BACTERIAL FOODBORNE PATHOGENS OF CONCERN

Mati Roasto[1], Berit Reinmüller[2], Raivo Vokk[3], Ayşe Handan Dinçer Baysal[4], Julijana Polanc[5], Tiina Veskus[3], Kadrin Juhkam[1], Margarita Terentjeva[6] & Elżbieta Maćkiw[7]
[1] Estonian University of Life Sciences, Tartu, Estonia
[2] KTH, Stockholm, Sweden
[3] Tallinn University of Technology, Tallinn, Estonia
[4] İzmir Institute of Technology, İzmir, Turkey
[5] Fructal, Ajdovscina, Slovenia
[6] Latvian University of Agriculture, Jelgava, Latvia
[7] National Food and Nutrition Institute, Warszawa, Poland

INTRODUCTION

A high level of protection of public health is one of the fundamental objectives of food law as laid down in regulations (EC) No 178/2002 and 852/2004. Throughout the European Union (EU) consumers are requiring the food industry to provide them with an increasing range of safe, nutritious and healthy foods of high sensory quality and increased shelf life. To meet the demand for healthier food of high sensory quality, the use of additives and preservatives is being reduced or eliminated and minimal processing techniques introduced. To increase food safety and quality considerable amount of time, effort and money has been spent to food safety control and management (ISO 22000:2005) systems including better packaging methods and improved new pathogen detection methods. Nevertheless there is still little sign within official statistics of significant reductions in the incidence of foodborne illnesses within EU countries. Todd (1997) reported that in the beginning of this decade 73 to 100% of all European outbreaks with known aetiology were caused by bacteria. Particular priority areas are species such as *Listeria monocytogenes, Campylobacter jejuni, Salmonella,* and *Escherichia coli* O157:H7. Biofilm formation and other problems in production environment have been in focus lately. Wirtanen *et al*. (2003) reported that pathogens such as *Listeria*

monocytogenes, *Salmonella* Typhimurium, and *Yersinia enterocolitica* can readily produce biofilms, causing severe disinfection and cleaning problems on surfaces in the food industry. Yeasts belonging to *Saccharomyces*, *Candida*, and *Rhodotorula* have been related with contamination of production environment as well as bacteria like *Legionella pneumophila*, *Pseudomonas* spp., *Gallionella* spp., and fungi of the *Aspergillus*, *Mucor*, and *Penicillium* strains (Wirtanen *et al*., 2003). The aims of the present report are to concisely discuss the most important bacterial pathogen contaminants with respect to associated foods, contamination sources and routes. Results of certain studies in some project related countries and specific aspects of the new European Food Law and the future needs in scientific and industrial research are emphasized.

BACTERIAL FOODBORNE PATHOGENS

LISTERIA MONOCYTOGENES

L. monocytogenes is a Gram-positive and motile bacterium that is commonly present in the environment and occurs in almost all food raw materials from time to time. According to current knowledge the genus *Listeria* contains six clearly distinguishable species (McLauchlin, 2006). The most commonly occurring species in food are *L. monocytogenes and L. innocua,* however *L. monocytogenes* is the only 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 found in 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). However, the poultry meat is contaminated during slaughtering and processing (Rørvik *et al*., 2003). It can be isolated from water, soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. (Johansson, 1999; EuropeAid, 2004). *L. monocytogenes* is transmitted via three main routes: contact with animals, cross-infection of newborn babies in hospital and foodborne infection. The latter two sources result in the majority of cases of listeriosis in humans. Listeriosis is an uncommon but the serious foodborne disease that can be life-threatening to the elderly, people with weakened immune system and pregnant women (Lyytikäinen *et al*., 2000; Frye *et al.,* 2002).

Associated foods: *L. monocytogenes* has been associated with food sources such as raw milk, supposedly pasteurised fluid 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). Its ability to grow at temperatures as low as 3 °C permits multiplication in refrigerated foods (Roasto, 2004). It can survive or even grow at pH values as low as 4.4 and at salt concentrations of up to 14%.

In the study of Praakle *et al*. (2007) a total of 240 raw broiler legs (120 of Estonian and 120 of foreign origin) from 12 retail stores in two biggest cities (Tallinn and Tartu) of Estonia were investigated from January to December 2002. Of the raw broiler legs, 70% were 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 *et al*. concluded 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 risk of transmission of *L. monocytogenes* (Farber and Peterkin, 1991). Prevalence of *L. monocytogenes* in cold smoked, sliced, vacuum packaged pork products during 15-month period from 2003 until 2004 was studied by Bērziņš *et al*. Samples originated from 8 Latvian and 7 Lithuanian manufacturers. The prevalences of *L. monocytogenes* in cold-smoked pork varied from 0 to 67% in Latvian products and 10 to 73% in Lithuanian products (Bērziņš *et al*., 2007). In order to identify the main risk factors associated with *L. monocytogenes* contamination, all production steps were studied separately in each meat processing plant. Bērziņš *et al*. suggested that brining by injection was a significant ($P < 0.05$) factor in contamination. Moreover, long cold-smoking times (12 h) had a significant ($P < 0.014$) predictive value for a sample to test positive for *L. monocytogenes*. The cold-smoking temperatures between 24 and 30 °C can provide an inhibitory effect on presence of *L. monocytogenes*. Low number of *L. monocytogenes* at the end of shell-life (< 100 cfu/g) can be explained by use of starter cultures during processing, which have an antilisterial effect, and affect multiplying of *L. monocytogenes* in pork products.

It is recognised that presence of *L. monocytogenes* in almost all raw foods cannot be completely eliminated, but through the application of effective hygienic measures, it is possible to reduce its incidence and levels 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 as to reliably control the growth of *Listeria monocytogenes* and to prevent from multiplying to potentially harmful levels, >100/g (Commission Regulation, 2005).

CAMPYLOBACTER JEJUNI

Campylobacter jejuni is a Gram-negative slender, curved, non-sporing motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen. It is relatively fragile, and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, and acidic conditions). Because of its microaerophilic characteristics the organism requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth conditions. This bacterium is now recognized as an important enteric pathogen. Before 1972, when methods were developed for its isolation from faeces, it was believed to be primarily an animal pathogen causing abortion and enteritis in sheep and cattle. Surveys have shown that *Campylobacter* spp. is the most common registered bacterial causes of human intestinal infections in many developed countries (Hänninen *et al*., 2003). *Campylobacter jejuni* subsp. *jejuni* and *C. coli* are the main cause of *Campylobacter* enteritis in human (Nachamkin and Blaser, 2000). *C. jejuni* is responsible for 80–90% of campylobacteriosis. It causes more disease than *Shigella* spp. and *Salmonella* spp. combined (Nachamkin and Blaser, 2000). Although *C. jejuni* is not carried by healthy individuals in the US or Europe, it is often isolated from healthy cattle, chickens, birds and even flies. It is sometimes present in non-chlorinated water sources such as streams and ponds. In industrialized countries, including Western Europe, US, Canada, Australia and New Zealand, the rate of human *Campylobacter* infections has been increasing steadily. In 2004 a total of 183 961 human cases of campylobacteriosis were reported from 25 Member States of European Union. The Community incidence was 47.6 cases per 100 000 population. (EFSA, 2006.) 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 result of foodborne transmission (Bhaduri and Cottrell, 2004).

Associated foods and environment: *Campylobacter* spp. is widespread in nature, not only in wildlife but also among food animals such as cattle, sheep, swine, and avian species as commensally organisms (Friedman *et al*., 2000). The avian species are the most common host for *Campylobacter*, probably because of their higher body temperature (Skirrow, 1977). Monitoring studies indicate that most chicken flocks are colonised with *C. jejuni*. Intestinal colonisation usually leads to contamination of the final product, which cannot be prevented in the processing plant.

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 and Robach, 2003). *Campylobacter* spp. colonization in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalences ranking from 18 to over 90%, with northern countries showing a lower proportion of positive flocks (Barrios *et al*., 2006). A recent monitoring study in Poland showed that 75.4% of chicken carcasses is contaminated with *Campylobacter* species. It is well established that poultry products are a vehicle for foodborne campylobacteriosis and they are suspected to be an important source of infection (Roasto *et al*., 2005).

Other foods (mainly of animal origin) must be considered as potential sources of infection. *Campylobacter* have also been isolated from such food items as raw milk, pork, beef, lamb, and seafood (Duffy *et al*., 2001). The presence of *Campylobacter* spp. in raw materials and products of animal origin may represent a source of infections, however, 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 hygienic 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).

Effective quality-control programme in Estonian large-scale poultry processing plant accounted for the lower contamination levels of fresh chicken meat compare to contamination level with the same type of products of 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. All chicken meat samples from market halls were sold fresh and unpacked. Of the raw chicken products of Estonian origin, 15.8% were 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 originated from the large-scale company (6.3%) ($P<0.001$). In order to reduce the incidence of campylobacteriosis in humans a number of preventive measures are needed throughout the way from farm to table.

SALMONELLA

Salmonella spp. are facultatively anaerobic, Gram-negative, straight, non-spore-forming small rods, which are usually motile with peritrichous flagella. *Salmonella* is a genus within the family *Enterobacteriaceae* in which approximately 2200 serotypes are recognised. Some of these strains are specifically adapted to hosts and largely restricted to them, e.g. *S.* Typhi in man and *S.* Dublin in cattle. The growth range for salmonellae is 5–47 °C at pH 4.0–9.0, with optimum growth at 35–37 °C and pH 6.5–7.5. Salmonellae are not particularly salt-tolerant, although growth can occur in the presence of 4% sodium chloride. The lower limit of water activity (a_w) permitting growth is 0.93 (Mead, 1993).

Associated foods: A wide variety of foods have been implicated in outbreaks of illness caused by many different 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, and chocolate. Various *Salmonella* serotypes have long been isolated from the outside of eggshells. The present situation with *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* still is 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. This is true especially considering the most important meats from pig and poultry. In areas such as Scandinavia measures against this pathogen have been traditionally more thoroughly endeavoured, finally resulting in a lower prevalence of *Salmonella* in these

countries compared to Continental Europe (EuropeAid, 2004). 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, consumer handling.

ESCHERICHIA COLI O157:H7

E. coli is a facultatively anaerobic, non-spore forming, Gram-negative rod within the family *Enterobacteriaceae.* They form part of the natural gastro-intestinal microflora of man and warm-blooded animals. Because many microbes from faeces are pathogenic in animals and humans, the presence of the intestinal bacterium *E. coli* in water and foods indicates a potential hygiene hazard. Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. Although most *E. coli* are harmless commensal organisms, there are many pathogenic strains capable cause a variety of illness in humans. There are six recognized groups of pathogenic *E. coli* (EPEC, ETEC, EIEC, EaggEC, EHEC, NTEC). Each group has different virulence traits and mechanisms of pathogenity (Duffy, 2006). Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins (verotoxin (VT), shiga-like toxin) are closely related or identical to the toxin produced by *Shigella dysenteriae*.

Associated Foods: Undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks, however *E. coli* O157:H7 outbreaks have implicated alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, game meat, and cheese curds. Raw milk was the vehicle in a school outbreak in Canada.

LEGISLATION

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs constitutes that foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. 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 (hazard analysis and critical control points) based procedures and other hygiene control measures. According to Article 4 of 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 Regulation (EC) No 2073/2005 is laying down specific rules for testing and sampling, according to which the ISO standard 18593 shall be used as a reference method. Food business operators manufacturing ready-to-eat foods, which may pose a *L. monocytogenes* risk for public health, shall 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 the Commission Regulation (EC) No 2073/2005 for microbiological criteria for foodstuff.

FUTURE NEEDS

There is research need for more sensitive, reliable, and cost-effective tools, particularly sampling methodologies, for analysing food and environmental samples (*e.g.,* high priority commodities include produce, eggs, and seafood) for microbial pathogens, where frequency and extent of contamination are expected to be low, for identification and evaluation of relevant characteristics of different forms of product packing and handling on the safety of a variety of foods. Developing modelling techniques to assess microbial behaviour in various foods, human exposure and dose-response relations to certain foodborne pathogens (*e.g.,* enumerative detection methods for pathogens), potential risk of those pathogens causing human illness, and the setting of safety performance standards to regulate microbial content of food, determining the population trends with respect to food safety knowledge, attitudes, and practices, especially

behaviours that may be significant risk factors for foodborne illness (*e.g.,* food consumption, in-home food preparation and handling) are the other aspects needed research. The microbiological safety of food has been advanced substantially by the introduction and implementation of HACCP. 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 foodborne pathogens. There is a need to re-examine how food is produced, processed, marketed, and prepared to identify conditions that contribute to emergence. For example, organic acids are used extensively throughout the food industry to control spoilage and pathogenic microorganisms (Baysal and Unluturk, in press).

The changing epidemiology of foodborne disease calls for improved surveillance including rapid sub-typing methods, cluster identification, and collaborative epidemiological investigation (including case-control studies). Also examined was the need for better integrated, coordinated, and standardized animal disease surveillance and health monitoring programs. The new problems of foodborne 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 foodborne 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., DNA hybridisation and polymerase chain reaction) to examine emerging foodborne 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.

CONCLUSION

It is a long way from the new borne food animal to the consumer's table. On one hand, there exist several stages independent from each other, where the persons involved do not always contact. On the other hand, there are a lot of

circumstances and 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 the primary production, immunisation, logistic slaughter or measures in cleaning and disinfection the site. The horizontal and vertical transfer of pathogens must become under tighter control: the routes of the agent via transport to the abattoir are not at all safe. There is not point, where *Salmonella* or other pathogens would be safely prohibited to invade the human food chain. It is obvious, that the inspection service by the authorities cannot afford the total of surveillance in every production process. The hygienic 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 practice and checks must be carried out frequently. As a consequence the authorities have to rely more in the responsibility of the plant. So the role of the authorities is presently in reconsideration 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 husbandry, which possibly demands more personnel in the future. It should be emphasized that the producer is responsible for the product and should do everything to guarantee it.

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