Yersinia in food: recommendations for protection against infections

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The consumption of food contaminated with *Yersinia* can lead to gastrointestinal infections. The cause for this so-called yersiniosis are the species *Y. enterocolitica* and *Y. pseudotuberculosis*. *Yersinia* are rod-shaped bacteria which are very common in our environment. The main reservoir for *Yersinia enterocolitica* are pigs, meaning that the bacteria can be found in raw pork. For *Yersinia pseudotuberculosis* wild animals are probably the most important reservoir.

In the year 2011, roughly 3,400 *Yersinia* infections were reported in Germany. Most of the infections were caused by *Y. enterocolitica*. This makes yersiniosis – after infections with *Campylobacter* and *Salmonella* – the third most common bacterial gastrointestinal disease in Germany. Children up to the age of three are especially frequently affected, since the immune system is not fully developed at that age. The biggest risk factor for an infection with *Yersinia* is the consumption of raw pork products, for example in the form of ground pork and seasoned minced meat.

Yersinia can multiply even at a temperature of 4 °C, meaning that if a contaminated product is stored in the fridge, the number of germs and hence the risk of infection can increase. For this reason, ready-to-eat foods should not contain any pathogenic *Yersinia*. In order to reduce the contamination of foods with *Yersinia* to the greatest possible extent, very high hygienic standards should be observed during slaughtering and processing of pigs. The bacteria are notably contained in the tonsils, the lymph nodes and the intestine of pigs. For this reason, contamination from there to body parts intended for consumption should be avoided during the slaughtering process.

Consumers can protect themselves against infections with *Yersinia* by adhering to the rules of kitchen hygiene during food preparation: you should heat meat to 70 °C for at least two minutes and avoid spreading bacteria from raw meat to other foods. Cross-contamination of food can occur, for example, through the hands, chopping boards or from knives. Especially vulnerable groups of persons, including infants, pregnant women, the elderly and persons with a weakened immune system, should refrain from consuming raw meat.

1 Subject of the Assessment

The Federal Institute for Risk Assessment (BfR) hereby expresses its opinion on possible threats resulting from the consumption of foods contaminated with *Yersinia enterocolitica (Y.)* and *Y. pseudotuberculosis*.

2 Findings

The consumption of foods contaminated with *Y. enterocolitica* can pose a threat to consumer health. To ensure that any health risk for consumers are avoided, in particular for especially sensitive risk groups such as children, the BfR is therefore of the opinion that ready-to-eat foods should not contain any *Y. enterocolitica* that are pathogenic to humans. This recommendation also applies to all other ready-to eat foods contaminated with *Y. pseudotuberculosis*.

4 Statement of Reasons

4.1 Risk Assessment

4.1.1 Possible Source of Danger

Yersinia are gram-negative, facultatively anaerobic, non-spore-forming bacilli. The genus of *Yersinia* (*Y.*) currently comprises 17 species of which three (*Y. pestis*, *Y. enterocolitica, Y. pseudotuberculosis)* can trigger infection diseases in humans (Drummond et al., 2012). Whereas *Y. pestis* is the pathogen responsible for the plague, the syndromes caused by *Y. enterocolitica* and *Y. pseudotuberculosis* are referred to as yersiniosis. These are predominantly gastrointestinal diseases caused by the consumption of contaminated food, notably pork products, but also contaminated drinking water (Tauxe et al., 1987). In the year 2011, 3,397 cases of yersiniosis were reported in Germany (RKI, 2012a). Most types of yersiniosis are caused by *Y. enterocolitica* (Long et al., 2010). The main reservoir of this species which is very common in the environment are pigs (Fredriksson-Ahomaa et al., 2006; Virtanen et al., 2012). The species *Y. enterocolitica* consists of six biotypes and over 50 different serotypes which are heterogeneous with regard to their pathogenic potential. Strains of biotypes 1B, 2, 3, 4 and 5 are classified as human pathogens. Out of these, Biotype 4 (Serotype O:3) and Biotype 2 (Serotype O:9) are, in Europe, most frequently detected in connection with infections in humans. 86% of *Yersinia* cases in humans reported in Germany for which information on the serotype is available are attributable to Bio / Serotype 4/O:3 (RKI, 2012a). Strains of biotype 1A (Serotype O:5) are often isolated from environmental samples, faeces from humans and animals and food samples. However, they are only rarely detected in connection with human infections (Tennant et al., 2003).
Y. pseudotuberculosis is prevalent in the environment where it can survive for a long time. All strains of this species are potentially pathogenic to humans and many animal species. Serotype I is by far the most common serotype in Europe. It is found in connection with infections of both animals and humans. The second-most prevalent serotype is Serotype III. Wild animals are probably the most important reservoir for *Y. pseudotuberculosis* in Europe. The pathogen has additionally been detected in untreated surface water. Vegetables can be contaminated with this pathogen through direct contact with the faeces of wild animals or via contaminated water, for example during irrigation, harvest or transport (EFSA, 2007a).

All pathogenic *Yersinia* possess a similarly large virulence plasmid (pYV) of 70 kbp (kilobase pairs) on which genes for virulence factors (including Yops, "*Yersinia* outer proteins") are located (Cornelis et al., 1987). They play a major role in the pathogenicity of the bacteria, for example by enabling attachment of the bacteria to epithelial cells, by exerting a toxic effect on host cells or by passing on resistance to the immune system (e.g. macrophages). Other virulence genes are located on the chromosome of the bacteria, e.g. for invasines inducing entry of *Yersinia* into eukaryotic cells.

Y. enterocolitica and *Y. pseudotuberculosis* grow at temperatures between 0°C and 42°C, the optimal temperature being 28°C. Since the bacteria can multiply at 4°C, fridge temperatures are generally not sufficient to efficiently suppress the growth of these bacteria. Even in frozen food, *Yersinia* can survive and remain infectious for several weeks. Common heating methods such as boiling and pasteurising kill the pathogens. Heating to at least +70°C for two minutes is deemed to be sufficient to kill *Yersinia* provided that the heat gets to the inside of the food as well (BfR, 2012).

No reliable data are available as yet for defining a possible minimal infection dose for the bacteria which would permit estimation of the dose / effect relationship. The Public Health Agency of Canada states an infection dose of 10^6 cells (Public Health Agency of Canada). However, no other health agency (e.g. the US Centers for Disease Control and Prevention, CDC) has published such a figure, nor can it be verified through any other sources. In addition, it is to be assumed that the minimum infection dose is, similarly to *Salmonella*, dependent on the food matrix and the immune status of exposed consumer groups.

4.1.2 Detection Methods for Enteropathogenic *Yersinia* in Foods and Animals

Enteropathogenic *Yersinia* can be detected and differentiated using a wide range of cultural, molecular and immunological methods. According to § 64 of the Food and Feed Code (LFGB) the L00.00-90 method of the official collection of testing procedures, which is identical with DIN EN ISO 10273 (ISO, 2003), the cultural detection is initially based on an enrichment of the bacteria in liquid media with subsequent fractioned streak on solid selective media (e.g. CIN-Agar). However, it has been shown that other bacteria too (notably apathogenic *Yersinia* species) can grow on these selective media which makes it harder to identify pathogenic *Yersinia*. *Yersinia* can alternatively be detected by means of PCR methods. To identify pathogenic strains, virulence genes located on the virulence plasmid or in the chromosome of the bacteria are targeted by PCR. However, the results obtained through PCR do not provide any information on the number of living pathogens, since they can only be detected indirectly via their nucleic acids sequences. By means of serological methods (ELISA), testing for *Yersinia* antibodies can be performed at the population level. For this purpose, either blood serum (live animal population) or meet juice (following slaughter) can be tested for antibodies. On the basis of a purified lipopolysaccharide (LPS) from *Y. enterocolitica* O:3, an indirect ELISA detection method was developed. The antibodies in the blood serum of pigs were detectable after three weeks at the earliest, and they persisted up to the time of slaughter (seven weeks after the infection) (Nielsen et al., 1996). However, seropositive results do not necessarily correlate with active carriers in the population (Nesbakken et al., 2006). In addition, the serotype is not a reliable marker for the pathogenicity of *Y. enterocolitica.*

Yersinia isolates are usually differentiated by means of bio- and serotyping. As part of this process, specific biochemical properties of the bacteria (e.g. synthesis of specific enzymes) and / or their surface structures (O-antigen of the LPS) are tested for. A fine distinction of bacteria can be made by means of the "Multilocus Variable-Number Tandem-Repeat Analysis" (MLVA) and pulsed field gel electrophoresis (PFGE) (Sihvonen et al., 2011).

4.1.3 Hazard Characterisation

Yersiniosis typically occurs sporadically following consumption of contaminated food (EFSA, 2007b; RKI, 2012b). After contracting an infection, small children usually have acute self-limiting gastroenteritis (fever, watery to bloody diarrhoea, vomiting etc.), whereas mesenteric lymphadenitis with abdominal pain usually manifests itself in school-aged children and youths. These symptoms can mimic appendicitis. In adults, symptoms can be similar to those of influenza infections with pharyngitis. If underlying diseases (diabetes mellitus, liver cirrhosis, immunosuppression) are already present, extramesenteric conditions such as liver abscesses, endocarditis, pericarditis, pleuritis etc. can occur. Other sequelae without direct pathogen detection include reactive arthritis, persistent ileitis (pseudo-Crohn's disease) and erythema nodosum (Heesemann, 1998).

In Germany, intestinal infections in humans caused by *Y. enterocolitica* must be reported. In contrast, other (extraintestinal) diseases triggered by *Y. enterocolitica* and infections with *Y. pseudotuberculosis* are not as yet subject to reporting requirements. Acute yersiniosis is, after *Campylobacter* and *Salmonella* infections, the third-most commonly reported gastrointestinal disease caused by bacteria in Germany and Europe (EFSA 2007b). According to age-specific information provided by the Robert Koch Institute (RKI), the incidence of yersiniosis is most common in small children aged between 1 and 3, the highest incidence being found in one-year-olds. As their immune system is not fully developed, small children are probably especially susceptible to infection with *Y. enterocolitica* . Incidence decreases with age and stays at a constantly low level throughout adulthood (RKI, 2012a and b).

4.1.4 Exposure Estimation

In Europe, pigs are often asymptomatic carriers of *Y. enterocolitica* strains which are pathogenic to humans, notably strains of Biotype 4 (Serotype O:3) and, less often, Biotype 2 (Serotype O:9 and O:5,27). Using methods of molecular genetics such as PFGE it has been shown that the same *Y. enterocolitica* types are present in humans as in pigs (Fredriksson-Ahomaa et al., 2006). The bacteria are found in the oral cavity of the animals, especially the tonsils and submaxillary lymph nodes but also in their intestine and faeces (Fredriksson-Ahomaa, 2012). Strains of Biotype 4 (Serotype O:3) are frequently detected on the surface of freshly slaughtered pigs, since they can, during the slaughtering process, be spread via the intestine content and tonsils. A study conducted in Germany revealed that for 38.4% of slaughtered pigs, the tonsils tested positive for pathogenic *Y. enterocolitica* (Gürtler et al., 2005). In a similar study from Switzerland, 34% of pigs' tonsils were positive (Fredriksson-Ahomaa et al., 2007). In both studies, almost all isolates belonged to Bio/Serotype 4/O:3.

Pathogenic *Y. enterocolitica* have been detected in raw pork (pigs' tongues and innards) on several occasions (De Boer und Nouws, 1991; De Boer, 1995; Doyle et al., 1981; Fredriksson-Ahomaa et al., 1999; Fredriksson-Ahomaa et al., 2001). In a study from the years 2008 and 2009, a series of untreated raw foods offered for sale were tested for *Y. enterocolitica* in Bavaria (Messelhäusser et al., 2011). While milk samples (goat, mare, cow) were culture-negative, real-time PCR detection for pathogenic *Y. enterocolitica* showed positive results in 3 (6 %) of 51 game meat samples and in 81 (18 %) of 446 raw pork samples. In a total of 46 (approx. 10%) of the pork samples *Y. enterocolitica* were also identified by means of cultural methods. The high detection rate for *Y. enterocolitica* in this study was attributable to the fact that 129 samples from raw pigs' tongues were tested. Of these samples, 58 (45 %) were PCR-positive and 34 (26 %) were confirmed by cultural

detection methods. In the opinion of the authors, cross-contamination of the tongue meat from the affected tonsils occurs when the pigs are slaughtered. In samples from minced meat and other raw pork products contamination with *Y. enterocolitica* was significantly lower; of 255 samples, 15 (6 %) were PCR-positive and 8 (3 %) were shown to be positive using cultural methods. The same study also attempted to quantify contamination for some *Yersinia*-positive tongue meat samples. 28 samples were investigated and the test showed that for 18 of the samples *Y. enterocolitica* could be detected only after the bacteria were allowed to multiply overnight, meaning that the original titre of the colony-forming units (CFU) was below 10 CFU per gram of tongue meat. The highest concentration in a sample was 2.3 x 10^5 CFU per gram of meat (Messelhäusser et al., 2011).

Another study conducted in abattoirs in Lower Saxony in 2007 and 2008 investigated whether the livers of slaughtered pigs were superficially contaminated in the course of the slaughtering process. In 4.7% of the swab samples taken (1,500 samples in total), humanopathogenic *Y. enterocolitica* of bio / serotype 4/O:3 were detected using cultural methods (Altrock et al., 2010).

For the annual zoonosis trend report, the federal states report their study findings to the BfR. Accordingly, three Laender investigated the presence of *Y. enterocolitica* in foods in 2011. The studies were conducted by means of PCR (Mäde et al., 2008) and in some cases using cultural methods. Of 106 meat samples (94 pork) 5 (5%) were PCR-positive and 2 (2%) were culturally positive. Of 313 minced pork preparations, 13 (4%) were PCR-positive and 10 (3%) were culturally positive. In the zoonosis reports of the BfR it was reported that while pathogenic *Y. enterocolitica* were isolated from a range of different foods, they predominantly came from pork including minced pork. In 2010, it was found that 5.1 % of the routine samples contained *Y. enterocolitica*, whereas in 2009 the figure was 9.4 %. In raw meat products made from pork *Y. enterocolitica* was detected in 4.4 % (2009: 5.2%) of samples in 2010. In bulk milk (raw milk for dairy plants) *Y. enterocolitica* was found in 9 % (2009: 9% of samples. One single finding from certified raw milk was reported.

Overall, successful detection of *Y. enterocolitica* pathogenic to humans in pork products from the retail trade is rather rare (EFSA, 2007a). For this reason, it is difficult, within the framework of disease outbreak studies, to identify the causative vehicle. In addition, reliable quantification of the pathogens in suspicious foods is not possible in practice as yet due to testing procedures (especially cultural detection) which are still in need of optimisation. It is to be expected, however, that owing to the insufficient selectivity of the internationally standardised methodology for the detection of potentially pathogenic *Y. enterocolitica* in foods, the prevalence of the pathogen is often higher in foods than the detection results suggest. Since the same strains are frequently identified in affected humans as in pigs, it is assumed that infections with *Y. enterocolitica* are caused predominantly by the consumption of contaminated pork and pork products as well as milk and dairy products (Ackers et al., 2000; Grahek-Ogden et al., 2007; EFSA, 2007a und 2009a).

Little is currently known about the spread of *Y. pseudotuberculosis* in food, since food is not routinely tested for this germ. For the culture of food and environmental samples which is very difficult, there is currently no standardised testing procedure.

In a recently published epidemiological study of the RKI it was stated that "the most important risk factor for acquiring yersiniosis is the consumption of raw minced pork, probably in the form of ground pork or seasoned minced meat." This is especially true for children under the age of 5 who have the highest yersiniosis incidence in Germany. Even the relatively high incidence of yersiniosis in Laender of the east can be explained in terms of

regional consumption differences. The study results suggest that more raw minced pork is eaten in Brandenburg, Saxony-Anhalt and Thuringia than in Bavaria and Hesse (Rosner et al., 2012).

4.1.5 Risk Characterisation

The available findings demonstrate that pigs are often infected with *Y. enterocolitica*. This can lead to contamination of pork products during slaughtering and further processing and hence to yersiniosis in consumers following raw consumption of such products. Since the detection methods for *Y. enterocolitica* are still in need of improvement, it can be assumed that the effective prevalence of *Y. enterocolitica* along the food chain is higher than established so far. Due to the lack of information on the infection dose for humans and the different infection risks for different risk groups (notably small children), it is too early to conduct a final assessment of the probability of illness following consumption of food contaminated with pathogenic *Y. enterocolitica*. However, the prevalence data in pork and minced meat and the study on risk factors for yersiniosis published by the RKI indicate that most of these illnesses are caused by the consumption of raw or not sufficiently cooked pork products or other foods contaminated with these bacteria. Since *Y. enterocolitica* is capable of multiplying even at 4°C thus leading to an increase in the number of germs in foods stored in the fridge, it is important to minimise contamination of foods with this pathogen. To ensure that any health risks for consumers are avoided, in particular for especially sensitive risk groups such as children, ready-to-eat foods should therefore not contain any pathogenic *Y. enterocolitica*. This recommendation also applies to all other ready-to-eat foods that may be contaminated with *Y. pseudotuberculosis*.

4.2 Framework of Action, Measure Recommendations:

Risk assessment shows clearly that in order to reduce possible hazards from enteropathogenic *Yersinia* along the food chain, there is still considerable need for action. To achieve this goal, it is necessary, first off, to improve the detection methods for these bacteria. This applies in the first instance to the cultural detection of *Y. enterocolitica* in food through which it must become possible to quantify the pathogen. With the help of an improved detection method, it will then be possible to collect additional data on the spread of the bacteria in the environment, primary production (animal populations) and in different ready-to-eat foods. In addition, a quantitative detection method is required to determine the minimum infection dose for enteropathogenic yersinia more precisely. For this reason, EFSA too demands that the existing cultural detection method in particular be improved. EFSA is of the opinion that while PCR diagnostics can be helpful, it certainly requires cultural confirmation to be able to characterise the biotype of the pathogen as well (EFSA, 2007a). At the level of the European Committee for Standardisation (CEN), a task force was created in 2012 which is concerned with the review and modification of DIN EN ISO 10273. New studies on the validation of the detection method are planned for the year 2013.

To collect valid data on the prevalence of bacteria in the porcine reservoir, it has been suggested that a basic study investigating the presence of pathogenic *Y. enterocolitica* in pigs' tonsils at the time of slaughtering be conducted at the EU level. For this purpose, a harmonised procedure has been developed by the EFSA in order to obtain comparable data from all member states (EFSA, 2009b). A decision on the execution of the study at EU level is still outstanding. If applicable, monitoring on the prevalence of *Yersinia enterocolitica* in pigs should be conducted in Germany, as soon as improved detection methods are available.

Irrespective of the need for research, courses of action to minimise the risk of contracting yersiniosis already exist. In order to reduce the level of contamination of the food chain with *Yersinia*, observance of high hygienic standards during the pig slaughtering process are of special importance. In particular, the currently common practice of splitting pigs' heads should be dispensed with, since the tonsils can be damaged as a result. An alternative method is severing the heads from the bodies before the former are split. That way cross-contamination from contaminated tonsils is prevented. The required testing of lymph nodes in the pharyngeal area is still possible with this method: instead of the mandibular, the retropharyngeal lymph nodes are tested as part of official meat testing. If, as is currently necessary in accordance with the legally required examination technique, the lymph nodes of the tonsillar ring are cut using the two-knife technique, the meat inspection knives should be disinfected between the individual test steps in order to avoid cross-contamination. When an exclusively visual examination without feeling and cutting of organs is carried out, as is already the case in abattoirs authorised to use this method, the risk of cross-contamination resulting from meat inspections can be reduced. The studies of Altrock et al. (2010) furthermore indicate that shortcomings exist in the process of cutting up carcasses. The currently common practice involves taking out the organs located in the breast cavity, throat and head including the liver in a single operation step as part of which the so-called pluck is removed from the carcass in its anatomic context. However, no knife change or separate removal takes place. Studies based on swab samples of livers in which *Y. enterocolitica* was detected show that this practice leads to cross-contamination.

By implementing these measures, it would be possible to counteract the spread of pathogenic *Y. enterocolitica* to the meat and innards. At the processing level, it would make sense henceforth only to use the meat of the head of the pig mask, cheek meat and tongue in meat products that are heated before consumption (pre-cooked sausages) in order to ensure that the pathogen is effectively inactivated and consumer exposure minimised accordingly.

Last but not least, consumers themselves can make a contribution to reducing the risk of an infection with pathogenic *Yersinia* by preventing cross-contamination of other foods through strict observance of the rules of kitchen hygiene when processing pork. This is especially important in the preparation of liver. In addition, consumers should refrain from eating raw pork. This notably applies to risk groups, i.e. small children, pregnant women, the elderly and persons with a weak immune system.

5 References

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