CONTAMINATION ROUTES OF PATHOGENIC *Yersinia* spp. IN PIG SLAUGHTER AND CUTTING

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Background.

Yersinia enterocolitica causes gastro-enteritis in humans, with symptoms such as self-limiting diarrhoea, mild fever, abdominal pain and sometimes arthritis or skin complaints *(Schiemann, 1989)*. About 10 cases of yersiniosis per 100,000 inhabitants are reported annually in several countries, i.e. Belgium, Denmark, the Netherlands, Norway and Sweden. Only *Salmonella* spp. and *Campylobacter* spp. cause more, mandatory, reported cases of human foodborne disease in the Nordic countries *(Borch et al., 1996)*. The pig is considered to constitute the most important source of pathogenic *Y. enterocolitica* serotypes O:3 and O:9 *(Schiemann, 1989; Ostroff et al., 1994)*. *Y. enterocolitica* O:3 is often found in the intestinal contents and faeces of pigs, and spreads to the carcass during the slaughter and dressing operations *(Borch et al., 1996)*.

Objective.

To evaluate the contamination routes of pathogenic/non-pathogenic *Yersinia* spp. during pig slaughter and cutting.

Material and Methods.

Samples from pig faeces, pig carcasses, pork and the environment in a slaughterhouse were analysed for the presence of pathogenic and non-pathogenic *Yersinia* spp., using a multiplex Polymerase Chain Reaction (PCR)-method according to *Lantz et al., 1998*. Prior to PCR, swab samples were enriched in Yersinia Selective Enrichment medium (YSE, Merck) at 25°C for 18 hours, and subsequently centrifuged in Percoll (Pharmacia Biotech, Sweden) in order to remove PCR inhibitory factors. Two pairs of primers were used for the recognition of the plasmid associated virulence gene *yadA* and a part of the 16S rRNA gene specific to *Yersinia* spp. (*Yersinia enterocolitica*, *Yersinia intermedia, Yersinia kristensenii* and *Yersinia pseudotuberculosis*). Only *Y. pseudotuberculosis* and *Y. enterocolitica* are associated with the virulence plasmid. The detection of only the 16S rRNA fragment indicated the presence of non-pathogenic *Yersinia* spp. The occurrence of both the *yadA* gene and the 16S rRNA fragment indicated the presence of pathogenic *Yersinia* spp., or pathogenic plus non-pathogenic *Yersinia* spp.

Samples were taken at one slaughterhouse, upon two visits separated by one week. The slaughter rate was 360 per hour. Moistured cotton swabs were used for sampling. The carcasses were sampled at three positions (Figure 1). An area of 10 cm x 15 cm at the medial hind leg on the carcass, the total exposed meat surface of neck area on the carcass and the pelvic/ham incision were swabbed. Samples were taken at the end of the slaughter line, and after chilling the carcasses (rapid chiller: -12°C, 60-70 minutes; 1st cold storage room: 0-2°C, 2-3 hours; 2nd cold storage room: 4°C, 24 hours). The cut meat (inner flank and pork collar; corresponding to the areas sampled at the carcass) was swabbed directly after cutting, and after subsequent aerobic storage at 8°C for 7 days. On each occasion, 60 carcasses or pieces of meat were analysed. The sampling area was, in general, 10 cm x 10 cm for the environmental samples. In total, 950 samples were analysed, of which 230 were from the environment.

Results and Discussion.

The pigs harboured pathogenic *Yersinia* spp. (*Y. enterocolitica* and/or *Y. pseudotuberculosis*) in their faeces (28% positive samples, n=60) and tonsils (15% positive, n=60). A similar prevalence of *Y. enterocolitica* O:3 in faeces is reported from Denmark *(Andersen, 1988)* and Japan *(Shiozawa et al., 1991)*. Non-pathogenic *Yersinia* spp. (*Y. enterocolitica*, *Y. intermedia,* and/or *Y. kristensenii*) were found in 3% of faecal samples (n=60) but in none of the tonsil samples (n=60).

At the end of the slaughter-line, before chilling (Table 1), no *Yersinia* spp. was recovered from the neck or from the medial hind leg. However, pathogenic *Yersinia* spp. were recovered from the pelvic duct/ham incision (25% positive, n=60). The occurrence of non-pathogenic *Yersinia* spp. was low at this sampling position (2%, n=60). At the time of investigating, there was a problem with rupturing of the rectum when using the bung cutter, which is directly reflected in the contamination of the pelvic duct. The slaughtering technique is decisive in spreading the bacterium to the carcass *(Nesbakken et al., 1994; Borch et al., 1996)*.

After chilling for 24 hours, pathogenic *Yersinia* spp. were found on 2% of the carcasses (n=60; sampling position: pelvic duct/ham incision (Table 1). This was considerably lower than the 25% positive carcasses found before the chilling operation. The chilling of pork carcasses is reported to kill *Campylobacter* spp. *(Oosterom, et al., 1985)*. No information has been found in the literature on the tolerance of *Yersinia* spp. to chilling alone, or in combination with drying. The hypothesis that pathogenic *Yersinia* spp. are killed during chilling should be further evaluated.

Pathogenic *Yersinia* spp. were found on 3% of the inner flanks (Table 1). In comparison, on the chilled carcasses, a similar level (2%) of contamination was found at the corresponding sampling.

The occurrence of non-pathogenic *Yersinia* spp. increased during cold storage of the inner flank (Table 1). No increase was found for pathogenic *Yersinia* spp.

No *Yersinia* spp. were detected on the polishing equipment (n=20), i.e. before evisceration. Pathogenic *Yersinia* spp. were retrieved from bung cutters, trimming knives and the carcass splitter/saw, i.e. from equipment directly in contact with the carcass. Only non-pathogenic *Yersinia* spp. were found in samples taken from the chill rooms (17 positive out of 50 samples) such as the floor, drains, pole, crate wheel, and on one cutting board.

The differences observed in the distribution between pathogenic and non-pathogenic samples may have several explanations. (i) The non-pathogenic strains could outgrow the pathogenic ones in the slaughterhouse environment and on the cold stored pork. *Goverde (1994)* showed that *Y. enterocolitica* harbouring the virulence plasmid have a lower growth rate at chill temperatures than strains without the plasmid. (ii) The number of plasmid copies decreases below the detection limit of PCR when a strain is exposed to a hostile environment outside the pig intestine. (iii) The enrichment medium used prior to PCR may favour non-pathogenic strains.

Conclusions.

Pathogenic *Yersinia* spp. were detected on the pig carcasses, and on equipment in direct contact with the carcasses.

Non-pathogenic *Yersinia* spp. were detected in the environment in the chilling rooms (floor, drains, pole, crate wheels) and on the cutting board.

The occurrence of pathogenic *Yersinia* spp. decreased during the chilling of the pig carcasses.

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Table 1. Occurrence of *Yersinia* spp. on carcasses and cut pork at different sampling positions along the slaughter line and further processing, *n*=60 at each sampling site.

Sampling site	Occurrence of *Yersinia* spp. (%)							
	Before chilling		After chilling		Unstored		Cold stored	
	Non-pathogenic[1]	Pathogenic[2]	Non-pathogenic	Pathogenic	Non-pathogenic	Pathogenic	Non-pathogenic	Pathogenic
Pelvic duct/ham incision	2	25	0	2	NA[3)]	NA	NA	NA
Outer ham	0	0	0	0	NA	NA	NA	NA
Neck	0	0	0	2	NA	NA	NA	NA
Inner flank	NA	NA	NA	NA	3	3	42	0
Pork collar	NA	NA	NA	NA	0	0	2	2

[1] Detection of only the 16S rRNA fragment indicated the presence of non-pathogenic *Yersinia* spp.

[2] Detction of both the *yadA* gene and the 16S rRNA fragment indicated the presence of pathogenic *Yersinia* spp., or pathogenic plus non-pathogenic *Yersinia* spp.

[3] NA, not analysed.

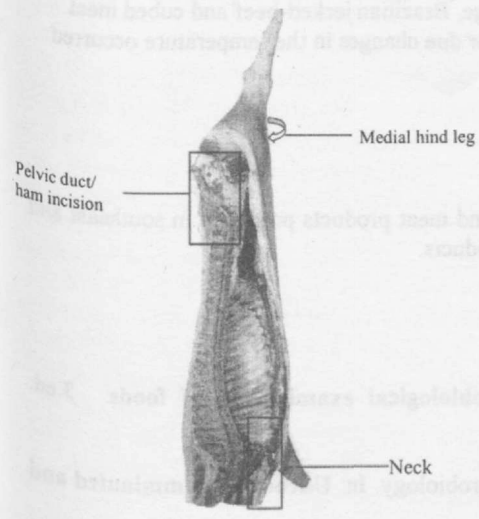

Figure 1. Pig carcass sampling sites: medial hind leg; pelvic duct/ham incision; neck.