12. Jemeļjanovs, A., Vētra, J., Blūzmanis, J., Konošonoka, I.H., Duļbinskis, J., Pūce, B. (2004) Augu valsts ārstniecības līdzekļu pielietošana govju tesmeņa slimību profilaksē. *Veterinārmedicīnas Raksti 2004*, lpp.94-99.
13. Kaeoket, K. (2003) Infiltration by cells of the immune system in the sow endometrium. *Acta Universitatis Agriculturae Sueciae Veterinaria 136*, Uppsala, 184 pp
14. Kask, K. (2005) Normal and impaired uterine function in cow. *NOVABA Postgraduate Course Bovine reproduction and swine reproduction.* Tartu, Estonia.
15. Kirks, J., Oertons, M., (2000) Postcalving uterine infections, ttp://www.vetmed.ucdavis.edu Resurss aprakstīts 25.06.2004.
16. Kornmatitsuk, B., (2002) Endocrine and Clinical Studies of Late pregnancy and Parturition in Dairy Cattle with Special Emphasis on Stillbirth. *Acta Universitatis Agriculturae Sueciae Veterinaria 140*, Uppsala, 126.pp
17. Liepa, L. (2000) *Asiņu bioķīmisko rādītāju klīniskā interpretācija govīm*. Jelgava, 44 lpp.
18. Liepa, L., Krūmiņa, D. (2002) Brīvo taukskābju un glikozes koncentrācijas izmaiņas serumā govīm pārejas periodā. *Veterinārmedicīnas raksti 2002*. 125- 128 lpp.
19. Meglia, G., E. (2004), Nutrition and Immune Response in periparturient Dairy Cows. *Acta Universitati. Agriculturae Sueciae Veterinaria 170*, Uppsala**,** 116.pp
20. Meyer, D., Harwey, J. (2004) *Veterinary laboratory medicine*. Saunders, pp.82-88.
21. Paura, L., Arhipova, I. (2002) *Neparametriskās metodes*. Jelgava, LKC 148 lpp.
22. Purviņš, I. (1994) *Praktiskā farmakoloģija*. Medikamentu informācijas centrs. Rīga, 582 lpp.
23. Reksen, O., Havrevoll, Ø. Gröhn, T.Y., Bolstad, T. (2002) Relationships among body condition score, milk constituents, and postpartum luteal function in Norwegian dairy cows. *J.Dairy Science*, 85:1406-1415, American Dairy Association.
24. Мозгов, И.Е. (1988) *Ветеринарная рецептура с основами терапии и профилактики*. «Агропромиздат» Москва, 384 стр.
25. Зверева, Г.В., Хомин, С.П., (1976) *Гинекологические болезни коров*. «Урожай» Киев, 152 стр.

# *YERSINIA* ĢINTS BAKTĒRIJU SASTOPAMĪBA LATVIJAS IZCELSMES NOKAUTO CŪKU MANDELĒS

# OCCURRENCE OF *YERSINIA* SPECIES IN SLAUGHTERED PIGS' TONSILS OF LATVIAN ORIGIN

**Terentjeva Margarita, Bērziņš Aivars, Liepiņš Edgars**
LLU Veterinārmedicīnas fakultāte, Latvija
Faculty of Veterinary Medicine, LUA, Latvia
Margarita.Terentjeva@llu.lv, Aivars.Berzins@llu.lv, Edgars.Liepins@llu.lv

**ANOTĀCIJA**

Kopā 88 Latvijas izcelsmes nokauto cūku mandeļu paraugi tika savākti no piecām saimniecībām vienā lieljaudas kautuvē. *Yersinia* ģints baktērijas tika konstatētas visos ganāmpulkos. To sastopamība saimniecībās bija robežās no 25 līdz 55%. No pozitīvajiem paraugiem tika izolētas potenciāli patogēnās sugas - *Y.enterocolitica* (31%) un *Y.pseudotuberculosis* (8%), un viena nepatogēnā *Y.kristensenii* kultūra (1%). Patogēnā *Y.enterocolitica* bija dominējoša *Yersinia* ģints baktēriju suga visās piecās saimniecībās. Augsta potenciāli patogēno *Yersinia* sastopamība norāda, ka cūku mandeles var kļūt par blakusproduktu un liemeņa kontaminācijas avotu eviscerācijas un *post-mortem* inspekcijas veikšanas laikā.
**KEY WORDS**: *Yersinia*, tonsils, occurrence

## INTRODUCTION

Genus *Yersinia*, a member of *Enterobacteriaceae* family, consists of 11 species. Three of them- *Yersinia pestis*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are human pathogenic (Bottone, 1999). Another eight species are considered to be non-pathogenic. However, their epidemiological significance and pathogenic properties are still discussed (Falcao et. al., 2004, Sulakvelidze, 2000, Tennant et. al.2003)

*Y.pseudotuberculosis* and *Y.enterocolitica* cause a human infection- yersiniosis. The primary route of infection is the food- borne. Disease is characterized with gastro- intestinal disorders, sometimes with severe immunological sequelae like reactive arthritis and erythema nodosus (Bottone, 1999). Yersiniosis is one of the most actual food-borne infections in various European Union (EU) members- Belgium, Czech Republic, Denmark, Finland, Germany, Lithuania and Sweden. Disease is recognized in Latvia as well. The incidence of yersiniosis was 2.27 cases per 100 000 inhabitants during last five years with a trend to increase (EFSA, 2006, Public Health Agency).

Transmission of pathogenic *Yersinia* spp. via foods, especially with meat products is well documented (Asplund et al., 1998). The contamination of meat products may often occur at the slaughterhouse level. Regardless of numerous hosts of the microorganism- farm animals, pets, variety of wild animals, pathogenic *Yersinia*, particularly *Y.enterocolitica*, more often are isolated from pigs (Bottone, 1997, Fredriksson- Ahomaa et al., 2001). Clinical healthy animals carry pathogenic *Yersinia* in their tonsils and intestines (Asplund et al, 1998). Offal and carcass easily become contaminated with bacteria during the evisceration at the slaughterhouse (Andersen, 1988, Fredriksson-Ahomaa et al., 2001). Properties of *Y.enterocolitica* and *Y. pseudotuberculosis* allow them to survive and multiply in the environment of the meat processing plant, on offal and surface of carcass (Logue et. al., 1998). Thus, contaminated pork can be an important source of pathogenic *Yersinia* species to human. Investigations on *Yersinia* spp. occurrence in pigs are needed to detect potential sources of bacteria to consumers.

The aim of this study was to determine occurrence of *Yersinia* spp. in Latvian in pigs slaughtered in Latvia.

## MATERIALS AND METHODS

A total of 88 samples of Latvian origin pigs' tonsils were collected from one slaughterhouse from January until March in 2006. Investigations were performed at Latvia University of Agriculture, Faculty of Veterinary Medicine, Laboratory of Food Hygiene.

Amount of 10 g from each sample was homogenized in 90 ml of buffered peptone water (Oxoid, Basingstoke, Hampshire UK). After resuscitation for 1 h at 20 °C, 0.1 ml of prepared sample material was transferred onto cefsulodin-irgasan-novobiocin agar (CIN agar, Oxoid, Basingstoke, Hampshire, UK). Homogenized sample in buffered peptone water was cold enriched for 2 weeks at 4 °C. Plating onto selective agar plates was carried out at $8^{th}$ and $15^{th}$ incubation day. A volume of 0.5 ml sample material before the streaking onto selective agar plates was previously treated with 4.5 ml of 0.05% KOH for 20 seconds at the $15^{th}$ day of cold enrichment.

CIN agar plates were incubated for 18- 24 hours at 30 °C. Presumptive colonies with bull's eye like appearance with bright red center and transparent surrounded outer zone were examined with oxidase test and for urea reaction. Oxidase- negative, urea- positive isolates were confirmed with API 20 kit (BioMérieux, Marcy l'Etoile, France).

## RESULTS AND DISCUSSION

*Yersinia* genus species were isolated from all farms (Table 1). The highest incidence of *Yersini*a spp. was observed in the farm 4.

1.tabula / Table 1

**_Yersinia_ ģints mikroorganismu izplatība dažādās Latvijas saimniecībās**
**Distribution of _Yersinia_ species in Latvian pigs herds**

| Farm<br>Saimniecība | Geographical localization<br>Saimniecības novietojums | No. of samples<br>Paraugu skaits | No. of positive (%)<br>Pozitīvo paraugu skaits (%) |
|---|---|---|---|
| 1 | Ziemeļzemgale | 20 | 5 (25) |
| 2 | Dienvidrietumu Latgale | 20 | 6 (30) |
| 3 | Dienvidaustrumu Latgale | 20 | 9 (45) |
| 4 | Ziemeļzemgale | 20 | 11 (55) |
| 5 | Ziemeļvidzeme | 8 | 4 (50) |

Both pathogenic and non-pathogenic *Yersinia* species are distributed in Latvian pigs herds (Table 2).

2.tabula / Table 2

**Occurrence of _Yersinia_ species in Latvian pig herds**
***Yersinia* sugu sastopamība dažādās Latvijas saimniecībās**

| | *Yersinia* species<br>*Yersinia* ģints baktēriju sugas | | |
|---|---|---|---|
| | *Y.enterocolitica* | *Y.pseudotuberculosis* | *Y.kristensenii* |
| Farm<br>Saimniecība | No.of isolates(%)<br>Izolēto kultūru skaits(%) | No.of isolates(%)<br>Izolēto kultūru skaits(%) | No.of isolates(%)<br>Izolēto kultūru skaits(%) |
| 1 | 4 (11) | 1 (3) | - |
| 2 | 5 (14) | 1 (3) | - |
| 3 | 9 (26) | - | - |
| 4 | 6 (17) | 5 (14) | - |
| 5 | 3 (9) | - | 1(3) |
| Total<br>Kopā | 27 (77) | 7 (20) | 1 (3) |

*Yersinia* spp. were recovered from 35 samples of Latvian origin pigs' tonsils (40%). Prevalence of *Y.enterocolitica* and *Y.pseudotuberculosis* were 31% and 8%, respectively. One culture of non-pathogenic *Yersinia* species- *Y.kristensenii* (1%) was identified.

*Y.enterocolitica* was found in all 5 five farms. Prevalence of *Y.enterocolitica* varied from 15 to 45%. *Y.pseudotuberculosis* recovered from 3 farms at level 5% at farms 1, 2 and 25% at farm 4. Prevalence of *Y.pseudotuberculosis* at farm 4 was found to be the highest. Total prevalence of *Y.pseudotuberculosis* in our study was 2 times higher comparing with Finnish survey (Niskanen et.al., 2005).

Although not all *Yersinia enterocolitica* bioserotypes are associated with a disease, pathogenic *Yersinia enterocolitica* strains are often isolated at slaughter (Nesbakken et al., 2003; Fredriksson-Ahomaa *et al*, 2001) from clinically healthy pigs.

Our results showed that at least three animals in each herd were carriers of potentially pathogenic *Yersinia* in their tonsils. As the bacteria may establish reservoir at pig herds (Nesbakken et al., 2006), these data indicate problem significance in Latvian pig's husbandry.

Piglets are easy become *Yersinia* carriers from the adult animals such as sows at the birth time or fattening pigs during regrouping, thus possibly establishing long-term reservoir for pathogenic *Yersinia*. Animals can become carriers of bacteria during transportation to the slaughterhouse or separation at lairage during *ante-mortem* examination (Skjerve *et al*., 1998). As the pigs do not show any clinical manifestations (Pilon et al., 2000), *Yersinia* genus bacteria can easy reach slaughterhouse environment. If hygiene level is inappropriate and cross-contamination occurs, there are wide possibilities for raw and processed pork contamination with the pathogenic *Yersinia* species.

High prevalence of potentially pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in tonsils of Latvian pig's herds indicate importance of this study.

Further epidemiological investigations are needed on *Y.enterocolitica* and *Y.pseudotuberculosis* biogroups and/or serotypes, pathogenic properties evaluation and the role of slaughtering and evisceration techniques under Latvian condition with a particular significance in the spreading of pathogenic yersinia through pork to consumer.

## CONCLUSIONS

1. Presence of potentially pathogenic *Yersinia* genus species in pigs indicates that a possibility for contamination with bacteria occurs during evisceration and processing of offal and carcasses.
2. Special precautions should be undertaken with a removal of palatine tonsils during pluck and carcass *post- mortem* examination.
3. Information about *Y.enterocolitica* and *Y.pseudotuberculosis* positive herds should be collected by competent authorities in order to minimize contact of *Yersinia* carriers with *Yersinia* free herds during transportation to the slaughterhouses and separating at the lairages.

## REFERENCES


1. Andersen, J.K. Contamination of freshly slaughtered pig carcasses with human pathogenic *Yersinia enterocolitica*. International Journal of Food Microbiology, 1988, 7:193- 202
2. Asplund, K., Hakkinen, M., Okkonen, T., Vanhala, P., Nurmi, E. Effects of growth-promoting antimicrobials on inhibition of *Yersinia enterocolitica* O:3 by porcine ileal microflora. Journal of Applied Microbiology, 1998, 85: 164- 170.
3. Bottone, E.J. *Yersinia enterocolitica*: the charisma continues. Clinical Microbiology Reviews, 1997, 10:257-276
4. Bottone, E.J. *Yersinia enterocolitica*: overview and epidemiological correlates. Microbes and Infection. 1999. 1:323-333
5. European Food Safety Authority. Trends and sources of zoonoses, zoonotic agents and microbial resistance in the European Union in 2004, 2006
6. Falcao, J.P., Brocchi, M., Proenca- Modena, J.L., Acrani, G.O., Correa, E.F., Falcao, D.P. Virulence characteristics and epidemiology of *Yersinia enterocolitica* and Yersiniae other than *Y.pseudotuberculosis* and *Y.pestis* isolated from water and sewage. Journal of Applied Microbiology, 2004, 96: 1230-1236
7. Fredriksson –Ahomaa, M., Bucher, M., Hank, C., Stolle, A., and Korkaela, H. High prevalence of *Yersinia enterocolitica* 4:O3 on pig offal in Southern Germany: a slaughtering technique problem. Systematic and Applied Microbiology, 2001. 24: 457-463
8. Fredriksson –Ahomaa, M., Korte, T., Korkeala, H. Transmission of *Yersinia enterocolitica* 4/O:3 to pets via contaminated pork. Letters in Applied Microbiology, 2001. 32, 375- 378

9. International Organization of Standardization. Microbiology of food and animal feedings stuffs- Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica* (ISO 10273: 2003).
10. Logue, C.M., Sheridan, J.J., McDowell, D.A., Blair, I.S., Harrington, D. A study of the growth plasmid bearing and plasmid cured strains of antibiotic resistant *Yersinia enterocolitica* serotype O:3 on refrigerated beef, pork and lamb. Food Microbiology, 1998, 15: 603-615.
11. Nesbakken, T., Eckner, K., Høidal, K.H., Røtterud, O.L. Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. International Journal of Food Microbiology, 2003. 80:231- 240
12. Nesbakken, T., Iversen, T., Eckner, K., Lium, B. Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic of infection. International Journal of Food Microbiology, 2006, 111: 99- 104
13. Niskanen, T., Fredriksson- Ahomaa, M., Korkeala, H. *Yersinia pseudotuberculosis* with genetic diversity is a common finding in tonsils of fattening pigs. Journal of Food Protection, 2005, 65: 540- 545
14. Pilon. J., Higgins, R., Quesy, S. Epidemiological study of *Yersinia enterocolitica* in swine herds in Québec. Canadian Veterinary Journal., 2000, 41: 383- 387
15. Public Health Agency, Latvia. www.sva.lv
16. Skjerve, E., Lium, B., Nielsen, B., Nesbakken, T. Control of *Yersinia enterocolitica* in pigs at herd level. International Journal of Food Microbiology, 1998, 45:195- 203
17. Sulakvelidze, A. *Yersinia* other than *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y.pestis*: the ignored species, Microbes and Infection, 2000, 2:497- 513
18. Tennant, S.M., Grant, T.H, Robins- Browne, R.M. Pathogenicity of *Yersinia enterocolitica* biotype 1A. FEMS Immunology and Medical Microbiology, 2003, 38: 127- 137