

# DAŽĀDA JERSĪNIJU SASTOPAMĪBA CŪKU LIEMENOS TRIJĀS KAUTUVĒS LATVIJĀ

## DIFFERENT PREVALENCE OF YERSINIAE ON PIG CARCASSES IN THREE SLAUGHTERHOUSES IN LATVIA

**Margarita Terentjeva, Edgars Liepiņš, Aivars Bērziņš**

LLU, Veterinārmedicīnas fakultāte, Pārtikas un vides higiēnas institūts, Latvija  
LUA, Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene,  
Latvia

[Margarita.Terentjeva@llu.lv](mailto:Margarita.Terentjeva@llu.lv)

### ABSTRACT

The aim of the present study was to detect yersinia species and their prevalence on carcasses of slaughter pigs. A total amount of 90 surface swabs of pig carcass were collected in three slaughterhouses in Latvia. All the samples were tested with direct plating, selective enrichment and cold enrichment according to the ISO and NCFA methods. Colonies, which resembled yersinia on CIN agar plates, were confirmed with API 20E kit. *Y. enterocolitica* strains were bio-serotyped. In total, non-pathogenic yersinia – *Y. enterocolitica* biotype 1A, *Y. kristesenii* and *Y. fredriksenii*, and pathogenic yersinia – *Y. enterocolitica* bioserotype 4/O:3 species and/or bioserotypes were isolated from pig carcasses. The prevalence of non-pathogenic and pathogenic yersinia on pig carcasses was 52 and 9 %, respectively. Non-pathogenic yersinia were isolated in all three slaughterhouses, while pathogenic *Y. enterocolitica* 4/O:3 in two slaughterhouses. In total, the prevalence of yersinia on carcasses was 56 %, 66 % and 60 % in slaughterhouses A, B and C, respectively. The prevalence of non-pathogenic yersinia in slaughterhouses B and C was significantly higher than in slaughterhouse A ( $p<0.05$ ). The prevalence of pathogenic yersinia was significantly higher in slaughterhouse A, than in slaughterhouses B and C ( $p<0.05$ ).

**KEY WORDS:** non-pathogenic, pathogenic yersinia, pig head, costal region.

### INTRODUCTION

*Yersinia* genus is a member of Enterobacteriaceae family, which consists from 11 non-pathogenic and 3 pathogenic species. Among the pathogenic species, *Y. pseudotuberculosis* and certain bioserotypes of *Y. enterocolitica* may cause foodborne yersiniosis, while non-pathogenic species are frequently isolated from ill patients and their epidemiological significance is still unclear. Pathogenic yersinia are transmitted to consumers by infected food vehicles, but the case control studies show that in the majority of yersiniosis cases pork is implicated (Ostroff et al, 1994).

A principal source of pathogenic yersinia, especially *Y. enterocolitica* bioserotype 4/O:3 are pigs, which are carrying pathogen in their lymphatic tissues, especially in palatine tonsils at the age of slaughter. Due to cross-contamination from yersinia positive tonsils, pathogenic *Y. enterocolitica* may spread to pig carcasses at the slaughter (Fredriksson-Ahoma et al., 2000). In contrast, non pathogenic yersinia species are distributed in the environment and are isolated from fresh water, fish and food samples, which can serve as a source of them to animals. Due to widespread occurrence of these yersinia in the environment, they can easily enter the

slaughterhouse with slaughtered animals and spread to carcasses of pigs during slaughtering procedures, indicating the hygiene status of certain plant (Harmon et al., 1984). As pathogenic yersiniae – *Y. enterocolitica* 4/O:3 and *Y. pseudotuberculosis* were isolated from pigs in previous studies in Latvia with the prevalence 35 % and 3 %, respectively, (Terentjeva and Bērziņš, 2010), possible that they could spread from tonsils and to contaminate carcasses at the slaughter .

The aim of the present study was (i) to detect yersiniae species and/or their bioserotypes, distributed on pig carcasses and (ii) to evaluate differences in the prevalence of non-pathogenic and pathogenic yersiniae depending on sampling site and slaughterhouse, where samples were collected.

## **MATERIAL AND METHODS**

A total amount of 90 surface swabs of pig carcasses were collected in three slaughterhouses - A, B and C, located in Zemgale, Vidzeme and Latgale. Samples were collected during July 2007 to November 2009. A total amount of 30 surface samples from each slaughterhouse were obtained. All slaughterhouses were large scale plants with slaughter capacity up to 50 pigs per hour. Slaughtering process included the following operations – stunning, bleeding, scalding, flaming, evisceration, carcass splitting, rinsing and cooling.

Carcass surface swabs were collected from pig carcasses after evisceration and splitting, but prior to *post-mortem* inspection. An area of approximately 20cm<sup>2</sup> of head and costal regions was swabbed with sterile gauze tampon (5 cm x 5 cm), moistured in 0.9% of saline, placed in sterile sample transporting bags and delivered to the laboratory on ice within 2 h after collection. Samples were diluted with 90 ml PMB broth (peptone-mannitol-bile salt broth) immediately after arrival to the laboratory.

Samples were tested using the direct plating, the selective enrichment and the cold enrichment according to the ISO and NMKL methods. Prior to testing, swabs in PMB and were left for one hour at 22°C for resuscitation. For the direct plating, 10µl of suspension were streaked on CIN Agar. For the selective enrichment, 0.1ml of suspension was transferred into ITC (Irgasan Ticarcillin Chlorate) enrichment broth (Fluka, Switzerland) and CIN agar (Cefsulodin-Irgasan-Novobiocin agar, Yersinia selective agar, OXOID, Basingstoke, Hampshire, UK) and incubated at 25°C for 48 h. For the cold enrichment, samples in PMB broth were plated out onto CIN agar after one, two and three weeks of incubation at 4°C with alkali treatment with 0.25% KOH in case no positive isolates were obtained during the first or second weeks of cold enrichment.

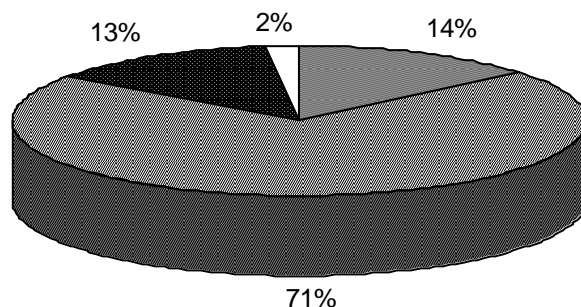
A quantity of 10µl of suspension from ITC broth after incubation, and PMB after cold enrichment was streaked onto CIN agar plates. CIN agar was incubated at 30°C for 48 h. CIN plates were evaluated after incubation in order to detect bacterial colonies with yersiniae-like appearance. Presumptive colonies with a “bull eye” like appearance - red centre and transparent surrounded margins, from CIN agar were tested for oxidase reaction and urea hydrolysis. Differentiation of species was carried out with API 20E system (BioMérieux, Marcy l’Etoile, France).

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 enterocolitica* O:3 antisera (Sifin, Berlin, Germany).

The Chi-square tests were used to detect differences in the prevalence of non-pathogenic and pathogenic yersiniae on pig carcasses in different slaughterhouses.

## RESULTS AND DISCUSSION

Both non-pathogenic and pathogenic yersinia were isolated from slaughtered pig carcasses. From the isolated species or/and their bioserotypes *Y. enterocolitica* biotype 1A, *Y. kristensenii* and *Y. frederiksenii* belonged to non-pathogenic, while *Y. enterocolitica* 4/O:3 to pathogenic yersinia (Figure 1).



■ *Y. enterocolitica* 4/O:3 ■ *Y. enterocolitica* 1A ■ *Y. kristensenii* □ *Y. frederiksenii*

### 1. attēls. Jersīniju sugu izplatība cūku liemeņos

Figure 1. The prevalence of yersinia on pig carcasses

*Y. enterocolitica* 1A was the most frequently isolated from yersinia species, whereas *Y. frederiksenii* was isolated less frequently. In total, the percentage of isolated non-pathogenic species were significantly higher than percentage of pathogenic *Y. enterocolitica* 4/O:3 on pig carcasses.

Pig heads and costal region of the carcasses were found to be contaminated with *Yersinia* spp., there the highest prevalence of yersinia were observed on pig head, while the lowest on costal region (Table 1).

1.tabula/Table 1

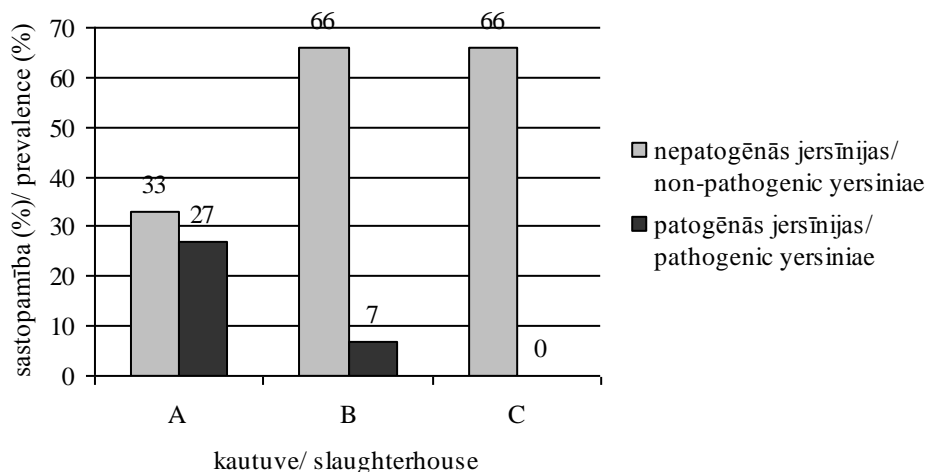
**Nepatogēno un patogēno jersīniju sastopamība cūku liemeņos**  
The prevalence of non-pathogenic and pathogenic yersinia on pig carcasses

Parauga noņemšanas vieta Sampling site	Paraugu skaits No. of samples	Pozitīvo paraugu skaits (%) No. of positive samples (%)	
		Nepatogēnās jersīnijas Non-pathogenic yersinia	Patogēnās jersīnijas Pathogenic yersinia
Galvas reģions Head region	45	25 (56)	5 (11)
Ribu reģions Costal region	45	22 (46)	3 (7)
Kopā/ Total	90	47 (52)*	8 (9)

\* the prevalence of non-pathogenic yersinia was significantly higher than the prevalence of pathogenic yersinia ( $p < 0.05$ )

In total, the prevalence of non-pathogenic yersinia on pig carcasses was significantly higher than pathogenic yersinia ( $p < 0.05$ ). However, we did not find significant differences in the prevalence of non-pathogenic and pathogenic yersinia on

pig head and costal regions ( $p < 0.05$ ). The prevalence of non-pathogenic and pathogenic yersiniae on pig heads varied depending on slaughterhouse, where samples were collected. Non-pathogenic yersiniae were observed in all three slaughterhouses, while pathogenic only in slaughterhouses A and B. The highest prevalence of non-pathogenic yersiniae was found in slaughterhouse C, but the lowest – in slaughterhouse B, 66% and 33% of positive cases, respectively (Figure 3). The highest prevalence of pathogenic yersiniae were observed in slaughterhouse A, but the lowest in slaughterhouse C, 27% and 0% of positive cases, accordingly.

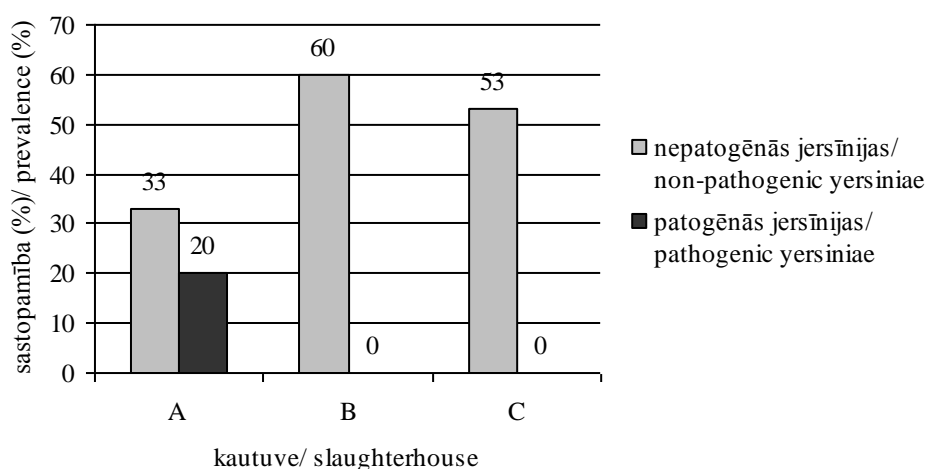


## 2.attēls. Jersīniju sastopamība cūku galvās dažādās kautuvēs

Figure 2. The prevalence of yersiniae in pig heads in different slaughterhouses

The prevalence of non-pathogenic yersiniae was significantly higher in slaughterhouse C, than in slaughterhouses A and B ( $p < 0.05$ ). In spite, the prevalence of pathogenic yersiniae, was significantly higher in slaughterhouse A, than in slaughterhouses B and C ( $p < 0.05$ ).

The prevalence of non-pathogenic and pathogenic yersiniae, isolated from costal region differed from slaughterhouse where samples were collected. Non-pathogenic yersiniae were observed in all three slaughterhouses, while pathogenic only in slaughterhouse A. The highest prevalence of non-pathogenic yersiniae was found in slaughterhouse B, but the lowest in the slaughterhouse A – 33% and 53%, respectively (Figure 3).



## 3.attēls. Jersīniju sastopamība ribu reģionā dažādās kautuvēs

Figure 3. The prevalence of yersiniae in costal region in different slaughterhouses

In total, the prevalence of non-pathogenic yersiniae in slaughterhouses B and C was significantly higher than in slaughterhouse A ( $p < 0.05$ ). In contrast, the prevalence of pathogenic yersiniae was significantly higher in slaughterhouse A, than in slaughterhouses B and C ( $p < 0.05$ ).

The isolation of non-pathogenic and pathogenic *Yersinia* species from the pig carcasses was in agreement with previous studies (Harmon et al., 1984, Fredriksson-Ahomaa et al., 2000, Bonardi et al., 2007, Laukkanen et al., 2009). However, the prevalence of non-pathogenic yersiniae in our study was higher than 1% of positive samples reported by Bonardi et al., 2007 and the prevalence of *Y. enterocolitica* 4/O:3 was higher than 0% and 6% of contaminated carcass reported by Bonardi et al., 2003 and Fredriksson-Ahomaa et al., 2000. These data indicate that yersiniae are widespread on pig carcasses in large scale slaughterhouses in Latvia, but serious concerns should be taken due to distribution of pathogenic yersiniae on them. As pigs are known to be the carriers of pathogenic yersiniae, especially *Y. enterocolitica* 4/O:3 in Latvia (Terentjeva, Bērziņš, 2010), the finding of same bioserotypes both in pig tonsils and pig carcasses, indicate that the source of this pathogen on pig carcasses of was pig tonsils.

## CONCLUSIONS

1. Non-pathogenic yersiniae were the most widely isolated in our study, indicating that carcasses were mainly contaminated from the outside environment or during processing due to insufficient hygiene procedures in the slaughterhouse.
2. High rates of isolation of *Y. enterocolitica* 4/O:3 from carcasses maybe due to tonsil tissues, which partially may remain on pig heads after splitting and remain surrounded tissues, because they often are carrying pathogen.
3. Finding of the same *Y. enterocolitica* bioserotypes 4/O:3 both in pig tonsils and pig carcasses, indicates that carcass became contaminated from pig tonsils.

## REFERENCES

1. Anonymous. Microbiology of food and animal feedings stuffs- Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica* (ISO 10273: 2003). – International Organization of Standartization. 2003; 1-15.
2. Anonymous. *Yersinia enterocolitica*. Detection in foods. Method no. 117, 3rd ed. - Nordic Committee on Food Analysis (NMLK), Espoo, Finland. 1996; 1-8.
3. Bonardi, S., Brindani, F., Pizzin, G., Lucidi, L., D'Incau M., Liebana E., Morabito S. Detection, of *Salmonella* spp., *Yersinia enterocolitica* and Verocytotoxin-Producing *Escherichia coli* O157 in Pigs at Slaughter in Italy. - International Journal of Food Microbiology. 2003;85: 101-110.
4. Bonardi, S., Paris, A, Bacci, C., D'Incau M, Ferroni L., Brindani F. Detection and characterization of *Yersinia enterocolitica* from pigs and cattle. - Veterinary Research Communications. 2007; 31: 347-350.
5. Fredriksson-Ahomaa, M., Korte, T., Korkeala, H. Contamination of carcasses, offal, and the environment with *yadA*-positive *Yersinia enterocolitica* in a pig slaughterhouse. -Journal of Food Protection. 2000; 63: 31-35.
6. Harmon, M.C., Swaminathan, B, Forest J.C. Isolation of *Yersinia enterocolitica* and related species from porcine samples obtained from an abattoir. - Journal of Applied Bacteriology. 1984; 56: 421-427.

7. Laukkanen, R., Martinez, P. O., Siekkinen, K. M., Ranta, J., Maijala, R., Korkeala, H. Contamination of Carcasses with Human Pathogenic *Yersinia enterocolitica* 4/O:3 Originates from Pigs Infected on Farms. *Foodborne Pathogens and Disease*. 2009; 6(6): 681-688.
8. Ostroff, S.M., Kapperud, G., Huteagner, L.C., Nesbakken, T., Bean, N.H. Sources of sporadic *Yersinia enterocolitica* infection in Norway: a prospective case-control study. - *Epidemiology and Infection*. 1994; 112: 133-141.
9. Terentjeva, M., Bērziņš, A. Prevalence and antimicrobial resistance of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in pigs in Latvia. - *Journal of Food Protection*. 2010; 73: 1135-1138.
10. Wauters, E., Kandolo, K., Janssens, M. Revised biogrouping scheme of *Yersinia enterocolitica*. - *Contributions in Microbiology and Immunology*. 1987; 9: 14-21.