High bacterial contamination of pig tonsils at slaughter

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Food-borne zoonoses have a major health impact in industrial countries. Campylobacter spp., Salmonella enterica, Yersinia enterocolitica and Listeria monocytogenes are high-risk food-borne zoonotic hazards in finishing pigs. The objectives of this work were (1) to study the isolation rate of pathogenic Y. enterocolitica, Salmonella spp., Campylobacter spp. and L. monocytogenes in the tonsils and feces and (2) to determine the number of mesophilic aerobic bacteria (MAB) and Escherichia coli in the tonsils of fattening pigs at slaughter. The samples, which were collected from one slaughterhouse on five occasions, originated from 50 pigs and 15 farms. The number of MAB varied from 6.40 to 7.82 log10 CFU/g and E. coli from 4.38 to 6.53 log10 CFU/g. Additionally, 31 (62%) of the tonsils were colonized with Y. enterocolitica and 16 (32%) with L. monocytogenes. Campylobacter spp. were more frequently excreted in feces and only 3 (6%) of the pigs carried Campylobacter spp. in the tonsils. No Salmonella spp. were isolated. The pig tonsils were shown to be colonized with a high number of bacteria including E. coli, which is the most important indicator for fecal contamination, and with Y. enterocolitica and L. monocytogenes, which are important food-borne pathogens. This study demonstrates that the tonsils are highly contaminated with micro-organisms and can be a very important source of contamination in the slaughterhouse.

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1. Introduction

Food-borne zoonoses are infectious diseases of major health and economic significance in developed countries. They are most often acquired through ingestion of contaminated foods but they can also be acquired directly from animals. The two most frequently reported zoonotic diseases in humans in the EU in 2007 were Campylobacter and Salmonella infections with incidences of 120 and 31 cases per 100,000 inhabitants, respectively (EFSA, 2009). Yersinia and especially Listeria monocytogenes had lower incidences of 2.0 and 0.3 cases per 100,000 inhabitants, respectively. However, L. monocytogenes infection may be severe, especially in immunocompromised individuals with rather high fatality (Ramaswamy et al., 2007). A high proportion of human campylobacteriosis, salmonellosis, yersiniosis and listeriosis is likely to originate from pigs (Fosse, Seeger, & Magras, in press).

Pigs are mostly asymptomatic carriers of Salmonella enterica, Campylobacter spp., Yersinia enterocolitica and L. monocytogenes. All these bacterial pathogens have been isolated from the intestinal tract of pigs (Fosse et al., in press). However, pathogenic Y. enterocolitica has been shown to be a more frequent inhabitant in pig tonsils (Bucher et al., 2008). L. monocytogenes is a ubiquitous organism occasionally present in the intestinal tract of various animal species but this pathogen has also been isolated from tonsils of fattening pigs (Autio et al., 2004). These pathogens can not be detected by macroscopic examination of carcasses during meat inspection.

S. enterica, Campylobacter spp., Y. enterocolitica and L. monocytogenes are food-borne zoonotic hazards which can be transmitted from pigs to humans through the consumption of pork which is today the most frequently consumed meat in Europe (Fosse et al., in press). Fosse, Seeger, and Magras (2008) have demonstrated that S. enterica, Y. enterocolitica and Campylobacter spp. are the three hazards most frequently reported in human clinical cases related to the consumption of pork. L. monocytogenes showed the highest severity score with high hospitalization and lethality rates (Fosse et al., 2008). In 2004, the prevalence rates of Campylobacter spp., Salmonella spp. and Yersinia spp. in pigs in the EU varied between 0.4–79.6%, 0.4–29.4% and 0.9–10.4%, respectively (Nørrung & Buncic, 2008). There was insufficient data available on the prevalence of L. monocytogenes in pigs (Nørrung & Buncic, 2008).

The objectives of this work were to (1) study the isolation rate of enteropathogenic Y. enterocolitica, Salmonella spp., Campylobacter spp. and L. monocytogenes in the tonsils and feces and (2) determine the number of mesophilic aerobic bacteria (MAB) and Escherichia coli in the tonsils of fattening pigs at slaughter.

2. Materials and methods

Tonsil and fecal samples were collected from 50 fattening pigs at slaughter in 2004. A slaughterhouse in Munich, Germany, was visited five times during January and March. At each visit, tonsil
and fecal samples were taken from 10 pigs from at least three to four farms. In total, the pigs originated from 15 farms. The tonsils were collected using sterile plastic gloves and knives, and the fecal samples from the rectum in sterile plastic gloves. The samples were transported to the laboratory in chilled insulated boxes and studied promptly on arrival. A 10-g sample of the tonsil was homogenized in a stomacher for 2 min with 90 ml of buffered peptone water (BPW) (Merck, Darmstadt, Germany) and a 1-g sample of feces was mixed in 9 ml of BPW with a vortex mixer.

The number of MAB was determined using the drop plating method according to DIN 10161-2 (Anonymous, 1984) and E. coli with the fluorescence optical colony count method according to DIN 10110 (Anonymous, 1994). The homogenate, which was the dilution of $10^{-1}$, was further diluted in BPW to $10^{-6}$. About 50 µl from the homogenate and subsequent 10 fold dilutions were inoculated on plate count agar (PCA, Merck) and on E. coli direct (ECD) agar (Fluorocult® ECD, Merck). The PCA and ECD plates were incubated at 30 °C for 48 to 72 h and at 42 °C for 24 h aerobically, respectively, to calculate the number of MAB and E. coli, respectively. All colonies on PCA and the light blue fluorescent colonies on ECD, which showed indole formation, were counted.

Prevalence of Salmonella, Y. enterocolitica, thermoduric Campylobacter and L. monocytogenes were studied using (1) direct plating on a selective agar plate and (2) selective enrichment according to ISO 6579 (Anonymous, 2002), ISO 10273 (Anonymous, 2003), ISO 10272 (Anonymous, 1995) and ISO 11290-1 (Anonymous, 1996), respectively, with some modifications.

For direct plating (1), 100 µl of the homogenate was inoculated on xylose lysine deoxycholate (XLD, Merck), cefsulodin irgasan novobiocin (CIN, Merck), modified CCDA-Preston (mCCDA, Oxoid, Wesel, Germany) and Oxford (Merck) selective agar plates for isolation of Salmonella, Yersinia, Campylobacter and Listeria, respectively. The inoculated XLD and Oxford plates were aerobically incubated at 37 °C for 24 and 48 h, respectively and the CIN plates at 30 °C for 18–24 h. The mCCDA plates were incubated for 48 h at 42 °C in an anaerobic jar in microaerobic conditions (85% N₂, 10% CO₂, 5% O₂) using filter-paper bags (Aerocult®C, Merck).

For selective enrichment (2), 1 ml of the homogenate was inoculated into 9 ml of Rappaport and Vassiliadis (RVS Merck), modified CCDA-Preston (mCCDA, Oxoid, Wesel, Germany) and Oxford (Merck) selective agar plates for isolation of Salmonella, Yersinia, Campylobacter and Listeria, respectively. The inoculated XLD and Oxford plates were aerobically incubated at 37 °C for 24 and 48 h, respectively and the CIN plates at 30 °C for 18–24 h. The mCCDA plates were incubated for 48 h at 42 °C in an anaerobic jar in microaerobic conditions (85% N₂, 10% CO₂, 5% O₂) using filter-paper bags (Aerocult®C, Merck).

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Up to four typical colonies from each agar plate were plated on blood agar (Oxoid) for pure culture. Identification of pure culture was done using API 20E (bioMérieux, Marcy-Étoile, France) for Salmonella and Yersinia, API Campy (bioMérieux) for Campylobacter and API Listeria (bioMérieux) for L. monocytogenes. Y. enterocolitica isolates were further bio and serotyped, and the pathogenicity was studied using congo-red magnesium oxalate (CR-MOX) agar plates (Fredriksson-Ahomaa, Bucher, Hank, Stolle, & Korkeala, 2001).

### 3. Results and discussion

The palatine tonsils are a well-known portal of entry and a site of multiplication and persistence for several micro-organisms in humans and animals including pigs (Salles & Middleton, 2000). In this study, the number of MAB and E. coli was very high in the tonsils of fattening pigs (Table 1). The mean number of MAB and E. coli was 7.12 log₁₀ CFU/g and 5.52 log₁₀, respectively. There was no significant difference in the mean number of MAB ($p > 0.1$, t-test) and E. coli ($p > 0.05$, t-test) between the sampling days. These findings demonstrate that pig tonsils are an important source of E. coli contamination in the slaughterhouse. Gill and Jones (1998) reported a mean E. coli count of 4.41 log₁₀ CFU/100 cm² in pig mouths by swabbing the tongue. High number of E. coli has also been recovered from the oral cavities of beef cattle at various stages of production (Aslam, Greer, Nattress, Gill, & McMullen, 2004). Keen and Elder (2002) have suggested that the oral cavities of cattle may be a natural reservoir of E. coli.

During the traditional slaughter procedure, which involves the removal of the tongue with tonsils attached together with pluck set (trachea, lungs, liver and heart, sometimes kidneys), the carcass and the pluck set will easily be contaminated with bacteria from the tonsils. Gill and Jones (1997) demonstrated that pig carcasses were heavily contaminated with E. coli as a result of opening of the throat and the floor of the mouth. They recommended that opening of the throat and floor of the mouth, and freeing of the esophagus, trachea and tongue, which are operations associated with heavy bacterial contamination, should be regarded as critical control points in a hazard analysis.

Food-borne zoonoses have a major health impact in industrial countries. Fosse et al. (in press) characterized Campylobacter spp., S. enterica, Y. enterocolitica and L. monocytogenes as high-risk food-borne zoonotic hazards in finishing pigs having the highest risk for pork consumers. In the present study, almost all (98%) fattening pigs at slaughter were carriers for at least one of these food-borne pathogens in their tonsils (76%) or were shedding them in feces (72%). Pathogenic Y. enterocolitica and L. monocytogenes were frequently isolated from tonsils while Campylobacter spp. were rarely found (Table 2). Ten pigs were colonized with both pathogenic Y. enterocolitica and L. monocytogenes, and 2 animals with both L. monocytogenes and Campylobacter spp. Campylobacter spp. were the only pathogen frequently isolated from feces. Five animals were either shedding both Campylobacter spp. and Y. enterocolitica (3) or both Campylobacter spp. and L. monocytogenes (2).

### Table 1

<table>
<thead>
<tr>
<th>Pigs</th>
<th>Mesophilic aerobic bacteria</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean log₁₀ CFU/g (range)</td>
<td>Mean log₁₀ CFU/g (range)</td>
</tr>
<tr>
<td>Tonsils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–10</td>
<td>7.11 (6.40–7.68)</td>
<td>5.55 (4.38–6.49)</td>
</tr>
<tr>
<td>11–20</td>
<td>7.13 (6.81–7.32)</td>
<td>5.30 (4.58–5.88)</td>
</tr>
<tr>
<td>21–30</td>
<td>7.12 (6.76–7.67)</td>
<td>5.43 (4.48–6.53)</td>
</tr>
<tr>
<td>31–40</td>
<td>7.08 (6.60–7.63)</td>
<td>5.71 (5.00–6.12)</td>
</tr>
<tr>
<td>41–50</td>
<td>7.16 (6.65–7.82)</td>
<td>5.62 (5.08–5.98)</td>
</tr>
<tr>
<td>1–50</td>
<td>7.12 (6.40–7.82)</td>
<td>5.52 (4.38–6.53)</td>
</tr>
</tbody>
</table>

CFU, colony forming unit.

### Table 2

<table>
<thead>
<tr>
<th>Pigs</th>
<th>Tonsils</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>LM</td>
</tr>
<tr>
<td>1–10</td>
<td>6</td>
<td>3</td>
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<tr>
<td>11–20</td>
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<tr>
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<td>5</td>
</tr>
<tr>
<td>41–50</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>1–50</td>
<td>38</td>
<td>16</td>
</tr>
</tbody>
</table>

P, tonsil positive for at least one pathogen.
LM, Listeria monocytogenes.
YE, Yersinia enterocolitica 4/O:3.
S, Salmonella spp.
C, Campylobacter spp.
Y. enterocolitica was the most frequently isolated pathogen in the tonsils of fattening pigs at slaughter (Table 2). It was isolated from 62% of the pigs when tonsils were studied but only from 16% of the pigs when fecal samples were studied. Only 4% of the animals, which excreted yersinia in feces, were negative in their tonsils. From 13% (2/15) of the farms no enteropathogenic Y. enterocolitica was isolated. One possible reason was the low number of pigs studied from the negative farms. All isolates showing congo-red uptake and calcium-restricted growth on CR-MOX plates belonged to bioserotype 4/O:3, which is the most common type found in both humans and pigs (Fredriksson-Ahomaa, Stolle, & Korkeala, 2006). This shows that fattening pigs are frequently colonized with pathogenic Y. enterocolitica in tonsils but may also excrete this pathogen in feces. For prevalence studies, tonsils will give a more reliable estimate than feces.

L. monocytogenes was also isolated more often from tonsils compared to feces; 32% of fattening pigs were positive in tonsils and only 4% in feces at slaughter (Table 2). These two feces-positive animals were also colonized with L. monocytogenes in the tonsils. L. monocytogenes was isolated from 46% (7/15) of the farms, which could indicate that some risk factors on the farms may have an influence on the prevalence. Among the risk factors described in the literature, wet feed has shown to increase the risk of pig infection by L. monocytogenes (Fosse et al., in press). Autio et al. (2004) also demonstrated that L. monocytogenes is common in tonsils of slaughter pigs isolating L. monocytogenes more frequently in fattening pigs (22%) than in sows (6%). In USA, the prevalence of L. monocytogenes in culled sows was only 0.6% and 0.18% in tonsils and feces, respectively (Wesley et al., 2008). Esteban, Oporto, Aduriz, Juste, and Hurtado (2009) studied fecal samples from 30 pigs from 17 herds in Spain and reported all herds to be L. monocytogenes negative. The tonsils may give a better estimate of L. monocytogenes prevalence than feces.

Campylobacter spp. were rare in pig tonsils; only 6% of the animals were colonized with this pathogen in their tonsils (Table 2). However, the prevalence of Campylobacter spp. was high in feces; 62% of fattening pigs excreted this pathogen in feces at slaughter. Only one animal was positive in tonsils but negative in feces. This indicates that fecal samples give a good estimate for campylobacter prevalence. Campylobacter spp. were isolated from most (13/15) of the farms. It has been shown that antibiotic treatment at the beginning of the fattening period decreases the detection of Campylobacters (Fosse et al., in press). The most common species was C. coli isolated from 73% of the animals. C. lari, C. fetus and C. jejuni were only isolated from 3%, 9% to 18% of the animals, respectively. One pig was C. coli positive in both tonsils and feces and one pig excreted both C. coli and C. fetus in feces. The prevalence of C. coli has been shown to be very high in fattening pigs world wide (Fosse et al., in press).

Surprisingly no Salmonella spp. were isolated from tonsils or feces of fattening pigs at slaughter. One reason can be that the prevalence of Salmonella in Bavarian fattening pigs is less than 2%. The prevalence of salmonella in feces has been reported to be between 1% and 34% in developed countries (Fosse et al., in press). The highest prevalences have been obtained when at least 25 g of feces have been studied. Furthermore, pooled fecal samples have proved to be more sensitive than individual samples. Fecal samples have been more frequently studied than tonsils, however, a high salmonella prevalence has been reported in tonsils of slaughter pigs in the Netherlands (Swanenburg, van der Wolf, Urlings, Snijders, & van Knapen, 2001).

This study demonstrates that L. monocytogenes and especially Y. enterocolitica are frequently isolated from pig tonsils at slaughter in Bavaria. The tonsils colonized with pathogens may play an important role in the contamination of the pluck set, the carcass and the slaughterhouse environment during slaughter (Fredriksson-Ahomaa, Korte, & Korkeala, 2000). The contamination in the slaughterhouse could be effectively reduced by changing the slaughter technique by not splitting the head and by leaving the tongue and tonsils in the oral cavity.

References