

Carriage of *Campylobacter* by sows and spread to fattening pigs in farrow-to-finish farms

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Abstract

We carried out a one-year study, in 2008, at 53 farrow-to-finish farms in Brittany, France, to determine the proportion of sows excreting *Campylobacter* and to determine whether *Campylobacter* excretion by fattening pigs on these farms was related to transmission from sows. We also determine the genotypes of the *Campylobacter* isolates.

Ten samples of feces from sows were collected from randomly selected sites (maternity, service and gestation areas) on the 53 farrow-to-finish farms. Sampling was also carried out during the fattening stage (four samples per farm) on 27 of the 53 farms. Feces were 10 fold diluted and direct streaking was done on Karmali plate. Plates were then placed at 37°C during 48h in microaerobic atmosphere. *Campylobacter* isolates were identified by PCR and typed by PFGE.

Campylobacter was detected in 25.1% of the 530 samples from sows, and 69.8% of the 53 pig farms had at least one positive sample (of 10 taken). *Campylobacter* was detected in 15.4% of the 168 samples from fattening pigs and 62.9% of the 27 farms studied had at least one positive sample (of 4 taken). All the *Campylobacter* isolates belonged to the *C. coli* species. They displayed a very high level of genetic diversity, also inside farms and few genotypes were common to several farms. Only few genotypes were common to both fattening pigs and sows. However, samples from fattening pigs at which *Campylobacter* had been detected in feces from sows were more likely to have a positive feces sample than those from farms at which the bacterium had not been detected in feces from sows.

This study provided recent valuable information on the occurrence of *Campylobacter* in farrow-to-finish farms and on its spread between sows and fattening pigs.

Introduction

Campylobacter sp. is one of the most frequent causes of human enteritis in industrialized countries. The main source of human *Campylobacter* infections, as highlighted by many epidemiological studies, is the consumption of contaminated food — particularly raw or insufficiently cooked poultry products (Moore et al., 2005). Pork meat has also been implicated in human *Campylobacter* infection. Friedman et al. (2004) in the USA identified the consumption of non poultry meats, such as hamburgers, pork roasts and sausages, as a high risk factor for sporadic *Campylobacter* infections. Pigs are a natural reservoir of *Campylobacter*, with a prevalence of infection superior to 50%, (Minvielle et al., 2007), with *Campylobacter coli* the predominant species.

The goals of this study were to determine the proportion of sows excreting *Campylobacter* at farrow-to-finish pig farms, to determine whether *Campylobacter* excretion by fattening pigs on these farms was related to transmission from sows and to analyze the species and genotype diversity of the *Campylobacter* population found on these farms.

Material and Methods

Samples. 53 farrow-to-finish farms from Brittany, France, were sampled from January to December 2008. Ten samples of feces (representing each at least 10 sows in the room) were realized randomly at different sites (maternity, service area, gestation) in each farm; each site being represented at least one time in a farm. When sows were in individual housing in a room; at least 10 sows were considered in the sample. Sampling was also carried out during the fattening stage (four samples per farm) on 27 of the 53 farms.

***Campylobacter* detection.** We carried out only direct streaking tests with our fecal samples. For each sample, 25 g of feces was diluted 1:10 in peptone-buffered water and 1 ml was streaked directly on three Karmali plates. Plates were incubated at 37°C in a microaerobic atmosphere for 48 h. Typical colonies on Karmali were then sub-cultured on blood agar plates for 24h at 37°C for *Campylobacter* confirmation as described in the ISO 10272 method and for species identification and genotyping.

Species identification. DNA extraction was done by blowing the cells at 95°C for 10 min. Multiplex-PCR (Wang et al. 2002) was used to confirm the genus of the bacterial isolates and to identify them to species level (*Campylobacter jejuni*, *C. coli*, *C. lari*, *C. fetus*, and *C. upsaliensis*). PCR products were visualized by the electrophoresis of 10 µl aliquots of each amplification product, for 3 hours at 100 V, in a 2% agarose gel stained with ethidium bromide.

Pulsed-field gel electrophoresis (PFGE) and analysis of electrophoretic profiles. DNA preparation, restriction endonuclease digestion and PFGE were carried out as described by the Campynet protocol (Rivoal et al., 2005). Two profiles, corresponding to the restriction profiles obtained with SmaI and KpnI, were obtained for each isolate. Electrophoretic patterns were compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium). Similarities between profiles were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for KpnI- and SmaI-digested DNA (KS) was constructed. Strains were clustered by the unweighted pair-group method using the arithmetic mean (UPGMA) (Struelens et al., 1996). The Simpson's index (D) was determined as described by Hunter (1990).

Results

Finally, 25.1% 95%CI [20.8-29.3] of the 530 samples from sows were tested positive for *Campylobacter* and at least one of the ten samples taken was positive in 37 farms among the 53 farms (69.8% 95%CI [56.3-83.2]). Low levels of contamination were found within the positive farms, with 71.7% of the farms for which a positive result was obtained having no more than three positive samples. The excretion of *Campylobacter* by sows was not associated with the stage of the sows at which samples taken; 18.8%, 28.2%, and 22.2% of the fecal samples carried the bacteria at the service area stage, the gestation stage and the maternity stage, respectively. For fattening pigs, 15.4% 95%CI [7.8-23.0] of the 168 samples tested positive for *Campylobacter* and at least one of the four samples taken tested positive for *Campylobacter* on 62.9% 95%CI [44.0-81.8] of the 27 farms.

All the *Campylobacter* isolates belonged to the *C. coli* species. Simpson's index was high, $D=0,998$ 95%CI [0,997-1,000], consistent with a high degree of genetic diversity in the *Campylobacter* population from pig. They displayed a very high level of genetic diversity, also inside farms and few genotypes were common to several farms. In 12 cases, isolates shared the same genotype. In 10 of these cases, the isolates with identical genotypes were obtained from the same farm. In only two cases isolates with identical genotypes came from different farms: isolates 08MD0081, 08MD0082 (farm no.75) and isolate 08MD0388 (farm no. 260), on the one hand, and isolate 08MD0139 (farm no. 120) and isolate 08MD0169 (farm no. 122), on the other. Diversity of genotypes from sows inside farm could be high. The number of genotypes varied from one to eight. In 16 farms, only one or two genotypes were found. In 14 farms, 3 to 5 genotypes were identified, and in 7 farms more than 6 genotypes.

Only few genotypes were common to both fattening pigs and sows. However, excretion of *Campylobacter* by sows was also recorded at 14 of the 17 farms (of 27 tested) on which *Campylobacter* excretion by fattening pigs was detected (table 1). The risk of fattening pigs excreting *Campylobacter* in their feces was higher on farms at which *Campylobacter* excretion by sows was observed.

Discussion

In our study, 25.1% of feces samples from sows tested positive for *Campylobacter*. *C. coli* was the only *Campylobacter* species present. Our results are similar to those of previous French studies (Magras et al., 2004; Minvielle et al., 2007). Leblanc Maridor et al., (2008) showed that if pigs were orally inoculated simultaneously with several species of *Campylobacter*, *C. coli* was the species with the strongest colonizing capacity.

Sows in France are thus a reservoir of *Campylobacter* and could be a source of contamination of the piglets. Wehebrinck et al. (2008) reported, for a farm in Germany, that 33.8% of the sows and 64.7% of the fattening pigs excreted *Campylobacter*. Finally, *Campylobacter* was detected in 77% of the 1448 feces samples from sows taken at American farms (Wright et al., 2008). In our study, 69.8% of the farrow-to-finish farms exhibited at least one positive sample which is closed to 52.9% reported by Oporto et al. (2007) however, 71.7% of our positive farms had no more than three positive samples. This situation may result from effective control through the use of sanitary barriers within farms, limiting propagation of the bacterium between different areas of the farm.

The *C. coli* isolates from our pig farms displayed a high level of genetic diversity which is similar to other studies in which PFGE was used for typing (Laroche et al., 2008). Only in three cases isolates with the same genotype come from two different farms. This high level of diversity makes it difficult to identify a common origin of contamination for pig farms

affected by *Campylobacter*. Our work highlighted for some farms several genotypes indicating that numerous *Campylobacter* can circulate in the pig buildings of a farm and suggesting several sources of contamination. Soultos and Madden (2007) previously reported that piglets were initially contaminated with bacteria of the same genotype as those infecting their mothers. These authors considered the sows to be a source of piglet contamination. Magras et al., (2004) reached the same conclusion following the isolation of *Campylobacter coli* from 79% of fecal samples taken from sows on nine French farms.

Conclusion

This study provided recent valuable information on the occurrence of *Campylobacter* in farrow-to-finish farms and on its spread between sows and fattening pigs. Sows are a reservoir of *Campylobacter* and risk that fattening pigs excrete the bacteria increases if *Campylobacter* excretion by sows was observed in the same farms.

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Table 1: Relationship between *Campylobacter* shedding by fattening pigs and sows from same farms (27 farrow-to-finish farms)

	No of farms with <i>Campylobacter</i> -negative fattening pigs	No of farms with <i>Campylobacter</i> -positive fattening pigs	total	% positive
No of farms with <i>Campylobacter</i> -negative sows	3	3	6	50.0
No of farms with <i>Campylobacter</i> -positive sows	7	14	21	66.6
Total	10	17	27	62.9

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