



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

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OPEN

DATA DESCRIPTOR

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

Benjamin Félix *et al.*[#]

Listeria monocytogenes (*Lm*) is a ubiquitous bacterium that causes listeriosis, a serious foodborne illness. In the nature-to-human transmission route, *Lm* can prosper in various ecological niches. Soil and decaying organic matter are its primary reservoirs. Certain clonal complexes (CCs) are over-represented in food production and represent a challenge to food safety. To gain new understanding of *Lm* adaptation mechanisms in food, the genetic background of strains found in animals and environment should be investigated in comparison to that of food strains. Twenty-one partners, including food, environment, veterinary and public health laboratories, constructed a dataset of 1484 genomes originating from *Lm* strains collected in 19 European countries. This dataset encompasses a large number of CCs occurring worldwide, covers many diverse habitats and is balanced between ecological compartments and geographic regions. The dataset presented here will contribute to improve our understanding of *Lm* ecology and should aid in the surveillance of *Lm*. This dataset provides a basis for the discovery of the genetic traits underlying *Lm* adaptation to different ecological niches.

Background & Summary

Listeria monocytogenes (*Lm*) is a facultative intracellular pathogen responsible for listeriosis, a serious disease affecting both humans and animals. *Lm* is a ubiquitous bacterium that is found in various ecological niches, including the natural and farm environments^{1,2}. In particular, soil is a primary ecological niche of *Lm* and may thus be important in its transmission from natural/farm environment to food and food-processing environment (FPE)^{1,2}. Farm animals, in particular ruminants, are also an additional important reservoir for *Lm* and contribute to contamination of the farm environment through fecal shedding^{3,4}. In addition, *Lm* can persist for a long time in soil and the farm environment. Increasing amounts of information are also available on the prevalence of *Lm* in wildlife, showing that various animal species (e.g., deer, wild boars, bears, foxes, monkeys, rodents, hedgehogs, snails, slugs and birds) can act as a vehicles for this pathogen^{5–11}. These findings point to an ecological role of wildlife as a reservoir of *Lm* and its potential importance in *Lm* infection cycle.

Lm is genetically heterogeneous species divided into four phylogenetic lineages, of which lineages I and II are the most frequently encountered. Multilocus sequence typing (MLST) classifies *Lm* into clonal complexes (CCs) and sequence types (STs), which are systematically used to describe its population structure^{12–14}. Certain epidemiological clones account for the majority of outbreaks and sporadic cases in humans¹⁵ and animals¹⁶, worldwide^{13,17}. The CCs that are commonly found in food and FPE, such as the most common CC9 and CC121, but also CC1, CC2, CC4, CC5, CC6, CC8 and CC37¹⁸, pose a serious challenge in food industry^{15,18,19}. Moreover, they can persist in FPE for several years^{20–24}. Remarkably, CC9 and CC121 are rarely reported in animals or natural/farm environments^{18,25}.

In order to improve surveillance and the management of health risks associated with *Lm*, a deeper understanding of the genetic make-up of strains adapted to food and FPE is required. As part of the Horizon 2020 “One Health” European Joint Programme, the 3-year research project “LISTADAPT” (Adaptive traits of *Listeria*

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monocytogenes to its diverse ecological niches - <https://onehealthjp.eu/jrp-listadapt/>) aimed to identify the genetic mutations and mobile genetic elements underlying the adaptation of *Lm* to different ecological niches. With this objective in mind, strains were collected from i) farm environment and animals and ii) natural environment and wild animals to study their genetic make-up and to compare this background with that of strains isolated from food products and FPE. This work was made possible due to the LISTADAPT consortium which included (i) seven national reference laboratories (NRLs) for surveillance of *Lm* in food, animals and the environment (AT, CZ, DK, FR, IT, NO and SE) and (ii) three research laboratories at INRAE (the French National Research Institute for Agriculture, Food and Environment). Out of the seven NRLs, two are also national public health laboratories (AT and CZ) that are in charge of the surveillance of clinical strains isolated in outbreaks and sporadic cases. In addition, 14 institutes from 12 countries participated as external partners providing isolates.

In this data descriptor, we present a dataset of 1484 high-quality draft genomes originating from *Lm* strains isolated in 19 European countries within the framework of the LISTADAPT project. The constructed dataset cover a wide genetic diversity of *Lm* since it includes about 79 different CCs and singleton STs including the most prevalent CCs in Europe¹⁵ and worldwide^{13,17}. The strains were collected from natural environment (wild animals and natural environment), primary production (farm environment and farm animals with or without listeriosis symptoms) until FPE and food products.

The constructed dataset provides a better understanding of the *Lm* transmission routes from the farm/natural environment to food and FPE and improves our understanding of its ecology. The dataset may also help to assess the importance of animal and food strains for human infection. Moreover, it can be used by the scientific community (i) to improve our understanding of the *Lm* population structure and the *Lm* evolutionary history, (ii) to facilitate the detection of the emerging *Lm* clones and (iii) to identify genetic traits related to the adaptation of *Lm* to particular ecological niches (ecophysiology). Such genetic traits could be used in the development of molecular assays for screening of food/FPE, animal and soil reservoirs.

Methods

Construction of the LISTADAPT dataset (n = 1484). In order to build a dataset of *Lm* draft genomes suitable for investigating the adaptive traits of *Lm* to diverse ecological niches, we gathered a curated collection of *Lm* draft genomes. Strains isolated over the period 2010–2020 were preferred, regardless of their origin of isolation. We considered two geographic levels, (i) the 27 EU countries including Norway and Switzerland, heterogeneous in size, population, climate, ecology and economical activities and (ii) based on country borders four European regions roughly equal in terms of surface area without consideration for other criteria (South-West, Central-South, Eastern and Northern). We included strains that were distributed evenly among these four European regions. The strain were gathered from already available strain collections and extensive sampling campaigns (Fig. 1). The LISTADAPT dataset was divided into two main ecological compartments: (i) C1 compartment, which included strains from animals and the natural/farm environment (n = 756), and (ii) C2 compartment, which included strains from food (n = 728) (Table 1).

Strains selected from the initial collection of the LISTADAPT consortium. At the beginning of the LISTADAPT project, the consortium had access to a collection of about 8000 food and animal *Lm* strains obtained from collaborative projects or national surveillance. Most of these strains were isolated from food, whereas the remainder were isolated from animals (C1 compartment: animal and environmental strains) with a substantial under-representation of certain animal species. This compartment mainly included strains isolated from animals showing listeriosis-related symptoms. Few strains were available from asymptomatic animals, soil and the agricultural environment, originating from three European countries (France, Italy and the Czech Republic).

Animal and environmental strains included in the collection during the LISTADAPT project (n = 756). We collected isolates from animals showing listeriosis associated symptoms, asymptomatic animals, soil and the environment, in a large number of countries across Europe. These strains were isolated between 1978 and 2019. Regarding environmental niches, the consortium selected strains from continental environments remote to cities, large rivers and estuaries or marine environment to avoid the selection of human or food strains released in the environment, detailed strain information were provided in Figshare File 1²⁶. However, the six strains described by Szymczak *et al.*²⁷ (Table 2) were isolated from city outskirts parks in Poland, distant from the city center. Similarly, the 47 strains from birds (mainly seagulls) (Hellström *et al.*)¹⁰ were isolated from localities from on the outskirts of Helsinki, Finland (Table 2).

Strains obtained from existing microbial collections (n = 648) To increase the size and representativeness of the *Lm* genome dataset the LISTADAPT consortium performed an extensive review of all recent collections of published and unpublished *Lm* strains and then contacted researchers in charge of these collections. Finally, 14 external partners, food and veterinary laboratories and research institutes, all dealing with *Lm* hazards in Europe, collaborated with the LISTADAPT consortium (Tables 2 and 3).

The initial collection included more strains from animals with listeriosis-associated clinical symptoms than without symptoms. In order to reduce the number of strains originating from animals with listeriosis while maintaining maximum diversity of the dataset, we adopted an original method to select the strains based on metadata (e.g., type of sample, geographic location, time of isolation, molecular typing data such as PFGE profiles, animal species and geographic sampling location). This method relies on Gower's coefficient (GC), which is a dissimilarity measure: the "distance" between two units is the sum of all the variable-specific distances (associated with metadata categories). The GC metric enables the combination of numeric and categorical data and enables applying weights to each variable, effectively altering the importance of each metadata category

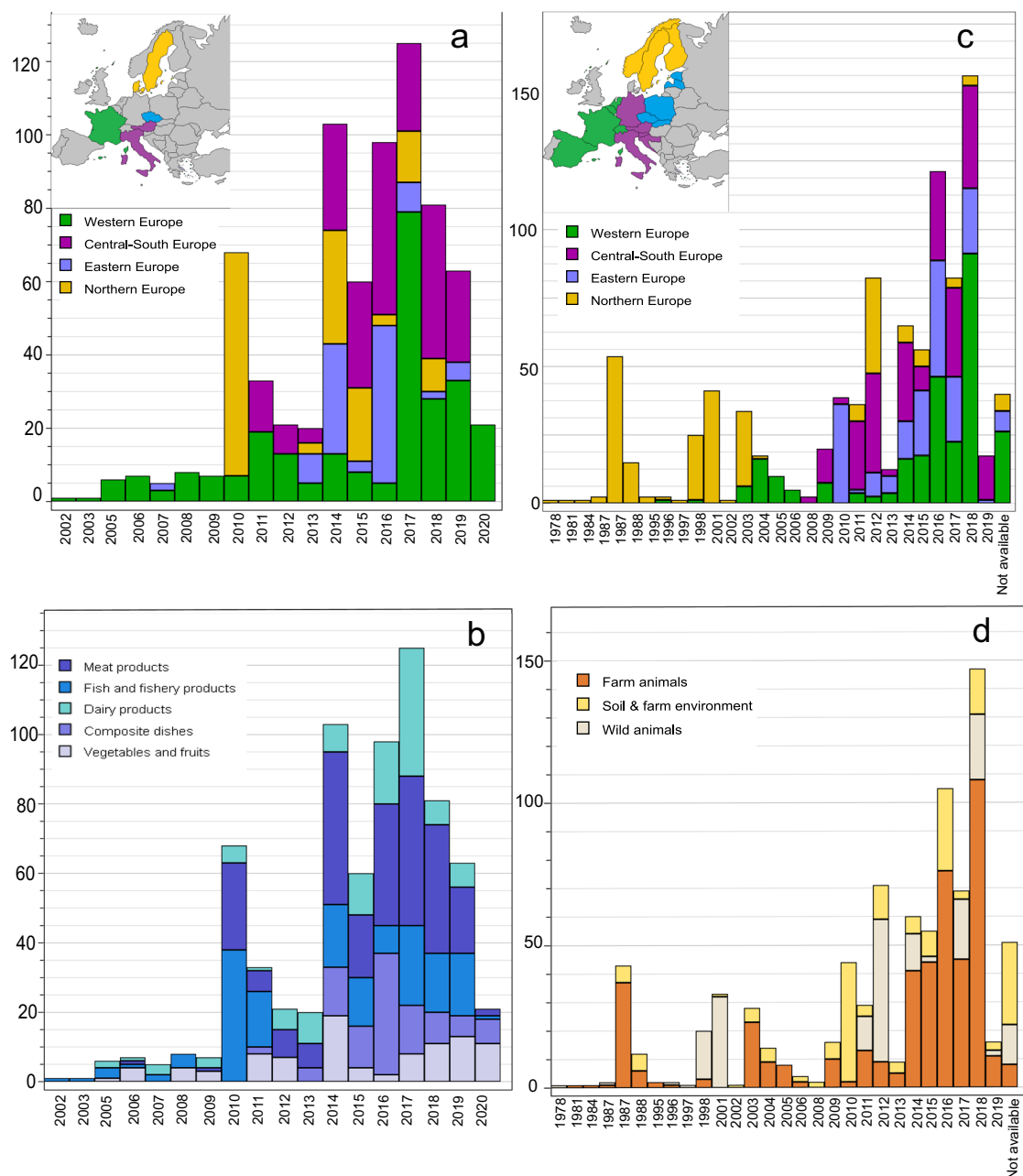


Fig. 1 Distribution of the LISTADAPT collection of *Listeria monocytogenes* strains (n = 1484) by time, geographic region and origin of isolation. (a) and (b) show the distribution of food strains by geographic region and food type, respectively. (c) and (d) show the distribution of environmental strains by geographic region and subcompartment, respectively.

Animal and environment (C1)				Food products and food production environment (C2)					
Farm animals	Wild animals	Soil and farm environment	Total	Meat	Fish	Dairy	Vegetables and fruits	Composite dish	Total
411	179	166	756	246	165	119	95	103	728

Table 1. Repartition by compartments and sub-compartments of strains from the whole LISTADAPT collection (1,484).

(e.g., geographical region as a more important category than year of isolation). The three steps are: (i) calculating the dissimilarity matrix based on Gower's distance (ii) clustering the dissimilarity matrix with hierarchical clustering (agglomerative bottom-up approach of clustering) and (iii) assessing clusters with the "Silhouette" method. The silhouette plot displays a measure of how close each point in one cluster is to the points in the

Partner	Country (country code)	Category	Origin of isolation	No. of strains	Isolation year	References
Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine of Helsinki (n = 131)	Finland (FI)	Wild animals	Wild birds, hare, reindeer [NS]	49	1998, 2001	10,14
		Farm animals	Cow, cow milk in bulk tank and pigs [NS]	61	1981–2011	14,47–50
			Cow (aborted fetus) [CS]	4	1984–1987	14,48,49
		Soil and farm environment	Silage ¹ and soil	17	1987–2004	
Faculty of Veterinary Medicine, University of Munich (n = 31)	Germany (DE)	Wild animals	Deer and wild boar [NS]	31	2011–2012	6
Norwegian Veterinary Institute (n = 28)	Norway (NO)	Wild animals	Slugs	28	2012	11
Department of Applied Microbiology and Human Nutrition ZUT (n = 36)	Poland (PL)	Soil and farm environment	Soil from agricultural area	30	2010–2012	27
			Soil from park on city outskirts	6	2015–2016	
Department of Animal Health, NEIKER (n = 49)	Spain (ES)	Farm animals	Cow, sheep and poultry feces [NS]	49	2004–2005, 2014–2016	4,51,52
Veterinary Faculty, University of Ljubljana (n = 26)	Slovenia (SI)	Farm animals	Cow and sheep [CS]	19	2011–2015	25
		Soil and farm environment	Farm environment, water, pond	2	2008, 2014	
		Wild animals	Fox brain, deer [NS]	5	2014	

Table 2. List of 301 animal and environmental *Listeria monocytogenes* strains from published microbial collections. CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk. NS, No listeriosis-associated Symptoms. ¹Strains isolated from silage were considered as originating from the farm environment since silage mainly includes fermented forage crops collected directly from fields.

neighboring clusters. An R script available at <https://github.com/lguillier/LISTADAPT/tree/master/metadata2association> was used to perform the selection of strains based on this method. This script takes as input a Comma Separated Values (CSV) file that includes strain ID and metadata information, then outputs a CSV file of selected strains.

In the present study, we constructed a large dataset comprising 301 animal and environmental *Lm* strains from six European countries and published collections (Table 2), as well as 347 animal and environmental *Lm* strains from 12 European countries that were obtained from non-published collections (Table 3). Strains collected from sampling campaigns (n = 108) Soil, farm, and wild animal samples were collected in nine European countries (Table 4). For the collection of soil samples, the LISTADAPT project members raised awareness and organised crowd-sampling campaigns. All the soil samples were collected from agricultural or wild areas according to a common procedure provided to the samplers based on the existing recommendations reported in the literature^{2,28–30}. The integration of feedback from samplers enabled a continuous improvement of the sampling protocol. The sampling campaigns were conducted in 17 areas in seven EU member states, Norway and Switzerland (Figs. 1 and 2, Table 4), namely AT, CH, CZ, FR, IT, NO, SE, SI and SK, resulting in the isolation of 58 *Lm* strains. Out of the 1752 available sampling records, the overall prevalence was 3%. We confirm in the present study the low prevalence of *Lm* in soil reported in the literature (below 1% and up to 6% depending on soil type)^{2,29}. Soil strains from AT, FR, SI and SE were isolated by employing a two-step specific enrichment: the first enrichment was performed with modified *Listeria* Enrichment Broth for 24 h at 30 °C, followed by enrichment in University of Vermont Medium (UVM) enrichment broth for 48 h at 30 °C. Detection of *Listeria* spp. and *Lm* was then achieved by specific SYBR Green real-time PCR targeting *prs2* and *inlA* genes, respectively. The samples positive for the presence of *Listeria* spp. and/or *Lm* were spread on RAPID[®] L.Mono agar plates (BioRAD, France). After 24 h incubation at 37 °C, colonies characteristic of *Lm* and other *Listeria* species were picked, purified and stored at –80 °C in Tryptone Soya Broth supplemented with 25% (v/v) glycerol. Strains from CH, CZ, IT and SK were isolated with the EN ISO 11290-1:2017 protocol (Horizontal method for the detection and enumeration of *Lm* and of *Listeria* spp.).

Regarding the subcompartments of farm and wild animal, 50 *Lm* strains were isolated from sampling campaigns. Three campaigns targeting shelled gastropods sampled in IT, SK and CH resulted in the isolation of six strains (Figs. 1 and 2, Table 4). Sampling campaigns were also carried out for wild deer and reindeer feces in Southern Norway, and from cattle, roe deer, wild boar, wolf, bear and fox feces in the Abruzzo and Molise regions of Italy (Fig. 1, Table 4). Of the 2577 samples collected from vertebrates during the campaign conducted in IT and NO 41 isolates were detected, with an overall prevalence of 1.6%.

Food strains included in the collection during the LISTADAPT project (n = 728). The food strains (C2 compartment) were classified according to the five main categories of risk food matrices for *Lm* defined by the European Food Safety Authority (EFSA)³¹: dairy products (n = 119), fish and fishery products (n = 165), meat products (n = 246), vegetables and fruits (n = 95), and composite dishes (food products combining several food categories) (n = 103). Six NRL project partners (AGES, ANSES, DTU, IZSAM, SLV and VRI) were instructed to target a maximum of 30 strains per food category from their strain collections, preferring strains isolated in the last

Partner	Country	Category	Origin of isolation	No. of strains	Isolation year
Not communicated by the authors (n = 87)	Belgium (BE)	Farm animals	Cow [NS]	87	2017–2018
Veterinary Research Institute (n = 14)	Czech Republic (CZ)	Farm animals	Cow, pig [NS]	6	2013–2017
		Soil and farm environment	Mud, algae from pond, soil from farm, decaying vegetation	8	2010, 2014
State veterinary institute (n = 7)	Czech Republic (CZ)	Wild animals	Gerbil, mouflon [NS]	3	Unknown
		Farm animals	Cow, sheep [NS]	4	Unknown
Veterinary and Food Laboratory (n = 25)	Estonia (EE)	Farm animals	Cow, sheep, goat [CS]	24	2014–2018
		Wild animals	Deer [CS]	1	2018
Faculty of Veterinary Medicine/ Department of Food Hygiene and Environmental Health, Helsinki (n = 24)	Finland (FI)	Farm animals	Cow, pork, goat milk, sheep [NS]	7	1987, 1995, 1998
		Wild animals	Hare, birds feces [NS]	4	1986, 1987
		Soil and farm environment	Silage ¹ and farm environment	13	2003, 2014–2015
Laboratory for Food Safety ANSES (n = 25)	France (FR)	Farm animals	Cow, poultry [NS], horse [CS]	8	2003, 2014, 2015, 2018
		Wild animals	Hare [NS]	3	1986, 1996, 2015
		Soil and farm environment	Manure, soil	14	2004, 2006, 2009, 2012
Research Unit OPAALE INRAE (n = 15)	France (FR)	Soil and farm environment	Soil, compost, pasture	15	2011, 2012, 2013
Institute of Food Safety and Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin (n = 15)	Germany (DE)	Farm animals	Pig and sheep at slaughterhouse retention area or immediately after slaughter [NS]	15	2009, 2018–2019
Institute of Food Safety, Animal Health and Environment BIOR (n = 25)	Latvia (LV)	Farm animals	Cow, goat, sheep, pig [CS]	25	2013–2018
Veterinary Faculty, University of Ljubljana (n = 51)	Slovenia (SI) (n = 28)	Farm animals	Cow, sheep, goat [CS]	20	2011–2015, 2018–2019
		Wild animals	Fox [NS]	7	2015
		Soil and farm environment	Cattle farm environment	1	2013
	Croatia (HR) (n = 23)	Farm animals	Cow, sheep, goat [CS]	23	2010, 2016–2017
Department of Biology, Swedish Food Agency (n = 5)	Sweden (SE)	Wild animals	Deer, rook, moose, wild boar [NS]	4	Unknown
		Farm animals	Poultry [NS], goat, sheep [CS]	1	Unknown
State Veterinary and Food Institute Dolny Kubin (n = 22)	Slovakia (SK)	Farm animals	Sheep, goat [CS]	20	2016–2018
		Soil and farm environment	Feed	2	2015, 2017
Laboratory Feed and Food and Product Safety VWA (n = 32)	The Netherlands (NL)	Farm animals	Cow, sheep, goat, poultry [NS]	32	2016–2018

Table 3. List of 347 animal and environment *Listeria monocytogenes* strains from non-published collections. CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk. NS, No listeriosis-associated Symptoms ¹ Strains isolated from silage were considered as originating from the farm environment since silage mainly includes fermented forage crops collected directly from fields.

10 years. This time period was extended to the under-represented categories (vegetables and fruits); the final dataset included strains originating from the 2002–2020 period. We excluded raw materials from the selection based on the assumption that they could be contaminated by strains originating from farms or animals. The 728 strains from C2 compartment were isolated along the food chain, from food processing plants to food retail in several EU countries (Table 1), detailed strain information were provided in Figshare File 1²⁶

Complete LISTADAPT dataset (n = 1484). The final LISTADAPT strain dataset that we constructed in collaboration with external partners was balanced with regard to the two main compartments: C1 (animals/ environment, n = 756) and C2 (food/FPE, n = 728) (Table 1). The geographic distribution covered 19 of the 27 EU countries plus Norway and Switzerland (Figs. 1 and 2).

Although the C1 compartment (n = 756) covered a 41-year period (1978–2019), most of the strains (75%) were isolated since 2010. This panel covered all successive years between 2009 and 2019 in at least three European regions (Fig. 1c). Between 2008 and 2019, except for the year 2013, the C1 compartment covered all successive years in the following three categories of subcompartments: farm animals, wild animals and natural/ farm environment (Fig. 1d).

Partner	Country (country code)	Category	Origin of isolation	No. of strains	Isolation year
Austrian Agency for Health and Food Safety AGES (n = 1)	Austria (AT)	Soil and farm environment	Meadow	1	2018
Veterinary Research Institute (n = 21)	Czech Republic (CZ)	Natural and farm environment	Soil, river bank, pond, decaying vegetation, manure	18	2016–2018
	Slovakia (SK)	Natural and farm environment	Soil, river bank	3	2016
Research Unit OPAALE INRAE (n = 13)	France (FR)	Soil and farm environment	Soil, compost, pasture	13	2018–2019
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale (n = 59)	Italy (IT)	Farm animals	Cow, goat, sheep [CS]	7	2014–2018
		Soil and farm environment	Soil and river water	16	2016–2018
		Wild animals	Fox, wolf, porcupine, badger, bear, snail, crayfish, roe deer, wild boar [NS]	36	2014, 2016–2018
Norwegian Veterinary Institute (n = 5)	Norway (NO)	Wild animals	Deer and reindeer [NS]	5	2017–2018
Veterinary Faculty, University of Ljubljana (n = 3)	Slovenia (SI)	Soil and farm environment	Water pond and soil	3	2018
State Veterinary and Food Institute Dolny Kubin (n = 1)	Slovakia (SK)	Wild animal	Snail	1	2019
Department of Microbiology, National Food Agency (n = 1)	Sweden (SE)	Soil and farm environment	Pasture	1	2018
Agroscope (n = 4)	Switzerland (CH)	Soil and farm environment	Pasture soil	3	2019
		Wild animal	Snail	1	2019

Table 4. List of 108 animal and environment strains from sampling campaigns. CS, Clinical Symptoms. The reported clinical symptoms included rhombencephalitis, abortion, septicemia and mastitis/subclinical mastitis. The type of clinical samples included cerebellum/brain tissue, aborted fetus, fetal membrane, liver, internal organs, feces and milk. NS, No listeriosis-associated Symptoms

Although the C2 compartment (n = 728) covered an 18-year period (2002–2020), most of the strains (78%) were recent, i.e. having been isolated between 2013 and 2019 (Fig. 1b). This panel covered all successive years between 2013 and 2019, as well as the five major categories in at least three European regions (Fig. 2b).

Strains from C1 compartment were isolated from more countries (n = 18) than strains from C2 compartment (n = 6). Finally, the majority (1135 of 1484 strains, 76%) of strains from both compartments originated from the period 2011–2019 (Fig. 1a,c).

Overall, the 1484 strains clustered into 137 MLST STs, which belonged to 54 CCs and 25 singleton STs (Fig. 3). For 22 strains, the allele profile was unknown (novel ST) or incomplete (When six out of seven MLST alleles were present, a CC was assigned when possible).

Standard strain nomenclature. In order to facilitate data sharing between partners, we adopted a standard nomenclature for strain identification (ID). This nomenclature was used as metadata codification to allow for fast identification of the geographic region and detailed isolation source of the strains (e.g., wild animal, food product or farm environment). In more detail, the LISTADAPT code has between 10 and 15 characters; the first two letters (level 1) correspond to the country code (ISO 3166-1-alpha-2 code), which is followed by a code detailing the origin of the strain and the sample type (level 2 to 4, depending on the nature of the source). Briefly, level 2 details the type of sample (e.g., animal species, environment and food categories) and level 3 details the nature of the sample (e.g., type of animal sample, type of food and nature of environmental sample). The level 4 gives additional information about the sample (e.g. type of preparation for aliments and health status of the animals). The code ends with a sequential number for each country, generated when the strain was added to the collection. For example: the strain DE-RDE-CP-13 was isolated in Germany (DE) from a roe deer (RDE) as a clinical strain (CP) and it was the 13th strain isolated from Germany included in the dataset. The Supplementary Table S2 provides a detailed overview of the employed LISTADAPT codification.

Whole Genome Sequencing and genomes data analysis. The next generation sequencing (NGS) paired-reads (2 × 150 bp) were generated during the project with Illumina platforms. Four LISTADAPT partners (AGES, IZSAM, ANSES and DTU) mainly performed the sequencing. Figshare File 1²⁶ lists the sequencing technology and the center which performed the library preparation and produced the sequences.

The genomes were all *de novo* assembled and annotated with a harmonized in-house workflow named ARTwork (Assembly of reads and typing workflow)³² used in the ANSES Laboratory for Food Safety. In addition to *de novo* assembly, the ARTwork pipeline also performs genome annotation using Prokka³³. This whole genome sequencing (WGS) workflow has been described in detail in previous publications^{32,34–36}, including the integrated bioinformatics tools and their corresponding versions, enabling repeatability and comparability of the results² (Table 5). Assembled genome files were made publicly available in FASTA format through Figshare³⁷.

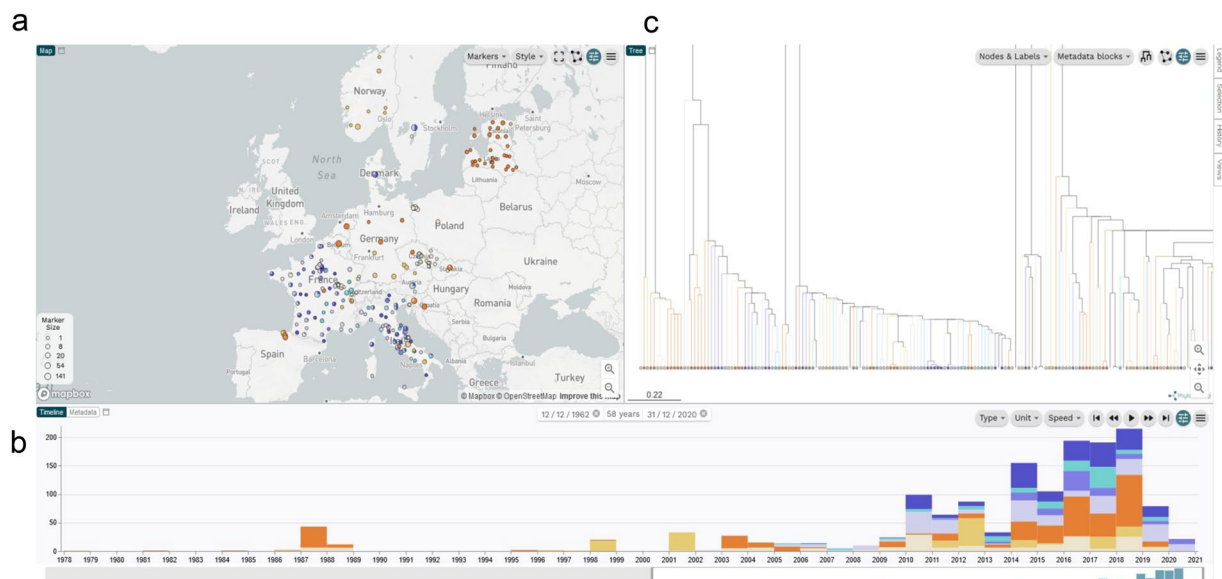


Fig. 2 Microreact screenshot representing the distribution of the whole LISTADAPT dataset ($n = 1484$) by geographic region (a) and time (b). The k-mer-based phylogenomic clustering of the complete dataset is shown in (c). Interactive access to strain metadata and MLST types is available through Microreact⁴⁴, a recently developed online tool for visualizing and sharing spatio-temporal and genetic distributions of strains (Fig. 2, accession link: <https://microreact.org/project/8YtGBqEqhos/tySXTVY79M-figure-2-distribution-of-the-whole-listadapt-dataset-n1484-by-geographic-region-time-and-genetic-diversity>). The dataset interactive map was generated using either the exact GPS coordinate, regional GPS coordinate or national GPS coordinate according to the level of details available for each strain. An annual timescale was used. The core genome MLST (Moura *et al.*) tree was generated from the draft genome assemblies using pairwise categorical difference and single linkage method in BioNumerics. The tree revealed three main clades corresponding to *Lm* phylogenetic lineages. Each clade included several clusters corresponding to MLST types (CC and singleton ST). Circles in shade of blue show food product isolates (clear blue: fish product, greenblue: dairy products, blue: composite dishes, deep blue: meat products). Circles in shade of orange show animal and environment isolates (beige: soil & farm environment, golden: wild animal, deep orange: farm animals). Circles size is proportional to the number of strains included.

Quality control of WGS data. Poor-quality reads or assemblies as well as contaminations can significantly affect gene prediction and cluster analyses^{38,39}. Different WGS metrics and quality criteria were thus employed in the ARTwork pipeline to ensure high-quality WGS data. Reads with an estimated depth of coverage $< 30\times$ (as estimated by BBmap⁴⁰) as well as contigs and scaffolds with a length of < 200 bp were excluded ($n = 22$). Draft genomes with a total length outside the range of 2.7–3.3 Mb and with a total number of scaffolds > 200 ($n = 46$) were also excluded. In addition, inter- and intra-species contamination of reads was determined using the recently developed ConFindr software (v0.5.1)⁴¹. Since recently demonstrated, inter-and-intra species contamination of 10 single nucleotide variants (SNVs) assessed by ConFindr in the conserved core genes does not significantly impact cluster analysis³⁹. We decided to exclude all genomes presenting SNVs lower than this cut-off ($n = 12$) as well as various read- or assembly-related errors ($n = 34$).

The employed WGS metrics and quality criteria of the complete LISTADAPT genome dataset are reported in Figshare File 1²⁶. In total, 114 sequenced genomes were of unsatisfactory quality after quality control and were thus excluded from the final dataset. After quality control of NGS and WGS data, the final LISTADAPT dataset included 1484 genomes.

Metadata and WGS data sharing. All metadata and WGS data collected herein were centralized and processed with standardized criteria for common nomenclature and NGS/WGS quality control before sharing between project partners. Reads normalized to $100\times$ coverage, draft assemblies (contigs and scaffolds) and annotated genomes (Genome Feature Format, GFF, and Genbank format, GBK) were also centralized at the MongoDB database located at ANSES (Maisons-Alfort Laboratory for Food Safety) providing quickly available, ready-to-use data.

Raw (non-normalized) reads for all the *Lm* strains sequenced in the LISTADAPT collection ($n = 1484$) were submitted to the NCBI Sequence Read Archive (SRA) for sharing with the LISTADAPT project's partners. Raw (non-normalized) reads for 67 *Lm* food strains obtained from previous publications^{19,42} were submitted to the NCBI Sequence Read Archive (SRA) database and were linked to their existing accession numbers in Figshare File 1²⁶.

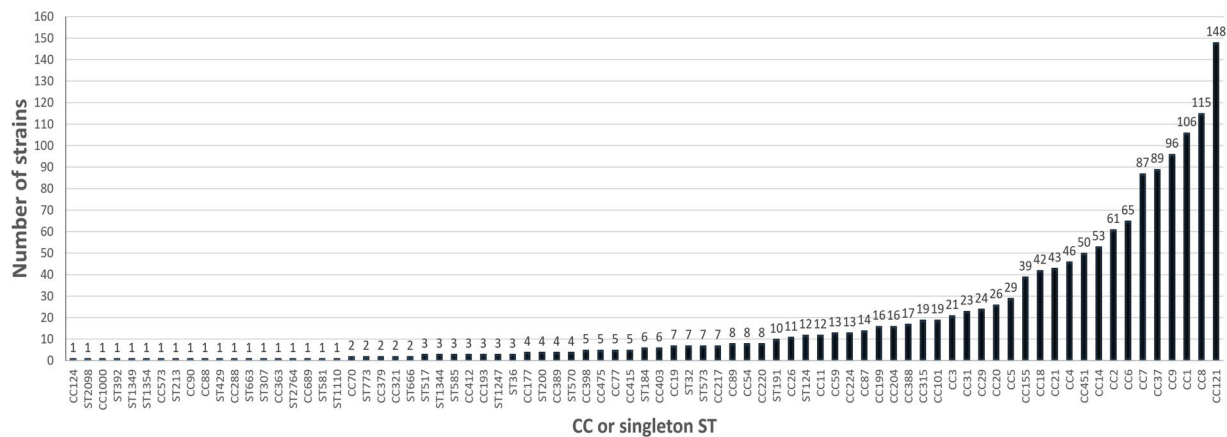


Fig. 3 Distribution of the LISTADAPT dataset of *Listeria monocytogenes* genomes by multilocus sequence typing clonal complex (CC) or singleton sequence type (ST).

Application	Software	Version
ARTwork		
Read mapping	BBMap	38.22-0
Read normalization	BBNorm	38.22-0
Quality assessment of reads	FastQC	0.11.8
Trimming of low-quality reads	Trimmomatic	0.38
De novo assembly	SPAdes	3.13.0
MLST prediction	MLST	2.16.1
Retrieval of the closest reference	Mash	2.0
Reference-based scaffolding	MeDuSa	1.3
Gap closing	GapCloser	2.04
Trimming of contigs < 200 bp	Biopython	
Quality assessment of the assembly	QUAST	5.0.2
Genome annotation	Prokka	1.13.3
Licensed software		
Core genome MLST	BioNumerics	V7.3
WGS based molecular serotyping	SeqSphere +	V7.0.4

Table 5. Bioinformatics tools used and their versions.

Data Records

All high-quality WGS data from this data descriptor are available for download at SRA/ENA public repository, including the sequences already available at the beginning of this study⁴³. Assembly and annotation files are available through Figshare⁴⁴. Complete metadata and quality check parameters are here reported in Figshare File 1²⁶.

Technical Validation

Redundant strains. The LISTADAPT dataset was analyzed by core-genome MLST (cgMLST) analysis, using BioNumerics (Table 5) according to a fixed cgMLST scheme consisting of 1748 Moura *et al.* loci⁴⁵. All strains with genomes presenting less than < 7 allele differences (AD), isolated in the same year, as well as sharing the same source of isolation and sharing identical geographic location (same region or country) were considered as redundant. When the latter information was not available, the provider was used instead. Although year of isolation was unknown for four strains, they were marked as redundant because of similar cgMLST (< 7 AD). Among the 1484 strains, 157 were identified as redundant. These strains were maintained in the dataset and marked accordingly (Figshare File 1²⁶)

Consistency analysis. The present study includes 648 strains from existing collections and 108 strains isolated in the framework of this study. The strains from historical collections were provided from 19 different laboratories. The management of large strain collections may lead to storage issue such as the isolation of two strains in the same tube. Furthermore, the sequencing of the strains involved several handling that may lead to human error.

For 380 of the 648 strains provided by partners, historical typing data were available. We established links between these typing data provided and the sequence obtained. These typing data were either, conventional serotyping data, molecular serotyping or MLST obtained by individual allele sequencing or mapping from PFGE. For conventional serotype the correspondence with the MLST type obtained from WGS was established following correspondence based on Ragon *et al.*¹². The correspondence with molecular serotyping was established based on Hyden *et al.*⁴⁶ mapping system using the Software SeqSphere (Table 5). For the strains isolated in Belgium (Table 3) the correspondence with PFGE was applied by our partner, based on the methodology described in Félix *et al.*¹⁸. For the strains isolated in Finland (Tables 2 and 3), the correspondence with PFGE was applied by our partners according to their in-house mapping methodology. The observed discordances were investigated with the partners. The concerned strains were re-sequenced if needed and discarded when unresolved. All results were reported in the Figshare File 1²⁶.

Code availability

The ARTwork pipeline, described in the WGS quality control section is publicly available at <https://github.com/afelten-Anses/ARTWORK>. The employed bioinformatics tools and their versions are specified in Table 5.

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References

- Piveteau, P., Depret, G., Pivato, B., Garmyn, D. & Hartmann, A. Changes in Gene Expression during Adaptation of *Listeria monocytogenes* to the Soil Environment. *PLOS ONE* **6**, e24881 (2011).
- Vivant, A. L., Garmyn, D. & Piveteau, P. *Listeria monocytogenes*, a down-to-earth pathogen. *Front Cell Infect Microbiol* **3**, 87 (2013).
- Buncic, S. *et al.* Microbial pathogen control in the beef chain: recent research advances. *Meat Sci* **97**, 288–97 (2014).
- Hurtado, A., Ocejó, M. & Oporto, B. *Salmonella* spp. and *Listeria monocytogenes* shedding in domestic ruminants and characterization of potentially pathogenic strains. *Vet Microbiol* **210**, 71–76 (2017).
- Yoshida, T., Sugimoto, T., Sato, M. & Hirai, K. Incidence of *Listeria monocytogenes* in wild animals in Japan. *J Vet Med Sci* **62**, 673–5 (2000).
- Weindl, L. *et al.* *Listeria monocytogenes* in Different Specimens from Healthy Red Deer and Wild Boars. *Foodborne Pathog Dis* **13**, 391–7 (2016).
- Parsons, C. *et al.* *Listeria monocytogenes* at the human-wildlife interface: black bears (*Ursus americanus*) as potential vehicles for *Listeria*. *Microb Biotechnol* **13**, 706–721 (2020).
- Lyautey, E. *et al.* Distribution and characteristics of *Listeria monocytogenes* isolates from surface waters of the South Nation River watershed, Ontario, Canada. *Appl Environ Microbiol* **73**, 5401–10 (2007).
- Hydeskov, H. B. *et al.* *Listeria Monocytogenes* Infection of Free-Living Western European Hedgehogs (*Erinaceus Europaeus*). *J Zoo Wildl Med* **50**, 183–189 (2019).
- Hellstrom, S., Kiviniemi, K., Autio, T. & Korkeala, H. *Listeria monocytogenes* is common in wild birds in Helsinki region and genotypes are frequently similar with those found along the food chain. *J Appl Microbiol* **104**, 883–8 (2008).
- Gismervik, K. *et al.* Invading slugs (*Arion vulgaris*) can be vectors for *Listeria monocytogenes*. *J Appl Microbiol* **118**, 809–16 (2015).
- Ragon, M. *et al.* A new perspective on *Listeria monocytogenes* evolution. *PLoS Pathog* **4**, e1000146 (2008).
- Chenal-Francisque, V. *et al.* Worldwide distribution of major clones of *Listeria monocytogenes*. *Emerg Infect Dis* **17**, 1110–2 (2011).
- Haase, J. K. *et al.* The ubiquitous nature of *Listeria monocytogenes* clones: a large-scale Multilocus Sequence Typing study. *Environ Microbiol* **16**, 405–16 (2014).
- Painset, A. *et al.* LiSEQ - whole-genome sequencing of a cross-sectional survey of *Listeria monocytogenes* in ready-to-eat foods and human clinical cases in Europe. *Microb Genom* **5**(2019).
- Dreyer, M. *et al.* *Listeria monocytogenes* sequence type 1 is predominant in ruminant rhombencephalitis. *Sci Rep* **6**, 36419 (2016).
- Cantinelli, T. *et al.* “Epidemic clones” of *Listeria monocytogenes* are widespread and ancient clonal groups. *J Clin Microbiol* **51**, 3770–9 (2013).
- Félix, B. *et al.* Population Genetic Structure of *Listeria monocytogenes* Strains Isolated From the Pig and Pork Production Chain in France. *Front Microbiol* **9**, 684 (2018).
- Henri, C. *et al.* Population Genetic Structure of *Listeria monocytogenes* Strains as Determined by Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing. *Appl Environ Microbiol* **82**, 5720–8 (2016).
- Holch, A. *et al.* Genome sequencing identifies two nearly unchanged strains of persistent *Listeria monocytogenes* isolated at two different fish processing plants sampled 6 years apart. *Applied and environmental microbiology* **79**, 2944–2951 (2013).
- Rychli, K. *et al.* Comparative genomics of human and non-human *Listeria monocytogenes* sequence type 121 strains. *PLOS ONE* **12**, e0176857 (2017).
- Stoller, A., Stevens, M. J. A., Stephan, R. & Guldemann, C. Characteristics of *Listeria Monocytogenes* Strains Persisting in a Meat Processing Facility over a 4-Year Period. *Pathogens* **8**, 32 (2019).
- Ortiz, S., Lopez, V. & Martinez-Suarez, J. V. Control of *Listeria monocytogenes* contamination in an Iberian pork processing plant and selection of benzalkonium chloride-resistant strains. *Food Microbiol* **39**, 81–8 (2014).
- Pasquali, F. *et al.* *Listeria monocytogenes* Sequence Types 121 and 14 Repeatedly Isolated Within One Year of Sampling in a Rabbit Meat Processing Plant: Persistence and Ecophysiology. *Frontiers in microbiology* **9**, 596–596 (2018).
- Papic, B., Pate, M., Félix, B. & Kusar, D. Genetic diversity of *Listeria monocytogenes* strains in ruminant abortion and rhombencephalitis cases in comparison with the natural environment. *BMC Microbiol* **19**, 299 (2019).
- Félix, B. Complete dataset, epidemiological informations, genomic quality assessment data and raw reads accession number. *figshare* <https://doi.org/10.6084/m9.figshare.17696738> (2022).
- Szymczak, B., Szymczak, M., Sawicki, W. & Dabrowski, W. Anthropogenic impact on the presence of *L. monocytogenes* in soil, fruits, and vegetables. *Folia Microbiol (Praha)* **59**, 23–9 (2014).
- Dowe, M. J., Jackson, E. D., Mori, J. G. & Bell, C. R. *Listeria monocytogenes* Survival in Soil and Incidence in Agricultural Soils (dagger). *J Food Prot* **60**, 1201–1207 (1997).
- Linke, K. *et al.* Reservoirs of *Listeria* species in three environmental ecosystems. *Appl Environ Microbiol* **80**, 5583–92 (2014).
- Weller, D., Wiedmann, M. & Strawn, L. K. Spatial and Temporal Factors Associated with an Increased Prevalence of *Listeria monocytogenes* in Spinach Fields in New York State. *Appl Environ Microbiol* **81**, 6059–69 (2015).
- Nielsen, E.M. *et al.* Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 3, the comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS) analysis. *EFSA Supporting Publications* **14** (2017).

32. Vila Nova, M. *et al.* Genetic and metabolic signatures of *Salmonella enterica* subsp. *enterica* associated with animal sources at the pangenomic scale. *BMC Genomics* **20**, 814 (2019).
33. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068–9 (2014).
34. Palma, F. *et al.* Dynamics of mobile genetic elements of *Listeria monocytogenes* persisting in ready-to-eat seafood processing plants in France. *BMC Genomics* **21**, 130 (2020).
35. Radomski, N. *et al.* A Simple and Robust Statistical Method to Define Genetic Relatedness of Samples Related to Outbreaks at the Genomic Scale - Application to Retrospective *Salmonella* Foodborne Outbreak Investigations. *Frontiers in microbiology* **10**, 2413–2413 (2019).
36. Felten, A. *et al.* First gene-ontology enrichment analysis based on bacterial coregenome variants: insights into adaptations of *Salmonella* serovars to mammalian- and avian-hosts. *BMC Microbiology* **17**, 222 (2017).
37. Li, W. *figshare* <https://doi.org/10.6084/m9.figshare.6025748> (2018).
38. Sims, D., Sudbery, I., Illott, N. E., Heger, A. & Ponting, C. P. Sequencing depth and coverage: key considerations in genomic analyses. *Nat Rev Genet* **15**, 121–32 (2014).
39. Pightling, A. W., Pettengill, J. B., Wang, Y., Rand, H. & Strain, E. Within-species contamination of bacterial whole-genome sequence data has a greater influence on clustering analyses than between-species contamination. *Genome biology* **20**, 286–286 (2019).
40. Bushnell, B. BBMap: A Fast, Accurate, Splice-Aware Aligner <https://www.osti.gov/servlets/purl/1241166> (2014).
41. Low, A. J., Koziol, A. G., Manninger, P. A., Blais, B. & Carrillo, C. D. ConFindr: rapid detection of intraspecies and cross-species contamination in bacterial whole-genome sequence data. *PeerJ* **7**, e6995 (2019).
42. Fritsch, L. *et al.* Insights from genome-wide approaches to identify variants associated to phenotypes at pan-genome scale: Application to *L. monocytogenes* ability to grow in cold conditions. *Int J Food Microbiol* **291**, 181–188 (2019).
43. Felix, B. *NCBI Sequence Read Archive/European Nucleotide Archive*. <https://www.ebi.ac.uk/ena/browser/view/PRJEB38828> (2022). ListAdapt complete high-quality WGS data.
44. Felix, B. ListAdapt complete *Listeria monocytogenes* de novo assemblies. *figshare* <https://doi.org/10.6084/m9.figshare.17315597> (2022).
45. Moura, A. *et al.* Whole genome-based population biology and epidemiological surveillance of *Listeria monocytogenes*. *Nat Microbiol* **2**, 16185 (2016).
46. Hyden, P. *et al.* Whole genome sequence-based serogrouping of *Listeria monocytogenes* isolates. *J Biotechnol* **235**, 181–6 (2016).
47. Hellstrom, S. *et al.* *Listeria monocytogenes* contamination in pork can originate from farms. *J Food Prot* **73**, 641–8 (2010).
48. Hsu, J. R. Epidemiological studies on the occurrence of *Listeria monocytogenes* in the feces of dairy cattle. *Zentralbl Veterinarmed B* **37**, 276–82 (1990).
49. Hsu, J. R., Seppanen, J. T., Sivela, S. K. & Rauramaa, A. L. Contamination of raw milk by *Listeria monocytogenes* on dairy farms. *Zentralbl Veterinarmed B* **37**, 268–75 (1990).
50. Ruusunen, M. *et al.* Pathogenic bacteria in Finnish bulk tank milk. *Foodborne Pathog Dis* **10**, 99–106 (2013).
51. Esteban, J. I., Oporto, B., Aduriz, G., Juste, R. A. & Hurtado, A. Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Vet Res* **5**, 2 (2009).
52. Esteban, J. I., Oporto, B., Aduriz, G., Juste, R. A. & Hurtado, A. A survey of food-borne pathogens in free-range poultry farms. *Int J Food Microbiol* **123**, 177–82 (2008).

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Author contributions

All authors read and approved the final manuscript. Benjamin Félix contributed substantially to the study design, soil sampling, acquisition of strains and the corresponding genomes, data analysis as well as writing and editing of the manuscript. Yann Sévellec contributed extensively to the acquisition of strains and the corresponding genomes, data analysis, quality control of the WGS dataset as well as writing and editing the manuscript. Federica Palma contributed to the acquisition of WGS data, data analysis and quality control of the WGS dataset and to writing and revision of the manuscript. Arnaud Felten, Nicolas Radomski and Ludovic Mallet performed the assembly centralized in a meaningful database. Pierre Emmanuel Douarre contributed substantially to the technical validation of the dataset. Pascal Piveteau participated in the study design and collected strains from natural environment in France. Pascal Piveteau and Eliette Ascensio performed the detection of *Lm* for part of the soil samples collected by the partners. Christophe Soumet, Arnaud Bridier and Michel Hébraud contributed to the study design and the manuscript revision. Marina Torresi and Francesco Pomilio collected strains from natural environment and provided strains and genomes from animals and food in Italy. Cesare Cammà and Adriano di Pasquale were responsible for sequencing part of the strains. Taran Skjerdal collected strains from natural environment and provided strains from wild animals and food in Norway. Ariane Pietzka and Werner Ruppitsch performed soil sampling and provided WGS data of strains isolated from food in Austria. Renáta Karpíšková collected and provided strains from soil, ruminants and food isolated in the Czech Republic. Tereza Gelbíčová contributed to the selection and characterization of strains, DNA extraction in the Czech Republic and partly to data sequencing. Monica Riccio collected strains from soil and provided strains from wild animals and food isolated in Sweden. Bojan Papić collected strains from soil, provided strains from ruminants and natural environment in Slovenia and partly contributed to data sequencing. Bart Wullings provided strains and genomes from ruminants previously isolated from the Netherlands. Hana Bulawova provided strains from wild animals and ruminants isolated in Czech Republic. Hanna Castro, Miia Lindström and Hannu Korkeala collected and provided strains from wild and farm animals from Finland. Žaneta Šteingolde and Toomas Kramarenko provided strains from farm animals isolated from Latvia and Estonia. Lenka Cabanova provided strains from ruminants and wild animals isolated from Slovakia. Barbara Szymczak provided strains from soil isolated from Poland. Verena Oswaldi provided strains from farm animals isolated from Germany. Manfred Gareis provided strains from wild animals isolated from Germany. Anne-Mette Seyfarth provided genomes from food strains isolated

from Denmark. Ana Hurtado provided strains from farm animals isolated from Spain. Thomas Berger and the team from Agroscope collected strains from soil and wild animals, and provided the genomes of these strains isolated from Switzerland. Jean-Charles Leblanc contributed to the revision of the manuscript and the technical validation of the dataset. Laurent Guillier participated in coordination of the study, selection of strains for sequencing and in drafting the manuscript. Sophie Roussel designed and coordinated the study and contributed to the writing and editing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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