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# *Campylobacter* from sows in farrow-to-finish pig farms: Risk indicators and genetic diversity

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ABSTRACT

Sows have been identified as a source of *Campylobacter* contamination in piglets. 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*. We also determined the genotypes of the *Campylobacter* isolates. Moreover, Generalized Estimating Equations including repeated effects were used to assess the association between management practices and farm characteristics, and risk of *Campylobacter* shedding by sows. Per farm, 10 feces samples from sows were collected from selected sites (maternity, service area, gestation area) on the farms. *Campylobacter* isolates were identified by PCR and typed by PFGE. *Campylobacter* was detected in 25.1% of the 530 samples from sows, and 67% of the 53 pig farms had at least one positive sample (of 10 taken). All the *Campylobacter* isolates belonged to the *Campylobacter coli* species. They displayed a very high level of genetic diversity, also inside farms and few genotypes were common to several farms. Warmer months, large farms, and individual housing for sows were identified as risk indicators of *Campylobacter* shedding by sows. A short delay between sampling and treatment of the samples should be considered, to improve the detection of the bacterium in the feces samples.

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## 1. Introduction

*Campylobacter sp.* is one of the most frequent causes of human enteritis in industrialized countries. The main source of human *Campylobacter jejuni* and *Campylobacter coli* infections, as highlighted by many epidemiological studies, is the consumption of contaminated food – particularly raw or insufficiently cooked poultry products (Mazick et al., 2005; Moore et al., 2005). Pork meat has also been implicated in human *Campylobacter* infection. Human *Campylobacter* infections have been associated with the consumption of sausages (Kapperud et al., 1992) and pork pâté (Gillespie et al., 2002). 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. The incidence of human cases of *Campylobacter* infection attributable to the consumption of pork meat was recently estimated at 2.17 cases per 100,000 inhabitants per year in Europe (Fosse et al., 2008), just after *Salmonella* (3.37) and *Yersinia* (2.82). Pigs are a natural reservoir of *Campylobacter*, with a prevalence of infection superior to 50% (Von Altrock et al., 2006; Minvielle et al., 2007; Varela et al., 2007), with *C. coli* the predominant species present. Only a few epidemiological studies investigating the *Campylobacter* infection status of pigs at farm level have been carried out. Sows have been identified as a source of *Campylobacter* contamination in piglets (Soultos and Madden, 2007). Magras et al. (2004) isolated *Campylobacter* from fecal samples from 79% of sows. The piglets

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**Table 1**
Definition and distribution of explanatory variables at the holding level after variable analysis of *Campylobacter* shedding by sows (quantitative variable were divided in categories) (530 samples from 53 farrow-to-finish farms in Brittany, France, 2008).

| Definition of variables concerning the holding | % of samples per level | % of positive samples per level |
|---|---|---|
| Number of sows on the farm on the day of sampling | | |
| <130 | 34.0 | 12.7 |
| 130–190 | 32.0 | 29.4 |
| >190 | 34.0 | 33.3 |
| Number of fattening pigs on the farm on the day of sampling | | |
| <2500 | 34.0 | 18.3 |
| 2500–4000 | 32.0 | 20.0 |
| >4000 | 34.0 | 36.6 |
| Gilt replacement policy | | |
| More than 90% of gilts purchased | 81.1 | 24.4 |
| More than 10% of gilts came from the farm | 18.9 | 28.0 |
| Systematic antibiotic treatment of sows | | |
| No | 54.1 | 24.5 |
| Yes | 45.9 | 25.8 |
| Production stage at which antibiotics were administrated to sows | | |
| No antibiotic treatment | 54.1 | 24.5 |
| Gestation area | 10.0 | 32.5 |
| Maternity | 15.1 | 20.0 |
| At several stages | 20.8 | 32.7 |

seemed to become contaminated early in their lives (Young et al., 2000) and genetic typing showed that the strains isolated from sows and their piglets had similar profiles (Soultos and Madden, 2007).

The goals of this study were to see the proportion of sows excreting *Campylobacter* in farrow-to-finish pig farms, to identify farm-level risk indicators for *Campylobacter* excretion by sows, and to analyze the species and genotype diversity of the *Campylobacter* population found on these farms.

## 2. Materials and methods

### 2.1. Samples

53 farrow-to-finish farms were sampled from January to December 2008. These farms were selected among the farrow-to-finish farms implied in the European baseline study which occurred in 2008 (EFSA, 2009). They were all from Brittany in France, because more than 54% of all French pig herds are located in this French area (ITP, 2000). Sampling was done by technicians of the veterinary services.

A sample consisted of fresh feces collected from at least 10 sows located in one room. A total of ten samples of feces was realized per farm. The rooms where feces were sampled could be at different sites (maternity, service area, gestation area) in the farm; each site had to be represented at least by one sample in a farm. When this condition was checked (one sample in maternity, one sample in service area and one sample in gestation area), the 7 other samples were randomly made on the 3 sites considered in this study.

The day on which the sample was taken was noted.

### 2.2. Data collection

General data relating to the farm and management of pigs were recorded (total numbers of sows and of fattening pigs, etc., Table 1). Data concerning pigs related to a sample at the day of the sampling were collected (type of feed, origin of feed, antibiotic treatment, age of the sows, etc., Table 2). The questionnaire used in this study was extracted from the European baseline questionnaire. It was completed at the pig farm by technicians of the veterinary services with the farmer.

*Campylobacter* colonization is asymptomatic in pigs and no routine bacteriological tests are carried out on farms. Thus, the farmers participating in the study were unaware of the *Campylobacter* infection status of their breeding pigs. There was therefore no risk of a change in pig management being introduced as a function of the infection status of the farm.

### 2.3. Campylobacter sp. isolation and identification

#### 2.3.1. Bacteriological analysis

The day of analysis was recorded so that we could determine the time elapsed between the day of sampling and the day of analysis of the samples for *Campylobacter sp.* isolation.

The level of *Campylobacter* excretion by French pigs has been reported to be high (Minvielle et al., 2007). We therefore carried out only direct streaking tests from 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 (5% $O_2$, 10% $CO_2$, 85% $N_2$) for 48 h. We decided to use a temperature of 37 °C, as opposed to 41.5 °C, for this test, because 37 °C is closer to the body temperature of the pig and we also wanted to see whether incubation at this temperature would result in species other than the *C. coli* typically found in French pig.

Presence of colonies on Karmali plate with the following characteristics (small curved bacilli and spiraling "corkscrew" motility) was checked and isolates were then sub-cultured on blood agar plates for 24 h at 37 °C for *Campylobacter* confirmation as described in the ISO 10272 method and for species identification and typing.

**Table 2**
Definition and distribution of explanatory variables at the sample level after variable analysis of *Campylobacter* shedding by sows (quantitative variable were divided in categories) (530 samples from 53 farrow-to-finish farms, in Brittany, France, 2008).

| Definition of variables for the sample | % of samples per level | % of positive sample per level |
|---|---|---|
| Season | | |
| Winter | 35.8 | 14.7 |
| Spring | 20.8 | 30.0 |
| Summer | 22.6 | 33.3 |
| Fall | 20.8 | 29.1 |
| Number of sows potentially sampled | | |
| 10 | 59.1 | 24.9 |
| More than 10 | 40.9 | 25.3 |
| Sows in individual housing | | |
| No | 15.8 | 11.9 |
| Yes | 84.2 | 27.9 |
| Age of the animal sampled | | |
| At least one gilt among the sampled sows | 53.2 | 26.2 |
| No gilt | 46.8 | 23.8 |
| Stage of sows at which samples taken | | |
| Gestation area | 65.1 | 28.2 |
| Maternity | 20.4 | 22.2 |
| Service area | 14.5 | 18.8 |
| Floor | | |
| Fully slatted floor | 73.0 | 26.9 |
| Partially slatted floor or solid floor | 27.0 | 20.2 |
| Room managed according to an “all-in-all-out” system | | |
| No | 72.3 | 25.8 |
| Yes | 27.7 | 23.1 |
| Feed | | |
| Granules or pellets | 13.2 | 25.7 |
| Meal | 39.6 | 22.4 |
| Soup | 47.2 | 27.2 |
| Origin of feed | | |
| Commercial compounds | 83.2 | 24.9 |
| Home milled or mixed | 16.8 | 25.8 |
| Feed/water supplement (ex: organic acid) | | |
| No | 72.8 | 22.0 |
| Yes | 27.2 | 33.3 |
| Use of antibiotic in the last 3 months | | |
| No treatment | 79.2 | 25.0 |
| Yes in water or by injection | 20.8 | 25.4 |
| Time between sampling and bacteriological analysis | | |
| One day | 47.2 | 30.8 |
| Two days | 32.1 | 18.2 |
| More than 2 days | 20.8 | 22.7 |

A few colonies from the bacterial culture were suspended in 200 μl of TE buffer (10 mmol $l^{-1}$ Tris–HCl, 1 mmol $l^{-1}$ EDTA, pH 7.6) for PCR analysis. The remaining colonies were used for genotyping by pulsed-field gel electrophoresis (PFGE), as indicated below.

*2.3.2. PCR analysis*

DNA extraction was done by blowing the cells out by placing the samples at 95 °C for 10 min. After low centrifugation (5000 g for 2 min), 10 μl of the supernatant were diluted in 90 μl TE buffer.

Multiplex-PCR, as described by Wang et al. (2002), was used to confirm the genus of the bacterial isolates and to identify them to species level. This multiplex-PCR was used in our study for identification of the following five *Campylobacter* species: *C. jejuni*, *C. coli*, *C. lari*, *C. fetus*, and *C. upsaliensis*. Five μl of DNA were used for amplification. PCR products were visualized by the electrophoresis of 10 μl aliquots of each amplification product, for 3 h at 100 V, in a 2% agarose gel stained with ethidium bromide.

*2.3.3. 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, based on band positions, were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for *Kpn1*- and *Sma1*-digested DNA (KS) was constructed, to reflect the similarities between the strains in the matrix. Strains were clustered by the unweighted pair-group method using the arithmetic mean (UPGMA) (Struelens, 1996).

The Simpson's index ($D$) was determined as described by Hunter (1990), and is given with a 95% confidence interval, as described by Grundmann et al. (2001). This index was used to assess the genetic diversity of the *Campylobacter* populations:

$$D = \frac{1 - 1}{N(N-1)} \sum_{j=1}^{s} nj(nj - 1)$$

$N$ is the number of isolates tested; $S$ is the number of different genotypes; $nj$ is the number of isolates belonging to type $j$.

Isolates displaying high levels of similarity were considered to originate from the same parental strain and were clustered together using a threshold of 80% (Denis et al., 2008).

*2.4. Identification of risk indicators*

*2.4.1. Definition of the outcome variable*

The epidemiological unit was the farm, whereas the statistical unit was the sample, the farm being considered as a repeated effect. Data from farm were individually associated with the 10 samples from the same farm. The outcome variable was thus dichotomous (positive versus negative sample).

*2.4.2. Definition of explanatory variables*

The explanatory variables studied are listed in Tables 1 and 2.

In the first step, quantitative variables were transformed into categorical variables. All variables were coded categorically. The number of categories per variable was limited so that each category had a frequency ≥10%.

In the second step, Generalized Estimating Equations (Liang and Zeger, 1986) including repeated effects was used to assess the relation between the *Campylobacter* status of the sample to each explanatory variable. Only

factors associated ($p < 0.20$) with the outcome variable were selected.

Then, all bilateral relationships between variables were checked ($p < 0.05$). For bilateral relationships between variables displaying strong structural collinearity, one of the two variables of interest was chosen (the one we believed easier to explain in relation to the outcome variable).

*2.4.3. Statistical procedure*

Generalized Estimating Equations including repeated effects were used, with a binomial probability distribution and a logit link function. Each explanatory variable was introduced in the model as a fixed effect. The farm number was incorporated in the model as a repeated measurement factor (ten times because 10 samples per farm) in order to take into account the within-farm covariability. Moreover, overdispersion was checked and all the statistics were adjusted appropriately.

The overall significance of the link between each explanatory variable and the outcome variable was performed through Wald statistics for Type III GEE analysis. Odds ratio estimation was calculated. Generalized Estimating Equations were computed with the GENMOD procedure of the SAS software (SAS, 2004).

## 3. Results

*3.1. Campylobacter shedding*

In this study, 530 feces samples were analyzed and 133 samples were tested positive for *Campylobacter*. The multiplex-PCR described by Wang et al. (2002) identified all isolates as *C. coli* isolates. No isolates of *C. jejuni*, *C. lari*, *C. fetus* subsp fetus, or *C. upsaliensis* were identified.

Finally, 25.1% $_{95\%CI}$ [20.8–29.3] of the 530 samples from sows were tested positive for *C. coli* and at least one of the ten samples taken was positive for *C. coli* in 37 farms among the 53 farms (70% $_{95\%CI}$ [56–83]). 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. In 8 farms, only one sample was positive in *Campylobacter*. The bacteria was detected in 18.8%, 28.2%, and 22.2% of the fecal samples collected at the service area, the gestation area and the maternity, respectively. Doing sampling at these 3 sites allowed to have the real situation of excretion of *Campylobacter* by the sows in these farms.

*3.2. Risk indicators*

Four risk indicators were identified. Three of these indicators were significantly associated with *Campylobacter* shedding by sows and the remaining factor was associated with the bacteriological analysis of the samples (Table 3).

The sampling season of the farm was concerned. Fecal samples collected in spring, summer and fall, were more likely to be positive for *Campylobacter* than those collected in winter.

**Table 3**
The final model for risk indicators for *Campylobacter* shedding by sows (530 samples from 53 farrow-to-finish farms, in Brittany, France, 2008).

| Variables | OR | 95% CI |
|---|---|---|
| Season | | |
| Winter | 1.0 | – |
| Spring | 2.5 | 1.6–4.1 |
| Summer | 2.3 | 1.6–3.2 |
| Fall | 1.9 | 1.5–2.4 |
| Number of sows on the farm on the day of sampling | | |
| <130 | 1.0 | – |
| 130–190 | 2.7 | 1.8–4.0 |
| >190 | 2.5 | 1.6–3.9 |
| Sows in individual housing | | |
| No | 1.0 | – |
| Yes | 2.6 | 1.6–4.3 |
| Time between sampling and bacteriological analysis | | |
| One day | 1.0 | – |
| Two days | 0.6 | 0.4–0.7 |
| More than 2 days | 0.7 | 0.5–0.9 |

One indicator was related to the size of the holding. Farms with more than 130 sows and farms with more than 2500 fattening pigs had a greater risk of *Campylobacter* excretion by sows than farms with fewer sows and fattening pigs. Because number of sows was correlated with number of fattening pigs in a farm, only results for sows are presented in Table 3.

We also found that individually housed sows were 2.6 $_{95\%CI}$ [1.6–4.3] times more likely to excrete *Campylobacter* than sows kept in groups.

Finally, the time between sampling and the start of the *Campylobacter* detection process also played an important role. If this interval was longer than one day, the chance of detecting *Campylobacter* in the samples was almost halved.

*3.3. Genetic diversity*

Typing by pulsed-field gel electrophoresis generated 119 *Kpn1*- and *Sma1*-digested DNA profiles from the 133 *C. coli* isolates (Fig. 1). Among the isolates, the genome of 12 isolates resisted to restriction by *Kpn1* and that of one isolate resisted to restriction by *Sma1*. These profiles were coded ND for "not digested" (data not shown).

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.

In 10 cases, isolates shared the same genotype. In 7 of these cases, the isolates with identical genotypes were obtained from the same farm. In only 3 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, isolate 08MD0437 (farm no. 272) and isolate 08MD0452 (farm no. 282), on the other, and finally, isolate 08MD0139 (farm no. 120) and isolate 08MD0169 (farm no. 122).

With a cut-off value of 80%, 54.6% of the isolates were grouped into 11 clusters (indicated by black spots on the dendrograms). These clusters bring together isolates from different farms not associated with a particular farm characteristic or particular management practices.

Diversity of genotypes from sows inside a farm could be high. The number of genotypes varied from one to eight. In

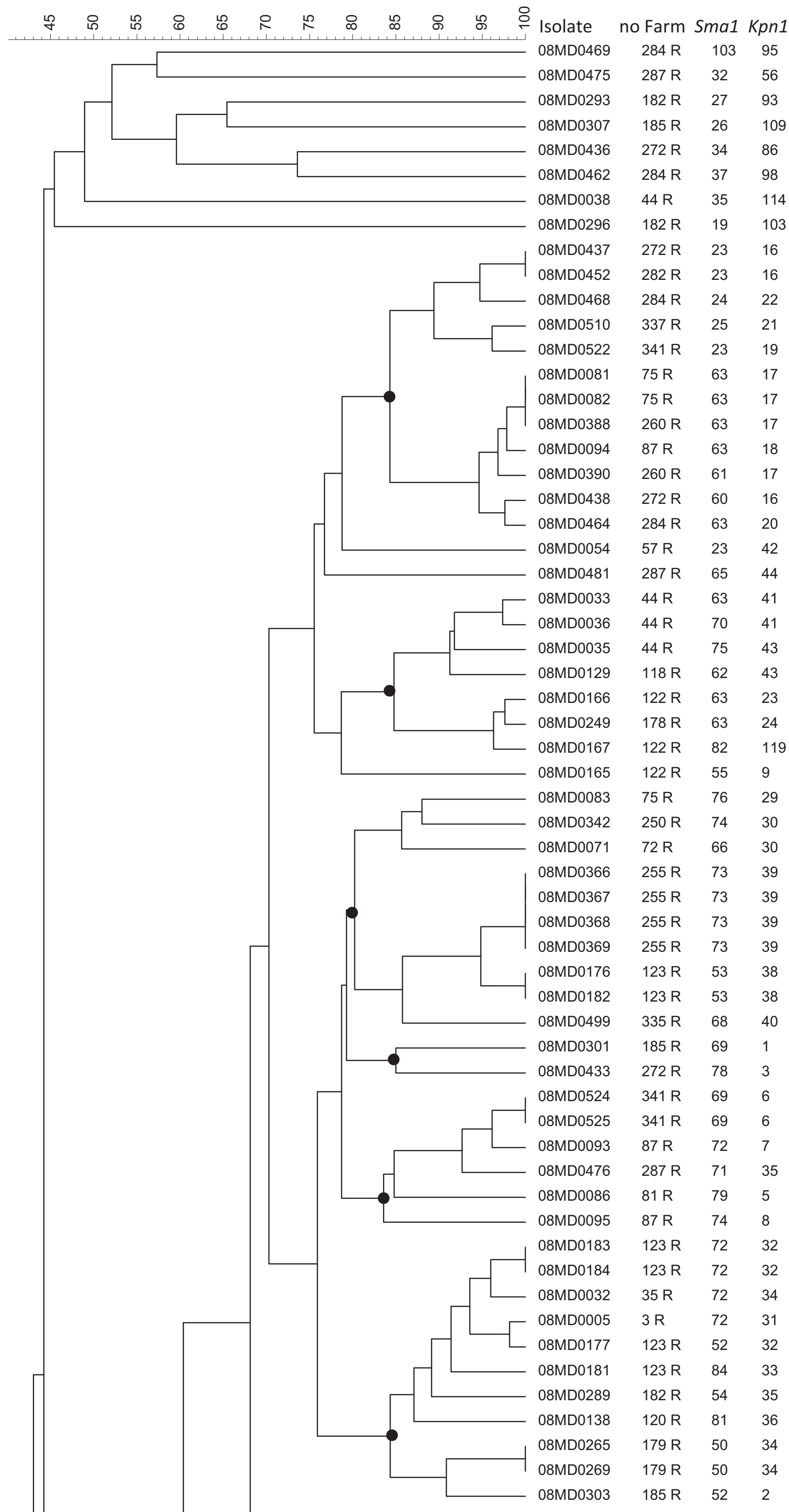


**Fig. 1.** Dendrogram of the *Sma1-Kpn1* profiles of *Campylobacter coli* from sows. No farm: code of the farm from where isolate was detected.

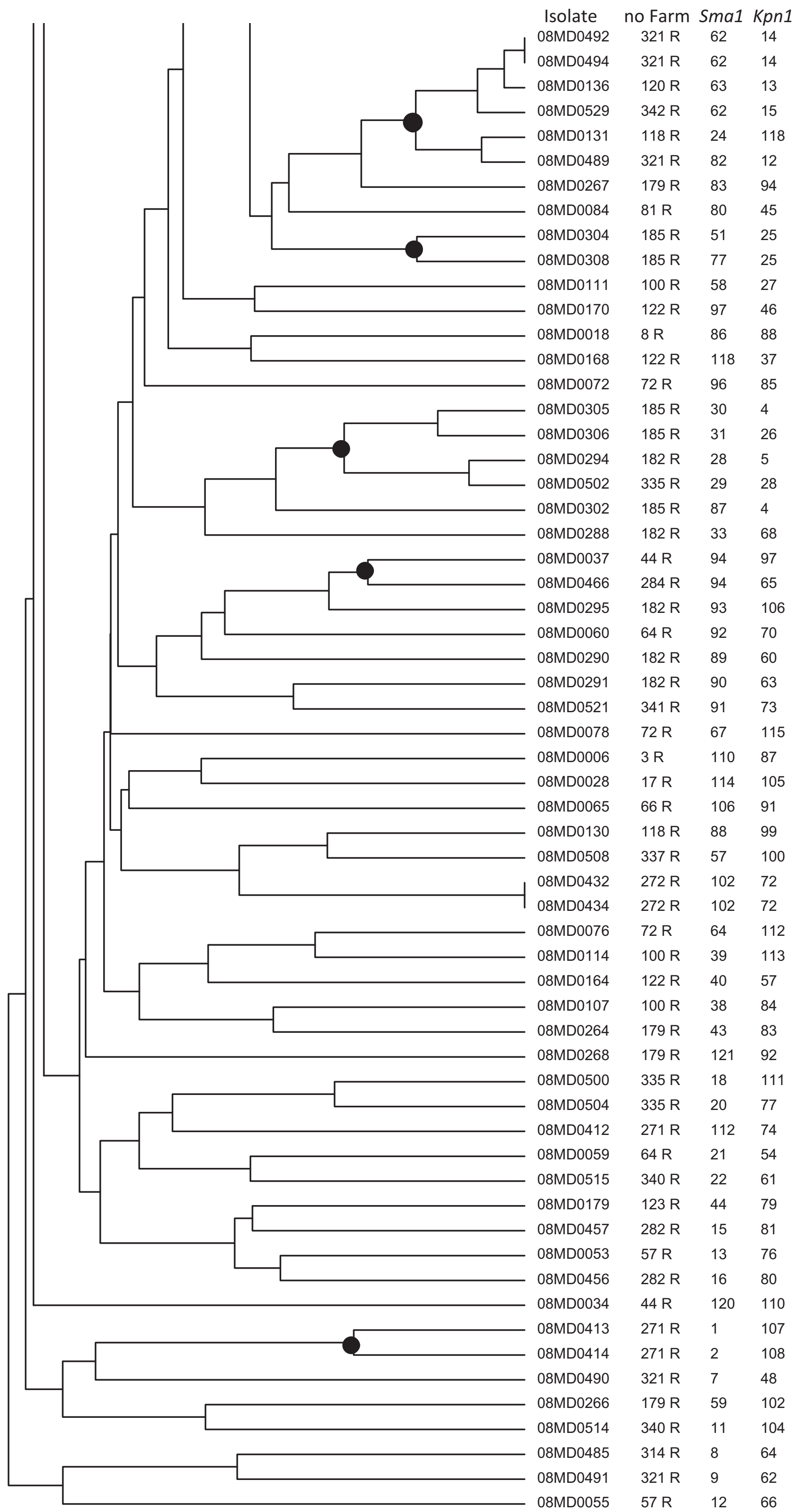


Fig. 1. (*Continued*).

16 farms, only one or two genotypes were found. In 14 farms, 3–5 genotypes were identified, and in 7 farms more than 6 genotypes.

## 4. Discussion

In our study, 25.1% of feces samples from sows were positive for *Campylobacter*. *C. coli* was the only *Campylobacter* species present. Our results are similar to those of previous French studies (Payot et al., 2004; Magras et al., 2004; Minvielle et al., 2007). Leblanc-Maridor et al. (2008) showed that if pigs were orally inoculated simultaneously with *C. jejuni* and *C. coli*. *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. Wehebrink 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 close to 52.9% as 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; Denis et al., 2009). Only in three cases isolates with the same genotype came 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 *C. coli* from 79% of fecal samples taken from sows on nine French farms.

An effect of season on the contamination of farms with *Campylobacter* was observed, the results for winter being significantly different from those of the other seasons. This factor was identified as a risk factor by Réfregier-Petton et al. (2001) and Huneau-Salaün et al. (2007) in poultry farms located in Brittany, France. The presence of a large number of pig animals on the farm was identified as a risk indicator for *Campylobacter* colonization. Similar findings have been reported for poultry farms (Réfregier-Petton et al., 2001) with several buildings. One of the assumptions emitted is that it is more difficult to respect hygiene practices and to be rigorous in the procedures of cleaning and disinfection of the rooms in farms which contain a great number of pigs.

The housing of sows in individual housing, rather than in group housing, was also identified as a significant risk indicator for *Campylobacter* contamination. One study (Weijtens et al., 1999) showed that pigs excrete *Campylobacter* intermittently. When sows are housed individually, this separation, together with the intermittent nature of excretion, might prevent the spread of *Campylobacter*. However, a recent study (Leblanc-Maridor et al., 2008) showed that separating *Campylobacter*-free animals from animals excreting *Campylobacter* intermittently did not prevent the passage of the bacterium between these two groups of animals. These findings provide support for the fact that animals housed in individual stalls can excrete the bacterium, but do not explain why individual housing is a risk indicator for *Campylobacter* excretion. This variable is perhaps related to a practice in the farms which was not listed in our questionnaire or was significant by chance.

If there were at least two days between sampling and sample analysis, the likelihood of a positive result was found to be only half that for an interval of one day. The transfer and maintenance of samples at room temperature (corresponding to a stressful situation) may play on our results. Indeed, Buswell et al. (1998) observed that the survival of *Campylobacter* in water varied considerably, from a few days at room temperature to weeks, or even months, at 4 °C. The transfer and storage of samples at low temperature should therefore be considered for studies of this type.

This study provides recent valuable information on the occurrence of *Campylobacter* in sows and first tentative explanations of excretion of *Campylobacter* by sows in farrow-to-finish farms. This study limited to 53 farms in Brittany, France. A larger study would be required to confirm these findings and to identify other risk indicators, taking into account the transport conditions of the samples.

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