Dynamic of *Campylobacter* infection within pig farms from sows to fattening pigs

Leblanc-Maridor M. [*,1,2], Denis M. [3,4], Chidaine B. [3,4], Belloc C. [1,2]

[1]*INRA, UMR1300 Biology, Epidemiology and Risk Analysis in animal health, F-44307 Nantes, France*

[2]*LUNAM Université, Oniris, Nantes-Atlantic College of veterinary medicine and food sciences and engineering, UMR Bio-EpAR, BP 40706, F-44307 Nantes, France*

[3]*Anses, Unité Hygiène et Qualité des Produits Avicoles et Porcins, BP 53, 22440 Ploufragan, France*

[4]*Université Européenne de Bretagne, France*

**Corresponding author* : mily.leblanc-maridor@oniris-nantes.fr

Abstract

This work aimed at (i) describing *Campylobacter* excretion by conventional pigs in field conditions from sows to fattening pigs, and (ii) assessing the role of the environment as a source of pig contamination. Five sows and six piglets per sow were individually followed in two farrow-to-finish farms. Faecal shedding, contamination of pens (empty or with animals), feed and water were monitored from birth to finishing for pigs and during one production cycle for sows. *Campylobacter*, mainly *C. coli,* was highly prevalent for sows and their piglets. All the sows excreted *Campylobacter*. Piglets became infected early: 84 and 86% of them excreted *Campylobacter* three to five days after their birth. The prevalences increased progressively through the rearing period with some young pigs becoming punctually negative. The amount of *Campylobacter* in faeces was highly variable between pigs (from 0 to 10^9 CFU/g of faeces) and between sampling times for a given pig, with sometimes no detection. Samples of the pigs' environment during the down period were always negative and became positive when pigs were housed in the pens. Nevertheless, no correlation was established between the excretion level of the pigs and the contamination level of their environment. Some samples of feed, initially free of *Campylobacter*, became positive due to contamination by faecal material. Finally, our study underlines the role of the sows as a *Campylobacter* contamination source for their piglets.

Introduction

Campylobacter, a major cause of food-borne human infection, is commonly carried in the intestinal tract of a wide range of animals, including livestock animals, without causing clinical signs (EFSA, 2007). Pigs are known to be frequently infected by *Campylobacter* with prevalence between 50% and 100% and excretion levels ranging from 10^2 to 10^7 Colony Forming Units (CFU) of *Campylobacter* per gram of faeces (Alter *et al.*, 2005). For the implementation of control measures in farms, dynamics of *Campylobacter* excretion and sources of contamination have to be known. Previous studies describe that piglets became infected by their mothers within the first weeks of life (Hume *et al.*, 2002). Other sources could be suspected among which the environment (Alter *et al.*, 2005). This work aimed at (i) describing *Campylobacter* excretion (presence/absence, main species and quantity) by conventional pigs in field conditions along one production cycle from sows to fattening pigs and (ii) assessing the role of the environment as a potential source of pig contamination.

Material and Methods

Farms and animals individual follow up

This study was carried out in two farrow-to-finish farms. In each farm, five sows from the same batch have been followed along one production cycle (before farrowing and until the slaughter of their offspring). After farrowing, 6 piglets per sow have been individually followed from their first week of life until their departure to the slaughterhouse. Sows were monitored weekly 2 weeks before farrowing. Piglets and sows were followed weekly from the first week to 5 weeks of age then growing pigs were monitored every three weeks until slaughter whereas the sows have been sampled at the 8th, 11th and 17th weeks after farrowing.

Samples and analysis

Fresh faecal samples from sows and young pigs were collected individually and 10 g of each were diluted to 1:10 in Preston broth for detection and enumeration. For the environment, surface swabs (floors, walls, structures) were collected from each pen and from the floors and walls around each pen (i) in empty rooms after cleaning/disinfection (during the down period) and (ii) in presence of the animals. Surfaces swabbed were measured to detect and quantify the number of *Campylobacter* per square meter. Swabs were humidified with 45ml Preston broth. Enumeration for all the samples was done using a 10-fold serial dilution on Karmali plates.

Presence/absence of *Campylobacter* was checked in feed and water. Feed samples were collected before the distribution to the animals (in bags or silos) and in presence of pigs (into the trough of each studied pens).Water samples were collected upstream to the distribution network and at the faucet in each unit (service, gestation, farrowing, post-weaning, fattening).

Species of isolates were identified by real-time PCR (Leblanc-Maridor *et al.*, 2011).

For the statistical analysis, the correlation between the contamination level of the environment and the excretion level of *Campylobacter* by the animals was estimated.

Results

For one farm (Farm I), all the faecal samples from sows were positive for *Campylobacter* whatever the sampling times while in the second farm (Farm II), no *Campylobacter* were detected in the faecal samples of one sow at three different sampling times. Level of *Campylobacter* excretion varied from less than 10^2 to 10^7 CFU of per gram of faeces, and varied between sows at one sampling time and also for a given animal at different sampling times.

At 3 to 5 days after birth (first faecal sample), 84% and 86% of the piglets (respectively Farm I and Farm II) excreted *Campylobacter* (Figure 1). These prevalences increased progressively through the rearing period except at three weeks of age where only 60 % of the piglets excreted *Campylobacter*. Afterwards, prevalences were above 90 % with some pigs becoming punctually negative.

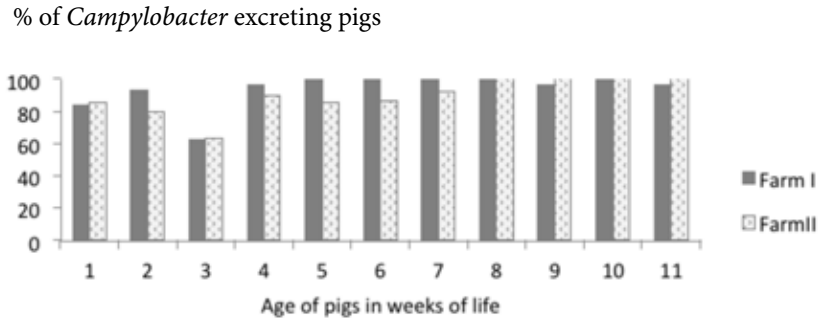

Figure 1: Evolution of the percentage of young pigs excreting Campylobacter in their faeces

The *Campylobacter* faecal excretion of young pigs was on average 10^5 to 10^7 CFU of *Campylobacter* per gram of faeces with a high variability in the quantities between two different samples for a given animal or between pigs at the same sampling time (from 0 to 10^9 CFU/g) (Figure 2). A decrease of the quantity of *Campylobacter* excreted by piglets was observed between the 4th and the 5th weeks of age for both farms after treatment with tylosine given to the piglets at the entrance into the post-weaning unit.

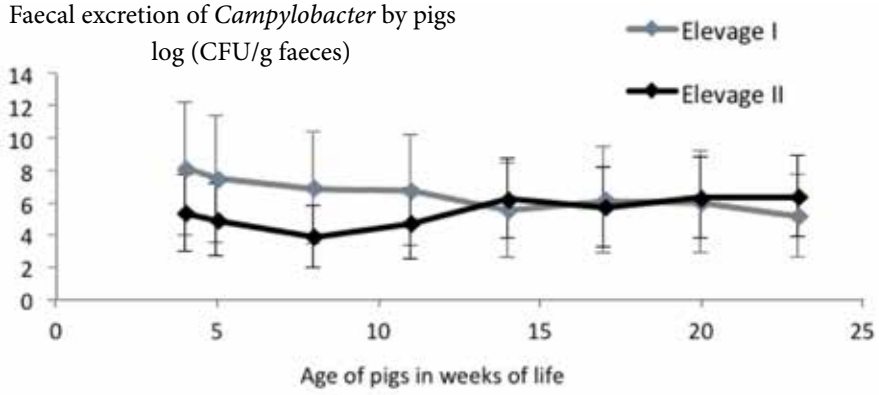

Figure 2: Faecal excretion of Campylobacter by young pigs

No *Campylobacter* was ever found in the environmental samples taken after the cleaning-disinfection process (without animals) as well as in the feed samples taken before distribution to the animals or in the water. When the animals were in the pens, feed samples and environmental samples were positive with a high variability of the contamination level (from less than 100 to 10^9 CFU/square meter). Concerning the environmental samples (walls or floors), the number of positive samples

increased with the age of the pigs. There is no significant correlation between the level of *Campylobacter* excretion by pigs and the contamination level of the environment, whatever the samples (walls or floors of the pens and food) (P>0.05).

All *Campylobacter* isolates from faecal samples of sows and pigs, environmental samples and feed were identified as *C. coli*.

Discussion

The objective of this study was to describe the dynamics of *Campylobacter* excretion in a conventional farrow-to-finish farm and to assess the role of the environment as a potential source of contamination. The originality of this study was the individual follow-up of the same animals all along a production Indeed, in the other studies, the comparisons of prevalence or excretion levels of *Campylobacter* between pigs at various stages have been done on different animals, at one sampling time or on a grouped way (Alter *et al.*, 2005; Weijtens *et al.*, 1993; Weijtens *et al.*, 1999).

In this survey, the high prevalence observed for sows throughout a production cycle is close to that found in the literature. Nevertheless, this result has to be taken with caution considering the low number of individuals sampled (5 sows per farm). Regarding the piglets, 84 and 86% of them are contaminated at the first sampling time, 3 to 4 days after their birth. This early contamination of piglets was similar to the results previously described (Weijtens *et al.*, 1997; Weijtens *et al.*, 1999).

In our study, the early contamination of the piglets seems to be due to the contact with their mothers as the environment was negative during the down period (after the cleaning and disinfection process) as well as feed and water of the farrowing unit. Moreover, sows excrete high quantities of *Campylobacter* which could promote the contamination of the piglets. The role of the sows as the first contamination source of *Campylobacter* for their piglets was previously described (Weijtens *et al.* 1999; Hume *et al.*, 2002; Alter *et al.* 2005).

Besides, the progressive increase of the prevalence of young pigs' infection is in accordance with the results of Weijtens *et al.* (1997). However, like Alter *et al.* (2005) we did not observed a decrease of the percentage of carrier pigs during the fattening period as observed by Weijtens *et al.* (1993). To our knowledge, the punctual decrease of prevalence (60% of the piglets) observed in both farms at the third week of life has not been reported previously. The origin of this decrease is difficult to explain and not linked to bias of bacteriological method as the positive sows samples have been treated at the same time.

For sows and for piglets, the amount of *Campylobacter* in faeces was highly variable (from 0 to 10^9 CFU/g). After the 5^{th} week of life, all young pigs became shedders until the end of the study with a level of excretion similar to those found in previous studies, except for one animal. Nevertheless, similar to previous findings (Weijtens *et al.*, 1999), variations in the average colony count of *Campylobacter* in the faeces between both animals and samples from a given animals were observed in our trial. *Campylobacter* could not be detected in one animal at one time point whereas high counts were observed in faeces from the same pigs at previous and following sampling times. This situation was previously described in an experimental trial (Leblanc-Maridor *et al.*, 2008). These observations suggest an intermittent excretion of *Campylobacter* or elimination followed by re-contamination of pigs by *Campylobacter* (Weijtens *et al.*, 1999).

No significant correlation has been found in our study between the contamination level of the environment and the excretion level of the pigs. However, the environment can play a role as source of indirect contamination for pigs, especially due to pig having frequent oral contacts with their environment. In this work, the resistance of *Campylobacter* in the environment in a pig farm seems limited as shown by a high number of negative environmental samples in the presence of the animals on dirty soil. In the same way, no positive environmental samples have been observed after the down period showing that good measures of hygiene between two batches (cleaning, disinfection, down period) allow the elimination of *Campylobacter* in the concerned rooms. The epidemiological role of the environment in the infection dynamics and the excretion pattern of *Campylobacter* by pigs might be limited if this environment is not constantly recontaminated by pigs faeces. Weijtens *et al.* (2000) underlined in a study that *Campylobacter* infection in pig farm can be reduced even eliminated through the implementation of strict hygienic measures associated with a repopulation with *Campylobacter* non-carriers pigs. Among the feed samples taken into the troughs in presence of animals, few were positive due probably to a contamination *via* the faecal material (the presence of faeces into the trough has been sometimes observed or strongly suspected). These results highlight that feed could play a role in indirect transmission of *Campylobacter* between pigs.

Conclusion

Our study confirmed the high prevalence of *Campylobacter* infection for sows and pigs in conventional farms all along a production cycle. The early contamination of the piglets could be mostly due to the contact with their mothers. Even if no significant correlation has been shown between the environment contamination and the excretion levels of *Campylobacter*

by the animals, the environment appeared frequently contaminated notably by faecal material (walls, floor and structures of the pen/room, feed in the troughs) and is probably an element of *Campylobacter* transmission between animals.

References

Alter, T., Gaull, F., Kasimir, S., Gurtler, M., Mielke, H., Linnebur, M., Fehlhaber, K., 2005, Prevalences and transmission routes of *Campylobacter* spp. strains within multiple pig farms. Vet. Microbiol. 108, 251-261.

EFSA 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006, Journal, T.E., ed. (EFSA), p. 130.

Hume, M.E., Droleskey, R.E., Sheffield, C.L., Harvey, R.B., 2002, *Campylobacter coli* pulsed field gel electrophoresis genotypic diversity among sows and piglets in a farrowing barn. Curr. Microbiol. 45, 128-132.

Leblanc Maridor, M., Denis, M., Lalande, F., Beaurepaire, B., Cariolet, R., Fravalo, P., Federighi, M., Seegers, H., Belloc, C., 2008, Experimental infection of specific pathogen-free pigs with *Campylobacter*: excretion in faeces and transmission to non-inoculated pigs. Vet. Microbiol. 131, 309-317.

Leblanc-Maridor, M., Beaudeau, F., Seegers, H., Denis, M., Belloc, C., 2011, Rapid identification and quantification of *Campylobacter coli* and *Campylobacter jejuni* by real-time PCR in pure cultures and in complex samples. BMC Microbiol. 11, 113.

Weijtens, M.J., Bijker, P.G., Van der Plas, J., Urlings, H.A., Biesheuvel, M.H., 1993, Prevalence of *Campylobacter* in pigs during fattening; an epidemiological study. Vet. Q 15, 138-143.

Weijtens, M.J., van der Plas, J., Bijker, P.G., Urlings, H.A., Koster, D., van Logtestijn, J.G., Huis in't Veld, J.H., 1997, The transmission of C*ampylobacter* in piggeries; an epidemiological study. J. Appl. Microbiol. 83, 693-698.

Weijtens, M.J.B.M., Reinders, R.D., Urlings, H.A.P., Van der Plas, J., 1999, *Campylobacter* infections in fattening pigs; excretion pattern and genetic diversity. J. Appl. Microbiol. 86, 63-70.

Weijtens, M.J.B.M., Urlings, H.A.P., Van der Plas, J., 2000, Establishing a *Campylobacter*-free pig population through a top-down approach. Letters in Appl. Microbiol. 30, 479-484.