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Prevalence of *Yersinia enterocolitica* 4/O:3 in raw pork at retail market in Latvia

Prävalenz von Yersinia enterocolitica 4/O:3 in rohem Schweinefleisch aus dem Einzelhandel in Lettland

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Summary

The aim of the present study was to determine the prevalence of pathogenic *Y. enterocolitica* in raw pork products at retail market in Latvia. Overall, 247 samples, including raw pork (n = 93), minced meat (n = 52) and edible offal (n = 102), were collected at supermarkets, farmer markets and butcher shops. Both non-pathogenic yersiniae (131/ 247/ 53%) and *ail*-positive *Y. enterocolitica* 4/O:3 (4/ 247/ 2%) were found at retail level. *Yersinia enterocolitica* 4/O:3 was isolated from raw pork (1/93) and edible offal (3/102), but not from minced meat samples (0/51). Non-pathogenic yersiniae were recovered from raw pork (35/93), minced meat (30/52) and edible offal samples (66/102). *Yersinia enterocolitica* 4/O:3 was found in raw pork originated from supermarkets and in edible offal, including tongue, liver and kidney samples, purchased in butcher shops. The pathogen was isolated from a raw pork sample in one out of four supermarkets, whereas edible offal samples were positive in two out of three butcher shops. Pathogenic *Y. enterocolitica* 4/O:3 is present in raw pork at the retail level in Latvia and as far as raw pork and edible offal samples are found to be contaminated, they may pose a risk to public health.

Keywords: *Y. enterocolitica,* butcher shop, raw pork, edible offal, Latvia

Zusammenfassung

Das Ziel dieser Studie war es, die Prävalenz von pathogenen *Y. enterocolitica* in Produkten aus rohem Schweinefleisch aus dem Einzelhandel in Lettland zu untersuchen. Insgesamt wurden 247 Proben in Supermärkten, auf Bauernmärkten und in Fleischereien genommen, einschlieblich rohem Schweinefleisch (n = 93), Hackfleisch (n = 52) und essbare tierische Nebenprodukte (n = 102). Es konnten sowohl nicht-pathogene Yersinien (131/ 247/ 53%) als auch *ail*-positive *Y. enterocolitica* 4/O:3 (4/ 247/ 2%) nachgewiesen werden. *Y. enterocolitica* 4/O:3 wurde aus Proben von rohem Schweinefleisch (1/93) und essbare tierische Nebenprodukte (3/102) isoliert, aber nicht aus Hackfleischproben (0/51). Nicht-pathogene Yersinien konnten sowohl in Proben von rohem Schweinefleisch (35/93) und Hackfleisch (30/52) als auch von essbare tierische Nebenprodukte (66/102) nachgewiesen werden. *Y. enterocolitica* 4/O:3 wurde in Proben von rohem Schweinefleisch aus Supermärkten und in essbare tierische Nebenprodukte einschlieblich Zunge, Leber und Nieren aus Fleischereien gefunden. Der Erreger wurde aus einer rohen Schweinefleischprobe isoliert, die aus einem von vier untersuchten Supermärkten stammte, während in zwei von drei beprobten Fleischereien die Proben von essbare tierische Nebenprodukte positiv waren. Pathogene *Y. enterocolitica* 4/O:3 ist also in Produkten aus rohem Schweinefleisch im lettischen Einzelhandel nachzuweisen. In den Fällen, in denen rohes Schweinefleisch und essbare tierische Nebenprodukte kontaminiert sind, kann dies ein Risiko für die öffentliche Gesundheit darstellen.

Schlüsselwörter: *Y. enterocolitica,* Fleischereien, rohes Schweinefleisch, essbare tierische Nebenprodukte, Lettland

Introduction

Yersinia (Y.) enterocolitica is a foodborne pathogen, which may cause yersiniosis in susceptible individuals. Disease characterizes with gastro-intestinal symptoms, but extraintestinal manifestations and post-infection sequelae as reactive arthritis or erythema nodosum may occur (Bottone, 1997). Yersiniosis is recognized as a significant foodborne illness in Latvian population with an incidence varying from 1.02 to 4.10 cases per 100 000 inhabitants during 2006-2011 (LIC, 2012).

Y. enterocolitica is very heterogeneous, and due to its biochemical and antigenic properties is divided in various bioserotypes, but only few of them, as 1B:O8, 2/O:9, 2/O:5,27, and 4/O:3 are associated with human disease (Bottone, 1997). *Y. enterocolitica* bioserotype 4/O:3 is most often implicated in clinical cases in Europe (Bottone, 1997, Rosner et al., 2010).

Epidemiology of yersiniosis is still poorly understood because disease occurs mostly sporadically without clear source of infection (Fredriksson-Ahomaa et al., 2006). However, consumption of raw or undercooked pork has been identified as an important risk factor for sporadic yersiniosis (Ostroff et al., 1994, Huovinen et al., 2010). Porcine and clinical isolates were indistinguishable using PFGE method, supporting the observation that *Y. enterocolitica* 4/O:3 contaminated pork is an important source of human yersiniosis (Fredriksson-Ahomaa et al., 2006).

Raw pork, especially edible offal, were found to be contaminated with *Y. enterocolitica* 4/O:3 at retail market, representing public health concerns (Fredriksson-Ahomaa et al., 1999, Bucher et al., 2008). Pork may become contaminated with pathogenic yersiniae during slaughter of pathogenic *Y. enterocolitica*-positive pigs. *Y. enterocolitica* 4/O:3 can be found in pigs lymphatic tissues, especially in tonsils, and transmission to raw pork may occur through cross-contamination from *Y. enterocolitica* 4/O:3-positive tissues to non-infected areas as carcasses and offal (Laukkanen et al., 2009). Prevalence studies on *Y. enterocolitica* 4/O:3 at retail level are important to understand possibilities for introduction of this pathogen into the food chain, especially when the prevalence of *Y. enterocolitica* 4/O:3 is high in the pig population. *Y. enterocolitica* 4/O:3 has been isolated from tonsils of slaughtered pigs in Latvia, and the prevalence varied from 34 % to 64 % (Ortiz Martínez et al., 2009, Terentjeva and Bērziņš, 2010), indicating that contamination of pork with pathogenic yersiniae could be the current issue for Latvian retail market. The aim of the present study was to investigate the prevalence of pathogenic *Y. enterocolitica* in raw pork at retail level in Latvia.

Material and methods

Sampling

A total of 247 of raw pork samples were obtained at retail in Latvia in 2008 and 2009. Samples were collected at supermarkets (n = 125), farmer markets (n = 42) and butcher shops (n = 80), located in Jelgava, Rīga, Liepāja and Daugavpils, representing four major towns of Latvia. Raw pork, including ham, chop and brisket samples (n = 93), minced meat (n = 52) and edible offal, including tongue, heart, liver and kidney (n = 102) were collected from retail. Samples were put into sterile single-use plastic bags and transported on ice to the laboratory for bacteriological testing. Testing was initiated immediately after delivery. A quantity of 25 g of each sample were added to 225 ml of peptone-mannitol-bile salt broth (PMB) and homogenized before further testing.

Bacteriological testing

Yersinia was cultured by direct plating, selective enrichment and cold enrichment. For direct plating, 100 µl of tested material suspension in PMB was plated on cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid, Basingstoke, UK) plate. For selective enrichment, 9 ml of irgasan-ticarcillin-potassium chlorate broth (ITC, Fluka, Buchs, Switzerland) was inoculated with 1 ml of suspension and incubated at +25 °C for 48 h. A quantity of 100 µl of enriched suspension from ITC broth was plated onto CIN agar after incubation. For cold enrichment, sample-PMB-suspension was incubated at +4 °C for 21 days. A quantity of 100 µl of enriched suspension was transferred onto CIN agar after 7, 14 and 21 day of cold incubation. Alkali treatment with 0.25 % KOH solution was used before plating on CIN agar after 21 day of cold incubation. Inoculated CIN agar was incubated at +30 °C for 24–48 h. CIN agar plates were examined for the presence of typical colonies with a "bull's eye" appearance – red centre and surrounded transparent zone. Presumptive colonies were screened for urea hydrolysis (Urea Agar Base, 40 % Urea supplement, Oxoid, UK), and confirmed with API 20E kit (BioMérieux, Marcy l'Etoile, France).

Isolates of *Y. enterocolitica* were biotyped. Serotypes were detected by slide agglutination with commercially available antisera O:3 according to the manufacturer instruction (Sifin, Berlin, Germany).

Confirmation of pathogenicity of *Y. enterocolitica*

Pathogenicity of *Y. enterocolitica* was detected targeting *ail*-gene according to Nakajima et al. (1992). A 1 µl loop with *Y. enterocolitica* culture was transferred into 100 µl of sample preparation reagent (Prepman® ultra sample preparation reagent, Applied Biosystems, USA). Suspension was placed in thermoblock at +100 °C for 10 min. After incubation, the suspension was vortexed and centrifuged for 3 min at the highest speed and the supernatant was used for testing. A quantity of 5 µl of supernatant was resuspended in 45 µl PCR mix, containing RNase free water (Qiagen, France), 1 x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTP mix (Sigma, Switzerland), primers *ail*-forward (5'-ACTCGATGATAACTGGGGAG-3') and *ail*-reverse (5'-CCCCCAGTAATCCATAAAGG-3') 0.1 µM each (Invitrogen, USA) and 0.5 U recombinant *Taq* polymerase (Fermentas, Lithuania).

DNA was amplified under the following conditions: first denaturation at +94 °C for 1 min continued by 25 cycles of 30 sec at +94 °C, 1 min at +55 °C and 2 min at +70 °C followed by a final extension at +70 °C for 1 min (termocycler PCR system 9700, Applied Biosystems, UK). The PCR products were analysed by agarose gel electrophoresis and in case of positive reaction, *ail*-gene was seen as 170 bp amplicon.

Statistical analysis

Chi-square test was used to detect differences in the prevalence of *Yersinia* spp. in raw pork, minced meat and edible offal.

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Results

Overall, the prevalence of *Y. enterocolitica* 4/O:3 and non-pathogenic yersiniae in raw pork samples was 2 % (4/ 247) and 53 % (131/ 247), respectively. *Y. enterocolitica* bioserovar 4/O:3 was the only pathogenic identified. The presence of *ail*-gene was confirmed in all *Y. enterocolitica* 4/O:3 isolates. Among non-pathogenic *Yersinia* spp. *Y. enterocolitica* 1A, *Y. kristensenii, Y. frederiksenii* and *Y. intermedia* were isolated with a prevalence of 45 %, 5 %, 2 % and 1 %, accordingly.

Pathogenic *Y. enterocolitica* 4/O:3 was isolated from raw pork (1 %) and edible offal samples (3 %), whereas raw pork (38 %), minced meat (58 %) and edible offal samples (65 %) were found to contain non-pathogenic *Yersinia* spp.

Y. enterocolitica 4/O:3 was isolated from raw pork (1 %) and edible offal (3 %) samples originated from supermarkets and butcher shops, respectively. Non-pathogenic *Yersinia* spp. were recovered from raw pork samples, including raw pork, minced meat and edible offal, purchased at supermarkets, farmer markets and butcher shops with a prevalence of 41 %, 81 % and 58 %, respectively. The prevalence of *Y. enterocolitica* 4/O:3 varied from 1 % in raw pork in supermarkets to 6 % in edible offal samples obtained in butcher shops, whereas the prevalence of non-pathogenic *Yersinia* spp. varied from 35 % to 83 % in raw pork and in edible offal samples originated from supermarkets and farmer markets, respectively (Tab. 1).

Y. enterocolitica 4/O:3 was isolated from edible offal samples purchased in butcher shops (6 %) more frequently than from raw pork samples (1 %) obtained in supermarkets. The highest prevalence of non-pathogenic yersiniae was observed in raw pork (75 %), minced meat (78 %) and edible offal (83 %) samples purchased at farmer market. In contrast, the lowest prevalence of non-pathogenic yersiniae was detected in raw pork (35 %), minced meat (44 %) and edible offal (54 %) samples bought in supermarkets. The prevalence of non-pathogenic yersiniae in supermarkets was significantly lower than the prevalence in farmer markets and butcher shops ($p < 0.05$) also.

Y. enterocolitica 4/O:3 was recovered from raw pork samples originated from one out of four supermarkets. Raw pork, minced meat and edible offal samples obtained from three farmer markets were *Y. enterocolitica* 4/O:3-negative. *Y. enterocolitica* 4/O:3 was isolated from edible offal samples originated from two out of three butcher shops (Tab. 2).

Y. enterocolitica 4/O:3 was isolated from raw pork samples only in supermarket B (8 %). Pathogen was recovered from edible offal samples in butcher shops H and I with the prevalence 20 % and 5 %, accordingly.

Discussion

Non-pathogenic *Yersinia* spp. and *Y. enterocolitica* 4/O:3 was found in raw pork at retail in Latvia, and the prevalence of non-pathogenic *Yersinia* spp. (53 %) was higher than the prevalence of *Y. enterocolitica* 4/O:3 (2 %). High prevalence of non-pathogenic yersiniae in raw pork could be linked to wide distribution of non-pathogenic yersiniae in the environment. Non-pathogenic yersiniae may be present in the surrounding of slaughterhouse, processing and retail facilities that enhance introduction of yersiniae in retail pork products. In contrast, contamination of raw pork with *Y. enterocolitica* 4/O:3 may occur during the slaughter of *Y. enterocolitica* 4/O:3-positive pigs, when raw pork may become contaminated from infected material – tonsils or intestinal content. Also in previous studies non-pathogenic yersiniae were more widely distributed in retail raw pork than pathogenic yersiniae (Logue et al., 1996, Ramírez et al., 2000). Moreover, *Y. enterocolitica* biotype 1A was more often isolated from raw pork comparing with other non-pathogenic *Yersinia* species in studies of Logue et al. (1996), Ramírez et al. (2000) and Bonardi et al. (2010). The presence of *Y. enterocolitica* 1A in raw meat should be taken into account, because the pathogenicity of *Y. enterocolitica* 1A is still disputable and bacteria may establish infection in immunosuppressed patients (Batzilla et al., 2011).

Non-pathogenic *Yersinia* spp. was isolated from raw pork (38 %), minced meat (58 %) and edible offal (65 %) samples. Our results show that raw products are predisposed to contamination with non-pathogenic yersiniae because non-pathogenic *Yersinia* spp. may be distributed in pork during retail cutting and handling of the product. The prevalence of non-pathogenic *Yersinia* spp. in raw meat in our study was significantly less than 89 %, reported by Logue et al. (1996), but higher than 40 % in the study of Ramírez et al. (2000).

Y. enterocolitica 4/O:3 was isolated from raw pork (1 %) and edible offal (3 %) samples, but all minced meat samples were *Y. enterocolitica* 4/O:3-negative. Among edible offal *Y. enterocolitica* 4/O:3 was isolated from tongue (7 %), liver (10 %) and kidney (8 %) samples. Edible offal are predisposed to contamination with *Y. enterocolitica* 4/O:3 due to the traditional slaughtering technique, when edible by-products are removed along with tonsils as a pluck set (Fredriksson-Ahomaa et al., 2006). Pathogen could be easily introduced on plucks as a result of close contact between tonsils and pharyngeal tissues, which frequently are contaminated with human pathogenic *Y. enterocolitica* 4/O:3, with pluck surfaces. Subsequent meat and edible offal processing procedures are no effective measures for

TABLE 1: *Prevalence of Yersinia in raw pork samples at different types of retail outlets in Latvia.*

Type of sample	Non-pathogenic *Yersinia* spp.			*Y. enterocolitica* 4/O:3		
	Supermarket	Farmer market	Butcher shop	Supermarket	Farmer market	Butcher shop
	No. of samples / No. of positive samples (%)					
Raw pork	74 / 26 (35)	4 / 3 (75)	15 / 6 (40)	74 / 1 (1)	4 / 0 (0)	15 / 0 (0)
Minced meat	27 / 12 (44)	9 / 7 (78)	16 / 11 (69)	27 / 0 (0)	9 / 0 (0)	16 / 0 (0)
Offal	24 / 13 (54)	29 / 24 (83)	49 / 29 (59)	24 / 0 (0)	29 / 0 (0)	49 / 3 (6)
tongue	13 / 11 (85)	16 / 16 (100)	15 / 12 (80)	13 / 0 (0)	16 / 0 (0)	15 / 1 (7)
heart	1 / 0 (0)	5 / 3 (60)	12 / 6 (50)	1 / 0 (0)	5 / 0 (0)	12 / 0 (0)
liver	9 /2 (22)	4 / 3 (75)	10 / 7 (33)	9 / 0 (0)	4 / 0 (0)	10 / 1 (10)
kidney	1 / 0 (0)	4 / 2 (50)	12 / 4 (33)	1 / 0 (0)	4 / 0 (0)	12 / 1 (8)
Total	125 / 51 (41)a	42 / 34 (81)	80 / 46 (58)	125 / 1 (1)	42 / 0 (0)	80 / 3 (3)

[a] the prevalence of non-pathogenic yersiniae in samples at supermarkets was significantly lower than the prevalence in samples at farmer markets and butcher shops ($p < 0.05$)

TABLE 2: *Prevalence of Yersinia enterocolitica 4/O:3 in raw meat samples originated from supermarkets, farmer markets and butcher shops.*

Sampling place	No. of samples	No. of samples / No. of positive samples (%)					
		Raw pork	Minced meat	Offal			
				Tongue	Heart	Liver	Kidney
Supermarket							
A	65	35 / 0 (0)	15 / 0 (0)	10 / 0 (0)	n/a	5 / 0 (0)	n/a
B	16	12 / 1 (8)	3 / 0 (0)	1 / 0 (0)	n/a	n/a	n/a
C	10	5 / 0 (0)	4 / 0 (0)	1 / 0 (0)	n/a	n/a	n/a
D	34	22 / 0 (0)	5 / 0 (0)	1 / 0 (0)	1 / (0)	4 / 0 (0)	1 / 0 (0)
Subtotal	125	74 / 1 (1)	27 / 0 (0)	13 / 0 (0)	1 / 0 (0)	9 / 0 (0)	1 / 0 (0)
Farmer market							
E	8	1 / 0 (0)	3 / 0 (0)	2 / 0 (0)	n/a	n/a	2 / 0 (0)
F	18	n/a	4 / 0 (0)	11 / 0 (0)	1 / 0 (0)	2 / 0 (0)	n/a
G	16	3 / 0 (0)	2 / 0 (0)	3 / 0 (0)	4 / 0 (0)	2 / 0 (0)	2 / 0 (0)
Subtotal	42	4 / 0 (0)	9 / 0 (0)	16 / 0 (0)	5 / 0 (0)	4 / 0 (0)	4 / 0 (0)
Butcher shop							
H	12	2 / 0 (0)	5 / 0 (0)	5 / 1 (20)	n/a	n/a	n/a
I	52	4 / 0 (0)	6 / 0 (0)	10 / 0 (0)	12 / 0 (0)	8 / 1 (13)	12 / 1 (8)
J	16	9 / 0 (0)	5 / 0 (0)	n/a	n/a	2 / 0 (0)	n/a
Subtotal	80	15 / 0 (0)	15 / 0 (0)	15 / 1 (7)	12 / 0 (0)	10 / 1 (10)	12 / 1 (8)
Total	247	93 / 1 (1)	51 / 0 (0)	44 / 1 (2)	18 / 0 (0)	23 / 1 (4)	17 / 1 (6)

n/a – samples were not collected

elimination of pathogen from pork that leads to the distribution of contaminated products via the food chain.

The prevalence of pathogenic *Y. enterocolitica* found in raw pork in our study (2 %) complies with the prevalences of 2 % and 4 % in Norway and in the United States reported by Johannesen et al. (2000) and Boyapalle et al. (2001), respectively. Besides different types of edible offal, the presence of *Y. enterocolitica* 4/O:3 mostly in tongues was reported previously (De Boer and Nouws, 1991, De Guisti et al., 1995, Fredriksson-Ahomaa et al., 1999). The prevalence of *Y. enterocolitica* 4/O:3 in tongues in the present study was comparable with results of De Giusti et al. (1995; 5 %), but significantly lower than 15 % and 78 % reported by De Boer and Nouws (1991) and Fredriksson-Ahomaa et al. (1999) for Netherlands and Finland. Minced meat samples were *Y. enterocolitica* 4/O:3-positive (2 %) in the study of Fredriksson- Ahomaa et al. (1999), but not in reports of Boyapalle et al. (2001) and Arnold et al (2004), what is in line with our results.

The highest prevalence of non-pathogenic yersiniae was observed in raw pork (75 %), minced meat (78 %) and edible offal (83 %) samples obtained at farmer markets. Farmer markets are intended for farmers to offer home-made foods directly to consumers and such farmer markets are common in Latvia. Since there are difficulties to maintain a proper hygiene level due to limited possibility for cleaning and disinfection of surfaces and facilities, contamination from outdoor/outside environment and unsatisfactory storage condition of products, pork may become contaminated with non-pathogenic yersiniae during the whole working day. In contrast, pork samples purchased in supermarkets were significantly less contaminated with non-pathogenic yersiniae (41 %) comparing with samples obtained from butcher shops (58 %) and farmer markets (81 %) ($p < 0.05$). In our mind, hygiene level in supermarkets is higher than in farmer market due to well implemented GHPs and HACCP systems. Observations as mentioned above may explain the differences in the prevalence of non-pathogenic yersiniae between different meat retail outlets in Latvia.

Y. enterocolitica 4/O:3 was isolated from raw pork samples obtained from one out of four supermarkets. Likewise, *Y. enterocolitica* 4/O:3-positive edible offal samples were found in two out of three butcher shops. It is important to emphasize that a previous study showed the high prevalence of *Y. enterocolitica* 4/O:3 in pig tonsils, sampled in the same slaughterhouses that provides raw meat to the butcher shops H and I (Terentjeva and Bērziņš, 2010). Those butcher shops are the small scale enterprises where slaughterhouses sell raw meat directly to consumers. Therefore, the results of the present study revealed that possibilities for contamination of edible by-products exist during slaughter of *Y. enterocolitica* 4/O:3-positive pigs. Contamination of edible offal with human pathogenic *Y. enterocolitica* can be explained also by overloaded chilling rooms and low throughput of products in small scale business as described previously by Fredriksson-Ahomaa et al. (2004) in Germany, and similarly observed in small scale plants in Latvia. Fredriksson-Ahomaa et al. (2004) showed that *Y. enterocolitica* 4/O:3 could easily be transmitted from slaughterhouse to retail environment with contaminated carcasses and edible offal. The contamination of pork samples with human pathogenic *Y. enterocolitica* 4/O:3 was higher in butcher shops comparing with supermarkets with a butchery departments in the study of Fredriksson-Ahomaa et al. (2004), and this is comparable with our results.

In conclusion, human pathogenic *Y. enterocolitica* 4/O:3 is present in raw pork at the retail outlets in Latvia and as far as edible offal and raw pork samples are found to be contaminated, they may pose a risk to public health. Despite the treatment of raw pork before consumption, the presence of *Y. enterocolitica* 4/O:3 may result in cross-contamination that may lead to foodborne infection. The different distribution patterns of non-pathogenic yersiniae and *Y. enterocolitica* 4/O:3 found at different types of retail outlets during our studies will help to better understand the epidemiology of yersiniosis in Latvia. This in turn will lead to subsequent introduction of preventive and control measures in the pork production system, distribution chain and at retail level.

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