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1 Historical aspects and contemporary problems

The genus *Yersinia* of the family *Enterobacteriaceae* includes three well-established pathogens (*Yersinia pestis, Yersinia pseudotuberculosis* and *Yersinia enterocolitica*) and several non-pathogens (Mollaret et al., 1979). *Y. pestis* was isolated by Alexandre Yersin in 1894 (Yersin, 1894). The most important *Yersinia* infection, plague, caused by *Y. pestis*, is one of the most ancient recognized human diseases. Disease due to *Y. pseudotuberculosis* (first described in 1884) has been recognized since the beginning of the twentieth century, and *Y. enterocolitica* was first shown to be associated with human disease in 1939 (Mollaret, 1995).

The current interest in *Y. enterocolitica* started in 1958 following a number of epi-zootics among chinchillas and hares (Mollaret et al., 1979; Hurvell, 1981), and after the establishment of a causal relationship with abscedizing lymphadenitis in man. The similarity between the human and animal isolates was established in 1963 (Knapp and Thal, 1963), and in 1964 the species name *Y. enterocolitica* was formally proposed by Frederiksen (1964). Over the past 30 years the bacterium has been found with increasing frequency as a cause of human disease, and from animals and inanimate sources.
Yersinia enterocolitica is an important cause of gastroenteritis in humans, especially in temperate countries (Mollaret et al., 1979; World Health Organization, 1983, 1987). Evidence from large outbreaks of yersiniosis in the US, Canada, and Japan (Cover and Aber, 1982) and from epidemiological studies of sporadic cases has shown that Y. enterocolitica is a foodborne pathogen, and that in many cases pork is implicated as the source of infection (Morris and Feeley, 1976; Hurvell, 1981; Tauxe et al., 1987; Lee et al., 1991; Ostroff et al., 1994). Due to the relative lack of information on Y. pseudotuberculosis as a foodborne pathogen (Schiemann, 1989), this bacterium will be considered less extensively than Y. enterocolitica. Y. pseudotuberculosis mainly causes epizootic disease, especially in rodents, with necrotizing granulomatous disease of liver, spleen and lymph nodes (Aleksic and Bockemühl, 1990; Aleksic et al., 1995). In humans it may cause acute abdominal disease, septicemia, arthritis and erythema nodosum (Ahvonen, 1972a; Knapp, 1958).

2 Classification

The genus Yersinia was proposed in 1944 by Van Loghem (1944) for bacteria that were related to the genus Pasteurella and that were pathogenic. Thal (1954) drew attention to evidence relating Yersinia to the Enterobacteriaceae. A general numerical taxonomic study from 1958 placed Yersinia between Klebsiella and Escherichia coli (Sneath and Cowan, 1958). The allocation of Yersinia to the family Enterobacteriaceae was further supported by Frederiksen (1964).

3 Differentiation of Y. enterocolitica from other Yersinia species

A range of strains of Yersinia variants has been isolated from animals, water, and food (Mollaret et al., 1979; Hurvell, 1981; Lee et al., 1981). Many of these bacteria have characteristics that deviate considerably from the typical pattern shown by Y. enterocolitica, but can be classified as belonging to the genus Yersinia (Mollaret et al., 1979). Such Y. enterocolitica-like bacteria have now been divided on a genetic basis into seven new species (Aleksic et al., 1987; Bercovier et al., 1980a, 1980b, 1984; Brenner et al., 1980a, 1980b, Ursing et al., 1980; Brenner, 1981; Wauters et al., 1988a): Yersinia frederiksenii, Yersinia kristensenii, Yersinia intermedia, Yersinia aldovae, Yersinia rohdei, Yersinia mollaretii and Yersinia bercovieri.

Y. enterocolitica is a Gram-negative, oxidase-negative, catalase-positive, nitrate reductase-positive, facultative anaerobic rod (occasionally coccoid), 0.5–0.8 × 1–3 μm in size (Bercovier and Mollaret, 1984). It does not form a capsule or spores. It is non-motile at 35–37°C, but motile at 22–25°C with relatively few, peritrichous flagellae. Some human pathogenic strains of serovar O:3 are, however, non-motile at both temperatures. In addition, the bacterium is urease positive, H₂S negative, ferments mannitol, and produces acid (but not gas) from glucose.
3.1 Phenotypic characterization

3.1.1 Biotyping
Wauters et al. (1987) described a revised biotyping scheme that differentiates between pathogenic (biovars 1B, 2, 3, 4, 5) and non-pathogenic (only biovar 1A) variants. The proposed biovar 6 (Wauters et al., 1987) is re-classified into two new species: *Y. bercovieri* and *Y. mollaretii* (Wauters et al., 1988a).

3.1.2 Serotyping using O- and H-antigens
*Y. enterocolitica* can be divided into serovars using O-antigens. Seventy-six different O-factors have been described in both *Y. enterocolitica* and *Y. enterocolitica*-like bacteria (Wauters, 1981; Wauters et al., 1991). A few strains, however, cannot be typed by this system, and the number of described antigen factors is therefore likely to increase in the future. Fifty-four H-factors have been recognized (Wauters et al., 1991; S. Aleksic, personal communication, 1995), but H-antigen determination is rarely carried out and most studies are limited to the O-antigens.

3.1.3 Correlation between biovars and serovars and pathogenicity
Strains of biovar 1B belong to a small number of pathogenic serovars, the most frequent being O:8. Biovar 2 only includes two serovars, O:9 and O:5,27, which are pathogenic for man. Biovar 4/serovar, O:3, is the main pathogenic serovar for man.

3.1.4 Phage typing
Phage typing requires a battery of phages and indicator strains. Two European phage-typing systems are described, but are not used in many laboratories (Mollaret and Nicolle, 1965; Niléhn, 1969; Nicolle, 1973). A bacteriophage typing system that allows greater differentiation of American O:8 strains has also been described (Baker and Farmer, 1982).

3.2 Genotyping
Methods based on the characterization of the genotype include plasmid profile analysis, restriction enzyme analysis of plasmid and chromosomal DNA (DNA fingerprinting), pulsed field gel electrophoresis (PFGE) (Buchrieser et al., 1994; Najdenski et al., 1994; Saken et al., 1994), and the use of DNA or RNA probes (Wachsmuth, 1985; Mayer, 1988; Tenover, 1988; Andersen and Saunders, 1990). Methods based on the characterization of the genotype, within some sero-/biovar combinations like serovar O:8/biovar 1B, often result in a number of different patterns. However, within serovar O:3/biovar 4, the diversity of patterns is limited (Nesbakken et al., 1987). Though several pulsotypes are found among O:3/biovar 4 strains, most of the strains belong to one or two dominating pulsotypes (Buchrieser et al., 1994; Najdenski et al., 1994; Saken et al., 1994; Asplund et al., 1998; Frederiksson-Ahoma et al., 1999).
4 Virulence factors

4.1 The virulence plasmid

Human pathogenic strains of *Yersinia* spp. *enterocolitica* possess a special plasmid 40–50 MDa in size (Brubaker, 1979; Portnoy and Martinez, 1985). The presence of this plasmid is an essential, though not sufficient, prerequisite for the bacterium to be able to induce disease. The presence of this virulence plasmid has been associated with several properties, most of which are phenotypically expressed only at elevated growth temperatures of 35–37°C (Portnoy and Martinez, 1985). The list of such plasmid-mediated and temperature-regulated properties includes Ca$^{2+}$-dependent growth (Gemski et al., 1980), production of V and W antigens (Perry and Brubaker, 1983), spontaneous autoagglutination (Laird and Cavanaugh, 1980), mannose-resistant haemagglutination (Kapperud et al., 1987), serum resistance (Pai and DeStephano, 1982), binding of Congo red dye (Prpic et al., 1985), hydrophobicity (Lachica et al., 1984), mouse virulence (Ricciardi et al., 1978; Nesbakken et al., 1987), and production of a number of proteins (Portnoy et al., 1984; Bölin et al., 1985; Portnoy and Martinez, 1985), of which one is a true outer membrane protein (YadA, previously termed Yop1) (Michiels et al., 1990). This true outer membrane protein forms a fibrillar matrix on the bacterial surface and mediates cellular attachment and entry (Bliska and Falkow, 1994). It also confers resistance to the bactericidal effect of normal human serum, and inhibition of the anti-invasive effect of interferon.

4.2 The chromosome

Elements encoded by the chromosome are also necessary for maximum virulence. The pathogenic yersiniae share at least two chromosomal loci, *inv* and *ail*, that play a role in their entry into eukaryotic cells (Miller et al., 1988). The *inv* and *ail* gene products can be classified as adhesins since they mediate adherence to the eukaryotic surface. Unlike other previously characterized bacterial adhesins, they also mediate entry into a variety of mammalian cells. A high pathogenicity island in pathogenic species of *Yersinia* encodes genes for three yersiniabactin (Ybt) transport proteins, six Ybt biosynthetic enzymes, one transcriptional regulator (YbtA) and one protein of unknown function (YbtX) (Perry et al., 2001).

5 Ability to survive and grow

*Y. enterocolitica* is a facultative organism able to multiply in both aerobic and anaerobic conditions. The ability of *Y. enterocolitica* to multiply at low temperatures is of considerable concern to food producers. Optimum temperature is 28–29°C, and the reported growth range is −2–42°C (Bercovier and Mollaret, 1984). The minimum pH for growth has been reported as being between 4.2 and 4.4 (Kendall and Gilbert, 1980). The ability to propagate at refrigeration temperature in vacuum-packed foods with a prolonged shelf-life (Hanna et al., 1976, 1979) is of considerable significance in
food hygiene. *Y. enterocolitica* may survive in frozen foods for long periods (Schiemann, 1989).

*Y. enterocolitica* is not able to grow at pH < 4.2 or > 9.0 (Kendall and Gilbert, 1980; Stern et al., 1980a), or at salt concentrations greater than 7% (aw < 0.945) (Stern et al., 1980a). The organism does not survive pasteurization or normal cooking, boiling, baking, and frying temperatures. Heat treatment of milk and meat products at 60˚C for 1–3 minutes effectively inactivates *Y. enterocolitica* (Lee et al., 1981). D-values determined in scalding water were 96, 27 and 11 seconds at 58˚C, 60˚C and 62˚C respectively (Sörqvist and Danielsson-Tham, 1990).

According to many reports, the ability of *Y. enterocolitica* to compete with other psychrotrophic organisms normally present in food may be poor (Stern et al., 1980b; Fukushima and Gomyoda, 1986; Schiemann, 1989; Kleinlein and Untermann, 1990). In contrast, a number of studies have shown that *Y. enterocolitica* is able to multiply in foods kept under chilled storage and might even compete successfully (Hanna et al., 1977; Stern et al., 1980a; Grau, 1981; Lee et al., 1981; Gill and Reichel, 1989; Brocklehurst and Lund, 1990; Lindberg and Borch, 1994; Borch and Arvidsson, 1996; Bredholt et al., 1999).

### 6 Nature of the infection in man

#### 6.1 Clinical symptoms of *Y. enterocolitica* infection

Gastroenteritis is by far the most common symptom of *Y. enterocolitica* infection (yersiniosis) in humans (Bottone, 1977; Mollaret et al., 1979; Wormser and Keusch, 1981; Cover and Aber, 1989). The clinical picture is usually one of a self-limiting diarrhea associated with mild fever and abdominal pain (Wormser and Keusch, 1981). Nausea and vomiting occur, but less frequently. The portion of the gastrointestinal tract usually involved is the ileocaecal region (Sandler et al., 1982). The colon may also be affected and the infection may simulate Crohn’s disease, which has a different prognosis (Vantrappen et al., 1977; Gleason and Patterson, 1982). Occasionally the infection is limited to the right fossa iliaca in the form of terminal ileitis or mesenteric lymphadenitis, with symptoms that can be confused with those of acute appendicitis. In several studies of patients with the appendicitis-like syndrome, *Y. enterocolitica* has been found in up to 9% of patients (Niléhn and Sjöström, 1967; Ahvonen, 1972a; Jebsen et al., 1976; Pai et al., 1982; Samadi et al., 1982; Attwood et al., 1987; Megraud, 1987). Infections with serovars O:3 or O:9 are, in some patients, followed by reactive arthritis (Aho et al., 1981), which is most common in patients possessing the tissue type HLA-B27. Often, although not always, the patient has shown prior gastrointestinal symptoms. Other complications seen with *Y. enterocolitica* infection are reactive skin complaints, erythema nodosum being the most common. Many such patients have no history of prior gastrointestinal involvement. Septicemia due to *Y. enterocolitica* is seen almost exclusively in individuals with underlying disease (Bottone, 1977), while those with cirrhosis and disorders associated with excess iron are particularly predisposed to infection and increased mortality.
Gastroenteritis dominates in children and young people, while various forms of reactive arthritis are most common in young adults, and most patients with skin manifestations are adult females (Wormser and Keusch, 1981). In Scandinavia there is a relatively high incidence of both reactive arthritis (10–30%) (Winblad, 1975) and erythema nodosum (30%) (Ahvonen, 1972b), caused by serovars O:3 and O:9.

6.2 Pathogenesis and immunity

Human infection due to *Y. enterocolitica* is most often acquired by the oral route. The minimal infectious dose required to cause disease is unknown. In one volunteer, ingestion of $3.5 \times 10^9$ organisms was sufficient to produce illness (Szita et al., 1973). The incubation period is uncertain, but has been estimated as being between 2 and 11 days (Szita et al., 1973; Ratnam et al., 1982).

Enteric infection leads to proliferation of *Y. enterocolitica* in the lumen of the bowel and in the lymphoid tissue of the intestine. Adherence to and penetration into the epithelial cells of the intestinal mucosa are essential factors in the pathogenesis of *Y. enterocolitica* infection (Portnoy and Martinez, 1985; Cornelis et al., 1987; Miller et al., 1988; Bliska and Falkow, 1994). When the bacteria reach the lymphoid tissues in the terminal ileum, a massive multiplication and inflammatory response takes place in the Peyer’s patches. Reactive arthritis and erythema nodosum appear to be delayed immunologic sequelae of the original intestinal infection.

7 Nature of infection or carrier state in animals

7.1 Food animals

7.1.1 Pigs

Newborn piglets are easily colonized and become long-term healthy carriers of *Y. enterocolitica* in the oral cavity and intestines (Schiemann, 1989). In one study (Skjerve et al., 1998), an enzyme-linked immunosorbent assay (ELISA) (Nielsen et al., 1996) was used to detect IgG antibodies against *Y. enterocolitica* O:3 in sera from 1605 slaughter pigs from 321 different herds. Positive titers were found in 869 (54.1%) of the samples. Healthy pigs are often carriers of strains of *Y. enterocolitica* that are pathogenic to humans, in particular strains of serovar O:3/biovar 4 and serovar O:9/biovar 2) (Hurvell, 1981; Schiemann, 1989). The organisms are present in the oral cavity (especially the tongue and tonsils), the submaxillary lymph nodes, the intestine and feces (Nesbakken et al., 2003a, 2003b; Figure 8.1).

Strains of O:3 have been found frequently on the surface of freshly slaughtered pig carcasses, in frequencies up to 63.3% (Nesbakken and Kapperud, 1985; Nesbakken, 1988). This is probably the result of spread of the organism via feces and intestinal contents during slaughter and dressing operations.

Other pathogenic strains do not appear to be as closely associated with pigs, and may have a different ecology. In western Canada, O:8 and O:5,27 strains have
been found most commonly in humans, but only O:5,27 strains were found in the throats of slaughter-age pigs (Schiemann, 1989). In the US, O:5,27 strains were isolated from the cecal contents and feces of 2 out of 50 pigs at slaughter (Kotula and Sharar, 1993). Serovar O:8/biovar 1B, until recently considered to be the most common human pathogenic strain of _Y. enterocolitica_ in the US (Ostroff, 1995) and in western Canada (Toma and Lafleur, 1981), has seldom been reported in pigs.

### 7.1.2 Cattle

Positive tests, in serological control programs for brucellosis in cattle, have in some cases proved to be cross-reactions against _Y. enterocolitica_ serovar O:9 (Nielsen _et al._, 1996; Wauters, 1981; Weynants _et al._, 1996a). However, cattle are generally not considered to be carriers of human pathogenic _Y. enterocolitica_.

### 7.1.3 Sheep and goats

In Norway, Krogstad (1974) demonstrated outbreaks of _Y. enterocolitica_ infection in goat herds in which serovar O:2/biovar 5 was implicated. He also described a case in which an animal attendant was infected by the same serovar. Biovar 5 has also been isolated from goats in New Zealand (Lanada, 1990). Enteritis in sheep and goats due to infection of _Y. enterocolitica_ O:2,3, biovar 5 is also seen in Australia (Slee and Button, 1990). Serovar O:3 was isolated from the rectal contents in two (3.0%) of 66 lambs in New Zealand (Bullians, 1987).

![Figure 8.1](image-url)  
7.1.4 Poultry

Stengel (1985) isolated Y. enterocolitica serovars O:3 ($n = 3$), O:9 ($n = 3$), and non-virulent Y. enterocolitica ($n = 13$) from 130 samples of poultry. This is probably the first time these virulent serovars have been isolated from poultry, and there was no obvious opportunity for cross-contamination from pigs or pork.

7.2 Deer

Surveys in New Zealand have found deer to carry both O:5,27/biovar 2 and O:9/biovar 2 (S. Fenwick, personal communication, 1996).

7.3 Other animals

Dogs, cats, and rodents, such as rats, may also occasionally be fecal carriers of O:3 and O:9 (Hurvell; 1981; Fukushima et al., 1984; Fenwick et al., 1994; Hayashidani et al., 1995). The relatively intimate contact between man and pets suggests a potential reciprocal transmission, although such an epidemiological link has not been clearly confirmed (Nesbakken et al., 1991a; Fenwick et al., 1994; Ostroff et al., 1994). O:3 has been isolated from 1 (0.9%) of 117 crows in Japan, and O:9 from 1 (0.7%) of 156 Japanese serows (Kato et al., 1985).

It has been suggested that rodents may be reservoirs of O:8 and O:21 strains in North America (Schiemann, 1989), and evidence has been published that this is indeed the case in Japan (Hayashidani et al., 1995). Serovars O:8 and O:21 are closely related in many ways (biochemical profile, H antigens and animal virulence). Serovar 21 (‘O:Tacoma’) has been isolated from wild rodent fleas in the western US (Quan et al., 1974).

As is the case with human pathogenic variants, the animal pathogenic strains also belong to particular combinations of serovars and biovars (Mollaret et al., 1979; Hurvell, 1981). Serovar O:1/biovar 3 was responsible for widespread outbreaks in chinchilla both in Europe and the US during the period 1958–1964 (Mollaret et al., 1979; Hurvell, 1981). During the same period, epizootics were observed among hares along the French–Belgian frontier, caused by serovar O:2/biovar 5 (Mollaret et al., 1979; Hurvell, 1981).

8 Foods most often associated with sporadic cases and outbreaks

8.1 Pork

In contrast to the frequent occurrence of the bacterium in pigs and on freshly slaughtered carcasses, pathogenic Y. enterocolitica have only exceptionally been found in pork products at the retail sale stage, with the exception of fresh tongues (Vidon, 1985; Nesbakken et al., 1985; DeBoer et al., 1986; Ternström and Molin, 1987; World Health Organization, 1987; Wauters et al., 1988b; Schiemann, 1989; Delmas and Nesbakken et al., 1991b).
Because of the high prevalence of *Y. enterocolitica* in pig herds, strict slaughter hygiene will remain an important means to reduce carcass contamination with *Y. enterocolitica* as well as other pathogenic microorganisms (Skjerve et al., 1998). During lairage, pathogenic *Y. enterocolitica* may spread from infected to non-infected pigs (Fukushima et al., 1990).

Technological solutions have already been found which allow removal of the rectum without soiling of the carcass. 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). Meat inspection procedures concerning the head also seem to represent a cross-contamination risk: incision of the submaxillary lymph nodes is a compulsory procedure according to the EU regulations (European Commission, 1995). In a screening of 97 animals, 5.2% of samples from the submaxillary lymph nodes were positive; when sampling 24 of these lymph nodes in a follow-up study, 12.5% of the samples were positive (Nesbakken et al., 2003b; Figure 8.1). The compulsory incision of submaxillary lymph nodes may result in the bacterium being 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., 2003a).

### 8.2 Milk and dairy products

Worldwide studies indicate that *Y. enterocolitica* is fairly common in raw milk (Lee et al., 1981). *Y. enterocolitica* was also isolated from ice cream (Mollaret et al., 1972) and pasteurized milk (Sarrouy, 1972; Zen-Yoji, 1973) as early as 1970. However, it is almost solely in connection with outbreaks caused by contaminated pasteurized milk (Tacket et al., 1984; Greenwood and Hooper, 1990; Alsterlund et al., 1995), reconstituted powdered milk (Morse et al., 1984) and contaminated chocolate milk (Black et al., 1978) that the pathogenic strains have been found.

### 8.3 Water

Shallow wells in particular, and also rivers and lakes, are susceptible to contamination by surface runoff from rain or snow melt. Such runoff may become fecally contaminated by wild or domestic animals, or by leakage from septic tanks or open latrines in the surrounding areas. Water is a significant reservoir of *Y. enterocolitica* (Lassen, 1972; Harvey et al., 1976; Kapperud and Jonsson, 1978; Saari and Jansen, 1979; Langeland, 1983; Brennhovd, 1991). However, most isolates of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria obtained from water are variants with no known pathogenic significance to man.

### 9 Sporadic cases

*Y. enterocolitica* has been isolated from humans in many countries of the world, but it seems to be found most frequently in cooler climates (North America; the western coast of South America; Europe; northern, central and eastern Asia; Australia; New Zealand;
and South Africa) (Mollaret et al., 1979; World Health Organization, 1983, 1987; Aleksic and Bockemühl, 1990). The widespread nature of Y. enterocolitica has been well documented; by the mid-1970s Mollaret et al. (1979) had compiled reports of isolates from 35 countries on six continents. Y. enterocolitica infections are an important cause of gastroenteritis in the developed world, occurring particularly as sporadic cases in northern Europe (Black and Slome, 1988; Cover and Aber, 1989), where a clustering of cases during the autumn and winter has been reported (World Health Organization, 1983).

There are appreciable geographic differences in the distribution of the different phenotypes of Y. enterocolitica isolated from man (Mollaret et al., 1979; Wauters, 1991). There is also a strong correlation between the serovars isolated from humans and pigs in the same geographical area (Esseveld and Goudzaard, 1973; Pedersen, 1979; Wauters, 1979; Bercovier et al., 1980a; Schiemann and Fleming, 1981). Serovar O:3 is widespread in Europe, Japan, Canada, Africa, and Latin America. Sometimes, but not always, phage typing makes it possible to distinguish between European, Canadian and Japanese strains (Mollaret et al., 1979; Kapperud et al., 1990b). 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 78.8% of the cases in Belgium. Serovar O:9/biovar 2 is the second most common in Europe, but its distribution is uneven; while it still accounts for a relatively high percentage of the strains isolated in France, Belgium and the Netherlands, only a few strains have been isolated in Scandinavia (World Health Organization, 1983). Until recently, the most frequently reported serovars in the US were O:8 followed by O:5,27 (Mollaret et al., 1979; Bisset et al., 1990; Ostroff, 1995; World Health Organization, 1995). In recent years, serovar O:3 has been on the increase in the US; O:3 now accounts for the majority of sporadic Y. enterocolitica isolates in California (Bisset et al., 1990). In 1989, the estimated cost of yersiniosis in the US was $138 million (World Health Organization, 1995). Principal foodborne infections, as estimated for 1997, are ranked by estimated number of cases caused by foodborne transmission each year in the United States. Y. enterocolitica is number 10 in the list (among the bacteria in the list, Y. enterocolitica is number 7) (Mead et al., 1999). The appearance of strains of serovars O:3 and O:9 in Europe and Japan in the 1970s (Anon., 1976), and in North America by the end of the 1980s (Lee et al., 1990, 1991), is an example of a global pandemic (Tauxe, 2002).

The first Japanese case of Y. enterocolitica O:8 infection was linked to consumption of imported raw pork (Ichinohe et al., 1991), although O:8 infections from raw water have also occurred in Japan (Hayashidani et al., 1995).

The incidence of Y. enterocolitica infection in patients with acute endemic enterocolitis ranges from 0% to 4%, depending on the geographic location, study method, and population (Kapperud and Slome, 1998). Only a few epidemiological studies have been performed to investigate the sources of sporadic human infections. A 1985 study of Y. enterocolitica in Belgium identified consumption of raw pork as a risk factor for disease (Tauxe et al., 1987). The following variables were found to be independently related to an increased risk of yersiniosis in a case-control study conducted in Norway: drinking untreated water, general preference for meat to be prepared raw or rare, and frequency of consumption of pork and sausages (Ostroff et al., 1994).
10 Outbreaks

In the United States, chocolate milk (Black et al., 1978), pasteurized milk (Tacket et al., 1984), soybean curd (tofu) (Tacket et al., 1985) and bean sprouts (Aber et al., 1982) have been implicated as sources in outbreaks of *Y. enterocolitica* infection. These outbreaks, all of which occurred before 1983, were caused by *Y. enterocolitica* serovars that have been infrequently associated with human disease (serovars O:13, O:18), or which no longer predominate in the US (serovar O:8). More recently, the preparation of raw pork intestines (chitterlings) was associated with an outbreak of *Y. enterocolitica* O:3 infections among black US infants in Georgia (Lee et al., 1990); the organism was isolated from samples of the pork intestines. Also, in outbreaks in Buffalo, New York, between 1994 and 1996 (Kondracki et al., 1996), chitterlings were the vehicle.

The milkborne outbreak in Sweden in 1988 (Alsterlund et al., 1995) was probably caused by recontamination of pasteurized milk because of lack of chlorination of the water supply. In the multistate outbreak in 1982 (Tacket et al., 1984), milk cartons were contaminated with mud from a pig farm (Aulisio et al., 1982). In the case of the outbreak described by Greenwood and Hooper (1990), post-pasteurization contamination may have occurred from bottles. Previous studies have shown that milk-associated *Y. enterocolitica* outbreaks have been linked to the addition of ingredients after pasteurization (Black et al., 1978; Morse et al., 1984).

In 1981, an outbreak of infection due to *Y. enterocolitica* O:8 in Washington State occurred in association with the consumption of tofu packed in untreated spring water (Tacket et al., 1985). The outbreak serovar was isolated from the spring water samples. Another outbreak caused by serovar O:8 was traced to ingestion of contaminated water used in manufacturing or preparation of food (Schiemann, 1989). Two other *Yersinia* outbreaks have been associated with well water. One occurred among members of a Pennsylvania girl-scout troop after they ate bean sprouts grown in contaminated well water (Aber et al., 1982); the other was a familial outbreak of yersiniosis in Canada (Thompson and Gravel, 1986).

The epidemiology of yersiniosis in the US seems to have evolved into a pattern similar to the picture in Europe (Bottone et al., 1987; Bisset et al., 1990; Ostroff, 1995), where foodborne *Yersinia* outbreaks are rare, and where serovar 3 predominates (Mollaret et al., 1979; Prentice et al., 1991; Verhaegen et al., 1991). Although yersiniosis appears to be more common in Europe than in the United States, only five foodborne outbreaks have been reported in Europe (Toivanen et al., 1973; Olsovsky, et al., 1975; Greenwood and Hooper, 1990; Alsterlund et al., 1995; Swedish Institute for Infectious Disease Control, 1995).

In Japan, several outbreaks connected to schools (Zen-Yoji et al., 1973; Maruyama, 1987) and communities (Asakawa et al., 1973) have been reported. In all cases the vehicle was unknown. Often a few hundred persons were ill out of several hundred who were at risk. Serovar O:3 was the agent involved in all cases. In one outbreak in China, caused by serovar O:3 from pickled vegetables, 351 persons were ill (Anon., 1987).
11 Principles of detection

The analytical methods available today for the isolation of pathogenic *Y. enterocolitica* suffer from limitations such as insufficient selectivity and, in particular, inadequate differentiation between pathogenic and non-pathogenic strains.

11.1 Specific principles for isolation

A three-step method, based on a combination of cold enrichment in a non-selective medium with subsequent inoculation onto a highly selective medium, has been developed for the Nordic Committee on Food Analysis (1987).

Wauters *et al.* (1988b) developed a method for isolation of serovar O:3 from meat and meat products. The procedure is based on a 2- to 3-day selective enrichment period in irgasan-ticarcillin-potassium chlorate (ITC) enrichment broth at room temperature, and therefore saves time compared with the method described above (Figure 8.2).

Both *Y. enterocolitica* and *Y. pseudotuberculosis* seem to be more tolerant of alkaline conditions than do most other *Enterobacteriaceae*, and treatment of food enrichments with potassium hydroxide (KOH) may be used to selectively reduce the level of background flora (Aulisio *et al*., 1980) (Figure 8.3). Elements of the methods from the Nordic Committee on Food Analysis (1987), Schiemann (1982) and Wauters *et al.* (1988b), and KOH treatment (Schiemann, 1983), are incorporated into the International Organization for Standardization (ISO) method (ISO 10273) (Figures 8.2, 8.3; International Organization for Standardization, 1994).

![Diagram](image.png)

**Figure 8.2** Method for recovery of *Y. enterocolitica* from foods according to the International Organization for Standardization (1994). This element of the method is recommended for serovar O:3 in particular. ITC, irgasan-ticarcillin-potassium chlorate enrichment broth; SSDC, Salmonella-Shigella + sodium deoxycholate, CaCl₂ agar.
11.2 Detection by DNA colony hybridization

Genetic probes can also be used in DNA colony hybridization to demonstrate virulent *Y. enterocolitica* strains (Wachsmuth, 1985; Tenover, 1988; Kapperud et al., 1990a). Isolation plus hybridization increased the detection rate from 16% to 38% for the method according to Wauters et al. (1988a), and from 10% to 48% for the Nordic method. The results of this investigation (Nesbakken et al., 1991b) support the supposition that conventional culture methods lead to underestimation of virulent *Y. enterocolitica* in pork products.

11.3 Detection by polymerase chain reaction

Polymerase chain reaction (PCR) methods often use primers targeting the *virF* (Thisted-Lambertz et al., 1996; Weynants et al., 1996b) or *yadA* (Kapperud et al., 1993) gene, but the *IcrE* gene (Viitanen et al., 1991) and the *yopT* gene (Arnold et al., 2001) from the virulence plasmid have also been used. *Y. enterocolitica* may lose the virulence plasmid during culture, subculture or storage (Blais and Philippe, 1995). Accordingly, PCR methods based on chromosomal virulence genes, often the *ail* gene, have been developed. Often a combination of genes from the virulence plasmid and the chromosome are used. A common gene combination in such a multiplex PCR assay is the *virF* and *ail* genes (Kaneko et al., 1995; Nilsson et al., 1998).

Rasmussen et al. (1995) detected *Y. enterocolitica* O:3 in fecal samples and tonsil swabs from pigs using IMS and PCR based on the *inv* gene. O:3 cells were detected after pre-enrichment, but direct detection needed further optimization of the sample preparation procedures. By combining *inv*, *virF* and *ail* genes in a multiplex PCR assay, Weynants et al. (1996b) could differentiate between *Y. pseudotuberculosis*, virulent *Y. enterocolitica* and *Y. enterocolitica* O:3.