Investigating the genetic diversity of *Listeria monocytogenes* strains at the pig slaughterhouse to understand the source of strains isolated in the pork meat processing sector.

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Abstract
Listeria monocytogenes (*Lm*) is a pathogenic bacterium responsible for Listeriosis. In France, the genetic diversity of *Lm* is known in the pig farming and finished pork meat products compartment only. The aim of the study was (i) to investigate the genetic diversity at the slaughterhouse, based on MLST-clonal complexes (CCs) (ii) to compare it to the genetic diversity observed in the other compartments and better understand the strain contamination routes through the pig and pork chain. A total of 290 environmental swabbing in warm and cold areas, on food contact and non-food contact surfaces were performed in two slaughterhouses of the West of France. Overall, 115 strains were isolated and belonged to twelve different CCs. According to the virulence classification, four hypervirulent strains (CC1, CC4, CC54, CC207), five medium virulent strains (CC8, CC20, CC21, CC59, C155) and three hypovirulent strains (CC9, CC121, CC31) were identified. No hypervirulent CC strains were detected on non-food contact surfaces. Strain CC diversity was higher in warm areas (H'-index=1.83) compared to cold areas (H'-index=1.53). The strains of CC9 (38%) and CC121 (17%) were the most detected in both slaughterhouses. CC9 and CC121 strains, either rarely or never detected at pig farming, were shown to appear at the slaughterhouse, and strongly set up in cold areas (cutting plants and finished pork products processing). CC21 and CC59 strains were associated to warm areas while CC31 and CC155 to cold areas. Additional sampling campaigns are ongoing to consolidate these results.

Introduction
Listeria monocytogenes (*Lm*) is a ubiquitous pathogenic bacterium, transmissible to humans through the consumption of contaminated food. *Lm* is a major hazard along the value chain from pig farming to the pork industry. Contamination of pork products can occur at various stages, from primary production up to processing. Understanding the origin of these contaminations thus remains an important public health challenge. *Lm* is a genetically heterogeneous species, and most strains can be classified into major MLST-clonal complexes (CCs). Hypervirulent, medium virulent and hypovirulent CCs have been identified by combining epidemiological, clinical and experimental approaches (Maury et al., 2016) or risk assessment approaches (Fritsch et al., 2018). In a large –scale study, Felix et al (2018) analysed the population genetic structure of 687 strains isolated over the last 20 years in 86 of the 101 French departments. The two most prevalent strain CCs in the food-processing environment and finished food products were CC9 and CC121, rarely or never detected in pig farming. No strain isolated from the slaughterhouse and meat-cutting stage was part of the study panel. Complementary studies are then needed to understand the main source of contamination by these strains. The objective of the study was (1) to investigate the genetic diversity of *Lm* at the slaughterhouse and to compare it to other compartments of the pig and pork chain, (2) to identify the source of contamination of the CC9 and CC121 strains.

Materials and methods

Sampling was performed during production in two slaughterhouses A and B, located in the West of France, both including raw meat cutting plants areas. Each slaughterhouse was sampled three times over a 11-month period between March 2020 and February 2021. A total of 290 environmental swabbing in warm (n= 62) and cold areas (n=228), on food contact (cold n=112, warm n=50) and non-food contact (cold n=116, warm n=12) surfaces were analysed for the detection of *Lm.* Sampling sites in warm areas included the bleeding conveyor, floor drains, the white offal gutter, the red offal platform and the carcass opener. Sampling sites in cold areas included conveyors, knifes, blades, mesh gloves, floor drains, cold rooms walls and poles. The strains were isolated from Fraser half enrichment of the swab and then isolated on AL agar medium (BRD-07-16-01/09). One strain per sample was selected. The CC was obtained (i) by PFGE using the mapping PFGE/CC described in Félix et al. 2018 (ii) by a newly developed real-time PCR CC identification method (Félix et al. 2023). Strain virulence classification was based on the studies of Maury et al. 2016 and Fritsch et al. 2018.

Results and discussion

Overall, 115 strains were isolated and belonged to twelve different CCs: CC1, CC4, CC8, CC9, CC20, CC21, CC31, CC54, CC59, CC121, CC155 and CC207. Four hypervirulent strains CC1 (n=4), CC4 (n=1), CC54 (n=4) and CC207 (n=1), five medium virulent strains CC8 (n=3), CC20 (n=4), CC21 (n=1), CC59 (n=3) and C155 (n=14) and three hypovirulent strains CC9 (n=44), CC31 (n=11) and CC121 (n=20) were isolated. Five *Lm* strains could not be attributed with a CC. The strains of CC9 (38%) and CC121 (17%) were the most detected in both slaughterhouses. CC9 strains, almost exclusively associated with strains of molecular serotype IIc (Félix et al., 2018), were detected early in the slaughtering process at the carcass opening step, confirming existing data (Fravalo et al. 2013, Lariviere-Gauthier et al. 2014, Neira et al. 2015). CC121 strains were detected at the slaughtering in floor drains just before chilling. The strains CC31 (n=11) and CC155 (n=14) were abundant in cold environments in slaughterhouse A. Strain CC diversity was higher in warm areas (H'-index=1.83, n-strain=26, n-CC=8) compared to cold areas (H'-index=1.53, n-strain=89, n-CC=7). The chilling temperature in cold areas probably acts as a selective factor depleting the CC strains diversity. The average percentage of strain belonging to hypervirulent CCs in warm and cold areas in both processing plants were of 20.8% [9.3% -40.7%] and 4,6% [1.9% -11.3%], respectively. To visualise the transfer of CCs through the pig and pork chain, CCs genetic diversity observed at the slaughterhouse was compared to the one observed in pig farming and finished food products compartments (Félix et al. 2018) (Table 1). CC20, CC54 and CC207 strains were almost exclusively found in the slaughterhouse compartment. CC21 and CC59 strains were only observed in the warm areas (pig farming and slaughterhouse). On the contrary, CC31 and CC155 strains were only observed in cold areas (cutting plant and finished food products compartment). CC9 and CC121 strains, either rarely or never observed at pig farming, appeared at the slaughterhouse, and strongly set up in cold areas. CC9 strains have been described to be associated to the meat sector (Martin et al. 2014, De Cesare et al. 2017). Contrary to CC9 strains, CC121 strains have been described to be the most prevalent in all food sectors, except milk and milk products sector (Félix et al. 2018).

Hypervirulent CC1 strains appeared at pig farming and remained detected at low level in the following compartments. On the contrary, CC2 strains were only detected in the finished food products compartment. CC54 strains were only detected in warm areas of the slaughterhouse. At last, CC6 strains detected in the pig farming compartment were absent at the slaughterhouse but detected at low level in the finished food products compartment. No hypervirulent CC strains were detected on non-food contact surfaces. Additional sampling campaigns are ongoing to consolidate these results.

MLST CC mapped from PFGE cluster	Pig farming compartment	slaughterhouse/cutting plant		Finished food product compartment	
		Warm areas	Cold areas	Food production environement compartment	Finished food products
CC121		7,8	20,2	25	22,4
CC9	1,2	30,7	40,5	10,7	23
CC8	10,6		3,4	10,7	7,5
CC1	9,4	3,8	3,4	3,6	4,8
CC6	7,1			1,2	3,3
CC5	2,4			7,1	4,2
CC2					4,1
CC4			1,1		
CC4-CC217	1,2			1,2	2,7
CC54		15,4			
CC37	12,9			2,4	2,7
CC31			12,3	2,4	1,2
CC155			15,7	6	1,4
CC204				2,4	0,6
CC3				1,8	1,9
CC207		3,8			
CC59	8,2	11,5			0,6
CC7	1,2			3,6	1
CC14	1,2				1,9
CC77	11,8			1,2	1,2
CC224	8,2			2,4	0,8
CC11	3,5			4,8	0,8
CC18					0,4
CC193				1,2	1,5
CC101-CC90	1,2				0,4
CC21	2,4	3,8			
CC91					0,8
CC204					
CC20		15,4			0,4
Not assigned	1,2	7,8	3,4		0,6
Other PFGE clusters	16,5			9,5	10
Total	85	26	89	84	518

Table 1: Distribution of the mapped clonal complexes (CCs) through the pig and pork production chain

Conclusion

This study allowed to better understand the transmission of CCs of *Lm* through the pig and pork chain. The study of Félix et al. 2018 showed that CC9 and CC121 were almost absent in the pig farming compartment while they become highly prevalent in the pork food products. Here we showed that the settlement of these two CCs in the pig and pork chain starts in warm areas at the slaughterhouse and set up strongly in cold areas (cutting and cold storage rooms). This data raise questions regarding the source of CC9 and CC121 within the warm processing areas and how to hinder their settlement. Finally, the detection of hypervirulent clones in the slaughterhouse environment calls out on the transient or resident nature of these strains.

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