



**A preliminary risk assessment of *Yersinia enterocolitica* in
the food chain:
some aspects related to human health in Norway**

Norwegian Scientific Committee for Food Safety

Panel on Biological Hazards

CONTENTS

Summary	3
Background	4
Scope.....	4
Hazard identification	4
Hazard characterisation.....	5
Isolation, identification and epidemiological typing.....	6
Characteristics of the disease	8
Acute intestinal infections.....	8
Extra-intestinal and systemic infections.....	8
Post-infectious sequelae	8
Antimicrobial resistance.....	9
The public health problem in Norway and the Nordic region	10
Exposure assessment	12
Transmission via food.....	12
Some aspects of <i>Y. pseudotuberculosis</i>	13
Prevention and control	13
At the farm level.....	14
Slaughter hygiene and meat inspection.....	15
Packaging.....	16
Water.....	17
Some general hygiene aspects	17
Risk assessment needs and questions.....	17
Major data gaps	17
Conclusions.....	18
References	19

Summary

This preliminary risk assessment is a result of self-tasking by the Panel on Biological Hazards, Norwegian Scientific Committee for Food Safety. The suggestion was offered to the Norwegian Food Safety Authority (Mattilsynet), which responded and requested a risk profile, or a preliminary risk assessment, to evaluate whether a full risk assessment would be needed at a later date.

Yersinia enterocolitica is one of a few zoonotic bacteria that have a stable reservoir within the domestic animal population in Norway. This bacterial species has been isolated from human patients with acute enteritis, who sometimes exhibit symptoms resembling appendicitis. *Y. enterocolitica* has attracted considerable attention due to its ability to cause serious post-infectious complications. Serious clinical consequences occur relatively often with *Y. enterocolitica* as a relatively high frequency of people in Norway possess the tissue type HLA-B27. A severe sequela linked to this tissue type is reactive arthritis. The cold climate in Norway may enhance growth of *Y. enterocolitica*. Although the predominant cause of yersiniosis in Norway is *Y. enterocolitica* O:3, and the pig is considered the main source of infection, the relative contribution of pork consumption compared with other risk factors, for example drinking untreated water, is unknown. In Norway, a decline in human cases of yersiniosis has been recorded since the beginning of the 1990s. This decline has been attributed to implementation of improved slaughtering methods, including enclosure of the anus into a plastic bag after rectum-loosening. In Norway, most fattening pigs are slaughtered at the age of 150 to 180 days. By this age the tonsils may be an even more significant source of human pathogenic *Y. enterocolitica* than intestinal contents, since the occurrence in the intestinal tract and faeces is reduced at the time of slaughter. Accordingly, hygienic handling of the head and the plucks during slaughter and dressing is very important to avoid contamination of the carcass. The most efficient way to limit the spread from tongue and tonsils is probably decapitation early on in the carcass dressing procedure. In such a procedure, the head, including tongue and tonsils, should be removed on a separate line. Also, avoidance of incision of the sub-maxillary lymph nodes might reduce the spread. Epidemiological data suggest that it is possible to reduce the herd prevalence of *Y. enterocolitica* O:3 by minimising contact between infected and non-infected herds. Further, attempts to reduce the prevalence at the top levels of the breeding pyramids may be beneficial for the industry as a whole. The meat industry might be able to categorise herds using serological methods, and use these results in its strategy to reduce the risks for consumers. However, such a strategy has to be evaluated in a cost benefit context. The apparently low prevalence of pathogenic *Y. enterocolitica* in food may be due to lack of suitable selective methods. The culturing methods, which are used routinely in microbiological laboratories, are insufficiently sensitive. There is a need for a standardised DNA-based technique, with improved sensitivity, for the detection of *Y. enterocolitica* in clinical, food and environmental samples.

Background

The document presented here is a result of self-tasking by the Panel on Biological Hazards, Norwegian Scientific Committee for Food Safety. The suggestion was offered to the Norwegian Food Safety Authority, which responded and requested a risk profile, or a preliminary risk assessment, to evaluate whether a full risk assessment would be needed at a later date.

The risk profile contains several features of an interim qualitative risk assessment based on the available data at the time. According to the Codex Alimentarius, Codex Committee on food safety (CODEX COMMITTEE ON FOOD HYGIENE 2005), the risk profile is a decision making tool that

- presents the current state of knowledge related to a food safety issue,
- describes various potential microbiological risk management options that have been identified to date, and
- will influence further possible options in a food safety policy context.

The New Zealand Food Safety Authority has performed a number of risk profiles for various microorganisms in various food products e.g. *Bacillus* spp. in rice, *Campylobacter jejuni/coli* in poultry, Shiga toxin-producing *Escherichia coli* in red meat and meat products, Shiga toxin-producing *Escherichia coli* in uncooked comminuted fermented meat products, *Listeria monocytogenes* in ice cream, *L. monocytogenes* in processed ready-to-eat meats, *Mycobacterium bovis* in milk, Norwalk-like virus in mollusca (raw), *Salmonella* (non-typhoid) in poultry (whole and pieces), *Toxoplasma gondii* in red meat and meat products, *Vibrio parahaemolyticus* in seafood and *Y. enterocolitica* in pork (www.nzfsa.govt.nz/science-technology/risk-profiles/index.htm?print). To our knowledge, Codex Alimentarius Commission is the only other organization that has published a risk profile. That document addressed *Enterobacter sakazakii* in powdered infant formula (Codex Committee on Food Hygiene and Codex Alimentarius commission 2003).

Scope

The purpose of this preliminary risk assessment of *Y. enterocolitica* in the Norwegian food chain is to provide a discussion paper that lays out the key elements of microbial risk management concerns in order to facilitate decision-making on the part of the risk manager (the Norwegian Food Safety Authority). This preliminary risk assessment presents:

- the current state of knowledge related to *Y. enterocolitica* in the food chain and some aspects related to human health in Norway, and
- Discuss possible actions in the food chain.

Hazard identification

Yersinia forms a genus within the family *Enterobacteriaceae*. The cells are small rods, sometimes coccoid in shape, and Gram-negative. *Y. enterocolitica* has been divided into more than 70 serovars (Wauters *et al.* 1991), of which only a few have been conclusively associated with human or animal disease. *Y. enterocolitica* has been the focus of growing interest during the past couple of decades. Worldwide this

bacterial species has been isolated from human patients with acute enteritis, who sometimes exhibit symptoms resembling appendicitis. *Y. enterocolitica* has attracted considerable attention due to its ability to cause serious post-infectious complications. The organism has been isolated from humans in many countries of the world, but it seems to be found most frequently in cooler climates. In developed countries, *Y. enterocolitica* can be isolated from 1 - 4 % of all human cases of acute enteritis. Worldwide, there appears to have been a real and general increase in incidence during the past 30 years (Bottone, 1999; Tauxe, 2002). However, in Denmark, Norway and Sweden, the incidence of yersiniosis has decreased over the last 10 – 15 years. In many countries, *Y. enterocolitica* is not routinely looked for by medical laboratories and is therefore likely to be under-diagnosed (Fredriksson-Ahomaa and Korkeala, 2003).

Hazard characterisation

Simplistically, *Y. enterocolitica* may be divided into three groups according to clinical significance; each group comprises different serovars:

The human pathogens serovars O:3, O:5,27, O:8, and O:9 are the most important causative agents in man (Bottone 1999). Although other serovars may occasionally cause infection, these variants are completely dominant.

The animal pathogenic strains also belong to particular serovars. O:2 has been associated with disease in goats, sheep, and hares, while O:1 caused widespread epizootics among chinchillas in the early 1960s. With few exceptions, O:1 and O:2 have not been implicated in human disease.

The environmental strains usually lack clinical significance and comprise a wide range of variants, which are ubiquitous in terrestrial and freshwater ecosystems. A number of closely related *Yersinia* species are also frequently encountered in nature (*Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia*, *Y. aldovae*, *Y. rohdei*, *Y. mollaretii*, and *Y. bercovieri*), all of which are apathogenic (Bercovier and Mollaret 1984).

There are appreciable geographic differences in the distribution of the pathogenic serovars. O:3 is the most widespread in most parts of the world, including Europe, Japan, and Canada. Previously, the most frequently reported variants in the United States were O:8 followed by O:5,27. In recent years, serovar O:3 has been on the increase in the United States and now accounts for the majority of isolates in certain states.

Y. enterocolitica is able to multiply at temperatures approaching 0°C, which means that it can grow in properly refrigerated foods. However, some results indicate that *Y. enterocolitica* competes poorly with other psychrotolerant organisms. *Y. enterocolitica* can survive in frozen foods for long periods. The heat resistance, salt tolerance, and pH tolerance are comparable to that of other *Enterobacteriaceae*. The bacteria are inactivated during pasteurisation processes or normal cooking at boiling, baking, and frying temperatures. Heat-treatment of milk and meat products at 60° C for 1-3 min effectively inactivates the bacteria (Lee *et al.* 200). The minimum pH for growth is in the range 4.2-4.8, depending on temperature and the acidulant. Maximum pH for growth is around 10.0, and optimum is 7.2-7.4.

Y. enterocolitica causes enteritis by adherence to, and penetration of, the epithelial cells in the terminal ileum, followed by invasion of the intestinal mucosa, and

multiplication in the lymphoid tissue of the intestine. *Y. enterocolitica* undergoes a temperature adaptation in the human host prior to the initiation of an infectious process. To achieve this, *Y. enterocolitica* uses both chromosomal and plasmid associated virulence determinants that are temperature-dependent (Table 1). Virulent strains harbour a particular plasmid (size 40-50 megadaltons). The plasmid encodes a series of proteins, several of which are important virulence determinants. At least two chromosomal gene loci are also necessary for expression of virulence.

TABLE 1. *Y. enterocolitica* chromosomal and plasmid-encoded virulence determinants operative in establishing gastrointestinal infections (Bottone 1999)

Genomic origin	Determinant	Function	Expressed temperature
Chromosomal			
<i>Inv locus</i>	Invasion	Attachment + invasion	28°C
<i>Ail locus</i>	AiL	Attachment + invasion; serum resistance	37°C
<i>yst locus</i>	Yst (enterotoxin)	Fluid secretion in intestine	28°C
<i>Hem</i>	Hem R and other proteins	Haem receptor- removes iron bound to haem proteins	37°C
<i>Irp2</i>	HMWP 1 and 2	Synthesized under iron starvation by high pathogenicity strains involved in iron or siderophore uptake	37°C
Plasmid			
<i>Yad loci</i>	YadA	Attachment + invasion	37°C
	YopH	Resistance to pathogenesis by macrophages, phosphorylation of host cell proteins	37°C
	YopB	Suppresses tumour necrosis factor α . Evasion of immune and inflammatory responses	37°C
	YopE	Translocated into target cell at zone of contact between <i>Y. enterocolitica</i> and eukaryotic cell; leads to cytotoxicity	

Isolation, identification and epidemiological typing

Diagnosis of *Y. enterocolitica* infection is best achieved by isolation of the bacteria from clinical specimens from infected individuals (Fredriksson-Ahomaa and Korkeala 2003). By using antigens prepared from purified plasmid-encoded outer-membrane proteins of *Y. enterocolitica*, serology as a diagnostic tool has become more specific.

In food one can expect to find a broad spectrum of *Yersinia*, the vast majority of which are of no medical importance. However, the development of isolation media and procedures that clearly differentiate pathogenic from non-pathogenic variants has been difficult. Lack of proper selective methods may underestimate prevalence rates of pathogenic *Y. enterocolitica* (Fredriksson-Ahomaa and Korkeala 2003). The threshold for detection of pathogenic *Y. enterocolitica* has been estimated to be 10^3 - 10^6 CFU or higher per g faeces or pork samples. Thus, the culturing methods are relatively insensitive. Non-pathogenic *Y. enterocolitica* has the same appearance as pathogenic strains, therefore selection of the relevant colonies for further confirmation can be difficult (Fredriksson-Ahomaa and Korkeala 2003).

A number of isolation procedures are currently in use. Most methods require time-consuming resuscitation and enrichment, and no single method provides optimal isolation of all pathogenic serovars. However, Wauters *et al.* (1988) developed an efficient method for isolation of serovars O:3 (in particular) and O:9 from meat and meat products. The procedure is based on a two-day selective enrichment period in irgasan-ticarillin-potassium chlorate (ITC) enrichment broth at room temperature, and is therefore very timesaving compared with the many of the previous methods. This approach is now used in the International Organization for Standardization method (ISO 10273). Both Cefsulodin-irgasan-novobiocin (CIN) agar and modified Salmonella-Shigella agar with 1 % sodium deoxycholate and 0.1 % CaCl₂ (SSDC) agar are differential selective media that are more effective than routine enteric media for the recovery of *Y. enterocolitica* from food.

Identification of *Y. enterocolitica* is based on cultural-biochemical characterization, including biotyping {Wauters, 1987}. Serotyping is conducted by slide agglutination against specific O-antigen sera (Wauters *et al.* 1991). Since the majority of strains capable of causing disease belong to only a few serovar-biovar combinations, serotyping and biotyping are sufficient to differentiate pathogenic strains from non-pathogenic ones for practical purposes. In addition, a series of *in vitro* virulence assays has been described. DNA-based methods, including PCR, enable rapid, sensitive, and specific detection of all pathogenic variants. However, probes have a limited application in modern microbiological laboratories.

To investigate the association between *Y. enterocolitica* and pigs in Norway, 152 raw and cooked pork products were examined (Nesbakken *et al.* 1985). The results indicated that *Yersinia* spp. are more likely to be isolated from food with a high level of coliforms than from food with low coliform counts. Only one strain of O:3 / biovar 4, which is the predominant human pathogen in Norway, was isolated. The sensitivity of conventional isolation techniques (Nordic Committee on Food Analysis 1987, Wauters *et al.* 1988) and a colony DNA hybridization was compared for the detection of *Y. enterocolitica* in samples of raw pork products in Norway (Nesbakken *et al.* 1991). The results of this investigation (Nesbakken *et al.* 1991) support the supposition that conventional culture methods result in the occurrence of virulent *Y. enterocolitica* in pork products being underestimated.

PCR, single or multiplex, using primers from virulence-associated genes like *ail*, *inv*, *yst*, *virF* has been shown to increase the sensitivity in detecting virulent *Y. enterocolitica* in foods compared to the traditional culture methods (Fredriksson-Ahomaa and Korkeala 2003). However, PCR is seldom used as a routine diagnostic tool for detection of *Y. enterocolitica*, although one PCR assay showed a high sensitivity for detection of pathogenic *Y. enterocolitica* in pork samples as compared with culturing technique (Johannessen *et al.* 2000).

A number of methods like biotyping, serotyping, antibiogram typing, phage typing, multi-locus enzyme electrophoresis, restriction enzyme analysis of plasmid DNA or chromosomal DNA, and pulsed-field gel electrophoresis (PFGE) have been used to differentiate pathogenic *Y. enterocolitica* for epidemiological purposes (Nesbakken 2000). However, none of these methods (PFGE included) demonstrate sufficient discriminatory power for differentiation of serovar O:3. Although PFGE has identified several pulsotypes among O:3/biovar 4 strains, most of the strains belong to one or

two dominant pulsotypes (Asplund *et al.* 1998; Buchrieser *et al.* 1994; Fredriksson-Ahomaa *et al.* 1999; Najdenski *et al.* 1994; Saken *et al.* 1994).

During the last ten years, multi-locus variable–number tandem repeats analysis (MLVA) has been used for molecular typing of several bacterial species, including *Y. pestis* (Klevytska *et al.* 2001; Pourcel *et al.* 2004). The availability of the whole genome sequences of *Y. enterocolitica* has enabled the development of MLVA as an epidemiological tool for this bacterial species.

Characteristics of the disease

Illness caused by *Y. enterocolitica* is referred to as yersiniosis. *Y. enterocolitica* is associated with a spectrum of clinical syndromes in man (Table 2) (Bottone, 1999; Ostroff *et al.* 1992):

Acute intestinal infections

Acute, non-complicated enteritis is by far the most frequently encountered manifestation. In 3-15% of cases, the infection causes mesenteric lymphadenitis, terminal ileitis, or both, which results in symptoms resembling appendicitis. The incubation time for *Y. enterocolitica* enteritis ranges from 1 to 11 days and clinical disease typically persists for 1 to 2 weeks, but may occasionally last for several months. The minimum infective dose has not yet been determined. The organism may be excreted in the stools for a long period after symptoms have resolved. It is generally unnecessary to treat acute, non-complicated enteritis with antibiotics. However, patients with systemic or extra-intestinal infections should be treated.

Extra-intestinal and systemic infections

Septicaemia and localized extra-intestinal infections are rare manifestations that are almost exclusively seen in patients with underlying illness for such cases, therapy with doxycycline or trimethoprim-sulphamethoxazole has been recommended.

Post-infectious sequelae

Although a range of post-infectious sequelae has been reported, reactive arthritis and cutaneous manifestations like erythema nodosum are the most common. The two latter complications occur mainly in adults and are caused by serovars O:3 and O:9. Reactive arthritis following *Y. enterocolitica* infection typically persists for 1- 4 months, but follow-up studies indicate that prolonged symptoms may occur in a significant proportion of cases. These possible consequences make *Y. enterocolitica* infection a public health and economic problem of greater magnitude than the actual number of recorded cases would suggest.

The ability of *Y. enterocolitica* to proliferate at low temperatures poses a problem in blood transfusion, if it is present during transient bacteraemia in blood donors, it may multiply in blood products stored at 4°C and produce septic shock upon transfusion (Mollaret *et al.* 1979).

The persistence of certain bacterial antigens in the host and the ability of some of them to result in a prolonged antibody response is a central issue in the pathogenesis of *Yersinia*-induced arthritis. Reactive arthritis during yersiniosis is more common in people who are HLA (human lymphocyte antigen)-B27 positive. This tissue type is common in the Nordic population (Ostroff *et al.* 1992). Bacterial lipopolysaccharide (LPS) is found in the synovial fluid and synovial membranes of

patients with *Yersinia*-induced reactive arthritis (Granfors *et al.* 1989), and these patients have a vigorous and persistent antibody response to LPS (Laesmaa-Rantala *et al.* 1989). This has the potential to provide a localised mitogenic stimulus for the B cells.

TABLE 2. Spectrum of *Y. enterocolitica* infections (Bottone 1999)

Type of infection	Manifestation/population
Gastrointestinal	Enterocolitis: predominantly in young children; concomitant bacteraemia may also be present in infants Pseudoappendicitis syndrome (children older than 5 years; adults) Acute mesenteric lymphadenitis Terminal ileitis
Septicaemia	Especially in immunosuppressed individuals and those in iron overload or being treated with deferoxamine Transfusion related (usually leads to septic shock syndrome)
Metastatic	Focal abscesses; liver, kidney, spleen, lung Cutaneous manifestations; cellulites, pyomyositis, pustules, and bullous lesions Pneumonia, cavitary pneumonia Meningitis Panophthalmitis Endocarditis, infected myotic aneurysm Osteomyelitis
Post-infection sequelae	Arthritis (associated with HLA-B-27), myocarditis, glomerulonephritis, erythema nodosum
Pharyngitis (common after oral ingestion of <i>Y. enterocolitica</i>)	

Antimicrobial resistance

In Norway, all *Yersinia* isolates are examined for resistance against antimicrobial agents. The prevalence of resistance against antimicrobial agents in Norway has been quite stable between 2001-2003 (NORM and NORM-VET 2004). Resistance determination of human clinical isolates in 58 isolates of *Y. enterocolitica* O:3 against tetracycline, chloramphenicol, ampicillin, Trimethoprim/sulphamethoxazole, ciprofloxacin and nalidixic acid showed that none of the isolates were resistant against ciprofloxacin. Two isolates were classified as resistant against nalidixic acid and one of these was also demonstrated intermediate susceptibility to ciprofloxacin. All isolates showed reduced susceptibility to ampicillin, which is considered as an intrinsic resistance in *Y. enterocolitica* serovar O:3 (NORM and NORM-VET 2004). One study concluded that antimicrobial treatment does not alter the course or duration of localized enteritis in children less than 6 years of age (Hoogkamp-Korstanje and Stolk-Engelaar 1995). Treatment of older children with trimethoprim-sulphamethoxazole may prevent some of the complications of *Y. enterocolitica* enteritis, e.g. pseudoappendicular syndrome, mesenteric adenitis, ileitis, and extramesenteric complications (Hoogkamp-Korstanje and Stolk-Engelaar 1995). Quinolones are the drugs of choice in immunocompromised patients where the potential for bacteraemia is a real threat.

The public health problem in Norway and the Nordic region

Y. enterocolitica may cause a variety of clinical syndromes in humans (Table 2). Yersiniosis in humans is a notifiable disease, under the Norwegian Surveillance System for Communicable Disease (MSIS) (www.msis.no). Verification and typing of isolates are carried out at The National Reference Laboratory at the Norwegian Institute of Public Health. In Norway and Denmark the occurrence of *Y. enterocolitica* is relatively low; it is higher in Sweden and highest in Finland. In Nordic countries, in 2003, the number of reported cases of yersiniosis was as follows: Norway 86 (1.9) (www.fhi.no), Denmark 245 (4.5) (Ministry of Food 2003), Finland 647 (12.4) (The National Public Health Institute 2003), and Sweden was 714 (National Veterinary Institute 2003). The number given in parentheses is the number of yersiniosis per 100,000 inhabitants. The majority of the infections in Norway were domestically acquired (Figure 1). Yersiniosis in Finland is caused by both *Y. enterocolitica* and *Y. pseudotuberculosis*. The latter species has been involved in three outbreaks during the last 10 years; 1998, 2001 and 2003 (Hallanvuori *et al.* 2003; Jalava *et al.* 2004; Nuorti *et al.* 2004).

The serovar most frequently involved in human disease in Norway is serovar O:3. The majority of cases of yersiniosis are sporadic and without identifiable source (Kapperud 1991). In 2004, only 43 (27%) of 158 yersiniosis cases were classified as imported. According to surveillance data for 1984 - 2004, the incidence of the disease was higher in males than females (1824 vs. 1626). Young children (age group 1-9 years old) were more frequently affected than other age groups. The reasons for this sex and age distribution require further investigation. The highest incidence is during the cold season. Although the majority of yersiniosis cases are sporadic, a few outbreaks of *Y. enterocolitica* have been reported in Norway (Jørgen Lassen and Jørn Weidemann, personal communication). There are also some examples of food-borne outbreaks in Sweden. A milk-borne outbreak occurred in Kristianstad in 1988 (Alsterlund *et al.* 1995) and was probably caused by recontamination of pasteurized milk due to lack of chlorination of the water supply, and 75 persons were infected with *Y. enterocolitica* O:3. In 1994, 13 persons were also infected with O:3 in Västernorrland (Swedish Institute of Infectious Disease control 1995). The source was unknown, but pork was probably the vehicle (B. de Jong, personal communication).

According to data from MSIS, the incidence of yersiniosis is similar in the different counties of Norway. A steady decline in human cases started in 1995 (Figure 1). The reason for this decline is probably improvement in slaughtering technique, including enclosure of the anus in a plastic bag after rectum loosening, and improved slaughter hygiene during slaughtering and dressing of pigs in the abattoirs in general (Nesbakken *et al.* 1994). This procedure reduces the risk of faecal contamination of carcasses. Most Nordic countries started to improve slaughter hygiene by implementation of the plastic bag technique during the period 1990 to 1995. However, in Finland the plastic bag technique was not implemented. This may have contributed to the level of yersiniosis being higher in Finland than in the other Nordic countries.

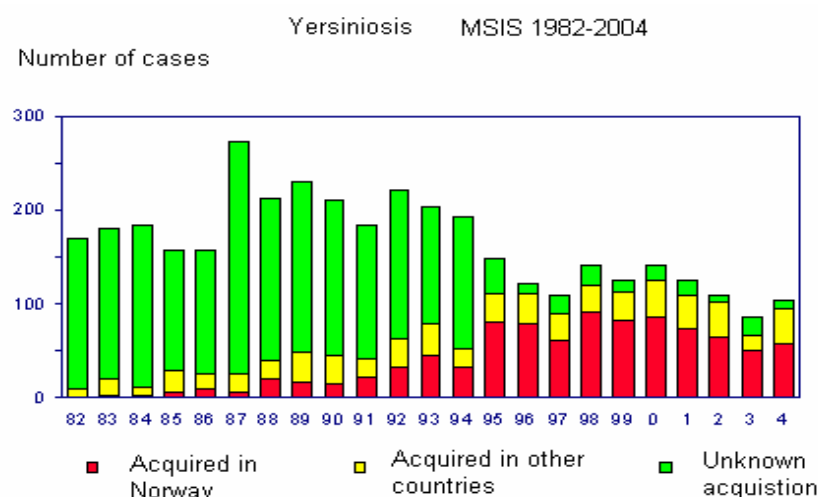


FIGURE 1. Laboratory-confirmed cases of *Y. enterocolitica* infection in Norway (both by bacteriological examination and detection of antibodies against *Y. enterocolitica*), Norwegian Surveillance System for Communicable Diseases, 1990-2004. From 1995, more detailed information on the place of infection was requested, resulting in a considerable drop in the number of patients from whom such data is lacking.

The importance of yersiniosis is further emphasised by its economic impact. Based on the data obtained during the Norwegian case-control study conducted in 1988-1990, it was estimated that the 275 expected annual cases would result in a minimum of 5681 days of illness, 316 days of hospitalisation, 493 physician consultations, and 1197 days lost from work. When long-term sequelae were included, at least 9535 days of illness and 2858 lost work days could be expected per year. The data presented were estimated minimum figures, since only the estimated number of culture-confirmed cases was taken into account.

A comparison between the incidence of yersiniosis in Norway and some other countries is presented in Table 3. Serovar O:3 is widespread in Europe, Japan, Canada, Africa and Latin America. Sometimes, but not always, phage typing enables distinction between European, Canadian and Japanese strains (Kapperud *et al.* 1990; Mollaret *et al.* 1979). Serovar O:3 seems to be responsible for more than 90% of the cases in Denmark, Norway, Sweden and New Zealand, and as many as 79% of the cases in Belgium (Table 3). In general, however, the data originating from different surveillance programmes, national statistics and even estimates are not directly comparable. According to Tauxe, the emergence of serovars O:3 and O:9 in Europe, Japan in the 1970s, and in North America by the end of the 1980s, is an example of a global pandemic (Tauxe 2002).

TABLE 3. Incidence of yersiniosis in some countries. Adapted from Nesbakken (2005a)

Country	Total number of cases (year)	Cases per 100,000 inh.	References
Verified cases			
Belgium	829 ¹ (1994)	8.5	(Ministere des Affaires Sociales 1995)
Denmark	245 ² (2003)	4.5	Danish Zoonosis Centre, Copenhagen www.dfvf.dk
Finland	647 (2003)	12.4	National Public Health Institute, Helsinki, www.ktl.fi
Germany	7113 (2001)	8.7	(Robert Koch Institute 1995)
Norway	86 ² (2003)	1.9	Norwegian Institute of Public Health, Oslo www.fhi.no
Sweden	714 ² (2003)	8	Swedish Institute for Infectious Disease Control, Stockholm www.smittskyddsinstitutet.se
Switzerland	95 (1993)	1.4	Swiss National Reference Laboratory for Foodborne Diseases, Berne
The European Union	7385 ³ (2000)		European Commission, Health & Consumer Protection Directorate-General, Brussels http://www.euro.who.int/hen/resources/eusanco/20030723_1
Estimated cases			
New Zealand	3,000 ² (1994)	84	(Wright J 1995)
United States	87,000 (1997)	33.4	(Mead <i>et al.</i> 1999)

¹ Serovar O:3: 78.8%; serovar O:9: 5.9%

² Serovar O:3: >90%

³ Figures from nine countries in the European Union

Exposure assessment

Transmission via food

Y. enterocolitica is frequently encountered in healthy animal carriers, among warm- and cold-blooded animals, in foods, and in the environment. However, the vast majority of the strains isolated from these sources are apathogenic variants. Pets may occasionally be faecal carriers, and raw pork might be an important source of *Y. enterocolitica* O:3 infections in dogs and cats (Frederiksson-Ahomaa *et al.*, 2001). These animals might be vehicles for infections in man (Frederiksson-Ahomaa *et al.*, 2001) but were not identified as risk factors in case-control studies (Tauxe *et al.*, 1987; Ostroff *et al.*, 1994). However, the pig is the only animal consumed by man, which regularly harbours the pathogenic serovars O:3 and O:9. In addition to being faecal commensals, these serovars inhabit the oral cavity of swine, especially the tongue and tonsils. As a result of present slaughter techniques, they are also frequently encountered as surface contaminants on freshly slaughtered pig carcasses. Pathogenic *Y. enterocolitica* have only infrequently been recovered from pork products at the stage of retail sale. This might be explained by the lack of appropriate selective methodology for isolation of pathogenic strains. Studies using DNA-based detection methods, including PCR, have indicated that such strains are more common in pork products than previously documented (Fredriksson-Ahomaa and Korkeala, 2003).

Epidemiological investigations have supported the role of pork as a vehicle for *Y. enterocolitica*. Case-control studies of sporadic cases conducted in Belgium (Tauxe *et al.* 1987) and Norway (Ostroff *et al.* 1994) have identified consumption of pork as an important risk factor for infection. Following a yersiniosis outbreak due to serovar O:3 among children in Atlanta, USA, a case-control study showed that household preparation of chitterlings (raw pork intestines), was significantly associated with illness.

In contrast to O:3 and O:9, serovar O:8 appears to be rare in swine. O:8 may have an entirely different reservoir and ecology. In Japan, small rodents have been identified as a reservoir for O:8. Outbreaks and sporadic cases due to this serovar have been traced to ingestion of contaminated drinking water, water used in manufacturing or preparation of food (e.g. bean sprouts, tofu), and milk products, which probably became contaminated subsequent to pasteurization. Consumption of untreated drinking water was also identified as a risk factor for infection with serovar O:3 in a case-control study conducted in Norway (Ostroff *et al.* 1994).

Some aspects of *Y. pseudotuberculosis*

Y. pseudotuberculosis is less ubiquitous than *Y. enterocolitica* and may be found in association with animals (wild animals as well as domestic animals), rarely from soils, water, and foods (Fukushima *et al.* 1989; Fukushima *et al.* 1991; Tsubokura *et al.* 1987; Tsubokura *et al.* 1989). Serologically, the *Y. pseudotuberculosis* isolates are classified into six groups, each serovar containing pathogenic isolates. Both chromosomal (Isberg *et al.* 1988) and plasmid encoded virulence factors have been identified in *Y. pseudotuberculosis* and are mainly similar to those harboured by *Y. enterocolitica*. The *inv* gene, which encodes for an invasion factor for mammalian cells, is homologous in *Y. pseudotuberculosis* and *Y. enterocolitica*. *Y. pseudotuberculosis*, like *Y. enterocolitica*, is also isolated most frequently in cooler climates (Aleksic *et al.* 1986). Despite *Y. pseudotuberculosis* being reported as a source of outbreaks in a Northern country (Finland) (Hallanvuori *et al.* 2003; Jalava *et al.* 2004; Nuorti *et al.* 2004), the bacterium has not been reported as a cause of foodborne illness, or other diseases, in Norway.

Prevention and control

Preventive measures, which reduce contamination and improve hygiene, during all stages of pig production and pork processing, are essential to reduce infection with serovars O:3 and O:9. The putative effects of preventive measures at different stages in the food chain are shown in Table 4.

TABLE 4. The putative effects of preventive action on occurrence of *Y. enterocolitica* in the food chain (+++=great effect, ++=good effect, +=limited effect, -=probably no effect) (Nesbakken 2005b)

Herd level	Slaughter hygiene	Meat inspection	Cutting and deboning	Processing	Preparation and consumption
+++	++	-	-	+++*	+++*

* Reduction/elimination by heat treatment. However, the main problem at these stages is cross-contamination.

At the farm level

In herds highly contaminated with *Y. enterocolitica*, suckling piglets are protected by maternal antibodies during the first weeks of their lives. Young pigs become carriers in tonsils and faeces when they are about 60 to 80 days old, and seropositive shortly thereafter (Nesbakken *et al.* 2005). In a study in Norway (Skjerve *et al.* 1998), an enzyme-linked immunosorbent assay (ELISA) was used to detect IgG antibodies against *Y. enterocolitica* O:3 in sera from 1605 slaughter pigs from 321 different herds. Positive titres were found in 869 (54.1%) of the samples. In the final epidemiological study 182 (63.4%) of 287 herds were defined as positive. Among the positive herds, there were significantly fewer combined herds of piglets and fatteners than fattening herds. Combined herds (farrow-to-finish production) represent an important protective factor (Odds ratio = 0.15; 95% confidence interval 0.05 – 0.33). Other risk factors identified were using an own farm vehicle for transport of slaughter pigs to abattoirs, daily observations of a cat with kittens at the farm, and using straw bedding for slaughter pigs. In conclusion, the epidemiological data suggest that it is possible to reduce the herd prevalence of *Y. enterocolitica* O:3 by minimising contact between infected and non-infected herds. Further, attempts to reduce the prevalence at the top levels of the breeding pyramids may also reduce the prevalence of *Y. enterocolitica* in the general pig population. Such preventive measures may be beneficial to the industry and may significantly reduce the occurrence of human yersiniosis in Norway. The meat industry might categorise herds using serological methods, and use these results in its strategy to reduce the risks for consumers (Figure 2).

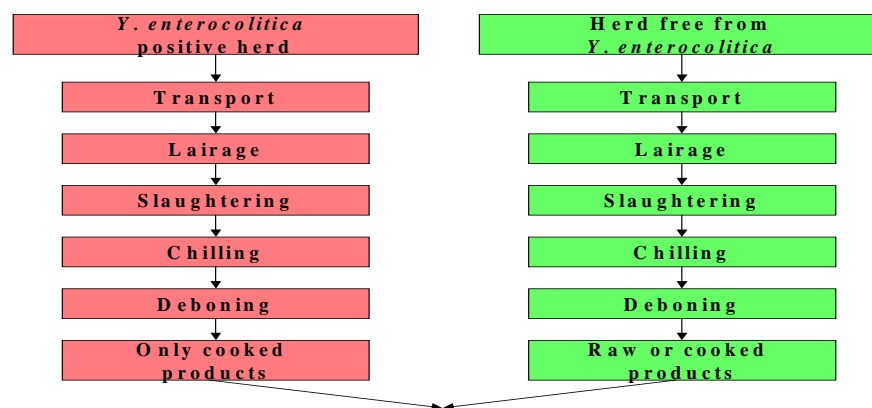


FIGURE 2. Flow diagram illustrating production of *Y. enterocolitica* free pork. To avoid contamination from carrier animals pigs from negative and positive herds should not be mixed. Pig carcasses from positive herds have to be handled separately and only be used for production of heat-treated products within the plant. Categorisation might be based on serological tests of the herds. Adapted after (Nesbakken 2004).

Slaughter hygiene and meat inspection

During the slaughtering process, bacteria from the oral cavity or intestinal contents may easily contaminate the carcasses and the environment in the slaughterhouse. Improved hygiene at critical control points should be attempted (Figure 3).

Because of the high prevalence of *Y. enterocolitica* in pig herds, strict slaughter hygiene is an important means by which to reduce carcass contamination with *Y. enterocolitica*, as well as other pathogenic micro-organisms (Skjerve *et al.* 1998). Pig slaughter is an open process with many opportunities for the contamination of the pork carcass with *Y. enterocolitica*, and there is no point where hazards are completely eliminated (Borch *et al.* 1996). It is not possible to sort out pigs contaminated with *Y. enterocolitica* at post-mortem meat inspection.

HACCP (Hazard Analysis Critical Control Point) and GMP (Good Manufacturing Practice) in pig slaughter must focus upon limiting this spread (Borch *et al.* 1996). As a guide, attention should be given to the establishment of control measures and identification of critical control points by considering different steps during slaughter and dressing including: lairage, killing, scalding, dehairing, singeing/flaming, scraping, circum-anal incision and removal of the intestines, excision of the tongue, pharynx, and in particular the tonsils, splitting, post mortem meat inspection procedures, and de-boning of the head (Borch *et al.* 1996).

The results presented by Nesbakken *et al.* (Nesbakken *et al.* 1994) indicate that it is important to modify procedures for removal of the guts in order to avoid contamination of the carcass by intestinal contents from the rectum. Technological solutions have already been found which allow removal of the rectum without soiling the carcass. This can be done, *inter al.*, by insertion of a pre-frozen plug into the anus prior to rectum-loosening and gut removal. The sealing off of the rectum with a plastic bag immediately after it has been freed, can significantly reduce the spread of *Y. enterocolitica* to pig carcasses (Nesbakken *et al.* 1994). According to data from the Norwegian Institute of Public Health, the occurrence of human yersiniosis dropped by about 30 – 40 % after the plastic bag technique was introduced in the pig slaughterhouses in Norway (Figure 1).

In a study of the dynamics of natural infection with *Y. enterocolitica* in pig herds (Nesbakken *et al.*, submitted), the proportion of animals with *Y. enterocolitica* O:3 in faeces decreased from about 135 days of age. These results indicate that animals younger than 135 days represent a greater risk for contamination of carcasses with *Y. enterocolitica* than older animals. In contrast, many of the tonsils remained positive for *Y. enterocolitica* up to the time for slaughter of fattening pigs in Norway (150 – 180 days of age). When pigs are slaughtered at the age of 135 days or more, the tonsils may present a more significant source of human pathogenic *Y. enterocolitica* than faeces (Nesbakken *et al.*, submitted). In this context, the possibility of decapitation early on in the carcass dressing procedure has been considered and investigated. In such a procedure, the head, including tongue and tonsils, would be removed on a separate line (Christensen and Lühtje, 1994; Petersen *et al.*, 2002).

Meat inspection procedures of pigs that involve examination of the head also represent a cross-contamination risk: incision of the sub-maxillary lymph nodes in order to detect tuberculosis is a compulsory procedure according to the EU

regulations (European Commission, 1995). In a Norwegian study, 12.5% of these lymph nodes were positive for virulent *Yersinia* (Nesbakken *et al.* 2003a; Nesbakken *et al.* 2003b). Consequently, the bacteria may be transported from the medial neck region to other parts of the carcass by the knives and hands of the meat inspection personnel (Nesbakken 1988; Nesbakken *et al.* 2003b).

In view of the fact that the incidence of tuberculosis in pigs has been reduced to a very low level in Norway, it may be possible to re-consider regulations that require incision of the sub-maxillary lymph nodes by meat inspectors. Also incision of the mesenteric lymph nodes might represent a cross-contamination risk since 8.3% of the samples were positive (Figure 3). According to the new EU regulation (Regulation (EC) No 854/2004) that are going to be implemented 1/1/2006 the possibility exists that herds with an integrated pig production with sufficient Good Manufacturing Practice (GMP) and Good Hygienic Practice (GHP) may avoid the traditional post-mortem inspection. Pigs from such herds might only be investigated visually (no palpations or incisions).

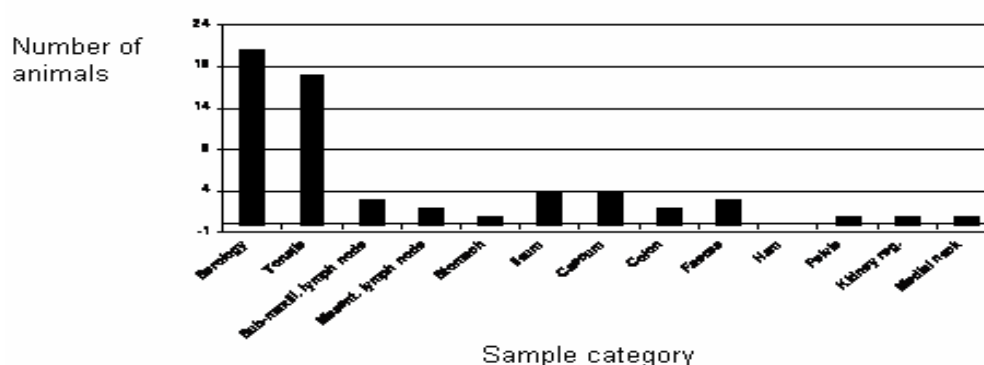


FIGURE 3. Antibodies against *Y. enterocolitica* O:3 in blood samples, and detection of *Y. enterocolitica* O:3 in lymphoid tissues, intestinal contents and on 24 pig carcasses. With permission of Kluwer Academic Publishers (Nesbakken *et al.* 2003a).

Packaging

Growth of *Y. enterocolitica* was compared in ground beef packed in modified atmospheres of 60% CO₂/ 40% N₂ /0.4% CO (high CO₂/ low CO mixture), 70% O₂/ 30% CO₂ (high O₂ mixture) and in chub packs (Nissen *et al.* 2000). The ground beef was inoculated with *Y. enterocolitica* (final concentration 10²-10³ bacteria/g) and stored at 4 and 10°C for up to 14 days. Growth of *Y. enterocolitica* was nearly totally inhibited both at 4 and 10°C in the high CO₂/ low CO mixture, while the bacterial numbers in the samples packed in the high O₂ mixture increased from about 5x10² bacteria/g at day 0 to about 10⁴ at day 5 at 4°C and to 10⁵ at 10°C. Growth in the

chub packs was even higher. The study showed that prolongation of shelf life for up to two weeks at 4°C did not increase growth of *Y. enterocolitica* in ground beef stored in the high CO₂/ low CO mixture. However, this packaging technique was prohibited by the EU Commission in 2004.

Water

Although *Y. enterocolitica* isolated from water are not usually pathogenic for humans, a risk of transmission through water is present as demonstrated by a case-control study conducted in Norway (Ostroff *et al.* 1994). Since *Y. enterocolitica* is sensitive to chlorination and UV-irradiation, proper treatment of drinking water and water used for food processing should eliminate the risk of infection from this source.

Some general hygiene aspects

Preventive and control measures should also focus on informing of all categories of people involved in production, processing, and final preparation of food, about the importance of good hygienic practices. Strict hygiene is particularly necessary because *Y. enterocolitica* is able to propagate at refrigeration temperatures. Therefore, chilling of food products should not be considered as an effective control measure for this microbe. Consumption of undercooked pork should be discouraged. The need to adhere to preventive measures, such as pasteurization of milk and kitchen hygiene practices which reduce recontamination and cross-contamination after heat treatment, should be emphasized. Avoidance of contact with faeces from pigs or domestic pets, as well as being an obvious standard hygiene procedure, may also reduce transmission.

Risk assessment needs and questions

The key questions in relation to the risk posed by the presence of *Y. enterocolitica* in food chains are as follow:

- At what stages in the food chain would it be most efficient to allocate resources to prevent contamination with *Y. enterocolitica*?
 - Is the price paid for possible interventions at herd level so high that this might be an unrealistic approach?
 - Is decapitation early on in the carcass dressing procedure together with enclosure of the anus into a plastic bag after rectum-loosening the most efficient way to limit the spread in a cost benefit context?
- What is the significance of improved slaughter hygiene in relation to the stable low level of human cases of yersiniosis since 1994?
- What is the relative importance of pork consumption versus other risk factors for yersiniosis, including the use of untreated drinking water?

Major data gaps

- The relative importance of pork as a risk factor compared to untreated drinking water is unknown.
- Due to insufficient detection methods, the real occurrence of *Y. enterocolitica* O:3 in foods are unknown.

- Molecular typing methods with high discriminatory power are missing, since most of the human pathogenic *Y. enterocolitica* O:3 strains belong to one or two dominating epitypes.

Conclusions

- *Y. enterocolitica* is one of a few zoonotic bacteria that have a stable reservoir within the domestic animal population of Norway. The predominant cause of yersiniosis in Norway is *Y. enterocolitica* O:3, and the pig is considered to be the main source of infection,
- *Y. enterocolitica* might have serious clinical consequences since a relatively high frequency of the people in Norway possess the tissue type HLA-B27. A severe sequela linked to this tissue type is reactive arthritis,
- A relatively high proportion of the Norwegian populations drink untreated water which is a well recognised risk factor. However, the relative contribution of this risk factor is unknown.

In Norway, a decline in human cases of yersiniosis has been recorded since the beginning of the 1990s. This decline has been attributed to implementation of improved slaughtering methods, including enclosure of the anus into a plastic bag after rectum-loosening. In Norway, most fattening pigs are slaughtered at the age of 150 to 180 days. By this age, the tonsils may be a more significant source of human pathogenic *Y. enterocolitica* than intestinal contents, since the occurrence in the intestinal tract and faeces is reduced at the time of slaughter. Accordingly, hygienic handling of the head and the plucks during slaughter and dressing is very important to avoid contamination of the carcass. The most efficient way to limit the spread from tongue and tonsils is probably decapitation early on in the carcass dressing procedure. In such a procedure, the head, including tongue and tonsils, should be removed on a separate line. Also, avoidance of incision of the sub-maxillary lymph nodes might reduce the spread.

The apparently low prevalence of pathogenic *Y. enterocolitica* in food may be due to lack of suitable selective methods. The culturing methods, which are used routinely in microbiological laboratories, are not sensitive enough. There is a need for a standardised DNA-based technique, with improved sensitivity, for the detection of *Y. enterocolitica* in clinical, food and environmental samples.

Epidemiological data suggest that it is possible to reduce the herd prevalence of *Y. enterocolitica* O:3 by minimising contact between infected and non-infected herds. Further, attempts to reduce the prevalence at the top levels of the breeding pyramids may be beneficial for the industry as a whole. The meat industry might be able to categorise herds using serological methods, and use these results in its strategy to reduce the risks for consumers. However, such a strategy has to be evaluated in a cost benefit context.

References

- Aleksic,S., Bockemuhl,J. and Lange,F. (1986) Studies on the serology of flagellar antigens of *Yersinia enterocolitica* and related *Yersinia* species. *Zentralbl. Bakteriol. Mikrobiol. Hyg. [A]* **261**, 299-310.
- Alsterlund,R., Danielsson-Tham,M.L., Karp,G., Eden,T., De Jong,B., Nilsson,P.O. and Ransjo,U. (1995) [An outbreak of *Yersinia enterocolitica* infection on the Bjarred peninsula. Indications for risks of refrigerated food]. *Lakartidningen* **92**, 1213-1214.
- Asplund,K., Johansson,T. and Siitonen,A. (1998) Evaluation of pulsed-field gel electrophoresis of genomic restriction fragments in the discrimination of *Yersinia enterocolitica* O:3. *Epidemiol. Infect* **121**, 579-586.
- Bercovier, H. and Mollaret, H. H. (1984) Genus XIV. *Yersinia*. In *Bergey's Manual of Systematic Bacteriology* ed. Krieg,N.R. pp. 498-506. Baltimore: Williams & Willkins.
- Borch,E., Nesbakken,T. and Christensen,H. (1996) Hazard identification in swine slaughter with respect to foodborne bacteria. *Int. J. Food Microbiol.* **30**, 9-25.
- Bottone,E.J. (1999) *Yersinia enterocolitica*: overview and epidemiologic correlates. *Microb. Infect* **1**, 323-333.
- Buchrieser,C., Weagant,S.D. and Kaspar,C.W. (1994) Molecular characterization of *Yersinia enterocolitica* by pulsed-field gel electrophoresis and hybridization of DNA fragments to ail and pYV probes. *Appl. Environ. Microbiol.* **60**, 4371-4379.
- Christensen, H. and Luhtje, H. Reduced spread of pathogens as result of changed pluck removal technique. Proceeding of the 40th International Congress of Meat science and Technology, August 28 to September 2 1994. paper 5 III.06. 1994. The Hague, The Netherland.
Ref Type: Conference Proceeding
- Codex Committe on Food Hygiene and Codex Alimentarius commission. Risk profiles of *Enterobacter sakazaki* in powdered infant formula. 2003.
Ref Type: Report
- Codex Committe on Food Hygiene. Proposed draft principles and guidelines for the conduct of microbiological risk management (MRM). 2005.
Ref Type: Report
- European Commission, 1995. *Council directive 64/433/EEC on health condition for the production and marketing of fresh meat*. Brussels, Belgium, 34pp.
- Fredriksson-Ahomaa,M., Autio,T. and Korkeala,H. (1999) Efficient subtyping of *Yersinia enterocolitica* bioserotype 4/O:3 with pulsed-field gel electrophoresis. *Letters Appl. Microbiol.* **29**, 308-312.

- Fredriksson-Ahomaa,M. and Korkeala,H. (2003) Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. *Clin. Microbiol. Rev.* **16**, 220-229.
- Frederiksson-Ahomaa,M., Korte,T. and Korkeala,H. (2001). Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. *Lett. Appl. Microbiol.* **32**, 375-378.
- Fukushima,H., Gomyoda,M., Ishikura,S., Nishio,T., Moriki,S., Endo,J., Kaneko,S. and Tsubokura,M. (1989) Cat-contaminated environmental substances lead to *Yersinia pseudotuberculosis* infection in children. *J. Clin. Microbiol.* **27**, 2706-2709.
- Fukushima,H., Gomyoda,M. and Kaneko,S. (1991) Wild animals as the source of infection with *Yersinia pseudotuberculosis* in Shimane Prefecture, Japan. *Contrib. Microbiol. Immunol.* **12**, 1-4.
- Granfors,K., Jalkanen,S., von Essen,R., Lahesmaa-Rantala,R., Isomaki,O., Pekkola-Heino,K., Merilahti-Palo,R., Saario,R., Isomaki,H. and Toivanen,A. (1989) *Yersinia* antigens in synovial-fluid cells from patients with reactive arthritis. *N. Engl. J. Med.* **320**, 216-221.
- Hallanvuori,S., Nuorti,P., Nakari,U.M. and Siitonen,A. (2003) Molecular epidemiology of the five recent outbreaks of *Yersinia pseudotuberculosis* in Finland. *Adv. Exp. Med Biol.* **529**, 309-312.
- Hoogkamp-Korstanje,J.A. and Stolk-Engelaar,V.M. (1995) *Yersinia enterocolitica* infection in children. *Pediatr. Infect. Dis. J.* **14**, 771-775.
- Isberg,R.R., Swain,A. and Falkow,S. (1988) Analysis of expression and thermoregulation of the *Yersinia pseudotuberculosis* *inv* gene with hybrid proteins. *Infect. Immun.* **56**, 2133-2138.
- Jalava,K., Hallanvuori,S., Nakari,U.M., Ruutu,P., Kela,E., Heinasmaki,T., Siitonen,A. and Nuorti,J.P. (2004) Multiple outbreaks of *Yersinia pseudotuberculosis* infections in Finland. *J. Clin. Microbiol.* **42**, 2789-2791.
- Johannessen,G.S., Kapperud,G. and Kruse,H. (2000) Occurrence of pathogenic *Yersinia enterocolitica* in Norwegian pork products determined by a PCR method and a traditional culturing method. *Int. J. Food Microbiol.* **54**, 75-80.
- Kapperud,G. (1991) *Yersinia enterocolitica* in food hygiene. *Int. J. Food Microbiol.* **12**, 53-65.
- Kapperud,G., Nesbakken,T., Aleksic,S. and Mollaret,H.H. (1990) Comparison of restriction endonuclease analysis and phenotypic typing methods for differentiation of *Yersinia enterocolitica* isolates. *J. Clin. Microbiol.* **28**, 1125-1131.
- Klevytska,A.M., Price,L.B., Schupp,J.M., Worsham,P.L., Wong,J. and Keim,P. (2001) Identification and characterization of variable-number tandem repeats in the *Yersinia pestis* genome. *J Clin. Microbiol.* **39**, 3179-3185.

Laheesmaa-Rantala,R., Heesemann,J., Lehtonen,O.P., Granfors,K. and Toivanen,A. (1989) Avidity of antibodies against released proteins of *Yersinia* spp: comparison of patients with or without reactive arthritis. *Ann. Rheum. Dis* **48**, 1003-1006.

Lee, W. H., Vanderzant, C. and Stern, N. (200) The occurrence of *Yersinia enterocolitica* in foods. In *Yersinia enterocolitica* ed. Bottone,E.J. pp. 161-171. Boca Raton, Fl.: CRS Press Inc.

Mead,P.S., Slutsker,L., Griffin,P.M. and Tauxe,R.V. (1999) Food-related illness and death in the united states reply to Dr. Hedberg. *Emerg. Infec. Dis.* **5**, 841-842.

Ministere des Affaires Sociales,de la Sante Publique et de l'Environnement.Institut d'Hygiene et d'Epidemiologie *Surveillance van Infectieuze Aandoeningen door een Netwerk van Laboratoria voor Microbiologie 1994 + Retrospectieve 1983 - 1993.*

1995. Brussels.

Ref Type: Report

Ministry of Food, Agriculture.and Fisheries. 2003. Annual report on zoonoses in Denmark.

Ref Type: Report

Mollaret,H.H., Bercovier,H. and Alonso,J.M. (1979) Summary of the data received at the WHO Reference Center for *Yersinia enterocolitica*. *Contr. Microbiol. Immunol.* **5**, 174-184.

Najdenski,H., Iteman,I. and Carniel,E. (1994) Efficient subtyping of pathogenic *Yersinia enterocolitica* strains by pulsed-field gel electrophoresis. *J. Clin. Microbiol.* **32**, 2913-2920.

National Veterinary Institute. Zoonoses in Sweden. 2003.

Ref Type: Report

Nesbakken,T. (1988) Enumeration of *Yersinia enterocolitica* O:3 from the porcine oral cavity, and its occurrence on cut surfaces of pig carcasses and the environment in a slaughterhouse. *Int. J. Food Microbiol.* **6**, 287-293.

Nesbakken, T. (2000) *Yersinia* species. In *The microbiological safety and quality of food* ed. Lund,B.M., baird-Parker,T.C. and Gould,G.W. pp. 1363-1393. Gaithesburg, Maryland: Aspen Publisher, Inc.

Nesbakken,T. (2004) Moderne kjøttkontroll. *Nor. Vet. Tidsskr.* **116**, 794-801. (In Norwegian).

Nesbakken, T. (2005b) *Yersinia enterocolitica*. In *Emerging Food Pathogens* ed. Motarjemi,Y. and Adams,M. Cambridge, UK: Woodhead Publishing Ltd.

Nesbakken, T. (2005a) *Yersinia enterocolitica*. In *Foodborne pathogens: Microbiology and Molecular Biology.* ed. Fratamico,P.M., Bhunia,A.K. and Smith,J.L. pp. 228-249. Norwich UK: Caister Academic Press.

Nesbakken,T., Eckner,K., Høidal,H.K. and Røtterud,O.J. (2003a) Occurrence of *Y. enterocolitica* in slaughter pigs and consequences for meat inspection, slaughtering and dressing procedures. *Adv. Exp. Med Biol.* **529**, 303-308.

Nesbakken,T., Eckner,K., Høidal,H.K. and Røtterud,O.J. (2003b) Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. *Int. J. Food Microbiol.* **80**, 231-240.

Nesbakken,T., Gondrosen,B. and Kapperud,G. (1985) Investigation of *Yersinia enterocolitica*, *Yersinia enterocolitica*-like bacteria, and thermotolerant campylobacters in Norwegians pork producta. *Int. Food Microbiol.* **1**, 311-320.

Nesbakken, T., Iversen, T., Eckner, K. and Lium, B. The dynamic of natural infection with *Yersinia enterocolitica* in pig herds. 2005. (Submitted to Veterinary microbiology).

Nesbakken,T., Kapperud,G., Dommarsnes,K., Skurnik,M. and Hornes,E. (1991) Comparative study of a DNA hybridization method and two isolation procedures for detection of *Yersinia enterocolitica* O:3 in naturally contaminated pork products. *Appl. Environ. Microbiol.* **57**, 389-394.

Nesbakken,T., Nerbrink,E., Røtterud,O.J. and Borch,E. (1994) Reduction of *Yersinia enterocolitica* and *Listeria* spp. on pig carcasses by enclosure of the rectum during slaughter. *Int. J. Food Microbiol.* **23**, 197-208.

Nissen,H., Alvseike,O., Bredholt,S., Holck,A. and Nesbakken,T. (2000) Comparison between the growth of *Yersinia enterocolitica*, *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques. *Int. J. Food Microbiol.* **59**, 211-220.

Nordic Committee on Food Analysis. *Yersinia enterocolitica*,. Detection in food. Method no. 117, 2nd. ESBO. 1987.
Ref Type: Report

NORM and NORM-VET. 2004. Usage of antimicrobial agents and chemotherapy and occurrence of antimicrobial resistance in Norway-2003. Oslo / Tromsø, Norway.
Ref Type: Report

Nuorti,J.P., Niskanen,T., Hallanvuori,S., Mikkola,J., Kela,E., Hatakka,M., Fredriksson-Ahomaa,M., Lyytikäinen,O., Siitonen,A., Korkeala,H. and Ruutu,P. (2004) A widespread outbreak of *Yersinia pseudotuberculosis* O:3 infection from iceberg lettuce. *J. Infect. Dis.* **189**, 766-774.

Ostroff,S.M., Kapperud,G., Hutwagner,L.C., Nesbakken,T., Bean,N.H., Lassen,J. and Tauxe,R.V. (1994) Sources of sporadic *Yersinia enterocolitica* infections in Norway: a prospective case-control study. *Epidemiol. Infect* **112**, 133-141.

Ostroff,S.M., Kapperud,G., Lassen,J., Aasen,S. and Tauxe,R.V. (1992) Clinical features of sporadic *Yersinia enterocolitica* infections in Norway. *J. Infect. Dis* **166**, 812-817.

Petersen,J.V., Andersen,J.K., Sorensen,F. and Knudsen,H. (2002) Food safety on the slaughterline: inspection of pig heads. *Vet. Rec.* **150**, 782-784.

Pourcel,C., Andre-Mazeaud,F., Neubauer,H., Ramisse,F. and Vergnaud,G. (2004) Tandem repeats analysis for the high resolution phylogenetic analysis of *Yersinia pestis*. *BMC Microbiol.* **4**, 22.

Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Official Journal of the European Union, L155/206, 30.4.2004.

Robert Koch Institute. Epidemiologisches Bulletin 29. 1995. Berlin, Germany.
Ref Type: Magazine Article

Saken,E., Roggenkamp,A., Aleksic,S. and Heesemann,J. (1994) Characterisation of pathogenic *Yersinia enterocolitica* serogroups by pulsed-field gel electrophoresis of genomic NotI restriction fragments. *J. Med. Microbiol.* **41**, 329-338.

Skjerve,E., Lium,B., Nielsen,B. and Nesbakken,T. (1998) Control of *Yersinia enterocolitica* in pigs at herd level. *Int. J. Food Microbiol.* **45**, 195-203.

Swedish Institute of Infectious Disease control. Smittskyddinstitutets Epidemiologiska Årsrapport 1994. 27. 1995. Stockholm-Sweden.
Ref Type: Report

Tauxe,R.V. (2002) Emerging foodborne pathogens. *Int. J Food Microbiol.* **78**, 31-41.

Tauxe,R.V., Vandepitte,J., Wauters,G., Martin,S.M., Goossens,V., De Mol,P., Van Noyen,R. and Thiers,G. (1987) *Yersinia enterocolitica* infections and pork: the missing link. *Lancet* **1**, 1129-1132.

The National Public Health Institute. Infectious Diseases in Finland. 2003.
Ref Type: Report

Tsubokura,M., Otsuki,K., Sato,K., Ouchi,K., Tanaka,M., Hongo,T., Fukushima,H. and Inoue,M. (1987) [Distribution of *Yersinia pseudotuberculosis* in Japan and epidemiology of human infection]. *Kansenshogaku Zasshi* **61**, 737-745.

Tsubokura,M., Otsuki,K., Sato,K., Tanaka,M., Hongo,T., Fukushima,H., Maruyama,T. and Inoue,M. (1989) Special features of distribution of *Yersinia pseudotuberculosis* in Japan. *J. Clin. Microbiol.* **27**, 790-791.

Wauters,G., Aleksic,S., Charlier,J. and Schulze,G. (1991) Somatic and flagellar antigens of *Yersinia enterocolitica* and related species. *Contr. Microbiol. Immunol.* **12**, 239-243.

Wauters,G., Goossens,V., Janssens,M. and Vandepitte,J. (1988) New enrichment method for isolation of pathogenic *Yersinia enterocolitica* serogroup O:3 from pork. *Appl. Environ. Microbiol.* **54**, 851-854.

Wauters, G., Kandolo, K. and Janssen, M. (1987) Revised biotyping scheme of *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. **9**, 14-21.

Wright J, Fenwick S and McCarthy M. (1995). Yersiniosis: an emerging problem in New Zealand. N Z Public Health Rep 2, 65-66.

Scientific Panel Members

Panel on Biological Hazards

Hilde Kruse (chair), Georg Kapperud, Jørgen Lassen, Bjørn Tore Lunestad, Truls Nesbakken, Espen Rimstad, Lucy Robertson, Eystein Skjerve, Yngvild Wasteson.

ACKNOWLEDGMENTS

The Chair and members of the Working Group on this risk assessment are acknowledged for their valuable contributions to this mandate. The members of the Working Group are; Truls Nesbakken (chair), Georg Kapperud, and Jørgen Lassen.

Scientific coordinator from the Secretariat; Siamak Yazdankhah.