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
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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-

**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. 2000). 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)

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

**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 10³ - 10⁶ 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-ticarcillin-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 % CaCl$_2$ (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 pulsortypes 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-Ahoma et al. 1999; Najdenski et al. 1994; Sakon 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 (Lahesmaa-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)

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Manifestation/population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td>Enterocolitis: predominantly in young children; concomitant bacteraemia may also be present in infants; Pseudoappendicitis syndrome (children older than 5 years; adults)</td>
</tr>
<tr>
<td></td>
<td>Acute mesenteric lymphadenitis \nTerminal ileitis</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>Especially in immunosuppressed individuals and those in iron overload or being treated with deferrioxamine; Transfusion related (usually leads to septic shock syndrome)</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Focal abscesses; liver, kidney, spleen, lung \nCutaneous manifestations; cellulites, pyomyositis, pustules, and bullous lesions \nPneumonia, cavitary pneumonia \nMeningitis \nPanophthalmitis \nEndocarditis, infected myotic aneurysm \nOsteomyelitis</td>
</tr>
<tr>
<td>Post-infection sequelae</td>
<td>Arthritis (associated with HLA-B-27), myoccarditis, glomerulonephritis, erythema nodosum</td>
</tr>
<tr>
<td>Pharyngitis (common after oral ingestion of <em>Y. enterocolitica</em>)</td>
<td></td>
</tr>
</tbody>
</table>

**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](http://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](http://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 ([Hallanvuo et al. 2003;Jalava et al. 2004;Nuorti et al. 2004](http://www.msis.no)).

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](http://www.msis.no)). 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](http://www.msis.no)). There are also some examples of food-borne outbreaks in Sweden. A milk-borne outbreak occurred in Kristianstad in 1988 ([Alsterlund et al. 1995](http://www.msis.no)) 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](http://www.msis.no)). 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](http://www.msis.no)). 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)
<table>
<thead>
<tr>
<th>Country</th>
<th>Total number of cases (year)</th>
<th>Cases per 100,000 inh.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verified cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>829^1 (1994)</td>
<td>8.5</td>
<td>(Ministere des Affaires Sociales 1995)</td>
</tr>
<tr>
<td></td>
<td>245^2 (2003)</td>
<td>4.5</td>
<td>Danish Zoonosis Centre, Copenhagen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><a href="http://www.dfvf.dk">www.dfvf.dk</a></td>
</tr>
<tr>
<td>Germany</td>
<td>7113 (2001)</td>
<td>8.7</td>
<td>(Robert Koch Institute 1995)</td>
</tr>
<tr>
<td>Norway</td>
<td>86^2 (2003)</td>
<td>1.9</td>
<td>Norwegian Institute of Public Health, Oslo website aficionado</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><a href="http://www.fhi.no">www.fhi.no</a></td>
</tr>
<tr>
<td>Sweden</td>
<td>714^2 (2003)</td>
<td>8</td>
<td>Swedish Institute for Infectious Disease Control, Stockholm website aficionado</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><a href="http://www.smittskyddsinstitutet.se">www.smittskyddsinstitutet.se</a></td>
</tr>
<tr>
<td>Switzerland</td>
<td>95 (1993)</td>
<td>1.4</td>
<td>Swiss National Reference Laboratory for Foodborne Diseases, Berne website aficionado</td>
</tr>
<tr>
<td>Estimated cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>3,000^2 (1994)</td>
<td>84</td>
<td>(Wright J 1995)</td>
</tr>
<tr>
<td>United States</td>
<td>87,000 (1997)</td>
<td>33.4</td>
<td>(Mead et al. 1999)</td>
</tr>
</tbody>
</table>

^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) (Hallanvuo 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)

<table>
<thead>
<tr>
<th>Herd level</th>
<th>Slaughter hygiene</th>
<th>Meat inspection</th>
<th>Cutting and deboning</th>
<th>Processing</th>
<th>Preparation and consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++*</td>
<td>+++*</td>
</tr>
</tbody>
</table>

* 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).

![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).](image-url)
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).


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 $10^2-10^3$ 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 $5 \times 10^2$ bacteria/g at day 0 to about $10^4$ at day 5 at 4°C and to $10^6$ 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 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.
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