Nature and sources of Yersinia enterocolitica and Yersinia pseudotuberculosis

Main microbiological characteristics

Yersinia pseudotuberculosis and the enteropathogenic strains of Yersinia enterocolitica are enteric bacteria responsible for the zoonotic disease yersiniosis.

These Gram-negative rods, often in the form of an unencapsulated coccobacillus, non-motile at 37°C and motile at 30°C, can multiply in either the presence or absence of oxygen. They contain highly active urease, which provides the key for bacteriological diagnosis, and they form minute colonies (<1 mm) in 24 hours. Although their optimum temperature for growth is 28-29°C, they are psychrotrophic bacteria capable of multiplying at temperatures found in refrigerators and of surviving for several months in freezers.

The species enterocolitica may be subdivided into 5 biotypes (1A/1B, 2, 3, 4, 5) and 76 serotypes. What are known as the "American" strains (biotype 1B) belong to the subspecies enterocolitica of the species enterocolitica whereas the "European" strains (biotypes 1A, 2, 3, 4, 5) belong to the subspecies palearctica. These biotypes are pathogenic to humans, with the exception of biotype 1A, although its association with diarrheic forms is still a matter of debate. This bacterium’s pathogenicity factors are coded by genes located on its chromosome and on the pYV virulence plasmid and, in the specific case of bioserotype 1B/O:8, on a high pathogenicity island. The serotype alone is not a reliable marker for pathogenicity, as several serotypes are common to the biotypes of both pathogenic and non-pathogenic strains. In certain conditions, especially temperature conditions, some strains of Y. enterocolitica can produce a thermostable enterotoxin (YST).

Sources of the hazard

The main animal reservoirs of human enteropathogenic Y. enterocolitica are domestic pigs and wild boar. Because of the structure of pig production (a pyramidal system: holdings for selection, multiplication and then production), the circulation of animals carrying these microorganisms can contribute to propagation between holdings. Other species have been reported as potential asymptomatic carriers of Y. enterocolitica: rodents, rabbits, cattle, sheep, horses, poultry, seagulls, dogs, and cats. In pigs, carriage occurs via the tonsils, with intermittent shedding by faecal matter. The bioserotype 4/O:3 has mainly been isolated in France and Belgium from pig tongues and tonsils; to a lesser extent, bioserotype 2/O:5,27 has been isolated in cow’s milk. In France, prevalence in pig tonsils is estimated to be 14% [10-17] individually and 74% [65-84] in batches (holdings). On carcasses examined in two slaughterhouses, zero frequency of contamination was found, whereas a frequency of 9% and 18% respectively was found in faecal matter and also 5% and 27%

Yersinia enterocolitica © Isabelle Grand and Murielle Naïtali, UMR1319 Micalis - B2HM - Photo: Thierry Meylheuc, INRA MIMA2 Platform

Table 1. Growth and toxinogenesis characteristics of Y. enterocolitica strains

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Growth</th>
<th>Production of YST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>4.2</td>
<td>7.2 – 7.4</td>
</tr>
<tr>
<td>A&lt;sub&gt;n&lt;/sub&gt;</td>
<td>0.96</td>
<td>1</td>
</tr>
<tr>
<td>NaCl (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(1) Biotype is determined by a set of biochemical characteristics.
(2) Bioserotype expresses the joint characteristics of the biotype and serotype.

Data sheet on foodborne biological hazards

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respectively in the tonsils. Regarding seasonal variation, studies in France and abroad show higher prevalence in the colder seasons.

*Y. pseudotuberculosis* is a bacterium found in animal species and occasionally in humans and can also infect birds and wild and domesticated mammals. The French Wildlife Disease Surveillance Network (SAGIR), run by the National Office for Hunting and Wildlife, investigates the causes of mortality in wild animals. Since 1998, French cases of yersiniosis in wild animals associated with *Y. pseudotuberculosis* have been found mainly in hares (an average of 154 cases/year), common rabbits (average of 6 cases/year) and roe deer (average of 5 cases/year); these numbers only represent cadavers collected and sent to a laboratory. They should not be taken as indicators of prevalence.

To date, there is no known vector for these bacteria. However, flies may play a role in dispersion. Surface water, sludge and soil contaminated by the droppings of shedding animals can be sources of the hazard for humans.

**Transmission routes**

The most common route for transmission is the faecal-oral route; this occurs via the ingestion of contaminated food or water, or via contact with infected animals or people.

One case of nosocomial transmission via contaminated thermolabile blood products has been described in France, but it was controlled by the transfusion safety procedures.

**Recommendations for primary production**

- Strict application of measures to prevent contamination and combat pests, particularly following good hygiene practice, especially in pig farms, contribute to controlling the risk of infection and dissemination of *Y. enterocolitica* in production sectors.

**Human foodborne illness**

**Nature of the disease**

*Y. enterocolitica* is responsible for acute febrile gastroenteritis, sometimes leading to complications (Table 2). Most cases of yersiniosis are sporadic and the few clustered cases reported in France occurred in families.

**Susceptible population group(s)**: there is a higher than average probability that children under the age of 10 will develop gastroenteritis caused by *Y. enterocolitica*. The occurrence of other symptoms is linked to the HLA group (septicemia and arthritis in HLA-B27 patients) or suffering from autoimmune disorders. Septicaemia is most frequently observed in the elderly.

**Dose-effect relationship**

Yersiniosis has been observed after ingestion of food containing $10^6$ *Y. enterocolitica* cells. Little work has been done on the possibility of intoxication by the toxin preformed in the food, so it is not possible to determine a dose-effect relationship.

**Epidemiology**

Yersiniosis is not a notifiable disease. In France, a national surveillance network for enteropathogenic *Yersinia*, run by the National Centre of Reference for plague and other yersinioses, was set up in 2003. Clustered cases of yersiniosis may be declared in the framework of the mandatory declaration of foodborne outbreaks. In the European Union, The European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) publish an annual epidemiology report as part of surveillance for foodborne zoonoses. There is no international surveillance network but the Institut Pasteur in Paris, which is a WHO collaborating Centre for enteropathogenic Yersinia, undertakes international surveillance.

In Europe, the strains of *Y. enterocolitica* belonging to bioserotypes 4/O:3 and 2/O:9 are those mainly associated with human infections and, less frequently, 2/O:5,27, 3/O:3 and 3/O:5. The main bioserotypes observed in France are consequently, by frequency of isolation, 4/O:3, 2/O:9 and 2/O:5,27.

In France, the incidence of yersiniosis has been estimated at 16 cases/100,000 in 2003; in 2009, 208 cases were confirmed (or 0.32 cases/100,000). The difficulty of defining a case lies in the fact that isolating a strain in a patient’s stool may be related to transitory carriage and not infection. The proportion of positive stool tests for *Yersinia* spp. was estimated to be 0.5% between 1994 and 1997. Seroprevalence of the different enteropathogenic *Yersinia* in France is unknown. The number of hospitalisations for enteritis attributable to *Y. enterocolitica* is estimated at 172 per year, and lethality is unknown.

**Table 2. Characteristics of the disease**

<table>
<thead>
<tr>
<th>Target population</th>
<th>Mean incubation period</th>
<th>Main symptoms</th>
<th>Duration of symptoms</th>
<th>Duration of infectious period (shedding)</th>
<th>Complications (including lethality)</th>
<th>Asymptomatic forms</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>7 days (1-14 days)</td>
<td>- Enterocolitis: fever, usually moderate, abdominal cramps, watery or bloody diarrhoea, headaches, anorexia, vomiting.</td>
<td>1-3 days to 3 weeks</td>
<td>Up to 2-3 months in the absence of treatment</td>
<td>• In subjects with a predisposition (liver damage including alcoholic cirrhosis, diabetes, iron overload including haematochromatosis); Septicaemia (lethality: 34-50%), osteomyelitis, hepato-splenic abscesses, skin ulcers (such as Sweets’s syndrome), conjunctivitis, meningitis, pharyngitis, urinary infections.</td>
<td>Yes (proportion unknown)</td>
</tr>
<tr>
<td>Particularly children below the age of 10, adolescents, young adults, and immuno-compromised individuals consuming implicated foods</td>
<td></td>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Y. pseudotuberculosis</em></td>
<td>1-11 days</td>
<td>- Abdominal cramps, fever, anorexia and nausea, vomiting.</td>
<td>2-3 days to a few weeks</td>
<td>Up to 2-3 months in the absence of treatment</td>
<td>• Erythema nodosum, arthritis</td>
<td>Deep abscess associated with septicemia</td>
</tr>
</tbody>
</table>
* Inflammation of the terminal part of the ileum

(3) Susceptible population group: people with a higher than average probability of developing symptoms of the disease, or severe forms of the disease, after exposure to a foodborne hazard [definition used in ANSES data sheets].

(4) Relationship between the dose (the quantity of microbial cells ingested during a meal) and the effect on an individual.
According to the report by EFSA (2011), 7,595 cases of human infection by *Y. enterocolitica* were declared and confirmed in the European Union in 2009; the figures are declining (8,988 cases in 2007, 9,142 in 2006, 9,508 in 2005).

Outbreaks have been described, mainly in the United States for *Y. enterocolitica*, following the consumption of milk (bioserotype 1B/O:8) and pig tripe (biotype 4/O:3), and in Scandinavian countries (Finland) for *Y. pseudotuberculosis* where the source was waterborne or soilborne.

### Role of foods

#### Main foods to consider

*Y. enterocolitica* is associated, for sporadic cases, with the consumption of pork (tongue and minced meat) and, to a lesser extent, of unpasteurised cow’s milk, fresh produce (carrots, tomatoes, lettuces, beetroot, radishes, parsley, red cabbage, mushrooms, celery, bananas, mixed salads), tofu and, less commonly, eggs, snails, oysters, mussels, shrimps or fish. Dairy products (chocolate flavoured milk, pasteurised milk, powdered milk), tofu and plant-based foods have been responsible for outbreaks.

When pigs are slaughtered, in the absence of specific measures, there is a risk that the carcass may become contaminated by the tongue and tonsils especially by faecal matter during evisceration. In facilities where the meat is prepared, the incorporation of cranial muscle and the pharynx mucosa into products, especially minced products, is a contamination risk factor.

In France, unpasteurised milk can be contaminated by strains of *Y. enterocolitica* of biotype 1A, but the likelihood of observing them in the cheese industry is very slight. The contamination of dairy products can be the consequence of defective pasteurisation or may occur subsequent to thermal treatment when other ingredients are added or during handling. Enteropathogenic *Yersinia* can survive in soft cheeses if the acidification stage is not performed correctly.

Lastly, water and those handling it (whether symptomatic or asymptomatic carriers) can be sources of contamination of foods by enteropathogenic *Yersinia*.

#### Table 3. Inactivation treatment in industrial environments

<table>
<thead>
<tr>
<th>Disinfectants/Preservatives</th>
<th>Effects of temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible to all disinfectants authorised in the food-processing sector, on condition that the recommended procedures for use are followed. Potassium sorbate and sodium nitrite hinder the growth of enteropathogenic <em>Yersinia</em>.</td>
<td>Pasteurisation is an effective thermal treatment against enteropathogenic <em>Yersinia</em>. Value of D** for enteropathogenic <em>Yersinia</em></td>
</tr>
<tr>
<td><strong>High pressures</strong></td>
<td></td>
</tr>
<tr>
<td>500 MPa/2 min/10 or 25°C. &gt; 7 log₁₀ reductions (skimmed milk) and &gt; 5 log₁₀ reductions (orange juice pH 4.2)</td>
<td>T</td>
</tr>
<tr>
<td>55°C</td>
<td>2 to 7.7 min</td>
</tr>
<tr>
<td>60°C</td>
<td>1.2 to 1.6 min</td>
</tr>
<tr>
<td>65°C</td>
<td>2 to 10 sec</td>
</tr>
<tr>
<td><strong>Ionisation</strong></td>
<td>YST toxin remains stable after deep-freezing and after being heated for 15 min to 121°C, with the same level of pathogenicity in mice as in the absence of thermal treatment.</td>
</tr>
</tbody>
</table>
| D₁₀** (25°C) = 0.1-0.2 KGy | **D** is the dose (in KGy) needed to reduce a population to 10% of its initial strength.

* Monitoring in foods

There are no microbiological criteria for *Yersinia* enteropathogens but Article 14 of Regulation (EC) No 178/2002 applies to the “sale or supply of safe food to the consumer”. There is no active monitoring of foods for *Yersinia* in France.

For the detection of *Y. enterocolitica* in foods, there is a horizontal European standard, NF EN ISO 10273, based solely on conventional microbiology, and the NMKL¹¹¹ 117 standard, which uses a PCR screening method followed, if positive, by isolation and characterisation of the strain. The difficulty is to isolate the strain with its virulence plasmid in order to qualify it as an enteropathogen.

#### Recommendations for operators

- As always, good hygiene practice must be applied and observed, at every stage of the food chain.
- Operations carried out on the oral cavity in pigs (inspection, removal of tonsils and tongue, splitting the skull) present a risk of contaminating the meat and must be performed with great care.
- Faecal contamination of the carcass must be avoided, by the use of appropriate techniques, especially during evisceration.
- Operations carried out on foods, especially milk, after any inactivation treatment, must be performed under controlled conditions.
- Water intended for human consumption must be used during processing.

#### Domestic hygiene

#### Recommendations to consumers

- Pork should never be eaten raw or undercooked.
- Wash vegetables carefully with drinking water before eating.
- Wash hands thoroughly after using the toilet and before preparing or eating food, and also after contact with animals or children suffering from diarrhoea.
- People in a household known to be infected with *Y. enterocolitica* or *Y. pseudotuberculosis* should not handle food.

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References and links

General references


Useful links

• http://foodsafety.govt.nz/elibrary/industry/Yersinia_Enterocolitica-Science_Research.pdf