Wild boars as an important reservoir for food-borne pathogens

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Abstract

One hundred fifty-three wild boars shot in the canton of Geneva, Switzerland, were studied for the occurrence of foodborne pathogens. Tonsils and fecal samples of the animals were examined using real-time polymerase chain reaction, enzyme-linked fluorescent immunoassay, and cultural methods. The detection rate of Salmonella spp., Yersinia enterocolitica, Yersinia pseudotuberculosis, stx-positive Escherichia coli, and Listeria monocytogenes was 12%, 35%, 20%, 9%, and 17%, respectively, when tonsil samples were studied. Only Y. enterocolitica (5%) and L. monocytogenes (1%) were detected in fecal samples. None of the samples was positive for Campylobacter spp. Females (71%) and young animals (61%) carried more frequently one or more pathogens than males (53%) and older ones (44%). In total, 8 Salmonella spp., 14 Y. enterocolitica, 4 Y. pseudotuberculosis, and 26 L. monocytogenes strains were further characterized. Most of the Salmonella spp. strains were of serotype Salmonella Enteritidis (75%) followed by serotypes Salmonella Stourbridge (13%) and Salmonella Veneziana (13%). L. monocytogenes strains belonged to serotypes 1/2a (42%), 1/2b (19%), and 4b (38%). Serotypes O:3 (36%), O:5,27 (21%), and O:9 (29%) were identified among Y. enterocolitica strains and serotypes O:1 (75%) and O:2 (25%) among Y. pseudotuberculosis strains. This study shows that wild boars are frequent carriers of foodborne pathogens. High wild boar densities and increasing popularity of outdoor ranging of pigs may intensify the risk of transmission of these pathogens to fattening pigs.
Wild Boars as an Important Reservoir for Foodborne Pathogens

Silke Wacheck, Maria Fredriksson-Ahomaa, Martin König, Andreas Stolle, and Roger Stephan

Abstract
One hundred fifty-three wild boars shot in the canton of Geneva, Switzerland, were studied for the occurrence of foodborne pathogens. Tonsils and fecal samples of the animals were examined using real-time polymerase chain reaction, enzyme-linked fluorescent immunoassay, and cultural methods. The detection rate of *Salmonella* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *stx*-positive *Escherichia coli*, and *Listeria monocytogenes* was 12%, 35%, 20%, 9%, and 17%, respectively, when tonsil samples were studied. Only *Y. enterocolitica* (5%) and *L. monocytogenes* (1%) were detected in fecal samples. None of the samples was positive for *Campylobacter* spp. Females (71%) and young animals (61%) carried more frequently one or more pathogens than males (53%) and older ones (44%). In total, 8 *Salmonella* spp., 14 *Y. enterocolitica*, 4 *Y. pseudotuberculosis*, and 26 *L. monocytogenes* strains were further characterized. Most of the *Salmonella* spp. strains were of serotype *Salmonella Enteritidis* (75%) followed by serotypes *Salmonella Stourbridge* (13%) and *Salmonella Veneziana* (13%). *L. monocytogenes* strains belonged to serotypes 1a=2a (42%), 1b=2b (19%), and 4b (38%). Serotypes O:3 (36%), O:5,27 (21%), and O:9 (29%) were identified among *Y. enterocolitica* strains and serotypes O:1 (75%) and O:2 (25%) among *Y. pseudotuberculosis* strains. This study shows that wild boars are frequent carriers of foodborne pathogens. High wild boar densities and increasing popularity of outdoor ranging of pigs may intensify the risk of transmission of these pathogens to fattening pigs.

Introduction
During the last decades, wild boar populations have increased in Europe and spread over the entire continent. In Switzerland, the increase in wild boar population has shown to correlate with warming temperatures and improved food supply through increased cultivation of maize (Geisser and Reyter, 2005). The population densities of wild boars in Switzerland are among the highest reported in western Europe (Hebeisen et al., 2008). High wild boar densities and increasing popularity of outdoor ranging of pigs may intensify the risk of contacts between wild boars and domestic pigs and therefore the transmission of microorganisms and parasites (Gortázar et al., 2006; Köppel et al., 2007; Gebreyes et al., 2008).

Domestic pigs and wild boars share common pathogenic agents (Al Dahouk et al., 2005; Gortázar et al., 2007; Woeste and Grosse Beilage, 2007). Some of these pathogenic agents like *Salmonella* spp. and hepatitis E virus are foodborne pathogens (Boyen et al., 2008; de Deus et al., 2008). Thus, wild boars could constitute a reservoir of pathogens for domestic pigs, as de Deus et al. (2008) previously described for hepatitis E virus.

In this study, the distribution of six pathogens including *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, shiga toxin–producing *Escherichia coli* (STEC), and *Listeria monocytogenes* was examined. These foodborne pathogens are prevalent in domestic pigs or their environments, and the animals acquire those mainly by fecal–oral transmission (Butzler, 2004; Naylor et al., 2005; Jemmi and Stephan, 2006; Fredriksson-Ahomaa et al., 2007; Boyen et al., 2008). Humans are infected by consuming any contaminated food or water, through smear infections or through close contact with asymptptomatically infected farm animals (Anonymous, 2004).

Causing gastroenteritis in humans, most of these foodborne pathogens are notifiable diseases in the European Union (EU) and in Switzerland. The reported cases have been diagnosed by either bacterial isolation or detection of specific antigens and toxins. Campylobacteriosis ranked first on the list of the notifiable diseases 2007 in the EU and Switzerland, showing an incidence of 45.2 confirmed cases per 100,000 inhabitants in the EU and 79.5 cases per 100,000 inhabitants in Switzerland. Salmonellosis ranked second with an incidence...
The rate of 31.1 and 23.9 cases per 100,000 inhabitants in the EU and in Switzerland, respectively. STEC infections ranked third, with 0.6 and 0.9 reported cases per 100,000 inhabitants in the EU and Switzerland, respectively. Listeriosis was reported with an incidence of 0.3 and 0.8 cases per 100,000 inhabitants in the EU and Switzerland, respectively (EFSA, 2009a, 2009b). There were 2.8 reported cases per 100,000 inhabitants of enteropathogenic yersiniosis in the EU. Although this foodborne disease was deleted from the register of notifiable diseases in 1999 in Switzerland and thus no data on the morbidity in Switzerland are available after that time, there is an obligation to notify the authorities in a case of enteropathogenic yersiniosis in Germany. In 2007, the incidence rate was 6.1 cases (per 100,000 inhabitants), thus being the fourth most often acquired foodborne disease in Germany (EFSA, 2009a).

The most common enteropathogenic agents in slaughtering pigs are Salmonella spp., Campylobacter spp., enteropathogenic Yersinia, L. monocytogenes, and STEC (Milnes et al., 2008; Fosse et al., 2009). In contrast to the abundance of literature available on the prevalence of foodborne pathogens in humans and also in slaughtering pigs, little is known about the occurrence of foodborne pathogens in wild boars.

With this background and with the background of increasing per capita consumption of wild boar meat and the high per capita consumption of pork, knowledge of the situation of the foodborne pathogens circulating in wildlife population is an important public health issue (Anonymous, 2006, 2008). Thus, the aim of this study was to determine the occurrence of foodborne pathogens in wild boars from Switzerland and to further characterize isolated strains.

Materials and Methods

Animals, sampling, and sample preparation

Since 1974 hunting is prohibited by law in the canton of Geneva, Switzerland, and thus the regulation of the population is done by governmentally employed gamekeepers. This regulation is not appointed to a specific season, rather the gamekeepers are allowed to cull and shoot the wild boars whenever there is reason such as agricultural damages. In the time frame of October 2007 to December 2008, 624 wild boars have been shot in this region. In whole Switzerland, there was a hunting bag of 8748 wild boars in 2008. But estimating the population size of wild boars in the canton of Geneva is hard because the animals use this protection zone as a pullback during hunting season in France. Between October 2007 and March 2008, 153 wild boars were shot within regulation measures in the canton of Geneva and these animals were taken for this study.

The sex of the shot animals was equally distributed—51% (78) were males, and 48% (73) were females (Table 1). The sex of two animals was not known. The eviscerated weight of most (120/153) of the animals was between 20 and 60 kg (Table 1). Tonsils from 153 animals were removed and stored in a sterile bag. Additionally, fecal specimens from 73 animals were taken. The entire sample material was stored at −20°C until transport to Germany and until examination. The samples were taken from a veterinarian at the slaughterhouse in the canton of Geneva, Switzerland. The examination was conducted in the Faculty of Veterinary Medicine, Institute of Food Hygiene and Technology, Ludwig-Maximilians-University, Munich, Germany.

### Table 1. The Sex and Slaughter Weight of 153 Wild Boars Shot in the Canton of Geneva in Switzerland Between October 2007 and March 2008

<table>
<thead>
<tr>
<th>Sex</th>
<th>&lt;20</th>
<th>20–40</th>
<th>40–60</th>
<th>&gt;60</th>
<th>NK</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1</td>
<td>28</td>
<td>39</td>
<td>8</td>
<td>2</td>
<td>78</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>25</td>
<td>28</td>
<td>10</td>
<td>1</td>
<td>73</td>
</tr>
<tr>
<td>NK</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>53</td>
<td>67</td>
<td>18</td>
<td>5</td>
<td>153</td>
</tr>
</tbody>
</table>

NK, not known.

All of the laboratory analyses were based on sample enrichment in tryptic soy broth (TSB) (CASO; Merck, Darmstadt, Germany). Therefore, 10 g of the tonsil material and 1 g of the fecal material were added to 90 mL TSB and were enriched separately. After overnight enrichment at 37°C, Salmonella, pathogenic Y. enterocolitica, Y. pseudotuberculosis, and STEC were screened with real-time polymerase chain reaction (PCR), and Campylobacter and L. monocytogenes were screened with immunoassay. Additionally, culturing was done from all the positive samples.

**Screening using real-time PCR and immunoassay**

**Real-time PCR.** One hundred microliters of the overnight enrichment of TSB was used for DNA extraction using Instagene® (BioRad, Munich, Germany). For PCR screening, JE0420-1 of Salmonella spp. (Aabo et al., 1993), ail of Y. enterocolitica (Nakajima et al., 1992), inv of Y. pseudotuberculosis (Thoerner et al., 2003), and stx1 and stx2 of STEC (Karch and Meyer, 1989) were amplified. Real-time PCR based on SYBRGreen was used to detect all these pathogens. The total reaction volume for PCR was 25 μL containing 1 × ready-to-use mix (iQ™SYBRGreen Supermix; BioRad), 200 nM of primers, and 2 μL of template. A three-step protocol (denaturation at 95°C for 10 s, annealing at 56°C for 20 s, and elongation at 72°C for 10 s) with 40 cycles followed by melting curve analysis was used. The PCR fluorescence was detected using the iQ™5 Multicolour Real-Time PCR Detection System (BioRad). The sample was considered positive when the threshold cycle (cT) was under 38 and a specific melting curve was observed.

**Automated enzyme-linked fluorescent immunoassay (VIDAS®)**

For VIDAS screening, tonsils of four to five animals were pooled. Thus, there were 31 pool samples for immunoassay analyses. The pooling volume was 1 mL, consisting of 200 μL of each overnight TSB enrichment taken for pooling. Five hundred microliters of the pooling volume was used as the reaction volume for VIDAS analysis, and this volume was directly used for L. monocytogenes and after-heat treatment (10 min at 99°C) for Campylobacter spp. The enzyme immunoassay test was performed in an automated VIDAS 30 instrument (BioMérieux, Nueratingen, Germany) using VIDAS Campylobacter CAM-kit for detection of Campylobacter antigens and VIDAS LM II-kit for detection of L. monocytogenes antigens. The further proceeding followed the manufacturer’s instructions.
FOODBORNE PATHOGENS IN WILD BOARS

Table 2. Distribution of Foodborne Pathogens Among 153 Wild Boars of Different Sexes and Slaughter Weights Shot in the Canton of Geneva in Switzerland Between October 2007 and March 2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of positive animals</th>
<th>No. of positive animals of different sexes</th>
<th>No. of positive animals of different weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (78)</td>
<td>Female (73)</td>
<td>NK (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;20 (10) 20–40 (53) 40–60 (67) &gt;60 (18) NK (5)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>19</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>53</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>30</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>STEC</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>26</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>94</td>
<td>41</td>
<td>52</td>
</tr>
</tbody>
</table>

*Number of samples studied.

Confirmation of positive results by screening methods

Positive results by either real-time PCR or VIDAS were confirmed by cultural methods. Thus, 100 µL of overnight enrichment was plated onto selective agar plates. Rambach (Merck) and xylose-lysine-desoxycholate agar plates were used to isolate Salmonella, cefsludin-irgasan-novobiocin (Merck) agar plates for Y. enterocolitica and Y. pseudotuberculosis, Oxford and chromogenic L. monocytogenes-specific plating medium (ALOA²; Merck) for L. monocytogenes, and Sorbitol–MacConkey agar (SMAC; Merck) and chromogenic E. coli O157:H7-specific plating medium (Fluorocult® E. coli O157:H7; Merck) for STEC. Cefsludin-irgasan-novobiocin plates were incubated at 30°C; Rambach, xylose-lysine-desoxycholate, Oxford, and ALOA plates at 37°C; and SMAC and Fluorocult E. coli O157:H7 plates at 42°C for 24–48 h. Up to four characteristic colonies on selective agar plates were further characterized. Salmonella spp. and Yersinia spp. were identified with the API 20 E system (BioMérieux) incubated for 18–20 h at 37°C and 30°C, respectively. L. monocytogenes was identified using the API Listeria system (BioMérieux) incubated at 37°C for 20–24 h. The isolates were identified using the database provided by the manufacturer (APILAB V4.0; BioMérieux). Pathogenic Y. enterocolitica was identified using real-time PCR targeting the ail, Y. pseudotuberculosis targeting the inv, and STEC targeting the stx1 and stx2 as described earlier. DNA was released from the colonies by heat treatment (10 min at 99°C). The identified isolates were stored in Cryobank tubes (MastGroup, Bootle, United Kingdom) at −20°C until typing.

Serotyping

Serotyping of Salmonella and L. monocytogenes isolates was performed by the Swiss National Reference Labs. Pathogenic Y. enterocolitica (ail-positive) isolates were serotyped using commercial Y. enterocolitica O3, O5, O9, and O:27 antisera (Sifin, Berlin, Germany) and Y. pseudotuberculosis (inv-positive) isolates using commercial antisera O:1-O:4.

Results and Discussion

Distribution of foodborne pathogens among 153 wild boars of different sex and weights

It is known that wildlife populations constitute a source of infection for domestic animals. Examining wild boars from Switzerland gave an overall frequent contamination in the tonsils of the studied animals with foodborne pathogens. In the majority (61%) of the animals, at least one foodborne pathogen was found (Table 2). Further, 27 animals (18%) were bearing 2 pathogens, 8 animals (5%) 3 pathogens, and 2 animals (1%) 4 pathogens. Multispecies exposure to infectious agents, probably due to the external factors such as stress, cold and long winters, and population density, was also found by Al Dahouk et al. (2005) testing 763 sera from wild boars in northern Germany.

Although in our study female animals (71%) carried significantly (Fisher’s exact test, p = 0.0201) more often foodborne pathogens than the males (53%), Al Dahouk et al. (2005) were not able to find a difference in the susceptibility of the sexes in their study (Table 2). The difference in susceptibility of the sexes observed in this study might be due to the social behavior of the animals where the females live in a family community, whereas the males are loners unless during breeding times. Thus, females are more prone to become infected due to their social contacts, making transmission very easily possible.

Comparing the detection rate of foodborne pathogens in the animals having a slaughter weight less than 40 kg to those weighing more than 40 kg, the younger wild boars seem to be more frequently the carrier of foodborne pathogens than the older ones (Table 2). However, this difference in the carriage was not statistically significant (Fisher’s exact test, p = 0.3071) in this study. Kranner et al. (2003) found a similar spreading behavior of Salmonella spp. in pigs. Studying the culture- and seroprevalence in blood and fecal samples of different age groups until slaughter and in sows and gilts, they isolated Salmonella predominantly from weaners, growers, and finishers and only occasionally from sows and gilts. They concluded that stress is a major factor for susceptibility and horizontal transmission. However, another reason could be a lower immunity of these age groups against these pathogens. Drawing the connection to wild boars, the same might hold true considering their social behavior living in families consisting of the leading mother and her previous year offspring guiding their own offspring. During times where there is little food available, the family members separate making horizontal transmission beyond the offspring under these stressful conditions possible.

There was no statistical association between sex and weight for the animals with a slaughter weight less than 40 kg.

No. of positive animals of different sex and weights

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of positive animals</th>
<th>No. of positive animals of different sexes</th>
<th>No. of positive animals of different weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (78)</td>
<td>Female (73)</td>
<td>NK (2)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>&lt;20 (10) 20–40 (53) 40–60 (67) &gt;60 (18) NK (5)</td>
</tr>
<tr>
<td>Salmonella spp.</td>
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<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>53</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>30</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>STEC</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>26</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>94</td>
<td>41</td>
<td>52</td>
</tr>
</tbody>
</table>
Table 3. Detection of Salmonella, Campylobacter, Yersinia enterocolitica, Yersinia pseudotuberculosis, Shiga Toxin–Producing Escherichia coli (STEC), and Listeria monocytogenes in Tonsils of 153 Swiss Wild Boars Shot in the Canton of Geneva in Switzerland Between October 2007 and March 2008

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>No. of positives</th>
<th>No. of positive animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR  (153)</td>
<td>VIDAS (31)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>19</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>53</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEC</td>
<td>14</td>
<td>ND</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>ND</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aNumber of samples studied.  
bTonsils of four to five animals were pooled for one VIDAS sample.  
cOnly PCR or VIDAS-positive samples were studied.  
dAt least one positive animal was detected from each VIDAS-positive sample.  
ND, not done; PCR, polymerase chain reaction.

(Fisher’s exact test, p = 0.2852) and not quite a statistical significant association (Fisher’s exact test, p = 0.0815) for those having a slaughter weight more than 40 kg.

Detected foodborne pathogens in the tonsils of 153 wild boars using different detection methods

Analyzing the tonsils using real-time PCR screening, Salmonella spp., Y. enterocolitica, Y. pseudotuberculosis, and STEC were detected in 12%, 35%, 20%, and 9% animals, respectively (Table 3). Campylobacter spp. could not be found in any tonsil using VIDAS, but this screening method gave positive results for tonsils being tested for L. monocytogenes. Isolation of L. monocytogenes was possible in 26 (17%) wild boars. Salmonella spp., Y. enterocolitica, and Y. pseudotuberculosis were isolated only in 5%, 9%, and 3% animals, respectively (Table 3). No STEC strain could be isolated on selective agar media.

To our best knowledge, there have been no studies conducted on the distribution of foodborne pathogens in the tonsils of wild boars in Switzerland. Vicente et al. (2002) analyzed the antibody titers in 78 blood samples from wild boars in Spain. They found a seroprevalence of 4% Salmonella serogroup B (4%) and of 3% Salmonella serogroup C. Seroprevalence of Salmonella spp. in 178 wild boars from Slovenia was 47% (Vengust et al., 2006). Kanai et al. (1997) isolated Salmonella spp. from two meat samples from wild boars in Japan. Yet, in Austria, Paulsen and Winkelmayer (2004) were not able to isolate Salmonella spp. from game carcasses. Analyzing 1430 fattening pigs at slaughter in Switzerland gave 22 seropositive pigs, while no Salmonella spp. was isolated from retail pork (Ledergerber and Zychowska, 2003).

Using real-time PCR, Y. enterocolitica and Y. pseudotuberculosis were identified in 35% and 20% of the animals, respectively. Thus, wild boars appear to be an important reservoir for both Yersinia species. In Switzerland, Y. enterocolitica was detected in 88% of the tonsils of slaughterhog pigs using PCR (Fredriksson-Ahomaa et al., 2007), whereas there are no data available on the occurrence of Y. pseudotuberculosis in these animals from Switzerland. The high detection rate of Y. pseudotuberculosis in wild boars in this study might be due to the close contact with other wild animals as was shown by Laukkanen et al. (2008) for organically held fattening pigs. Besides herd size and herd management, mixing of batches appears to be a major risk factor for the increase of prevalence of Y. enterocolitica in fattening pigs (Fosse et al., 2009). Because wild boars stay in their family community, the lower distribution rate of Y. enterocolitica might be explained.

STEC was detected in 9% of the examined animals using real-time PCR. However, no STEC strain could be isolated on selective agar media. In Sweden, Wahlström et al. (2003) tested ~1% of the fecal samples taken from wild boars as STEC positive. The fecal shedding among slaughtering pigs appears to be higher. Kaufmann et al. (2006) detected 22% STEC-positive fecal samples by testing 630 slaughtered lighter pigs using PCR methods in Switzerland.

In accordance to the study of Wahlström et al. (2003), no positive sample for Campylobacter spp. could be detected in this study. Thus, the prevalence of Campylobacter spp. in wild boars appears to be negligible, whereas the prevalence in pigs appears higher. In Switzerland, the prevalence of Campylobacter spp. in pig fattening farms was 98.9% (Ledergerber and Zychowska, 2003).

In this study, isolation of L. monocytogenes was possible in 17% of the animals examining the tonsils. Even there are only a few reports on the occurrence of L. monocytogenes in wild boars, the data are similar to the data collected in this study. Analyzing wild boar meat for the occurrence of zoonotic pathogens, Kanai et al. (1997) isolated five L. monocytogenes strains out of 100 retail wild boar meats, whereas Paulsen and Winkelmayer (2004) were not able to isolate L. monocytogenes from carcass swabs. Unfortunately, no data on the prevalence of L. monocytogenes in fattening or slaughter pigs are available from Switzerland. But Fantelli and Stephan (2001) were able to isolate L. monocytogenes from minced pork samples.

Detected foodborne pathogens in the tonsils and feces of 73 wild boars

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Tonsil (%)</th>
<th>Feces (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR (%)</td>
<td>Culture (%)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>4 (5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>26 (36)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Y. pseudotuberculosis</td>
<td>12 (16)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>STEC</td>
<td>1 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>ND</td>
<td>6 (8)</td>
</tr>
</tbody>
</table>

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Table 4. Detection of Foodborne Pathogens in Tonsils and Feces of 73 Wild Boars Shot in the Canton of Geneva in Switzerland Between October 2007 and March 2008
Y. enterocolitica, 16% for Y. pseudotuberculosis, 1% for STEC, and 8% for L. monocytogenes in the tonsils. These results indicate that wild boars are frequent carriers of foodborne pathogens in tonsils, but shedding in feces occurs rarely. The different detection rates in tonsils and fecal samples agree with studies in slaughtering pigs where the prevalences for digestive carriage of foodborne diseases were higher than for shedding (Bucher et al., 2008; Fosse et al., 2009).

Serotyping of the isolated strains

Serotyping of the eight Salmonella strains resulted in Salmonella Enteritidis (six), Salmonella Veneziana (one), and Salmonella Stourbridge (one). The detection of Salmonella strains of serotype Enteritidis as the predominant serotype was surprising since Salmonella Enteritidis is mainly found in poultry and game birds and not in pigs. To our knowledge, there is no report on Salmonella Enteritidis in wild boars. Pérez et al. (1999) reported a case of salmonellosis caused by Salmonella Cholerasuis, and Eccó et al. (2006) found Salmonella Saintpaul to be responsible for illness and death in wild boars. Recently, Mainar-Jaime et al. (2008) described Salmonella Enteritidis as the second most frequently isolated serotype following Salmonella Derby investigating the Salmonella prevalence in slaughter pigs in Canada. Salmonella Enteritidis is the main serotype causing gastroenteritis in humans in Europe (EFSA, 2009a).

The serotypes O:5,27, O:9, and O:3 were identified for Y. enterocolitica in three (21%), four (29%), and five (36%) strains, respectively, and serotypes O:1 and O:2 were identified for Y. pseudotuberculosis in three (75%) and one (25%) strain, respectively. These serotypes are associated with human disease and are also found in domestic pigs and wild animals. In Bulgaria, Y. enterocolitica serotype O:3 and Y. pseudotuberculosis serotype O:2 could be isolated from the viscera of wild boars (Nikolova et al., 2001). However, the serotype O:3 is the most frequently isolated type for both Yersinia species among pigs at slaughter (Fredriksson-Ahomaa et al., 2009).

Of the 26 strains identified as L. monocytogenes, 11 (42%) belonged to serotype 1/2a, 10 (38%) to serotype 4b, and 5 (19%) strains belonged to serotype 1/2b. All these serotypes are commonly associated with sporadic illness in humans (Dowmith et al., 2004). In a previous study, L. monocytogenes serotype 4b could be isolated in rectal contents taken from euthanized wild boars (Hayashidani et al., 2002).

Conclusions

Wild boars are frequent carriers of foodborne pathogens in their tonsils (Salmonella spp., Y. enterocolitica, Y. pseudotuberculosis, STEC, and L. monocytogenes). These results have two implications.

First, carriers represent a source of carcass contamination. This is mainly to be considered by the hunters, who may be handling carcasses under minimal hygiene conditions. Even in the light of processing (cooking) of wild boar meat, this is not a significant public health problem; handling and preparing wild boar meat can lead to cross-contamination problems with processed food mainly on the kitchen level. Moreover, abstaining from eating uncooked or medium done wild boar meat is advisable.

Second, outside rearing of domestic pigs and close contact with wild boars may increase the risk of transmission of these foodborne pathogens between these animal groups.

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