Ecosystem Health and Sustainable Agriculture

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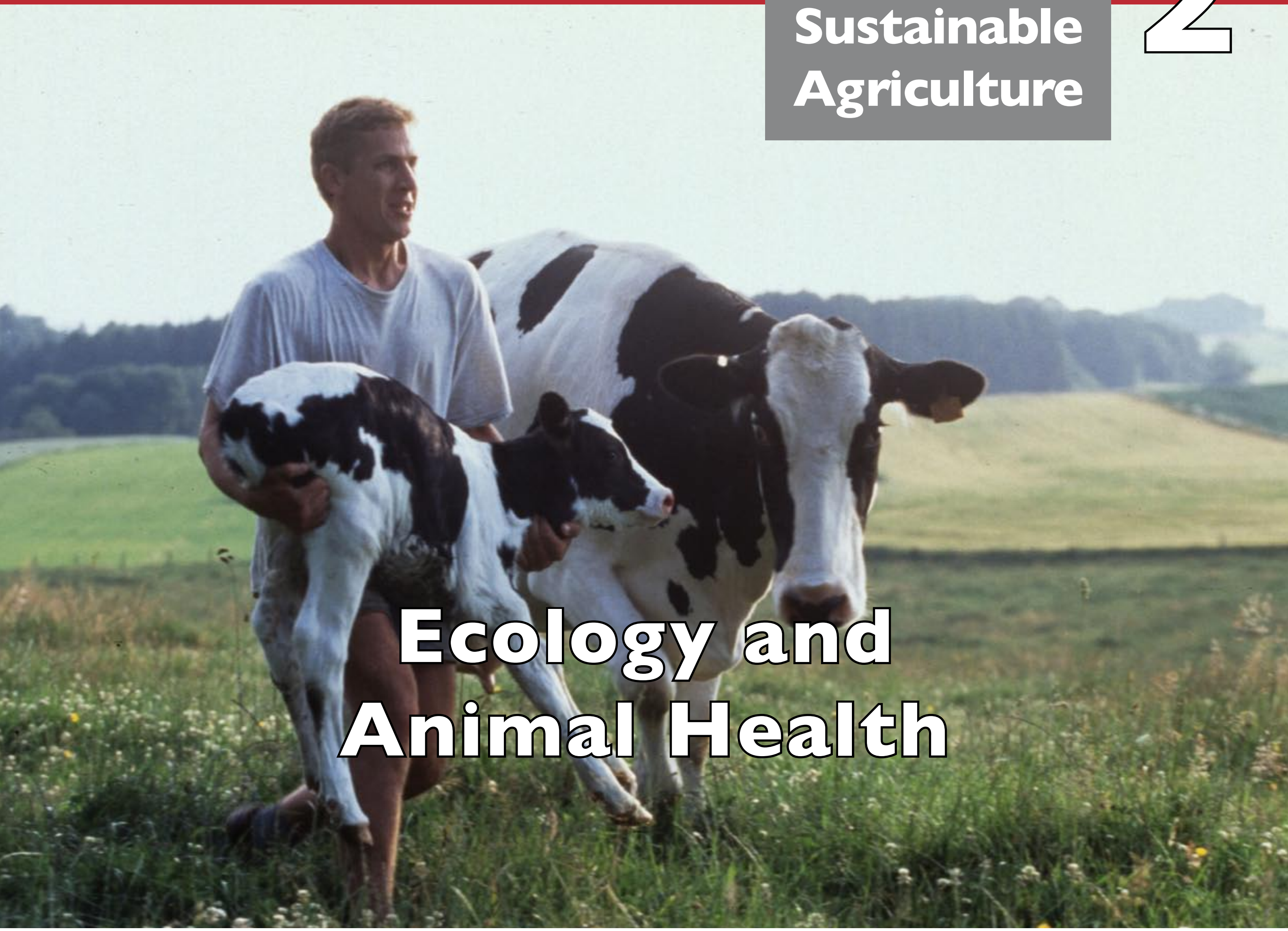

Ecology and Animal Health

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Food-borne Pathogens and Public Health

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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-

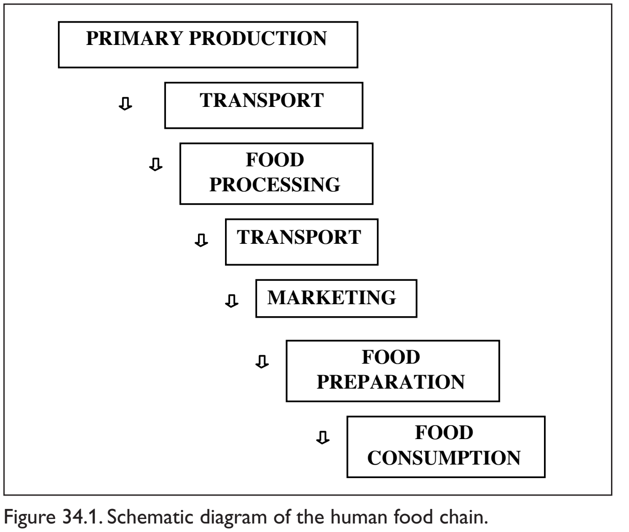

Figure 34.1. Schematic diagram of the human food chain.

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% N_2, 10% CO_2 and 5% O_2), 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* ssp. *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-

Table 34.1. Food-borne disease outbreaks and associated foods.

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

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 45ºC. 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 food-borne zoonoses (SCVMPH, 2000). In this opinion the Committee identified *Campylobacter* spp. as one of the 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

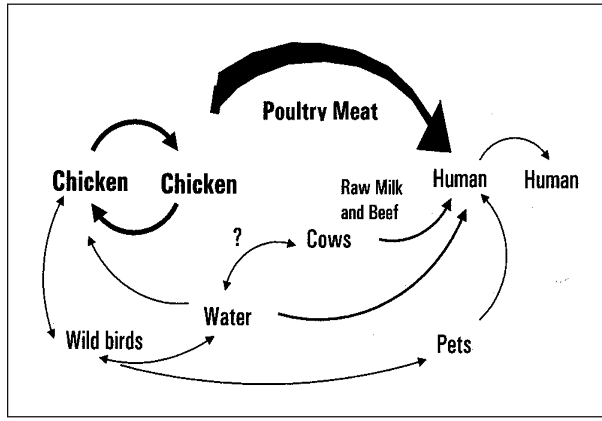

Figure 34.2. Campylobacter jejuni transmission. Source: Hänninen, 1999.

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 34.2. Campylobacter spp. isolated from fresh poultry meat.

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

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 S*almonella*: 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).

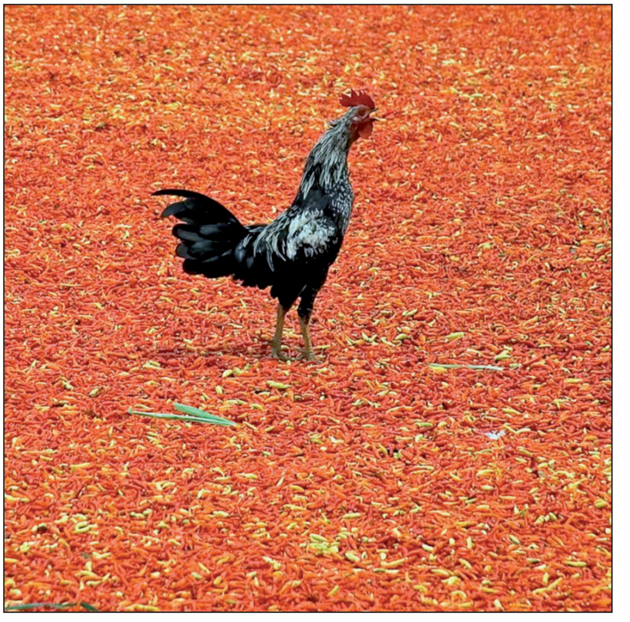

Figure 34.3. Possible route of contamination of chillies with Salmonella during drying. Photo: Gul Chotrani, Lombok, Indonesia, 2005.

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 37ºC. 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.

There are six recognised groups of pathogenic *E. coli* (EAEC, EPEC, ETEC, EIEC, EHEC, VTEC). Each group has different virulence features and mechanisms of pathogenity (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).

Associated Foods

Strains of EAEC have been isolated from the contents of infant feeding bottles and outbreaks have been associated with food. Disease due to EPEC occurs predominantly in developing countries, with vehicles including different foods or infant formula, contaminated hands and contaminated utensils such as linen, scales, toys, etc. ETEC strains have been implicated in several large water-borne outbreaks, while a wide range of foods (various meats and poultry, mashed potatoes, milk products) have been implicated as sources. Many outbreaks have been attributed to EIEC strains in cheeses, milk, meats, potato salads and other foods. Outbreaks of the most well-known EHEC strain, *E. coli* O157:H7, have mostly been attributed to undercooked or raw minced beef meat. However, *E. coli* O157:H7 outbreaks have also resulted from contaminated alfalfa sprouts, unpasteurised fruit juices, drinking water, dry-cured salami, lettuce, game meat and cheese curds. Raw milk was the vehicle in a school outbreak in Canada (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 Looveren 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 transfer. 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

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™.

Table 34.3. Antimicrobial susceptibility of C. jejuni isolates (n = 131) from broiler chickens in Estonia, 2005-2006.

Antimicrobial agent[a]	Antimicrobial concentration range (µg/ml) VetMICTMCamp	Breakpoint (µg/ml)	No. of resistant strains (%)
Am	0.5-64	32	10 (7.6)
Ef	0.03-4	1	96 (73.3)
Em	0.12-16	16	26 (19.8)
Gm	0.25-8	8	25 (19.1)
Nal	1-128	32	99 (75.6)
Tc	0.25-32	4	42 (32.1)

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

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

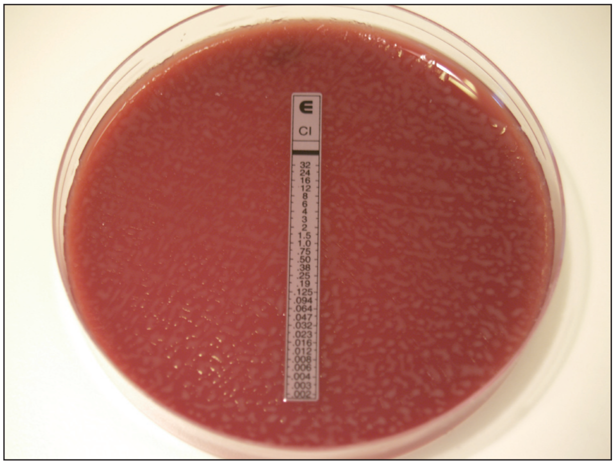

Figure 34.4. Campylobacter jejuni strain resistant to ciprofloxacin in E-test™. Photo: Mati Roasto, 2005.

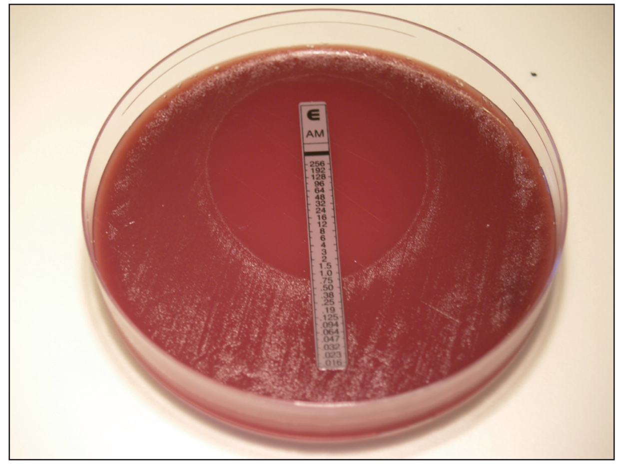

Figure 34.5. Campylobacter jejuni strain susceptible to ampicillin in E-test™. Photo: Mati Roasto, 2005.

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.

natural recovery (MNR) as a contaminated sediment management option. In: *Proceedings: 2nd International Conference on Remediation of Contaminated Sediments*, Venice Italy (September 30, 2003). Columbus OH: Battelle.

National Research Council, Committee on Sediment Dredging at Superfund Megasites. 2007. *Sediment Dredging at Superfund Megasites: Assessing the Effectiveness*. Washington DC: The National Academies Press.

USEPA. 1994. *Assessment and Remediation of Contaminated Sediments (ARCS) Program Remediation Guidance Document*. EPA/905/R-94/003. Chicago IL: USEPA.

USEPA. 1996. *Estimating Contaminant Losses from Components of Remediation Alternatives for Contaminated Sediments*. EPA/905/R-96/001. Chicago IL: USEPA.

USEPA. 1998. *Assessment and Remediation of Contaminated Sediments (ARCS) Program Guidance for In-Situ Subaqueous Capping of Contaminated Sediments*. EPA/905/B-96/004. Chicago IL: USEPA.

USEPA. 2001. *Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual*. EPA 823-B-01-002. Washington DC: USEPA.

USEPA/USACE. 2004. *Evaluating Environmental effects of Dredged Material Management Alternatives- A Technical Framework*. EPA842-B-92-008. Washington DC: USEPA/USACE.

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Ambio, 1998. Special Issue about The Järnsjön Project, Sweden. Vol 27, No 5, August 1998.

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Abbott, S.L., Waddington, M., Lindquist, D., Ware, J., Cheung, W., Ely, J. and Janada, J.M. 2005. Description of *Campylobacter curvus* and *C. curvus*-like strains associated with sporadic episodes of bloody gastroenteritis and Brainerd´s diarrhea. In: *Journal of Clinical Microbiology*, 43, 585-588.

Aho, M. and Hirn, J. 1988. Prevalence of campylobacteria in the Finnish broiler chicken chain from the producer to the consumer. In: *Acta Veterinaria Scandinavica*, 29, 451-462.

Anonymous. 1993. The National Advisory Committee on Microbiological Criteria for Foods. *Campylobacter jejuni/coli*. In: *Journal of Food Protection*, 57, 1101-1121.

Barrios, P. R., Reiersen, J., Lowman, R., Bisaillon, J-R., Vala Fridriksdottir, P., M., Gunnarsson, E., Stern, N., Berke, O., McEwen, S. and Martin, W. 2006. Risk factors for *Campylobacter* spp. colonization in broiler flocks in Iceland. In: *Preventive Veterinary Medicine*, 74, 264-278.

Berziņš, A., Hörman, A., Lunden, J. and Korkeala, H 2007. Factors associated with *Listeria monocytogenes* contamination of cold- smoked pork products produced in Latvia and Lithuania. International In: *Journal of Food Microbiology*, 115(2):173-179.

Bhaduri, S. and Cottrell, B. 2004. Survival of Cold-Stressed *Campylobacter jejuni* on Ground Chicken and Chicken Skin during Frozen Storage. In: *Applied and Environmental Microbiology*. 70, 7103-7109.

Bolton, F.J., Sails, A.D., Fox, A.J., Wareing, D.R.A. and Greenway, D.L.A. 2002. Detection of in foods by enrichment culture and polymerase chain reaction enzyme-linked immunosorbent assay. In: *Journal of Food Protection*, 65, 760-767.

Buzby, J.C., Allos, B.M. and Roberts, T. 1997. The economic burden of *Campylobacter*-associated Guillain-Barre syndrome. In: *Journal of Infectious Diseases*, 176, S192-S197.

Catteau, M. 1995. The genus *Listeria*. In: Bourgenois, C.M. and Leveau, J.-Y. (eds.), translated by S. Davids, editor for the English-language edition D.Y.C. Fung, *Microbiological control for foods and agricultural products*. New York: VCH Publishers, Inc. Chapter 23, pp. 373-382.

CDC. Centers for Disease Control and Prevention. 2004. Protecting health for life. The statute of the CDC, fiscal year 2004. Centers for Disease Control and Prevention, Atlanta, Ga. http://www.cdc.gov/cdc.pdf.Accessed 5 June 2007.

Colomba, C., Saporito, L., Infurnari, L., Tumminia, S., Titone, L. 2006. Typhoid fever as a cause of opportunistic infection: case report. BMC Infectious Diseases, 6, 38.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union.

Commission Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Commission Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

Connor, B.A., Johnson, E. and Soave, R. 2001. Reiter syndrome following protracted symptoms of *Cyclospora* infection. In: *Emerging Infectious Diseases*, 7, 453-454.

Daelman, W. 2002. The EU food safety action plan. In: Smulders, J.M., Collins, J.D. (eds.), *Food safety assurance and veterinary public health, Volume 1, Food safety assurance in the pre-harvest phase*, Wageningen Academic Publishers, The Netherlands, pp. 17-22.

De Boer, P., Duim, B., Rigter, A., van Der Plas, J., Jacobs-Reitsma, W.F. and Wagenaar, J.A. 2000. Computer-assisted analysis and epidemiological value of genotyping methods for *Campylobacter jejuni* and *Campylobacter coli*. In: *Journal of Clinical Microbiology*, 38, 1940–1946.

De Jong, B., Andersson, Y. and Ekdahl, K. 2006. The comparative burden of salmonellosis in the European Union member states, associated and candidate countries. In: *BMC Public Health* 6, 4.

Domingues, C., I. Gomez and J. Zumalacarregui 2002. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. In: *International Journal of Food Microbiology*, 72**,** 165-168.

Dubos, R. 1998. Chapter 10. Victory over Disease. In: Brock, T.D. (ed.). *Pasteur and modern science*. Washington, D.C: ASM Press.

Duffy, E.A., K.E. Belk, J.N. Sofos, G.R. Bellinger, A. Pape, and G.C. Smith. 2001. Extent of microbial contamination in United States pork retail products. In: *J. Food Prot*. 64**,** 172-178.

Duffy, G. 2006. Emerging pathogenic *E. coli*. In: Motarjemi, Y. and Adams, M. (eds) *Emerging foodborne pathogens*. CRC Press Woodhead Publishing Limited, pp. 253-272.

EFSA. 2004. Scientific Report on *Campylobacter* in animals and foodstuffs, 173, 1-105.

EFSA. 2006. Trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004. European Food Safety Authority. 96-116.

EFSA. 2009a. Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2007. Summary Report. *Campylobacter* 3.2. In: *The EFSA Journal* (January 2009), 109–133.

EFSA. 2009b. Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2007. Summary Report. *Listeria* 3.3. In: *The EFSA Journal* (January 2009), 135–159.

Eggertson, L. 2005. *C. difficile* strain 20 times more virulent. In: *Canadian Medical Association Journal*, 172, 1279.

Erkmen, O. 1996. Survival of virulent Yersinia enterocolitica during the manufacture and storage of Turkish feta cheese. In: *International Journal of Food Microbiology*, 33, 285-292.

European Commission. 2004. *Trends and sources of zoonotic agents in animals, feeding stuffs, food and man in the European Union and Norway in 2002*. European Commission, health and consumer protection directorate-general. Sanco/29/2004. http://europa.eu.int/comm/food/food/biosafety/salmonella/zoonoses reps 2002 en.htm

Euzeby, J.P. 2006. *Dictionnaire de bacteriologie veterinaire*. http://www.bacdico.net.

Farber, J.M. and Peterkin, P. 1991. *Listeria monocytogenes*, a foodborne pathogen. In: *Clinical Microbiology Reviews*, 55, 476-511.

Farmer, J.J., Asbury, M.A., Hickman, F.W. and Brenner, D.J. 1980. Enterobacter sakazakii: a new species of „*Enterobacteriaceae*“ isolated from clinical specimens. In: *International Journal of Systematic Bacteriology*, 30, 569-584.

Fernandez, H. and Pison, V. 1996. Isolation of thermotholerant species of *Campylobacter* from commercial chicken livers. In: *International Journal of Food Microbiology*, 29, 75-80.

Flynn, O.M.J., Blair, I.S. and McDowell, D.A. 1994. Prevalence of *Campylobacter* species on fresh retail chicken wings in Northern Ireland. In: *Journal of Food Protection*, 57, 334-336.

Forsythe, S.J. and Hayes, P.R. 1998. Food poisoning and other foodborne hazards. In: Forsythe, S.J. and Hayes, P.R (eds.) *Food hygiene, microbiology and HACCP*, Third edition, Gaithersburg, MD: Aspen Publication, pp. 21-85.

Fratamico, P.M., Smith, J.L. and Buchanan, R.L. 2002. *Escherichia coli*. In: Cliver, D.C. and Riemann, H.P (eds.), *Foodborne diseases*, Second edition, Academic Press, California, USA, pp. 79-101.

Friedman, C.R., Neimann, J., Wegener, H.C. and Tauxe, R.V. 2000. Epidemiology of *Campylobacter jejuni* in the United States and other industrialized nations. In: Nachamkin, I. and Blaser, M.J. (eds.) *Campylobacter*, 2nd ed., ASM Press, Washington, USA, 121-138.

Frye, D.M., Zweig, R., Sturgeon, J., Tormey, M., LeCavalier, M., Lee, I., Lawani, L. and Mascola, L. 2002. An outbreak of febrile gastroenteritis associated with delicatessen meat contaminated with *Listeria monocytogenes.* In: *Clinical Infectious Diseases*, 35, 943-949.

Hald, T., Wegener, H.C. and Beck Joergensen, B. 1998. *Annual Report on Zoonoses in Denmark 1998*. Danish Ministry of Food, Agriculture and Fisheries. Danish Zoonosis Centre, Copenhagen, Denmark.

Hakanen, A.J., Lehtopolku, M., Siitonen, A., Huovinen, P. and Kotilainen, P. 2003. Multidrug resistance in *Campylobacter jejuni* strains collected from Finnish patients during 1995-2000. In: Journal *of Antmicrobial Chemotheraphy*, 52, 1035-1039.

Harris, L.J. 2002. *Listeria monocytogenes*. In: Cliver, D.C. and Riemann, H.P (eds.), *Foodborne diseases*, Second edition, Academic Press, California, USA, pp. 137-150.

Hazeleger, W., Arkesteijn, C., Toorop-Bouma, A. and Beumer, R. 1994. Detection of the coccoid form of *Campylobacter jejuni* in chicken products with the use of the polymerase chain reaction. In: *International Journal of Food Microbiology*, 24, 273-281.

Health Protection Inspectorate of Estonia. In: *Estonian Communicable Disease Bulletin* 2009, pp. 46.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. 1994. *Bergey´s Manual of Determinative Bacteriology*. Ninth Edition. pp. 41, 58-61. Baltimore, MD: Williams & Wilkins.

Hänninen, M.-L., Haajanen, H., Pummi, T., Wermundsen, K., Katila, M.-L., Sarkkinen, H., Miettinen, I. and Rautelin, H. 2003. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. In: *Applied Environmental Microbiology* 69**,** 1391-1396.

ICMSF. 1996. Microorganisms in Foods 5. Charachteristics of microbial pathogens. London: Blackie Academic & Professional. pp. 45-65.

INFOSAN. 2005. Antimicrobial-resistant *Salmonella,* International Food Safety Authorities Network. In: *INFOSAN information Note No. 3/2005 – Salmonella*.

ISO 22000:2005. *Food safety management systems. Requirements for any organization in the food chain*.

Johansson, T. 1999. *Tracking of Listeria monocytogenes in foods and industrial environments*. Academic Dissertation. Department of Food Microbiology. Helsinki: National Veterinary and Food Research Institute pp. 119.

Kwiatek, K., Wojton, B. and Stern, N.J. 1990. Prevalence and distribution of *Campylobacter* spp. on poultry and selected red meat carcasses in Poland. In: *Journal of Food Protection*, 53, 127-130.

Käferstein, F.K. 2004. Food Safety – a worldwide public health issue. Actions to reverse the upward curve of foodborne illness. In: Smulders, F.J.M. and Collins, J.D. (eds.) *Food safety assurance and veterinary public health, Volume 2, Safety aasurance during food processing*, Wageningen Academic Publishers, The Netherlands, pp. 19-33.

Meremäe, K., Elias, P., Tamme, T., Kramarenko, T., Lillenberg, M., Karus, A., Hänninen, M-L. and Roasto, M. 2010. The occurrence of *Campylobacter* spp. in Estonian broiler chicken production in 2002-2007. In: *Food Control*, 21, 272-275.

On, S.L.W. 1996. Identification methods for campylobacters, helicobacters and related organisms. In: *Clinical Microbiology Reviews*, 9, 405-422.

On, S.L.W. and Jordan, P.J. 2003. Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli*, In: *Journal of Clinical Microbiology*, 41, 330-336.

Ono, K. and Yamamoto, K. 1999. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. In: *International Journal of Food Microbiology*, 47, 211-219.

Praakle-Amin, K., Roasto, M., Korkeala, H. and Hänninen, M-L. 2007. PFGE genotyping and antimicrobial susceptibility of *Campylobacter* in retail poultry meat in Estonia". In: *International Journal of Food Microbiology*, 114, 105-112.

Praakle-Amin, K., Hänninen, M-L. and Korkeala, H. 2006. Prevalence and genetic characterization of *Listeria monocytogenes* in retail broiler meat in Estonia. In: *Journal of Food Protection*, 69, 436-440.

Roasto, M., Praakle, K., Korkeala, H., Elias, P. and Hänninen, M-L. 2005. Prevalence of *Campylobacter* in raw chicken meat of Estonian origin. In: *Archiv für Lebensmittelhygiene* 56, (49-72). 61-62.

Roasto, M. 2006. *Salmonella*. In: Roasto, M., Tamme, T. and Juhkam, K. (eds.). *Toiduhügieen ja ohutus (Food Hygiene and Safety), Teine, parandatud ja täiendatud trükk*. Tartu: Eesti Maaülikool, ISBN: 9985-9582-0-9. pp. 42-46.

Roasto, M., Juhkam, K., Tamme, T., Hörman, A., Häkkinen, L., Reinik, M., Karus, A. and Hänninen, M.-L. 2007. High level of antimicrobial resistance in *Campylobacter jejuni* isolated from broiler chickens in Estonia in 2005-2006. In: *Journal of Food Protection*, 70(8), 1940-1944.

Roasto, M. 2009. *Listeria monocytogenes* toidupatogeenina ja tootmishügieeni mõjutava faktorina. In: *Veterinaarmeditsiin 2009*, 68-72 (in Estonian).

Rosenquist, H., Nielsen, N.L., Sommer, H.M., Norrung, B. and Christensen, B.B. 2003. Quantitative risk assessment of human campylobacteriosis associated with thermophilic Campylobacter species in chickens. In: *International Journal of Food Microbiology*, 83, 87-103.

Rørvik, L.M., Aase, B., Alvestad, T. and Caugant, D.A. 2003. Molecular epidemiological survey of *Listeria monocytogenes* in broilers and products. In: *Journal of Applied Microbiology* 94, 633-640.

SCVMPH. 2000. *Scientific committee on veterinary measures relating to public health (SCVMPH). Opinion on foodborne zoonoses*. European Commission.

Skirrow, M.B. 1977. *Campylobacter* enteritidis: a new disease. In: *British Medical Journal* 2, 9-11.

Skirrow, M.B. and Blaser, M.J. 2000. Clinical aspects of *Campylobacter* infection, in: Nachamkin, I. and Blaser, M.J. (eds.) *Campylobacter*, Washington, DC: American Society for Microbiology Press pp. 69-88.

Skovgaard, N. and Morgen, C.A. 1988. Detection of *Listeria spp*. in faeces from animals, in feeds, and in raw foods of animal origin. In: *International Journal of Food Microbiology*, 6, 229-242.

Smith, J. L., D. J. V. Drum, Y. Dai, J. M. Kim, S. Sanchez, J. J. Maurer, C. L. Hofacre, and M. D. Lee. 2007. Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. In: *Applied Environmental Microbiology* 73:1404-1414.

Snowdon, J.A., Buzby, J.C. and Roberts, T. 2002. Epidemiology, costs, and risk of foodborne disease, In: Cliver, D.O. and Riemann, H.P. (eds.) *Foodborne diseases*. 2nd Edition. Academic Press, An imprint of Elsevier Science. pp. 31-51.

Stern, N.J. and Robach, M.C. 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. In: *Journal of Food Protection*, 66, 1557-1563.

Teuber, M. 2004. Veterinary use and antibiotic resistance. In: Smulders, F.J.M. and Collins, J.D. (eds.) *Food safety assurance and veterinary public health, Volume 2, Safety aasurance during food processing*, Wageningen Academic Publishers, The Netherlands, pp. 229-241.

Todd, E.C.D. 1997. Epidemiology of foodborne diseases: a worldwide review. In: *World Health Statistics Quarterly*, 50, 30-50.

Van Looveren, M., Daube, G., De Zutter, L., Dumont, J-M., Lammens, C., Wijdooghe, M., Jouret, M., Cornelis, M. and Goossens, H. 2001. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. In: *Journal of Antimicrobial Chemotherapy*, 48, 235-240.

Van Nierop, W., Duse, A.G., Marais, E., Aithma, N., Thothobolo, N., Kassel, M., Stewart, R., Potgieter, A., Fernandes, B., Galpin, J.S. and Bloomfield, S.F. 2005. Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. In: *International Journal of Food Microbiology*, 99, 1-6.

Wesley, I. 2009. Public health impact of food-borne illness: impetus for the international food safety effort. In: Heredia, N., Wesley, I. and Garcia, S. (eds.) *Microbiologically safe foods*. John Wiley & Sons, Inc. pp 3–13.

WHO. 2004. *1-st joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: Scientific assessment, Geneva, 1-5 December 2003*. http://www.who.int/foodsafety/publications/micro/nov2003/en/

Wirtanen, G., Storgards, E. and Mattila-Sandholm, T. 2003. Biofilms. In: Caballero, B., Trugo, L. and Finglas, P. (eds.) *Encyclopedia of food science and nutrition*. London: Academic Press. pp. 484-489. ISBN: 0-12-227055-X.

Further Reading:

Fries, R 2002. Reinigung und Desinfektion in der Fleischwirtschaft. In: Strauch, D. and Böhm, R. *Reinigung und Desinfektion in der Nutztierhaltung und Veredelungswirtschaft*. Stuttgart: Verlag Enke S. pp. 239-267

Gray, J.T. and Fedorka-Cray, P.J. 2002. Salmonella, In: Cliver, D.C. and Riemann, H.P (eds.), *Foodborne diseases*, Second edition, Academic Press, California, USA, pp. 55-68.

Park, S.F. 2002. The physiology of Campylobacter species and its relevance to their role as foodborne pathogens. In: *International Journal of Food Microbiology*, 74, 177-188.

Perko-Mäkelä, P., Isohanni, P., Katzav, M., Lund, M., Hänninen, M-L. and Lyhs, U. 2009. A longitudinal study of Campylobacter distribution in a turkey production chain. In: *Acta Veterinaria Scandinavica*, 51:18.

Chapter 35

Annan, K. 2000. *'We the Peoples' The Role of the United Nations in the 21st Century*. Millenium Report of the Secretary-General. United Nations, New York, NY, US.

Ashbolt, N., Grabow, W.O. and Snozzi, M. 2001. Indicators of microbial water quality, p. In: Fewtrell, L. and Bartram, J. (eds.) *Water*