INCIDENCE OF *YERSINIA ENTEROCOLITICA* 4/O:3 IN PIGS OF LATVIAN ORIGIN AT SLAUGHTERING

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Abstract

A total amount of 90 pig tonsil samples were investigated to detect presence of *Y.enterocolitica* 4/O:3. Samples were collected in two large-scale Latvian slaughterhouses from five pig herds during February and March, 2006. Samples were investigated with direct plating onto selective CIN agar plates according to ISO 10273 method, with subsequent cold enrichment for 2 weeks at 4 °C in accordance with NMKL 117 method. *Y.enterocolitica* positive isolates were investigated for pyrazinamidase and lipase activity, indole production and salicine, xylose, trehalose fermentation. Slide agglutination reaction with commercial antisera O:3 was carried out for detection of *Y.enterocolitica* sevovars. Pathogen was isolated from all five herds with 30% mean prevalence. Incidence of *Y.enterocolitica* 4/O:3 depending on herd varied from 20 to 45%. High incidence of *Y.enterocolitica* in pig tonsils indicates that animals are important reservoir of this pathogen in Latvia. Further studies about incidence of *Y.enterocolitica* pathogenic bioserotypes in pig population and contamination pattern with pathogen in slaughterhouses are needed.

Keywords: bioserotype, tonsil, herd.

Introduction

Yersinia enterocolitica is a highly heterogenic bacterium, depending on its biochemical and antigenic properties divided into six biotypes and various serotypes. It is well documented that *Y.enterocolitica* bioserovars correlate with virulence properties, ecological and geographical distribution of microorganism. Only few of them, such as 1B/O:8, 2/O:9, 2/O:5, 27, 3/O:3 and 4/O:3, are pathogenic to human (Bottone, 1999).

Pathogenic *Y.enterocolitica* bioserotypes cause the yersiniosis, a human food-borne disease, characterized by gastro-intestinal disorders and severe post-infection complication such as erythema nodosum, glomerulonephritis, myocarditis or arthritis (Bottone, 1999). Disease is recognized in Latvia with an average incidence 2.3 cases per 100 000 inhabitants during 2001-2005 with a recent trend to increase (Anonymous, 2006).

While bioserotypes 1B/O:8, 2/O:9, O:5, 27, as a causative agent of yersiniosis, are mostly described in the United States and Japan, the most common biovar for Northern and Central Europe is 4/O:3 (Fredriksson-Ahomaa et al., 2006), infection with 2/O:5, 27, 3/O:5, 27, 2/O:9 and 1B/O:8 is rarely reported (Bottone, 1999; Nowak et al., 2006; Schubert et al., 2003).

Epidemiology of yersiniosis is still poorly understood, because *Y.enterocolitica* is seldom recovered from food (De Boer, 1995). However, M. Fredriksson-Ahomaa et al., (1999) reported high prevalence of pathogen on pork offal and minced meat at retail level in Finland. Consumption of pork was identified as a source of sporadic yersiniosis in Norway (Ostroff et al., 1994).

Pigs seem to be the most important reservoir of *Y.enterocolitica* (Kapperud, 1991). Healthy animals harbour pathogen in the lymphatic tissues, especially in tonsils, during life-time without clinical signs (Nowak et al., 2006). T. Nesbakken et al., (2003) showed that bacterium is usually introduced in food chain due to initial contamination with *Y.enterocolitica* in slaughterhouses. Presence of bacterium in pigs at slaughtering opens various opportunities for contamination with *Y.enterocolitica* 4/O:3 to offal and meat with further transmission of pathogen to retail level. No previous studies on occurrence of pathogenic *Yersinia* bioserotypes and epidemiology of *Y.enterocolitica* were undertaken in Latvia. However, detection of *Y.enterocolitica* carriers, especially pathogenic, is important factor for understanding the epidemiology of yersiniosis (Asplund et al., 1998).

The aim of study was to detect incidence of *Y.enterocolitica* 4/O:3 in pigs of Latvian origin at slaughtering.

Materials and Methods

A total amount of 90 pig tonsil samples were collected from five pig herds in two large-

Table 1

Incidence of *Y.enterocolitica* 4/O:3 in pigs at slaughtering

Herd localization	No. of samples	*Y.enterocolitica* 4/O:3 positive samples (%)
East Kurzeme	10/3(30)	3(30)
Ziemeļzemgale	20/4 (20)	4(20)
Dienvidrietumu Latgale	20/5 (20)	5(20)
Dienvidaustrumu Latgale	20/9 (45)	9(45)
Ziemeļzemgale	20/5 (25)	5(25)

scale slaughterhouses, located in different parts of Latvia during February – March, 2006. All samples were taken aseptically from randomly selected pluck sets. Tonsils were put into sterile stomacher bag and were transported to the Faculty of Veterinary Medicine, Latvia University of Agriculture.

Samples were investigated during April-September, 2006. An amount of 10 g of sample were homogenised in peptone buffered water (Oxoid, Basingstoke, Hampshire, UK), resuscitated for 1 h at 22 °C, and plated out on to cefsulodin-irgasan-novobiocin agar plates (CIN, Yersinia selective agar, Oxoid, Basingstoke, Hampshire, UK) according to ISO method 10273:2003. Agar plates were incubated for 48 h at 30 °C. Three presumptive colonies with typical 'bull-eye like' appearance - red centre and transparent surrounded margins were tested for urea hydrolysis and oxidase reaction. Urea-positive and oxidase-negative isolates were confirmed with API 20E kit (BioMérieux, Marcy l'Etoile, France). *Y.enterocolitica*-negative samples were cold enriched in agreement with NMKL method, Nr. 117 at 4 °C for 2 weeks with plating out on CIN agar at 8^{th} and after alkali treatment with potassium hydroxide at 15^{th} day of incubation with subsequent confirmation with API 20E.

Confirmed *Y.enterocolitica* isolates were tested for pyrazinamidase activity, indole production, tween-esterase reaction, fermentation of xylose, trehalose and salicin according to G. Wauters et al., 1987. Indole, tween-esterase, xylose, salicine and pyrazinamidase activity negative and trehalose positive isolates were investigated for slide agglutination with commercial *Y.enterocolitica* antisera O:3 (Sifin, Berlin, Germany).

The mean prevalence for each herd was calculated.

Results and Discussion

Y.enterocolitica 4/O:3 was isolated from all five investigated herd. Prevalence of *Y.enterocolitica* was varied from 20% in herd located in Ziemeļzemgale and Dienvidrietumu Latgale to 45% in Dienvidaustrumu Latgale (Table 1).

The mean prevalence – 26 (28)% for *Y.enterocolitica* 4/O:3 is close to previously reported on high prevalence of pathogenic *Y.enterocolitica* bioserotypes in slaughtered pigs, especially 4/O:3 in Europe (Table 2).

Almost all *Y.enterocolitica* isolates belong

Table 2

Reported incidence of *Y.enterocolitica* 4/O:3 in pig tonsils at slaughtering

Country	No. of samples	Incidence	Reference
Norway	24	15(63)	Nesbakken et al., 2003
	50	30(60)	Fredriksson-Ahomaa et al., 2000
Netherlands	86	33(38)	De Boer and Nouws, 1991
Italy	150	22(14.7)	Bonardi et al., 2003
	106	43(41)	De Guisti et al., 1995
Germany	210	60(28.8)	Nowak et al., 2006
	50	30(60)	Fredriksson-Ahomaa et al., 2001

to bioserogroup 4/O:3, with exception of herd located Ziemeļzemgale, where one indol, xylose, salicine, trehalose positive, Tween-esterase negative isolate was found. Pattern of biochemical reaction is typical to biotype 2, but slide agglutination reaction with antisera was not performed, so it was not possible to detect this isolate serotype.

Presence of biotype 2 indicates that other pathogenic bioserovars can be found in Latvia; however, initial reservoir of this pathogen is still unclear (Fredriksson-Ahomaa, 2006).

High prevalence of *Y.enterocolitica* indicates that pig tonsils can serve as the potential source of contamination of by-products and meat during evisceration and dressing in the abattoirs with following introduction of pathogen into retail market. Presence of *Y.enterocolitica* 4/O:3 is frequently reported on carcasses and offal at the slaughterhouse (Gurtler et al., 2005; Fredriksson-Ahomaa et al., 2001; Nesbakken et al., 2003). It is not possible to avoid completely from contamination with pathogen during routine slaughtering and hygienic procedures, but is more difficult to prevent distribution of *Y.enterocolitica* from tonsils in herds with high prevalence (Nesbakken et al., 2006). Further epidemiological studies on contamination pattern in abattoirs in Latvia are needed.

Previous observations indicate that *Y.enterocolitica* 4/O:3 can establish the long-term reservoir within pig herd (Nesbakken et al., 2006; Skjerve et al., 1998). An animal may become the carrier of pathogen within short-time contact with infected pigs from *Yersinia*-positive herd just before slaughtering: during transportation, staying in the waiting pens or at the ante-mortem inspection at the slaughterhouse, resulting in positive tonsils at slaughtering. In our study none of the herds was free from *Yersinia*. As samples were taken from pluck set on the slaughter line, it is not excluded that animals were carriers before slaughtering because pigs were held at the lairage in the same room where animals from other herds stayed.

Yersinia – negative and positive herds should be recognized by an official veterinarian in order to avoid contact between animals and provide adequate plant sanitation after slaughtering. Information about presence of pathogen should be delivered to the slaughterhouse with food chain according to European Community Regulation EC 854/2004. The elimination of *Y.enterocolitica* from the herd is long and cost-expensive process (Nesbakken et al., 2006), thus the improvement of slaughtering techniques and meat hygiene practices are essential.

Our study on occurrence of *Y.enterocolitica* should be continued with many Latvian herds involved to get more reliable information about epidemiological situation in pig population in Latvia. However, it is obvious that pathogenic *Y.enterocolitica* 4/O:3 is the most frequently isolated from pigs of Latvian pig at slaughtering.

Conclusions:

1. High prevalence of *Y.enterocolitica* 4/O:3 in pigs of Latvian origin at slaughtering indicate that further contamination form pig tonsils may occur during slaughtering and processing of the pig offal and carcasses in slaughterhouses;
2. Information about *Yersinia*- positive herds should be provided before the animal slaughtering as food chain information in accordance to EC Regulation 854/2004;
3. Investigation should be continued to get more reliable information about incidence of pathogenic *Y.enterocolitica* bioserovars in Latvian pigs.

References

1. Anonymous (2006) Zoonoses Report of Latvia 2004/2005. Ministry of Agriculture, Food and Veterinary Service, pp. 31-32.
2. Asplund K., Hakkinen M., Okkonen T., Vanhala P., Nurmi E. (1998) Effects of Growth-promoting Antimicrobials on Inhibition of *Yersinia enterocolitica* O:3 by Porcine Ileal Microflora. Journal of Applied Microbiology, 85, pp. 164-170.
3. Bonardi S., Brindani F., Pizzin G., Lucidi L., D'Incau M., Liebana E., Morabito S. (2003) Detection of *Salmonella* spp., *Yersinia enterocolitica* and Verocytotoxin-producing *Escherichia Coli* O157 in Pigs at Slaughter in Italy. International Journal of Food Microbiology, 85, pp. 101-110.
4. Bottone E.J. (1999) *Yersinia enterocolitica*: Overview and Epidemiological Correlates. Microbes and Infection, 1, pp. 323-333.
5. De Boer E., Nouws J.F.M. (1991) Slaughter Pigs and Pork as a Source of Human Pathogenic *Yersinia enterocolitica*. International Journal of Food Microbiology, 12, pp. 375-378.

6. De Boer E. (1995) Isolation of *Yersinia enterocolitica* from Foods. Contribution to Microbiology and Immunology, 13, pp. 71-73.
7. De Giusti M., de Vito E., Serra A., Quattrucci B., Boccia A., Pacifico L., Ranucci A., Ravagnan G., Chiesa C. (1995) Occurrence of Pathogenic *Yersinia enterocolitica* in Slaughtered Pigs and Pork Products. Contribution in Microbiology and Immunology, 13, pp. 126-129
8. Gurtler M., Alter T., Kasimir S., Linnebur M., Fehlhaber K. (2005) Prevalence of *Yersinia enterocolitica* in Fattening Pigs. Food Protocols, 68, pp. 850-854
9. International Organization of Standardization (2003) Microbiology of Food and Animal Feedings Stuffs- Horizontal Method for the Detection of Presumptive Pathogenic *Yersinia enterocolitica* (ISO 10273: 2003), pp. 1-15.
10. Fredriksson-Ahomaa M., Hielm S., Korkeala H. (1999) High Prevalence of yadA - positive *Yersinia enterocolitica* in Pig Tongues and Minced Meat at Retail Level in Finland. Journal of Food Protection, 62, pp. 123-127.
11. Fredriksson-Ahomaa M., Björkroth J., Hielm S., Korkeala H. (2000) Prevalence and Characterization of Pathogenic *Yersinia enterocolitica* in Pig Tonsils from Different Slaughterhouses. Food Microbiology, 17, pp. 93-101.
12. Fredriksson-Ahomaa M., Bucher M., Hank C., Stolle A., Korkaela H. (2001) High Prevalence of *Yersinia enterocolitica* 4:O3 on Pig Offal in Southern Germany: a Slaughtering Technique Problem. Systematic and Applied Microbiology, 24, pp. 457-463.
13. Fredriksson-Ahomaa M., Stolle A., Korkeala H. (2006) Molecular Epidemiology of *Yersinia enterocolitica* Infections. FEMS Immunology and Medical Microbiology, 47, pp. 315-329.
14. Kapperud G. (1991) *Yersinia enterocolitica* in Food Hygiene. International Journal of Food Microbiology, 12, pp. 53-66.
15. NCFA (Nordic Committee on Food Analysis) (1996) *Yersinia enterocolitica*. Detection in Food. Method NMKL No. 117., Nordic Committee on Food Analysis, Espo, Finland
16. Nesbakken T., Eckner K., Høidal H.K., Røtterud O.J. (2003) Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in Slaughter Pigs and Consequences for Meat Inspection, Slaughtering and Dressing Procedures. International Journal of Food Microbiology, 80, pp. 231-240.
17. Nesbakken T., Iversen T., Eckner K., Lium B. (2006) Testing of Pathogenic *Yersinia enterocolitica* in Pig Herds Based on the Natural Dynamic of Infection. International Journal of Food Microbiology, 111, pp. 99-104.
18. Nowak B., Mueffling T., Caspari K., Hartung J. (2006) Validation of a Method for the Detection of Virulent *Yersinia enterocolitica* and their Distribution in Slaughter Pigs from Conventional and Alternative Housing Systems. Veterinary Microbiology, 117, pp. 219-228.
19. Ostroff S.M., Kapperud G., Huteagner L.C., Nesbakken T., Bean N.H. (1994) Sources of Sporadic *Yersinia enterocolitica* Infection in Norway: a Prospective Case-control Study. Epidemiology and Infection, 112, pp. 133-141.
20. Schubert S., Bockemühl J., Brendler U., Heesemann J. (2003) First Isolation of Virulent *Yersinia enterocolitica* O:8, Biotype 1B in Germany. European Journal of Clinical Microbiology and Infection Diseases, 22, pp. 66-68.
21. Skjerve E., Lium B., Nielsen B., Nesbakken T. (1998) Control of *Yersinia enterocolitica* in Pigs at Herd Level. International Journal of Food Microbiology, 45, pp. 195-203.
22. Wauters E., Kandolo K, Janssens M. (1987) Revised biogrouping scheme of Yersinia enterocolitica. Contributions in Microbiology and Immunology, 9, pp. 14-21.