PREVALENCE OF *YERSINIA ENTEROCOLITICA* IN THE ENVIRONMENT OF SLAUGHTERHOUSE

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Abstract

A total amount of 64 surface swabs from slaughterhouse rooms and equipment, work tools and clothes were collected in three large scale slaughterhouses between January 2006 and January 2009 during pig slaughter. Samples were tested according to ISO 10273 standard requirements, with subsequent cold enrichment for three weeks in peptone mannitol bile salt broth. Isolated cultures were confirmed with API 20E, after that all *Y. enterocolitica* isolates were biotyped and serotyped. In general, the prevalence of *Y. enterocolitica* in the slaughterhouses was 37% (24/64), where 34% (22/64) and 3% (2/64) comprised *Y. enterocolitica* 1A and *Y. enterocolitica* 4/O:3, accordingly. *Y. enterocolitica* 1A was recovered in slaughterhouses A, B and C with the prevalence 42% (8/19), 34% (9/26) and 26% (5/19) of positive cases, while *Y. enterocolitica* 4/O:3 was observed only in slaughterhouse A with the prevalence 11% (2/19). *Y. enterocolitica* 1A was found on sink (4/4/100%), door (2/4/50%), meat inspection platform (2/4/50%), floor (5/12/42%), work surface (2/5/40%), table for work equipment (1/3/33%), box for cold storage of products (1/4/25%), apron (1/4/25%), gloves (1/4/25%), footwear (1/4/25%), hook (1/5/20%), box for offals (1/7/14%) samples. *Y. enterocolitica* 4/O:3 was found on work surface (1/5/20%) and floor samples (1/12/8%). No significant differences ($p>0.01$) were observed in the prevalence of *Y. enterocolitica* in environmental samples between slaughterhouses A, B and C. The presence of *Y. enterocolitica*, bioserotype 4/O:3 in environmental samples, indicated that environment of the slaughterhouse can be a cause of contamination of slaughter products with yersiniae, and greater efforts should be made to maintain hygiene in slaughterhouse on acceptable level.

Key words: non-pathogenic yersiniae, pathogenic yersiniae, hygiene, pig

Introduction

Yersiniosis is a foodborne infection, caused by pathogenic *Y. enterocolitica* bioserovars (Bottone, 1997). Pathogenic *Y. enterocolitica* bioserovar 4/O:3 has been frequently isolated from pork and pork products at retail level and in clinical cases in Europe (Fredriksson-Ahomaa et al., 2006). During the case control studies, pork was recognized as a most important source of pathogen, thus meat and meat products could be responsible for transmission of *Y. enterocolitica* 4/O:3 to consumers (Fredriksson-Ahomaa et al., 2006). The presence of non-pathogenic *Y. enterocolitica* biovar 1A was often reported on meats also, but its clinical significance is still discussible (Logue et al., 1996). *Y. enterocolitica* 4/O:3 is distributed in clinically healthy pigs and pathogen could be introduced in pork and the environment of slaughterhouse during the slaughter of *Y. enterocolitica* 4/O:3 positive animals (Nesbakken, 1988). Sites where *Y. enterocolitica* could be recovered are important to identify in the plant environment, because they represent possibilities for contamination of slaughter products (Sammarco et al., 1997). As presence of *Y. enterocolitica* 4/O:3 was detected in pig and in slaughter products in Latvia, possible that pathogen could spread to the slaughterhouse environment (Terentjeva, Bērziņš, 2010). The aim of present study was to detect the prevalence of *Y. enterocolitica* in the slaughterhouse environment.

Materials and Methods

A total amount of 64 environmental samples were collected in three slaughterhouses in Latvia between January 2006 and January 2009 during pig slaughter. The slaughter capacity of the selected slaughterhouses was 50 pigs per hours. The slaughtering process was similar in the selected plants, and consisted from stunning, bleeding, scalding, dehairing, polishing and evisceration steps.

Samples were collected from the following sampling sites: work surfaces (n=5), doors (n=4), tables for work equipment (n=3), floors (n=12), sinks (n=4), boxes for offal (n=7), meat

inspection platforms (n=4), boxes for cold storage of products (n=4), hooks (n=5), knives (n=4), gloves (n=4), aprons (n=4), footwear (n=4).
An area of approximately 20cm^2 of selected sampling site was swabbed with sterile gauze tampon (5cm X 5cm), moistured in 0.9 % of NaCl, 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 (Anonymous, 1996, Anonymous, 2003). Prior to testing, swabs in PMB and were left for one hour at 22 °C for resuscitation. For the direct plating, 10 µl of suspension were streaked on CIN Agar. For the selective enrichment, 0.1 ml 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 10 µl of suspension from ITC broth after incubation, and PMB fater 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 between the prevalence of *Y. enterocolitica* in slaughterhouses.

Results and Discussion

Y. enterocolitica was isolated from the slaughterhouse environment with the prevalence 37% (24/64) positive cases, where 34% (22/64) and 3% (2/64) comprised *Y. enterocolitica* 1A and *Y. enterocolitica* 4/O:3, respectively Table 1.

Table 1

Prevalence of *Y. enterocolitica* in the environment of slaughterhouse

Sampling site	No. of samples	No. of positive samples (%)	
		***Y. enterocolitica* 1A**	***Y. enterocolitica* 4/O:3**
Work surface	5	2 (40)	1 (25)
Door	4	2 (50)	0 (0)
Table for work equipment	3	1 (33)	0 (0)
Floor	12	5 (42)	1 (8)
Sink	4	4 (100)	0 (0)
Box for offals	7	1 (14)	0 (0)
Meat inspection platform	4	2 (50)	0 (0)
Box for cold storage of products	4	1 (25)	0 (0)

Continue of Table 1

Sampling site	No. of samples	No. of positive samples (%)	
		***Y. enterocolitica* 1A**	***Y. enterocolitica* 4/O:3**
Hook	5	1 (20)	0 (0)
Knife	4	0 (0)	0 (0)
Gloves	4	1 (25)	0 (0)
Apron	4	1 (25)	0 (0)
Footwear	4	1 (25)	0 (0)
Total	64	22 (34)	2 (3)

Table 1 shows that *Y. enterocolitica* 1A was found on work surfaces, doors, tables for work equipment, floor, sinks, boxes for offal, meat inspection platforms, boxes for cold storage of products, hooks, knives, gloves, aprons and footwear, while *Y. enterocolitica* 4/O:3 on work surface and floor samples. *Y. enterocolitica* 1A was not recovered from knives, but *Y. enterocolitica* 4/O:3 from doors, tables for work equipment, sinks, boxes for offal, meat inspection platforms, boxes for cold storage of products, hooks, knives, gloves, aprons and work footwear samples. *Y. enterocolitica* 1A was observed in slaughterhouse samples due to wide appearance of microorganism in nature. *Y. enterocolitica* 1A could enter the environment of slaughterhouse from outside sources (Harmon et al., 1984, Sammarco et al., 1997). The principal source of *Y. enterocolitica* 4/O:3 are pigs, and the environment of slaughterhouse could become contaminated with pathogen from pig faces and tonsils (Kapperud, 1991, Fredriksson-Ahomaa et al., 2000). The results on the presence of non-pathogenic *Y. enterocolitica* was in agreement with Sammarco et al., 1997, who found work surfaces and floor samples to be contaminated with bacteria, however, no yersiniae-positive samples were revealed in case slaughtering wall, hand wash basin, handles, hooks, knives and abattoir worker clothing were tested. The presence of *Y. enterocolitica* 4/O:3 in the environment of slaughterhouse was also in agreement with Nesbakken, 1988, who found pathogen on the floor of eviscerating area and viscera table. The highest prevalence of *Y. enterocolitica* 1A was found on sink, where 4/4 (100%) samples were positive, while the lowest on box for offal – 1/7 (14%) positive samples. In our mind, high prevalence on sink was observed due to it contamination with yersiniae from highly contaminated material, such as worker clothing and contaminated equipment. In contrast, Sammarco et al., 1997 reported, that the highest prevalence of non-pathogenic *Y. enterocolitica* was observed on slaughterhouse floor samples, where 3 out of 18 samples were positive (17%), but did not find contamination with *Y. enterocolitica* on hand-wash basin. *Y. enterocolitica* 4/O:3 were found in two samples, and the most probably pathogen was introduced on floor sample with blood from slaughtered animals, and on work surface due to direct contact with contaminated material (Nesbakken, 1988). *Y. enterocolitica* was isolated from the slaughterhouse environment samples in slaughterhouses A, B and C, and the prevalence is shown in Table 2.

Table 2

Prevalence of *Y. enterocolitica* in different slaughterhouses

Slaughterhouse	No. of samples	No. of positive samples (%)	
		***Y. enterocolitica* 1A**	***Y. enterocolitica* 4/O:3**
A	19	8 (42)*	2 (11)
B	26	9 (34)*	0 (0)
C	19	5 (26)*	0 (0)
Total	64	22 (34)	2 (3)

* differences in the prevalence of *Y. enterocolitica* 1A between slaughterhouses A, B and C were not significant ($p>0.01$).

The highest prevalence of *Y. enterocolitica* 1A was found in slaughterhouse A, while the lowest- in slaughterhouse C, however, without significant differences (p>0.01). Our findings are similar to Sammarco et al., 1997, who found presence of non-pathogenic *Y. enterocolitica* in the environment of two out of 11 slaughterhouses, but without statistical differences. *Y. enterocolitica* 4/O:3 was detected only in slaughterhouse A. Prevalence of *Y. enterocolitica* 1A in the environment of three slaughterhouses is shown in table 3.

Table 3

Prevalence of *Y. enterocolitica* 1A in the environment of slaughterhouse

Sampling site	No. of samples / No. of positive samples (%)		
	Slaughterhouse		
	A	B	C
Work surface	3/ 1 (33)	2/ 1 (50)	0/ 0 (0)
Doors	1/ 0 (0)	2/ 2 (100)	1/ 0 (0)
Table for work equipment	1/ 0 (0)	1/ 1 (100)	1/ 0 (0)
Floor	2/ 1 (50)	7/ 2 (29)	3/ 2 (66)
Sink	2/ 2 (100)	1/ 1 (100)	1/ 1 (100)
Box for offals	1/ 0 (0)	3/ 0 (0)	3/ 1 (33)
Meat inspection platform	0/ 0 (0)	2/ 1 (50)	2/ 1 (50)
Box for cold storage of products	1/ 1 (100)	2/ 0 (0)	1/ 0 (0)
Hoof	1/ 1 (100)	2/ 0 (0)	2/ 0 (0)
Knife	2/ 0 (0)	1/ 0 (0)	1/ 0 (0)
Gloves	1/ 1 (100)	2/ 0 (0)	1/ 0 (0)
Apron	2/ 0 (0)	1/ 1 (100)	1/ 0 (0)
Work footwear	2/ 1 (50)	1/ 0 (0)	1/ 0 (0)
Total	19/ 8 (42)	26/ 9 (34)	19/ 5 (26)

Table 3 shows that variations in the prevalence of non-pathogenic *Y. enterocolitica* exist between slaughterhouses. In our mind, these variations in the prevalence of *Y. enterocolitica* between certain sampling sites as work surfaces, doors, table for work equipment, floor, box for offal and meat inspection platform was observed due to the differences between plants in their structural characteristics, the slaughtering practices and the sanitation practices (Sammarco et al., 1997).

Conclusions

1. The presence of *Y.enterocolitica*, especially of pathogenic biosetype 4/O:3 in environmental samples, indicated that the environment of slaughterhouse can be a cause of contamination of slaughter products with yersiniae, and greater efforts should be made to maintain hygiene in slaughterhouse on acceptable level.
2. Our study revealed sites in the environment of the slaughterhouse where contamination with yersiniae occurs more often, therefore cleaning and sanitation procedures should be performed more carefully.

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