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Monitoring and identification of human enteropathogenic *Yersinia* spp.[1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-037)

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PANEL MEMBERS

Olivier Andreoletti, Herbert Budka, Sava Buncic, Pierre Colin, John D Collins, Aline De Koeijer, John Griffin, Arie Havelaar, James Hope, Günter Klein, Hilde Kruse, Simone Magnino, Antonio, Martínez López, James McLauchlin, Christophe Nguyen-The, Karsten Noeckler, Birgit Noerrung, Miguel Prieto Maradona, Terence Roberts, Ivar Vågsholm, Emmanuel Vanopdenbosch.

SUMMARY

Following a request from EFSA, the Panel on Biological Hazards was asked to (i) identify the serotypes of *Yersinia enterocolitica* which are pathogenic to humans, (ii) give advice regarding the analytical methods to be used to detect and identify the human pathogenic *Yersinia enterocolitica* serotypes from food and animals, (iii) consider the need to monitor *Yersinia pseudotuberculosis* in animals and food and (iv) recommend the monitoring methods for *Yersinia* spp. in animal populations and foodstuffs that are most optimal from the public health point of view.

There are several species of the genus *Yersinia. Y. enterocolitica* and *Y. pseudotuberculosis* are widespread in Europe and have the characteristics of typical enteropathogens.

Not all *Yersinia enterocolitica* strains are pathogenic. The BIOHAZ Panel concludes that the best and most reliable indicator of *Y. enterocolitica* pathogenicity is the biotype as the various biotypes are either pathogenic or non-pathogenic. The serotype is not a reliable marker of *Y. enterocolitica* pathogenicity because several serotypes are common to both pathogenic and non-pathogenic strains. Strains of biotype 4 (serotype O:3) and biotype 2 (serotypes O:9) are commonly associated with human infections in Europe. Biotype 4 predominates in most Member States. However, biotype 2 might predominate in a few other Member States. These biotypes are seldom reported to be isolated from the environment. Animals (pigs and cattle) are

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the main reservoir and human cases are typically sporadic. Strains of biotype 1A are widely spread in the environment and are often isolated from animal and human stools and from foods, but they are considered non-pathogenic. With regard to *Y. pseudotuberculosis*, all strains are potentially pathogenic for humans and a wide range of animal species. Serotype I is by far the most common serotype associated with human and animal infections in Europe, followed by serotype III. Wild animals are probably the principal reservoir of *Y. pseudotuberculosis* in Europe.

While several culture methods have been described for the isolation and characterisation of *Y. enterocolitica* from foods, the environment and animals, no single isolation procedure appears to be optimal for recovery of all human-pathogenic strains of *Y. enterocolitica* in foods. However, the International Standard Organization method for the detection of pathogenic *Y. enterocolitica* in foods can also be applied equally well to lymphatic tissues such as tonsils, and includes the parallel use of two isolation procedures. The isolation of *Y. pseudotuberculosis* in food and environmental samples is relatively difficult and no single selective medium is available that can be used for all strains of this species. Polymerase Chain Reaction (PCR) could be a useful method for preliminary screening for pathogenic *Y. enterocolitica* in animal, food or environmental samples. An enrichment step prior to PCR is essential to increase the sensitivity and to decrease the risk of false-positive results due to detection of dead cells. PCR could be a useful method to use in parallel with culture methods to screen *Y. pseudotuberculosis* in animal, food or environmental samples, but its performance first needs to be carefully evaluated.

The BIOHAZ Panel also concludes that a reporting system for *Y. enterocolitica* and *Y. pseudotuberculosis* cannot rely only on the presence of genetic traits. Isolation of the strains is essential for confirmation and to enable characterisation. Therefore, when PCR methods are used, positive results should be confirmed with culture methods.

Monitoring and surveying of human pathogenic *Yersinia* in animal populations and food should rely on information on human yersiniosis. The BIOHAZ Panel concludes that routine EU-wide monitoring of human-pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in animals and foods is not recommended. However, more comparable data are needed on the prevalence of pathogenic *Y. enterocolitica* in the porcine reservoir. These could be obtained, depending on the risk management priorities, by a EU-wide baseline survey on pathogenic *Y. enterocolitica* in the pig population, or by national surveys on pathogenic *Y. enterocolitica* in the pig population following a harmonised design. Sampling of pig tonsils at the time of slaughter would provide data on the prevalence at a relevant point in the food chain. If specific biotypes/serotypes represent a serious problem in human yersiniosis, other animal reservoir may be surveyed. When large numbers of animals are to be tested, a pre-screening by serological testing at the time of slaughter could be used to identify infected herds from which pathogenic *Y. enterocolitica* can be detected thereafter by culture methods. Depending on the human disease situation, consumption patterns and prevalence of pathogenic *Y. enterocolitica* in pigs, focused surveys in foods of concern in the individual Member State could be considered, e.g. in fresh pork meat.

With regard to *Y. pseudotuberculosis*, the BIOHAZ Panel recommends that in the event of an increased incidence of human *Y. pseudotuberculosis* infections or if outbreaks occur, focused surveys could be considered in individual Member States guided by the results of epidemiological data.

Although the current ISO method for the detection of pathogenic *Y. enterocolitica* in foods is not optimal for the isolation of all human-pathogenic strains, it is currently recommended as the

method of choice for monitoring and survey purposes. Efforts should be made at EU level to improve the current isolation methods for *Y. enterocolitica* and to develop a better and standardised *Y. pseudotuberculosis* isolation medium.

Finally, the BIOHAZ Panel recommends that only pathogenic *Yersinia* strains should be included when reporting on the occurrence of *Yersinia* spp. in animals, foods, and human cases of yersiniosis. These strains are (i) all *Y. pseudotuberculosis* strains (with an indication of their serotype) and (ii) all *Y. enterocolitica* strains, except those of biotype 1A, with indication of their biotype (and preferably also their serotype).

Key words: ***Yersinia enterocolitica, Yersinia pseudotuberculosis,*** **monitoring, survey, detections, characterisation methods, biotype, serotype, yersiniosis**

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BACKGROUND AS PROVIDED BY EFSA

The Directive 2003/99/EC[2] lays down the Community system for monitoring and collection of information on zoonoses, which obligates the Member States to collect relevant, and where applicable, comparable data of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks. In addition, Member States shall assess trends and sources of these agents and outbreaks in their territory, and transmit to the European Commission a report covering the data collected every year. The European Food Safety Authority (EFSA) is assigned the tasks of examining the data collected and preparing the Community Summary Report.

Data collected in the framework of Directive 2003/99/EC relate to the occurrence of zoonotic agents isolated from animals, food and feed, as well as to antimicrobial resistance in these agents. The information concerning zoonoses cases in humans and related antimicrobial resistance is derived from the structures and/or authorities referred to in Article 1 of Council Decision No 2119/98/EC[3].

EFSA published its second Community Summary Report[4] on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005 on 14 December 2006. For the first time, the European Centre for Disease Prevention and Control (ECDC) provided the data on cases of zoonoses in humans and also the analysis of these data in this report. The data used for analysis derived from several disease networks; the Basic Surveillance Network (BSN) and two Dedicated Surveillance Networks (DSN); Enter-Net and Euro-TB.

When the data received from the Member States were analysed, it came apparent that the information available on *Yersinia enterocolitica* was not sufficient to facilitate a proper analyses of the importance of the findings of *Yersinia* from foodstuffs and animal populations to the human yersiniosis cases. This information would be crucial in order to assess the potential sources of human infections, and measures to protect the public health.

There was lack of information on the *Y. enterocolitica* serotypes isolated from food and animals, and due to this it was often not possible to estimate whether these *Yersinia* findings were pathogenic to humans. Member States reported *Y. enterocolitica* findings from various animal species and foodstuff categories, including pig, bovine, and poultry meat, and milk, as well as pigs, cattle, sheep and goats. In 2005, yersiniosis was the third most frequently reported human zoonoses in EU with a total of 9,630 recorded cases. Most of the cases were caused by *Y. enterocolitica*, but a few cases due to *Y. pseudotuberculosis* were also reported (the Community Summary Report 2005).

There is a need for scientific advice on harmonising the monitoring of *Yersinia* spp. in animals and foodstuffs as well as the analytical and diagnostic methods used, and, specifying the information required for evaluating the association of the *Yersinia* findings from foodstuffs and animals with human yersiniosis cases.

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[2] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003 p. 31)

[3] Decision No 2119/89/EC of the European Parliament and of the Council setting up a network for the epidemiological surveillance and control of communicable diseases in the Community (OJ L 268, 3.10.1998, p.1)

[4] The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005, *The EFSA Journal* (2006), 94

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Biological Hazard Panel is asked to

- identify the serotypes of *Yersinia enterocolitica* which are pathogenic to humans;
- give advice regarding the analytical methods to be used to detect and identify the human pathogenic *Yersinia enterocolitica* serotypes from food and animals;
- consider the need to monitor *Yersinia pseudotuberculosis* in animals and food;
- recommend the monitoring methods for *Yersinia* spp. in animal populations and foodstuffs that are most optimal from the public health point of view. These recommendations may refer to, among other things, relevant animal species and food categories to be covered, the stages of food chain to be sampled, as well as the type of sample to be collected.

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ASSESSMENT

1. Introduction

The genus *Yersinia* of the family *Enterobacteriaceae* includes three well-established human pathogens (*Yersinia pestis, Yersinia pseudotuberculosis* and *Yersinia enterocolitica*) and several non-pathogenic species. *Y. pseudotuberculosis* and some types of *Y. enterocolitica* are considered enteropathogenic as they typically cause enteric infections in humans. The transmission mode of these bacteria to humans is usually foodborne. These enteropathogenic *Yersinia* spp. are the focus of this scientific opinion.

As current monitoring data presented in the annual Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union (EFSA, 2006a) are insufficient to facilitate proper analysis of the importance of the findings of *Yersinia* spp. from foodstuffs and animal populations to human yersiniosis cases, advice is needed on how to improve monitoring and reporting. Relevant monitoring data is crucial in order to assess the potential sources of human infections, and to suggest measures to protect public health. The following chapters review the classification of enteropathogenic *Yersinia* spp., the epidemiology of these bacteria, the current methodologies used for identification and characterisation, and in light of this information discuss monitoring of these bacteria in animals and foods. Finally, conclusions and recommendations will be presented.

2. Classification of *Y. enterocolitica* and *Y. pseudotuberculosis* according to pathogenicity (pathotypes)

2.1. The genus *Yersinia*

The genus *Yersinia* is currently composed of 12 species (*Y. pestis, Y. pseudotuberculosis, Y. enterocolitica, Y. frederiksenii, Y. kristensenii, Y. intermedia, Y. aldovae, Y. mollaretii, Y. bercovieri, Y. rohdei, Y. ruckeri* and *Y. aleksiciae*).

Only *Y. pestis*, *Y. pseudotuberculosis* and some *Y. enterocolitica* are human pathogens. *Y. pestis*, the etiologic agent of plague, is not found in Europe and is not a food-borne pathogen. *Y. enterocolitica* and *Y. pseudotuberculosis* are widespread in Europe and have the characteristics of typical enteropathogens (i.e. transmission by the faecal-oral route, with part of their life cycle in the environment and responsible for intestinal symptoms).

2.2. The two human enteropathogenic *Yersinia* species

Y. enterocolitica is by far the *Yersinia* species most frequent associated with human infections in most countries in Europe. *Y. pseudotuberculosis* infections are most frequently observed in the North-Eastern part of Europe (Finland and Russia) (Jalava *et al.*, 2004).

All pathogenic *Yersinia* harbour a virulence plasmid (pYV), which is essential for their virulence phenotype. In addition, a subset of these strains harbours the High-Pathogenicity Island (HPI), which confers the capacity to cause disseminated infection.

Subgroups of *Y. enterocolitica* are delineated based on biochemical characteristics, i.e. biotypes (see Table 2 of section 4.1.3), and O-antigen specificity, i.e. serotypes. *Y. pseudotuberculosis* strains are subdivided into serotypes, but not into biotypes.

The pathogenic potential of the various biotypes and serotypes of *Y. enterocolitica* and *Y. pseudotuberculosis* is summarised in Table 1.

Table 1. **Pathogenic potential of the various biotypes and serotypes of *Y. enterocolitica* and *Y. pseudotuberculosis*.**

Species	Biotype	Serotypes	Virulence for humans	Frequency in Europe	Pathogenicity determinants	
					pYV	**HPI**
Y. enterocolitica	4	O:3	P	++++[a]	Yes	No
	2	O:9; O:5,27	P	++ to +++	Yes	No
	3	O:3; O:5,27	P	+	Yes	No
	1B	O:8; O:21; O:13; O:7 (and others)	HP	≈ 0	Yes	Yes
	5	O:3; O:2,3; O:1,2,3	P	≈ 0	Yes	No
	1A	Numerous (including O:8; O:5; O:7; O:13;..)	NP	++++	No	No
Y. pseudotuberculosis	NA	I	HP	++ to +++	Yes	Yes
	NA	III	P to HP	++	Yes	Yes*
	NA	II, IV, V (and others)	P	+	Yes	No

NA: not applicable.
HP: Highly pathogenic, P: pathogenic, NP: non-pathogenic
*: truncated HPI.
a: From 0 to ++++ indicates the degree of frequency of the various subgroups.

2.2.1. *Yersinia enterocolitica*

Y. enterocolitica is subdivided into 6 biotypes (1A, 1B, 2, 3, 4 and 5), and into numerous serotypes (> 48). Some preferential, but not systematic, associations between certain biotypes and serotypes are observed.

Y. enterocolitica strains may be separated into three main groups of pathogenicity (pathotypes, Table 1):

- HP = High pathogenicity (pYV+, HPI+): biotype 1B.
- P = Moderate pathogenicity (pYV+, HPI-): biotypes 2 to 5.
- NP = No pathogenicity (pYV-, HPI-): biotype 1A.

The highly pathogenic biotype 1B isolates are mainly found in North America followed by Japan but are extremely rare in Europe. Their epidemiological characteristics are clearly

different from other pathogenic *Y. enterocolitica*: they can be found in the environment (including water) and are responsible for human outbreaks.

Strains of biotype 4 (serotype O:3) and biotype 2 (serotype O:9) are commonly associated with human infections in Europe. Biotype 4 predominates in most Member States. However, biotype 2 has been shown to be the major biotype in the UK and this might also be the case in a few other Member States. These biotypes are seldom reported to be isolated from the environment. Animals (pigs and cattle) are the main reservoir and human cases are typically sporadic.

Biotype 3 strains are not commonly isolated, and biotype 5 strains are extremely rare.

In contrast, the non-pathogenic strains of biotype 1A are widely spread in the environment and are often isolated from foods. They are often found in human stools but are not thought to colonise the human gastro-intestinal tract. Although it has been suggested that they may have some virulence properties, no solid data have been provided until now to support this assumption.

2.2.2. *Yersinia pseudotuberculosis*

Y. pseudotuberculosis is classically subdivided into 5 serotypes (I to V). Additional serotypes and subserotypes have been identified by Japanese scientists, but are quite uncommon in Europe.

Serotype I is by far the most common serotype associated with human and animal infections in Europe, followed by serotype III.

All *Y. pseudotuberculosis* strains are potentially pathogenic (pYV+) for humans and a wide range of animal species. Furthermore, strains of serotypes I and III have both an enhanced capacity to cause systemic infections (HPI+) (Table 1), with strains of serotype I being most frequently associated with the most severe forms of infections.

Some particular strains of *Y. pseudotuberculosis* cause a specific disease, the Far-East Scarlet-Like Fever (FESLF). This disease was initially described in the 1960s in the region of Vladivostock, but it is now commonly found in Western Russia (Saint Petersburg region). These types of strains might now be spreading to some Member States (Sweden). The strains possess some genetic markers that allow differentiation from classical strains.

3. Epidemiology of human enteropathogenic *Yersinia* spp.

Disease due to *Y. pseudotuberculosis* has been recognised since the beginning of the 20th century. *Y. enterocolitica* was first shown to be associated with human disease in 1939.

3.1. *Y. enterocolitica*

Since the 1960s, *Y. enterocolitica* has been identified as a frequent and important cause of human disease in developed countries, especially in temperate ones. As the potential complications of yersiniosis are severe (prolonged acute infections, pseudoappendicitis, and long-term sequelae such as reactive arthritis), the public health burden of yersiniosis are of greater magnitude than the actual number of reported cases would suggest.

Evidence from large yersiniosis outbreaks in the USA, Canada and Japan (Cover and Aber, 1989) 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

(Hurvell, 1981; Tauxe *et al.*, 1987; Ostroff *et al.*, 1994). Most cases in Europe are sporadic. There is often a strong correlation between the biotype/serotype of strains isolated from humans and strains isolated from pigs in the same geographical area (Wauters, 1991; Tauxe, 2002). Serotypes O:3 and O:9 infections emerged in Europe and Japan in the 1970s, and in North America by the end of the 1980s (Tauxe, 2002).

3.1.1. *Occurrence of human enteropathogenic Y. enterocolitica in animals and foods thereof*

3.1.1.1. Pigs and pork

In Europe, pigs are often asymptomatic carriers of human pathogenic strains of *Y. enterocolitica*, in particular strains of biotype 4 (serotype O:3) and less frequently biotype 2 (serotype O:9 and O:5,27). The organisms are present in the oral cavity, especially in the tonsils, submaxillar lymph nodes, and in the intestine and faeces.

Strains of biotype 4 (serotype O:3) have been found frequently on the surface of freshly slaughtered pig carcasses as a result of spread of the organism via faeces, intestinal contents and the tonsils during slaughter and dressing operations. Raw pork might be an important source of *Y. enterocolitica* biotype 4 (serotype O:3).

During slaughter, pork carcasses and edible offal can become contaminated with this pathogen. Slaughter techniques and slaughter hygiene may influence the contamination rate. Faecal contamination can be considerably reduced by sealing off the rectum with a plastic bag immediately after it has been freed (Andersen, 1988). Since the oral cavity is frequently contaminated, handling the head during slaughter (removal of the tongue, splitting of the carcass and post mortem inspection) may lead to the spreading of the contamination present in this part of the carcass. Muscles situated near the tonsils like *M. digastric* can be frequently contaminated (De Zutter and Van Hoof, 1987). Since tonsils or at least a part of these are removed along with the pluck set and then hung on a hook, contamination of the rest of the pluck set by the tonsils is unavoidable. As a consequence, edible offal such as tongues, hearts and livers are more frequently and to a greater extent contaminated than pig carcasses.

During cutting, further processing and distribution of fresh pork and offals, *Yersinia* contamination can further spread. However, human pathogenic *Y. enterocolitica* has only rarely been isolated from pork products at the retail sale stage, with the exception of fresh tongues. Moreover handling of contaminated pork in butcher shops was presumed to be the source for contamination of minced beef found in such shops (Andersen *et al.*, 1991).

Due to the psychrotrophic character of pathogenic *Y. enterocolitica*, strains present in meat have the potential to multiply during the storage of meat and meat products. However, the ability to compete with a high number of psychrotrophic organisms normally present in meat with a normal pH seems to be poor, especially at low temperatures. At higher temperatures (>5°C) and on meat with a high pH, *Yersinia* can multiply considerably. The organism does not survive pasteurisation or normal cooking.

During an outbreak in 2006, eleven cases of *Y. enterocolitica* biotype 2 (serotype O:9) infection were identified in Norway indicating a processed pork product ("julesylte"; Christmas brawn) as the probable source (Grahek-Ogden, 2007). One smaller family outbreak of yersiniosis caused by *Y. enterocolitica* biotype 4 (serotype O:3) occurred in brawn in 2006 (Stenstad *et al.*, 2007). The preparation of raw pork intestines was associated with outbreaks of *Y. enterocolitica* (serotype O:3) infections among infants in the USA (Lee *et al.*, 1991).

3.1.1.2. Ruminants and products thereof

Positive tests in serological control programs for brucellosis in brucellosis negative cattle have in some cases proved to be cross-reactions against *Y. enterocolitica* serotype O:9; meaning that cattle can be asymptomatic carriers of this serotype.

In Norway, outbreaks of *Y. enterocolitica* infection in goat herds were caused by biotype 5 (serotype O:2). An animal attendant was infected by a strain of biotype 5 (serotype O:2). Biotype 5 has also been isolated from goats in New Zealand. Enteritis in sheep and goats due to infection with *Y. enterocolitica* biotype 5 (serotypes O:2,3) was reported in Australia. Biotype 4 (serotype O:3) has been isolated from the rectal contents of lambs in New Zealand. In New Zealand, both biotype 2 (serotype O:5,27) and biotype 2 (serotype O:9) have been isolated from deer.

Y. enterocolitica strains have on several occasions been isolated from milk and dairy products, but most of the isolates were non-pathogenic. In such products pathogenic strains of *Y. enterocolitica* are mostly detected in connection with outbreaks. Contaminated pasteurised milk, reconstituted powered milk and contaminated chocolate milk have been implicated in outbreaks caused by *Y. enterocolitica* biotype 1B (serotype O:8) (Black *et al.*, 1978; Shayegani *et al.*, 1983; Morse *et al.*, 1984; Tacket *et al.*, 1984; Ackers *et al.*, 2000). Sources for the contamination included the addition of contaminated ingredients after pasteurisation, poor washing of bottles, contamination of the exterior of the milk crate, or contamination of the final product by raw milk. Pasteurised milk constitutes an ideal growth medium, allowing the proliferation of psychrotrophic *Y. enterocolitica* without competitors. One outbreak in Sweden in 1988 (Alsterlund *et al.*, 1995) was probably caused by recontamination of pasteurised milk due to lack of chlorination of the water supply.

3.1.1.3. Poultry

In Germany, *Y. enterocolitica* biotype 4 (serotype O:3) and biotype 2 (serotype O:9) have been isolated from poultry (Stengel, 1985). This is probably the only time that isolation of these pathogenic biotypes has been reported from poultry, and there was no obvious opportunity for cross-contamination from pigs or other animals.

3.1.1.4. Vegetables

In Finland, 8% of samples of pre-cut vegetables at processing were found positive for *Y. enterocolitica*, whereas 86% (31 out of 36) of non-pre-cut vegetables at retail were positive. All isolates were reported to be of non-pathogenic biotype 1A. In Norway, the presence of human pathogenic *Y. enterocolitica* was indicated in a few samples of lettuce using PCR, whilst no positive samples were found using a culture method (Johannessen *et al.*, 2002).

3.1.1.5. Other sources

Y. enterocolitica biotype 4 (serotype O:3) infections have occurred in dogs and cats, and these animals may occasionally be asymptomatic carriers of enteropathogenic *Yersinia* (Fredriksson-Ahomaa et al., 2001).

Rodents are a reservoir of biotype 1B (serotypes O:8 and O:21) in Japan, and potentially in North America. Biotype 1B (serotype O:21) ("O:Tacoma") has been isolated from wild rodent fleas in the western United States (Quan et al., 1974).

Biotype 3 is responsible for outbreaks in chinchilla both in Europe and the USA. During the 1960s, epizootics were observed among hares along the French-Belgium frontier caused by biotype 5 (serotype O:2) (Mollaret et al., 1979).

Wells, rivers and lakes are susceptible to contamination with faeces from wild or domestic animals, or by leakage from septic tanks or open latrines in the surrounding areas. Water is a possible source of *Y. enterocolitica*. However, most isolates of *Y. enterocolitica* obtained from water are characterised as belonging to *Y. enterocolitica* biotype 1A or as other non-pathogenic *Yersinia* species.

Consumption of untreated water was identified as a risk factor for *Y. enterocolitica* infection in US, as outbreaks of *Y. enterocolitica*, biotype 1B infection have been traced back to contaminated water from wells and rivers, either for consumption or used in food preparation (Keet, 1974; Tacket *et al.*, 1985; Thompson and Gravel, 1986). In Europe, very few water-associated cases of *Y. enterocolitica* infections have been reported (Christensen, 1979).

3.2. *Y. pseudotuberculosis*

In Europe, human cases of *Y. pseudotuberculosis* infection are rarely reported. Most reported cases are sporadic. However, several outbreaks due to strains of serotypes I and III, have been reported in Finland, mainly in children in day-care centres and schools (Jalava *et al.*, 2004; Jalava *et al.*, 2006). Russia and Japan are the countries where sporadic or epidemic *Y. pseudotuberculosis* infections are most frequently reported in humans.

3.2.1. *Occurrence of Y. pseudotuberculosis in animals and foods thereof*

3.2.1.1. Animals

A wide range of animals are potential reservoirs of *Y. pseudotuberculosis*. This bacterium has been recovered from diverse animal sources ranging from farm animals, pets and experimental animals to wild and captive animals (Fukushima *et al.*, 1998). *Y. pseudotuberculosis* infection in hares has been reported in Germany. Serotypes I and II predominate in hares (Wuthe *et al.*, 1995). Serotype III has frequently been isolated from asymptomatic pigs in Finland (Niskanen *et al.*, 2002). However, the principal reservoir hosts are believed to be rodents and wild birds. Most animals are asymptomatic carriers, but they may become ill and excrete the bacteria after a stress, such as cold and humid weather or starvation. It has been reported in Australia that *Y. pseudotuberculosis* is one of the most common infectious causes of death among farmed deer. Outbreaks of *Y. pseudotuberculosis* infection regularly occur among captive animals in zoos (predominantly monkeys) in Member States (Kageyama *et al.*, 2002).

3.2.1.2. Foods

Y. pseudotuberculosis has very rarely been isolated from foods. This pathogen has sporadically been isolated from fresh produce in Finland and Russia, and in Japan from fresh produce and pork (Fukushima *et al.*, 1997). Iceberg lettuce and raw carrots have been implicated in some food-borne outbreaks in Finland (Jalava *et al.*, 2004; Nuorti *et al.*, 2004; Jalava *et al.*, 2006), and fresh produce in an outbreak in Russia[5]. Fresh produce may become contaminated with *Y. pseudotuberculosis* during irrigation, harvesting, packing, shipping and processing. A

[5] ProMed 2007: ProMED-mail post: PRO/AH/EDR> Yersiniosis - Russia (Yamalo-Nenetsky), 01/10/2007

combination of direct contact with wildlife faeces during storage, and cross-contamination via the equipment are the most likely contributing factors.

3.2.1.3. Environment

Y. pseudotuberculosis is widely spread in the environment (soil, water, vegetables, etc) where it can survive for a long time. The environment can be contaminated by the faeces of infected animals, mainly wild animals such as deer, rodents and birds. *Y. pseudotuberculosis* has been isolated from fresh water such as river, well and mountain stream water at a considerable high rate in some Asian countries. Untreated mountain spring water contaminated with faeces of wild animals has been linked to human *Y. pseudotuberculosis* infection in Japan and Korea (Fukushima *et al.*, 1998). However, many factors related to the epidemiology of *Y. pseudotuberculosis*, like sources and transmission routes, which are still obscure, should be further studied using effective direct and indirect detection methods.

4. Methods for detecting and identifying human enteropathogenic *Yersinia* spp. from foods and animals

4.1. Methods for detection and identification of *Y. enterocolitica*

4.1.1. *Culture methods for detection and characterisation of Y. enterocolitica*

Various cultural methods for the isolation of *Y. enterocolitica* from foods have been described. These methods are also used for samples from animals and the environment. Many of these methods result in the isolation of non-pathogenic *Yersinia* strains. At present, no single isolation procedure appears to be optimal for recovery of all human-pathogenic strains of *Y. enterocolitica* in foods (De Boer, 2003).

As the numbers of *Y. enterocolitica* in foods are usually low and there is often a great variety of background flora, direct isolation on selective plating media is seldom successful. Isolation methods usually involve enrichment of the sample followed by plating onto selective agar media and confirmation of typical colonies. Isolated strains can be further characterised by biotyping, serotyping, antimicrobial susceptibility and testing for virulence properties.

4.1.1.1. Enrichment

As a psychrotrophic organism, *Y. enterocolitica* is able to multiply at 4ºC and enrichment at this temperature for 2-4 weeks has been used. The growth rate of competitive bacteria at 4°C is slowed sufficiently to enable *Y. enterocolitica* to multiply to numbers necessary for isolation on plating media. The long period required for cold enrichment is a serious disadvantage. In addition, the method has proven to have a low sensitivity for many foods (especially for refrigerated foods).

Cold enrichment in phosphate-buffered saline plus 1% sorbitol and 0.15% bile salts (PBSSB) and two-step enrichment with tryptone soy broth (TSB) and bile oxalate sorbose (BOS) broth are useful methods for the recovery of a wide spectrum of *Y. enterocolitica*. Enrichment in irgasan ticarcillin chlorate (ITC) broth is an efficient method for recovery of strains of biotype 4 (serotype O:3), the most prevalent clinical type of *Y. enterocolitica* in Europe. However, it is not a recommended method for biotype 2 (serotype O:9). As *Y. enterocolitica* strains are

relatively tolerant to alkaline solutions, post-enrichment alkali treatment often results in higher isolation rates.

4.1.1.2. Plating media

Cefsulodin irgasan novobiocin (CIN) agar and Salmonella-Shigella deoxycholate calcium chloride (SSDC) agar are the most frequently used plating media. The colonial appearance of *Yersinia* spp. on these isolation media is not always characteristic, making selection of colonies for confirmation is somewhat difficult.

Selection of the proper isolation procedure will depend on the bio/serotypes of *Yersinia* spp. sought and on the type of food to be examined. Use of more than one medium for both enrichment and plating will result in higher recovery rates of *Yersinia* spp. from foods.

4.1.1.3. Characterisation

For the differentiation of *Yersinia* from related genera the following tests may be used: Urease, motility at 25ºC and 37ºC, arginine dihydrolase, lysine decarboxylase, phenylalanine deaminase and H_2S production.

Biochemical differentiation within the genus *Yersinia* includes the following tests: indole production, Voges-Proskauer, citrate utilisation, L-ornithine, mucate, pyrazinamidase, sucrose, cellobiose, L-rhamnose, melibiose, L-sorbose, L-fucose. Commercial identification tests provide suitable alternatives to conventional tube tests.

Biotyping is essential for differentiation between pathogenic and non-pathogenic *Y. enterocolitica* strains. Serotyping is useful for subgrouping within the biotypes 2, 3, 5 and 1B. Additionally, *in vitro* virulence testing may be used.

For biotyping of *Y. enterocolitica* according to Wauters *et al.* (1987) the following tests are applied: Lipase (Tween-esterase), aesculin hydrolysis, acid production from salicin, xylose and trehalose, indole production, nitrate reduction and production of pyrazinamidase (Table 2). Testing for aesculin hydrolysis and/or acid production from salicin and production of pyrazinamidase is usually sufficient for the discrimination between pathogenic and non-pathogenic biotypes. Commercially available kits using these tests in one system are not currently available.

Table 2. Biotyping of *Y. enterocolitica* (Wauters *et al.*, 1987)

Test		Biotypes					
		1A	1B	2	3	4	5
Lipase (Tween-esterase)		+[a]	+	-	-	-	-
Aesculin hydrolysis		+	-	-	-	-	-
Salicin (acid production)		+	-	-	-	-	-
Pyrazinamidase	+	-	-	-	-	-	
Indole production		+	+	(+)	-	-	-
Xylose (acid production)		+	+	+	+	-	-
Trehalose (acid production)		+	+	+	+	+	-
Nitrate reduction		+	+	+	+	+	-

a + = positive; - = negative; (+) = often weak or delayed

Antisera to be used in agglutination tests are commercially available for the serogroups O:3, O:5, O:27, O:8 and O:9, which are predominant in human yersiniosis.

Several *in vitro* tests have been described to determine the potential virulence of *Y. enterocolitica* isolates and many of these tests are easy to perform in routine laboratories. Examples of these tests include: (i) calcium dependence, measured by growth restriction on magnesium oxalate agar; (ii) uptake of Congo red and crystal violet, and (iii) autoagglutination in certain media. However, these tests are not always easy to interpret and since these characteristics are plasmid-encoded, they may not be detected when the virulence plasmid is lost during laboratory manipulation.

4.1.1.4. Standardised methods

The International Standard Organization method for the detection of presumptive pathogenic *Y. enterocolitica* (ISO 10273:2003) in foods, which can be applied equally well to lymphatic tissues such as tonsils, includes parallel use of the following two isolation procedures: (i) enrichment in peptone, sorbitol and bile salts (PSB) broth for 2-3 days at 22-25ºC with agitation or 5 days without agitation; plating on CIN agar directly and after alkaline treatment and incubation for 24 h at 30ºC and (ii) enrichment in ITC broth for 2 days at 24ºC; plating on SSDC agar and incubation for 2 days at 30ºC. For confirmation, five colonies considered to be characteristic or suspect are taken from each plate of each selective medium.

A validation study of ISO 10273 will be carried out in 2008/2009, as part of a mandate for standardisation addressed to the European Committee for Standardisation (CEN) in the field of methods for microbiological analysis of foods.

The Nordic Committee on Food Analysis (NMKL) method No. 117 (1996) "*Yersinia enterocolitica*. Detection in foods" prescribes the use of cold enrichment in PSB for one and three weeks at 4°C. After one week a second enrichment step, using Modified Rappaport Broth for 4 days at 22-25, is carried out. Subcultivation is performed on either CIN- or SSDC agar (Christensen, 1979; NCFA, 1996).

The Bacteriological Analytical Manual (U.S. FDA) method for the isolation of *Y. enterocolitica* includes enrichment in peptone sorbitol bile broth (PSBB) at 10ºC for 10 days; plating on MacConkey agar and CIN agar directly and after alkaline treatment and incubation at 30ºC for 1 day. As an alternative, ITC broth may be used as the enrichment medium.

The enrichment and plating media which are currently used are not particularly selective for *Y. enterocolitica* as they support the growth of several other members of the *Enterobacteriaceae* family. This makes the isolation of low numbers of *Yersinia* in products containing many other contaminants rather difficult and may lead to false-negative results when testing foods for the presence of this organism. Moreover, non-pathogenic environmental *Yersinia* strains are very common in many raw foods and may greatly hinder the isolation of pathogenic *Yersinia* strains from these products.

For a more rapid and sensitive detection, further improvement of these methods specifically directed to the isolation of human pathogenic types of *Y. enterocolitica* and parallel use of DNA-based methods will be necessary.

4.1.2. *Molecular detection methods of Y. enterocolitica*

Polymerase Chain Reaction (PCR) is so far the most frequently used DNA-based method for the detection of virulence-associated genes in *Y. enterocolitica* in naturally contaminated samples and for the identification of pathogenic isolates (Fredriksson-Ahomaa and Korkeala, 2003).

PCR methods have some drawbacks such as a low reproducibility due to false-negative results when naturally contaminated samples are studied. The main reason for false-negative results is the presence of inhibitors in clinical, food and environmental samples. PCR might also be too sensitive, leading to false-positive results due to dead cells present in relatively high numbers (about 10^3 bacteria per gram), or to the presence of partly homologous target sequences in non-pathogenic *Yersinia* or in other bacterial species.

PCR has the advantage of being rapid, sensitive and easy to perform. This method could thus be used in parallel with culture methods for the detection of pathogenic *Y. enterocolitica* in clinical samples, or as a pre-screening method prior to bacterial isolation in animal, food or environmental samples. An enrichment step prior to PCR is essential to increase the sensitivity and decrease the risk of false-positive results due to detection of dead cells.

Several PCR methods use primers targeting the *virF* or *yadA* gene located on the virulence plasmid (pYV). Because of possible plasmid loss during culturing, PCR methods targeting chromosomal virulence genes have also been designed. The *ail* gene located on the chromosome of pathogenic *Y. enterocolitica* strains is the most frequently used target. In addition, some PCR assays have been designed to detect *inv* and *yst* genes in the chromosome of pathogenic *Y. enterocolitica* strains. The *inv* codes for an outer membrane protein Inv that allows bacteria to invade epithelial cells and the *yst* gene encodes a heat-stable enterotoxin Yst.

Two standardised PCR methods for detection of pathogenic *Y. enterocolitica* in food have been reported by the Nordic Committee on Food Analysis (NCFA, 1998). Method A is based on a

one-step PCR with primers targeting the chromosomal *ail* gene and method B uses a two-step PCR (nested PCR) with primers targeting the *yadA* gene on the pYV.

The prevalence of pathogenic *Y. enterocolitica* in pigs and pork has been determined by PCR after an enrichment step in several studies (Fredriksson-Ahomaa and Korkeala, 2003). In Switzerland, the prevalence of *ail*-positive *Y. enterocolitica* in tonsils of slaughter pigs was shown to be 88% by PCR and 34% by culture methods (Fredriksson-Ahomaa *et al.*, 2007). In the USA, *ail*-positive *Y. enterocolitica* were detected in 12% of pig faeces samples by PCR, and in 4% of them using culture methods. Similarly, 40% of the pig lymph nodes were positive by PCR, but none by culturing (Boyapalle *et al.*, 2001).

High detection rates of *yadA*-positive *Y. enterocolitica* from raw pork products in Norway were also achieved using PCR (Johannessen *et al.*, 2000). Recently, a relatively high prevalence (11%) of *ail*-positive *Y. enterocolitica* detected by PCR was reported in fermented pork sausages in Sweden (Lambertz *et al.*, 2007).

A comparison of PCR assays and culture methods showed that the latter also underestimate the occurrence of pathogenic *Y. enterocolitica* in samples from the environment (surface and sewage water) and from slaughterhouses (Fredriksson-Ahomaa and Korkeala, 2003).

PCR could be a useful method for preliminary screening of pathogenic *Y. enterocolitica* in animal, food or environmental samples since it is rapid and sensitive, and it can easily be applied to samples in high numbers.

A reporting system for *Y. enterocolitica* cannot rely only on the presence of genetic traits, due to possible false-negative and false-positive PCR results. Isolation of the strains is essential to enable characterisation (biotype, serotype, antimicrobial susceptibility, eventually genotype) and therefore, when PCR methods are used, positive results should be confirmed with culture methods.

Another possible molecular method for screening the pathogenicity of large numbers of isolated pathogenic *Y. enterocolitica* is colony hybridisation. This method has been used by the US Food and Drug Administration on pure cultures of *Y. enterocolitica* (FDA, 2001). The oligonucleotide probes in this method are specific for the chromosomal *ail* gene and the pYV-borne *virF* gene.

4.1.3. *Serological methods for detection of Y. enterocolitica*

An indirect ELISA based on purified LPS (lipopolysaccharide) from pathogenic *Y. enterocolitica* is another method for screening herds as demonstrated for biotype 4, serotype O:3 (Nielsen *et al.*, 1996). Serological testing of pigs could be performed at all ages from about 100 days, including at slaughter when the pigs are 150 - 180 days old (Nesbakken *et al.*, 2006).

4.2. Current methods for detection and identification of *Y. pseudotuberculosis*

4.2.1. *Bacteriological methods of Y. pseudotuberculosis*

4.2.1.1. Isolation

Isolation of *Y. pseudotuberculosis* in mono-microbial samples (blood, mesenteric lymph node or other organs) is usually quite easy by direct plating on conventional enteric media. In contrast, the organism is rarely isolated from specimens with a diverse background flora (stools, food and environmental samples). It has been reported that cold enrichment for 2 weeks in low-selective phosphate-buffered saline broth supplemented with 1% mannitol and 0.15% bile salts

is useful for the isolation of *Y. pseudotuberculosis* (Niskanen *et al.*, 2002). Cold enrichment followed by alkali treatment has also been proposed for *Y. pseudotuberculosis* isolation (Niskanen *et al.*, 2002).

Cefsulodin-irgasan-novobiocin (CIN) selective agar is the most commonly used medium for both *Y. enterocolitica* and *Y. pseudotuberculosis*. However, only some *Y. pseudotuberculosis* strains grow on this medium. The use of MacConkey agar along with CIN can increase the chances of *Y. pseudotuberculosis* recovery from samples with a diverse background flora (FDA, 2001).

The isolation of *Y. pseudotuberculosis* in food and environmental samples is difficult. No single selective medium is available that can be used for all strains of this species. There is thus a real need for a better and standardised *Y. pseudotuberculosis* isolation medium.

4.2.1.2. Identification

The commercially available API 20E identification system is widely used and gives a satisfactory identification of *Y. pseudotuberculosis* if the strips are incubated at a temperature between 25°C and 30°C (not 37°C) and read after 24 h and 48 h (Neubauer *et al.*, 1998). This kit has a high (90%) positive identification rate for *Y. pseudotuberculosis*.

4.2.1.3. Characterisation

Serotyping of the most commonly isolated strains, which belong to serotypes I to VI, can be done with commercial antisera. However, isolates having less common serotypes or rough strains cannot be characterised with these antisera. A PCR-based O-genotyping method has been developed to overcome this problem (Bogdanovich *et al.*, 2003). Although useful, this method is not yet widely used and it requires several multiplex reactions which are not easy for every laboratory to set up.

4.2.2. *Molecular methods of detection of Y. pseudotuberculosis*

So far, only two PCR methods have been reported for the detection of *Y. pseudotuberculosis* in naturally contaminated samples (Nakajima *et al.*, 1992; Kaneko *et al.*, 1995). Both methods use primers against the chromosomal *inv* gene and the pYV-borne *vir*F gene and can be used to detect *Y. pseudotuberculosis* in pork and water samples. Furthermore, a colony hybridisation method using oligonucleotide probes for *inv* on the chromosome and *virF* on the pYV has been developed by the US Food and Drug Administration for identification of *Y. pseudotuberculosis* isolates in pure culture (FDA, 2001).

PCR has not yet been widely used to detect *Y. pseudotuberculosis* in samples with a diverse background flora. It could represent a useful tool to use in parallel with culture methods to screen animal, food and environmental samples, but its performance first needs to be carefully evaluated.

5. Reporting of human yersiniosis cases

5.1. Current situation

In 2005, 23 member states notified a total of 9,662 cases (9,553 confirmed cases) of human yersiniosis (EFSA, 2006a). The incidence was calculated to be 2.6 per 100,000 for the whole

population. In 2006, 8,979 confirmed cases of yersiniosis were reported at the community level. This represented a 5.8% decrease from 9,533 cases in 2005, and a decrease in community incidence from 2.6 to 2.1 cases per 100,000 population. In the Community Summary Report for 2005 (EFSA, 2006a), information regarding species was available for 90% of the reported cases, and the majority (89 %) was found to be *Y. enterocolitica*. Only five countries reported cases of *Y. pseudotuberculosis*. However, many countries do not test specifically for this species.

These incidence data are believed to represent both under- and over-reporting, leading to major uncertainties regarding the true incidence and burden of disease due to human yersinosis. There are three main factors that in conjunction, lead to under-reporting:

i. Isolation of pathogenic *Yersinia* requires specific media and growth temperatures (different from other *Enterobacteriaceae*), especially when they are present in a sample with a diverse background flora (food, stools, etc). The number of isolated strains is thus likely to be much lower than the real number of contaminated samples.

ii. *Y. enterocolitica* infections are often mild in adults and a stool culture is not systematically requested and performed.

iii. Reporting of yersiniosis cases is not compulsory in most Member States and is done on a voluntary basis.

On the other hand, the numbers reported to ECDC also reflect an over-reporting of human yersiniosis cases. Indeed, non-pathogenic *Y. enterocolitica* strains are widespread in the environment and they are found with high frequencies in food products (vegetables, greeneries, etc). The presence of these strains in foodstuffs or in human stools has no consequence for human health. It is thus of key importance to differentiate them from the pathogenic isolates. In the absence of clear and uniform criteria to define a true yersiniosis case, the data collected from the various member states will not be interpretable.

5.2. Strain characteristics to be considered when reporting human cases

All *Y. pseudotuberculosis* strains (whatever their serotype) are potentially pathogenic for humans and animals. All *Y. pseudotuberculosis* strains isolated from clinical samples should therefore be reported. It is recommended to provide the serotype of each isolate because it gives an estimate of its level of potential pathogenicity, and may help identify possible sources of contamination.

Since *Y. enterocolitica* species comprise both pathogenic and non-pathogenic isolates, and since only pathogenic strains should be reported, there is a need for common criteria to define pathogenic *Y. enterocolitica*.

The best and most reliable indicator of *Y. enterocolitica* pathogenicity is the **biotype**:

- All strains of biotypes 1B, 2, 3, 4 and 5 are potentially pathogenic for humans.
- Strains of biotype 1A are non-pathogenic.

The serotype is not a reliable marker of *Y. enterocolitica* pathogenicity because:

- Several serotypes are common to both pathogenic and non-pathogenic strains (for instance serotypes O:8, O:5, O:3, etc.).
- Self-agglutinable and non-agglutinable strains are common and it is then impossible to attribute them a pathogenicity potential.

- The battery of all specific antisera is available only in a few reference laboratories. Strains that do not belong to the classical serotypes (O:3, O:9 and O:5,27) are thus not serotyped.

Although the serotype is not the most suitable indicator of *Y. enterocolitica* pathogenicity, it is common practice in many laboratories to refer to the serotype rather than the biotype to indicate whether a strain is pathogenic. Therefore, in the absence of biotyping, the serotype, although not reliable to determine the pathogenic potential, may be the only means to get some epidemiological information and also indication of the pathogenic potential of the isolates.

Some genetic traits (pYV plasmid, *ail* or *inv* choromosomal genes) are sometimes screened to estimate the pathogenic potential of *Y. enterocolitica* strains. However, this screening is used by a limited number of laboratories. Furthermore, the virulence plasmid is easily lost *in vitro* during subculturing and the chromosomal markers are not 100% specific of pathogenic strains. Therefore, the reporting system cannot rely on the presence of these genetic traits.

In order to get information usable for the surveillance of human cases of yersiniosis in Europe, only pathogenic *Yersinia* should be reported. These strains are (i) all *Y. pseudotuberculosis* strains, (with an indication of their serotype) and (ii) all *Y. enterocolitica* strains, except those of biotype 1A, with indication of their biotype (and preferably also their serotype).

6. Monitoring and surveying of human enteropathogenic *Yersinia* spp. in animal population and foodstuffs

The data gathered from monitoring and surveys should be relevant and to the greatest extent reliable and comparable in order to provide a sound basis for scientific evaluation and decision-making. This contributes to a major challenge for microbiological data collection in general, and for human enteropathogenic *Yersinia* spp. in particular.

Different regulations, data collection systems and diagnostic procedures in EU member states make it difficult to compare results between member states. Detection methods, use of biotyping and serotyping, and reporting systems vary greatly between member states. Furthermore, the distribution, frequency and the type of *Y. enterocolitica* and *Y. pseudotuberculosis* varies geographically.

As described earlier (Chapter 2.2.1) the genus *Y. enterocolitica* consists of subgroups with clear human pathogenic characteristics as well as subgroups with no human significance. The consequence of finding *Y. enterocolitica* strains is therefore completely unclear without proper identification of biotype and preferably also serotype. Biotyping is a very important part of the identification of *Y. enterocolitica*. Serotyping provides valuable additional information, but must always be accompanied by biotyping, since serotype by itself is not indicative of human pathogenicity.

Only pathogenic *Yersinia* strains should be included when reporting on the occurrence of *Yersinia* spp. in animals, foods, and human cases of yersiniosis. These strains are (i) all *Y. pseudotuberculosis* strains (with their serotype) and (ii) all *Y. enterocolitica* strains with their biotype (and preferably also the serotype), except those belonging to biotype 1A.

In the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005 (EFSA, 2006a), the problems of ascertaining the significance of the results reported from investigations

and monitoring in the EU are pointed out. It is strongly recommended that the effort on biotyping, and preferably also serotyping, be increased in the future.

6.1. *Y. enterocolitica*

6.1.1. *Monitoring of animal populations*

It is well-documented that pigs can harbour human pathogenic *Y. enterocolitica* with a very high prevalence, especially biotype 4 (serotype O:3). An unpublished recent Danish survey reported that 39% of 400 faecal samples and 58% of 578 carcass swabs contained *Y. enterocolitica* biotype 4, serotype O:3.

More comparable data are needed on the prevalence of pathogenic *Y. enterocolitica* in the porcine reservoir. These could be obtained, depending on the risk management priorities, by a EU-wide baseline survey on pathogenic *Y. enterocolitica* in the pig population, or by national surveys on pathogenic *Y. enterocolitica* in the pig population following an harmonised design.

Other reservoirs than pigs may play a role in the epidemiology of human yersiniosis. Evidence suggests that ruminants (e.g. cattle) may play a role as reservoirs for biotype 2 (serotypes O:9 and O:5,27). Therefore surveys in ruminants, primarily cattle, may be considered when biotype 2 dominates or increases among human yersiniosis. In cases when the source is unknown, case-control studies based on interviews of patients and controls might indicate the source.

Presence of *Y. enterocolitica* can be detected in several ways. Traditional culture methods may be used on pig tonsils, faecal material and carcasses. When large numbers of animals are to be tested, pre-screening serological testing (e.g. by ELISA) could be used to identify infected herds from which pathogenic *Y. enterocolitica* thereafter can be detected by culture methods. Serological testing may be performed on serum or meat juice, which may conveniently be collected at the time of slaughter.

Culture of faeces, tonsils or carcasses is expensive and time-consuming, as compared to serological testing. The obvious differences between culture and serology must, however, be taken into account in the planning of surveys and monitoring. Whereas culture provides data on the current status of infection and the isolated strains may be subjected to further investigation, serology relies on a delayed reaction, so that positive reactions does not necessarily mean that the animal is shedding the bacterium at the time of sampling, as shown by Nesbakken *et al.* (2006).

For detection and retrieval of the organism shed by the animal at the time of slaughter, culture detection from tonsils is recommended, as tonsils are found to be either most highly infected, or most easily cultured (or possibly both).

6.1.2. *Monitoring and surveying of foodstuffs*

6.1.2.1. Meat and meat products

Different food producing animals can be carriers of pathogenic *Y. enterocolitica*. Pigs seem to be more often infected than the other animal species. At the normal slaughter age of pigs the prevalence of pathogenic *Y. enterocolitica* is higher in the tonsils than in the faeces. Fresh pork is the type of meat expected to be most frequently contaminated. The highest contamination rate is suspected to be found on pig carcasses. Contamination of internal organs like tongues and to a lesser extent liver and heart is also expected to be high. However, such organs are heat-

treated before consumption, leading to the elimination of possible pathogenic *Y. enterocolitica*. For monitoring and surveying purposes, these considerations must be taken into account.

For sampling, sites which have a higher chance of contamination should be considered. Further in the production chain, the proportion of positive samples is decreasing. This means that sufficient samples must be taken in order to detect a possible low contamination rate. Fresh meat (such as minced pork), which is eaten raw or undercooked, allowing survival of pathogenic *Y. enterocolitica*, might be especially considered for surveys.

The prevalence level of *Y. enterocolitica* in fresh meat from other animal species is always considered to be very low. However, during handling and preparation of products, such as minced meat, they may be cross-contaminated from pork. Heat-treated meat products can be considered non-contaminated immediately after production. Only cross-contamination during production or distribution is a possible source of contamination of such products.

6.1.2.2. Raw milk, fresh produce, water

Y. enterocolitica is widely distributed in the environment, in aquatic and terrestrial ecosystems, as well as animal reservoirs. Most strains isolated from environmental samples are non-pathogenic. Non-pathogenic and pathogenic strains of *Y. enterocolitica* have been shown to survive for variable periods (up to several weeks) in the environment (wells, streams, vegetation, soil), especially at low temperature (Tashiro *et al.*, 1991; Terzieva and McFeters, 1991), even longer than the indicator organisms like *Escherichia coli*. The presence of *Y. enterocolitica* in water, raw milk and vegetables generally occurs as a consequence of direct or indirect faecal contamination, which may be distant in time, because of the ability of *Y. enterocolitica* to survive in the environment. Control measures (e.g. pasteurisation,) are effective in minimizing risks associated with the consumption of these products. Outbreaks caused by those products have been linked to the consumption of untreated water and by hygienic deficiencies in the food production systems, causing recontamination of processed foods (mostly pasteurised milk).

Considering all these factors, monitoring of water, raw milk, and fresh produce does not seem to be very practical/useful. Given the low, sporadic and fluctuating prevalences, monitoring of *Y. enterocolitica* in these foodstuffs is not recommended.

6.2. *Y. pseudotuberculosis*

Since the prevalence of *Y. pseudotuberculosis* has very seldom been studied in human, animals and environmental sources in Europe, the understanding of the epidemiology is limited. The animal reservoirs and contamination routes of *Y. pseudotuberculosis* are still unknown in Europe. However, *Y. pseudotuberculosis* infection in hares has been reported in Germany. Furthermore, few studies have been conducted to detect *Y. pseudotuberculosis* in foodstuffs. In Finland, iceberg lettuce and raw carrots have been implicated in two human foodborne outbreaks.

Monitoring of animal populations and foodstuffs for the presence of *Y. pseudotuberculosis* is not recommended. However, in the event of an increased incidence of human *Y. pseudotuberculosis* infections or if outbreaks occur, focused surveys could be considered in individual Member States guided by the results of epidemiological data. All *Y. pseudotuberculosis* isolated from animals and foods should be reported, preferably with the indication of their serotype.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Yersinia enterocolitica

- Not all *Y. enterocolitica* strains are pathogenic. The best and most reliable indicator of *Y. enterocolitica* pathogenicity is the biotype as the various biotypes are either pathogenic or non-pathogenic. The serotype is not a reliable marker of *Y. enterocolitica* pathogenicity because several serotypes are common to both pathogenic and non-pathogenic strains.
  - Strains of biotype 4 (serotype O:3) and biotype 2 (serotypes O:9) are commonly associated with human infections in Europe. Biotype 4 predominates in most Member States. However, biotype 2 might predominate in a few other Member States. These biotypes are seldom reported to be isolated from the environment. Animals (pigs and cattle) are the main reservoir and human cases are typically sporadic.
  - The non-pathogenic strains of biotype 1A are widely spread in the environment and are often isolated from animal and human stools and from foods.
- Several culture methods have been described for the isolation and characterisation of *Y. enterocolitica* from foods, the environment and animals. At present no single isolation procedure appears to be optimal for recovery of all human-pathogenic strains of *Y. enterocolitica* in foods. However, the International Standard Organization method for the detection of pathogenic *Y. enterocolitica* in foods (ISO 10273:2003) can also be applied equally well to lymphatic tissues such as tonsils and includes the parallel use of two isolation procedures.
- PCR could be a useful method for preliminary screening for pathogenic *Y. enterocolitica* in animal, food or environmental samples. An enrichment step prior to PCR is essential to increase the sensitivity and to decrease the risk of false-positive results due to detection of dead cells.
- A reporting system for *Y. enterocolitica* cannot rely only on the presence of genetic traits. Isolation of the strains is essential for confirmation and to enable characterisation (biotype, serotype, antimicrobial susceptibility, genotype) and therefore, when PCR methods are used, positive results should be confirmed with culture methods.
- Monitoring and surveying (see glossary) of pathogenic *Y. enterocolitica* in animals and foods should rely on information on human yersiniosis.
  - There is a lack of comparable data on the prevalence of pathogenic *Y. enterocolitica* in the porcine reservoir.
  - Monitoring of pathogenic *Y. enterocolitica* in the pig population is not considered necessary. However, surveys providing estimates on the prevalence of pathogenic *Y. enterocolitica* in the pig population would be useful.
  - Monitoring of foodstuffs for the presence of pathogenic *Y. enterocolitica* is not very practical and useful considering the low, sporadic and fluctuating prevalences. Fresh pork meat (such as minced pork), which allows the survival

of pathogenic *Y. enterocolitica* and is eaten raw or undercooked, could be considered for surveys.

Yersinia pseudotuberculosis

- Human pseudotuberculosis is a severe disease. The reported incidence is very low in most Member States. *Y. pseudotuberculosis* has only sporadically been isolated from animal populations (wild animals, pigs), foodstuffs (fresh produce, pork) and the environment (water, soil).
- Routine monitoring of animal populations and foodstuffs for the presence of *Y. pseudotuberculosis* is not justified.
- All *Y. pseudotuberculosis* strains are potentially pathogenic for humans and a wide range of animal species.
  - Serotype I is by far the most common serotype associated with human and animal infections in Europe, followed by serotype III.
  - Wild animals are probably the principal reservoir of *Y. pseudotuberculosis* in Europe.
- The isolation of *Y. pseudotuberculosis* in food and environmental samples is relatively difficult. No single selective medium is available that can be used for all strains of this species. There is a real need for a better and standardised *Y. pseudotuberculosis* isolation medium.
- PCR could be a useful method to use in parallel with culture methods to screen *Y. pseudotuberculosis* in animal, food or environmental samples, but its performance first need to be carefully evaluated.
- A reporting system for *Y. pseudotuberculosis* cannot rely only on the presence of genetic traits due to possible false-negative and false-positive PCR results. Isolation of the strains is essential to enable characterisation (serotype, antimicrobial susceptibility, genotype) and therefore, when PCR methods are used, positive results should be confirmed with culture methods.

RECOMMENDATIONS

Reporting system

- Only pathogenic *Yersinia* strains should be included when reporting on the occurrence of *Yersinia* spp. in animals, foods, and human cases of yersiniosis. These strains are (i) all *Y. pseudotuberculosis* strains (with an indication of their serotype) and (ii) all *Y. enterocolitica* strains, except those of biotype 1A, with indication of their biotype (and preferably also their serotype).

Methods

- Although the current ISO method (ISO 10273:2003) for the detection of pathogenic *Y. enterocolitica* in foods is not optimal for the isolation of all human-pathogenic strains, it is currently recommended as the method of choice for monitoring and survey purposes. Efforts should be made at EU level to improve the current isolation methods.
- It is recommended to develop a better and standardised *Y. pseudotuberculosis* isolation medium.

Monitoring/surveying

- A routine EU-wide monitoring of human-pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in animal populations and foods is not recommended.
- *Y. enterocolitica*
  - More comparable data are needed on the prevalence of pathogenic *Y. enterocolitica* in the porcine reservoir. These could be obtained, depending on the risk management priorities, by a EU-wide baseline survey on pathogenic *Y. enterocolitica* in the pig population, or by national surveys on pathogenic *Y. enterocolitica* in the pig population following a harmonised design.
  - Sampling of pig tonsils at the time of slaughter would provide data on the prevalence at a relevant point in the food chain.
  - If specific biotypes/serotypes represent a serious problem in human yersiniosis, other animal reservoir may be surveyed. For example, surveys in ruminants, primarily cattle, may be considered when biotype 2 dominates or increases among human yersiniosis cases.
  - When large numbers of animals are to be tested, a pre-screening by serological testing at the time of slaughter could be used to identify infected herds from which pathogenic *Y. enterocolitica* can be detected thereafter by culture methods.
  - Depending on the human disease situation, consumption patterns and prevalence of pathogenic *Y. enterocolitica* in pigs, focused surveys in foods of concern in the individual Member State could be considered, e.g. in fresh pork meat.

- *Y. pseudotuberculosis*
  - In the event of an increased incidence of human *Y. pseudotuberculosis* infections or if outbreaks occur, focused surveys could be considered in individual Member States guided by the results of epidemiological data.

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Glossary

According to the Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents[6], "**monitoring**" means a system of collecting, analysing and disseminating data on the occurrence of zoonoses, zoonotic agents and antimicrobial resistance related thereto.

According to the Report on Guidance on Good Practices for Design of Field Surveys, "**survey**" (EFSA, 2006b) is a study involving a sample of units selected from a study population. This type of study is often known as a *descriptive survey*. Its main objective is that of *estimating* the mean level of some characteristics in a defined population including a measure of the precision for those estimates. A secondary objective of surveys often is the measurement of the relationship between two or more variables measured at the same point in time. These are *analytical surveys*.

According to the Report on Guidance on Good Practices for Design of Field Surveys, "**surveillance**" (EFSA, 2006b), represents an extension of monitoring and consists of the close and continuous observation of the occurrence of infection for the purpose of active control (EFSA, 2006b).

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[6] O.J. L 325, 12.12.2003, p. 31