PATOGĒNO JERSĪNIJU SUGU (*YERSINIA* SPP.) SASTOPAMĪBA CŪKU BLAKUSPRODUKTOS, LIEMEŅOS UN MANDELĒS
PREVALENCE OF PATHOGENIC *YERSINIA* SPP. ON BY-PRODUCTS, CARCASSES AND TONSILS

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ABSTRACT
The prevalence of pathogenic *Yersinia* was studied from July to October 2007. A total of 105 pig tonsils, 105 by-products and 30 carcass swabs were collected at a large scale slaughterhouse. Samples of tonsils and by-products were obtained from the pluck sets in *post-mortem* inspection area. Samples were tested according to the method of the International Organization of Standardization (Anonymous, 2003). Cold-enrichment was carried out for two weeks if no positive samples were found during the first week of incubation. Overall, 54% of tonsils were tested positive for *Yersinia* spp., where *Y. enterocolitica* 4/O:3 and *Y. pseudotuberculosis,* accounted for 50% and 4%, respectively. Both *Yersinia* species were recovered from by-products (48%), while *Y.enterocolitica* (23%) only from carcasses. The prevalence of *Yersinia* on by-products was significantly higher in tongues and liver than in other parts of the pluck set ($p<0.05$). High prevalence of Yersiniae on by-products and carcasses may present concerns for public heath. The results of this study indicate that possibilities of *Yersinia* contamination of by-products and carcasses may occur during pig slaughtering from *Yersinia* positive tonsils. Further studies are needed to find the main sources of contamination at slaughter.
KEY WORDS: tonsils, evisceration, tongues, liver, cross-contamination

INTRODUCTION

Yersiniosis is a human food-borne disease caused by two pathogenic *Yersinia* species- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. Despite of high heterogenicity of *Y.enterocolitica*, only few of bacteria types or bioserotypes as 1B/O:8, 2/O:9, 2/O:5, 27, 3/O:3 and 4/O:3 are known to be human pathogenic (Bottone, 1997). The bioserovar 4/O:3 is the common for Northern and Central Europe, while another biovars are rare reported in the European Union (Fredriksson-Ahomaa et al., 2006, Fredriksson-Ahomaa et al., 2007).

The clinical manifestation of infection usually is a mild self-limited diarrhea, however severe immunological sequelae as reactive arthritis may occur (Bottone, 1997). Yersiniosis may mimic appendicitis, especially in case with *Y.pseudotuberculosis* infection (Jalava et al., 2006).

Epidemiology of yersiniosis is not completely understood due to low recover rates of pathogenic *Yersinia* from the food-stuffs at retail market (De Boer, 1995). However pork was recognized as the most important source of sporadic yersiniosis during the case-control studies in Belgium and Norway (Ostroff et al., 1994, Tauxe et al., 1987). Moreover, Fredriksson-Ahomaa, et al. (2006) proved that genotypes of *Y.enterocolitica* 4/O:3 strain obtained from pigs are undistinguishable from human isolates, which support the hypothesis that pork could be a vehicle for transmission of bacteria to consumers.

Pigs appear to be the most important carriers of yersinia (Kapperud, 1991). Healthy animals are carrying bacteria in their lymphatic tissues, especially in tonsils, during the life-

time without any signs of disease. Pig slaughtering is an open process where no microbial hazard can be completely excluded (Borch et al., 1996). Yersinia can easily be introduced on by-products and carcasses of pigs if cross-contamination from tonsils occurs (Fredriksson-Ahomaa et al., 2001). This process is promoted by traditional slaughtering and processing techniques, then offal are removed from the carcass as a pluck set with tongue included (Kapperud, 1991). Evisceration step might be crucial for the introduction of yersinia into food chain since low temperatures applied in meat industry do not inhibit the microorganism and bacteria can reach consumers via contaminated pork and its products (Andersen, 1991). Thus, the impact of slaughtering technique on introduction of pathogenic Yersinia should be evaluated in order to recognize and minimize existing problems in meat industry.

The aim of this study was to determine the prevalence of pathogenic *Yersinia* spp. on pig tonsils, by-products and carcasses.

MATERIAL AND METHODS

Sampling. Samples of pig tonsils, by-products and carcass surfaces were collected in one large scale Latvian slaughterhouse during pig slaughtering from July until October 2007. The slaughter capacity of the plant was 50 pigs per hour. After evisceration pluck sets with tonsils were hanged vertically on the processing line and forwarded to the *post-mortem* inspection area. Tonsils were cut out from the pluck sets. Surfaces of pluck set - tongue, heart, lungs, diaphragm, liver, kidney; pharyngeal and costal region of carcasses were swabbed with sterile gauze tampons, moistured in 0.9% saline. The sampling was performed after evisceration, and before routine *post-mortem* examination. Each sample of by-products and carcasses contained five swabs obtained from surfaces. A total of 135 by-products and carcasses, and 105 tonsil swabs were collected.

During the sample collection the visual evaluation of evisceration technique for one hour was undertaken.

Bacteriological examination. Swabs were diluted in PMB (Peptone-Mannitol-Bile salts broth) within two hours after sampling and were left for one hour at 22 °C for resuscitation. Suspension was transferred into ITC (Irgasan Ticarcillin Chlorate) enrichment broth (Fluka, Switzerland) and Cefsulodin-Irgasan-Novobiocin agar (Yersinia selective CIN agar, OXOID, Basingstoke, Hampshire, UK) and incubated at 25 °C and 30 °C in ITC broth and CIN agar, respectively. The suspension from ITC broth after two days of incubation was streaked onto CIN agar plates. Presumptive colonies with a “bull eye” like appearance - red centre and transparent surrounded margins, from CIN agar were tested for oxidase reaction and urea hydrolysis. Species differentiation were carried out with API 20E system (BioMérieux, Marcy l’Etoile, France). Samples in PMB broth were plated out onto CIN agar after one and two weeks of incubation at 4 °C with alkali treatment in case no positive isolates were obtained during the first week of cold enrichment with a subsequent confirmation as was described above.

Biotyping of *Y.enterocolitica* positive isolates was performed as follows: strains were tested for pyrazinamidase activity, salicin, xylose, trehalose fermentation and lipase hydrolysis as described by Wauters et al., (1987) Indole reaction was obtained from API 20E kit. Serotyping was carried out as described by the manufacturer with Yersinia O:3 antisera (Sifin, Berlin, Germany).

Y.enterocolitica and *Y.pseudotuberculosis* cultures were frozen and stored in liquid nitrogen for further tests.

Data analysis. The Chi-square tests were used to detect differences between the prevalence of *Yersinia* spp. on by-products.

RESULTS AND DISCUSSION

The prevalence - 54% of *Yersinia* spp. especially 50% of *Y.enterocolitica* 4/O:3 in pig tonsils is higher than previously reported in Latvia (Terentjeva et al., 2007). Results support observations on prevalence of this pathogen in pig tonsils at the age of slaughtering (Fredriksson-Ahomaa et al., 2001, Nesbakken et al., 2006).

The prevalence of bacteria on carcasses could be the result of cross-contamination during evisceration from *Yersinia* positive tonsils. Our observations of evisceration process showed that removal of tonsils together with a pluck set did cause additional contamination of carcasses with bacteria. Carcasses were vended fresh, so the prevalence of *Yersinia enterocolitica* 4/O:3 on carcasses present the hazard for consumers.

Obtained prevalence of pathogenic *Yersinia* species is shown in Table 1

1.tabula/Table 1

Patogēno jersīniju (*Yersinia* spp.) sugu sastopamība cūku mandelēs, blakusprodukstos un liemeņos

Prevalence of pathogenic *Yersinia* on pig tonsils, by-products and carcasses

Sampling site/ Parauga ņemšanas vieta	Kopējais paraugu skaits/ kopējais pozitīvo paraugu skaits (%) Total no. of samples/ total no. of positive samples (%)	Number of Positive Samples (%) Pozitīvo paraugu skaits/ (%)	
		Y.enterocolitica 4/O:3	*Y.pseudotuberculosis*
Cūku mandeles/ Pig tonsils	105/ 57 (54)	53/ 50	4/ 4
Liemenis/Carcass:			
Rīkles apvidus/ *Reg.pharyngea*	15/ 4 (27)	4 (27)	-
Krūšu kurvja 1.-5. ribas apvidus/ Thorax, I-V costae	15/ 3 (20)	3 (20)	3 (20)
Plūči/Pluck set:			
Mēle/Tongue	15/ 8 (53)	8 (53)	-
Plaušu kraniālā daiva/Lungs, *lobus cranialis*	15/ 8 (40)	4 (27)	2 (13)
Plaušu kaudālā daiva/ Lungs, *lobus caudalis*	15/ 1 (7)	1 (7)	-
Sirds/Heart	15/ 4 (27)	3 (20)	1 (7)
Aknas/Liver	15/ 8 (53)	5 (33)	3 (20)
Diafragma/ Diaphragm	15/ 3 (20)	3 (20)	-
Nieres/ Kidneys	15/ 3 (20)	3 (20)	-

The highest prevalence of *Y.enterocolitica* 4/O:3 - 15/ 8 (53) was detected on tongues and livers, and it was significantly higher for tongues and livers than for other parts of the pluck set ($p<0.05$).

High prevalence of *Y.enterocolitica* 4/O:3 on pig tongues could be explained by their location near the pig tonsils, which are thought to be the most important source of bacteria. Oral cavities of *Yersinia* positive pigs usually are contaminated with these microorganisms at high rates (Nesbakken et al., 1985, Andersen, 1991). Contamination of other offal, as heart, lungs and diaphragm, most probably occurred during removal of the pluck set at evisceration from *Y.enterocolitica* positive tonsils (Fredriksson-Ahomaa et al., 2001). High prevalence of *Yersinia* on the liver could interpreted as follows: it has the lowest position hanged together with the pluck set on the evisceration line, and bacteria could spread from the most contaminated areas, such as the tongue, with the rinsing water. However, our personal observations at the slaughterhouse indicate that *Y.enterocolitica* can be introduced onto liver directly from tonsils or pharyngeal region of the carcass due to their close contact with tonsils area during evisceration step.

Y.pseudotuberculosis was recovered only from lungs and liver, but not from other by-products. *Y.pseudotuberculosis* is rarely isolated from pigs, and the natural reservoirs of the bacteria are expected to be wild animals and birds (Niskanen et al., 2002).

Y.pseudotuberculosis positive by-products were originated only from one pig herd, which was found to be positive with 30% (3/10) the mean prevalence of *Y.pseudotuberculosis*. So the occurrence of pathogenic Yersiniae on the offal depends on the species which pigs are carrying. The failure to isolate *Y.pseudotuberculosis* from tongues, where the highest prevalence was expected, could be explained by the large amount of the background microflora since the oral cavity of a live animal is colonized by other microorganisms, and by lack of appropriate method for detection of bacteria, even CIN agar can cause an inhibitory effect on the growth of *Y.pseudotuberculosis* (Fukushima and Gomyoda, 1986).

Consequent palpation and incision of lymphatic nodes and organ surfaces may lead to distribution of bacteria from the contaminated to non-contaminated areas by the meat inspector's hands and knives. From this point of view the high prevalence of Yersinia on the liver should be taken into consideration, because a routine *post-mortem* examination of the pluck starts from the liver. Liver is expected to be free from bacteria, but as all samples were taken before *post-mortem* examination, cross-contamination from liver may occur.

The previous studies on distribution of Yersinia at slaughtering indicate that the pig tonsils serve as a source of contamination of by-products and carcasses. The results of this study show that pathogenic *Yersinia* can easily enter the food chain if animals are carriers, and mistakes in the slaughter technique occur during evisceration step.

More sensitive methods, such as pulsed gel electrophoresis (PFGE) should be applied for detection of genotypes of isolated *Yersinia* cultures to find the sources of contamination.

CONCLUSIONS

1. The prevalence of pathogenic *Yersinia* on by-products may indicate to the mistakes of slaughterhouse personnel which may enhance the distribution of bacteria.
2. Since each slaughterhouse may have its own contamination pattern with bacteria, studies should be continued to find out the possible slaughtering technique problems existing in Latvian meat industry.
3. Due to the high prevalence of pathogenic *Yersinia* in tonsils, long-term preventive measures should be developed for the meat industry to decrease the existing problems.

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