SOURCES OF *CAMPYLOBACTER SP.* CONTAMINATION OF PIGLETS IN FARROWING UNITS OF FARROW-TO-FINISH FARMS: FIRST RESULTS

Catherine Magras[1], Nathalie Garrec[1], Michel Laroche[1], Albert Rossero[1], Clémentine Mircovich[2], Marie-Hélène Desmonts[3],Michel Federighi[1]

UMR -INRA 1014 SECALIM, National Veterinary school of Nantes, Nantes, France ; [2]Institut Technique du Porc, La Motte au Vicomte, Le Rheu, France ; [3]Aérial, Schiltigheim, France.

Introduction

Campylobacter sp. is one of the most frequent cause of human enteritis with *Campylobacter jejuni* more commonly implicated than *C. coli*. *Campylobacter* sp. has been isolated from raw beef, pork, limb, chicken and cooked meats. Campylobacters are often found in digestive tract of pigs [2, 8, 12, 14, 10, 3, 15, 4, 6]. *C. coli* is the large predominant species [12, 14, 4, 6] but *C. jejuni* was also isolated in association with *C. coli* [3, 15, 9]. *Campylobacter* colonization of the pigs seems to occur at an early age [3, 13, 15]. In France, little information about intestinal carriage of *Campylobacter* sp. in pigs is available. The purpose of the present investigation was to improve our knowledge of the epidemiology of *Campylobacter* in pigs.

Materials and methods

Sampling (table 1): The samples were collected from 9 pigs farms, randomly selected, situated in the western part of France. The farms were confined farrow-to-finish operations of intensive type and managed using the batch procedure and an all-in/all-out hygiene policy for farrowing, post-weaning and fattening sections. Three batches per farm were tested over a year. For each batch, 10 nursing dams randomly selected and 4 piglets from litters were tested for Campylobacter. Rectal fecal samples were collected once from piglets and the nursing sows. In addition, in 6 farms at each visit, water and piglets feed samples were taken.

Bacteriological analysis : All samples were transported to the laboratory at < 10°C. For water and "food" samples, the presence or absence of *Campylobacter* in each sample was tested by selective enrichment in Preston broth. For all the samples, some drop of each suspension were plated in duplicate on each following media : Butzler agar, Karmali agar. Agar plates were incubated at 42 °C for 5 days in microaerophilic conditions (5% O_2, 10% CO_2, 85 % N_2), and examined daily for presumptive *Campylobacter* colonies. Suspect colonies were confirmed as a member of the genus by examining motility, Gram staining and morphology. Three *Campylobacter* colonies per sample (when present) were randomly selected for genetic typing and stored at -80°C until further use.

Molecular analysis : Each isolate was identified as *Campylobacter jejuni*, *C. coli* or *Campylobacter sp.* by PCR. Multiplex PCR was performed using primers and conditions previously described by van de Giessen *et al* [11] . The PCR-RFLP typing method was used in order to obtain additional information about infection cycles of *Campylobacter* on the farm. For this analysis, all the isolates of one farm (farm A) were typed. The *fla*A gene encoding flagellin A was amplified with a pair of primers previously described by Nachamkin *et al.* [5] and Chuma *et al.* [1]. The 1700 bp product was digested overnight at 37 °C with the restriction enzyme DdeI according to the manufacturer's instructions. The digest products mixed with loading buffer, and PCR 100 bp ladder, were then electrophoresed in TAE buffer for 45 min at 100 Volt, 400 mA, through a 2 p.100 agarose MS-8 type gel with 1µg/mL ethidium bromide (Euromedex). The analysis of the gel electrophoresis image was done with Bio 1D++ software (Vilbert Lourmat).

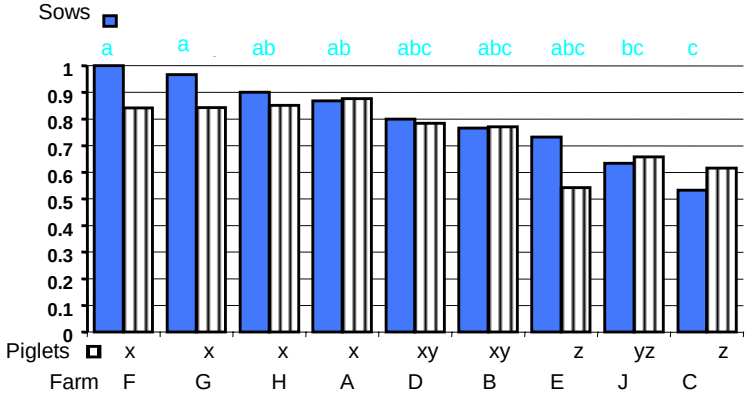
Statistical analysis : Data were analysed with SAS sofware (SAS Institute) using SAS analysis of variance (PROC GLM). A pig tested positive if it gave at least one isolate of *Campylobacter* using the isolation procedure described above. Data analysis were performed utilizing Chi square and Student-Newman-Keuls' test (SNK).

Table 1 : Characteristics of the 9 farrow-to-finish farms and of the sampling during the three visits in each farm.

	9 farms				
	F	D, I, B, H	C, E	G, A	
Number of sows	70	100 to 150	220 to 230	450	
Fattening pigs produced/year	1600	1700 to 2800	4000	9500	*Total samples analysed*
Sows tested/farm	3 X 10 (except farm F only 7 sows/batch)				**261**
Piglets tested/farm	3 X 4 X 10 (7) 24,9 (σ = 1,1) days				**1036**
Water tested/farm	3 X 2 (farms A, B, C, D, E, F)				**6 L/farm 6 farms**
3 dry feed samples/farm	3 x 3 x 25g (pellets, meal) (farms A, B, C, D, E, F)				**75 g/type of food/farm 6 farms**

Results

Campylobacter was not detected from water samples and feed samples. On all the 9 farms, pigs were heavily contaminated by *Campylobacter*. *Campylobacter* was recovered from 75 % of the faecal samples collected from the 1036 piglets and 79 % from the 261 sows. Nevertheless some differences between the 9 farms in the number of pigs tested positive for *Campylobacter* were statistically significant (p < 0.005 SNK test) (fig.1).



Values with different letters are significantly different (P<0.005)-SNK test

Fig. 1. Prevalence of *Campylobacter coli* in piglets and their nursing sows from [illegible] farms.

The contaminated sows had more contaminated piglets than negative sows (fig. 2). A total of 1.100 isolates were obtained. On the basis of identification with multiplex-PCR, *C. coli* was the only species recovered from the faecal samples. First results of PCR-RFLP subtyping showed a large diversity of *Campylobacter* subtypes isolated from the pigs (farm A). Nevertheless piglets and their nursing sows in a same batch often harboured *Campylobacter* isolates with identical genetic subtyping profiles (fig. 3).

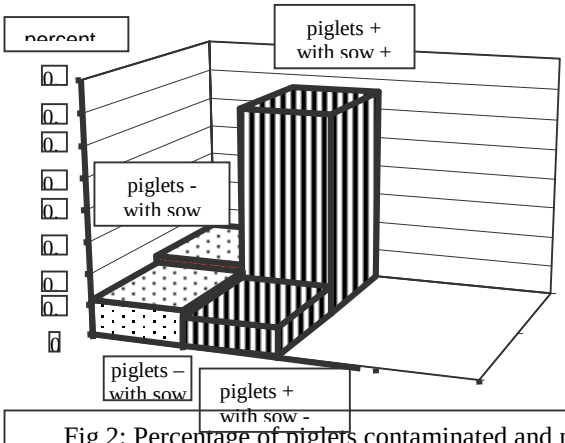


Fig 2: Percentage of piglets contaminated and not contaminated according to the status of their nursing

Discussion

The high prevalence rates reported in this study agree with other results indicating prevalence of *Campylobacter* of 85 % amongst piglets [14, 15] and at 75 to 100 % amongst sows [14, 15]. Young *et al.* [15] have described a predominant infection of pigs by *C. jejuni*. In our study and in agreement with Nesbakken *et al.* [6], *C. jejuni* had never been isolated from the faecal samples. These findings suggest that the prevalence of the respective species might differ considerably between breedings companies and countries. An other explanation may be the use of different identification procedures. This study confirms that piglets are already intestinal carriers of *C. coli* at the age of 25 days on the piggeries. The direct transmission of *C. coli* from the infected sows to piglets is attested by PCR-RFLP results. The similarities between genetic subtyping profiles of strains isolated from families of pigs (nursing sow and her piglets) and from subsequent groups of pigs housed in a same batch suggest that *C. coli* strains isolated are more dependent on the origin of contamination (sows) than on the farm [14]. Nevertheless, some genetic subtyping profiles are different between sow and her piglets in a same family of a batch [4, 14], and, some piglets were tested positive although their nursing sow was tested negative. This suggests that other sources of piglets contamination by *C. coli* than the nursing sows exist. Despite negative results in our water and feed samples analysed, the piglets environmental source (other sows of the batch and of the farm, hygienic practices of the farmer …) of contamination by *Campylobacter* sp. can not be exclude [7]. But adoption practices appear to be a major risk factor in the dissemination of *C. coli* into the farm [3, 13]. Contrary to other studies, focused on only 1 or 2 farms [14, 3], our survey reveals that there is a significant distinction between the level of contamination with *Campylobacter* of the pigs on these farms.

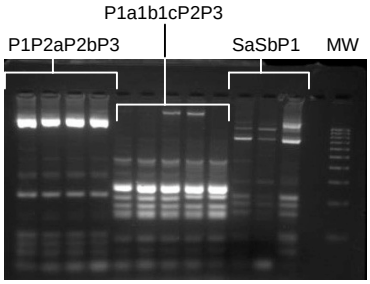


Fig. 3. Examples of PCR-RFLP *Campylobacter* subtypes isolated from piglets (P) and sows (S) of a same batch (legends: MW=molecular weight ; a to c= three isolates/animal)

Conclusion

Further studies are needed to identify risk factors in the dissemination of *Campylobacter sp*. in farms and to evaluate the impact of this infection of pigs on the meat and process contamination.

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