

030

## Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork meat production chain in France

Félix B.<sup>1</sup>, Feurer C.<sup>2</sup>, Mailliet A.<sup>1</sup>, Guillier L.<sup>1</sup>, Boscher E.<sup>3</sup>, Kerouanton A.<sup>3</sup>, Denis M.<sup>3</sup>, Roussel S.<sup>1</sup>

<sup>1</sup>ANSES, Laboratory for Food Safety Unit Salmonella & Listeria, Maisons-Alfort, France, <sup>2</sup>IFIP, French Institute for the Pig and Pork Industry, Le Rheu, France, <sup>3</sup>ANSES, Hygiene and Quality of Poultry and Pig Products Unit, Ploufragan, France

### Introduction

*Listeria monocytogenes* (*Lm*) is one of the main causative agents for foodborne infections in Europe in terms of severity of the illness and fatality rate (EFSA-ECDC, 2015). In France, listeriosis causes less than 0.1% of foodborne illnesses, but has the highest rate of mortality (20-30%) and hospitalizations (98.9%) among foodborne infections (Goulet et al., 2013; Van Cauteren et al., 2017). Meat products – and more specifically pork meat – are regularly reported as contaminated, with a prevalence of up to 12% in raw products (Roussel et al., 2010; Kerouanton et al., 2011). Understanding the origin of these contaminations remains an important public health issue.

*Lm* can survive for long periods of time in unfavorable environments that do not allow the strains to grow (Carpentier and Cerf, 2011). This factor makes its circulation difficult to trace. A better understanding of *Lm* genetic population structure may help to characterize the circulation routes.

In France, the major CCs responsible for clinical cases are present in food samples (Maury et al. 2016). In particular, CC1, CC2, CC4 and CC6 are strongly associated with a clinical origin and the most likely to cause disease, in particular human central nervous system infections or maternal-neonatal listeriosis (Maury et al., 2016). Other CCs, such as CC9 and CC121, are associated with food production sectors (Henri et al., 2016; Maury et al., 2016). The introduction sources of these CCs in the food supply chain are not well understood.

An overview of *Lm* genetic diversity, along the entire pig and pork production chain, is needed to improve food safety, identify the contamination routes and prevent human infections.

Here, we focused on 687 *Lm* strains isolated in France along the entire pig and pork production chain, from pig farming to finished food products. All strains were typed by pulsed-field gel electrophoresis (PFGE), and then assigned to an Multi-locus Sequence Typing Clonal complexes (CC), using a mapping

method specifically developed for this study. The distribution and prevalence of CCs in the different pig and pork production chain compartments were compared. Then, the CCs obtained were compared with those obtained from 1106 strains isolated from the other main food production sectors in France.

### Materials and Methods

#### Panel of 687 strains isolated from the pig and pork production chain

The strains were isolated, in 85 of the 101 French *départements*, from national studies (Roussel et al., 2010; Roussel et al., 2012, Roussel et al., 2014a) either research projects (Kerouanton et al., 2011) nor with corporate clients of IFIP or ANSES.

#### Strains from pig farming (PF), from the food processing environment (FPE) & from finished food products (FFP)

A total of 91 PF strains in majority isolated from the study of Boscher et al., 2012 in 2008 in the Brittany region (represented 58% of the French pig production in 2008). Eighty four FPE strains were isolated from surface sampling carried out in 37 food factories. Five hundred and eighteen FFP strains were isolated at the processing plant or at the point of sale. In this compartment, two groups were defined: (i) unprocessed meat (UM), including, fresh meat, minced meat and meat preparations (n=248); (ii) meat products (MP) including, non-heat-treated or heat-treated products (n=270).

#### Panel of 1106 strains isolated from other food production sectors

The strains came from five main food sectors: “Meat products” (excluding pork meat) (n=284), “Milk products” (n=287), “Fish and fishery products” (n=237), “Food products combining several food categories” (n=205) and “Fruit, vegetables, cereals and herbs” (n=67). Finally, 26 without assignment to a specific food sector.

#### PFGE typing & mapping MLST /PFGE

The PFGE and PFGE profile interpretation, PFGE MLST mapping & statistical analysis of distribution was performed according to Félix et al. 2018 methods.

### Results

#### Strain genetic diversity from pig farming and pork production (687 strains)

Comparison of the MLST clonal complex distributions between the three compartments

CC121 was not observed among the strains identified in the PF compartment, but was one of the most prevalent CCs in the FPE (25%) and FFP (22.4%) compartments (Figure 3). The distribution of CC121 was comparable in these two compartments (p-value > 0.27). CC9 was associated with the FFP, but not with PF or FPE compartments (p-value < 0.001). CC37,

CC77 and CC59 were associated with the PF, but not with the FPE or FFP compartments (p-value < 0.004). The distributions of CC8, CC1, CC5 (n > 30) and CC6, CC4-CC217 and CC7 (n < 30) were comparable in the three compartments (p-value > 0.038) (Figure 1).

**Finished food products**

The most prevalent CCs in the UM and MP groups were CC9 (29.0% and 17.4%), CC121 (20.4% and 24.4%) and CC8 (8.1% and 7.0%). The prevalence of CC9 significantly decreased between the UM and MP groups (p-value < 0.001).

**Strain genetic diversity compared between the pork sector and the other food production sectors**

The distributions of CC5, CC6 and CC2 were comparable between the Pork sector and the five other food production sectors (p-value > 0.038).

The distribution of the CC121 was comparable in the Pork sector and the Meat products, Food products combining several food categories and Fruit, vegetables, cereals and herbs sectors (p-value > 0.118). Compared with the Pork sector, the prevalence of CC121 was 10 times lower in the Milk products sector (p-value < 0.001), but one-third higher in the Fish and fishery products sector (p-value < 0.001) (Figure 2).

The distribution of CC9 was comparable between the Pork sector and the following two sectors: Meat products and Food product combining several food categories (p-value > 0.028). In contrast, CC9 was rarely found in the three other food sectors (p-value < 0.001) (Figure 2).

The prevalence of CC1, CC6 and CC4-CC217 was comparable in the Pork sector and in the four other production sectors (p-value > 0.026), except the Milk products sector in which these CCs were more abundant (p-value < 0.006) (Figure 2).

**Discussion**

First, this study aimed to understand the genetic diversity of *Lm* strains isolated along the pig and pork production chain in France and to compare it between the three compartments: pig farming (PF), food processing environment (FPE) and finished food product (FFP). To our knowledge, this study represents the largest and the most representative study ever performed in France.

One of the main results obtained here is that the major CCs of pork strains were not equally distributed among the three compartments. Three CC (CC37, CC59 and CC77) strains were rarely found in the FPE and FFP compartments, but were prevalent and associated with the PF compartment. CC37, the most prevalent CC in the PF compartment in our study, was frequently isolated in other studies dealing with primary production or wild environment (Linke et al., 2014, Dreyer et al., 2016, Haase et al., 2014).

CC37 is likely better adapted to pig farms than to the pork production environment.

Second, this study aimed to compare the genetic diversity between the Pork sector and the other food production sectors on a strain panel collected over 27 years of sampling, from hundreds of processing facilities and retail stores. Among the major CCs obtained, we distinguished three CCs (CC5, CC6 and CC2) considered ubiquitous, because they were found in comparable proportions in all sectors.

CC9 was predominantly isolated from meat products several European studies (Leong et al., 2017, Martin et al., 2014, Ebner et al., 2015 Nielsen et al., 2017, De Cesare et al., 2017). CC9 contamination was shown for mammalian meat production, regardless of meat type, suggesting that the contamination is likely not related to the primary contamination of livestock animals. Several studies report increased detection of CC9 strains at the slaughterhouse, after carcass dressing and prior to transfer to the ultraclean meat processing area (Fravalo et al., 2013; Lariviere-Gauthier et al., 2014; Neira et al., 2015). In contrast to CC9, CC121 was not associated with a given food sector. However, CC121 was the most prevalent in the Fish and fishery products, Pork and Meat products sectors, making this CC possibility related to the food processing after slaughtering that have in common ultra clean process.

**Conclusion**

The results obtained in this study led to a better understanding of the structure of the *Lm* population isolated from the pig and pork production sector. CC9 and CC121 are associated with food production, most likely because processing steps, such as slaughtering or stabilization treatments, favor their settlement and recontamination of the food produced. Both results indicate that processing steps are likely the source point of contamination.

Molecular serotype	85% PFGE cluster	MLST CC mapped from PFGE cluster	Pig farming compartment	Food production environment compartment	Finished food product compartment		
					Total	Unprocessed meat, including fresh meat, minced meat and meat preparations	Meat products including non-heat-treated and heat-treated products
Ila	A	CC121		25,0	22,4	20,2	24,4
Ilc	B	CC9	1,2	10,7	23,0	29,0	17,4
Ila	C	CC8	10,6	10,7	7,5	8,1	7,0
IVb	D	CC1	9,4	3,6	4,8	3,6	5,9
IVb	E	CC6	7,1	1,2	3,3	3,6	3,0
Ilb	F	CC5	2,4	7,1	4,2	3,6	4,8
IVb	G	CC2			4,1	2,4	5,6
IVb	H	CC4 - CC217	1,2	1,2	2,7	3,2	2,2
Ila	I	CC37	12,9	2,4	2,7	3,2	2,2
Ila	J	CC31		2,4	1,2	0,4	1,9
Ila	K	CC155		6,0	1,4	0,4	2,2
Ila	L	CC204		2,4	0,6	0,4	0,7
Ilb	M	CC3		4,8	1,9	1,2	2,6
Ilb	N	CC59	8,2		0,6	0,8	0,4
Ila	O	CC7	1,2	3,6	1,0	0,8	1,1
Ila	P	CC14	1,2		1,9	2,4	1,5
Ilb	Q	CC77	11,8	1,2	1,2	0,8	1,5
Ilb	R	CC224	8,2	2,4	0,8	1,2	0,4
Ila	S	CC11	3,5	4,8	0,8	0,8	0,7
Ila	T	CC18			0,4	0,8	
Ila	U	CC193		1,2	1,5		3,0
Ila	V	CC101 - CC90	1,2		0,4		0,7
Ila	W	Not assigned	1,2		0,6	0,8	0,4
Ila	X	CC21	2,4				
Ila	Y	CC91			0,8	1,2	0,4
Ila	Z	CC20			0,4	0,4	0,4
Other PFGE clusters			16,5	9,5	10,0	10,5	9,6
Total			85	84	518	248	270

Figure 1: Distribution of the 26 major mapped clonal complexes (CCs) within the pig and pork meat production chain

Molecular serotype	85% PFGE cluster	MLST CC mapped from PFGE cluster	Pork sector (excluding pig farming strains)	Meat products sector (excluding pork meat)	Milk products sector	Fish and fishery products sector	Food products combining several food categories sector	Fruit, vegetables, cereals and herbs sector	Without assignment to a specific food sector	Total	Total including pig farming
Ila	A	CC121	22,8	21,5	2,8	34,2	26,8	22,4	26,9	21,3	20,3
Ilc	B	CC9	21,3	21,8	3,5	3,8	15,1	1,5	11,5	14,3	13,7
Ila	C	CC8	8,0	6,3	2,8	5,5	6,3	14,9	3,8	6,5	6,7
IVb	D	CC1	4,7	5,6	9,1	2,5	5,4	6,0		5,3	5,5
IVb	E	CC6	3,0	3,9	7,0	3,0	3,4	7,5	7,7	4,1	4,2
Ilb	F	CC5	4,7	4,6	2,8	3,4	4,9	4,5		4,1	4,0
IVb	G	CC2	3,5	3,9	2,4	5,5	4,9	4,5	3,8	3,9	3,7
IVb	H	CC4 - CC217	2,5	3,2	9,1	0,8	1,5	3,0	7,7	3,5	3,3
Ila	I	CC37	2,7	1,4	4,5	0,4		6,0		2,2	2,7
Ila	J	CC31	1,3	4,6	1,0		3,9	6,0	7,7	2,7	2,6
Ila	K	CC155	2,0	3,2	1,7	4,6	2,0			2,4	2,3
Ila	L	CC204	0,8	1,4	0,7	6,3	4,9		3,8	2,2	2,1
Ilb	M	CC3	2,3	1,8	3,8	0,4	2,0		3,8	2,1	2,0
Ilb	N	CC59	0,5	1,1	2,8	4,2	0,5	1,5		1,5	1,8
Ila	O	CC7	1,3		3,8	2,5		3,0		1,7	1,7
Ila	P	CC14	1,7	2,1	1,0		2,0			1,7	1,7
Ilb	Q	CC77	1,2	0,4	1,7		0,5		3,8	0,9	1,4
Ilb	R	CC224	1,0	0,7	2,4		1,5			1,1	1,4
Ila	S	CC11	1,3		1,0	0,4	0,5	1,5	3,8	0,9	1,0
Ila	T	CC18	0,3	1,4	2,8	0,4	0,5			0,9	0,9
Ila	U	CC193	1,3	0,4	1,4	1,3				0,9	0,9
Ila	V	CC101 - CC90	0,5	0,4	2,1	1,3	0,5			0,8	0,8
Ila	W	Not assigned	0,5	0,4	3,5					0,8	0,8
Ila	X	CC21	0,4	0,4	2,8	0,4	0,5			0,6	0,7
Ila	Y	CC91	0,7	0,4	1,0	0,8		3,0		0,8	0,7
Ila	Z	CC20	0,3	0,4	1,4	0,8			3,8	0,7	0,7
	Other		10,0	9,2	26,9	11,4	10,2	14,9	11,5	12,1	12,3
Total			602	284	287	237	205	67	26	1708	1793

Figure 2: Distribution of the 26 major mapped clonal complexes (CCs) in the pork production sector and in other food production sectors