

# Shedding of *Listeria monocytogenes* by sows in French farrow-to-finish pig farms: prevalence, serotype and risk factors of contamination

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## Abstract

This work was undertaken in 2008 to estimate the prevalence of *L. monocytogenes* in French farrow-to-finish pig farms at the breeding pig level and to determine risk factors of contamination of sows by *L. monocytogenes*.

A total of 730 feces (10 per farm) were sampled from sows in 73 pig farms. 172 samples were also taken during the fattening stage, at 43 of the 73 farms (4 per farm). Detection of *L. monocytogenes* was carried out according to the ISO 11290-1/A1 method and isolates were serotyped. Generalized Estimating Equations were used in order to determine risk factors associated to contamination of sows by *L. monocytogenes*.

For sows, 46.6% of the farms and 11.3% of the samples were positive for *L. monocytogenes*. The 83 positive samples provided a total of 125 strains. Serotype 1/2a, 1/2b and 4b were the most prevalent serotypes with 41.6%, 36.0% and 20.8% of the strains, respectively. Of the remaining isolates, 1.6% were attributed to serotype 1/2c. Moreover, the serotype 1/2a, 1/2b and 4b were found in 21, 17 and 11 farms respectively. The serotype 1/2c was detected in only one farm. In 20 farms, only one serotype was found. In 11 farms, 2 serotypes were identified, and in 3 farms until 3 serotypes.

The prevalence in the fattening rooms was estimated at 25% and *L. monocytogenes* was confirmed in 14.5% of samples. The 33 strains collected belonged to four serotypes: 1/2a(30%), 1/2b(43%), 4b(24%) and 1/2c(3%). The risk of fattening pigs excreting *L. monocytogenes* in their feces was higher on farms at which *L. monocytogenes* excretion by sows was observed (OR=33.51).

Different factors were associated to contamination of sows by *L. monocytogenes*: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of *L. monocytogenes*. This survey also showed that the sows were source of contamination by *L. monocytogenes* of finishing pigs.

## Introduction

In 2009, there were 1,645 reported cases of human listeriosis in the European Union, making *L. monocytogenes* the fifth most important zoonotic agent implicated in human enteritis, in terms of the number of cases (EFSA and ECDC, 2011). The number of listeriosis cases in humans increased by 19.1 % compared to 2008. Serotypes 1/2a, 1/2b and 4b are to those usually involved in human listeriosis in Europe (Goulet et al. 2008).

Pig and pork products have been identified as the main source of human infection by *L. monocytogenes*. In various industrialized countries, pork products were specifically involved in listeriosis (Goulet et al., 1998; De Valk et al., 2001; Thevenot et al, 2006).

In 2000-2001, Beloeil et al. (2003) detected *L. monocytogenes* in 14% in fattening pigs of the 93 French farms investigated. In this study, no search for this germ was realized on sows of these same farms.

This work was undertaken in 2008 to estimate the prevalence of *L. monocytogenes* in French farrow-to-finish pig farms at the breeding pig level and to determine risk factors of contamination of sows by *L. monocytogenes*. Distribution of serotypes was also considered.

## Materials and methods

**Samples and questionnaire.** Seventy-three farrow-to-finish Brittany farms were sampled in 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. A total of 730 samples were collected for sows.

Samples were also taken during the fattening stage, in 43 of the 73 farms. Four feces samples (representing each at least 10 pigs in the room) were taken in 4 different fattening rooms chosen at random in the farm. A total of 172 samples were collected for fattening pigs.

General data relating to the farm and management of the pigs were recorded (total numbers of sows and of fattening pigs, etc.). 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.).

Detection and serotyping. All the samples were analyzed for *L. monocytogenes* detection according to a modified protocol based on the standard NF EN ISO 11290-1 published in 2005. All the collected strains were serotyped with sera purchased from Eurobio (Les Ulis, France)

Statistical analysis from the questionnaire. The overall significance of the link between each explanatory variable and the outcome variable (presence/absence of *L. monocytogenes*) was performed through Wald statistics for Type III GEE analysis (Horton and Lipsitz, 1999). Generalized Estimating Equations were computed with the GENMOD procedure of the SAS 9.1 software.

## Results

For sows, 46.6% of the 73 farms and 11.3% of 730 the samples were positive for *L. monocytogenes*. The 83 positive samples provided a total of 125 strains. Serotype 1/2a, 1/2b and 4b were the most prevalent serotypes with 41.6%, 36.0% and 20.8% of the strains, respectively. All the remaining isolates were of serotype 1/2c (1.6%). Moreover, the serotype 1/2a, 1/2b and 4b were found in 21, 17 and 11 farms respectively. The serotype 1/2c was detected in only one farm. The number of serotypes per farm varied from one to three (table 1). In 20 farms, only one serotype was found. In 11 farms, 2 serotypes were identified, and in 3 farms until 3 serotypes

The prevalence in the fattening rooms was estimated at 25% (11 positive farms on 43). *L. monocytogenes* was confirmed in 14.5% of samples (25/172). The 33 strains collected belonged to four serotypes: 1/2a (30%), 1/2b (43%), 4b (24%) and 1/2c (3%). Among the 10 farms in which sows and fattening pigs excreted *L. monocytogenes*, the different serotypes (1/2a, 1/2b, 1/2c and 4b) found in the sows were also recovered in the fattening pigs from the same farm except for 3 farms in which serotype 1/2b was found in sows but not in fattening pigs.

Excretion of *L. monocytogenes* by fattening pigs was also recorded at 10 of the 17 farms on which *L. monocytogenes* excretion by sows was detected (table 1). The risk of fattening pigs excreting *L. monocytogenes* in their feces was higher on farms at which *L. monocytogenes* excretion by sows was observed (OR=33.51). Moreover, we observed that more the number of positive samples is high among the sows higher is the probability that fattening pigs shed *L. monocytogenes* (Table 1).

Table 1: distribution of the 43 farms according their status in *L. monocytogenes* at the sows and fattening levels.

N° of farms		Status of farms at fattening pig level		Total
Status of farms at sows level		negative	positive	
Negative		25	1	26
positive		7	10	17
N° of samples + on the 10	1 to 3	7	6	13
	More than 3	0	4	4
		32	11	43

Different factors were associated to contamination of sows by *L. monocytogenes*: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of *L. monocytogenes*.

Table 2: the final GEE model for risk factor for *Listeria* shedding of sows

Variables	OR	95% CI
<b>Origin of feed</b>		
Commercial compounds	1.0	-
Home milled or mixed	3.18	1.34-7.56
<b>Stage of sows at which samples taken</b>		
Maternity	1.0	-
Service area	4.21	1.48-11.97
Gestation area	4.46	1.82-10.94
<b>Use of antibiotic in the last 4 weeks</b>		
Yes	1.0	-
No	2.19	1.05-4.57
<b>Season</b>		
Spring / Summer	1.0	-
Fall / Winter	3.76	1.48-9.51

## Discussion

Our study showed that sows are a reservoir of *L. monocytogenes*. At the breeding pig level (sows), we found *L. monocytogenes* in 46.6% of the 73 farms and, 11.36% of the fecal samples collected were positive. Few works described excretion of *L. monocytogenes* by sows. A recent Canadian study (Farzan et al., 2010) explored the contamination of *L. monocytogenes* in different stages of production (finisher, sows and weanlings). They indicated that *L. monocytogenes* was not recovered from sow fecal samples and only infrequently found in the feces of weanling pigs and finisher pigs. Their results are similar to those of Esteban et al., (2009); no *L. monocytogenes* was detected in the 17 swine herds studied.

At the fattening level, 25.6% of our 43 farms were positive of *L. monocytogenes*, with 14.5% of the collected samples positive. This prevalence is twice higher than that observed by Beloeil et al., (2003). In this previous French study realized in 2000-2001 on 93 farms, 14% of the farms had their finishing pigs' excreted *L. monocytogenes*. However, these results didn't reach the same conclusion than Fosse et al. (2011). No *L. monocytogenes* was detected in the 127 pooled fresh feces collected from 37 batches of fattening pigs originated from the 14 French farms considered in their work. Nevertheless, their study used only a direct streaking on ALOA which is less sensitive.

Four serotypes were identified in these farms; the most dominant serotype was 1/2a, followed by 1/2b, 4b and 1/2c. Among these serotypes, three of them (1/2a, 1/2b and 4b) were also recovered from French pork-processing plants (Chasseignaux et al., 2001). However, the percentage of isolates with serotype 1/2c is higher on food products (Thevenot et al, 2006). Among the 10 farms in which sows and fattening pigs excreted *L. monocytogenes*, the different serotypes found in the sows were also recovered in the fattening pigs from the same farm. Moreover, in our study, we showed that the risk of fattening pigs excreting *L. monocytogenes* in their feces was higher on farms at which *L. monocytogenes* excretion by sows was observed (OR=33.51). This survey showed that the sows were source of contamination by *L. monocytogenes* of finishing pigs. This relation was not observed by Farzan et al., (2010).

Our work highlighted different factors associated to contamination of sows by *L. monocytogenes*: a food completely or partly made in farm, the production stages "service area" and "gestation area" and the period "autumn/winter". An antibiotic treatment during the 4 weeks before the sampling reduces the shedding of *L. monocytogenes*. In previous study (Beloeil et al., 2003), wet feeding during the fattening period was identified as a risk factor. This factor was not revealed in our study but we observed that from samples collected from pig provided in "wet feeding" the number of positive samples was about 11% when "wet feeding" was from commercial origin and about 35.3% when "wet feeding" was "food completely or partly made in farm". Among the different production stages, "maternity" seems to be more protector than the stages "service area" and "gestation area". Good hygiene practices could explain this result. However, we showed that an antibiotic treatment during the 4 weeks before the sampling reduces the shedding of *L. monocytogenes*. In our study, these two variables were linked; indeed 51% of the sows in "maternity" received an antibiotic treatment in the last 4 weeks before the sampling. Some earlier studies have reported that antibiotic treatment decreases carriage of *Salmonella* by pigs (Beloeil et al., 2007) and *Campylobacter* by poultries (Réfregier et al., 2001). The season fall/winter was identified as another risk factor in this study. *L. monocytogenes* may be subsisting in the environment because of its ability to grow and to survive at low temperature (Brisabois, 2008).

## Conclusion

This study provided recent valuable information on the occurrence of *L. monocytogenes* in French farrow-to-finish pig farms. This microorganism is prevalent in the sows (46.6%) and in fattening pigs (25%). Our study showed that sows are a reservoir of *L. monocytogenes* and could contribute to contamination of fattening pigs. Our work highlighted for some farms several serotypes suggesting several sources of contamination. Furthermore, the serotypes found in this study are identical to those usually involved in human listeriosis in Europe. The definition of risk factors of contamination of sows by *L. monocytogenes* can reduce the prevalence of *L. monocytogenes* in these farms and contribute to the overall reduction of human risk from consumption of pork products.

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