Salmonella in breeding pig herds - differences between sows and weaned piglets

Ten Haghen B.1, Szabo I.1, Alc K.1, Weiser A.A.1, Kásbohrer A.1,2
German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany; Veterinary University Vienna, Vienna, Austria

Introduction
Salmonella spp. continue to be prevalent in the pig production chain in Germany. It was the purpose of this study to compare Salmonella isolates from sows and weaned piglets in breeding pig herds that were collected during a national monitoring program.

Methods
In the framework of a national monitoring program composite fecal samples were collected from sows and weaned piglets and tested for Salmonella according to ISO 6579. Isolates were serotyped and tested for antimicrobial resistance to 14 substances using broth microdilution in concordance with the prescriptions of Commission Implementing Decision 2013/652/EU. Only farms that provided samples from sows and weaners were included in the analysis.

Results
Overall, prevalence of Salmonella spp. in the herds was 14.4% (51/353 herds). It was higher in weaners to ISO 6579. Isolates were serotyped and tested for antimicrobial resistance to 14 substances using broth microdilution in concordance with the prescriptions of Commission Implementing Decision 2013/652/EU. Only farms that provided samples from sows and weaners were included in the analysis.

Conclusions
Using only two composite fecal samples probably provides only limited sensitivity for the detection of Salmonella in pig herds. Results indicate that the prevalence of Salmonella in breeding pig farms is a complex issue and that transmission of Salmonella from sows to weaners is not straightforward. Controlling Salmonella in breeding pig herds therefore requires a complex approach that addresses both, Salmonella in the sows and potentially independent circulation of Salmonella among weaners.

Characterization of Campylobacter coli isolated from pig, sheep, poultry, wild bird, river and shellfish using MALDI-TOF and comparison of their protein spectra to identify relationships between sources

Denis M.1, Rose V.1, Nagard B.1, Serghine J.1, Meunier M.1, Benoit F.2, Rincé A.1, Cavuin E.1, Gourmelon M.1
1Anses - Laboratoire de Ploufragan, Ploufragan, France; 2Ehren, Plouescat, France; 3Labéo, Saint Lô, France; 4Université de Caen, Caen, France

Introduction
The sanitary quality of shellfish-harvesting areas is a key issue in France, the leading shellfish producer in Europe. Campylobacter spp. was detected in coastal catchments and shellfish-harvesting areas in Brittany and Normandy, France (Rincé et al., 2013). This pathogen is excreted by many animals whether wild birds or livestock (Mughini-Gras et al., 2016). These participate in the contamination of the environment directly or via manure spreading. Comparisons of PFGE profiles or MLST types have proved their effectiveness in determining the origin of human cases of campylobacteriosis or surface water contamination (Denis et al., 2009; Denis et al., 2011a; Clark et al., 2011; Jonas et al., 2013; Mughini-Gras et al., 2016). Identify the sources of shellfish contamination with these techniques is possible but these latter are expensive and long to implement. An alternative may be the typing of strains by MALDI-TOF MS. Links of MALDI-types of Campylobacter to their MLST types were described (Zautner et al., 2013). Moreover, the MALDI-TOF MS technique is easy to perform and inexpensive.

In this study, we characterize Campylobacter coli isolated from pig, sheep, poultry and shorebird fecal samples, river water samples and shellfish batches using MALDI-TOF and compare their protein spectra to identify relationships between sources and the source of shellfish contamination. We focused on C. coli, the only species detected in pig in France (Denis et al., 2011b).

Material and Methods
We considered 144 C. coli isolated from feces of pigs (60), sheep (15), poultry (30), and shorebirds (10) and from river water samples (24) and shellfish batches (5). They were isolated in Brittany and Normandy, which are the two main areas of shellfish production in France with an important livestock production. After culture on blood agar (24h, 37°C) in microaerophilic conditions, proteins of each strain were extracted as recommended by Bruker. Then, 1 μl of each extract was deposited 8 times on spots of MSP 48 Target polished steel plate and included in 1 μl of Dvx matrix HCCA. The steel plates were sent to MALDI-TOF Platform of Anses where four reads per spot were realized (32 protein spectra per strain). Under BioNumerics, an average spectrum of protein was obtained for each strain, and all the average spectra were clustered in a dendrogram using upgma method and Pearson’s coefficient.

Results
The strains were distributed in 14 clusters (Tab1). Campylobacter coli of pigs were mostly distinguishable from C. coli of other animal reservoirs. They never clustered with C. coli from poultry. Seventy percent of strains clustered together; the others (28.3%) clustered with sheep (3 strains and, one pig strain (1.6%) with sheep (12), shorebird (4) and river water (3) strains. Sheep strains (80%) clustered with wild birds strains (4) and river water strains (3). Poultry strains (86.6%) clustered with shorebird (3), river (11) and shellfish (2) strains. Shorebird strains (90%) clustered with river water strains (19) and shellfish strains (2). Finally, two strains of shellfish were grouped with strains of river water, shorebird and poultry; the three other strains were only grouped with C. coli of river water.

Table 1: Clustering with 93% of similarity of average protein spectrum data bioNumerics

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Pig</th>
<th>Sheep</th>
<th>Poultry</th>
<th>Wild Bird</th>
<th>River</th>
<th>Shellfish</th>
<th>Total</th>
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<td></td>
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<td></td>
<td>2</td>
</tr>
<tr>
<td>C2</td>
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<td>3</td>
<td>20</td>
<td></td>
<td></td>
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<td>20</td>
</tr>
<tr>
<td>C3</td>
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<td>1</td>
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<td>3</td>
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<td>2</td>
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<td>76</td>
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<td>7</td>
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<tr>
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<td>2</td>
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<tr>
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<td>1</td>
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<td>Total</td>
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<td>15</td>
<td>30</td>
<td>24</td>
<td>5</td>
<td>114</td>
<td>185</td>
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</table>

Discussion and Conclusion
Although in France, C. coli is rarely isolated from shellfish, it is important to identify the animal reservoir that causes this C. coli contamination. Especially since the water, from rivers of the upstream catchments, arriving in these shellfish-harvesting areas mainly contains C. coli (Rincé et al., 2013).
With the use of MALDI-TOF, we observed that C. coli of pigs were mostly distinguishable from C. coli of other animal reservoirs, and particularly from C. coli isolated from poultry. PFGE already highlighted that C. coli of pigs differed genetically from C. coli of poultry in France (Denis et al., 2009) while MLST showed common STs in these two reservoirs in other countries (Mughini-Gras et al., 2016). This study suggests already that pig is very weakly involved in river contamination by C. coli as already described by PFGE or MLST in other studies (Denis et al., 2013a, Mughini-Gras et al., 2016). This may explain why C. coli of pigs was not linked to shellfish. C. coli of sheep clustered with pig strains. Another study showed that very few STs of C. coli of ruminants shared common STs with C. coli of pigs (Mughini-Gras et al., 2016).

Our study suggests that poultry, sharebirds and sheep could contribute to the contamination of rivers by C. coli. This is consistent with MLST results (Mughini-Gras et al. 2016) showing that Campylobacter in surface water were mostly attributed to wild birds and poultry followed by ruminants. The contamination of the rivers by C. coli can thus contribute to that of shellfish.

Acknowledgments

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References


Denis M., Henrique E., Chidaine B .., et al. (2011b) Campylobacter from 1,75 % in the control and 1.0 % in the PFA group. Significant reduction was observed (difference of PFA to control -28.2 %; P=0.465).

Discussion and Conclusion

The occurrence of PWD in piglets is frequently associated with the presence of F4-positive E. coli strains (Fairbrother et al., 2005). These fimbriae are important for intestinal adhesion and colonization of E. coli and previous studies demonstrated, that quorum sensing is involved in their expression (Sturbelle et al., 2015). The results of study 1 strongly suggest, that s-cells were associated with bacterial quorum sensing. Reduced adhesion of pathogenic E. coli protects the intestinal tract from loss of gut barrier integrity. For this purpose a PFA was formulated, using efficient substances from study 1 and evaluated regarding its effect on gut permeability in piglets. The reduction of FITC-4kDa in the ex vivo permeability assay suggests, that the prototypes were able to support the integrity of the piglets' small intestines. During maturation of the animals, this effect was nearly lost, although numerically lower permeability for FITC-4kDa was also seen at day 42. Using the same PFA in study 3 revealed its potential to protect piglets from the outbreak of PWD, indicated by both, assignment to two groups (200 male and 200 female animals per group). Whereas one group was fed an unsupplemented diet, a PFA was added to feed of the second group. Mortality, appearance of diseases and use of medication was recorded.

Results

Study 1: In untreated microtiter wells nearly 20% of radioactive labelled pathogens adhered to the mucus coating, indicating the occupation of all available receptor sites in the wells. Test substance 1 increased attachment to the mucus whereas substance 2 had no effect, and substances 3 and 4 reduced mucosal attachment compared to the control. Study 2: Permeability for the FITC-4kDa marker was reduced in the PFA group at day 14 by 69.3 % compared to the control group. At day 42, a non-significant reduction was observed (difference of PFA to control -28.2 %; P=0.465).

Study 3: Overall mortality was at a low level with 1.75 % in the control and 1.0 % in the PFA group. Occurrence of PWD (118/82 in control/PFA group) was reduced by 30.5 % (P=0.001). Respiratory disorders (46/44 in control/PFA group, P=0.833) and other diseases (7/6 in control/PFA group, P=0.761) were not affected by treatment. Antibiotic treatments against PWD (82/45 in control/PFA group) were reduced by 45.1 % with supplementation of the PFA.

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