Yersinia enterocolitica: A Dangerous, But Often Ignored, Foodborne Pathogen

Anna Zadernowska [a], Wioleta Chajęcka-Wierzchowska [a] & Łucja
Łaniewska-Trokenheim [a]

[a] Department of Industrial and Food Microbiology, Faculty of Food
Sciences , University of Warmia and Mazury , Olsztyn , Poland
Accepted author version posted online: 14 Oct 2013.Published
online: 16 Dec 2013.

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Yersinia enterocolitica: A Dangerous, But Often Ignored, Foodborne Pathogen

ANNA ZADERNOWSKA, WIOLETA
CHAJĘCKA-WIERZCHOWSKA, AND ŁUCJA
ŁANIEWSKA-TROKENHEIM

Department of Industrial and Food Microbiology, Faculty of Food Sciences,
University of Warmia and Mazury, Olsztyn, Poland

Yersinia enterocolitica *is listed in the annual reports of the European Food Safety Authority (EFSA) as the third-most-common enteropathogen. The highly pathogenic* Y. enterocolitica *bioserotype 1B/O8 is geographically limited to Northern America, although it has also emerged in Japan and Europe. Furthermore, the number of reports on the pathogenicity of serotype 1A (so far regarded as nonpathogenic) has been increasing. Humans are most often infected by consuming raw or inadequately thermally processed pork or milk as well as vegetable products and ready-to-eat meals. Identification of these bacteria in food presents considerable methodological problems.*

Keywords   Foodborne pathogens, Pathogen detection, *Yersinia enterolitica*

Introduction

In May 2012, the European Food Safety Authority (EFSA) published the annual e-report on the prevalence of zoonoses and foodborne pathogens (The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010). This report covers the period of 2010. Among zoonoses, yersiniosis is listed in third place after campylobacteriosis and salmonellosis (these data are convergent with information recorded in the previous years). According to the data from 2010 submitted by 27 European Union (EU) member countries, *Yersinia enterocolitica* was the cause of 6776 disease cases in humans. It is, however, suspected that these numbers are underestimated, since not all cases are reported to the appropriate authorities or services. The main reason may be associated with diagnostic problems, which prevents all cases being correctly diagnosed. Many cases of yersiniosis have also been reported outside Europe, for instance, in Australia, Japan, USA, Brazil, Iran, Bangladesh, and Nigeria.[1–3]

Yersinia enterocolitica is a gram-negative rod from the *Enterobacteriaceae* family. These bacteria were first described by L. Malasses and W. Vignal in 1883. A year later, A. Yersin and S. Kitasato isolated the rods—now named *Yersinia pestis*—during a plague outbreak in Hong Kong. *Yersinia enterocolitica* was discovered in 1939 when Schleifstein and Coleman studied some previously unidentified strains isolated from facial lesions and intestines of humans showing sign of enteritis.[4] In Europe, *Yersinia enterocolitica* was

Address correspondence to Wioleta Chajęcka-Wierzchowska, Department of Industrial and Food Microbiology, Faculty of Food Sciences, University of Warmia and Mazury, Plac Cieszyński 1, 10-726 Olsztyn, Poland. E-mail: wioleta.chajecka@uwm.edu.pl

first isolated from clinical material by A. Hässig in 1949. Thanks to W. Frederiksen's work in the 1960s, the classification of *Yersinia* genus was described and these rods were then classified into the *Enterobacteriaceae* family. The genus *Yersinia* is composed of several species, of which only *Y. enterocolitica, Y. pseudotuberculosis, Y. pestis*, and *Y. ruckeri* are known pathogens for humans and animals. The last one is the causative agent of enteric red mouth disease (ERM) in salmonid fish.[5]

Factors Influencing Growth and Survival in Foods

Yersinia enterocolitica is a short, gram-negative, facultative anaerobic, non-spore-forming straight rod or coccobacilli bacterium (0.5–1-2 µm).[6] The optimal temperature for its growth is 22–29 °C, but it is capable of growing in a wide range of temperatures. Scientists have reported different ranges of cardinal temperatures: from 0 to 45 °C[7,8] and from -1 to 40 °C.[9] It was also reported that some *Yersinia* strains can grow at temperatures as low as -5 °C, although growth is very slow below 0 °C.[10] All *Yersinia enterocolitica*, regardless of their pathogenicity or lack thereof, are capable of degrading urea and sugars such as sucrose and glucose without gas production; they are oxidase-negative and can decarboxylate ornithine, but do not ferment lactose, decarboxylate lysine, degrade citrate, nor produce H_2S or acetoin (Table 1). Pathogenic strains do not degrade aesculin and pyrazinamidase and show calcium-dependent growth at 37 °C.

Depending on the temperature, *Yersinia enterocolitica* can present different phenotypic features. Most *Yersinia enterocolitica* strains are immotile at 37 °C, whereas below

Table 1
Biochemical characteristics of *Yersinia enterocolitica*

Biochemical test	Reaction of *Yersinia enterocolitica* including different biotype					
	1A	1B	2	3	4	5
Esculin hydrolysis	+	−	−	−	−	−
Inositol fermentetion	+	+	+	+	+	+
Salicin fermentation	+	−	−	−	−	−
Sorbose fermentation	+	+	+	+	+	−
Trehalose fermentation	+	+	+	+	+	−
Ornithine decarboxylase activity	+	+	+	+	+	+
Lipase activity	+	+	−	−	−	−
Pyrazinamidase activity	+	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	−
Indole production	+	+	(+)	−	−	−
D-Xylose fermentation	+	+	+	+	−	s
Vogues Proskauer reaction	+	+	+	+	+	(+)
β-D-Glucosidase	+	−	−	−	−	−
Proline peptidase	s	−	−	−	−	−
DNase	−	−	−	−	+	+

Note. Modified from References [11–13].
(+) = delayed positive; s = reaction depends on bacterial strains.

30 °C they become motile with peritrichously distributed flagella. However, the role of flagella may differ for the various biotypes depending on the presence of the pYV virulence plasmid.[14] Freund *et al.*[15] found, by live-cell imaging, that the pYV-negative strain is motile at 27 °C as well as at 37 °C in the three-dimensional collagen environment. In contrast, the pYV-positive strain were immotile at 37 °C.

Because of its psychrotrophic nature, a cold temperature does not prevent growth, but only retards it. Studies have shown that at temperatures below 3 °C, *Yersinia enterocolitica* can multiply its population by 2 logarithmic units within 4 days. Moreover, *Yersinia enterocolitica* multiplies in cold conditions much faster than *Listeria monocytogenes,* the other important psychrotrophic foodborne pathogen.[16,17] Cold-adapted organisms such as *Y. enterocolitica* must alter the composition of lipids and change the protein contents in the cell membrane in order to maintain essential functions such as nutrient uptake, ion pumping, and electron transport.[18] Ilijev and Najdenski[19] found some differences in the survival and stability of the plasmid among different serotypes. It may be assumed that less virulent *Y. enterocolitica* serotypes O:9 and O:3 can survive longer during storage, also with a high level of accompanying microflora than highly virulent O:8 serotypes. *Yersinia enterocolitica* is relatively susceptible to heating, and does not survive pasteurization or typical cooking, boiling, baking, and frying temperatures. Heat treatment of milk and meat products at 60 °C for 1–3 min effectively inactivates *Y. enterocolitica*.[20] Heating susceptibility is strain-dependent: *D* values (the time required at a certain temperature to kill 90% of the organisms) in milk may range from 0.7 to 57.6 s.[21] The thermostable enterotoxin produced by these bacteria is degraded by heating at 100 °C for 20 minutes and at 120 °C for 15 minutes.[22,23] Considering the acidity of environment, the optimum for growth is between pH 7 and 8, whereas growth below pH 4 has not been reported, although the type of acidifier and temperature also play a certain role. It has been confirmed that with a decrease in temperature, *Yersinia* prefers a less acidic environment. The presence of organic acids significantly limits the growth of these bacteria, with the strongest inhibitor being acetic acid, followed by lactic acid and citric acid.[24,25] Due to the strong effect of lactic acid on these bacteria, they are not usually found in fermented milk products. Even if *Yersinia enterocolitica* is present in raw materials designed for fermentation, its growth is generally inhibited and it is totally inactivated because of the growth of lactic acid bacteria and the production of their metabolites.[26] However, as indicated by Lindqvist and Lindblad,[27] *Y. enterocolitica* may survive in fermented sausage for long periods as well. This pathogen shows good growth in the environment, with up to 5% of salt regardless of temperature, whereas it is inhibited at 7% salt concentration.[21] The minimum water activity allowing growth depends on the temperature.[28] Stern *et al.* tested four strains of *Y. enterocolitica* and reported that 7% salt (0.945 water activity) was bactericidal to all four strains tested, when incubated at 3 °C, but at 25 °C both bactericidal and bacteriostatic effects were observed. At 9% NaCl and 25 °C, all four strains were killed.[29] Some authors report that method of food packaging (vacuum packing or packaging at high CO_2 concentration) does not influence the growth of these bacteria, which grow very well in both conditions.[30−32] There is also evidence that modified atmosphere packaging at 100% N_2 and CO_2/N_2 gas mixers inhibited the growth of *Y. entericolitica* at refrigeration temperatures.[33] Potassium sorbate up to 5000 ppm inhibit the growth of *Y. entericolitica* at pH 6.5 but at pH 5.5 concentrations above 1000 ppm virtually eliminate growth or cause inactivation. Selma *et al.* found that treatments with ozone (1.4 and 1.9 ppm) and with ozonated water (1 minute exposure) reduce *Yersinia* loading.[34]

It has been proven that the increased growth capacity in different environmental conditions is clearly correlated with the presence of the virulence pYV plasmid.[18] The most

recent studies on the potential inhibition of these bacteria in food products have indicated the possibility of using virulent bacteriophages to effectively eliminate *Yersinia enterocolitica*. Orquera *et al.*[35] studied the efficacy of the *Yersinia* phage PY100 to reduce the numbers of *Y. enterocolitica* in meat at 4 °C, applying different multiplicities of infection. Initial experiments were carried out in broth at 4 °C and 37 °C to compare cell number reductions under chilling and optimized growth conditions, respectively. *Y. enterocolitica* cell numbers were reduced in broth at 4 °C (up to 3 $\log_{10}$ units after 24 hours) and 37 °C (5 $\log_{10}$ units after 1.5 hours) and also in pork meat at 4 °C (2 $\log_{10}$ units after 48 hours). The highest cell number reductions were obtained at the highest infection. This was the first report on an application of phage to reduce *Yersinia* cell numbers in food.

Taxonomy, Classification, and Pathogenicity

Among *Yersinia enterocolitica*, there are considerable differences in biochemical features and pathogenicity. Based on these differences, six biotypes were identified within this species: 1A, 1B, 2, 3, 4, and 5.[36] Potential pathogenicity is the main criterion of this classification. The biotypes 1B and 2–5 are regarded as pathogenic, since they have so-called "virulence" markers, such as enterotoxin Yst (*Yersinia* stable toxin), Myf antigen, Inv invasine, and Ail adhesin. The presence of pYV plasmid (*Yersinia* virulence) with approximately 70,000 bp is also one of the basic indicators of virulence.[37] This plasmid encodes YadA adhesin (i.e., the protein of the external membrane that allows adhering to the host cells), Yop outer proteins, which paralyze the immune system, and Ysc proteins composing the secretion system.[10,34] It has been established that the pathogenic strains with pYV plasmid have common properties: calcium-dependent growth, capability of absorbing Congo red, insensitivity to bacteriocidal activity of human serum, and autoagglutination.

The biotype 1B is thought to be the most pathogenic to humans. The strains belonging to this biotype have, apart from the above-mentioned pathogenicity markers, an additional virulence determinant: high pathogenicity island (HPI).[39,40] The biotype 1A is regarded as nonpathogenic, but this opinion is more and more frequently debated mainly due to reports on the isolation of this biotype from clinical materials.[12,41,42] The strains that belong to the biotype 1A do not have pYV plasmid or other virulence markers.[43]

Yersinia enterocolitica can be divided into 76 serological groups based on the structure of somatic O antigen. According to the classification proposed by Neubauer,[44] *Y. enterocolitica* subsp. *palearctica* includes strains of European origin that belong to the following bioserotypes: 4/O:3, 2/O:9,2 and 3/O:5,27, 1A/O:7,8, 1A/O:6,30, and 1A/O:5, whereas *Y. enterocolitica* subsp. *enterocolitica* includes strains of American origin from the biotype 1B and serotypes 1A/O:7,8. *Yersinia enterocolitica* strains that are most frequently the cause of disease in humans belong to the serotypes 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3, and 4/O:3, but less frequently to 3/O:5,27 and other serotypes of the biotype 1B (Table 2).

Despite the fact that *Yersinia enterocolitica* are found in all climatic zones, for many years the individual biotypes/serological groups were linked to specific geographical regions. Studies carried out since the end of the 1980s have indicated that *Y. enterocolitica* O:3 and O:9 are mainly isolated in Europe and serotype O:8 in the USA. It is probable that the unrestricted flow of raw materials, feedstuffs, products, and great interest in tourism have been responsible for the increase in disease cases caused by *Y. enterocolitica* O:8 in the climatic zones where this microorganism has not yet been isolated. The first reports of food poisoning caused by this serotype were recorded in Japan in 2004. The disease outbreak occurred in a school following ingestion of a salad containing apples, cucumbers, ham, potatoes, carrots, and mayonnaise.[45] In Europe, a fully virulent *Y. enterocolitica* O:8 strain

Table 2

Relationship between biotype, O serotype, and pYV carriage of *Y. enterocolitica*

Biotype	Serotypes
1A	O:4; O:5; O:6,30; O:6,31; O:7,8; O:7,13; O:10; O:14; O:16;O:21; O:22; O:25; O:36; O:37; O:41,42; O:46; O:47; O:57
1B	O:4,32[a]; O:8[a]; O:13[a]; O:16; O:18[a]; O:20[a]; O:2[a]; O:25; O:41,42
2	O:5,27[a]; O:9[a]; O:27
3	O:1,2,3[a]; O:3[a]; O:5,27[a]
4	O:3[a]
5	O:2,3[a]

Note. Modified from Tennant *et al.*[12]
[a]Serotypes that include strains that carry pYV.

was most probably isolated for the first time in Germany. This strain originated from clinical material isolated from a 4-year-old boy.[46] In Poland, the first case of *Y. enterocolitica* O:8 isolation from clinical material sampled from a 38-year-old woman was reported in 2004. Subsequent years have revealed a considerable increase in the percentage of cases caused by this *Y. enterocolitica* serotype in Poland.[47,48] This is very disturbing, since the bacteria that belong to the biotype 1B and serological group O:8 are thought to be the most dangerous and most virulent to humans. They may cause ulceration in the mucosa of the gastrointestinal tract and may even lead to death.[49] Yersiniosis frequently affects children and studies carried out in Germany have shown that this disease was most often diagnosed in children under 5 years of age.[50] Furthermore, Okwori *et al.*[51] found a more common occurrence of these bacteria from faeces of children than from adults. *Yersinia enterocolitica*, after entering the human body via the alimentary route, multiplies in the intestinal lumen and adheres to the mucosa of the small bowel. The clinical picture of yersiniosis is very diversified. The symptoms become noticeable after 4–7 days following infection and may persist for over 3 weeks. Food poisoning most often results from the ingestion of food products in which a thermostable toxin has been produced. By increasing the concentration of cyclic gaunosine monophosphate (GMP) in the intestinal mucosal cells, excessive amounts of water are excreted to the intestinal lumen and diarrhea develops. Fever, stomach contractions, vomiting, and hematuria may also be present.[52] In general, the symptoms are self-limiting and disappear after several days. Mesenteric lymphadenitis and inflammation of the distal small bowel and the caecum are also reported. Sometimes (particularly in children under 7 years of age) the symptoms may be mistaken for appendicitis. In extreme cases, *Yersinia enterocolitica* may cause bacteremia and sepsis leading to death.[50,53]

Reservoirs

Yersinia sp., similar to the other bacteria from the *Enterobacteriaceae* family, are prevalent in the environment due to their presence in companion (dogs, cats), feral (rodents, birds, wild boars), and domestic (pigs, sheep, poultry) animals.[54−56] Following excretion from the body, these bacteria may survive for a long time in the environment due to their low nutritional requirements and relatively high resistance to unfavorable conditions. In general, pigs are thought to be the major reservoir and main cause of the spreading of pathogenic

Yersinia enterocolitica; these bacteria are commonly isolated from the tongue and the gastrointestinal tract of pigs.[57,58] Fredriksson-Ahomaa *et al.*[59] and Falcao *et al.*[60] reported a significant overlap in phenotypes and genotypes of human and pig strains. Fredriksson-Ahomaa *et al.*[59] also showed that pigs were an important source of human *Y. enterocolitica* 4/O:3 infection in Germany and Finland.

However, studies carried out by Baumgartner *et al.*[61] in Switzerland did not confirm the thesis that pork was the main cause of infection with these bacteria. The antibiotic resistance of 386 *Yersinia enterocolitica* strains isolated from patients, pork, and swine feces was determined. The resistance to 16 commonly administered antibiotics and two growth promoters used in animal production (carbadox and olaquindox) was investigated. Despite using growth promoters in Switzerland for 25 years, all strains were sensitive to carbadox and olaquindox. This study revealed a significant difference in the antibiotic resistance of "animal" strains and those isolated from humans. Differences between the strains isolated from meat and swine faces were also detected in serotyping.[61]

Poultry meat, which is eagerly consumed in many countries because of its dietary properties, is indicated as a potential source of these pathogenic bacteria.[62−64] Sharifi *et al.*[65] tested 190 samples of poultry meat and 189 samples of beef meat and found that 42 samples of poultry and 18 samples of beef meat were positive for *Yersinia*. The prevalence of *Y. enterocolitica* with the highest incidence was 80%. The occurrence of *Y. enterocolitica* was slightly higher in chicken than beef meat.[65]

In Sweden, studies were carried out to determine whether sheep might be a reservoir of *Yersinia enterocolitica* pathogenic to humans. Despite detecting these bacteria in a large number of samples, no strains pathogenic to humans were found. The authors emphasized, however, the significant proportion of strains belonging to the biotype 1A that had been previously regarded as nonpathogenic despite an increasing number of scientific reports suggesting that the strains of this biotype may be pathogenic to humans.[66] *Yersinia enterocolitica* is found in the gastrointestinal tract of animals and the contamination of meat may result from improper slaughter and gutting techniques. Furthermore, cross-contamination (secondary) during production of food may occur. Carrasco *et al.*[67] referring to the data released by the World Health Organization (WHO), reported that 25% of all food poisoning cases are caused by cross-contamination due to violation of Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) standards. Secondary contaminations seem particularly important, because these bacteria may multiply in biofilms.[68] A biofilm generated within an installation in a production plant is difficult to remove with standard methods of washing and disinfection; pathogenic microorganisms may be intermittently released from a biofilm and may secondarily contaminate a product.[69] There are also reports of cases of yersiniosis due to the ingestion of vegetable products that were most probably manufactured from raw materials fertilized with organic fertilizers.[70] The risks posed by these bacteria found in vegetable food products have increased in recent years because of the higher numbers and wider availability of minimally processed products, wheat germ, or cold-pressed fruit juices that are not thermally processed. These products contain almost all microorganisms that were present on raw materials used for their production. In addition, secondary contamination may occur during harvesting and processing. The shelf life of these products is usually short and they require storage under refrigerated conditions. Low temperatures largely inhibit the growth of the *Enterobacteriaceae* except for *Yersinia*, which, due to its psychrotrophic nature, dominates the environment and may become predominant over time.[71] The storage of fish, fish products, meat, meat products, milk, and dairy products contaminated with *Yersinia enterocolitica* at 0–4 °C may, according to Tudor *et al.*[72] lead to intensive multiplication of these bacteria. Bracket *et al.*[73]

detected live *Yersinia enterocolitica* cells in food products with pH 4 stored for 21 days at 5 °C. Barbini de Pederiva and Stefanini de Guzmán showed that these bacteria also easily survived the process of freezing and storage at -18 °C.[74]

It has been pointed out that the majority of infections caused by *Yersinia enterocolitica* are seasonal and their number tends to increase in winter months and in colder climatic zones.[75] Furthermore, differences in serotypes may be found, depending on the climatic conditions.[76]

Foodborne Outbreaks

Foodborne outbreaks of *Y. enterocolitica* infection have been reported throughout the world (Table 3). The largest outbreak (affecting 1051 persons) related to this bacterium was reported in Japan in 1980 following the ingestion of milk contaminated with *Y. enterocolitica*. Subsequent cases of the disease were recorded in Canada, the USA, and Sweden. Outbreaks also occurred following the consumption of meat and meat products. In 2006, 11 people fell ill in Norway after ingesting traditional pork products consumed during the holidays.[93] Pork products have also been a source of food poisoning in the USA and Hungary. In Japan, an epidemiological investigation was undertaken in relation to a so-called "family outbreak" during which three persons from one household fell ill. Tests with pulsed-field electrophoresis were carried out, and based on their results, a genetic similarity between the strains isolated from three family members was confirmed, whereas these bacteria were not detected in the animals kept by this family. These authors suggested that pork could have been a source of food poisoning and pointed out that within this family, *Yersinia enterocolitica* was isolated from an 11-month-old child who had not consumed pork. Therefore, these authors assumed that the grandmother fell ill following ingestion of contaminated meat and she then infected the child. The results of studies suggest the possibility of *Yersinia enterocolitica* transmission not only via food, but also from human to human.[96] Similar conclusions were drawn by the authors who reported an outbreak of yersiniosis on a tanker. Of the 120 crewmembers and workers, 22 suffered from gastrointestinal symptoms. In 17 of the patients, *Y. enterocolitica* O:3 was isolated from stool samples. All available food and water samples were negative and the source of infection was not determined. Probably a foodborne transmission was involved, although person-to-person transmission could not be excluded.[97]

Detection of *Y. enterocolitica* in Food

The type of environment from which the bacteria are isolated and the presence of other microorganisms are the most important factors for detection of *Yersinia enterocolitica*. Cultures from blood and feces are performed during clinical studies. If *Yersinia enterocolitica* is predominant and forms a large population, its detection does not generally present any problems, but not all laboratories routinely perform such assays. Serological tests are often used in clinical investigations. Currently, enxyme-linked immunosorbent assay (ELISA) is the most common technique together with Western-immunoblotting with Yop proteins as the antigens. However, due to the antigenic affinity between strains belonging to different serological groups and different species, identification based on serological tests may be difficult.

Detection of *Y. enterocolitica* in food is an entirely different issue. These rods are not demanding and grow well on typical media for culturing Enterobacteriaceae (MacConkey, SS, Hektoen), but following standard incubation at 37 °C for 24 hours they form tiny

Table 3
Some foodborne outbreaks caused by *Y. enterocolitica*

Food vehicle	Year and country		Cases	Bioserotype	Reference
Chocolate milk	1976	New York, USA	38	O:8	(77)
Milk	1980	Japan	1051	O:3	(78)
Powdered milk, chow mein	1981	New York, USA	239	O:8	(79)
Tofu and untreated spring water used to wash tofu at plant	1981	Washington, USA	50	O:8	(80)
Bean sprouts immersed in contaminated well water	1982	Pennsylvania, USA	16	O:8	(81)
Not identified (probably food eaten in canteen)	1982	Finland	26	O:3	(82)
Brawn	1983	Hungary	8	O:3	(83)
Water	1984	Canada	2	4/O:3	(84)
Handling chitterlings	1988	Georgia, USA	15	O:3; O:1,2,3	(85)
Cream, milk	1988	Sweden	61	O:3	(86)
Pasteurized milk	1995	Vermont, USA	10	O:8	(87)
Water used to dilute buttermilk	1997	India	25	4/O:3	(88)
Chitterlings	2001	Tennessee, USA	12	4/O:3	(89)
Not identified (probably food eaten in canteen)	2003	Finland	12	4/O:3	(90)
Salads (apples, potatoes, carrots, cucumber, ham, mayonnaise)	2004	Japan	42	O:8	(45)
Homemade Christmas brawn	2005	Norway	4	4/O:3	(91)
Barbecue or roast pork	2009	Australia	3	Unknown	(92)
Homemade Christmas brawn /pork chops	2005/2006	Norway	11	2/O:9	(93)
Ready-to-eat salad mix	2011	Norway	21	2/O:9	(94)
Pasteurized cow milk	2011	Pennsylvania, USA	16	Unknown	(95)

colonies that can easily be omitted in the presence of other bacteria from this family (often forming large, mucous colonies). It is thus usually recommended to incubate at 22 °C, not at 37 °C, since it inhibits the growth of other bacteria. Samples are often incubated under refrigerated conditions, which significantly extends the time of detection (Table 4). A cold enrichment step is implemented: incubation is carried out at 4 °C for 3 weeks. Samples are taken every week and cultured on selective agar media. Although the lowered temperature favors the growth of psychrotrophic *Yersinia enterocolitica*, if a medium is not adequately selective, it may stimulate the growth of other *Yersinia* species and different bacteria present in a food sample.[43] In a study by Sihovonen *et al.*[107] 25% of the strains belonging to pathogenic bioserotypes of *Y. enterocolitica* were only detected after cold enrichment. However, cold enrichment also increased the number of isolates representing biotype 1A and *Y. enterocolitica*–like strains. Selective enrichment is an alternative to cold enrichment, but this method is more useful for outbreaks, because it allows for rapid confirmation of *Yersinia enterocolitica*. In such cases, 9 mL of ITC (irgasan, ticarcillin, and potassium chlorate) medium is enriched with 1 mL of medium for cold enrichment and incubation is performed at 25–30 °C for 48 hours.[104]

It should be remembered that food is a very specific environment for the growth of pathogenic microorganisms. Each type of thermal processing, addition of salt, sugar, etc., may cause sublethal damage to the cells, which significantly hinders their detection directly after manufacturing. Studies have been carried out in which *Y. enterocolitica* O:3, O:8, and O:17 were exposed to stress factors in 0.1 M phosphate-buffered saline (PBS), pH 7.0, at 47 °C for 70, 60, and 12 minutes. Over 99% of a live cell population subjected to stress factors were sublethally damaged. The damaged cells were able to form colonies on brain heart infusion medium, whereas they did not have such capability on trypticase soy agar plus bile salt medium.[108] However, under refrigerated conditions, even sublethally damaged *Yersinia enterocolitica* cells in food products were capable of recovering and multiplying to a level that posed a risk to human health.

Salmonella-Shigella-deoxycholate calcium chloride (SSDC) agar was one of the widely used selective media, originally developed for more efficient isolation of *Y. enterocolitica* from pork products.[103] Furthermore, better recovery rates of *Y. enterocolitica* than on SSDC or McConkey agar has been found with Cefsulodin-Irgasan-Novobiocin (CIN) agar.[109] CIN agar inhibits the growth of many other organisms of the family Enterobacteriaceae to the advantage of the more slowly growing *Yersinia* species. *Y. enterocolitica* forms distinctive colonies with a deep red center (bull's eye) with a sharp border surrounded by a translucent zone on CIN agar. Some of the competing Enterobacteriaceae genera (*Citrobacter, Enterobacter, Serratia, Klebsiella*) are able to grow on CIN agar and produce a little bit larger but similar colonies than *Yersinia*.[109,110] This creates a possible source of error when a limited number of presumptive colonies are picked for identification.

In accordance with International Organization for Standardization (ISO) method ISO 10273,[104] three stages of *Yersinia enterocolitica* detection in food, feedstuffs, and environmental samples are identified. The first stage involves multiplication in selective liquid media. Similar to the detection of other pathogens, this stage aims at creating optimal conditions for the growth of these bacteria in the broth with peptone, sorbitol, and bile salts (PBS) and in the broth with irgasan, ticarcillin, and potassium chlorate (ITC). Apart from the preenrichment media listed in the above-mentioned standard, other selective media such as Ossmer broth (YSEO) and Modified Tryptone Soya broth are also used.[105,106]

The next stage consists of culturing on solid differential media: cefsulodin-irgasan-novobiocin (CIN) agar and SSDC (agar with sodium deoxycholate and calcium chloride). In the third stage, biochemical and serological confirmation tests are performed. Difficulties

Table 4

Methods of isolation *Y. enterocolitica* from food

Preenrichment			Selective enrichment			Alkalization	Agar plate			Reference
Broth	Temperature	Time	Broth	Temperature	Time		Broth	Temperature	Time	
PSB	4°C	3–4 weeks	—			—	MAC	25°C	48 hours	(98)
							CIN	30°C	24 hours	
PBS	10°C	10 days	—			KOH	MAC	22–26°C	48 hours	(99)
							CIN			
PSB/PBS	25°C	1–3 days	—			KOH	MAC	25°C	48 hours	(100)
							CIN	30°C	24 hours	
—			SEL	22°C	3 days	—	MAC	25°C	48 hours	(20)
PSB	4°C	7 days	MRB	22°C	4 days	—	CIN	30°C	24 hours	(101)
YER	4°C	1 day	BOS	22°C	5 days	—	CIN	30°C	24 hours	(102)
TSB	22°C	10 days	BOS	22°C	7 days	—	CIN	30°C	24 hours	(103)
			ITC	24°C	2 days	—	SSDC	30°C	24 hours	(104)
PSB	25°C	2–3 days				—	CIN	30°C	24 hours	
PSB	25°C	2–3 days				KOH	CIN	30°C	24 hours	
—			ITC	25°C	2 days	—	SSDC	30°C	24–48 hours	
YSEO	30°C	24 hours	—			KOH	CIN	30°C	24 hours	(105, 106)

Note. PBS/PSB = phosphate-buffered saline; MAC = MacConkey agar; CIN = cefsulodin-irgasan-novobiocin agar; KOH = potassium hydroxide; SEL = selenite broth; YER = yeast extract–rosebengal broth; MRB = modified Rappaport broth with magnesium chloride, malachite, and carbenicilin; BOS = bile-oxalate-sorbose; TSB = tryptic soy broth; ITC = irgasan-ticarcillin-chlorate; YSEO = Ossmer broth; SSDC = *Salmonella-Shigella* deoxycholate calcium chloride agar.

62

with isolating pathogenic strains are associated with the fact that very often there are also nonpathogenic strains in the tested materials; furthermore, on standard media (CIN, SSDC) the colonies of nonpathogenic strains do not differ from the pathogenic strains. The main difficulty is with selecting proper colonies for further confirmation assays.

It has been found that enteropathogenic *Yersinia enterocolitica* strains have some phenotypic features that correlate with the presence of pYV plasmid in their cells. These include calcium-dependent growth and binding of Congo red. CRMOX medium (Congo red magnesium oxylate) is based on the mechanism of these features. The growth on this medium at 37°C (seen as small red colonies) indicates the presence of pYV plasmid in the bacterial cells. The strains that do not have this plasmid grow as large salmon-colored or cream colonies.[111] It should be remembered that *Yersinia*, when incubated for a longer period of time at 37°C, may lose the virulence plasmid. Weagant[112] proposed a chromogenic culture medium (*Yersinia enterocolitica* chromogenic medium) for the isolation of these bacteria. Incubation is performed at 30°C for 24–48 hours and potentially pathogenic strains grow as convex colonies with a red center, whereas the others form violet or blue colonies. This medium contains the mixture of cefsulodin, irgasan, and vancomycin as growth inhibitors. 5-Bromo-4-chloro-3-indolyl-β-D-glucopyranoside is a chromogenic substrate. Based on the color of colonies, pathogenic strains can be easily differentiated from the strains without virulence factors.[112]

Whereas phenotypic methods study the presence or absence of biological and metabolic activities for the characterization of bacteria, genotypic methods apply more specific characterization of bacteria at the nucleic acid level. Apart from plate cultures, there are a number of molecular methods used to detect these pathogens in food. Several polymerase chain reaction (PCR) assays have been developed to *Y. enterocolitica* in food samples.[43] Many of these samples use primers targeting the *yad*A or *vir*F gene located on the pYV. Because of possible plasmid loss, PCR methods targeting chromosomal virulence genes have also been created for natural samples. The *ail, inv*, and *yst* genes, located in the chromosome of pathogenic *Y. enterocolitica* strains, are the most frequently used chromosomal targets.[59] The methods based on DNA hybridization and PCR reaction are most commonly utilized. Studies have indicated significant differences in the level of detection of these bacteria depending on the method used. The PCR technique is more sensitive in comparison with plate cultures and it has been shown that *Yersinia enterocolitica*, despite its presence in tested materials, does not always form colonies on solid media.[33] The studies carried out by Vázlerová and Steinhauserová[113] with the PCR method showed that out of 2982 samples collected from pigs, 120 were contaminated with *Yersinia enterocolitica*. These samples were simultaneously cultured on plates and tested with standard biochemical assays, which yielded 111 positive results. Poultry meat was also examined: out of 929 samples tested with PCR, *Yersinia enterocolitica* was detected in 19 samples, whereas with the classical method it was only detected in 17 samples. Multiplex PCR were used for specific detection and differentiation of *Y. enterocolitica* serotype O:3 and other pathogenic *Y. enterocolitica*, obtained with *rfbC, inv, ail, virF yst*, and primers.[114] Recently, real-time PCR (qPCR) methods based on the virulence-associated genes, in particular *ail*, for detection of pathogenic *Y. enterocolitica* have been developed.[115,116] Real-time PCR assays, especially those using TaqMan-based probes, provide greater specificity and require less time (due to reduced cycle times) and labor to complete than conventional PCRs.[59] Lambertz *et al.*[116] have developed and evaluated in-house a TaqMan probe–based real-time PCR method for the detection of this pathogen. The complete method comprises overnight enrichment, DNA extraction, and real-time PCR amplification. The selected primer-probe set was designed

to use a 163-bp amplicon from the chromosomally located gene *ail*. Following the enrichment of 10 g of various food samples (milk, minced beef, cold-smoked sausage, fish, and carrots), the sensitivity ranged from 0.5 to 55 colony-forming unit (CFU) *Y. enterocolitica*. Good precision, robustness, and efficiency of the PCR amplification were also established. In addition, the method was tested on naturally contaminated food; in all, 18 out of 125 samples were positive for the *ail* gene. This novel method can be completed within one to two working days.[116] Other studies show that detection rate of *ail*-positive *Y. enterocolitica* in 200 pig tonsils was 88% and 35% with PCR and culture methods, respectively. When 100 raw pork samples were studied, 7 were positive with PCR and all were culture negative.[117]

A major goal for food microbiologists using PCR is to be able to apply the technique for direct detection of bacteria in the samples. However, it is difficult to reach this goal because inhibitory substances present in food may interfere with the amplification and need to be efficiently removed. The ability of PCR to detect viable and nonviable bacteria without distinction is also a problem.[116]

Conclusions

As documented by the results of numerous studies, *Yersinia enterocolitica* is an important etiological factor in food poisoning. Swaminathan *et al.* defined *Campylobacter* spp. and *Yersinia enterocolitica* as the "pathogenic bacteria of the 1980s."[118] However, the role and importance of *Yersinia enterocolitica* has risen in recent years due to the spreading of virulent serotypes in Europe that have so far been detected only in the USA. This is evidenced by the titles of some publications such as "A dramatic increase of *Yersinia enterocolitica* serogroup O:8 infections in Poland."[47] Furthermore, reports of the pathogenicity of serotype 1A that has been so far thought to be nonpathogenic to humans are disturbing.

These bacteria are highly resistant to unfavorable conditions during food processing, such as low pH, salinity, or disinfectants. The feature that clearly distinguishes these rods from the other *Enteriobacteriaceae* is the capacity to dominate the environment under refrigerated conditions. In addition, there are considerable problems with detecting pathogenic *Yersinia enterocolitica* in food due to limited sensitivity of the laboratory methods.

Despite the fact that these pathogens are mainly associated with food of animal origin, they have being increasingly isolated from vegetable products such as minimally processed fruits, vegetables, and water. Taking this into account, there is a need for intensification of control and supervision of food in the context of contamination with *Yersinia enterocolitica*.

In order to minimize the risk of infection with these pathogens, it is essential to observe basic hygiene rules. It is critical to avoid contamination of carcasses with feces at slaughter. Proper thermal processing of meat also plays a significant role. Moreover, in the prophylaxis of infections caused by *Yersinia enterocolitica*, it is important to avoid consumption of nonpasteurized milk and water from unknown sources and adequately process fruits and vegetables (especially those that have contact with organic fertilizers).

References

1. Rahman, A.; Bonny, T.; Stonsaovapak, S.; Ananchaipattana, C. *Yersinia enterocolitica:* Epidemiological studies and outbreaks. J. Pathog. **2011**, *2011*, 239391.

2. Okwori, A.E.J.; Martinez, P.O.; Fredriksson-Ahomaa, M.; Agina, S.E.; Korkeala, H. Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. Food Microbiol. **2009**, *26*, 872–875.

3. Sabina, Y.; Rahman, A.; Ray, R.C.; Montet, D. *Yersinia enterocolitica*; mode of transmission, molecular insights of virulence, and pathogenesis of infection. J. Pathog. **2011**, *2011*, 429069.

4. Schleifstein, J.I.; Coleman, M.B. An unidentified microorganism resembling *B. lignieri* and *Past. psedeudotuberculosis*, and pathogenic for man. New York State J. Med. **1939**, *34*, 1749–1753.

5. Fernandez, L.; Mendez, J.; Guijarro, J.A. Molecular virulence mechanisms of the fish pathogen *Yersinia ruckeri*. Vet. Microbiol. **2007**, *125*, 1–10.

6. Bottone, E.J.; Bercovier, H.; Mollaret, H.H. Genus XLI. *Yersinia*. In *Bergey's Manual of Systematic Bacteriology*; Garrity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T., Eds.; Springer: New York, 2005, pp 838–848.

7. Bresolin, G.; Neuhaus, N.; Scherer, S.; Fuchs, T. Transcriptional analysis of long-term adaptation of *Yersinia enterocolitica* to low-temperature growth. J. Bacteriol. **2006**, *188*, 2945–2958.

8. Olsvik, O.; Kapperud, G. Enterotoxin production in milk at 22 and 4 degrees C by *Escherichia coli* and *Yersinia enterocolitica*. Appl. Environ. Microbiol. **1982**. *43*, 997–1000.

9. Bottone, E.J. *Yersinia enterocolitica*: The charisma continues. Clin. Microbiol. Rev. **1997**, *10*, 257–276.

10. Bergann, T.; Kleemann, J.; Sohr, D. Model studies of psychrotrophia in *Yersinia enterocolitica*, Zentralbl. Veterinarmed. B **1995**, *42*, 523–531.

11. Białas, N.; Kasperkiewicz, N.; Radziejewska-Lebrecht, J.; Skurnik, M. Bacterial cell surface structures in *Yersinia enterocolitica*. Arch. Immunol. Ther. Exp. **2012**, *60*, 199–209.

12. Tennant, S.M.; Grant, T.H.; Robins-Browne, R.M. Patogenicity of *Yersinia enterocolitica* biotype 1A. FEMS Immunol. Med. Microbiol. **2003**, *38*, 127–137.

13. Bockemühl, J.; Roggentin, P. Enterale Yersiniosen. Bundesgesundeitsbl. Gesundheitsforsch. Gesundheitsschutz. **2004**, *47*, 685–691

14. Lepka, D.; Wilharm, G. Flagellin genes of *Yersinia enterocolitica* biotype 1A: Playground of evolution towards novel flagellin functions. Microbiol. Res. **2010**, *1*, 37–42.

15. Freund, S.; Czech, B.; Trulzsch, K.; Ackermann, N.; Heesemann, J. Unusual, virulence plasmid-dependent growth behavior of *Yersinia enterocolitica* in three-dimensional collagen gels. J. Bacteriol. **2008**, *190*, 4111–4120.

16. Bhaduri, S. Survival, injury, and virulence of freeze-stressed plasmid-bearing virulent *Yersinia enterocolitica* in ground pork. Foodborne Pathog. Dis. **2005**, *2*, 353–356.

17. Kowalik, J.; Lobacz, A.; Tarczynska, A.S.; Ziajka, S.; Graphic validation of growth models for *Listeria monocytogenes* in the milk during storage. Milchwissenschaft **2012**, *67*, 38–42.

18. Goverde, R.L.; Kusters, J.G.; Huis in 't Veld, J.H. Growth rate and physiology of *Yersinia enterocolitica*; influence of temperature and presence of the virulence plasmid. J. Appl. Bacteriol. **1994**, *77*, 96–104.

19. Iliev M.; Najdenski H. Monitoring of plasmid dissociation and pathogenic potential among *Yersinia enterocolotica* and *Yersinia pseudotuberculosis* during storage of refrigerated pork meat. Ann. Microbiol. **2008**, *58*, 623–632.

20. Lee, W.H.; Harris, M.E.; McClain, D.; Smith, R.E.; Johnston, R.W. Two modified selenite media for the recovery of *Yersinia enterocolitica* from meats. Appl. Environ. Microbiol. **1980**, *39*, 205–209.

21. Adams, M.R.; Moss, M.O. *Food Microbiology*, 2nd ed.; Royal Society of Chemistry: London, 2000; pp 264–269.

22. Okamoto, K.; Inoue, T.; Shimizu, K.; Hara, S.; Miyama, A. Further purification and characterization of heat-stable enterotoxin produced by *Yersinia enterocolitica*. Infect. Immun. **1982**, *35*, 958–964.

23. Verma, N.K.; Misra, D.S. Characterization of enterotoxin produced by four *Yersinia enterocolitica* strains of pig origin. Antonie Van Leeuwenhoek J. Microbiol. **1984**, *50*, 361–368.

24. Karapinar, M.; Gonul, S.A. Effects of sodium bicarbonate, vinegar, acetic and citric acids on growth and survival of *Yersinia enterocolitica*. Int. J. Food Microbiol. **1992**, *16*, 343–347.

25. Brocklehurst, T.F.; Lund, B. M. The influence of pH, temperature and organic acids on the initiation of growth of *Yersinia enterocolitica*. J. Appl. Microbiol. **1990**, *69*, 390–397.

26. Vereeckena, K.M.; Devlieghere, F.; Bockstaele, A.; Debevere, J.; Van Impe, J.F. A model for lactic acid-induced inhibition of *Yersinia enterocolitica* in mono- and coculture with *Lactobacillus sakei*. Food Microbiol. **2003**, *20*, 701–713.

27. Lindqvist, R.; Lindblad, M. Inactivation of *Escherichia coli, Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. Int. J. Food Microbiol. **2009**, *129*, 59–67.

28. Le Marc, Y.; Pin, C.; Baranyi, J. Methods to determine the growth domain in a multidimensional environmental space. Int. J. Food Microbiol. **2005**, *100*, 3–12.

29. Stern, N.J.; Pierson, M.D.; Kotula, A.W. Effects of pH and sodium chloride on *Yersinia enterocolitica* growth at room and refrigeration temperatures. J. Food Sci. **1980**, *45*, 64–67.

30. Nissen, H.; Alvseike, O.; Bredholt, S.; Holck, A.; Nesbakken, T. 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. **2000**, *59*, 211–220.

31. Fredriksson-Ahomaa, M.; Murros-Kontiainen A.; Säde, E.; Puolanne, E.; Björkroth, J. High number of *Yersinia enterocolitica* 4/O:3 in cold-stored modified atmosphere-packed pig cheek meat. Int. J. Food Microbiol. **2012**, *155*, 69–72.

32. Barakat, R.K.; Harris, L.J. Growth of *Listeria monocytogenes* and *Yersinia enterocolitica* on cooked modified-atmosphere-packaged poultry in the presence and absence of a naturally occurring microbiota. Appl. Environ. Microbiol. **1999**, *65*, 342–345.

33. Bari, L.; Hossain, A.; Isshiki, K.; Ukuku, D. Behavior of *Yersinia enterocolitica* in foods. J. Pathog. **2011**, *2011*, 420732.

34. Selma, M.V.; Beltrán, D.; Chacón-Vera, E.; Gil, M.I. Effect of Ozone on the inactivation of *Yersinia enterocolitica* and the reduction of natural flora on potatoes. J. Food Prot. **2006**, *69*, 2357–2363.

35. Orquera, S.; Golz, G.; Hertwig, S.; Hammerl, J.; Sparborth, D.; Joldic, A.; Alter, T. Control of *Campylobacter* spp. and *Yersinia enterocolitica* by virulent bacteriophages. J. Mol. Gen. Med. **2012**, *6*, 273–278.

36. Wauters, G.; Kandolo; K.; Janssens; M. Revised biogrouping scheme of *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. **1987**, *9*, 14–21.

37. Bhagat, N.; Virdi, J.S. Molecular and biochemical characterization of urease and survival of *Yersinia enterocolitica* biovar 1A In acidic pH in vitro. BMC Microbiol. **2009**, *9*, 262.

38. Cornelis, G.R. The *Yersinia* Ysc–Yop 'Type III' weaponry. Nat. Rev. Mol. Cell Biol. **2002**, *3*, 742–752.

39. Denis, E.; Osek, J. Occurrence of *Yersinia enterocolitica* in food and the threat to human health. Med. Wet. **2009**, *65*, 511–515.

40. Czerkies, M.; Raczkowska, A.; Brzostek, K. Quo vadis *Yersinia pestis*? Ewolucja patogennych gatunków z rodzaju *Yersinia*. Post. Mikrob. **2009**, *48*, 181–196.

41. Howard, S.L.; Gaunt, M.W.; Hinds, J.; Witney, A.A.; Stabler, R.; Wren, B.W. Application of comparative phylogenomics to study the evolution of *Yersinia enterocolitica* and to identify genetic differences relating to pathogenicity. J. Bacteriol. **2006**, *188*, 3645–3653.

42. Batzilla, J.; Heesemann, J.; Rakin, A. The pathogenic potential of *Yersinia enterocolitica* 1A. Int. J. Med. Microbiol. **2011**, *301*, 556–561.

43. Fredriksson-Ahomaa; M.; Korkeala, H. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: Methodological problem. Clin. Microbiol. Rev. **2003**, *16*, 220–229.

44. Neubauer, H.; Aleksic, S.; Hensel, A.; Finke, E.J.; Meyer, H. *Yersinia enterocolitica* 16S rRNA gene types belong to the same genospecies but form three homology groups. Int. J. Med. Microbiol. **2000**, *290*, 61–64.

45. Sakai. T.; Nakayama, A.; Hashida, M.; Yamamoto, Y.; Takebe, H. Imai S., Outbreak of food poisoning by *Yersinia enterocolitica* serotype O8 in nara prefecture: The first case report in Japan. Jpn. J. Infect. Dis. **2005**, *58*, 257–258.

46. Schubert, S.; Bockemuhl, J.; Brendler, U.; Heesemann, J. First isolation of virulent *Yersinia enterocolitica* O8, biotype 1B in Germany. Eur. J. Clin. Microbiol. Infect. Dis. **2003**, *22*, 66–68.

47. Rastawicki W; Szych, J.; Gierczyński, R.; Rokoszet, N. A dramatic increase of *Yersinia enterocolitica* serogroup O:8 infections in Poland. Eur. J. Clin. Microbiol. Infect. Dis. **2009**, *28*, 535–537.

48. Rastawicki, W.; Szych, J.; Rokosz, N.; Zacharczuk, K.; Gierczyński, R. Seasonality of *Yersinia enterocolitica* bioserotype 1B/O:8 infections in Poland. Epidemiol. Infect. **2012**, *6*, 1–4.

49. Truszyński; M. *Yersinia enterocolitica*—An important zoonotic human pathogen [in Polish]. Med. Wet. **2009**, *65*, 296–300.

50. Rosner, B.M.; Stark, K.; Werber, D. Epidemiology of reported *Yersinia enterocolitica* infections in Germany, 2001–2008. BMC Public Health **2010**, *10*, 337.

51. Okwori, A.E.J.; Agada, G.O.A.; Olabod, A.O.; Okpe, E.S.; Okopi, J. The prevalence of pathogenic *Yersinia enterocolitica* among diarrhea patients in Jos; Nigeria. Afr. J. Biotechnol. **2007**, *6*, 1031–1034.

52. Huovinen, E.; Sihvonen, L.M; Virtanen, M.J.; Haukka, K.; Siitonen, A.; Kuusi, M. Symptoms and sources of *Yersinia enterocolitica*-infection: A case-control study. BMC Infect. Dis. **2010**, *10*, 122.

53. Mielczarek, P.; Bagłaj, M. Jersinioza—rzadko rozpoznawana choroba układu pokarmowego. Gastroenterol. Pol. **2004**, *11*, 69–74.

54. Grattarola, C.; Gennero, M.S.; Bergagna, S.; Zoppi, S.; Chiavacci, L.; Dondo, A. Correlation between *Yersinia* spp. found in faeces and *Brucella suis* infection in wild boars. Epidémiol. Santé Anim. **2007**, *51*, 43–47.

55. Wesley, I.V.; Bhaduri, S.; Bush, E. Prevalence of *Yersinia enterocolitica* in market weight hogs in the United States. J. Food Prot. **2008**, *71*, 1162–1168.

56. James, S.; Tan, M.D. Human zoonotic infections transmitted by dogs and cats. Arch. Intern. Med. **1997**, *157*, 1933–1943.

57. Fredriksson-Ahomaa, M.; Gerhardt, M.; Stolle, A. High bacterial contamination of pig tonsils at slaughter. Meat Sci. **2009**, *83*, 334–336.

58. Atobla, K.; Karou, T.G.; Dadie, A.T.; Niamke, L.S; Dje, K.M. Isolation and characterization of pathogenic *Yersinia enterocolitica* from pigs in Abidjan, Côte d'Ivoire. J. Appl. Biosci. **2012**, *50*, 3540–3548.

59. Fredriksson-Ahomaa, M.; Stolle, A.; Korkeala, H. Molecular epidemiology of *Yersinia enterocolitica* infections. FEMS Immunol. Med. Microbiol. **2006**, *47*, 315–329.

60. Falcao, J.P.; Falcão, D.P.; Pitondo-Silva, A.; Malaspina, A.C.; Brocchi, M. Molecular typing and virulence markers of *Yersinia enterocolitica* strains from human, animal and food origins isolated between 1968 and 2000 in Brazil. J. Med. Microbiol. **2006**, *55*, 1539–1548.

61. Baumgartner, A.; Kuffer, M.; Suter, D.; Jemmi, T.; Rohner, P. Antimicrobial resistance of *Yersinia enterocolitica* strains from human patients, pigs and retail pork in Switzerland. Int. J. Food Microbiol. **2007**, *115*, 110–114.

62. Capita, R.; Alonso-Calleja, C.; Prieto, M.; Garcia-Fernandez, M.; Moreno, B. Incidence and pathogenicity of *Yersinia* spp. isolates from poultry in Spain. Food Microbiol. **2002**, *19*, 295–301.

63. Soltan Dallal, M.M.; Doyle, M.; Rezadehbashi, M.; Dabiri, H.; Sanaei, M.; Modarresi, S.; Bakhtiari, R.; Sharifiy, K.; Taremi. M.; Zali, M.R; Sharifi-Yazdi, M.K. Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, *Campylobacter* and *Yersinia* spp. isolated from retail chicken and beef, Tehran, Iran. Food Control **2010**, *21*, 388–392.

64. Aliyu R.M.; Egwu E.O.; Abubakar M.B.; Adamu A.Y.; Salihu M.D.; Dabai A.I.; Tambuwal F.M. Bacteriological quality of commercially prepared and self compounded poultry feeds in Sokoto metropolis, Sokoto, Nigeria. Int. J. Appl. Biol. Pharm. Technol. **2012**, *3*, 345–350.

65. Sharifi Yazdi, M.K.; Soltan-Dallal, M.M.; Zali, M.R.; Avadisians, S.; Bakhtiari, R. Incidence and antibiotic susceptibilities of *Yersinia enterocolitica* and other *Yersinia* species recovered from meat and chicken in Tehran; Iran. Afr. J. Microbiol. Res. **2011**, *5*, 2649–2653.

66. Söderqvist, K.; Boqvist, S.; Wauters, G.; Vågsholm, I.; Thisted-Lambertz, S. *Yersinia enterocolitica* in sheep—A high frequency of biotype 1A. Acta Vet. Scand. **2012**, doi: 10.1186/1751-0147-54-39.

67. Carrasco, E.; Morales-Rueda, A.; García-Gimeno, R.M. Cross-contamination and recontamination by *Salmonella* in foods: A review. Food Res. Int. **2012**, *45*, 545–556.

68. Kim, T.J.; Young, B.M.; Young, G.M. Effect of flagellar mutations on *Yersinia enterocolitica* biofilm formation. Appl. Environ. Microbiol. **2008**, *74*, 5466–5474.

69. Mattila-Sandholm, T.; Wirtanen, G. Biofilm formation in the industry: A review. **1992**, *8*, 573–603.

70. Beuchat, L.R. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Food Control **2002**, *7*, 223–228.

71. Jiang, G.C.; Kang, D.H.; Fung, D.Y.C. Enrichment procedures and plating media for isolation of *Yersinia enterocolitica*. J. Food Prot. **2000**, *63*, 1483–1486.

72. Tudor, L.; Ţogoe, I.; Pop, A.; Mitrănescu, E. The *Yersinia enterocolitica* species tolerance to temperature. Roumanian Biotechnol. Lett. **2008**, *13*, 17–22.

73. Bracket, R.E. Growth and suvival of *Yersinia enterocolitica* at acidic pH. Int. J. Food Microbiol. **1986**, *3*, 243–251.

74. Barbini de Pederiva, N.B.; Stefanini de Guzmán, A.M. Isolation and survival of *Yersinia enterocolitica* in ice cream at different pH values, stored at −18°C, bnr. Braz. J. Microbiol. **2000**, *31*, 174–177.

75. Smego, R.A.; Frean, J.; Koornhof, H.J. Yersiniosis I: Microbiological and clinicoepidemiological aspects of plague and non-plague *Yersinia* infections. Eur. J. Clin. Microbiol. Infect. Dis. **1999**, *18*, 1–15.

76. Wang, X.; Qiu, H.; Jin, D.; Cui, Z.; Kan, B.; Xiao, Y.; Xu, Y.; Xia, S.; Wang, H.; Yang, J.; Wang, X.; Hu, W.; Xu, J.; Jing, H. O:8 serotype *Yersinia enterocolitica* strains in China. Int. J. Food Microbiol. **2008**, *125*, 259–266.

77. Black, R.E.; Jackson, R.J.; Tsai, T.; Medvesky, M.; Shayegani, M.; Feeley, J.C.; MacLeod, K.I.; Wakelee, A.M. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. N. Engl. J. Med. **1978**, *298*, 76–79.

78. Maruyama, T. *Yersinia enterocolitica* infection in humans and isolation of the microorganism from pigs in Japan. Contrib. Microbiol. Immunol. **1987**, *9*, 48–55.

79. Shayegani, M.; Morse, D.; DeForge, I.; Root, T.; Parsons, L.M.; Maupin, P.S. Microbiology of a major foodborne outbreak of gastroenteritis caused by *Yersinia enterocolitica* serogroup O:8. J. Clin. Microbiol. **1983**, *17*, 35–40.

80. Tacket, C.O.; Ballard, J.; Harris, N.; Allard, J.; Nolan, C.; Quan, T.; Cohen, M.L. An outbreak of *Yersinia enterocolitica* infections caused by contaminated tofu (soybean curd). Am. J. Epidemiol. **1985**, *121*, 705–711.

81. Aber, R.; McCarthy, M.A.; Berman, R.; DeMelfi, T.; Witte, E. An outbreak of *Y. enterocolitica* gastrointestinal illness among members of a Brownie troop in Centre County, Pennsylvania. In *The 22nd International Conference on Antimicrobial Agents and Chemotherapy*, Miami Beach, Florida, October 3–6, 1982; American Society for Microbiology: Miami Beach, FL, **1982**; 1–245.

82. Tuori, M.R.; Valtonen, V. *Yersina enterocolitica* outpatient epidemic. Duodecim **1983**, *99*, 706–711.

83. Marjai, E.; Kalman, M.; Kajary, I.; Belteky, A.; Rodler, M. Isolation from food and characterization by virulence tests of *Yersinia enterocolitica* associated with an outbreak. Acta Microbiol. Hung. **1987**, *34*, 97–109.

84. Thompson, J.S.; Gravel, M.J.; Family outbreak of gastroenteritis due to *Yersinia enterocolitica* serotype 0:3 from well water. Can. J. Microbiol. **1986**, *32*, 700–701.

85. Lee, L.A.; Gerber, A.R.; Lonsway, D.R.; Smith, J.D.; Carter, G.P.; Puhr, N.D.; Parrish, C.M.; Sikes, R.K.; Finton, R.J.; Tauxe, R.V. *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. N. Engl. J. Med. **1990**, *322*, 984–987.

86. Alsterlund, R.; Danielsson-Tham, M.L.; Karp, G.; Eden, T.; De Jong, B.; Nilsson, P.O.; Ransjo, U. An outbreak of *Yersinia enterocolitica* infection on the Bjarred peninsula. Indications for risks of refrigerated food. Lakartidningen **1995**, *92*, 1213–1214.

87. Ackers, M.L.; Schoenfeld, S.; Markman, J.; Smith, M.G.; Nicholson, M.A.; DeWitt, W.; Cameron, D.N.; Griffin, P.M.; Slutsker, L. An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. J. Infect. Dis. **2000**, *181*, 1834–1837.

88. Abraham, M.; Pai, M.; Kang, G.; Asokan, G.V.; Magesh, S.R.; Bhattacharji, S.; Ramakrishna, B.S. An outbreak of food poisoning in Tamil Nadu associated with *Yersinia enterocolitica*. Indian J. Med. Res. **1997**, *106*, 465–468.

89. Jones, T.F. From pig to pacifier: Chitterling-associated yersiniosis outbreak among black infants. Emerg. Infect. Dis. **2003**, *9*, 1007–1009.

90. Anonymous. *Infectious Diseases in Finland 2003*. National Public Health Institute: Helsinki, Finland, 2004; pp 1–41.

91. Tafjord Heier, B.; Nygard, K.; Lassen, J. **2007**. Yersiniose i Norge 2006 [The Norwegian Institute of Public Health Web site]. http://www.fhi.no/eway/?pid=238 (accessed February 20, 2013).

92. Bell, R.; Bright, A.; Butow, B.; Combs, B.; Franklin, N.; Fullerton, K.; Gibbs, R.; Gradie, D.; Gregory, J.; Gunn, J.; Harlock, M.; Heilbronn, C.; Hogg, G.; Kardamanidas, K.; Kirk, M.; Knope, K.; Lalor, K.; Leader, R.; McCallum, .L; McKercher, C.; Miller, M.; Moffatt, C.; Munnoch, S.; Pingault, N.; Raupach, J.; Roper, K.; Shadbolt, C.; Stafford, R.; Stephens, N. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the Ozfoodnet Network; 2009. Commun. Dis. Intell. Q. Rep. **2010**, *34*, 396–426.

93. Grahek-Ogden, D.; Schimmer, B.; Cudjoe, K.S.; Nygard, K.; Kapperud, G. Outbreak of *Yersinia enterocolitica* serogroup O:9 infection and processed pork, Norway. Emerg. Infect. Dis. **2007**, *13*, 754-756.

94. MacDonald; E.; Heier; B.T.; Stalheim, T.; Cudjoe, K.S.; Skjerdal, T.; Wester, A.; Lindstedt, B.A.; Vold, L. *Yersinia enterocolitica* O:9 infections associated with bagged salad mix in Norway, February to April 2011. Eurosurveillance **2011**, *16* (19), 1–3.

95. Anonymous. *Yersinia enterocolitica* infections associated with pasteurized milk. MMWR Morb. Mortal. Wkly. Rep. **2011**, *60*, 1428.

96. Moriki, S.; Nobata, A.; Shibata, H.; Nagai, A.; Minami, N.; Taketani, T.; Fukushima, H. Familial outbreak of *Yersinia enterocolitica* serotype O9 biotype 2. J. Infect. Chemother. **2010**, *16*, 56–58.

97. Babić-Erceg, A,; Klismanić, Z.; Erceg, M.;Tandara, D.; Smoljanović, M. An outbreak of *Yersinia enterocolitica* O:3 infections on an oil tanker. Eur J. Epidemiol. **2003**, *18*, 1159–1161.

98. Mehlman, I.J.; Auliso, C.C.G.; Sanders, A.C. Problems in the recovery and identification of *Yersinia* from food. J. Assoc. Off. Anal. Chem. **1978**, *61*, 761–777.

99. Miliotis, M.D.; Feng, P. In vitro staining technique for determining invasiveness in food-borne pathogens. FDA Lab. Inform. Bull. **1992**, *9*, 3754.

100. Doyle, M.P.; Hugdahl, M.B. Improved procedure for recovery of *Yersinia enterocolitica* from meats. Appl. Environ. Microbiol. **1983**, *45*, 127–135.

101. Schiemann, D.A. Development of a two-step enrichment procedure for recovery of *Yersinia enterocolitica* from foods. Appl. Environ. Microbiol. **1982**, *43*, 14–20.

102. Schiemann, D.A. Comparison of enrichment and plating media for recovery of virulent strains of *Yersinia enterocolitica* from inoculated beef stew. J. Food Prot. **1983**, *46*, 957–964.

103. Wauters, G.; Goossens, V.; Janssens, M.; Vandepitte, J. New enrichment method for isolation of pathogenic *Yersinia enterocolitica* serogroup O:3 from pork. **1988**, *54*, 851–854.

104. Anonymous. *Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of Presumptive Pathogenic* Yersinia enterocolitica; EN ISO 10273:2003, International Organization for Standardization: Geneva, **2003**.

105. Hudson, J.A.; King, N.J.; Cornelius, A.J.; Bigwood, T.; Thom, K.; Monson, S. Detection, isolation and enumeration of *Yersinia enterocolitica* from raw pork. Int. J. Food Microbiol. **2008**, *123*, 25–31.

106. Premaratne, A.; Wilson, T.; King, N.; Hudson, J.A. Growth of *Yersinia enterocolitica* and *Y. pseudotuberculosis* in *Yersinia* selective enrichment broth according to Ossmer. J. Microbiol. Methods. **2012**, *89*, 198–200

107. Sihvonen, L.M.; Haukka, K.; Kuusi, M.; Virtanen, M.J.; Siitonen, A. *Yersinia enterocolitic*a and *Y. enterocolitica*-like species in clinical stool specimens of humans: Identification and prevalence of bio/serotypes in Finland. Eur. J. Clin. Microbiol. Infect. Dis. **2009**, *14*, 14.

108. Restaino, L.; Jeter, W.S.; Hill, W.M. Thermal injury of *Yersinia enterocolitica*. Appl. Environ. Microbiol. **1980**, *40*, 939–949.

109. Head, C.B.; Whitty, D.A.; Ratnam, S. Comparative study of selective media for recovery of *Yersinia enterocolitica*. J. Clin. Microbiol. **1982**, *16*, 615–621.

110. Harmon, M.C.; Yu, C.L.; Swaminathan, B. An evaluation of selective differential plating media for the isolation of *Yersinia enterocolitica* from experimentally inoculated fresh ground pork homogenate. J. Food Sci. **1983**, *48*, 6–9.

111. Riley, G.; Toma, S. Detection of patogenic *Yersinia enterocolitica* by using Congo red magnesium oxylate agar medium. J. Clin. Microbiol. **1989**, *27*, 213–214.

112. Weagant, S.D. New chromogenic agar medium for detection of potentially virulent *Yersinia enterocolitica*. J. Microbiol. Methods **2008**, *72*, 185–190.

113. Vázlerová, M.; Steinhauserová, I. The comparison of the methods for the identification of pathogenic serotypes and biotypes of *Yersinia enterocolitica*: Microbiological methods and PCR. Czech J. Food Sci. **2006**, *24*, 217–222.

114. Lambertz, S.T.; Danielsson-Tham, M.L. Identification and characterization of pathogenic *Yersinia enterocolitica* isolates by PCR and pulsed-field gel electrophoresis. Appl. Environ. Microbiol. **2005**, *71*, 3674–3681.

115. Mäde, D.; Reiting, R.; Strauch, E.; Ketteritzsch, K.; Wicke, A. A real-time PCR for Detection of pathogenic *Yersinia enterocolitica* in food combined with an universal internal amplification control system. J. Con. Prot. Food Saf. **2008**, *3*, 141–151.

116. Lambertz, S.T.; Nilsson, C.; Hallanvuo, S.; Lindblad, M. Real-time PCR method for detection of pathogenic *Yersinia enterocolitica* in food. Appl. Environ. Microbiol. **2008**, *74*, 6060–6067.

117. Fredriksson-Ahomaa, M.; Hartmann, B.; Scheu, P.; Stolle, A. Detection of pathogenic *Yersinia enterocolitica* in meat using real-time PCR. J. Verbr. Lebensm. **2006**, *1*, 202–208.

118. Swaminathan, B.; Harmon, M.C.; Mehlman, I.J. *Yersinia enterocolitica*. J. Appl. Bacteriol. **1982**, *52*, 151–183.