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Paper

Genomic Links between Listeria monocytogenes in Wild Animals and the Food Chain: Insights from Central and Southern Italy

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Abstract

Listeria monocytogenes (*L. monocytogenes*) is a significant foodborne pathogen, posing a threat to public health. This study investigated the prevalence and genomic diversity of *L. monocytogenes* in 466 wild animals sampled across Central and Southern Italy (2017–2023), including species such as wild boar, red fox, and wolf, to assess their role as reservoirs and potential links to the food chain. Overall, 22.5% of the animals tested positive, and 118 *L. monocytogenes* strains were isolated, predominantly from wild boar (n=46), red fox (n=20), and Italian wolf (n=15). Whole Genome Sequencing (WGS) analysis revealed high genomic diversity, classifying the strains into 27 Clonal Complexes (CCs) and 31 Sequence Types (STs). Both hypervirulent clones (e.g., CC1, CC6, CC207) and hypovirulent clones (e.g., CC9, CC19), known for their persistence, were identified, with wild boars harboring a majority of the hypervirulent isolates. All strains carried key virulence genes, and accessory virulence factors, particularly LIPI-3, were detected in hypervirulent strains. Persistence factors, such as the Stress Survival Islet 1 (SSI-1) and genes for metal/disinfectant resistance (*cadA*, *qacA*), were also detected, particularly in wild boar isolates. Crucially, core-genome MLST (cgMLST) analysis demonstrated direct genomic links between the wildlife isolates and the Italian National Reference Laboratory database. Multiple clusters were identified, connecting strains from wild animals (wild boars, foxes, wolves) with those from meat products, fresh salads, and food processing environments. A persistent CC9 cluster, circulating in the meat chain for seven years, was strongly correlated with wild boar isolates, underscoring the role of wildlife as a reservoir that continuously introduces both high-virulence and highly persistent strains into the food production system. These findings emphasize the necessity of integrating wildlife surveillance into public health strategies to mitigate the risk of zoonotic transmission, particularly through game meat consumption and handling.

Keywords

L. monocytogenes, Wildlife, hypervirulent, whole genome sequencing, food chain

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a ubiquitous foodborne pathogen that causes human listeriosis, a serious disease characterized by high rates of hospital admission and fatalities. This represents a substantial threat to public health and a significant economic burden on the food industry. In 2022, listeriosis was the fifth most reported foodborne disease in the European Union (EFSA and ECDC, 2022).

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L. monocytogenes is a heterogeneous species commonly divided into distinct categories based on clonal complexes (CCs) and virulence characteristics. According to Maury et al. (2016), the prevalent clones are grouped into three main categories: hypervirulent infection-associated (CC1, CC2, CC4, and CC6), hypovirulent food-associated (CC9 and CC121), and intermediate (CC8-16, CC5, CC3, CC37, CC155, and CC18). The hypervirulent clones are strongly associated with clinical cases in humans and animals due to their enhanced ability to colonize host cells and their increased virulence profile. This is largely attributed to the presence of the full-length *inlA* gene and accessory Listeria Pathogenicity Islands (LIPIs), such as LIPI-3 and LIPI-4, respectively (Guidi et al., 2021b; Ireton et al., 2021).

Conversely, hypovirulent clones are typically isolated from food, particularly meat and meat products (Maury et al., 2019), and from food processing environments. They are characterized by a reduced virulence profile, which is mainly linked to the *inlA* gene truncation. These clones are well-known for their capacity to persist in environments over years, thanks to stress-related factors such as Stress Survival Islets (SSI-1 and SSI-2) (Vilchis-Rangel et al., 2019; Wiktorczyk-Kapischke et al., 2023) and transposons, including *Tn6188_qac*, which confers tolerance to benzalkonium chloride, a quaternary ammonium compound used in food processing plants (Kim et al., 2018).

In animals, listeriosis is primarily reported in domestic ruminants, often associated with the consumption of poor-quality silage. Clinical signs include septicaemia, encephalitis, meningitis, meningoencephalitis, abortion, stillbirth, and gastroenteritis (Heiderich et al., 2024).

In contrast, listeriosis is uncommonly diagnosed in wild animals, and reports of detection in wildlife are limited.*L. monocytogenes* infection has been described in wild mammals (fallow deer, sika deer, red deer, wild boar, hares, and rodents) and birds. While *L. monocytogenes* can cause severe disease and death in wildlife (Rothenburger et al., 2015), certain infected animals, such as wild boars and birds, may remain asymptomatic and carry this pathogen as part of their normal microbiome (Schoder et al., 2022; Thomas et al., 2024). Furthermore, wild birds may also function as potential vectors and routes for longer-distance transmission of *L. monocytogenes* (Thomas et al., 2024).

A wild animal spreading a foodborne zoonotic pathogen can contaminate plants directly through faecal deposition or indirectly via faecal contamination of agricultural water or soil in contact with plants. Even low-level contamination by faecal pathogens poses a significant public health problem regarding pre-harvest contamination (Jay-Russel, 2013; Thomas et al., 2024).

Although the zoonotic transmission of *L. monocytogenes* has been reported (Hellstrom et al., 2008; McIntyre et al., 2015; Filippello et al., 2020), the role of wild animals is often overlooked during outbreak investigations. Another key concern in the spread of this foodborne pathogen is the frequent finding of *L. monocytogenes* on game meat, which presents a risk to consumers (Abel et al., 2020; Sauvala et al., 2021; Fredriksson-Ahomaa et al., 2022; Floris et al., 2024).

This study aims to elucidate the genomic population of *L. monocytogenes* in wildlife across selected regions of Central and Southern Italy and to identify potential transmission events between wildlife and the food chain using Whole Genome Sequencing (WGS) data.

Materials and methods

Sampling and microbiological analysis

Between 2017 and 2023, 466 wild animals were sampled across Central and Southern Italy. The regions encompassed in this study were Abruzzo, Campania, Calabria, Lazio, Marche, and Molise. The sampling of wild animals was part of regional epidemiological passive surveillance and monitoring plans for diseases in wild fauna, which are activated in the event of the discovery of dead or moribund wildlife, or animals that have been culled or recovered.

The sampled animals included 234 wild boars (*Sus scrofa*), 56 red foxex (*Vulpes vulpes*), 48 Italian wolves (*Canis lupus italicus*), 53 roe deer (*Capreolus capreolus*), 36 red deer (*Cervus elaphus*), 9 crested porcupines (*Hystrix cristata*), 20 European badgers (*Meles meles*), 2 European wildcats (*Felis silvestris*), 1 beech marten (*Martes foina*), 2 European hares (*Lepus europaeus*), 1 European hedgehog (*Erinaceus europaeus*), and 4 Marsican brown bears (*Ursus arctos marsicanus*).

For each animal, the following tissues were collected: brain, skeletal (carcass) and masseter muscle, intestine and intestinal content. No histological examination was performed on the collected tissues.

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The detection of *L. monocytogenes* strains in tissue samples was carried out according to the procedures detailed in ISO 11290-1:2017. Species confirmation was subsequently performed using MALDI-TOF mass spectrometry (Thouvenot et al., 2018).

Genomic characterization of *L. monocytogenes* genomes

DNA extraction and Whole Genome Sequencing (WGS) were performed following the protocols previously reported by Centorotola et al. (2023). For the analysis of WGS data, an accredited in-house pipeline (available at: https://github.com/genpat-it/ngsmanager/) was utilized, with quality control being assessed in accordance with ISO 23418:2022.

Multilocus Sequence Typing (MLST) was performed using the Pasteur reference schemes, which are accessible via the BIGSdb-Pasteur platform (https://bigsdb.pasteur.fr/). Virulence and resistance profiles, including Stress Islands, metal and disinfectants resistance, and antimicrobial resistance, were obtained from the *L. monocytogenes* genomes by employing the BLASTN algorithm as implemented on the BIGSdb-Lm platform (accessed on 12 April 2024). Furthermore, PlasmidFinder (Carattoli et al., 2014), integrated within ABRicate (Seeman), was utilized for the detection of plasmids in the genome assemblies.

The genomic correlation between the wildlife strains and the genomes contained within the Italian National Reference Laboratory for *Listeria monocytogenes* (NRL *Lm*) database was assessed using core-genome MLST (cgMLST) analysis. Specifically, the NRL *Lm* database comprised over seven thousand sequences obtained from strains isolated from food and/or environmental samples collected during official activities or epidemiological investigations conducted in response to listeriosis outbreaks.

A cut-off of seven alleles was applied to define genomic correlation and detect distinct clusters (Moura et al., 2016).

Results

Bacterial isolation

A total of 105 wild animals tested positive for *L. monocytogenes*, representing 22.5% of the animals examined. The positive animals were distributed across 12 species, including wild boar (n=46), red fox (n=20), Italian wolf (n=15), and roe deer (n=9), red deer (n=4), crested porcupine (n=3), European badger (n=2), European wildcat (n=2), beech marten (n=1), European hare (n=1), European hedgehog (n=1), and Marsican brown bear (n=1).

Based on the incidental findings of the animals, it was observed that wild boars were predominantly retrieved near urban areas characterized by high human population density. Conversely, the other positive wildlife species were primarily collected in the Apennine areas. Detailed information is provided in Supplementary Table 1, and the geographical distribution of the positive sampled animals is illustrated in Figure 1.

A total of 118 *L. monocytogenes* strains were isolated and confirmed from various sample types, including: brain (n=46), faeces (n=44), masseter muscle (n=22), intestine (n=4), and carcasses (n=2) (Supplementary Table 1).

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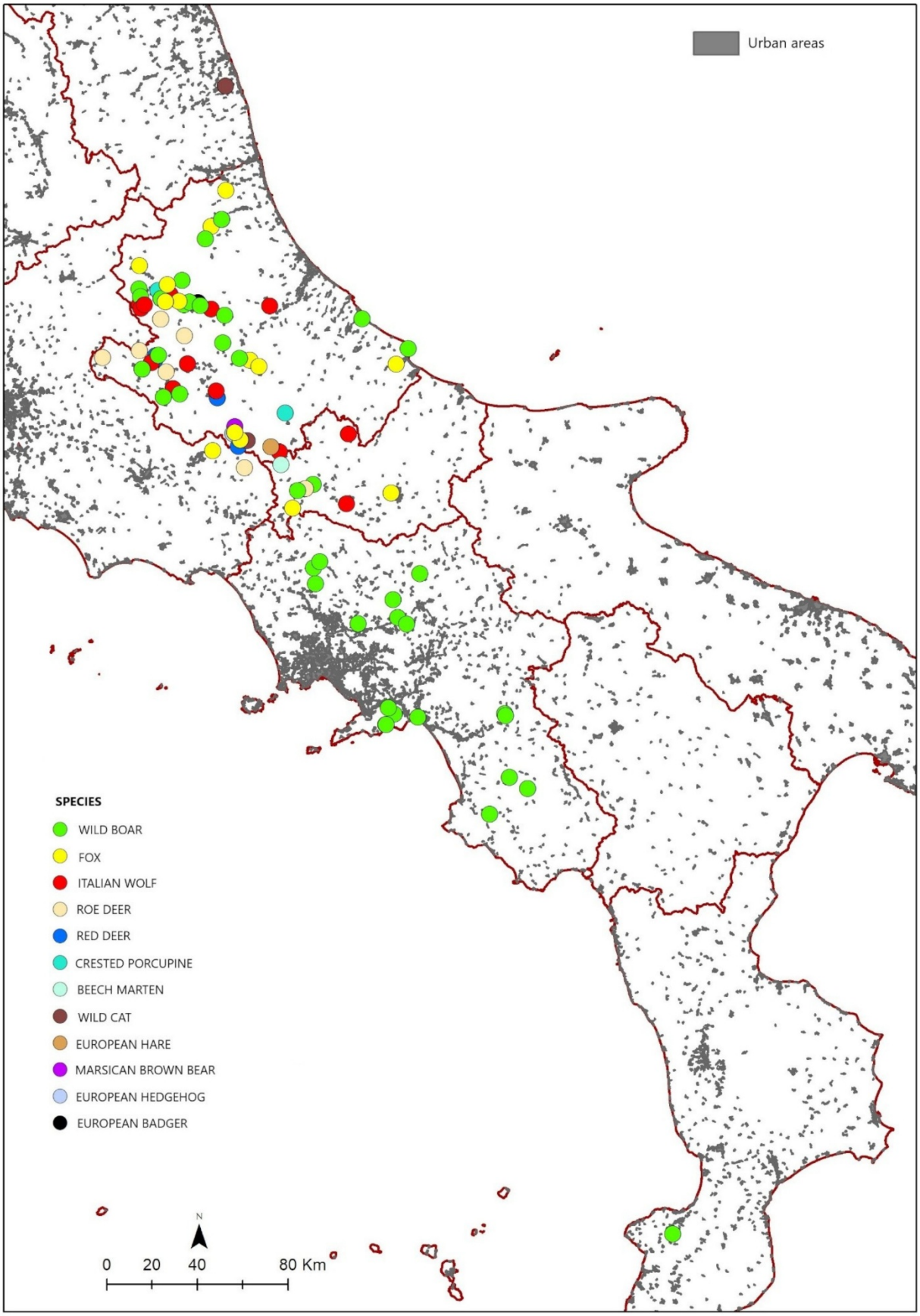

Figure 1. Spatial distribution of 105 selected wild animals collected from 2017 to 2023.

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Genomic characterization

MLST profiles calculated revealed the presence of a genetically heterogeneous population among the strains isolated in this study. *L. monocytogenes* genomes, indeed, were classified into 27 distinct Clonal Complexes (CCs) and 31 Sequence Types (STs) (Table S1) including hyper- and hypovirulent clones. The most prevalent CCs identified were CC1 (n=23), CC7 (n=10), CC6 (n=8), CC19 (n=8), and CC155 (n=7). Correspondingly, the dominant STs were ST1 (n=23), ST398 (n=8), and ST155 (n=7).

Hypervirulent clones (CC1, CC6, and CC4) were isolated from multiple species, including wild boars, foxes, wolves, roe deer, European hares, and crested porcupines. Additionally, the hypervirulent clone CC207 was identified in roe deer, wild cats, foxes, and wild boars (Figure 2). Hypovirulent clones (CC9, CC11, CC19, and CC204) were primarily isolated from an Italian wolf, foxes, roe deer, and wild boars. No strains belonging to the CC121 clone were identified.

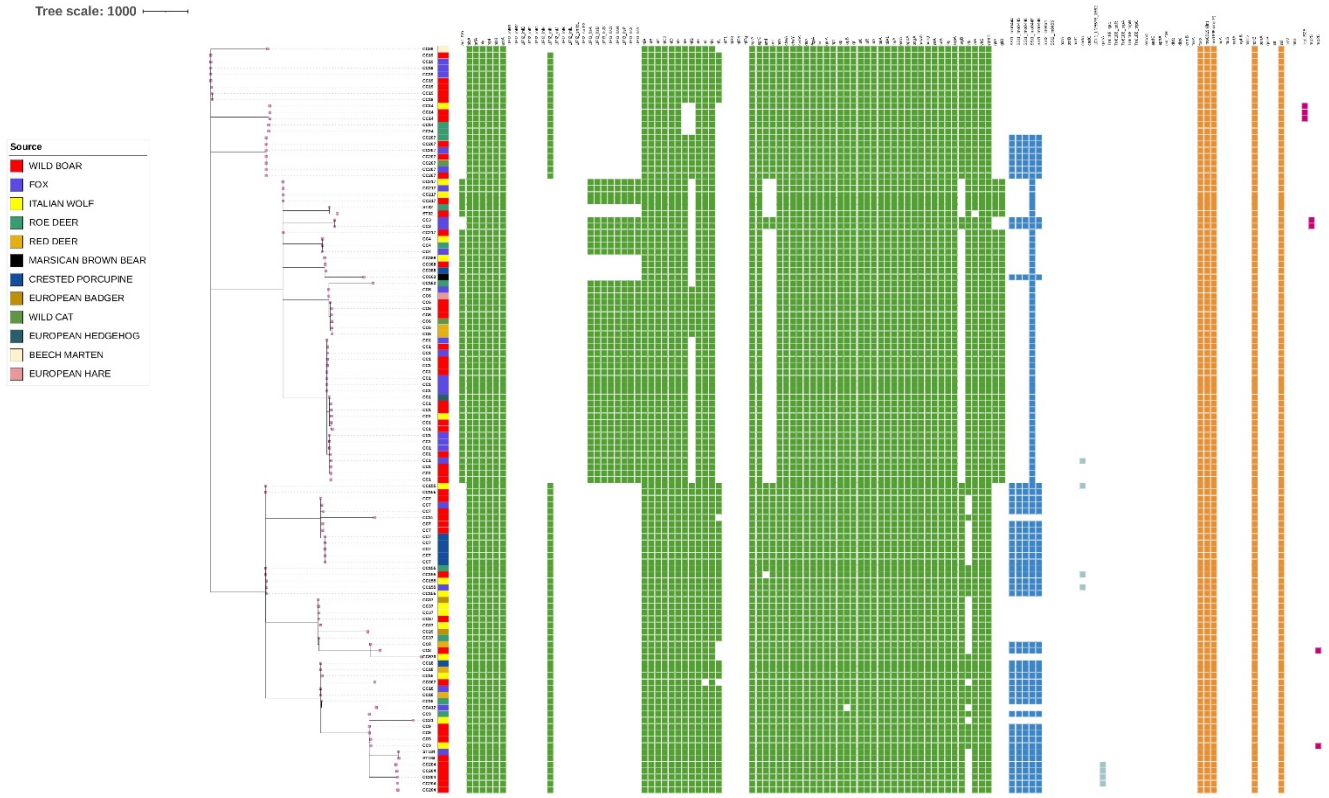

Figure 2. Phylogenetic tree obtained from cgMLST analysis of the 118 L. monocytogenes strains. The first layer represents MLST analysis results. Second layer represents the source of isolation as showed in the legend. Virulence (green square) and resistance genes (blue, light grey and orange squares) and plasmids (fuchsia suares) are shown in the heatmap.

The virulence traits and stress and resistance features identified across the 118 strains exhibited clone-specific distribution patterns (Figure 2).

The genes comprising the *L. monocytogenes* intracellular survival pathogenicity island 1 (LIPI-1)—namely *prfA*, *plcA*, *hly*, *mpl*, *actA*, and *plcB*—were present in all isolates. Similarly, the internalization loci *inlA*, *inlB*, *inlC*, *inlD*, *inlE*, *inlH*, and *inlK* were detected in all assemblies. The *inlJ* locus was absent in only one strain belonging to CC30. The *inlF* locus was absent in isolates belonging to ST399, and a premature stop codon was identified in one CC217 strain (2019.TE.1857.1.24). An incomplete LIPI-2 was mainly detected in CC7, CC19, and CC155 clones, while LIPI-3 was harbored by hypervirulent clones (CC1, CC4, and CC6) and strains belonging to CC217, CC3, and CC183. LIPI-4 was not found in any isolate.

The Stress Survival Islet (SSI-1) was present in 46 *L. monocytogenes* strains, primarily those belonging to CC155 and isolated from wild boars, foxes, roe deer, and wolves. SSI-2 was absent in all isolates.

Among the metal-resistance genes, *cadA* was detected in CC155 (n=3) and CC1 (n=1) strains isolated from foxes, wild boars, and a wolf. *QacA* was found in four CC204 strains isolated from wild boars. Meanwhile, intrinsic resistance traits—including the core genes *fosX*, *lin*, *mprF*, *norB*, and *sul*—were identified in all 118 strains, but no acquired

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antimicrobial resistance genes were detected.

Finally, only seven out of the 118 isolates harbored plasmids (repUS25, rep25, and rep26). These strains belonged to CC3, CC8, CC9, and CC14, and were isolated from wild boars (n=3), foxes (n=2), and wolves (n=2). All genomic results are summarized in Figure 2.

Genomic correlation with Italian National Reference Laboratory for *L. monocytogenes* database

CgMLST analysis, which compared the wildlife isolates with the Italian NRL *Lm* database, highlighted several genomic clusters, clearly indicating potential links between wildlife and the food chain.

In the hypervirulent CC1 clones, two distinct clusters were identified: the first cluster included three strains isolated from foxes and six *L. monocytogenes* strains from fresh and pork cured meat products (Figure 3a), and the second cluster linked two strains, one from a fox and the other from a sausage sample (Figure 3b).

Other identified clusters strongly supported connections between wild animals, particularly wild boars, and the food chain: specifically, one CC8 strain from a wild boar was correlated with four strains derived from pork meat processing environment samples (Figure 3c). Furthermore, two CC155 strains from wild boars clustered with five strains isolated from pork meat processing environment samples and one strain isolated from a wolf (Figure 4a).

A CC6 strain from a wild boar clustered with five strains isolated from fresh salad samples (Figure 4b). Additionally, a CC217 strain clustered with two strains isolated from a mixed food processing environment and dry vegetable soup mix samples (Figure 4c); in this cluster three strains isolated from two different foxes and one wolf were also included.

Finally, two CC9 isolates from a single wild boar were correlated with 102 strains isolated from various pork meat products and pork and bovine meat processing environment samples collected between 2017 and 2024 (Figure 4d).

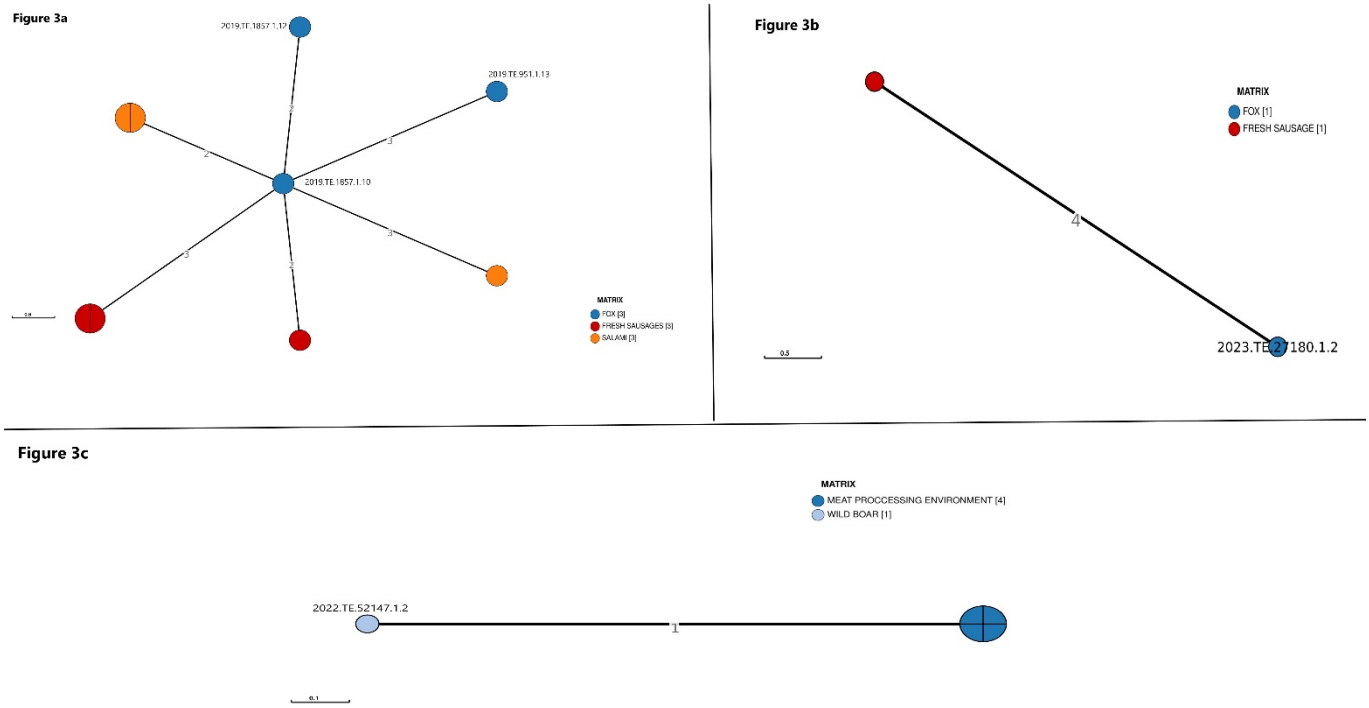

Figure 3. Minimum Spanning Tree representing genomic clusters identified among wildlife (foxes and wild boars) and Italian LNRLm database based on cgMLST analysis.

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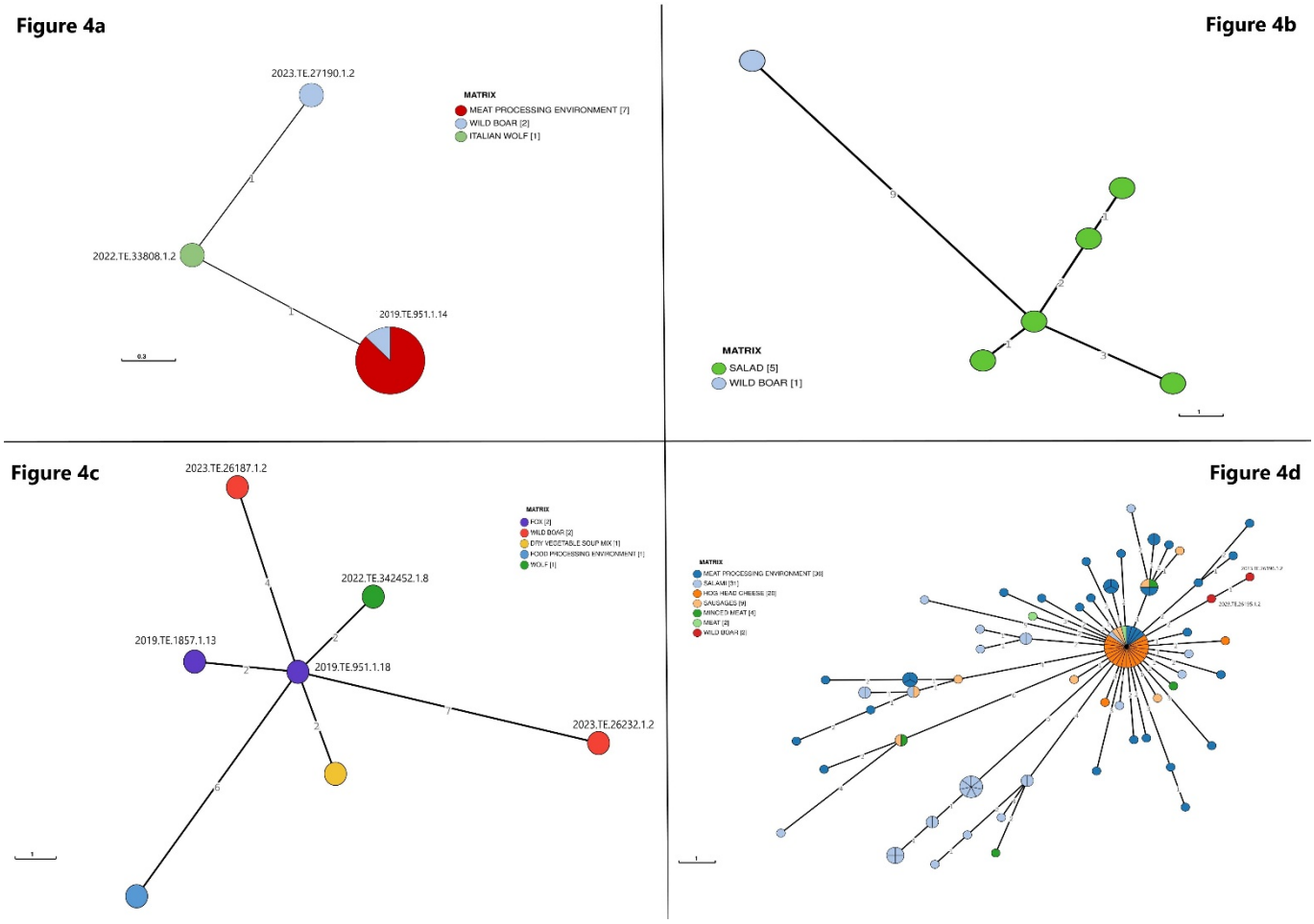

Figure 4. Minimum Spanning Tree representing genomic clusters identified among wildlife (wolves, foxes and wild boars) and Italian LNRLm database based on cgMLST analysis.

Discussion

The present study investigated the genomic diversity of *L. monocytogenes* strains isolated from diagnostic samples collected from a range of wild animal species across selected regions of Central and Southern Italy. Our findings confirmed the presence of *L. monocytogenes* within the wildlife population examined, with over 22% of the animals testing positive.

This study, however, presents several limitations. Notably, data on the tested animals did not allow us to confirm pathognomonic lesions directly caused by *L. monocytogenes* due to the lack of comprehensive histopathological examination, which prevented a definitive assessment of tissue lesions and limited our ability to correlate bacterial presence with clinical or subclinical disease. Although several strains were detected in the brain and masseter muscles, the isolations from the intestine and faeces strongly suggests a carrier function in these animals.

Furthermore, the geographically restricted and opportunistic sampling design may not fully capture the broader ecological diversity or the true prevalence of *L. monocytogenes* in the wider wildlife population.

Despite these limitations, the study offers valuable insights into the circulation and genomic heterogeneity of *L. monocytogenes* in wildlife. Indeed, while various authors have previously reported the detection of *L. monocytogenes* in wildlife (Palacios-Gorba et al., 2021; Chen et al., 2022; Floris et al., 2024), available genomic data remain scarce. For instance, a major European study by Félix et al. (2022) analyzing over 1,400 *L. monocytogenes* genomes from different ecological niches included only 10% of isolates from wildlife, such as deer, fallow deer, red deer, and wild boar.

Our study significantly expands the known host range, particularly highlighting a high prevalence in carnivores, where detection is not commonly expected (Heiderich et al., 2024). Specifically, several carnivore species, including beech martens, European wildcats, Marsican brown bears, and European badgers, were found positive, with particularly high

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rates in red foxes and wolves.

The red fox is highly common due to its adaptability (Cignini and Riga, 1997), and reports of other foodborne pathogens like Hepatitis E virus (HEV) and *Echinococcus* spp. and coagulase-positive *Staphylococcus* spp.in foxes are frequent (Ferrara et al., 2023; Hahaj-Siembida et al., 2024).

The Italian wolf, a major predator whose population is expanding (Crotti et al., 2023), has also been linked to various zoonotic pathogens (Greco et al., 2021; Ricchiuti et al., 2021; Gambi et al., 2022) and they could act as reservoirs of zoonotic diseases (Ortega et al., 2025). Unsurprisingly, wild boars were the most infected species, consistent with previous studies identifying them as important reservoirs of foodborne zoonoses (Fredriksson-Ahomaa, 2019; Aprea et al., 2020; Fredriksson-Ahomaa et al., 2022; Altissimi et al., 2024; Floris et al., 2024).

MLST analysis revealed a genetically heterogeneous population among the strains isolated in this study, underscoring the presence of multiple, genetically distinct clones that may influence patterns of transmission, virulence, or persistence. Hypovirulent clones, such as CC9, are generally associated with food and food processing environments and are known for their enhanced persistence in these settings (Maury et al., 2016; FAO and WHO, 2022). Conversely, hypervirulent clones like CC1, CC4, and CC6 are strongly associated to clinical cases and linked to dairy products (Maury et al., 2016). Furthermore, the recently described hypervirulent clone CC207 (Frisch et al., 2018) was also detected in the sampled wild animals.

Intriguingly, the wild boars collected in this study harbored most of the isolates associated with the hypervirulent clonal complexes. This finding suggests that these animals may serve as reservoirs for *L. monocytogenes* strains with a higher potential for causing human disease. The potential link between wild boars and the food chain makes this species particularly relevant for zoonotic pathogen surveillance.

The WGS analysis confirmed that all 118 *L. monocytogenes* strains harbored key virulence determinants, as demonstrated by the presence of genes encoding virulence factors like *inlA* and *inlB* (Chen et al., 2022). Crucially, none of the studied strains, including the hypovirulent ones, presented premature stop codons (PMSC) within key virulence genes, with the exception of the *inlF* locus in one CC217 strain, which may lead to virulence attenuation (Guidi et al., 2021a). Furthermore, complete LIPI-3 was found, which was previously associated with hypervirulence (Maury et al., 2016; Tavares et al., 2020) and encoding listeriolysin S (LLS)—a factor crucial for murine infection and polymorphonuclear-cell survival (Zakrzewski et al., 2023). LIPI-3 was found not only in typical hypervirulent clones but also in CC217, CC3, and CC183 clones. However, none of the isolates harbored LIPI-4, which has been associated with increased neural and placental tropism (Chen et al., 2022).

Adaptive mechanisms contributing to *L. monocytogenes* persistence were also identified. The SSI-1, which enhances growth during exposure to stressful food environments and is significantly associated with increased biofilm formation (Di Ciccio et al., 2022), was present in several strains, predominantly isolated from wild boars. The ability to form biofilms may enhance *L. monocytogenes* persistence in the environment protecting the pathogen from harvest conditions (Tuytschaever et al., 2023), representing a risk for of pre-harvest vegetables contamination. When biofilm-forming strains are introduced into food processing plants via raw materials, even a thin biofilm layer can persist in hard-to-reach niches, becoming a continuous source of food contamination (Guidi et al., 2021a). Another public health concern is represented by the introduction and manipulation of *L. monocytogenes* biofilm-forming strains through raw game meat in domestic environments where cleaning and sanitation procedures may be lacking or inadequate.

Interestingly, the metal-resistance genes *cadA* and *qacA*, associated with resistance to cadmium and quaternary ammonium compounds, respectively, were found in strains isolated primarily from wild boars.

The antimicrobial resistance (AMR) genetic profile was highly conserved across all studied strains, including core genes involved in antibiotic efflux (*norB*), antibiotic target alteration (*mprF*), and antibiotic inactivation (*lin*, *fosX*). The consistent presence of *fosX* and *lin* genes is attributed to the species' intrinsic resistance to fosfomycin and lincosamides. Importantly, no acquired AMR genes were detected. Although phenotypical expression was not tested, none of these intrinsic genes are known to confer resistance to the primary antibiotics used for treating listeriosis (Guidi et al., 2021b; Mafuma et al., 2021; Fredriksson-Ahomaa et al., 2022; Hanes et al., 2022; Parra-Flores et al., 2022).

Only Brown et al. (2023) investigated the presence of AMR determinants in *L. monocytogenes* strains isolated wild black bears, especially those involved in tetracycline resistance. None of tested strains was resistant to ampicillin, penicillin, trimethoprim, vancomycin, erythromycin, rifampicin, gentamicin, chloramphenicol, streptomycin, or kanamycin.

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A notable strength of the present study is the capacity to contextualize *L. monocytogenes* genomes obtained from wild animals within a broader national genomic database, thereby enhancing the resolution of strain comparison and supporting insights into potential transmission events. The comparative genomics approach strongly, based on cgMSLT analysis, highlighted the connection between wild animals (wild boars, foxes, and wolves) and the food chain, involving both hyper- and hypovirulent clones. Comparative genomics has allowed us to identify small clusters involving both vegetables and animal- based products.

Interestingly, a single large cluster of a hypovirulent CC9 clone was found circulating in different meat processing environments for a seven-year period, demonstrating a strict and persistent connection between wild boars and the meat chain. This supports the notion that wild boars can act as asymptomatic carriers, introducing the pathogen into the food chain (Schoder et al., 2022).

An asymptomatic animal spreading a foodborne zoonotic pathogen can contaminate plants directly or indirectly through fecal contamination of the environment, representing a significant public health risk for pre-harvest contamination (Jay-Russel, 2013; Thomas et al., 2024).

Human infections can occur through the consumption of undercooked game meat, or through hunting and handling infected carcasses (Navarro-Gonzalez et al., 2016; Fredriksson-Ahomaa, 2019, Aprea et al., 2020; Fredriksson-Ahomaa et al., 2022; Schoder et al., 2022). Given that wild boar is a major game food species in Central and Southern Italy, utilized for both food and sport hunting, it represents a plausible transmission route to humans through the consumption of raw meat products (Altissimi et al., 2024), consistent with contamination reports by other authors (Atassanova et al., 2008; Avagnina et al., 2012; Abel et al., 2020).

Overall, these findings underline the need to further investigate transmission pathways between wildlife and food-processing plants and emphasize the importance of establishing a national surveillance plan integrating wildlife and food chain monitoring to effectively mitigate the risk of human exposure.

Conclusion

This study provides valuable insights into the circulation and genomic heterogeneity of *L.monocytogenes* in wildlife. Several wildlife species, including wild boars, red foxes, and wolves, were found positive suggesting that they could act as a reservoirs for the spread of major zoonotic diseases.

Genomic investigations identified different CCs, encompassing both hypervirulent and persistent hypovirulent clones. The widespread presence of key virulence determinants (LIPI-1 and LIPI-3) alongside resistance-associated genes (SSI-1, *cadA*, *qacA*) underscores the circulation of strains with considerable pathogenic potential and enhanced environmental persistence.

Most significantly, cgMLST analysis demonstrated direct genomic links between *L. monocytogenes* strains found in wildlife and those isolated from the Italian food chain (including meat products and processing environments, and vegetables) supporting the occurrence of wildlife-to-food chain transmission events.

Integrating genomic surveillance with future systematic pathological investigations, broader and more structured sampling strategies, and longitudinal monitoring will enhance the characterization of transmission pathways, clarify the ecological role of wildlife reservoirs, and ultimately strengthen risk assessment efforts relevant to both animal and public health.

Acknowledgments

NA

Ethical approval

The diagnostic specimens were collected during national epidemiological passive surveillance and monitoring activities of wild fauna. For this reason, the ethical approval was not applicable.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

ACh conceptualized the study. FG, GC, MDE, ACr, MF, SB, VDM, AM, AS, SS, NS, BC, SC, LM, IDM, DP, RA, AA, MR, MCC, ACo, YP, GB and AP carried out sample collection, identification and isolation experiments. FG, GC, MA, CC carried out the WGS experiment. ACh, FG, GC carried out the bioinformatics analysis. ACh, FG, GC analyzed the data, organized the draft, and wrote the manuscript. FP supervised the entire work and acquired funding and revised the manuscript. All the authors contributed to editing the manuscript and reviewed the final version. All authors have read and approved the manuscript.

Data availability

The datasets analyzed for this study can be found in the National Centre for Biotechnology Information (NCBI) database under the BIOPROJECT PRJNA1197424

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References

Abel, T., Boulaaba, A., Lis, K., Abdulmawjood, A., Plötz, M., & Becker, A. (2020). Inactivation of Listeria monocytogenes in game meat applying sous vide cooking conditions. Meat Science, 167, 108164. https://doi.org/10.1016/j.meatsci.2020.108164.

Altissimi, C., Primavilla, S., Roila, R., Gavaudan, S., Morandi, B., Di Lullo, S., Coppini, M., Baldinelli, C., Cai, D., Branciari, R., Valiani, A., Paulsen, P., & Ranucci, D. (2024). Salmonella in wild boar meat: Prevalence and risk assessment in Central Italy (Umbria and Marche Region). Foods, 13(8), 1156. https://doi.org/10.3390/foods13081156.

Atanassova, V., Apelt, J., Reich, F., & Klein, G. (2008). Microbiological quality of freshly shot game in Germany. Meat Science, 78(4), 414–419. https://doi.org/10.1016/j.meatsci.2007.07.004.

Aprea, G., Scattolini, S., D'Angelantonio, D., Chiaverini, A., Di Lollo, V., Olivieri, S., Marcacci, M., Mangone, I., Salucci, S., Antoci, S., Cammà, C., Di Pasquale, A., Migliorati, G., & Pomilio, F. (2020). Whole genome sequencing characterization of HEV3-e and HEV3-f subtypes among the wild boar population in the Abruzzo Region, Italy: First report. Microorganisms, 8(9), 1393. https://doi.org/10.3390/microorganisms8091393.

Avagnina, A., Nucera, D., Grassi, M. A., Ferroglio, E., Dalmasso, A., & Civera, T. (2012). The microbiological conditions of carcasses from large game animals in Italy. Meat Science, 91(3), 266–271. https://doi.org/10.1016/j.meatsci.2012.01.025.

Brown, P., Hernandez, K., Parsons, C., Chen, Y., Gould, N., DePerno, C. S., Niedermeyer, J., & Kathariou, S. (2023). Tetracycline resistance in Listeria monocytogenes and L. innocua from wild black bears (Ursus americanus) in the United States is mediated by novel transposable elements. Applied and Environmental Microbiology, 89, e0120523. https://doi.org/10.1128/AEM.01205-23.

Carattoli, A., Zankari, E., Garcia-Fernandez, A., Voldby Larsen, M., Lund, O., Villa, L., Aarestrup, F. M., & Hasman, H. (2014). PlasmidFinder and pMLST: In silico detection and typing of plasmids. Antimicrobial Agents and

Chemotherapy, 58(7), 3895–3903. https://doi.org/10.1128/AAC.02412-14.

Centorotola, G., Ziba, M. W., Cornacchia, A., Chiaverini, A., Torresi, M., Guidi, F., Cammà, C., Bowa, B., Mtonga, S., Magambwa, P., D'Alterio, N., Scacchia, M., Pomilio, F., & Muuka, G. (2023). Listeria monocytogenes in ready-to-eat meat products from Zambia: Phenotypical and genomic characterization of isolates. Frontiers in Microbiology, 14, 1228726. https://doi.org/10.3389/fmicb.2023.1228726.

Chen, T., Orsi, R. H., Chen, R., Gunderson, M., Roof, S., Wiedmann, M., Childs-Sanford, S. E., & Cummings, K. J. (2022). Characterization of Listeria monocytogenes isolated from wildlife in central New York. Veterinary Medicine and Science, 8, 1319–1329. https://doi.org/10.1002/vms3.758.

Cignini, B., & Riga, F. (1997). Red fox sightings in Rome. Hystrix, the Italian Journal of Mammalogy, 9(1–2).

Crotti, S., Spina, S., Cruciani, D., Bonelli, P., Felici, A., Gavaudan, S., Gobbi, M., Morandi, F., Piseddu, T., Torricelli, M., & Morandi, B. (2023). Tapeworms detected in wolf populations in Central Italy (Umbria and Marche regions): A long-term study. International Journal for Parasitology: Parasites and Wildlife, 21, 11–16. https://doi.org/10.1016/j.ijppaw.2023.03.007.

Di Ciccio, P., Rubiola, S., Panebianco, F., Lomonaco, S., Allard, M., Bianchi, D. M., Civera, T., & Chiesa, F. (2022). Biofilm formation and genomic features of Listeria monocytogenes strains isolated from meat and dairy industries located in Piedmont (Italy). International Journal of Food Microbiology, 378, 109784. https://doi.org/10.1016/j.ijfoodmicro.2022.109784.

EFSA & ECDC (European Food Safety Authority & European Centre for Disease Prevention and Control). (2023). The European Union One Health 2022 Zoonoses Report. EFSA Journal, 21(12), e8442. https://doi.org/10.2903/j.efsa.2023.8442.

FAO & WHO. (2022). Listeria monocytogenes in ready-to-eat (RTE) foods: Attribution, characterization and monitoring – Meeting report (Microbiological risk assessment). Rome, Italy: FAO & WHO.

Félix, B., Sevellec, Y., Palma, F., Douarre, P. E., Felten, A., Radomski, N., Mallet, L., Blanchard, Y., Leroux, A., Soumet, C., Bridier, A., Piveteau, P., Ascensio, E., Hébraud, M., Karpíšková, R., Gelbíčová, T., Torresi, M., Pomilio, F., Cammà, C., … Roussel, S. (2022). A European-wide dataset to uncover adaptive traits of Listeria monocytogenes to diverse ecological niches. Scientific Data, 9(1), 190. https://doi.org/10.1038/s41597-022-01278-6.

Ferrara, G., Brocherel, G., Falorni, B., Gori, R., Pagnini, U., & Montagnaro, S. (2023). A retrospective serosurvey of selected pathogens in red foxes (Vulpes vulpes) in the Tuscany region, Italy. Acta veterinaria Scandinavica, 65(1), 35. https://doi.org/10.1186/s13028-023-00699-6.

Filipello, V., Mughini-Gras, L., Gallina, S., Vitale, N., Mannelli, A., Pontello, M., Decastelli, L., Allard, M. W., Brown, E. W., & Lomonaco, S. (2020). Attribution of Listeria monocytogenes human infections to food and animal sources in Northern Italy. Food microbiology, 89, 103433. https://doi.org/10.1016/j.fm.2020.103433.

Floris, I., Vannuccini, A., Ligotti, C., Musolino, N., Romano, A., Viani, A., Bianchi, D. M., Robetto, S., & Decastelli, L. (2024). Detection and characterization of zoonotic pathogens in game meat hunted in Northwestern Italy. Animals, 14(4), 562. https://doi.org/10.3390/ani14040562.

Fredriksson-Ahomaa, M., Sauvala, M., Kurittu, P., Heljanko, V., Heikinheimo, A., & Paulsen, P. (2022). Characterisation of Listeria monocytogenes isolates from hunted game and game meat from Finland. Foods, 11(22), 3679. https://doi.org/10.3390/foods11223679.

Fredriksson-Ahomaa, M. (2019). Wild boar: A reservoir of foodborne zoonoses. Foodborne Pathogens and Disease, 16(3), 153–165. https://doi.org/10.1089/fpd.2018.2512.

Fritsch, L., Guillier, L., & Augustin, J. C. (2018). Next generation quantitative microbiological risk assessment: Refinement of the cold smoked salmon-related listeriosis risk model by integrating genomic data. Microbial Risk Analysis, 10, 20–27. https://doi.org/10.1016/j.mran.2018.06.003.

Gambi, L., Ravaioli, V., Rossini, R., Tranquillo, V., Boscarino, A., Mattei, S., D'incau, M., Tosi, G., Fiorentini, L., & Donato, A. D. (2022). Prevalence of Different Salmonella enterica Subspecies and Serotypes in Wild Carnivores in Emilia-Romagna Region, Italy. Animals : an open access journal from MDPI, 12(23), 3368. https://doi.org/10.3390/ani12233368.

Greco, G., Zarea, A. A. K., Sgroi, G., Tempesta, M., D'Alessio, N., Lanave, G., Bezerra-Santos, M. A., Iatta, R., Veneziano, V., Otranto, D., & Chomel, B. (2021). Zoonotic Bartonella species in Eurasian wolves and other free-ranging wild mammals from Italy. Zoonoses and Public Health, 68(4), 316–326. https://doi.org/10.1111/zph.12827.

Guidi, F., Orsini, M., Chiaverini, A., Torresi, M., Centorame, P., Acciari, V. A., Salini, R., Palombo, B., Brandi, G., Amagliani, G., Schiavano, G. F., Massacci, F. R., Fisichella, S., Domenico, M. D., Ancora, M., Pasquale, A. D., Duranti, A., Cammà, C., Pomilio, F., & Blasi, G. (2021b). Hypo- and hyper-virulent Listeria monocytogenes clones persisting in two different food processing plants of Central Italy. Microorganisms, 9(2), 376. https://doi.org/10.3390/microorganisms9020376.

Guidi, F., Chiaverini, A., Repetto, A., Lorenzetti, C., Centorotola, G., Bazzucchi, V., Palombo, B., Gattuso, A., Pomilio, F., & Blasi, G. (2021a). Hyper-virulent Listeria monocytogenes strains associated with respiratory infections in Central Italy. Frontiers in Cellular and Infection Microbiology, 11, 765540. https://doi.org/10.3389/fcimb.2021.765540.

Hahaj-Siembida, A., Nowakiewicz, A., Korzeniowska-Kowal, A., Szecówka, K., Trościańczyk, A., Zięba, P., & Kania, M. G. (2024). Red foxes (Vulpes vulpes) as a specific and underappreciated reservoir of resistant and virulent coagulase-positive Staphylococcus spp. strains. Research in Veterinary Science, 166, 105111. https://doi.org/10.1016/j.rvsc.2023.105111.

Hanes, R. M., & Huang, Z. (2022). Investigation of antimicrobial resistance genes in Listeria monocytogenes from 2010 through to 2021. International Journal of Environmental Research and Public Health, 19, 5506. https://doi.org/10.3390/ijerph19105506.

Heiderich, E., Origgi, F. C., Pisano, S. R. R., Kittl, S., Oevermann, A., Ryser-Degiorgis, M. P., & Marti, I. A. (2024). Listeria monocytogenes infection in free-ranging red foxes (Vulpes vulpes) and Eurasian lynx (Lynx lynx) in Switzerland. Journal of Zoo and Wildlife Medicine, 55(1), 268–276. https://doi.org/10.1638/2022-0144.

Hellstrom, S., Kiviniemi, K., Autio, T., & Korkeala, H. (2008). Listeria monocytogenes is common in wild birds in Helsinki region and genotypes are frequently similar with those found along the food chain. Journal of Applied Microbiology, 104, 883–888. https://doi.org/10.1111/j.1365-2672.2007.03604.x.

Ireton, K., Mortuza, R., Gyanwali, G. C., Gianfelice, A., & Hussain, M. (2021). Role of internalin proteins in the pathogenesis of Listeria monocytogenes. Molecular Microbiology, 116(6), 1407–1419. https://doi.org/10.1111/mmi.14836.

ISO 11290-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. Part 1: Detection method.

ISO. (2022). ISO 23418:2022 – Microbiology of the food chain: Whole genome sequencing for typing and genomic characterization of bacteria – General requirements and guidance.

Jay-Russell, M. T. (2013). What is the risk from wild animals in food-borne pathogen contamination of plants? CAB Reviews, 8, 040. https://doi.org/10.1079/PAVSNNR20138040.

Kim, M., Weigand, M. R., Oh, S., Hatt, J. K., Krishnan, R., Tezel, U., Pavlostathis, S. G., & Konstantinidis, K. T. (2018). Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. Applied and Environmental Microbiology, 84(17), e01201-18. https://doi.org/10.1128/AEM.01201-18.

Mafuna, T., Matle, I., Magwedere, K., Pierneef, R. E., & Reva, O. N. (2021). Whole genome-based characterization of Listeria monocytogenes isolates recovered from the food chain in South Africa. Frontiers in Microbiology, 12, 669287. https://doi.org/10.3389/fmicb.2021.669287.

Maury, M. M., Bracq-Dieye, H., Huang, L., Vales, G., Lavina, M., Thouvenot, P., Disson, O., Leclercq, A., Brisse, S., &

Lecuit, M. (2019). Hypervirulent Listeria monocytogenes clones' adaptation to mammalian gut accounts for their association with dairy products. Nature Communications, 10, 2488. https://doi.org/10.1038/s41467-019-10380-0.

Maury, M. M., Tsai, Y. H., Charlier, C., Touchon, M., Chenal-Francisque, V., Leclercq, A., Criscuolo, A., Gaultier, C., Roussel, S., Brisabois, A., Disson, O., Rocha, E. P. C., Brisse, S., & Lecuit, M. (2016). Uncovering Listeria monocytogenes hypervirulence by harnessing its biodiversity. Nature genetics, 48(3), 308–313. https://doi.org/10.1038/ng.3501.

McIntyre, L., Wilcott, L., & Naus, M. (2015). Listeriosis outbreaks in British Columbia, Canada, caused by soft ripened cheese contaminated from environmental sources. BioMed Research International, 2015, 131623. https://doi.org/10.1155/2015/131623.

Moura, A., Criscuolo, A., Pouseele, H., Maury, M. M., Leclercq, A., Tarr, C., Björkman, J. T., Dallman, T., Reimer, A., Enouf, V., Larsonneur, E., Carleton, H., Bracq-Dieye, H., Katz, L. S., Jones, L., Touchon, M., Tourdjman, M., Walker, M., Stroika, S., Cantinelli, T., … Brisse, S. (2016). Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes. Nature Microbiology, 2, 16185. https://doi.org/10.1038/nmicrobiol.2016.185.

Navarro-Gonzalez, N., Ugarte-Ruiz, M., Domínguez, L., & Ruiz-Fons, F. (2016). A European Perspective on the Transmission of Foodborne Pathogens at the Wildlife–Livestock–Human Interface. In: Jay-Russell, M., Doyle, M. (eds) Food Safety Risks from Wildlife. Food Microbiology and Food Safety. Springer, Cham. https://doi.org/10.1007/978-3-319-24442-6_3.

Ortega, S., Figueiredo, A. M., Moroni, B., Abarca, N., Dashti, A., Köster, P. C., Bailo, B., Cano-Terriza, D., Gonzálvez, M., Fayos, M., Oleaga, Á., Martínez-Carrasco, C., Velarde, R., Torres, R. T., Ferreira, E., Hipólito, D., Barros, T., Lino, A., Robetto, S., Rossi, L., … Carmena, D. (2025). Free-ranging wolves (Canis lupus) are natural reservoirs of intestinal microeukaryotes of public health significance in Southwestern Europe. Zoonoses and Public Health, 72(3), 269–283. https://doi.org/10.1111/zph.13202.

Parra-Flores, J., Holý, O., Bustamante, F., Lepuschitz, S., Pietzka, A., Contreras-Fernández, A., Castillo, C., Ovalle, C., Alarcón-Lavín, M. P., Cruz-Córdova, A., Xicohtencatl-Cortes, J., Mancilla-Rojano, J., Troncoso, M., Figueroa, G., & Ruppitsch, W. (2022). Virulence and Antibiotic Resistance Genes in Listeria monocytogenes Strains Isolated From Ready-to-Eat Foods in Chile. Frontiers in microbiology, 12, 796040. https://doi.org/10.3389/fmicb.2021.796040.

Palacios-Gorba, C., Moura, A., Leclercq, A., Gómez-Martín, Á., Gomis, J., Jiménez-Trigos, E., Mocé, M. L., Lecuit, M., & Quereda, J. J. (2021). Listeria spp. isolated from tonsils of wild deer and boars: Genomic characterization. Applied and Environmental Microbiology, 87, e02651-20. https://doi.org/10.1128/AEM.02651-20.

Ricchiuti, L., Petrini, A., Interisano, M., Ruberto, A., Salucci, S., Marino, L., Del Riccio, A., Cocco, A., Badagliacca, P., & Pozio, E. (2021). First report of Trichinella pseudospiralis in a wolf (Canis lupus italicus). International Journal for Parasitology: Parasites and Wildlife, 15, 195–198. https://doi.org/10.1016/j.ijppaw.2021.05.002.

Rothenburger, J. L., Bennett, K. R., Bryan, L., & Bollinger, T. K. (2015). Septicemic listeriosis in wild hares from Saskatchewan, Canada. Journal of Wildlife Diseases, 51(2), 503–508. https://doi.org/10.7589/2014-06-159.

Sauvala, M., Woivalin, E., Kivistö, R., Laukkanen-Ninios, R., Laaksonen, S., Stephan, R., & Fredriksson-Ahomaa, M. (2021). Hunted game birds: Carriers of foodborne pathogens. Food Microbiology, 98, 103768. https://doi.org/10.1016/j.fm.2021.103768.

Schoder, D., Guldimann, C., & Märtlbauer, E. (2022). Asymptomatic carriage of Listeria monocytogenes by animals and humans and its impact on the food chain. Foods, 11(21), 3472. https://doi.org/10.3390/foods11213472.

Seemann, T. (n.d.). Abricate [Computer software]. GitHub. https://github.com/tseemann/abricate
Tavares, R. M., Silva, D. A. L. D., Camargo, A. C., Yamatogi, R. S., & Nero, L. A. (2020). Interference of the acid stress on the expression of llsX by Listeria monocytogenes pathogenic island 3 (LIPI-3) variants. Food research international (Ottawa, Ont.), 132, 109063. https://doi.org/10.1016/j.foodres.2020.109063.

Thomas, G. A., Paradell Gil, T., Müller, C. T., Rogers, H. J., & Berger, C. N. (2024). From field to plate: How do bacterial enteric pathogens interact with ready-to-eat fruit and vegetables, causing disease outbreaks? Food

Microbiology, 117, 104389. https://doi.org/10.1016/j.fm.2023.104389.

Thouvenot, P., Vales, G., Bracq-Dieye, H., Tessaud-Rita, N., Maury, M. M., Moura, A., Lecuit, M., & Leclercq, A. (2018). MALDI-TOF mass spectrometry-based identification of Listeria species in surveillance: A prospective study. Journal of Microbiological Methods, 144, 29–32. https://doi.org/10.1016/j.mimet.2017.10.003.

Tuytschaever, T., Raes, K., & Sampers, I. (2023). Listeria monocytogenes in food businesses: From persistence strategies to intervention/prevention strategies—a review. Comprehensive Reviews in Food Science and Food Safety, 22(5), 3910–3950. https://doi.org/10.1111/1541-4337.13219.

Vilchis-Rangel, R. E., Espinoza-Mellado, M. D. R., Salinas-Jaramillo, I. J., Martinez-Peña, M. D., & Rodas-Suárez, O. R. (2019). Association of Listeria monocytogenes LIPI-1 and LIPI-3 marker llsX with invasiveness. Current Microbiology, 76(5), 637–643. https://doi.org/10.

Wiktorczyk-Kapischke, N., Skowron, K., & Wałecka-Zacharska, E. (2023). Genomic and pathogenicity islands of Listeria monocytogenes—Overview of selected aspects. Frontiers in Molecular Biosciences, 10, 1161486. https://doi.org/10.3389/fmolb.2023.1161486.

Zakrzewski, A. J., Kurpas, M., Zadernowska, A., Chajęcka-Wierzchowska, W., & Fraqueza, M. J. (2023). A comprehensive virulence and resistance characteristics of Listeria monocytogenes isolated from fish and the fish industry environment. International Journal of Molecular Sciences, 24(4), 3581. https://doi.org/10.3390/ijms24043581.