Frequency of *Yersinia enterocolitica* O3 and *Listeria monocytogenes* on polished pig carcasses.

by

Mats Rasmussen, Henning Luthje and Anette Granly Larsen
Danish Meat Research Institute, Maglegårdsvej 2, DK-4000 Roskilde

Background

Yersinia enterocolitica O3 and *Listeria monocytogenes* are pathogenic bacteria that have been isolated from pig faeces at varying frequencies (Nesbakken et al., 1994 and Skovgaard and Nørrung, 1989). Andersen (1988) isolated *Yersinia enterocolitica* O3 from 24.7% of 1458 pigs. Skovgaard and Nørrung isolated *Listeria monocytogenes* from three out of 172 samples (1.7%) of pig faeces indicating a low frequency compared to that of *Yersinia enterocolitica* O3. It is well established that the pig tonsils also constitute a major and important reservoir for *Yersinia enterocolitica* O3 (Christensen, 1980; Szita et al. 1980; Andersen 1984).

In Denmark the rectum is enclosed in a plastic bag thus preventing contamination of the carcass with faeces from the rectum during rectum loosening (Sørensen and Kirk 1989). However, the rectum is not enclosed until the carcass enters the dressing floor, leaving the possibility of contamination of the carcass with *Yersinia enterocolitica* and *Listeria monocytogenes* during the operations prior to rectum loosening. When a carcass leaves the singeing kiln most of the bacteria on the surface have been destroyed. However, the skin is recontaminated with bacteria in the polishing process (Rahkio and Korkeala 1992). Some of these bacteria might be pathogenic.

Objectives

The aim of the study was to establish to which extent *Listeria monocytogenes* and *Yersinia enterocolitica* O3 are present on the carcasses after singeing and polishing.

Materials and Methods

Carcasses from three commercial slaughter plants each of which processes between 350 and 425 pig carcasses h^{-1} were examined. The carcasses are scalded, dehaired, singed, scraped and polished before they enter the dressing floor. Each slaughter plant was visited twice with an interval of one month between visits. Prior to the experiment the plants' singeing kilns were examined with regard to singeing time and temperature. Plant A and B were using high temperature (> 1000°C) singeing and plant C low temperature (<1000°C) singeing. Singeing time and temperature were recorded from the electronic panel in the monitoring room.

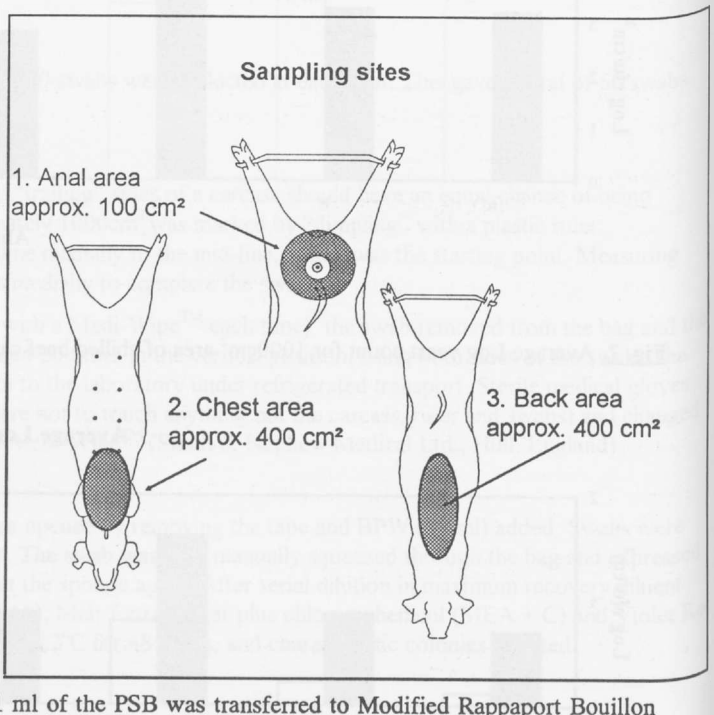
Sampling of carcasses.

Approximately every 15th carcass entering the dressing floor was chosen for sampling. Samples were taken from 100 carcasses. Each carcass was sampled in three locations: one sample from the area around anus, one sample from the chest area and one sample from the skin on the back of the carcass giving a total of 300 samples/slaughter plant/visit. Samples were obtained by swabbing an undelineated area of the carcass (figure 1) with a moist (0.9 % NaCl, 0.1% peptone) sterile gauze swab (10 x 10 cm, 16 ply, Smith and Nephew, France). Each swab was individually placed in a separate stomacher bag. From each sampling site 50 samples/slaughterhouse/visit were analysed for *Listeria monocytogenes* and 50 samples/slaughterhouse/visit for *Yersinia enterocolitica O3*.

Preparation of samples

Listeria monocytogenes

Examination for *Listeria monocytogenes* was carried out using a modified version of the Nordic Committee on Food Analysis (1990) method. Swabs to be analysed for *Listeria monocytogenes* were placed in 100 ml Listeria enrichment broth (Oxoid) and incubated at 30°C for 24h. 0.1 ml from each primary enrichment was transferred to 10 ml Listeria enrichment broth supplemented with Listeria secondary selective enrichment supplement (Oxoid) and incubated at 30°C for 24h. Loops of Listeria secondary enrichment broth were streaked out on Oxford-agar (Oxoid) and incubated at 30°C. After 24h and 48h plates were examined and colonies surrounded by a blackened zone were presumed to be Listeria. At least ten presumptive Listeria colonies were transferred onto blood agar (Merck) and incubated for 24-28h at 37°C. Colonies surrounded by a narrow clear zone of β-haemolysis were subjected to a confirmative test using Accuprobe (Geneprobe; San Diego, USA) or the API Listeria (Bio Merieux, France).



Yersinia enterocolitica O3

Examination for *Yersinia enterocolitica O3* was carried out using a modified version of the Nordic Committee on Food Analysis (1987) method. Swabs to be analysed for *Yersinia enterocolitica O3* were placed in 100 ml phosphate sorbitol buffer (PSB broth) and incubated at 22-25°C for 3h. The PSB broth was then incubated at 4°C for eight days (cold enrichment). After 4 days 0.1 ml of the PSB was transferred to Modified Rappaport Bouillon (MRB) and incubated at 22-25°C for four days. A loop of MRB was streaked onto Cefulodin-Irgasan-Novobiocin-agar (CIN) from Oxoid and incubated at 20°C for 20-22h. The PSB was also incubated further up to 21 days at 4°C after which 0.02 ml was inoculated onto CIN-agar and incubated at 20°C for 20-22h. Colonies with a clearly demarcated, deep red centre ("bull's eye") surrounded by an outer transparent zone were presumed to be Yersinia. At least four presumptive colonies from each of the CIN-agar plates were transferred to Bromothymol blue-saccharose agar (BS-agar) and incubated at 30°C for 24h. Saccharose-positive (yellow) colonies were agglutinated on a slide with specific *Yersinia enterocolitica* antiserum O3.

Results

Only one of the total 900 samples that were taken was found positive for *Listeria monocytogenes*. The sample was taken from the chest area in slaughter plant A. Of the total 900 samples examined for *Yersinia enterocolitica* O3, 19 were found to be positive.

The predominant sampling site for *Yersinia enterocolitica* O3 positive samples was the area around anus. Here 15 (5%) out of 300 samples were positive whereas the back area only yielded 4 (1.3%) positive samples out of 300. No positive samples were found from the chest area.

Singeing temperature and time

Plant A used a time/temperature combination of 7 seconds at 1100°C, whereas plant B used 10 seconds/1100°C and plant C 10 seconds/800°C. Plant A used a considerably smaller amount of water in the polishing machine than plant B and C.

Table 1. *Listeria monocytogenes (L.m.)* and *Yersinia enterocolitica* O3 (*Y.e.*) recovered from swabs from 300 carcasses (n=1800) at 3 pig slaughtering plants.

No. of samples positive for *Listeria monocytogenes* (*L.m.*) and *Yersinia enterocolitica* O3 (*Y.e.*)							
		Anal area		Chest area		Back area	
		L.m.	Y.e.	L.m.	Y.e.	L.m.	Y.e.
Plant A	1st visit	0	0	1	0	0	0
	2nd visit	0	2	0	0	0	0
Plant B	1st visit	0	1	0	0	0	0
	2nd visit	0	2	0	0	0	0
Plant C	1st visit	0	4	0	0	0	3
	2nd visit	0	6	0	0	0	1

Discussion

Although *Yersinia enterocolitica* O3 and *Listeria monocytogenes* have been demonstrated to be present in faeces from pigs at a varying frequency (Andersen, 1988 and Shiozawa et al., 1991) they are only present on the pork carcass prior to dressing at very low frequencies. This indicate that although the carcass is recontaminated in the polishing process the major part of this recontamination does not consist of *Listeria monocytogenes* or *Yersinia enterocolitica* O3. Furthermore the findings indicate that the contamination of carcasses with *Yersinia enterocolitica* O3 and *Listeria monocytogenes* takes place on the dressing floor and not in the polishing operation. The lower frequency of *Yersinia enterocolitica O3* and *Listeria monocytogenes* in plant A and B might be due to the higher singeing temperature compared to plant C.

It is important to choose sampling sites that are likely to be contaminated with *Yersinia enterocolitica* O3 or *Listeria monocytogenes*. Since faeces/intestinal contents and fluids from the mouth region are most likely to harbour *Yersinia enterocolitica* O3 or *Listeria monocytogenes*, the region around the rectum and the chest region were chosen. If *Yersinia enterocolitica* O3 or *Listeria monocytogenes* are transferred to the carcass during polishing, the bacteria are likely to be found here. The region on the back was chosen because occurrence of the two bacteria is less likely here. In this study the anal region was found to be the most frequent source of *Yersinia enterocolitica* O3, although all frequencies were very low.

Conclusion

The findings of this study show that pig carcasses that are singed and polished as normally done in large Danish slaughterhouses only very rarely are contaminated with *Yersinia enterocolitica* O3 and *Listeria monocytogenes*.

References

Andersen, J.K. (1984). Humanpatogene Yersinia enterocolitica i danske svinebesætninger. Ph.D. thesis, Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Copenhagen Denmark.

Andersen, J.K. (1988). Contamination of freshly slaughtered pig carcasses with human pathogenic *Yersinia enterocolitica*. Int. J. Food Microbiol. 7, 193–202.

Christensen, S.G. (1980). Yersinia enterocolitica in Danish pigs. J. Appl. Bacteriol. 48, 377–382.

Nesbakken T., Nerbrink, E., Røtterud, O–J. and Borch E. (1994). Reduction of *Yersinia enterocolitica* and *Listeria* spp. on pig carcasses by enclosure of the rectum during slaughter. Int. J. Food Microbiol. 23. 197–208.

Nordic Committee on Food Analysis (1987). *Yersinia enterocolitica*. Detection in food. Method No. 117, 2nd edition. Nordic Committee on Food Analysis, Esbo, Finland.

Nordic Committee on Food Analysis (1990). *Listeria* spp. Detection in food. Method No. 136. Nordic Committee on Food Analysis, Esbo, Finland.

Rahiko, M. and Korkeala, H. (1992). Effect of pre–scalding brudhing on contamination level of pork carcasses during the slaughtering process. Meat Sci. 32, 173–183.

Shiozawa, K., Nishina, T., Miwa, Y., Mori, T., Akahane, S. and Ito, K. (1991). Colonization in the tonsils of swine by *Yersinia enterocolitica*. Contrib. Microbiol. Immunol. 12, 63–67.

Skovgaard N. and Nørrung B. (1989). The incidence of *Listeria* spp. in faeces of Danish pigs and in minced pork meat. Int. J. Food Microbiol. 8, 59–63.

Sørensen, R. and Andersen, J.K. (1989). Yersinia contamination on pig carcasses in relation to slaughter technique. ICoMST 1989, Proceedings Vol. II, 371–375.