EcoHealth 20, 122–132, 2023
https://doi.org/10.1007/s10393-023-01625-y

EcoHealth

Original Contribution

Different Roles of Wild Boars and Livestock in *Salmonella* Transmission to Humans in Italy

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Abstract: Wild boar (*Sus scrofa*) is the most widely distributed large wildlife mammal worldwide. To investigate the transmission of *Salmonella enterica* amongst wild boars (*Sus scrofa*), humans, and livestock, we compared via pulsed-field gel electrophoresis and whole genome sequences the isolates of *S. enterica* serovar Typhimurium (biphasic and monophasic variants) and Enteritidis collected from wild boars, food-producing animals, and human patients in Emilia-Romagna region (Northern Italy) between 2017 and 2020. Specifically, we analysed 2175 isolates originated from human (1832), swine (117), bovine (128), poultry (76), and wild boar (22).The genomic analyses showed that wild boars shared most of their lineages of biphasic Typhimurium with bovines and most of Enteritidis with poultry, whilst we did not find any lineage shared with swine. Moreover, almost 17% of human biphasic Typhimurium and Enteritidis belonged to genomic clusters including wild boar isolates, but the inclusion of bovine and poultry isolates in the same clusters and the peculiar spatial distribution of the isolates suggested that human cases (and wild boar infections) likely originated from bovines and poultry. Consequently, wild boars appear not to play a significant role in infecting humans with these serovars, but seem to get infected themselves from livestock, probably through the environment.

Keywords: *Salmonella*, Wild boar, Human, Livestock, Whole genome sequencing, Source attribution

Introduction and Purpose

Salmonella is one of the most common zoonotic pathogens shared by wild animals and humans (Gortázar et al., 2007). In 2019, 87,923 confirmed cases of human salmonellosis were reported in the European Union (EU) with a notification rate of 20.0 cases per 100,000 inhabitants (EFSA and ECDC, 2021a). The impact of Covid-19 pandemic and the withdrawal of the United Kingdom from the EU reduced the number of cases reported in 2020, which were the lowest from 2007 (52,702 cases; notification rate of 13.7 per 100,000 population) (EFSA and ECDC, 2021b).

Wild boar (*Sus scrofa*) is amongst the most widely distributed large mammals in wildlife worldwide (Oliver et al., 1993), and during the last 30 years, its population has

Published online: March 15, 2023

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undergone a systematic increase in size and geographical distribution across Europe (Tack, 2018). This mammal is very adaptable and can be found in different habitats (Sjarmidi and Gerard, 1988) with deciduous and mixed forests, preferably composed of oak and beech enclosing marshes and meadows, as the favoured ones (Tack, 2018). They are versatile omnivores whose diet varies with their habitat and includes vegetables, crops, seeds, insects, earthworms, birds, mammals, amphibians, reptiles, and carrions (Heptner et al., 1988). Besides the generalist behaviour of wild boars in terms of wide feed and habitat selection, the main reason for this rapid increase is attributed to their high reproductive potential (Servanty et al., 2007). Hunting and trapping are commonly promoted to prevent overgrowth of the wild boar populations in Europe (EFSA, 2014).

In Italy, wild boars are amongst the most common wild ungulates and their density varies from 0.01–0.05 to 2.32–10.5 animals/km^2, with a density of 1.37–2.31 animals/km^2 in the area of the study (Emilia-Romagna region; 22,451 km^2) (Pittiglio et al., 2018). According to this estimate, the regional wild boar population should range between thirty- and fifty-thousand animals.

Due to their omnivorous diet, wild boars may easily ingest bacterial pathogens, including *Salmonella,* that may colonise their intestinal tract and lymphatic tissue, contaminate their meat, and be transmitted to humans (Wacheck et al., 2010; Chiari et al., 2013). In this study, we investigated, through whole genome sequencing (WGS), the strains of *Salmonella enterica* serovar Typhimurium (hereafter, Typhimurium), including its monophasic variant, and *Salmonella enterica* serovar Enteritidis (hereafter, Enteritidis) detected in wild boars and belonging to pulsed-field gel electrophoresis (PFGE) types in common with farmed animals (cattle, pigs, and poultry) and humans in the same study period (2017–2020) in order to: (*i*) assess clonality of isolates, (*ii*) investigate the likely pathways for wild boars to contaminate farmed animals or get contaminated by *Salmonella* strains shed by farmed animals, and (*iii*) compare the isolates from wild boars with the human isolates detected in Emilia-Romagna region to evaluate the role of wild boars in the epidemiology of human salmonellosis.

Methods

Sampling

The isolates of *Salmonella enterica* from wild boars used in the genomic analyses were obtained in two subsequent sampling campaigns and performed in the same geographical area of Northern Italy (see box in Figure 1).

First sampling campaign: from June 2017 to March 2019, 305 wild boars (*Sus scrofa*) hunted in the Parma and Piacenza provinces (NUTS3 levels ITH52 and ITH51, see light and dark grey areas in Figure 1) of Emilia-Romagna region in Northern Italy were tested for *Salmonella* spp. soon after shooting (less than 5 h). Mesenteric lymph nodes (MLNs) and faecal samples were collected immediately after evisceration and placed in sterile containers. The samples were stored at refrigeration conditions and analysed within 24 h.

Second sampling campaign: in 2020, 78 wild boars hunted in the same territory of the first sampling campaign (Parma and Piacenza provinces, Northern Italy) were tested for *Salmonella* contamination. Faeces were not tested due to the low prevalence of positive samples assessed during the first sampling campaign. Swabs from carcasses were collected from wild boar carcasses both after evisceration at the game collection centre as well as after transportation to the game-handling establishment. A 100 cm^2 area of the abdominal region in the inner part of the carcasses was swabbed with sterile sponges moistened with 10 ml of Buffered Peptone Water (BPW, Oxoid, Basingstoke, UK). MLNs were collected immediately after evisceration at the game collection centre and placed in sterile containers. The samples were transported to the laboratory under refrigeration and analysed on the day of arrival.

The epidemiological and serotyping results of the sampling campaigns in wild boar related to *Salmonella* serovars different than *S.* Typhimurium and *S.* Enteritidis were reported in Bonardi et al. (2019) and Bonardi et al. (2021).

Salmonella Detection and Typing in Wild Boars

MLNs, faecal samples, and sponges were tested for *Salmonella* following ISO 6579–1:2017 (ISO, 2017). MLNs were rinsed with sterile water and externally decontaminated with ethanol before being tested. Aliquots of lymph nodes ranging from 2.5 g to 27 g (average weight: 10.1 g) were diluted 1:10 in BPW for pre-enrichment. The variability in weight was due to size difference of the MLNs (young *versus* old animals). Faecal samples (10 g) and sponges were diluted 1:10 in BPW for pre-enrichment. After biochemical identification of the isolates by using the

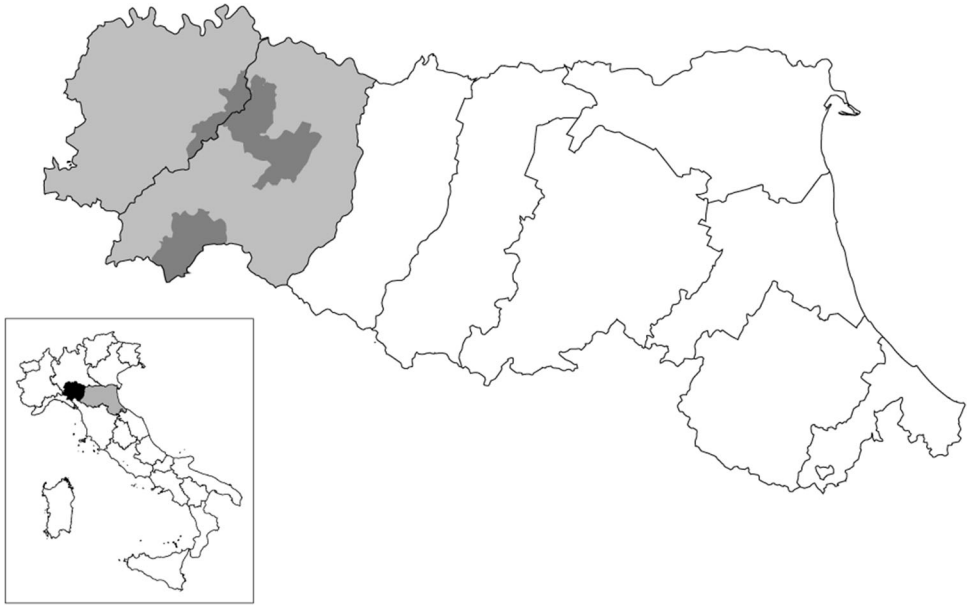

Figure 1. Hunting areas (dark grey) in the Piacenza and Parma provinces (light grey), Emilia-Romagna region, Northern Italy.

API 20 E ® system (bioMerieux, Marcy l'Etoile, France), *Salmonella* serotyping was performed following ISO/TR 6579–3:2014 (ISO, 2014). The monophasic/biphasic character of the Typhimurium isolates was evaluated by PCR as previously described in Barco et al. (2011). *Salmonella enterica* subsp. *enterica* isolates were typed by pulsed-field gel electrophoresis (PFGE) according to standard methods (PulseNet, 2017) with *Xba*I restriction of DNA. The genomic DNA underwent restriction before electrophoresis in a Chef Mapper XA system (Bio-Rad, CA, USA). Then, the PFGE patterns were analysed through BioNumerics Software version 7.5 (Applied-Maths, Sint-Martens-Latem, Belgium).

Comparison with Livestock and Human *Salmonella* Isolates

Data on isolates from domestic animals were taken from the Regional *Salmonella* Surveillance of Emilia-Romagna and derive from routine veterinary laboratory diagnostics and pre-slaughter surveys. A total of 1313 isolates were used for the study (290 swine, 198 bovine, and 825 poultry), collected from 2017 to 2020 from the regional population of swine (1,279,743 heads), bovine (558,695 heads) and poultry (26,277,294 heads) (National Statistics of Italy; Census, 2010).

Data on isolates from human cases of salmonellosis were taken from the Regional *Salmonella* Surveillance of Emilia-Romagna. A total of 2988 human isolates were used for the study, collected from 2017 to 2020. These human isolates represent the whole set of isolates derived from the microbiologically confirmed human salmonellosis cases of Emilia-Romagna, a region with a population around 4.5 million. All isolates originated from domestic animals and humans were serotyped and PFGE-typed during regular surveillance.

Whole Genome Sequencing

Sequencing libraries were prepared, starting from genomic DNA extracted with DNeasy Blood and Tissue kit (Qiagen), using DNA Prep (M) Tagmentation (Illumina) and run with Nextseq and Miseq platforms (Illumina) producing pair-end reads (2×150 bp and 2×250 bp, respectively). Quality and length of raw reads were checked with FastQC (Babraham, 2010), whilst species confirmation and possible contaminations were done with Kraken2 (Wood et al., 2019). Raw reads were then filtered with Trimmomatic ver. 0.38 (Bolger et al., 2014). High quality assemblies, obtained using Unicycler ver. 0.4.8 (Wick et al., 2017) and evaluated by QUAST ver. 4.2 (Gurevich et al., 2013), were retained only if coverage $>$ 30X and contig number $<$ 300 (Timme et al., 2020).

Starting from assemblies, in silico, Multi-Locus Sequence Typing (MLST) was determined using the Pasteur BIGSdb for *Salmonella spp.* (https://pubmlst.org/bigsdb?db=pubmlst_salmonella_seqdef)

based on Enterobase scheme whilst core-genome Multi-Locus Sequence Types (cgMLST) were assigned through BioNumerics Software ver. 7.6.3 (Applied-Maths, BioMerieux) according to the Enterobase cgMLST scheme (Achman et al., 2020; Alikhan et al., 2018). Single-linkage clustering, obtained through BioNumerics Software, was applied starting from cgMLST analysis. A cluster-defining threshold was identified through the maximum acceptable number of differing cgMLST loci computed by using single-linkage clustering. This number is commonly referred to as allelic distance (AD). The threshold value for AD adopted in this study was AD = 5, as proposed by the European Centre for Disease Prevention and Control and the European Food Safety Agency (ECDC and EFSA 2021, 2022).

Sequence accession numbers. Raw reads of the 81 newly sequenced isolates of the study were deposited at EBI under Project number PRJEB49128.

Results

Salmonella Enterica Subsp. *Enterica* Serovar Typhimurium

A total of 15 Typhimurium out of 51 (29.4%) *S. enterica* isolates were detected in wild boars in the two sampling campaigns. Fourteen Typhimurium isolates were detected from 383 MLN samples (apparent prevalence 3.7%; 95% CI 2.2 – 6.0) collected from 14 animals during the first sampling campaign. Eight positive animals (57.1%) belonged to class 0 (young), three (21.4%) to class 1 (subadult) and three (21.4%) to class 2 (adult). One Typhimurium isolate was found in one carcass out of 78 (apparent prevalence 1.3%; 95% CI 0.2–6.9) during the second sampling campaign. The MLN sample collected from the Typhimurium-positive carcass was negative for *Salmonella*. All the Typhimurium isolates from wild boar exhibited biphasic character (Table 1). Specifically, Typhimurium was found in 14/383 MLNs samples (apparent prevalence 3.7%; CI95% 2.2 – 6.0) and 1/93 wild boar carcasses (apparent prevalence 1.1%; CI95% 0.2 – 5.8), whilst any Typhimurium was found in the 305 faecal samples analysed. The 15 animals were hunted in a restricted area (36.4 square Km) belonging to two neighbouring municipalities (Sala Baganza and Collecchio, Parma province). The isolates belonged to three PFGE types (namely, T1, T2, and T3) and belonged to sequence type ST19 (Table 2).

In domestic animals, Typhimurium was detected in 116/290 swine (40.0%), in 125/198 bovine (63.1%), and 13/825 poultry (1.6%) in Emilia-Romagna region during the 2017–2020 period. The fraction of Typhimurium exhibiting biphasic character, like the wild boar isolates, was 13/116 (11.2%) in swine, 86/125 (68.8%) in bovine, and 6/13 (46.2%) in poultry (Table 1). The comparison of the PFGE profiles of swine isolates revealed no matches with the PFGE profiles found in wild boars. The comparison of the PFGE profiles of bovine isolates revealed that 16 bovine isolates (22.0% of the biphasic Typhimurium tested) matched with Typhimurium PFGE profiles found in wild boars. Specifically, the bovine isolates matching the PFGE profiles observed in wild boar belonged to profiles T1 (7 isolates), T2 (8 isolates), and T3 (1 isolate), see Table 2. The comparison of the PFGE profiles of poultry isolates revealed that one isolate matched with a Typhimurium PFGE profile found in wild boars, namely profile T3 (Table 2).

In humans, Typhimurium was identified as the causative agent of 1,541/2,988 cases of salmonellosis (51.6%) in Emilia-Romagna region during the 2017–2020 period. The fraction of Typhimurium isolated in humans exhibiting biphasic character, like the wild boar isolates, was 232/1,541 (15.1%), Table 1. Comparison of the PFGE profiles of human isolates showed that 18 human isolates (7.8% of the biphasic Typhimurium tested) matched with Typhimurium PFGE profiles found in wild boars. Matching human isolates belonged to profiles T1 (10/232; 4.3%), T2 (6/232; 2.6%), and T3 (2/232; 0.9%), Table 2.

Genomic analyses based on the cgMLST scheme proposed by Enterobase (Achman et al., 2020; Alikhan et al., 2018) were performed to assess the level of clonality amongst biphasic Typhimurium isolates belonging to ST19 from wild boars, livestock, and humans displaying the same PFGE profiles. A total of 42 isolates underwent WGS. For one human isolate WGS was not possible (not viable isolate). In addition, considering that all the 13 isolates from wild boar belonging to profile T2 originated from the same restricted area, we randomly included only six of them in the WGS analysis. The results of the genomic analysis for isolates with PFGE profile T1 are shown in Figure 2 reporting the minimum spanning tree (MST) obtained from cgMLST data. In the MST, green nodes represent isolates from wild boars, blue nodes represent isolates from bovine, and orange nodes represent isolates from humans. The MST shows that maximum between-node distance is 5 AD amongst isolates with T1 profile, suggesting a high level of clonality despite the diverse source of isolation. The

Table 1. Comparison of the number (and frequencies) of *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates from the wild boars in the study and isolates collected from 2017 to 2020 in swine, bovine, poultry, and human in Emilia-Romagna (Northern Italy). Percentages (%) in brackets.

Compartment	Total *Salmonella enterica* isolates	*S.* Typhimurim (%)			*S.* Enteritidis (%)
		Monophasic	Biphasic	Total	
Wild boar	51	0 (0)	15 (29.4)	15 (29.4)	7 (13.7)
Swine	290	103 (35.5)	13 (4.5)	116 (40.0)	1 (0.3)
Bovine	198	39 (19.7)	86 (43.4)	125 (63.1)	3 (1.5)
Poultry	825	7 (0.8)	6 (0.7)	13 (1.6)	63 (7.6)
Human	2988	1309 (43.8)	232 (7.8)	1541 (51.6)	291 (9.7)

Table 2. Comparison of the *XbaI* PFGE Profiles of *S.* Typhimurium ST19 (biphasic) and *S.* Enteritidis isolates from the wild boars in the study and isolates collected from 2017 to 2020 in swine, bovine, poultry, and humans in Emilia-Romagna (Northern Italy).

Serotype PFGE profile sequence type		*S.* Typhimurium			S. Enteritidis		
		T1 ST19	T2 ST19	T3 ST19	E1 ST3233	E2 ST11	E3 ST3406
Compartment	Wild boar	1	13	1	1	1	5
	Swine	0	0	0	0	0	0
	Bovine	7	8	1	0	0	0
	Poultry	0	0	1	2	8	0
	Human	10	6	2	17	10	0

spatial distribution of the isolates belonging to the genomic cluster showed that no isolates collected from bovine farms and humans originated from the province where the infected wild boar was sampled (see the map in Figure 2).

The results of the genomic analysis for isolates with PFGE profile T2 are summarised in the MST in Figure 3. The MST shows that isolates with T2 profile recovered from wild boars (green circles), bovine (blue circles) and four out of six human isolates (orange circles) display a high level of clonality (AD $\leq$ 5), see the grey area in Figure 3. The spatial distribution of the isolates belonging to the genomic cluster showed that only 5/8 of the isolates collected from bovine farms and 1/4 of the isolates collected from humans originated from the province where wild boars were sampled, whilst the others originated from other Emilia-Romagna provinces (see the map in Figure 3).

The results of the genomic analysis for isolates with PFGE profile T3 are summarised in the MST in Figure 4. The MST shows that the isolate with T3 profile recovered from wild boar (green circle) displays a very low level of clonality with respect to the isolates recovered from different compartments.

Salmonella Enterica Subsp. *Enterica* Serovar Enteritidis

A total of seven *S.* Enteriditis over 51 (13.7%) *S. enterica* isolates were detected in wild boars in the two sampling campaigns (Table 1). Specifically, Enteritidis was detected in 4/383 MNLs sample (apparent prevalence 1.0%; 95% CI 0.4 – 2.7), 1/305 faecal sample (apparent prevalence 0.3%; 95% CI 0.0 – 1.8), and 2/78 carcasses (apparent prevalence 2.6%: 95% CI 0.7–8.9) belonging to different animals hunted in both sampling campaigns. The faecal shedder belonged to class 1 (subadult), whilst the positive MLNs

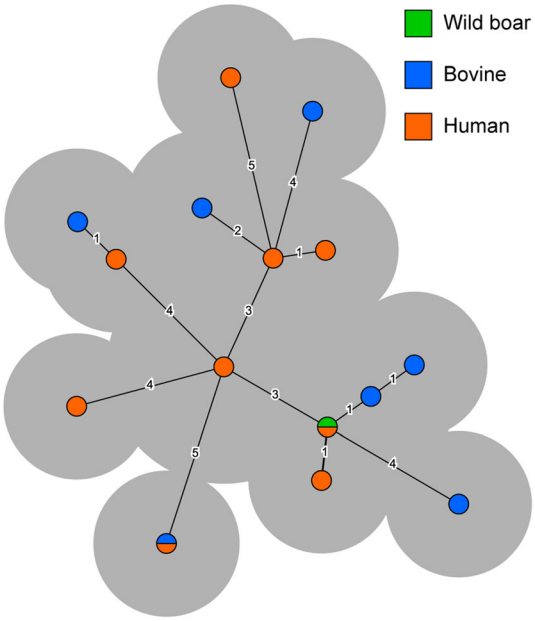

Figure 2. Minimum Spanning Tree (MST) obtained from cgMLST data for Typhimurium isolates with PFGE profile T1 and sequence type ST19. Green nodes represent isolates from wild boar, blue nodes represent isolates from bovine, and orange nodes represent isolates from humans. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences (AD) in cgMLST. Nodes included in the grey area belong to the same genomic cluster with AD $\leq$ 5. The Map represents the locations of the isolates in the cluster (Color figure online).

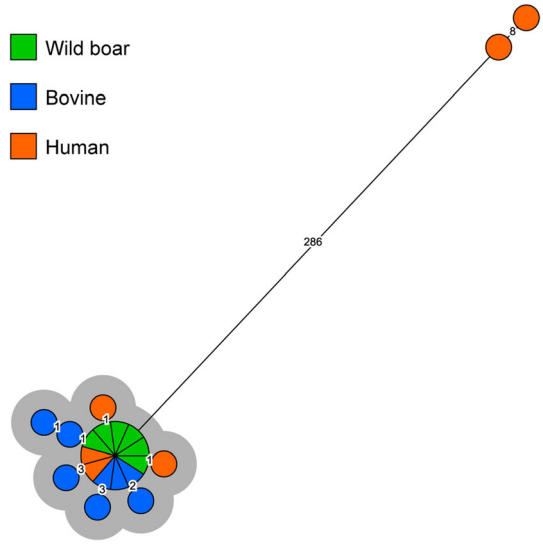

Figure 3. Minimum Spanning Tree (MST) obtained from cgMLST data for Typhimurium isolates with PFGE profile T2 and sequence type ST19. Green nodes represent isolates from wild boar, blue nodes represent isolates from bovine, and orange nodes represent isolates from humans. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences (AD) in cgMLST. Nodes included in the grey area belong to the same genomic cluster with AD $\leq$ 5. The Map represents the locations of the isolates in the cluster (Color figure online).

were collected from young animals. The isolates belonged to three PFGE types (namely, E1, E2, and E3), and belonged to sequence types ST3233, ST11, and ST3406, respectively.

In domestic animals, Enteritidis was detected in 1/290 swine (0.3%), in 3/198 bovine (1.5%), and 63/825 poultry (7.6%) in Emilia-Romagna region during the 2017–2020 period (Table 1). Comparison of the PFGE profiles of swine and bovine isolates revealed no matches with Enteritidis PFGE profiles found in wild boars. Comparison of the PFGE profiles of poultry isolates revealed that 10 isolates (15.9% of the Enteritidis tested) matched with Enteritidis PFGE profiles found in wild boars. Matching poultry isolates belonged to profiles E1 (2/63; 3.2%) and E2 (8/63; 12.7%), Table 2.

In humans, Enteritidis was identified as the causative agent of 291/2,988 cases of salmonellosis (9.7%) in Emilia-Romagna region during the 2017–2020 period (Table 1). Comparison of the PFGE profiles of human isolates revealed that 27 isolates (9.3% of the Enteritidis tested) matched with Enteritidis PFGE and MLST profiles found in wild boars. Specifically, Enteritidis profiles E1 (17/291; 5.8%) and E2 (10/291; 3.4%) were detected in human isolates (Table 2).

Genomic analyses based on the cgMLST scheme proposed by Enterobase (Achman et al., 2020; Alikhan et al., 2018) were performed to assess the level of clonality amongst Enteritidis isolates from wild boars, livestock, and humans with the same PFGE profile. The results for isolates with PFGE profile E1 are summarised in the MST of Figure 5, where green nodes represent isolates from wild boar, yellow nodes represent isolates from poultry, and orange nodes represent isolates from humans. The MST shows that maximum between-node distance is 5 AD amongst isolates with E1 profile, suggesting a high level of clonality amongst wild boar, poultry and human isolates. The spatial distribution of the isolates belonging to the genomic cluster showed that no isolates collected from poultry farms and humans originated from the provinces where infected wild boar was sampled (see map in Figure 5).

The results of the genomic analysis for isolates with PFGE profile E2 are summarised in Figure 6. The MST shows that isolates with E2 profile gather in three different cgMLST clusters characterised by AD $\leq$ 5 (grey areas la-

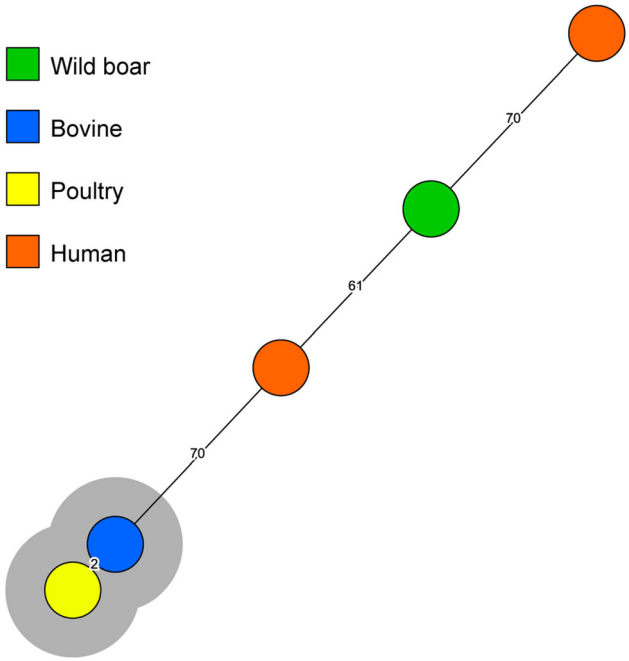

Figure 4. Minimum Spanning Tree (MST) obtained from cgMLST data for Typhimurium isolates with PFGE profile T3 and sequence type ST19. Green nodes represent isolates from wild boar, blue nodes represent isolates from bovine, yellow nodes represent isolates from poultry, and orange nodes represent isolates from humans. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences (AD) in cgMLST. Nodes included in the grey area belong to the same genomic cluster with AD $\leq$ 5 (Color figure online).

belled with letters A, B, and C). Specifically, cluster A grouped the isolate from wild boar with two isolates from poultry and six isolates from humans; cluster B grouped six isolates from poultry with two from humans; and cluster C grouped only isolates from human cases. The spatial distribution of the isolates of genomic cluster A showed that all isolates collected from poultry farms and human originated from the provinces where wild boars were sampled.

Discussion

The genomic analyses performed in this work showed that, in Northern Italy, wild boars share lineages of Typhimurium and Enteritidis with both livestock and humans. In the case of livestock, we found that wild boars share the lineages of Typhimurium with bovine and the lineages of Enteritidis with poultry. Conversely, we did not find lineages of either serotype shared by wild boars and swine, suggesting lack of or limited exchange of *Salmonella* be-

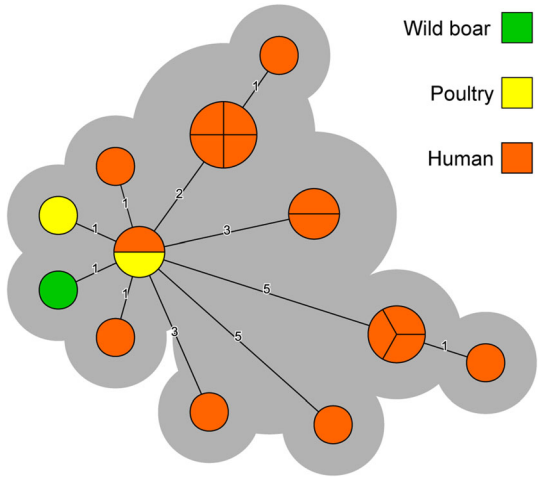

Figure 5. Minimum Spanning Tree (MST) obtained from cgMLST data for Enteritidis isolates with PFGE profile E1 and sequence type ST3233. Green nodes represent isolates from wild boar, yellow nodes represent isolates from poultry, and orange nodes represent isolates from humans. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences (AD) in cgMLST. Nodes included in the grey area belong to the same genomic cluster with AD $\leq$ 5. The Map represents the locations of the isolates in the cluster (Color figure online).

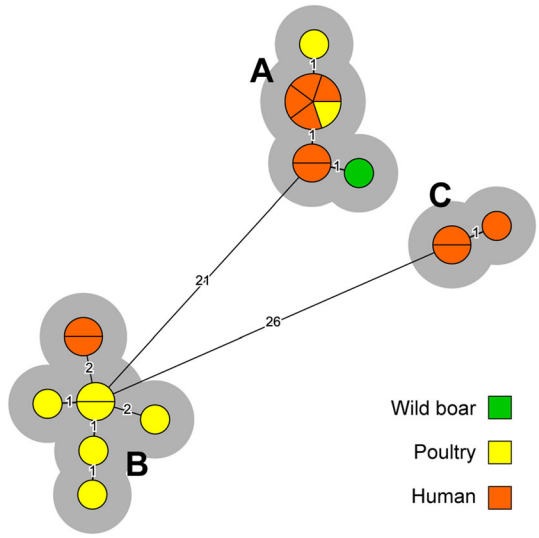

Figure 6. Minimum Spanning Tree (MST) obtained from cgMLST data for Enteritidis isolates with PFGE profile E2 and sequence type ST11. Green nodes represent isolates from wild boar, yellow nodes represent isolates from poultry, and orange nodes represent isolates from humans. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences (AD) in cgMLST. Nodes included in the grey areas (labelled A, B, and C) belong to the same genomic cluster with AD $\leq$ 5 (Color figure online).

tween the domestic swine and wild boar compartments. Even if wild boars were living in a territory where pig density was in line with the regional average density (Bonardi et al., 2019), the strict biosecurity measures applied to pig farming, including prevention of contact with wildlife as well as appropriate management of pig manure, prevented the environmental dissemination of *Salmonella* of pig origin.

Typhimurium was the most common serovar detected in the wild boars tested in the study. Typhimurium ranked second amongst the serovars reported in EU from human cases of salmonellosis in 2019–2020, confirming the trend observed in the last years (EFSA and ECDC, 2021a, 2021b). Pigs and broilers are the species more commonly associated with Typhimurium in the EU (EFSA and ECDC, 2021b). Moreover, several studies reported its detection from wild boars in EU countries, as Portugal (Vieira-Pinto et al., 2011), Spain (Diaz-Sanchez et al., 2013; Gil Molino et al., 2019), Austria (Glawishnig et al., 2018), Sweden (Sannö et al., 2014), Germany (Plaza-Rodriguez et al., 2021), and Italy (Chiari et al., 2013; Zottola et al., 2013).

Our study showed that about six percent (13/232) of biphasic Typhimurium isolates from humans affected by salmonellosis in Emilia-Romagna region (Northern Italy) were included in genomic clusters with wild boar isolates. Whilst this finding could suggest that wild boars may be responsible for a significant fraction of human infections, the simultaneous involvement of bovine isolates in the same clusters and the peculiar spatial distribution of the isolates from the three species suggest that human cases (and wild boar infections) very likely originate from bovines. Specifically, in wild boars, we found Typhimurium belonging to clusters related to human infections only in a confined area (Boschi di Carrega Regional Park, extension about 13 km^2), whilst human and bovine isolates belonging to the same clusters were detected in different provinces (including provinces outside the study area) of the Emilia-Romagna region.

The observed spatial confinement of Typhimurium in wild boars in the study area is consistent with the results previously obtained by investigating the prevalence of Hepatitis E in the same wild boar populations (Bonardi et al., 2020). In particular, that study detected a high prevalence of Hepatitis E (about 31%) in the Boschi di Carrega Regional Park wild boar population, whilst it did not detect any positive animal in a nearby wild boar population located 8 km apart.

The high level of clonality amongst Typhimurium strains detected in wild boars and cattle suggests a non-negligible interaction between the two species in the study area. In Northern Italy, intensive cattle farms commonly feature fenced outdoor resting areas for animals thus contributing to outdoor contamination with cattle manure. In addition, cattle farms in hilly and mountain areas are often close to the habitat of wild boar populations.

Even if Enteritidis was found at low rates, its detection from wild boar carcasses is of concern. Both in 2019 and 2020, Enteritidis represented the most frequently reported serovar from human cases of salmonellosis in the EU, being identified in 50.3% and 48.7% of the confirmed cases of disease, respectively (EFSA and ECDC, 2021a, 2021b). This serovar was also responsible for 72.4% (439/606) of the *Salmonella* outbreaks with available information on the serovar reported in 2019 (EFSA and ECDC, 2021a). Enteritidis is commonly associated with poultry and poultry products (EFSA and ECDC, 2021a, 2021b), but its detection at low rates in wild boars has been reported in Spain (Navarro-Gonzalez et al., 2012; Mentaberre et al., 2013; Gil Molino et al., 2019; Castillo-Contreras et al., 2022), Italy (Chiari et al., 2013), Switzerland (Wacheck et al., 2010), and Germany (Plaza-Rodriguez et al., 2021). Our study showed that nearly 7% of Enteritidis isolates from humans affected by salmonellosis in Emilia-Romagna region (Northern Italy) were included in genomic clusters with wild boar isolates. Similarly to what we found for Typhimurium, the simultaneous presence of the Enteritidis lineages responsible for human salmonellosis in both wild boar and poultry and the spatial distribution of the isolates in the three species suggest that human cases (and wild boar infections) very likely originate from poultry. Specifically, human and poultry isolates of Enteritidis belonging to the ST3233 cluster were found in different Emilia-Romagna provinces (included provinces outside the study area), whilst the human isolates belonging to the ST11 cluster were epidemiologically linked to the consumption of eggs of backyard poultry from which the same poultry cluster originated. The backyard flock was inside the study area, and the wild boar harbouring the Enteritidis belonging to the ST11 cluster was hunted about 16 km from the backyard flock. This last finding supports the hypothesis that contacts between wild boars and poultry in the study area originate from small backyard flocks rather than intensive poultry farms, which are located in different territories of the region. Small poultry flocks are common in habitats of wild boars and are easily accessible to wildlife.

Further support to the hypothesis that poultry and not wild boar were the source of Enteritidis infections in humans comes from the evidence that the PFGE/MLST types found in wild boars and absent in poultry (i.e., E3/ST3406) had no correspondence to human isolates.

Our results suggest caution in the interpretation of genomic data in the study of foodborne diseases' epidemiology. We remark that, in the absence of epidemiological support, high levels of genomic similarity between a potential source (in this case wild boar) and human cases do not imply definitive evidence of causality, per se.

The same observation applies to source attribution approaches based on population genetics models, which usually do not include non-genetic data such as prevalence and relative occurrence of the pathogen's subtypes in each source, food consumption data. Conversely, these non-genetic data are used to inform the so called "frequency-matching" models. Source attribution models are statistical approaches that use microbial subtyping data (e.g., serotype, PFGE, genomic typing) to ascribe human cases of foodborne infection to their putative sources by evaluating the similarity of pathogen's subtypes between humans and the sources (for a review of source attribution models of foodborne diseases see Mughini-Gras et al., 2018.) Applying source attribution models to our genomic dataset, without including epidemiological information, would have led to attributing human cases to wild boar since wild boar isolates are equally or more similar in cgMLST to some human cases compared to livestock isolates (see for instance Figs. 1, 2, and 5). Nevertheless, this attribution appears unlikely in the light of the actual epidemiological scenario. The ambiguity can only be solved by taking into consideration the epidemiological information about the context of infection transmission. An analogous scenario was apparent in Filipello et al. (2020), where population genetics models and frequency-matching models provided significantly different results in attributing human cases of listeriosis to wildlife (through game meat) compared to livestock sources. Specifically, population genetics models unexpectedly predicted wildlife as a larger reservoir of *Listeria monocytogenes* than swine, whilst frequency-matching models predicted swine as one of the largest *L. monocytogenes* reservoirs of human infections and a much larger reservoir than wildlife (Filipello et al., 2020).

Conclusions

The results of the study indicate that the clones of Typhimurium and Enteritidis shared by humans and wild boars are not transmitted to humans by wild boars, but by livestock. Consequently, wild boars appear not to have a very significant role in infecting humans with these serovars. Conversely, wild boars themselves seem to get infected from livestock, most probably by indirect contact through the environment. Most notably, no role was demonstrated for swine unlike bovine and poultry that were implicated.

Although these conclusions are conditioned by the limited number of isolates tested, they contribute to the understanding on experimental basis of the interaction of livestock, wild boars, and the environment in the epidemiology of *Salmonella* infections, including human salmonellosis, in the context of Northern Italy.

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