



GENOMIC DIVERSITY OF *LISTERIA MONOCYTOGENES* STRAINS THROUGHOUT THE AGRI-FOOD CHAIN : A CONTRIBUTION TO CONTAMINATION CONTROL

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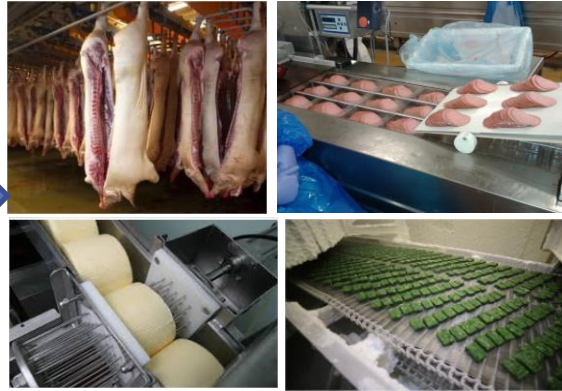
Laboratory for Food Safety, Maisons-Alfort

An ubiquitous pathogen

Soil, environnement, animal



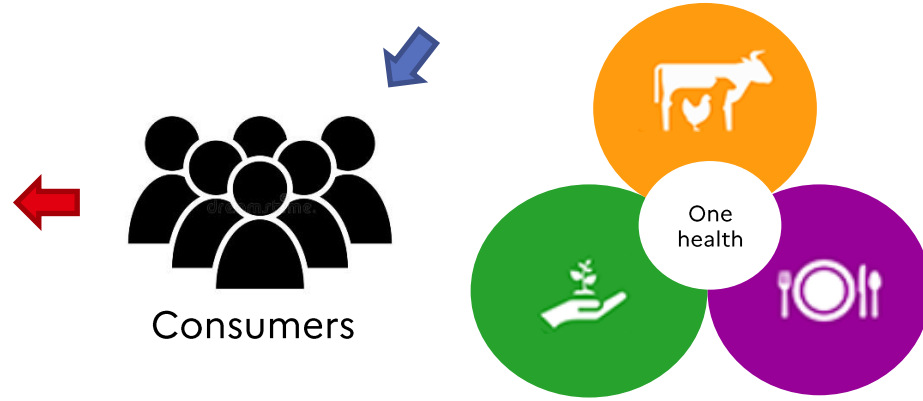
Food processing environnement



Final food product



Environnemental spread



A great diversity

Multi locus sequence typing (MLST)

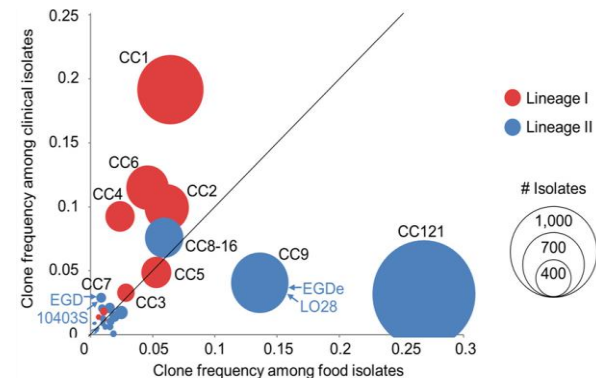
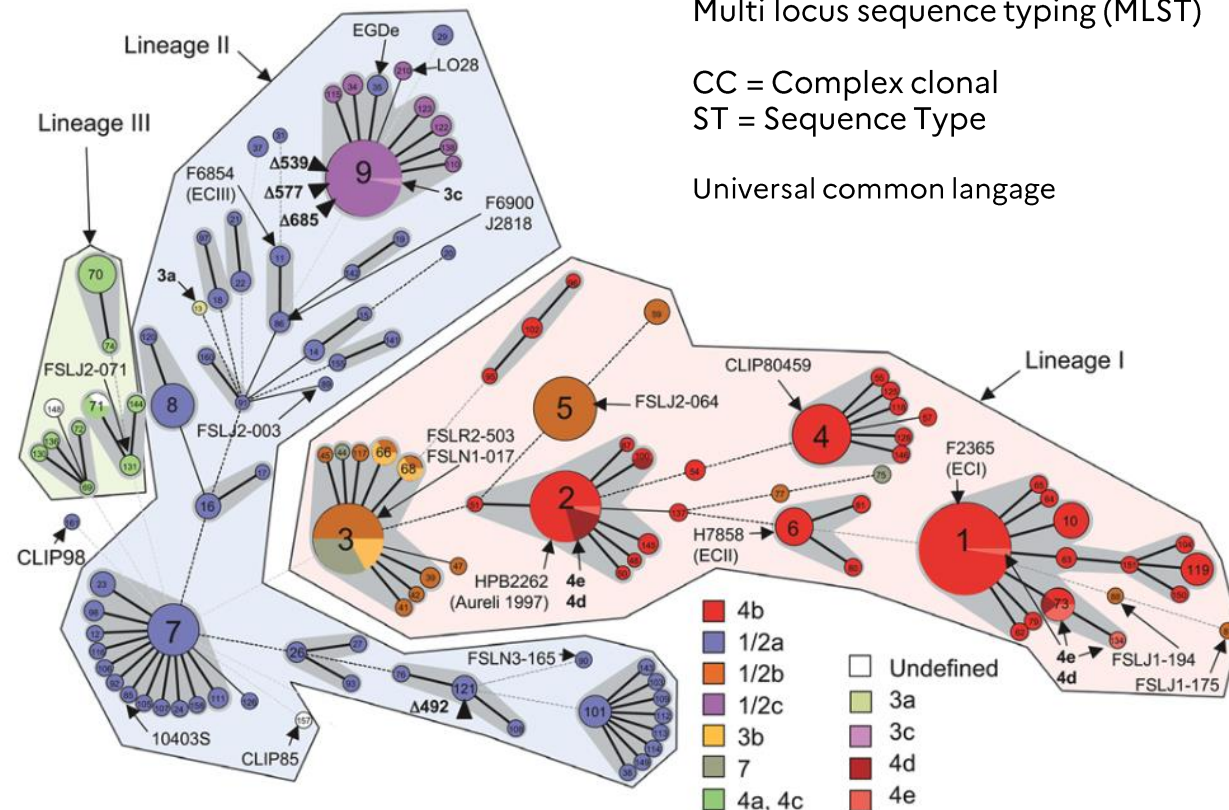
CC = Complex clonal
ST = Sequence Type

Universal common language

in January 2024

- Number of ST : 3220
- Number of CC : 262
- Single ST: 1055

<https://bigsdatabase.pasteur.fr/cgi-bin/bigsdatabase/>



From Maury et al. 2016 NG

From Ragon et al. 2008, *PLoS Pathogens*

Outbreak in France for 30 years

Molecular epidemiology start in 1990

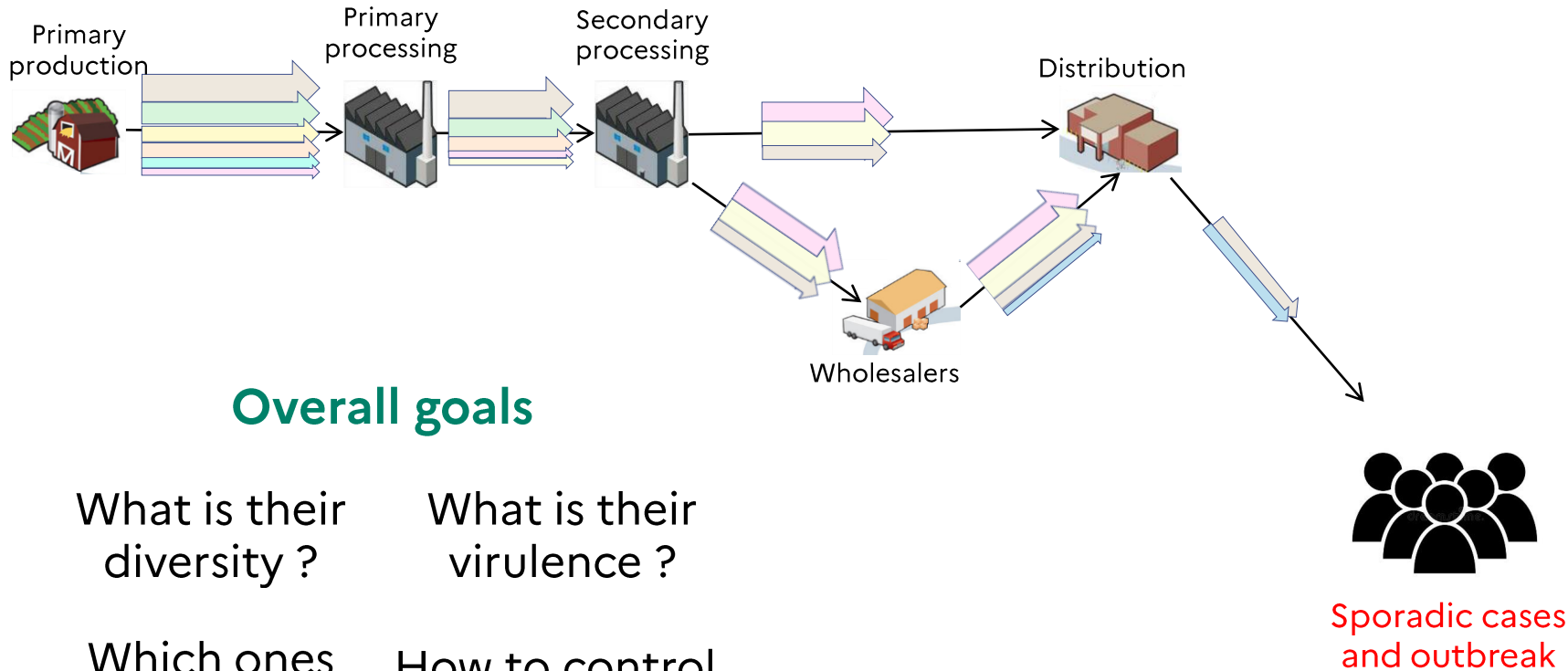
Definition: + two cases + food vehicle

Milk products 53% (n=26)

Pork meat products 30% (n=15)

Année	Nombre de cas	Aliments
1992	279	Langue de porc en gelée
1993	38	Rillettes
1995	36	Brie
1997	14	Pont-l'Évêque
1999	4	Époisses
2000	10	Rillettes
2000	32	Langue de porc en gelée
2002	11	Saucisse à tartiner (tartinette)
2003	4	Mortadelle
2012	11	Brie
2013	6	Brebis
2013	11	Quenelles
2013	3	Brebis
2013	2	Brebis
2013	7	Etablissement de soins
2014	2	Maroilles
2014	11	Charcuteries
2015	2	Saint-Nectaire
2015	3	Saint-Nectaire
2015	4	Saint-Nectaire
2015	13	Andouille
2015	2	Saint-Nectaire
2015	3	Fromage de vache
2015	2	Contamination d'environnement de production - Artisan
2015	3	Saint-Nectaire
2016	21	Reblochon
2016	2	Brie aux truffes
2016	2	Charcuterie
2016	19	Fromage de vache (TIAC)
2017	2	Reblochon
2018	2	Fromage de chèvre
2018	18	Brie au lait cru
2018	2	Produits traiteurs
2018	3	Boucherie
2018	3	Viande de cheval
2018	2	Céleri
2018	3	Brebis
2019	15	Brie au lait cru
2019	3	Charcuteries
2019	8	Yaourt bio
2019	2	Jambon
2019	2	Etablissement de restauration collective
2019	4	Fromages
2019	4	Etablissement de soins
2019	11	Fromages
2019	10	Charcuteries
2020	4	Charcuteries
2020	4	Truite fumée
2021	2	Fromage de chèvre

Contaminations flow throughout the food chain



Overall goals

What is their diversity ?

What is their virulence ?

Which ones reach us ?

How to control them ?

Partie 1 — CC distribution in the pork sector



Population Genetic Structure of *Listeria monocytogenes* Strains Isolated From the Pig and Pork Production Chain in France

Benjamin Félix^{1*}, Carole Feurer², Aurelien Maillet¹, Laurent Guillier¹, Evelyne Boscher³, Annaëlle Kerouanton³, Martine Denis³ and Sophie Roussel¹

¹ Maisons-Alfort Laboratory for Food Safety, Salmonella and Listeria Unit, University of Paris-Est, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France, ² The French Institute for Pig and Pork Industry, IFIP, Le Rheu, France, ³ Hygiene and Quality of Poultry and Pig Products Unit, Bretagne Loire University, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Ploufragan, France

Strain panel set up



Anses Maisons-Alfort laboratory (SEL unit)

National control plan

- Food {
Kerouanton *et al.* 2006 BE
Roussel *et al.* 2010-2012 BE
Roussel *et al.* 2014 BE
Henri *et al.* 2016 AEM

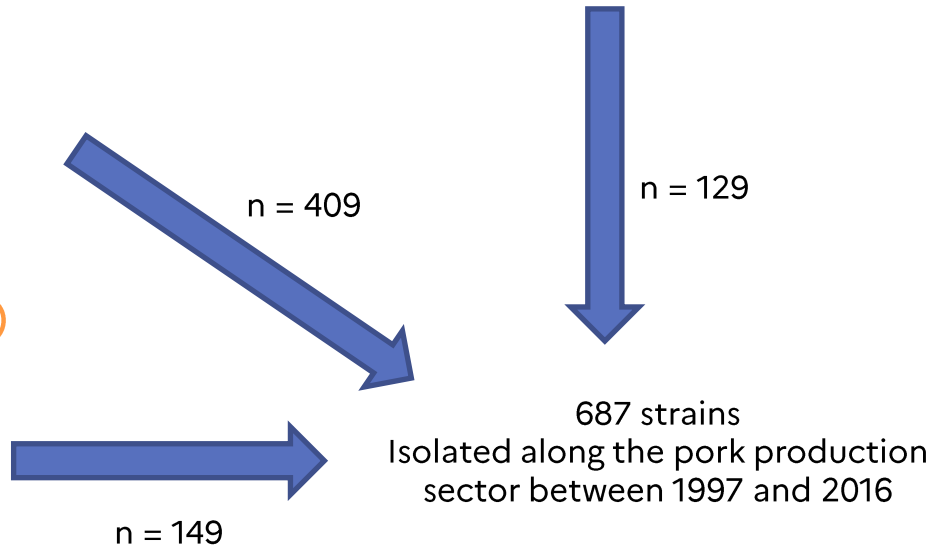
Anses Ploufragan laboratory (unit HQPAP)

Research project

- Farm { Boscher *et al.* 2010 JFP (n = 85)
Food { Kerouanton *et al.* Safepork2011 (n=64)

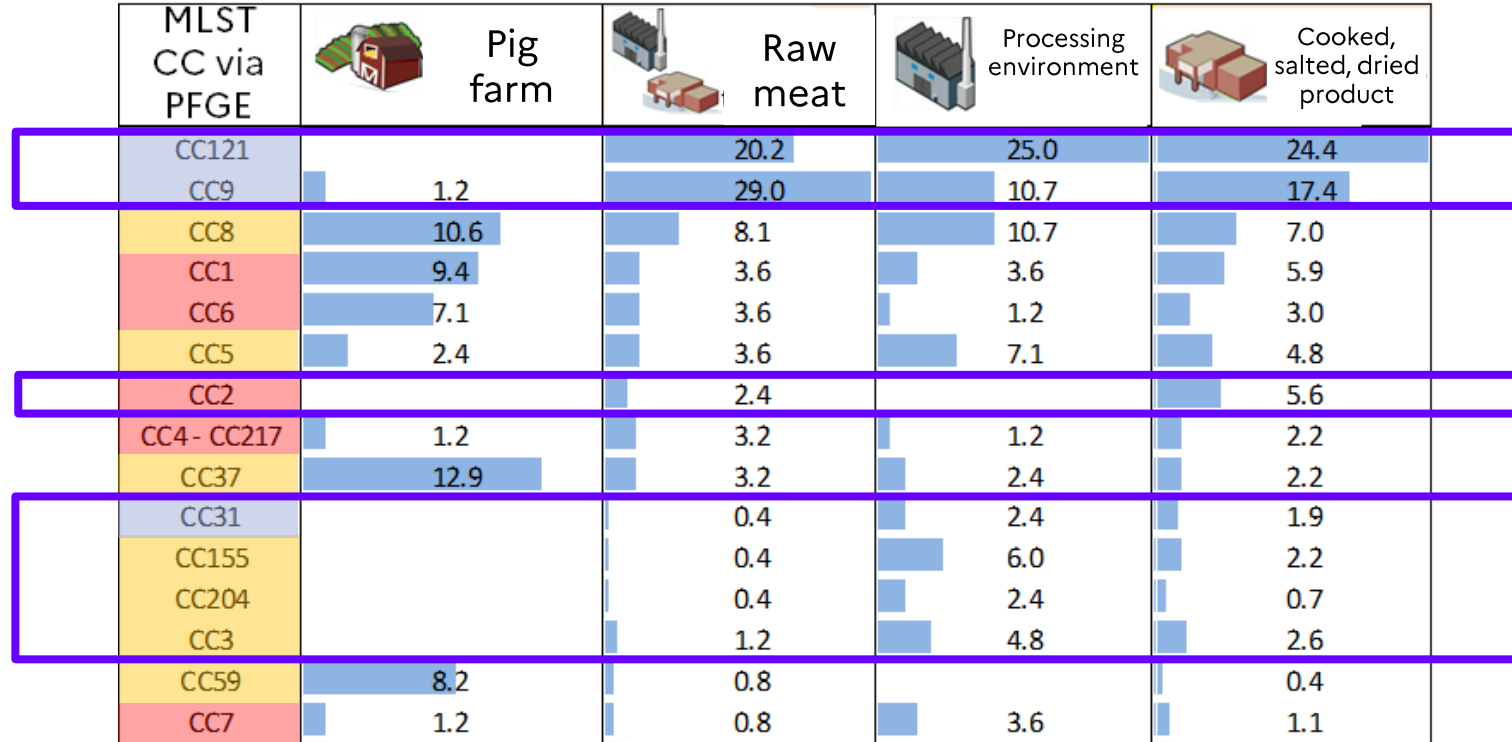
The French institute for pig and pork industry (IFIP)

Private contracting



CC distribution in the pork production sector

Hypervirulent
 Virulent
 Less virulent







Relative value expressed in percentage

CC121, CC9, CC2, CC3, CC31, CC155 and CC204 are « Food associated »

p-value < 0.025

CC distribution in the pork production sector

Hypervirulent
 Virulent
 Less virulent

MLST CC via PFGE	 Pig farm	 Raw meat	 Processing environment	 Cooked, salted, dried product
CC121		20.2	25.0	24.4
CC9	1.2	29.0	10.7	17.4
CC8	10.6	8.1	10.7	7.0
CC1	9.4	3.6	3.6	5.9
CC6	7.1	3.6	1.2	3.0
CC5	2.4	3.6	7.1	4.8
CC2		2.4		5.6
CC4- CC217	1.2	3.2	1.2	2.2
CC37	12.9	3.2	2.4	2.2
CC31		0.4	2.4	1.9
CC155		0.4	6.0	2.2
CC204		0.4	2.4	0.7
CC3		1.2	4.8	2.6
CC59	8.2	0.8		0.4
CC7	1.2	0.8	3.6	1.1

Relative value expressed in percentage

CC1, CC4, CC5, CC6, CC7 and CC8 are « Ubiquitous »

p-value > 0.025

Partie 2 — CC distribution in the milk production sector

Papić et al. *BMC Microbiology* (2019) 19:299
<https://doi.org/10.1186/s12866-019-1676-3>

BMC Microbiology

RESEARCH ARTICLE Open Access

Genetic diversity of *Listeria monocytogenes* strains in ruminant abortion and rhombencephalitis cases in comparison with the natural environment

Bojan Papić^{1*}, Mateja Pate¹, Benjamin Félix² and Darja Kušar¹





EURL Lm

European Union Reference Laboratory for
Listeria monocytogenes
<http://eurl-listeria.anses.fr>

What about ruminants and milk products ?

Lm responsible for pathologies in ruminants, unlike pigs

The milk production process is totally different from meat



Do strains isolated from ruminants pathology or carriage contaminate food ?

There were few strains from ruminant pathology in Anses collection

Strain panel set up

Anses Maisons-Alfort laboratory (unit SEL)

- National control plan
Milk product (Félix et al. 2018 FM)

OSCAR (Observatory and monitoring of ruminant abortion causes)

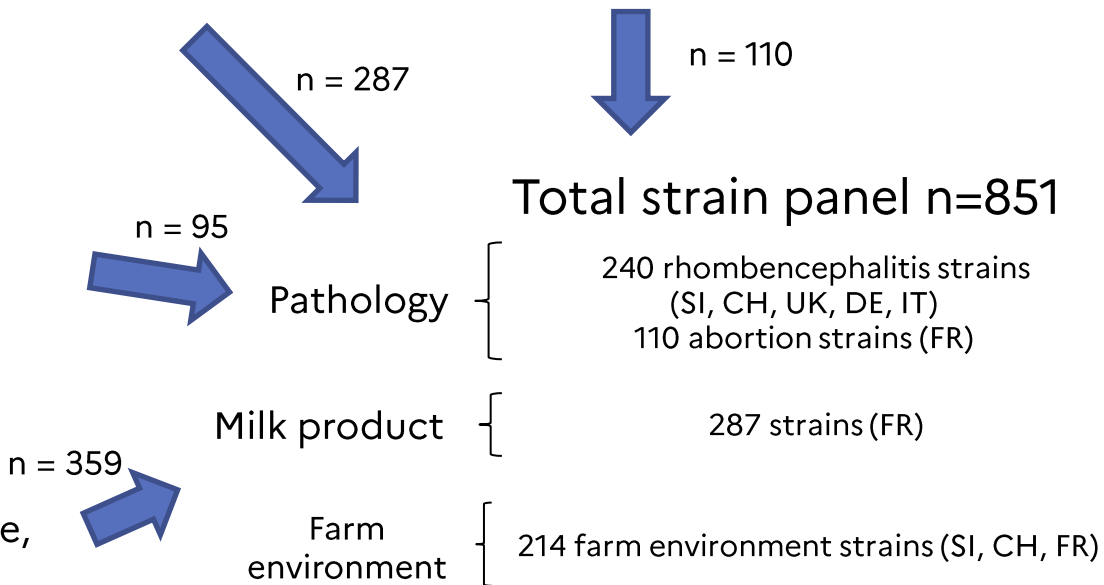
- Laboceva Ille-et-Vilaine departement
Ruminant abortion strains

Veterinary faculty of Ljubljana

- Rhombencephalitis strains (bovine, ovine, caprine) (n=55)
- Farm environnement strains (n=40)

Publications





- Rhombencephalitis strains (bovine, ovine, caprine) (n=185) (Dreyer et al., 2016 SR)
- Farm environnement strains (n=174)



CC distribution in the milk production sector

Hypervirulent
 Virulent
 Less virulent

Relative value expressed in percentage

CC WGS or PFGE mapping	 Rhombencephalitis strains	 Abortion strains	 Farm environment strains	 Milk product strains	
CC1	45,8	21,8	15,4	9,1	
CC4	10,4	14,5	10,3	9,1	
CC6	0,4	12,7	7,9	7,0	
CC37	0,4	18,2	8,4	4,5	
CC412	10,4	3,6	2,8	0,7	
CC59	1,3	4,5	3,7	2,8	
CC9		0,9	6,1	3,5	
CC29		0,9	5,6	3,5	
CC8	1,7	1,8	3,7	2,8	
CC7	0,4	5,5	1,9	3,8	
CC2		0,9	3,7	2,4	
CC18	0,4	3,6	2,3	2,8	
CC3				3,8	
CC121			2,3	2,8	
CC5	0,4	0,9	3,3	2,8	
	n=240	n=110	n=214	n=287	





p-value > 0.025
 p-value < 0.025

In contrast to the pig sector, most of the CC are « Ubiquitous »

Répartition des CCs dans la filière lait

Hypervirulent
 Virulent
 Less virulent

Relative value expressed in percentage

CC WGS or PFGE mapping	 Rhombencephalitis strains	 Abortion strains	 Farm environment strains	 Milk product strains	
CC1	45,8	21,8	15,4	9,1	
CC4	10,4	14,5	10,3	9,1	
CC6	0,4	12,7	7,9	7,0	
CC37	0,4	18,2	8,4	4,5	
CC412	10,4	3,6	2,8	0,7	
CC59	1,3	4,5	3,7	2,8	
CC9		0,9	6,1	3,5	
CC29		0,9	5,6	3,5	
CC8	1,7	1,8	3,7	2,8	
CC7	0,4	5,5	1,9	3,8	
CC2		0,9	3,7	2,4	
CC18	0,4	3,6	2,3	2,8	
CC3				3,8	
CC121			2,3	2,8	
CC5	0,4	0,9	3,3	2,8	
	n=240	n=110	n=214	n=287	

p-value > 0.025

p-value < 0.025

CC1, CC4, CC6, CC37 are responsible for the majority of pathologies
For the first time, CC6 and CC37 are associated with ruminant abortion

3. CC distribution in wild and agricultural animals or environments

www.nature.com/scientificdata

scientific **data**

 Check for updates

OPEN

DATA DESCRIPTOR

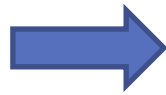
A European-wide dataset to
uncover adaptive traits of *Listeria*
monocytogenes to diverse
ecological niches

Benjamin Félix *et al.**

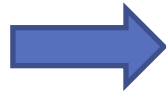
https://onehealthejp.eu/wp-content/uploads/2022/10/OHEJP-LISTADAPT-Project-Impact-Brochure_Oct2022.pdf



What are the prevalent CCs in outdoor environment ?



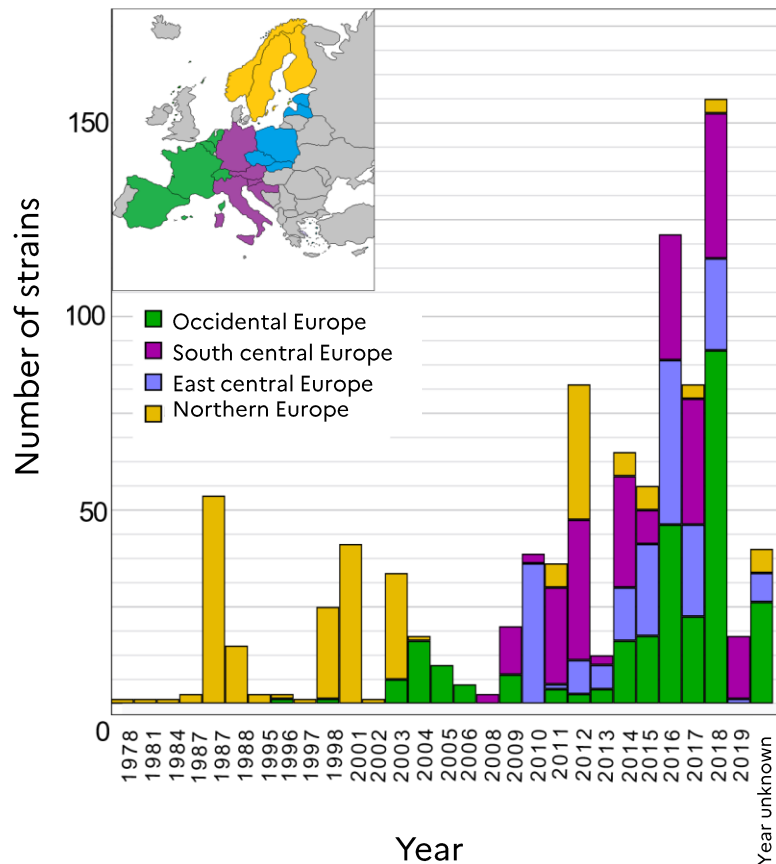
Are these CC different from food related CCs ?



Do some of them enter food processing environment and food products ?

Few data are available on outdoor CC distribution in the literature

Strain panel set up – « animal environment » compartment



756 environmental and animal strains

Strains from farm/wild animal without listeriosis symptom

Strains from farm/wild environments

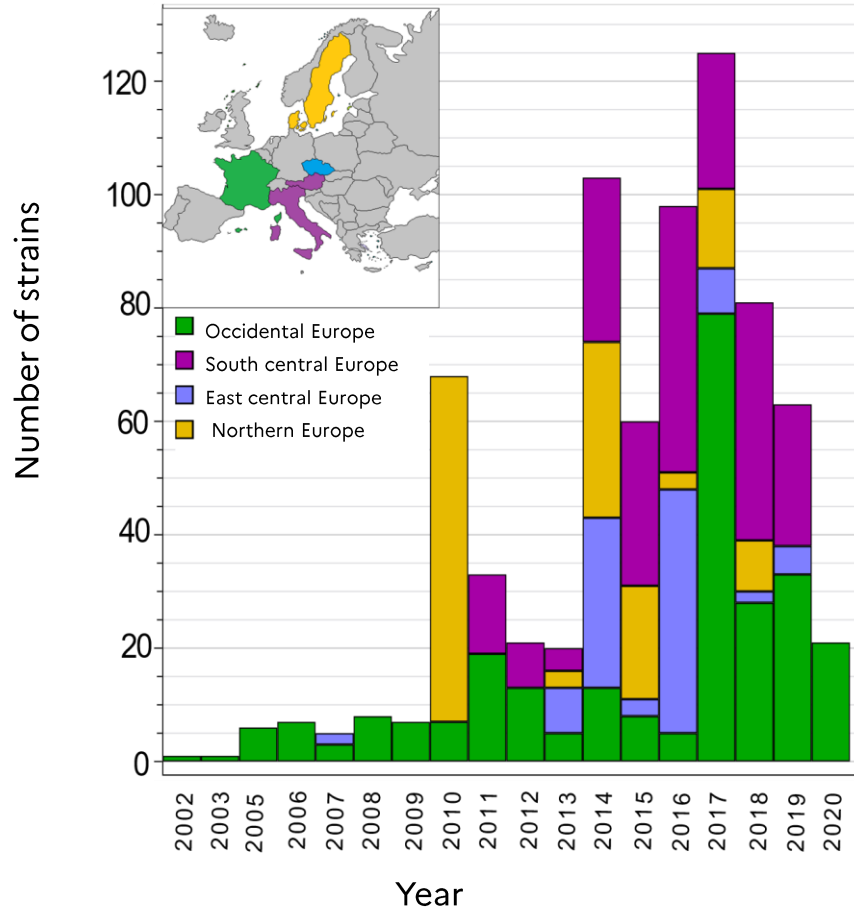
4 European regions

18 European countries

Mainly between 2010 and 2019

Avoid bias related to food strain reintroduction or pathology related strains

Strain panel set up – « food » compartment



728 food or food processing environment isolated strains

5 different sectors

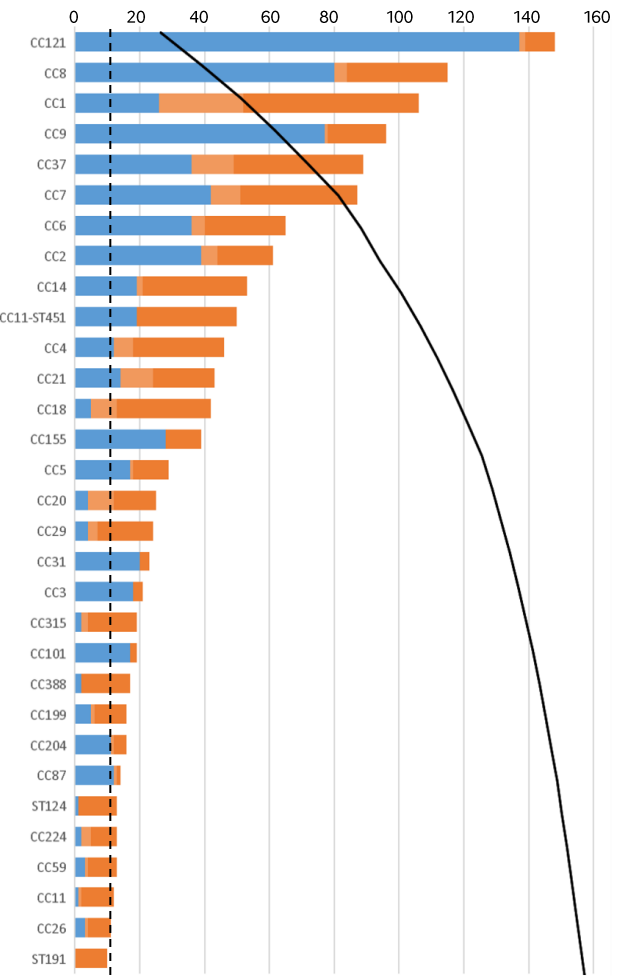
- meat
- fish
- dairy
- composite food
- fruit and vegetable

4 European regions

6 European countries

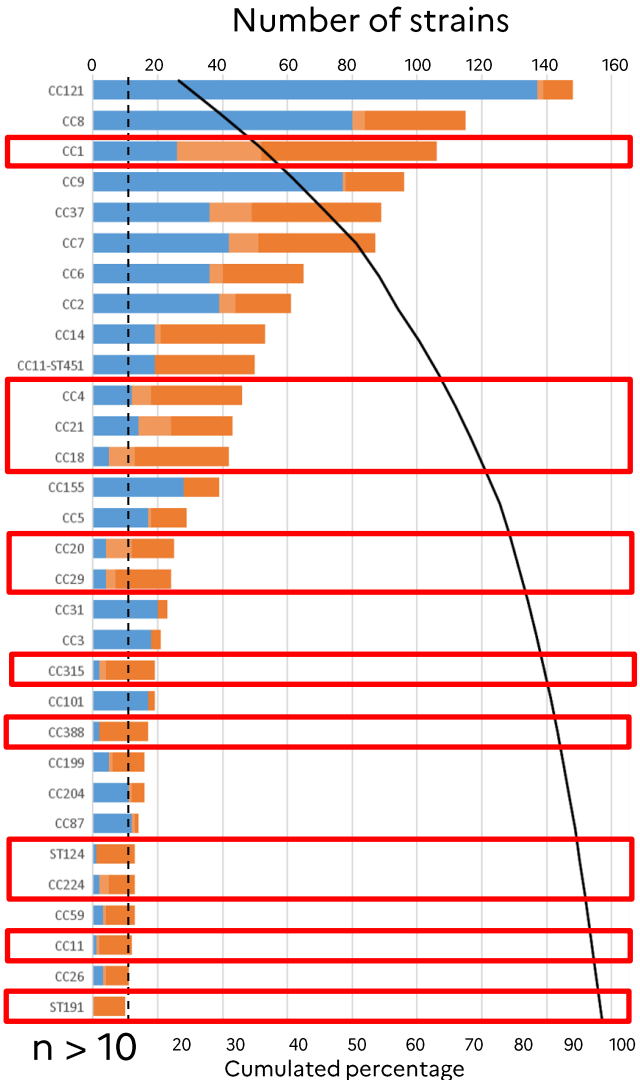
Mainly between 2010 and 2020

Number of strains



■ Farm/wild animal and environment
■ Food or food processing environment

n > 10
Cumulated percentage



12 CCs and STs were associated to the compartment « animal et environnement »



These 12 CCs include 375 strains

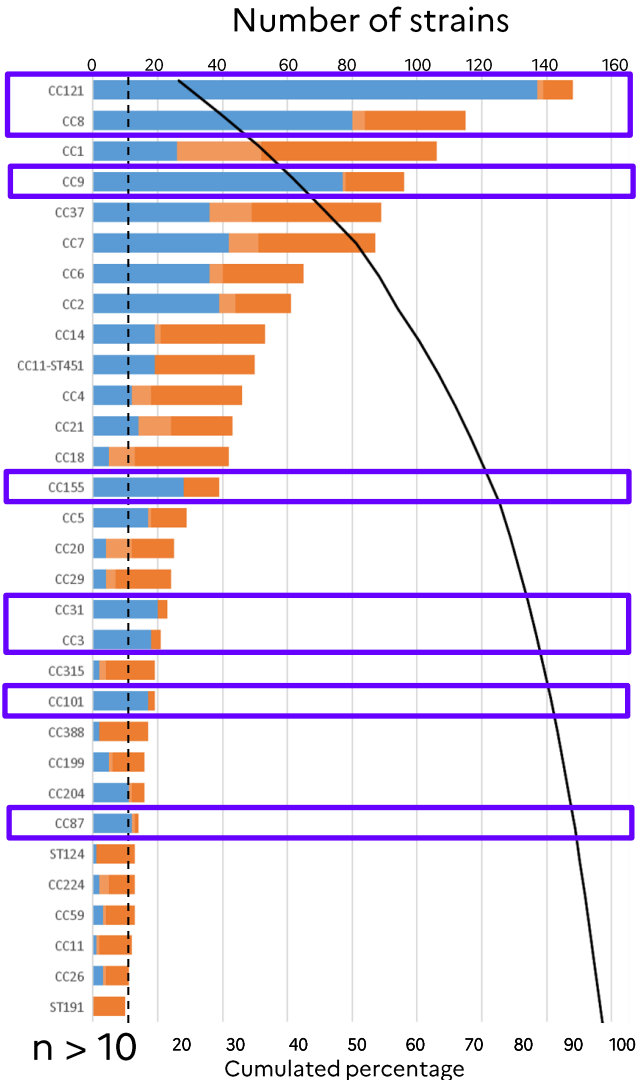
p-value < 0.025

-> Reported in the litterature :

- 15 international studies
- from animals et environnements
- in more then 10 countries

Haase et al., 2014 (multi)
 Kim et al., 2018 (US)
 Steckler et al., 2018 (US)
 Psareva et al., 2019 (multi)
 Demaitre et al., 2021 (BE)
 Palacios-Gorba et al., 2021 (multi)
 Steingolde et al., 2021 (LV)
 Terentjeva et al., 2021 (LV)
 Fagerlund et al., 2022 (NO)
 Fredriksson-Ahomaa et al., 2022 (FI)
 Gorski et al., 2022 (US)
 Varsaki et al., 2022 (ES)

 Farm/wild animal and environment
 Food or food processing environment



9 CCs were associated to the compartment « food »

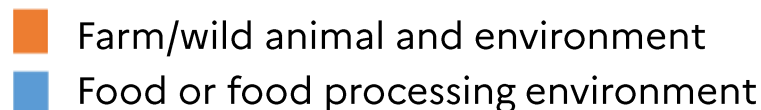
These 9 CCs include 536 strains

p-value < 0.025

-> Reported in the litterature :

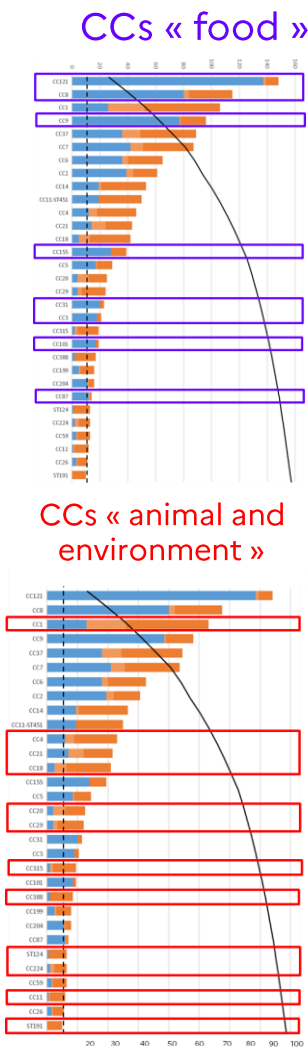
- 8 international studies
- from food
- in more than 6 countries

Haase et al. 2010 (multi)
Chenal-Francisque et al. 2011 (multi)
Maury et al., 2016 (FR)
Ebner et al., 2015 (CH)
Centorotola et al., 2021 (IT)
Chen et al. 2019 (US)
Chen et al. 2020 (CN)
Daeschel et al. 2022 (US)



Comparative genomics (GWAS*)

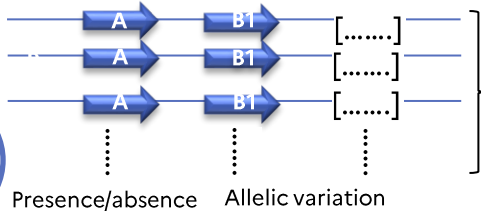
*: Genome wide association study



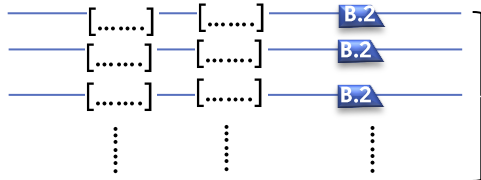
536 genomes

Software
Roary

Gene carriage « food »
vs. « animal et environment »



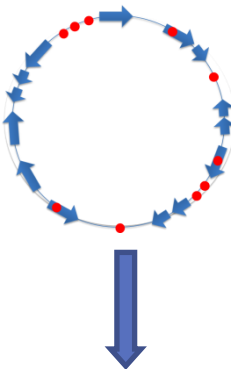
Presence/absence Allelic variation



« Food »
strains genomes

Software
Scoary

« Animal and
environments » strain
genomes



Genetic factor
related to each
compartment

375 genomes

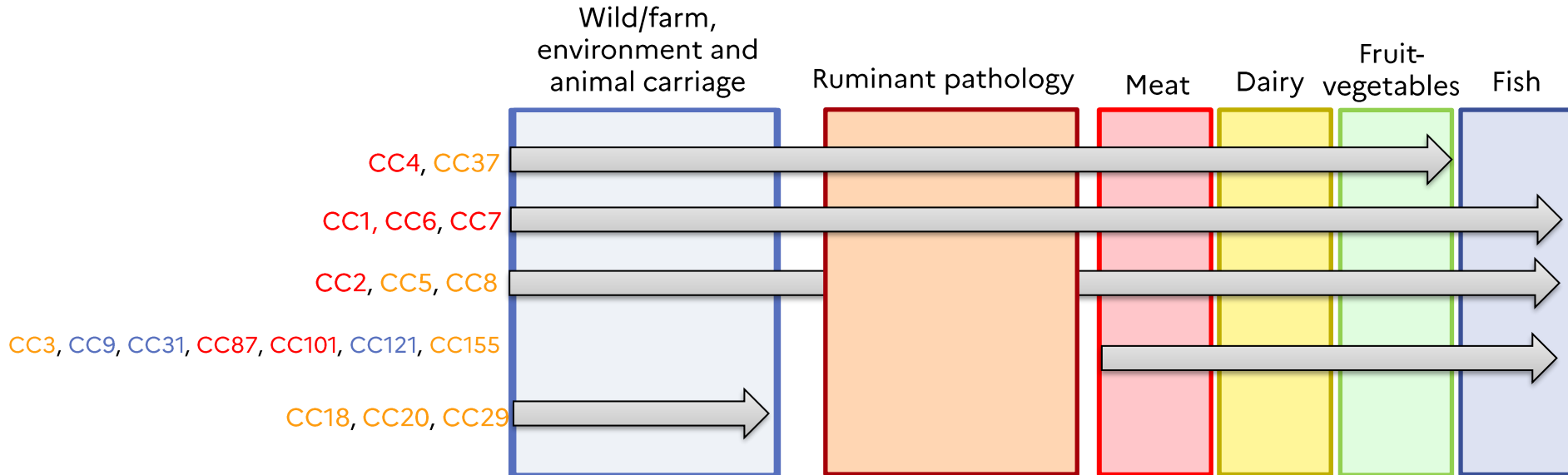
Annotation
EggNOG-mapper,
Conserved domain NCBI

- Checking for biases linked to the overabundance of certain CCs in the study panel
- Superimposition of GWAS tools: Pyseer
- Comparison with proline operons from other species (*Bacillus subtilis*, *Escherichia coli*)
- Reconstitution of a production environment
- Mutagenesis and comparison of phenotypes
- High-throughput phenotyping

Perspectives and conclusions



Distribution profil of the major observed CCs



Prediction based on CC:

- Probable contamination source
- Persistence potential in food processing environment



EURL Lm

European Union Reference Laboratory for *Listeria monocytogenes*
<http://eur-listeria.anses.fr>

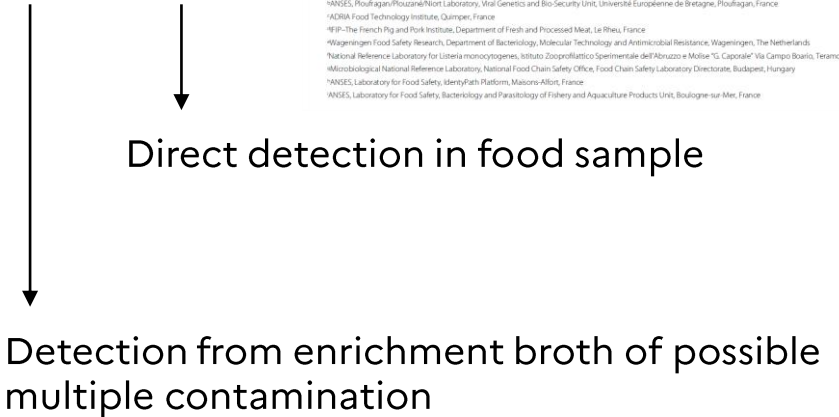
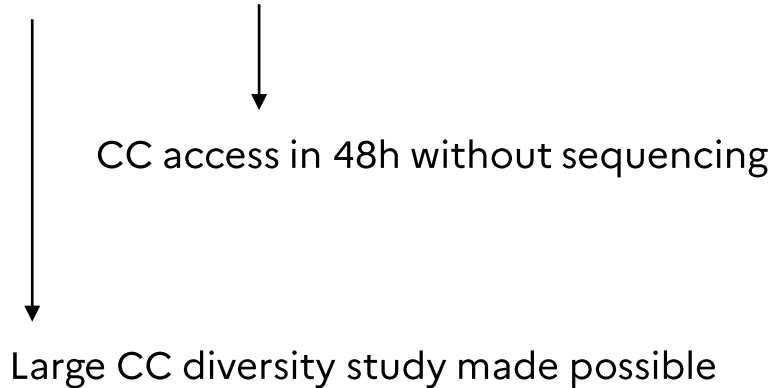
Conclusions and follow-up



Conclusions & perspectives



Rapid real-time PCR method for the identification of the 30 major CCs circulating in food in Europe



RESEARCH ARTICLE



Identification by High-Throughput Real-Time PCR of 30 Major Circulating *Listeria monocytogenes* Clonal Complexes in Europe

Benjamin Félix,* Karine Capitaine,* Sandrine Te,* Arnaud Felten,* Guillaume Gillot,* Carole Feurer,* Tijl van den Bosch,* Marina Torres,¹ Zsuzsanna Sréterné Lancz,² Sabine Delannoy,³ Thomas Brauge,⁴ Graziella Midelet,⁵ Jean-Charles Leblanc,⁶ Sophie Roussel⁷

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³IFIP - The French Pig and Poultry Institute, Department of Fresh and Processed Meat, Le Rheu, France
⁴ Wageningen Food Safety Research, Department of Bacteriology, Molecular Technology and Antimicrobial Resistance, Wageningen, The Netherlands
⁵National Reference Laboratory for *Listeria monocytogenes*, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale" Via Campo Boario, Teramo, Italy
⁶Microbiological National Reference Laboratory, National Food Chain Safety Office, Food Chain Safety Laboratory Directorate, Budapest, Hungary
⁷ANSES, Laboratory for Food Safety, Identity/Path Platform, Maisons-Alfort, France
⁸ANSES, Laboratory for Food Safety, Bacteriology and Parasitology of Fishery and Aquaculture Products Unit, Boulogne-sur-Mer, France

Acknowledgments

Unit SEL (ANSES)

- Pierre-Emmanuel Douarre
- Landry Tsoumtsas Meda
 - Karine Capitaine
 - Adrien Asséré
 - Sabrina Cadel-Six

Unit SBCL (ANSES)

- Noémie Vingadassalon

SPAAD (ANSES)

- Déborah Merda

DirDSP (ANSES)

- Bertrand Lombard

DER (ANSES)

- Laurent Guillier

Ploufragan Laboratory (ANSES)

- Martine Denis
- Evelyne Boscher
- Annaël Kerouanton

National reference

laboratories for *Lm*:

CY, CZ, CH, DE, IT, MK,
NLrvm, NLwfsr, NO, PT,
SE, SI, SK, UK

ADRIA Quimper

- Guillaume Gillot
- Nassim Mouhali

IFIP

- Carole Feuer
- Pauline Champigny

ACTALIA

- Valérie Michel
- Estelle Fontaine

Funding



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Listeria monocytogenes
<http://eurl-listeria.anses.fr>



FranceAgriMer

ÉTABLISSEMENT NATIONAL
DES PRODUITS DE L'AGRICULTURE ET DE LA MER



MINISTÈRE
DE L'ALIMENTATION,
DE L'AGRICULTURE
ET DE LA PÊCHE

avec la contribution financière du
compte d'affectation spéciale
«Développement agricole et rural»



Anses genomic platform (ANSES)

- Arnaud Felten

IdentyPath platform (ANSES)

- Patrick Fach
- Sabine Delannoy
- Mai-Lan Tran

Boulogne sur Mer Laboratory (ANSES)

- Thomas Brauge
- Guyline Leleu