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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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In France the prevalence of *Listeria monocytogenes* (Lm) in raw pork meat is estimated around 9%. Over the past two decades, pork meat and processed pork products, contaminated with Lm have been incriminated in several listeriosis outbreaks around the world. Thus, finding the origin of these contaminations is more than ever a major public health concern. This study aimed at completing the data obtained by Félix et al. (2018) at pig farming and finished food products compartments by investigating the Lm clonal complexes diversity at the pig slaughtering and pork cutting stages. This will help us to better understand the source of contamination of finished food products.

Conclusion

Less virulent CC9 and CC121 really settled at the slaughterhouse stage and were mostly related to cold areas including finished food products. Hypervirulent CCs were more related to warm areas including pig farming and slaughtering. Our results confirmed the presence of CC1 at the slaughterhouse stage as mentioned by Lagarde et al. 2023 (doi.org/10.1016/j.fm.2023.104430). This is the first time that *L. monocytogenes* CCs diversity is thoroughly described at the pig slaughterhouse in France.

Materials and Methods

- Four French slaughterhouses and cutting facilities in the West of France investigated from 2020 to 2022
- 569 environmental swabbing in warm and cold areas, on food contact and non-food contact surfaces
- Clonal complex determination by PFGE/CC mapping (Félix et al., 2018 – doi.org/10.3389/fmicb.2018.00684) and real-time PCR CC identification method (Félix et al., 2023 – doi.org/10.1128/spectrum.03954-22)
- Determination of the Shannon diversity index (H)
- Comparison to the diversity obtained in pig farming and finished food products compartments (Félix et al., 2018)

Main results

- 224 strains isolated : 60 at the slaughterhouse and 164 at the cutting plant
- 21 different clonal complexes (CCs) identified and classified according to their virulence ((Maury et al., 2016 - doi:10.1038/ng.3501; Fritsch et al., 2018 - doi.org/10.1016/j.mran.2018.06.003); Centorotola et al., 2021 - doi.org/10.3390/foods10081944):
 - 9 hypervirulent* CC1, CC4, CC54, CC6, CC7, CC101, CC191, CC207 and CC224
 - 8 moderately virulent CC5, CC8, CC20, CC21, CC37, CC59, CC77 and C155
 - 4 less virulent CC9, CC11, CC121 and CC31
- Higher CC diversity in warm areas (H'-index=2.48) compared to cold areas (H'-index=2.02)
- The most detected CC9 and CC121 appeared at the slaughterhouse and strongly set up in cold areas of the pig and pork sector
- CC21 and CC59 were related to warm areas, CC31 and CC155 to cold ones.

MLST CC mapped from PFGE cluster or obtained from real-time PCR CC	Pig farming compartment	Slaughterhouse/cutting plant		Finished food product compartment	
		Warm areas	Cold areas	Food production environment compartment	Finished food products
CC121		3,3	21,9	25	22,4
CC9	1,2	13,3	23,2	10,7	23
CC8	10,6	1,6	15,2	10,7	7,5
CC1*	9,4	16,6	3	3,6	4,8
CC6*	7,1	1,6		1,2	3,3
CC5	2,4	1,6		7,1	4,2
CC2					4,1
CC4*		5	1,1		
CC4-CC54*		3,3			
CC4-CC217*	1,2			1,2	2,7
CC54*		11,6	0,6		
CC37	12,9	5	1,8	2,4	2,7
CC31			8,5	2,4	1,2
CC155			10,4	6	1,4
CC204				2,4	0,6
CC3				1,8	1,9
CC207*		1,6			
CC59	8,2	6,6			0,6
CC7*	1,2		1,2	3,6	1
CC14	1,2				1,9
CC77	11,8		0,6	1,2	1,2
CC224*	8,2		0,6	2,4	0,8
CC11	3,5	1,6	1,2	4,8	0,8
CC18					0,4
CC193				1,2	1,5
CC101-CC90*	1,2	1,6			0,4
CC21	2,4	1,6			
CC91					0,8
CC191*		1,6			
CC20		6,6			0,4
Not assigned	1,2	15	2,4		0,6
Other PFGE clusters	16,5			9,5	10
Total	85	60	164	84	518

Diversity and transmission of CCs through the pig and pork sector