

Occurrence of human enteropathogenic *Yersinia* spp. in Belgian pigs and contamination of pork carcasses during slaughter

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Abstract

Human pathogenic *Yersinia enterocolitica* and *Y. pseudotuberculosis* typically cause enteric infections in humans, mainly young children. Pigs are the main animal reservoir for pathogenic *Y. enterocolitica* and infection in humans is often acquired by the consumption of contaminated pork. The aim of this work was to determine the contamination of pig carcasses with enteropathogenic *Yersinia* spp. in Belgium. Therefore, 180 pig carcasses were sampled in 9 different slaughterhouses. From each animal, tonsils, rectal content and carcass swabs were analysed for enteropathogenic *Yersinia* spp. using direct plating, selective enrichment and cold enrichment. All samples were taken after evisceration, but before chilling. Pathogenic *Y. enterocolitica* were isolated from the tonsils of 103 pigs (57.2%) and rectal contents of 36 pigs (20.0%). Twenty-eight pigs were positive for pathogenic *Y. enterocolitica* in both tonsils and rectal content, while 75 and 8 pigs were only *Y. enterocolitica* positive in tonsils and rectal content, respectively. All isolated *Y. enterocolitica* strains belonged to bioserotype 4/O:3. Tonsils and rectal content from 4 and 1 pig(s) were positive for *Y. pseudotuberculosis*, respectively. Regarding carcass samples, 76 (42.2%) pig carcasses were contaminated with enteropathogenic *Yersinia* spp. Pathogenic *Y. enterocolitica* were mostly recovered from the mandibular region (59/180), followed by the sternal region (31/180), medial site just before the sacrum (17/180), and pelvic duct (15/180). In conclusion, a high proportion of pigs carry pathogenic *Yersinia* spp. in their tonsils or intestines during slaughter. Moreover, a considerable number of pig carcasses is positive on one or more of the sampled carcass sites.

Introduction

Y. enterocolitica and *Y. pseudotuberculosis* are foodborne pathogens which generally cause enteric infections in humans (yersiniosis). Infections occur mainly in young children and usually manifest as acute gastroenteritis (Bottone 1997). Most yersiniosis cases are caused by *Y. enterocolitica* bioserotype 4/O:3 and 2/O:9. Pigs are regarded as the principal source of pathogenic *Y. enterocolitica* in the human food chain as they are the only food producing animals that regularly harbor these pathogenic types (Bucher et al. 2008; Fredriksson-Ahomaa et al. 2001). *Y. pseudotuberculosis* is also recovered from tonsils and intestines of healthy pigs, though to a lesser extent than pathogenic *Y. enterocolitica* (Laukkanen et al. 2008). The aim of this study was to determine the prevalence of enteropathogenic *Yersinia* spp. in tonsils and rectal content of Belgian pigs at slaughter. Moreover, carcass samples are taken to obtain qualitative and quantitative data on the contamination of pig carcasses with enteropathogenic *Yersinia* spp. during normal slaughter activities.

Material and Methods

Tonsils, rectal content and carcass swabs from 180 pigs were collected during 18 sampling visits in 9 different pig slaughterhouses (each slaughterhouse was visited twice). The annual number of slaughtered fattening pigs in these slaughterhouses varied from about 135,000 to 1,250,000. Each sampling visit, 10 animals were sampled (one every 15 minutes), starting from the beginning of slaughter activities. All samples were taken after evisceration, but before chilling. Carcasses were swabbed using cellulose sponges after splitting of the carcass. From each carcass, the following areas were swabbed: (1) pelvic duct, (2) split surface just before the sacrum, (3) sternal region (breast cut and surrounding skin), and (4) mandibular region (jowl). For the latter region, only the medial side of the mandibula was swabbed when the head was split, including submaxillary lymph nodes, but avoiding the area of the tonsils. When the head was intact and the skin (partly) removed, masseter muscles were also swabbed.

All samples were homogenized in peptone-mannitol-bile salts broth (PMB) and analyzed using (i) direct plating, (ii) selective enrichment and (iii) cold enrichment. (i) For direct plating, 500 µl of PMB homogenate was spread plated onto

a CIN plate, in duplicate. For tonsils and intestinal content, an additional CIN agar plate was inoculated with approximately 100 µl PMB homogenate using a spiral plater. (ii) For selective enrichment, 10 and 5 ml of PMB homogenate was transferred into 90 and 45 ml of irgasan-ticarcillin-potassium chlorate (ITC) broth for tonsils and rectal contents, and carcass samples, respectively. After 2 days enrichment at 25°C, a loopful was streaked onto CIN plates. Additionally, 100 µl was streaked onto another CIN agar plate after KOH treatment. (iii) For cold enrichment, the remaining PMB homogenate was incubated at 4°C for 7 and 14 days. After 7 days, the enriched culture was streaked onto a CIN agar plate. After 14 days enrichment, 100 µl was streaked onto a CIN agar plate after KOH treatment. All agar plates were incubated at 30°C for 24h and examined for *Yersinia* colonies using a stereo microscope with Henry illumination. Suspected colonies were streaked on a general nutrient agar and after 24h at 30°C transferred into urea broth, Kligler Iron Agar (KIA) and Tryptone Soy Broth (TSB).

The pathogenicity of *Y. enterocolitica* isolates was confirmed using a multiplex PCR with primers targeting the chromosomal virulence genes *ail* and *yst* and the plasmid virulence gene *virF* according to Harnett et al. (1996). Moreover, *Y. enterocolitica* serotype O:3 and O:9 were identified using primers targeting the *rfbC* and *per* gene, respectively (Jacobsen et al. 2005; Weynants et al. 1996). *Y. pseudotuberculosis* isolates were identified using a single PCR assay targeting the *inv*-gene according to Nakajima et al. (1992).

Results

Pathogenic *Y. enterocolitica* were isolated from the tonsils of 103 pigs (57.2%) and rectal content samples of 36 pigs (20.0%) (Table 1). Twenty-eight pigs were positive for pathogenic *Y. enterocolitica* in both tonsils and rectal content, while 75 and 8 pigs were only *Y. enterocolitica* positive in tonsils and rectal content, respectively. All isolated *Y. enterocolitica* strains belonged to bioserotype 4/O:3. Tonsil samples with countable numbers ($n=67$), were contaminated with a mean of $3.82 \pm 1.21 \log_{10}$ CFU/g and a maximum of $5.73 \log_{10}$ CFU/g tonsillar tissue. Rectal content samples that were positive by direct plating ($n=13$) were contaminated with a mean of $2.91 \pm 1.44 \log_{10}$ CFU/g and a maximum of $6.11 \log_{10}$ CFU/g.

Y. pseudotuberculosis was isolated from the tonsils of four pigs (2.2%). Tonsils from one pig were simultaneously infected with *Y. enterocolitica* and *Y. pseudotuberculosis* at concentrations of 4.20 and 4.43 \log_{10} CFU/g tonsillar tissue, respectively. From one pig, *Y. pseudotuberculosis* was isolated from the rectal content, while solely pathogenic *Y. enterocolitica* were isolated from its tonsils.

Regarding carcass samples, 76 pig carcasses (42.2%) were contaminated with enteropathogenic *Yersinia* spp. Thirteen out of 76 positive pig carcasses (17.1%) did not carry pathogenic *Yersinia* spp. in the tonsils or rectal content. From two animals, the carcass was positive for *Y. enterocolitica*, while only *Y. pseudotuberculosis* was isolated from the tonsils. Moreover, in 51 animals, enteropathogenic *Yersinia* spp. were recovered from tonsils and/or rectal content while no pathogenic *Yersinia* spp. were recovered from the carcass surface. In total, 53 pigs were negative for enteropathogenic *Yersinia* spp. in the tonsils, rectal content or any of the sampled carcass sites.

Pathogenic *Y. enterocolitica* were mostly recovered from the mandibular region (59/180), followed by the sternal region (31/180), split surface (17/180), and pelvic duct (15/180). The mandibular region from one pig was simultaneously contaminated with pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*.

Table 1. Isolations of pathogenic *Y. enterocolitica* using different isolation methods from tonsils, rectal content and carcass swabs (n=180).

| <i>Isolation method</i> | <i>Direct</i> | <i>Selective enrichment</i> | | <i>Cold enrichment</i> | | <i>Total (%)</i> |
|--------------------------|---------------|-----------------------------|-----------------|------------------------|-----------------------|------------------|
| | CIN | ITC + CIN | ITC + KOH + CIN | PMB + CIN (7d) | PMB + KOH + CIN (14d) | |
| Tonsils | 78 | 70 | 79 | 79 | 99 | 103 (57.2%) |
| Rectal content | 13 | 15 | 17 | 19 | 31 | 36 (20.0%) |
| Carcass swabs | | | | | | |
| <i>Pelvic duct</i> | 6 | 4 | 4 | 6 | 11 | 15 (8.3%) |
| <i>Split surface</i> | 3 | 5 | 6 | 2 | 14 | 17 (9.4%) |
| <i>Sternal region</i> | 1 | 3 | 10 | 2 | 26 | 31 (17.2%) |
| <i>Mandibular region</i> | 17 | 10 | 17 | 9 | 56 | 59 (32.8%) |

Most *Y. enterocolitica* and *Y. pseudotuberculosis* isolates were recovered after 14 days cold enrichment. For tonsil samples, direct plating detected 76% of positive tonsil samples. The sensitivities of direct plating for all other sample types (rectal content and different carcass swabs) varied from only 3 to 40%.

Discussion

A high proportion of pigs at slaughter carry enteropathogenic *Yersinia* spp. in tonsils and/or intestines, resulting in a high number of contaminated carcasses. Enteropathogenic *Yersinia* spp. are also isolated from several carcasses that are negative in either tonsils or rectal content, suggesting cross-contamination of carcasses during slaughter.

Contamination of the pelvic duct is most likely to occur during removal of the intestinal tract either directly from intestinal content or cross-contamination from a contaminated knife or bung cutter. Sealing of the rectum with a bag reduces carcass contamination (Laukkanen et al. 2010; Nesbakken et al. 1994); however, it is not a common practice in Belgium as none of the slaughterhouses applied this procedure. Moreover, in 8 pigs, the pelvic duct was positive while there were no pathogenic *Y. enterocolitica* isolated from the rectal content, which indicates cross-contamination.

The split surface of the carcass probably gets contaminated during splitting of the carcass. In this study, the head was split in 49% of the sampled carcasses. When splitting the head, the splitting machine inevitably also cuts the tonsils and can become contaminated, leading to the transfer of the pathogen to the split surface of the next carcass. However, during sampling it was observed that the splitting machine also makes contact with the tonsils if the head is not split. Eleven carcasses were positive at the split surface when the head was split (n=89). Considering carcasses from which the head was not split (n=91), 6 carcasses were positive at this site. In one slaughterhouse, tonsils were systematically cut out with the plug set and an incision was made in the neck to avoid contact of the splitting machine with the head. In this slaughterhouse, none of the 20 sampled pigs was positive at the split surface.

In 17% of the carcasses, pathogenic *Y. enterocolitica* were isolated from the sternal region. Four carcasses that were positive at this location were negative in both tonsils and rectum. The sternal region may get contaminated during opening of the thoracic cavity, evisceration, and removal of the plug set or following manipulation of the carcass.

The highest contamination was found in the mandibular region. This contamination might originate from the pig itself (such as tonsils, oral cavity or tongue) or may also be attributed to cross-contamination. Namely, in 12 animals, the mandibular region was contaminated with pathogenic *Y. enterocolitica* while the tonsils were negative. For instance, Nesbakken et al. (2003) indicated that incision of the submaxillary lymph nodes during veterinary inspection represents a risk for cross-contamination with enteropathogenic *Yersinia*.

Conclusion

A high proportion of pig carcasses are contaminated during slaughter. Taking into account that these pathogens are able to multiply at refrigerated temperatures, a considerable part of pork carcasses represent a potential risk for public health.

Therefore, further research is needed to elucidate the predominant factors related to the contamination of carcasses with enteropathogenic *Yersinia* spp. during slaughter.

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