may pose a *L. monocytogenes* risk for public health, must sample the processing areas and equipment for *L. monocytogenes* as part of their sampling scheme. Food safety and process hygiene criteria are given in chapter 1 and 2 of EU Commission Regulation (EC) No 2073/2005 for microbiological criteria for foodstuff.

The basic principles of food hygiene introduce a certain level of flexibility that is believed essential in order to take account of particular situations. This is in particular the case with regard to the implementation of the HACCP system, traditional ways of preparing certain food, and for certain small enterprises. It is clearly stated that this must not compromise food safety.

Finally, all the legislation alone is not able to guarantee the quality and safety of the food. Food hygiene legislation and detailed microbiological standards are meaningless if the legislation is impossible to apply in practice. Most important, however, is the care taken in the whole chain of food handling operations. The quality of raw materials, the hygiene environment within the food processing enterprise, the processing standards applied and the attitude of food enterprise personnel are all of crucial importance.

Future Needs

There is likely to be an increased need for attention to the safety of the food supply in relation to population growth, and therefore the need to provide food and to dispose safely of faecal and chemical waste will increase. There is a need for research on more sensitive, reliable and cost-effective tools, particularly sampling methodologies, for analysing food and environmental samples (e.g. high priority commodities including eggs and seafood) for microbial pathogens. This is especially needed where the frequency and extent of contamination are expected to be low and for identification and evaluation of relevant characteristics of different forms of product packing and handling on the safety of a variety of foods. Other areas where research is needed include: development of modelling techniques to assess microbial behaviour in various foods, human exposure and dose-response relations to certain food-borne pathogens (e.g. enumerative detection methods for pathogens), the potential risk of those pathogens causing human illness and the setting of safety performance standards to regulate microbial content of food, and determining the population trends with respect to food safety knowledge, attitudes and practices, especially behaviours that may be a significant risk factors for
food-borne illness (e.g. food consumption, in-home food preparation and handling).

The microbiological safety of food has been advanced substantially by the introduction and implementation of the hazard analysis of critical control point (HACCP) concept. HACCP provides a systematic conceptual framework for identifying hazards and focusing efforts on the proper functioning of key food production, processing and marketing steps. HACCP cannot be expected to control unknown hazards, such as emerging food-borne pathogens. There is a need to re-examine how food is produced, processed, marketed and prepared to identify conditions that contribute to emergence. The changing epidemiology of food-borne disease calls for improved surveillance, including rapid sub-typing methods, cluster identification and collaborative epidemiological investigation (including case-control studies). The new problems of food-borne disease require new control and prevention strategies to ensure that food in both domestic and international trade is safe. Topics included a need for multidisciplinary teams that can provide ‘just in time’ research; for basic research to explain factors associated with food production and processing that contribute to new food-borne microbial threats; for prompt evaluation and implementation of innovative preservation methods (e.g. food irradiation) to meet consumer demand for fresh foods; for the use of emerging molecular methods (e.g. polymerase chain reaction and molecular typing) to examine emerging food-borne disease organisms; and for models to predict the probability of a particular microbial event (e.g. growth and death), which may be useful in the design of HACCP programs and in defining processes, formulations and storage conditions to yield foods with acceptable shelf-life and safety characteristics.

**Conclusions**

There is a long and multi-step path taken by food of animal origin from the farm to the consumer’s plate. There are a lot of circumstances and potential hazards, which may or may not constitute a risk to humans. As a consequence, measures should be taken, especially where the prevalence of pathogens has been high, i.e. hygiene in primary production, immunisation, logistical slaughter or measures in cleaning and disinfection of the food processing site. It is obvious that the inspection service by the authorities cannot afford the total of surveillance in every production stage. The hygiene status of intermediate products and end products is particularly dependent on the circumstances of previous stages of production. In consequence, hygiene is an issue of day-to-day practices, and checks must be carried out frequently. Here, the authorities have to rely more on the responsibility of the processing plant. The role of the authorities is currently being reconsidered in order to focus the available resources on the essentials of surveillance. This is true also with respect to future additional tasks of surveillance in animal husbandry, which will possibly demand more personnel in the future. It should be emphasised that the producer is responsible for the product and should do everything to guarantee it. Consumers are important stakeholders in the food chain and as such they share equal responsibility. Among other things, all consumers should learn to understand and apply the basic rules of food hygiene. They should be able to discriminate between hygienic and unhygienic practices, and participate in improving food safety in the community.


Chapter 33


Chapter 34


Further Reading:


Chapter 35
