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Genomic characterization of *Listeria* spp. isolated from tonsils, udder, and feces of domestic dairy ruminants in Spain

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Running title: *Listeria* spp. from ruminants' organs

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Abstract

Two species of *Listeria* are pathogenic, *Listeria monocytogenes* and *Listeria ivanovii*. Although studies have shown that dairy ruminants shed *Listeria* spp. in feces, there is little information about ruminants that do not shed *Listeria* spp. in their feces but asymptotically carry them in organs. We evidence that ruminants can asymptotically carry *L. ivanovii* in udders and *L. monocytogenes* and *L. ivanovii* in tonsils without fecal shedding. Whole-genome sequence of *L. monocytogenes* and *L. ivanovii* contained known core genes involved in virulence and antibiotic resistance. This work highlights tonsils and udders as a *Listeria* intra-host site of colonization.

Keywords: *Listeria* spp.; dairy; ruminant; tonsil; udder; WGS

1. Introduction

Listeriosis is recognized as a significant public health issue due to the severity of the disease and its mortality rate (20% to 30%) [1,2]. Only two species of the genus *Listeria*, *Listeria monocytogenes* (*Lm*) and *Listeria ivanovii* (*Liv*), are considered facultative intracellular pathogens [2]. *Lm* can cause serious invasive disease (including central nervous system and fetal-placental infection) in animals and humans [1,2]. Although *Liv* is mainly an animal pathogen, its isolation from human patients with listeriosis symptoms has been reported [3]. *Listeria innocua* (*Lin*) is a close relative of *Lm* and is nonpathogenic to mammals, although atypical virulent isolates have been reported [4].

Healthy people and domestic ruminants tend to be asymptomatic carriers that shed *Lm*, *Liv* and non-pathogenic *Listeria* spp. in their feces [5,6]. Fecal shedding of *Lm* could lead to contamination of agricultural environments and raw produce which are then transported to food processing industries [7]. In dairy farms, *Lm* is usually transmitted to bulk tank milk as an outcome of environmental or fecal contamination of the udder [8].

While the incidence of listeriosis in humans is low compared with other foodborne infections, listeriosis is recognized as a significant public health issue due to its severity and high mortality rate [1]. *Lm* population is diverse, comprising 4 lineages (ref), and its population structure can be determined by using multilocus sequence typing (MLST) and core genome MLST (cgMLST). MLST, which analyzes seven housekeeping genes, groups *Lm* isolates in sequence types (STs) and clonal complexes (CCs or clones) (Ref Ragon2008). cgMLST, which analyzes 1748 core loci, allows *Lm* classification in cgMLST types (CTs) and

sublineages (SLs) [9]. *Listeria* spp. surveillance in animal production settings normally targets fecal or environmental samples, however, information on *Listeria* spp. presence in organs such as tonsils and udders is still scarce. Moreover, there is no information about asymptomatic carriage of *Listeria* spp. in ruminants' organs without fecal shedding. Here, we used whole-genome sequencing to subtype and characterize *Listeria* spp. isolated from feces, tonsils, and udders from domestic dairy ruminants. Increasing the understanding of the reservoirs and genomic characteristics of *Lm* and *Liv* will help to improve their control, enhancing human and animal disease prevention.

2. Materials and methods

2.1 Animals and sampling

In the time frame of December 2019 to May 2021, 93 dairy sheep (*Ovis orientalis aries* breed Lacaune, Manchega, Assaf, and crossbreed), 10 dairy goats (*Capra aegagrus hircus* breed Guadarrama and crossbreed), and 183 dairy cattle (*Bos primigenius taurus* breed Holstein-Friesian) were slaughtered in two slaughterhouses (slaughterhouse A located in Madrid for small ruminants and slaughterhouse B located in Valencia for cattle). Each slaughtering was followed by a systematic cleaning and disinfection protocol. Veterinary supervision was provided throughout the slaughter and Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SSOP), Hazard Analysis Critical Control Point (HACCP), and the traceability was strictly followed. Additionally, 29 dairy sheep and 1 dairy goat were culled for educational purposes to the authors' university. An intravenous injection of barbiturate was used to euthanize these additional animals, and complete necropsies were carried out immediately

following death. Sheep and goats originated from 36 farms located in the provinces (administrative division in Spain) from Valencia, Albacete, Cuenca, Toledo, Ciudad-Real, and Madrid (Table S1). Cattle originated from 13 farms located in the provinces of Murcia, Albacete, Teruel, Alicante and Valencia (Table S1 and Fig. S2). Approximately 10 g of tonsils and udders were removed aseptically and stored in sterile bags. Tonsils were sampled while the carcasses were hanging upside down which implies that surface contamination was not possible. Udders were sampled once they were removed from the animal and located on a tray. A sample from the inner part of the udder was obtained by applying a cut on a previous decontaminated part of the organ surface with alcohol. Veterinary inspectors assumed that slaughtered animals were healthy based on the lack of abnormal behavior, clinical signs and macroscopic abnormalities in the thoracic and abdominal cavities of the carcasses. Thoracic and abdominal organs were inspected to evaluate position, size, and color. Serosal surfaces were examined, and organs evaluated for any grossly visible abnormalities in structure and/or pathologic processes. The mandibular, the lung (cervical, mediastinal, tracheobronchial) and mesenteric lymph nodes, were also identified and evaluated. Collected feces from the rectum of each ruminant were stored in independent sterile bags. Each animal sample (tonsils, udder, and rectum) was handled with a new and independent sterile scalpel to prevent contamination. All samples were collected using disposable gloves under aseptic conditions. Within 24h, the entire sample material was transported to the laboratory at 5+/-3°C.

2.2 Listeria species isolation and identification.

Listeria spp. were isolated as previously described [6,10,11]. Briefly, 8 g of rectal fecal samples, 5 g of tonsils, and udders were disrupted and diluted 1/10 in Half-Fraser broth (Scharlab, Spain). An enrichment step of 24 h at 30 °C was performed on homogenized samples. Incubated suspensions were transferred into Fraser broth (Scharlab, Spain), and incubated for 24 h at 37 °C, before being transferred to RAPID'L. *mono* plates (BioRad, USA) and incubated at 37 °C for 24 h. RAPID'L. *mono* plates support growth of *L. monocytogenes* (PIPLC+/xylose-, blue colonies), *L. ivanovii* (PIPLC+/xylose+, blue-green colonies with yellow halo), and other *Listeria* spp. (white colonies). When more than one type of colony was present in RAPID'L. *mono* plates, one blue (no plate contained simultaneously *L. monocytogenes* and *L. ivanovii*) and one white *Listeria* spp. were picked and further examined in selective Oxford agar plates for *Listeria* (Scharlab, Spain). From each positive sample, one isolate colony was obtained (if in the same plate both pathogenic and non-pathogenic *Listeria* spp. were present, one colony of each was selected) and sent to the World Health Organization Collaborating Center *Listeria* (Institut Pasteur, Paris) for characterization. As described previously, *Listeria* isolates were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry applying the MicroFlex LT system with the last MBT library DB-7854 (Bruker Daltonics, Germany) [12] and by whole genome sequencing [10].

2.3 DNA extraction, genome sequencing and sequence analysis.

DNA was purified with the NucleoSpin Tissue DNA kit (Machenry-Nagel)

from 900 µL bacterial cultures grown overnight in 5 mL of brain heart infusion broth (Becton Dickinson, USA) at 37 °C.

DNA libraries were prepared with Nextera XT DNA Sample kit (Illumina, USA), following manufacturer's protocol, and run in NextSeq 500 platform (Illumina, USA) to obtain paired-end reads (2 x 150 bp run). Raw reads were trimmed with fqCleaner v.21.10 and assembled with SPAdes v.3.15.0, using automatic k-mers selection and the --only-assembler and --careful parameters.

In silico typing was performed from the assemblies using BIGSdb-*Listeria* v.1.33.1 [13] using the genoserogrouping, MLST, cgMLST profiles, resistance and virulence schemes implemented within [9], as previously described [6,9]. A maximum phylogeny was inferred from the core genome alignment built using Parsnp, v1.5.6 (as implemented in Harvest suite 1.3 [14]), using IQ-TREE/2.0.6 [15]. Trees were visualized with iTol v.4.2 [16].

2.4 Statistical analysis

IBM SPSS Statistics version 25 was used for all statistical analyses . Statistical tests were considered significant at $P < 0.05$. Chi-square (χ^2) tests were performed to determine the effect of geographical location of farms and animal species on the number of *Lm* or *Liv* carriage.

2.5 Data availability.

All sequences are publicly available at the European Nucleotide Archive (BioProject PRJEB51772) and BIGSdb-*Listeria* (<https://bigsdb.pasteur.fr/listeria>).

3. Results and discussion

A total of 948 tonsil, udder, and feces samples were collected from 316 dairy ruminants (183 cows, 122 sheep, and 11 goats) in two slaughterhouses and one necropsy room over 18 months. Three different *Listeria* spp. were detected in 35 animals (Table 1 and Table S1). *Lm* was isolated from 4 (1.3%) and 2 (0.6%) out of 316 animal tonsils, and feces samples, respectively (Table 1 and Table S1). *Liv* was isolated from 2 (0.6%), 1 (0.3%), and 13 (4.1%) out of 316 animal tonsils, feces, and udder samples, respectively (Table 1 and Table S1). *Lin* was isolated from 15 (4.7%) out of 316 feces samples but was not detected in any of the tonsils or udder samples (Table 1 and Table S1). Co-occurrence of different *Listeria* spp. (*Lm* and *Lin*) was detected in 0.6% (2/316) of individual animal feces (Fig. 1, Fig. S1, Table S1). *Lm* and *Liv* were detected more frequently in cattle (19/183 positive cows, prevalence 10.4%) than in small ruminants (2/122 positive sheep, prevalence 1.6%, and 0/11 positive goats, 0%) (χ^2 test, $P < 0.05$), in agreement with our previous longitudinal study performed in 19 dairy ruminant farms [6]. Regarding, non-pathogenic *Listeria* spp. the prevalence was significantly higher in small ruminants than in cattle (χ^2 test, $P < 0.05$), as previously shown [6]. These results show that dairy ruminants can silently carry *Lm* or *Liv* in udders or tonsils even without fecal shedding, as we previously reported in wild animals [11]. Other studies have shown that large numbers of *Lm* can be isolated in the tonsils of experimental orally infected sheep despite fecal excretion stopped [17], suggesting that tonsils are a location of *Lm* colonization. *Lm* is conveyed to milk through contamination of the udder surface or the equipment used for milking [8]. *Lm* is infrequently isolated as a cause of dairy ruminant mastitis, which tend to be

asymptomatic [18,19]. The rarity of listerial mastitis cases support the hypothesis that most of the *Listeria* milk contamination cases may arise from the udder surface or the milking equipment.

Liv positive cows originated more frequently from farms situated in the province of Murcia (14/82, prevalence 17.1%) than in the province of Valencia (2/88, prevalence 2.2%) ($P < 0.05$) (Fig. S2). According to official statistics, the province of Murcia has 77.346 bovines in a surface of 11.313 km², whereas the province of Valencia has 25.664 bovines in a surface of 10.841,82 km² suggesting that *Liv* prevalence could be influenced by animal density. However, since the number of animals that were slaughtered was not homogeneous across farms (Fig. S2), it could not be determined if the differences in the prevalence of *Liv* observed between Valencia and Murcia provinces could be attributed to a farm or a province effect. Previous environmental studies have shown that the occurrence of *Liv* is rare compared with *Lm* [20]. Despite the less frequent isolation of *Liv*, this species has been found in human feces, domestic ruminant feces and milk, farm environments, dairy processing facilities, and wild boar and deer tonsils [2,5,21,22]. In line with our results showing the presence of *Liv* in cow udders, a survey to evaluate the occurrence of *Liv* in food processing environments and foods in the Republic of Ireland, showed that a higher prevalence was detected for the dairy sector compared to the meat, seafood, and fresh-cut vegetable sectors (1.7 vs 0.2, 0.3, and 0%, respectively) [21]. Since a two-step enrichment protocol was used to enhance detection of *Listeria* spp., and the culled cows did not show mastitis clinical signs in our study, it is plausible that the number of *Liv* in the analyzed udders was low. The probable low numbers of *Liv* in the udders together with a restricted geographical distribution of *Liv* could explain why this

species is not commonly detected during surveillance of milk products and only few reports describe its higher prevalence in the milk sector compared to other food sectors [21]. Interestingly, *Liv* isolates obtained from varied geographical and temporal sources in the present study were mainly associated with udders.

Genome-based genotyping results indicated that *Lm* isolates belonged to CC9 ($n=2$), CC4 ($n=1$), CC6 ($n=1$), CC37 ($n=1$), and CC388 ($n=1$) (Table S1, Fig. 1 and Fig. S1). CC9 was previously reported as a CC associated with meat products [23]. CC4 and CC37 were previously reported as CCs associated with dairy products [23]. Importantly, CC4, CC6 and CC388 belong to lineage I which is significantly associated with a clinical origin both in cattle and humans [24]. CC4 and CC6 are more invasive and colonize better the intestinal lumen [23]. Moreover, CC4 and CC6 have been associated with human clinical cases some even without immunosuppressive conditions [24]. This and previous studies suggest that the population of *Liv* is genetically less diverse than *Lm* (Fig. S1) [11,25]. The significance of these observations and their relation to host adaptation will require further research.

The core virulence genes of LIPI-1 (necessary for the intracellular survival) (the term LIPI stands for *Listeria* pathogenicity island) and the *inlAB* locus (necessary for bacterial internalization into eukaryotic cells) were present in all *Lm* and *Liv* isolates (Fig. 1). *Listeria* pathogenic island 3 (LIPI-3), which promotes intestinal colonization by *Lm* [2], was present in 33.3% (2/6) *Lm* and 53.3% (8/15) *Lin*. *Listeria* pathogenic island 4 (LIPI-4), which increases *Lm* neural and placental tropism [24], was present in 33.3% (2/6) *Lm* and 93.3% (14/15) *Lin* (Fig. 1). LIPI-2, involved in virulence [2], was present in 100% (16/16) *Liv* (Fig. 1). Autolysins (*aut*, *aut_IVb*), and LPxTG proteins coding genes (*inlE*, *inlG*, *inlH*, *inlJ*, *inlK* and

vip) related to invasion were present in *Lm* (Fig. 1, Table S2). Other genes important for teichoic acids biosynthesis, adherence, regulation, and biofilm formation were present in *Lm*, *Liv* and *Lin* (Table S2). Intrinsic antibiotic resistance genes (*fosX*, *lin* (lmo0919), *norB*, and *sul*) were present in all *Lm*, *Liv* and *Lin* isolates. *tetM* and *InuG* (resistance to tetracyclines and lincosamides) genes were also present in *Lin* (5/15, 33.3%; 1/15, 6.7%, respectively). The SSI-1 stress survival islet, involved in *Lm* growth in high salt concentrations and low pH, was present in 2 out of 3 lineage II *Lm* isolates. The SSI-2 stress survival islet, which contributes to the alkaline and oxidative stress responses, was present in 14 out of 15 *Lin* isolates (Fig. 1).

The results of this study contributes to the understanding of the pathogenic potential and ecology of *Listeria* spp. in dairy ruminants' organs. We have revealed that (i) tonsils and udders constitute a reservoir of *Lm* or *Liv* in domestic dairy ruminants; (ii) dairy ruminants can silently carry *Lm* or *Liv* in udders or tonsils even without fecal shedding; and (iii) *Lm* and *Liv* are detected more frequently in cattle than in small ruminants. Dairy ruminants may contaminate milk or crops and the environment which can pass *Lm* and *Liv* to humans via food. The present discoveries could help public health authorities to better control the health risks associated with *Lm* and *Liv*.

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Figure legends

Fig 1. Genomic diversity of *Listeria* spp. found in this study (N=37). A) Midpoint rooted maximum likelihood phylogeny (GTR+F+I+G4 model, 1000 ultrafast bootstraps) inferred based on the core SNP alignment (327,775 nucleotides) using *L. ivanovii* subsp. *ivanovii* CLIP 2021/00402 arbitrary reference. *Listeria* species are shown in different colors. Information on the isolate's name, origin, date of isolation, CT, and SL are provided. Bold names highlight isolates from the same animal. B) resistance and virulence gene patterns. Color-filled boxes represent the presence of different genetic traits.

Supplementary Figures

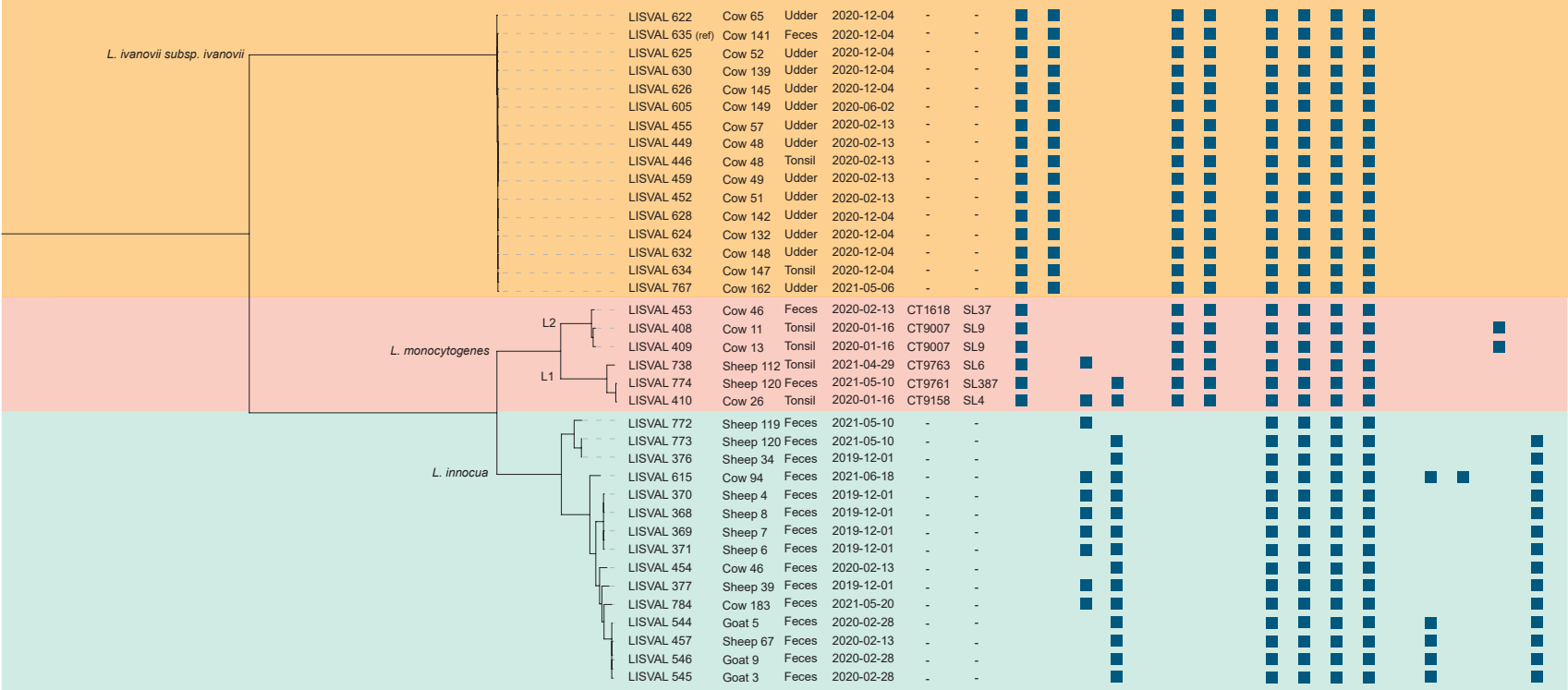
Fig S1. Unrooted core genome phylogenies of *Listeria* spp. found in this study. A) *L. monocytogenes* (2,506,493 nucleotides alignment). B) *L. ivanovii* subsp. *ivanovii* (2,791,218 nucleotides alignment). C) *L. innocua* (2,446,398 nucleotides alignment). Tips are colored by source type and sample origin. Dashed circles delimitate clonal complexes (CCs). Isolates obtained from the same animal are marked with a #.

Fig S2. Geographic map of Spain provinces and the *Liv* prevalence per cattle farm.

Figure 1

A)

Tree scale: 0.01



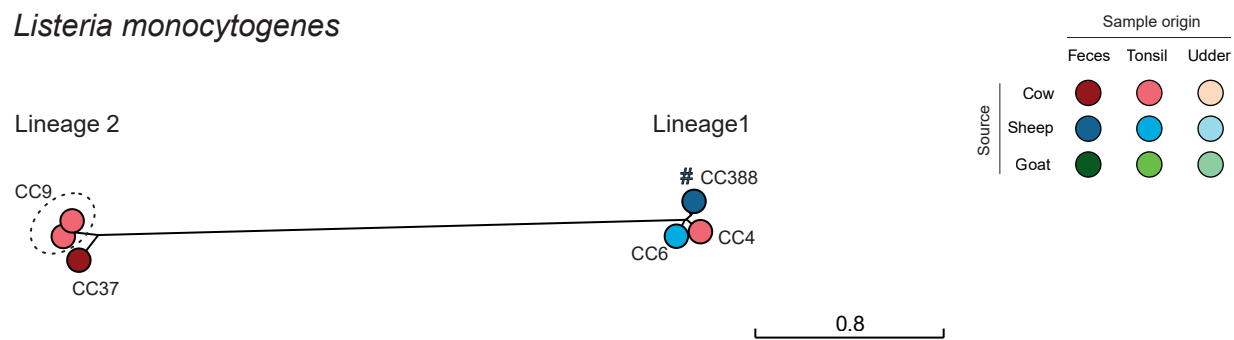
B)

LIPIS
inlAB
Intrinsic resistance
Acquired resistance

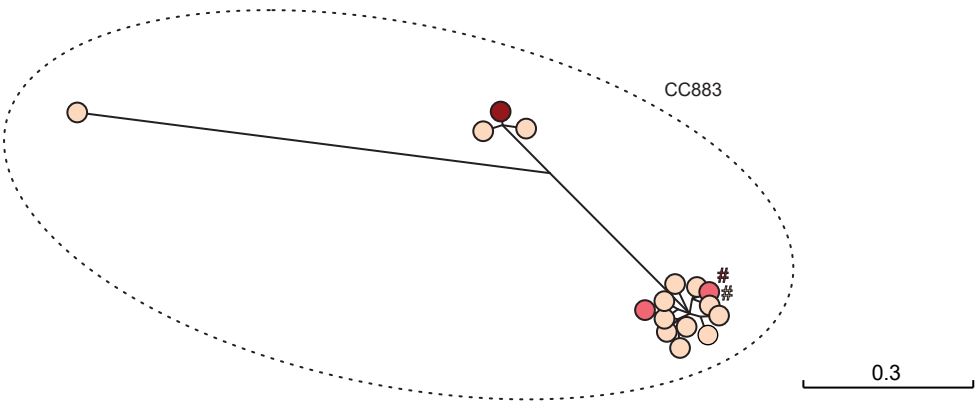
LIP1-1
LIP1-2
LIP1-3
LIP1-4
inlA
inlB
fosX
lin
norB
sui
tetM
truG
SSI-1
SSI-2

Supplementary figure 1

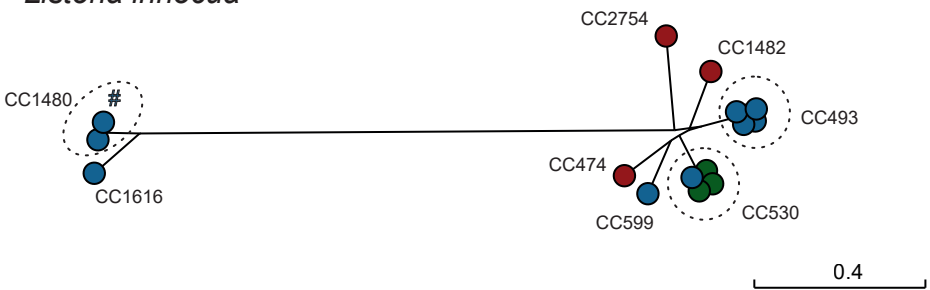
A *Listeria monocytogenes*



B *Listeria ivanovii* subsp. *ivanovii*



C *Listeria innocua*



Supplementary figure 2

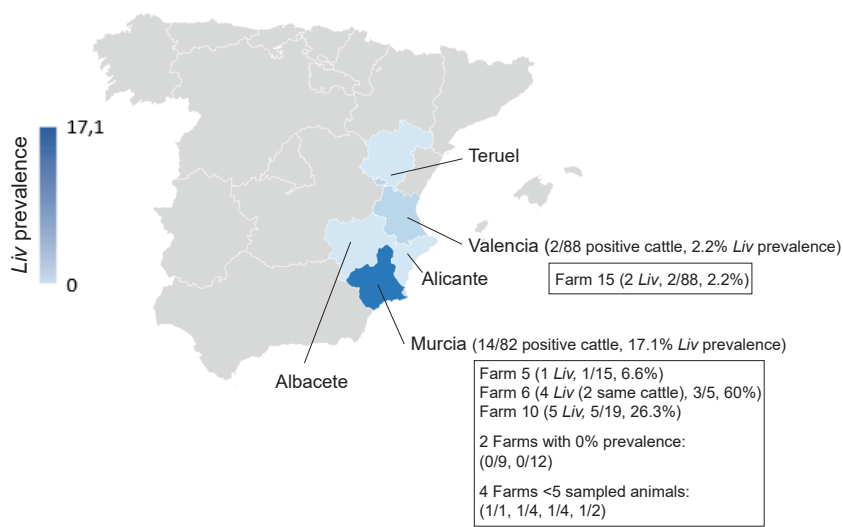


Table 1. Number of isolates obtained in this study (N =37).

<i>Listeria</i> spp.	Cow feces (n=183)	Cow tonsils (n=183)	Cow udder (n=183)	Sheep feces (n=122)	Sheep tonsils (n=122)	Sheep udder (n=122)	Goat feces (n=11)	Goat tonsils (n=11)	Goat udder (n=11)
<i>L. monocytogenes</i>	1 (0.5%)	3 (1.6%)	0	1 (0.8%)	1 (0.8%)	0	0	0	0
<i>L. ivanovii subsp. ivanovii</i>	1 (0.5%)	2 (1.1%)	13 (7.1%)	0 (%)	0 (0%)	0 (0%)	0	0	0
<i>L. innocua</i>	3 (1.6%)	0	0	9 (7.4%)	0	0	3 (27.2%)	0	0

Table S1. *Listeria* isolates characterized in this study.

BIGSdb id	isolate	aliases	species	isolation date	province	farm	source	source details	no. contigs	total length (bp)	N50 contig length (bp)	G+C content (%)	cgMLST loci called (%)	phylogenetic lineage	genosero group	CC (MLST)	ST (MLST)	SL (cgMLST)	CT (cgMLST)
77387	CLIP 2021/00361	LISVAL 368	<i>L. innocua</i>	2019-12-01	Toledo	Farm 16	Animal 8	sheep - feces	26	2893822	579505	37.3	n.a.	n.a.	L	CC493	ST493	n.a.	n.a.
77388	CLIP 2021/00362	LISVAL 369	<i>L. innocua</i>	2019-12-01	Toledo	Farm 16	Animal 7	sheep - fece	31	2894284	566880	37.3	n.a.	n.a.	L	CC493	ST493	n.a.	n.a.
77389	CLIP 2021/00363	LISVAL 370	<i>L. innocua</i>	2019-12-01	Toledo	Farm 16	Animal 4	sheep - feces	26	2894491	483337	37.3	n.a.	n.a.	L	CC493	ST493	n.a.	n.a.
77390	CLIP 2021/00364	LISVAL 371	<i>L. innocua</i>	2019-12-01	Toledo	Farm 16	Animal 6	sheep - feces	31	2894949	270613	37.3	n.a.	n.a.	L	CC493	ST493	n.a.	n.a.
77391	CLIP 2021/00365	LISVAL 376	<i>L. innocua</i>	2019-12-01	Cuenca	Farm 2	Animal 34	sheep - feces	17	2871184	543767	37.3	n.a.	n.a.	L	CC1480	ST1480	n.a.	n.a.
77392	CLIP 2021/00366	LISVAL 377	<i>L. innocua</i>	2019-12-01	Cuenca	Farm 3	Animal 39	sheep - feces	20	2784629	479655	37.4	n.a.	n.a.	L	CC599	ST2753	n.a.	n.a.
77422	CLIP 2021/00378	LISVAL 454	<i>L. innocua</i>	2020-02-13	Murcia	Farm 7	Animal 46	cow - feces	95	2822874	100830	37.4	n.a.	n.a.	L	CC474	ST474	n.a.	n.a.
77425	CLIP 2021/00381	LISVAL 457	<i>L. innocua</i>	2020-02-13	Cuenca	Farm 8	Animal 67	sheep - feces	67	2871346	144067	37.4	n.a.	n.a.	L	CC530	ST530	n.a.	n.a.
77426	CLIP 2021/00382	LISVAL 544	<i>L. innocua</i>	2020-02-28	Ciudad Real	Farm 9	Animal 5	goat - feces	46	2847186	140717	37.4	n.a.	n.a.	L	CC530	ST530	n.a.	n.a.
77427	CLIP 2021/00383	LISVAL 545	<i>L. innocua</i>	2020-02-28	Ciudad Real	Farm 9	Animal 3	goat - feces	45	2846589	188483	37.4	n.a.	n.a.	L	CC530	ST530	n.a.	n.a.
77428	CLIP 2021/00384	LISVAL 546	<i>L. innocua</i>	2020-02-28	Ciudad Real	Farm 9	Animal 9	goat - feces	48	2846234	219660	37.4	n.a.	n.a.	L	CC530	ST530	n.a.	n.a.
77432	CLIP 2021/00388	LISVAL 615	<i>L. innocua</i>	2020-06-18	Valencia	Farm 15	Animal 94	cow - feces	28	2971457	333301	37.3	n.a.	n.a.	L	CC2754	ST2754	n.a.	n.a.
79916	CLIP 2021/01137	LISVAL 772	<i>L. innocua</i>	2021-05-10	Castellón	Farm 13	Animal 119	sheep - feces	38	2815802	138611	37.3	n.a.	n.a.	L	CC1616	ST1616	n.a.	n.a.
79917	CLIP 2021/01138	LISVAL 773	<i>L. innocua</i>	2021-05-10	Castellón	Farm 13	Animal 120	sheep - feces	46	2864904	210079	37.3	n.a.	n.a.	L	CC1480	ST1480	n.a.	n.a.
79935	CLIP 2021/01149	LISVAL 784	<i>L. innocua</i>	2021-05-20	Valencia	Farm 14	Animal 183	cow - feces	13	2857362	459131	37.5	n.a.	n.a.	L	CC1482	ST1482	n.a.	n.a.
77393	CLIP 2021/00367	LISVAL 408	<i>L. monocytogenes</i>	2020-01-16	Murcia	Farm 4	Animal 11	cow - tonsil	35	3000468	477704	37.8	100	II	IIc	CC9	ST9	SL9	CT9007
77394	CLIP 2021/00368	LISVAL 409	<i>L. monocytogenes</i>	2020-01-16	Murcia	Farm 5	Animal 13	cow - tonsil	84	3003383	192819	37.8	99.6	II	IIc	CC9	ST9	SL9	CT9007
77395	CLIP 2021/00369	LISVAL 410	<i>L. monocytogenes</i>	2020-01-16	Murcia	Farm 4	Animal 26	cow - tonsil	37	2887721	501910	38	99.9	I	IVb	CC4	ST4	SL4	CT9158
77421	CLIP 2021/00377	LISVAL 453	<i>L. monocytogenes</i>	2020-02-13	Murcia	Farm 7	Animal 46	cow - feces	67	2991961	150603	37.9	99.9	II	IIa	CC37	ST37	SL37	CT1618
79286	CLIP 2021/01103	LISVAL 738	<i>L. monocytogenes</i>	2021-04-29	Castellón	Farm 13	Animal 112	sheep - tonsi	43	2926934	292321	37.9	99.9	I	IVb	CC6	ST6	SL6	CT9763
79918	CLIP 2021/01139	LISVAL 774	<i>L. monocytogenes</i>	2021-05-10	Castellón	Farm 13	Animal 120	sheep - feces	112	2927659	70512	37.9	99.7	I	IVb	CC388	ST388	SL387	CT9761
77396	CLIP 2021/00370	LISVAL 446	<i>L. ivanovii subsp. ivanovii</i>	2020-02-13	Murcia	Farm 6	Animal 48	cow - tonsil	56	2907510	323421	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77417	CLIP 2021/00373	LISVAL 449	<i>L. ivanovii subsp. ivanovii</i>	2020-02-13	Murcia	Farm 6	Animal 48	cow - udder	54	2926679	348239	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77420	CLIP 2021/00376	LISVAL 452	<i>L. ivanovii subsp. ivanovii</i>	2020-02-13	Murcia	Farm 6	Animal 51	cow - udder	70	2926607	181775	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77423	CLIP 2021/00379	LISVAL 455	<i>L. ivanovii subsp. ivanovii</i>	2020-02-13	Murcia	Farm 6	Animal 57	cow - udder	57	2929111	247159	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77424	CLIP 2021/00380	LISVAL 459	<i>L. ivanovii subsp. ivanovii</i>	2020-02-13	Murcia	Farm 7	Animal 49	cow - udder	55	2913115	352213	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77429	CLIP 2021/00385	LISVAL 605	<i>L. ivanovii subsp. ivanovii</i>	2020-06-02	Valencia	Farm 15	Animal 149	cow - udder	41	2900789	331742	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77433	CLIP 2021/00389	LISVAL 622	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 1	Animal 65	cow - udder	62	2929665	109996	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77435	CLIP 2021/00391	LISVAL 624	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 11	Animal 132	cow - udder	48	2901466	164327	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77436	CLIP 2021/00392	LISVAL 625	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 12	Animal 52	cow - udder	83	2954649	148669	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77437	CLIP 2021/00393	LISVAL 626	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 10	Animal 145	cow - udder	36	2944244	211155	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77439	CLIP 2021/00395	LISVAL 628	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 10	Animal 142	cow - udder	41	2901256	224777	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77441	CLIP 2021/00397	LISVAL 630	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 5	Animal 139	cow - udder	43	2944941	171888	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77443	CLIP 2021/00399	LISVAL 632	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 10	Animal 148	cow - udder	104	2895532	64194	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77445	CLIP 2021/00401	LISVAL 634	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 10	Animal 147	cow - tonsil	111	2894126	78390	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
77447	CLIP 2021/00402	LISVAL 635	<i>L. ivanovii subsp. ivanovii</i>	2020-12-04	Murcia	Farm 10	Animal 141	cow - feces	72	2947364	210627	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.
79911	CLIP 2021/01132	LISVAL 767	<i>L. ivanovii subsp. ivanovii</i>	2021-05-06	Valencia	Farm 15	Animal 162	cow - udder	34	2901547	239098	37	n.a.	n.a.	L	CC883	ST883	n.a.	n.a.

Table S2. Extended virulence gene patterns in *Listeria* isolates characterized in this study. Boxes with an “x” represent the presence of the genetic trait.

BIGSdb id	isolate	aliases	species	phylogenetic lineage	genoseregroup	CC (MLST)	ST (MLST)	SL (cgMLST)	CT (cgMLST)	gltA	gltB	tagB	aut	aut_lvb	inIE	inIG	inIH	inIJ	inIK	vip	oppA	agrC	mouR	mdrM	comK	bapL	ami	dtlA	fbpA	lap	lapB
77396	202100370	LISVAL 446	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77417	202100373	LISVAL 449	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77420	202100376	LISVAL 452	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77423	202100379	LISVAL 455	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77424	202100380	LISVAL 456	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77429	202100385	LISVAL 605	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77433	202100389	LISVAL 622	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77435	202100391	LISVAL 624	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77436	202100392	LISVAL 625	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77437	202100393	LISVAL 626	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77439	202100395	LISVAL 628	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77441	202100397	LISVAL 630	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77443	202100399	LISVAL 632	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77445	202100401	LISVAL 634	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77447	202100402	LISVAL 635	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
79911	202101132	LISVAL 767	<i>L. ivanovii</i> subsp. <i>ivanovii</i>	n.a.	L	CC883	ST883	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77393	202100367	LISVAL 408	<i>L. monocytogenes</i>	II	IIc	CC9	ST9	SL9	CT9007	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77394	202100368	LISVAL 409	<i>L. monocytogenes</i>	II	IIc	CC9	ST9	SL9	CT9007	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77395	202100369	LISVAL 410	<i>L. monocytogenes</i>	I	IVb	CC4	ST4	SL4	CT9158	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77421	202100377	LISVAL 453	<i>L. monocytogenes</i>	II	IIa	CC37	ST37	SL37	CT1618	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
79286	202101103	LISVAL 738	<i>L. monocytogenes</i>	I	IVb	CC6	ST6	SL6	CT9763	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
79918	202101139	LISVAL 774	<i>L. monocytogenes</i>	I	IVb	CC388	ST388	SL387	CT9761	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
77387	202100361	LISVAL 368	<i>L. innocua</i>	n.a.	L	CC493	ST493	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77388	202100362	LISVAL 369	<i>L. innocua</i>	n.a.	L	CC493	ST493	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77389	202100363	LISVAL 370	<i>L. innocua</i>	n.a.	L	CC493	ST493	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77390	202100364	LISVAL 371	<i>L. innocua</i>	n.a.	L	CC493	ST493	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77391	202100365	LISVAL 376	<i>L. innocua</i>	n.a.	L	CC1480	ST1480	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77392	202100366	LISVAL 377	<i>L. innocua</i>	n.a.	L	CC599	ST2753	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77422	202100378	LISVAL 454	<i>L. innocua</i>	n.a.	L	CC474	ST474	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77425	202100381	LISVAL 457	<i>L. innocua</i>	n.a.	L	CC530	ST530	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77426	202100382	LISVAL 544	<i>L. innocua</i>	n.a.	L	CC530	ST530	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77427	202100383	LISVAL 545	<i>L. innocua</i>	n.a.	L	CC530	ST530	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77428	202100384	LISVAL 546	<i>L. innocua</i>	n.a.	L	CC530	ST530	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
77432	202100388	LISVAL 615	<i>L. innocua</i>	n.a.	L	CC2754	ST2754	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
79916	202101137	LISVAL 772	<i>L. innocua</i>	n.a.	L	CC1616	ST1616	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
79917	202101138	LISVAL 773	<i>L. innocua</i>	n.a.	L	CC1480	ST1480	n.a.	n.a.	X	X										X	X	X	X	X	X	X	X	X	X	X
79935	202101149	LISVAL 784	<i>L. innocua</i>	n.a.	L	CC1482	ST1482	n.a.	n.a.						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X