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Salmonella in horses at slaughter and public health effects in Italy

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ABSTRACT

The study assessed the role of equids at slaughter as faecal carriers of *Salmonella enterica* and the occurrence of contaminated equid carcasses during the slaughter process in Northern Italy (Emilia-Romagna Region). From June to November 2021, 152 equids (146 horses, 5 donkeys and 1 mule) were tested for *Salmonella* both in caecal contents and through carcass swabs. Antimicrobial resistance (AMR) of recovered strains was tested against 15 antimicrobials. *Salmonella* was detected in 3/152 of the caecal contents (2.0 %), while all carcass samples were negative. *S. enterica* serovars Enteriditis, Typhimurium and Stanleyville were identified. The only AMR isolate was *S. Typhimurium* with AMR profile AmCStxT.

Considering the consumption of raw horse meat (i.e., minced raw meat named "pesto di cavallo" and dried and smoked strips named "sfilacci di cavallo") in different areas of Northern Italy, we also investigated the possible link between horse meat eating and salmonellosis cases in the human population in the same area. Specifically, we compared the *Salmonella* strains collected during the study with those routinely processed in the laboratory surveillance system for human salmonellosis in Emilia-Romagna (a region with about 4.5 million inhabitants). The comparison was based on whole genome sequencing data through core genome multi-locus sequence typing (cgMLST) used in routine surveillance. A genomic match in cgMLST was found between the strain of *S. enterica* serovar Enteritidis isolated from a horse caecal content and an enduring outbreak of 17 human cases in Emilia-Romagna during the study period. The consequent epidemiological investigation highlighted that a number of cases with known food history reported the consumption of horse meat and traced different batches of the consumed meat, released weeks apart from each other, to the slaughter investigated in the study. The results of the epidemiological investigation suggested the role of horses in the *S. enterica* serovar Enteritidis outbreak affecting raw horse meat consumers.

This study shows that, despite the low prevalence on equid carcasses, *S. enterica* in horse meat can represent a risk to consumers. From the perspective of the slaughter activities, this highlights the need to maintain a high level of hygiene during the entire process, starting from the hygiene at lairage up to the slaughtering phase and dressing of carcasses.

1. Introduction

Salmonella spp. is a Gram-negative bacillus, belonging to the *Enterobacteriaceae* family and divided in the two species *S. enterica* and *S. bongori*. The genus is classified in approximately 2600 serovars, most of which are pathogenic to humans (Issenhuth-Jeanjean et al., 2014). *Salmonella* is a food-borne pathogen of worldwide importance and is typically associated with symptoms of diarrhoea, fever, and abdominal

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cramps. Human salmonellosis is the second most frequently reported zoonosis after campylobacteriosis in the European Union (EU). In 2021, its notification rates varied among countries, ranging from 2.7 cases/100,000 population (Romania) to 93.7 cases/100,000 population (Czech Republic), with an average rate of 15.7 cases/100,000 in 27 countries (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022). In 2021, the top five *S. enterica* serovars responsible for human salmonellosis in the EU were Enteritidis (54.6 %), Typhimurium (11.4 %), monophasic variant of Typhimurium 1,4,[5],12:i:-(8.8 %), Infantis (2.0 %), and Derby (0.93 %) (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022).

Isolation of *Salmonella* in horses is not uncommon (Martelli et al., 2018). However, large variations in pathogenicity and epidemiology among serovars exist. Infection with the host-adapted *S.* Abortusequi causes equine paratyphoid, characterized by abortion in mares, and septicaemia and polyarthritis in the new-born foals. This serovar is sporadically reported in Europe but was responsible for a large outbreak among Murgese foals in Italy in 2016 (Grandolfo et al., 2018). *Salmonella* infections by other serovars may remain subclinical and self-limiting in healthy adult horses but might result in clinical disease, even evolving in invasive septicaemia with high mortality, in foals less than six month of age (Martelli et al., 2018).

Salmonella shedding by horses may be intermittent and influenced by seasonality, with higher prevalence in late summer and early autumn compared to spring- and wintertime (Smith et al., 1978; Traub-Dargatz et al., 2000). Nevertheless, equids seem to play a secondary role in *Salmonella* transmission to humans in comparison with other species especially because of the low consumption rate of soliped meat (then referred as "horse meat"), mainly limited to some local realities in a reduced number of countries, such as Belgium, France, Germany, Italy, The Netherlands, Spain, and Sweden (EFSA (European Food Safety Authority), 2013a). Horse meat is usually consumed as cooked fresh cuts, but raw minced meat as well as meat preparations are appreciated in some countries. Offal from solipeds is usually not consumed (EFSA (European Food Safety Authority), 2012). Given the restricted consumption in only a few countries, data on *Salmonella* prevalence in horse meat are scarce in the EU if compared with the most common sources of this microorganism, like eggs, poultry and pork. The only data commonly available at EU level derive from the monitoring of the Process Hygiene Criterion (PHC) set by Regulation (EC) No 2073/2005 (European Commission, 2005).

Some stress factors associated with horses brought to slaughter may contribute to *Salmonella* infection and enhance its shedding, such as transportation, overcrowding, food or water deprivation, new stable environments, and unfamiliar handlers (Martelli et al., 2018; Morse et al., 1976). Being located at the end of the primary production chain, slaughterhouses can play a key role in the control of meat contamination by *Salmonella*. Many studies have demonstrated that implementation of hygienic procedures during slaughtering strongly reduce contamination of carcasses with *Salmonella* (Argüello et al., 2013; Berends et al., 1997; Bottledoorn et al., 2003). This means that good hygienic practices at slaughterhouses are of critical importance in preventing *Salmonella* transmission to consumers, especially through meat not subjected to heat treatment before consumption, as the case of raw horse meat.

Based on these premises and since Italy is the EU country with the highest number of horses slaughtered for meat consumption (EFSA (European Food Safety Authority), 2013b) in this study we investigated *i*) the role of equids at slaughter as faecal carriers of *Salmonella*; *ii*) the occurrence of carcass contamination during slaughter; and *iii*) the possible link between horse meat contamination and salmonellosis cases in the human population. In addition, *iv*) we evaluated the ability of recently implemented on-health surveillance system based on whole genome sequencing to correctly attribute human infections to their sources.

2. Materials and methods

2.1. Sampling

From June to November 2021, 152 equids were tested for *Salmonella* in one slaughterhouse located in Emilia-Romagna Region in Northern Italy in cooperation with the local Food Safety Competent Authority (CA) of the National Health Service. A total of 304 samples were collected from the caecal content (n = 152) and carcasses (n = 152) during 17 sampling sessions. The average number of animals tested per session was 8.9, corresponding to one sixth of the animals slaughtered per day (ranging from 42 to 56). The equids to be tested were selected and discontinuously distributed along the slaughter day, so that consecutive processed equids/carcasses were not tested. The interval between the tested equids/carcasses ranged between three to seven units.

The samples of caecal content were collected by using a sterile spoon from the caecum immediately after evisceration and placed in sterile containers. Carcasses were swabbed by using sterile sponges moistened with Buffered Peptone Water (BPW, Oxoid, Basingstoke, UK) covering four sampling areas of 100 cm^2 (rump, flank, brisket, neck). The animals were represented mainly by horses (n = 146), followed by donkeys (n = 5) and mules (n = 1). The countries of origin were France (n = 84), Italy (n = 33), Poland (n = 17), Hungary (n = 11), Slovenia (n = 6), and Germany (n = 1). The equids were slaughtered two days a week (on Monday and Thursday) but they arrived at the slaughterhouse daily, thus frequently prolonging the lairage period prior to slaughter by two to four days.

2.2. Cultural methods

All samples were tested following ISO 6579-1: 2017 (International Organization for Standardization (ISO), 2017). The second isolation medium was represented by Chromatic *Salmonella* Agar (Liofilchem, Roseto degli Abruzzi, Italy). Plates of XLD and Chromatic *Salmonella* Agar were incubated at 37 °C for 24 ± 3 h. Suspect *Salmonella* colonies were seeded onto Tryptic Soy Agar (TSA, Oxoid) and incubated between 34 °C and 38 °C for 24 ± 3 h to obtain pure cultures. Biochemical confirmation of well-isolated colonies was performed by inoculating Triple Sugar Iron (TSI) agar, L-Lysin decarboxylation (LLDC) medium and Urea agar, followed by agglutination with omnivalent *Salmonella*-O antiserum (Biogenetics, Padua, Italy). Biochemical identification of the genus *Salmonella* was obtained by using the Microgen GN-A system (Biogenetics). *Salmonella* serotyping was performed following ISO/TR 6579-3:2014 (International Organization for Standardization (ISO), 2014).

2.3. Sensitivity to antimicrobials

Salmonella isolates were tested for sensitivity to the panel of antimicrobials recommended by the Commission Implementing Decision 2020/1729/EU (European Commission, 2020) by using Sensititre™ EUVSEC3 plates (ThermoFisher Scientific, East Grinstead, UK). The groups of tested antimicrobials were aminoglycosides (amikacin, gentamicin), penicillins (ampicillin), 3rd generation cephalosporins (cefotaxime, ceftazidime), carbapenems (meropenem), phenicols (chloramphenicol), quinolones (nalidixic acid), fluoroquinolones (ciprofloxacin), macrolides (azithromycin), polymixins (colistin), folate pathway antagonists (sulfamethoxazole, trimethoprim), tetracyclines (tetracycline) and glycylcyclines (tigecycline). Categorisation of the isolates was based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2020 breakpoints (EUCAST (European Committee on Antimicrobial Susceptibility Testing), 2020). *E. coli* ATCC 25922 was used as quality control microorganism.

2.4. Whole genome sequencing

Genomic DNA was extracted with Maxwell HT 96 gDNA Blood Isolation System (Promega, Madison Wisconsin, USA) and sequencing libraries were prepared using DNA Prep (M) Tagmentation (Illumina, San Diego, USA). Pair-end reads (2 × 150 bp and 2 × 250 bp respectively) were produced with Nextseq and Miseq platforms (Illumina, San Diego, USA), checked for quality with FastQC (Babraham, 2010) and filtered with Trimmomatic ver. 0.38 (Bolger et al., 2014). Species confirmation and possible contaminations were evaluated with Kraken2 (Wood et al., 2019). Filtered reads were assembled using Unicycler ver. 0.4.8 (Wick et al., 2017) and high-quality assemblies were evaluated by QUAST ver. 4.2 (Gurevich et al., 2013), holding assemblies with coverage >30× and contig number < 300 (Timme et al., 2020). Multi Locus Sequence Typing (MLST) was determined using the Pasteur BIGSdb for *Salmonella* spp. (https://pubmlst.org/bigsdb?db=pubmlst_salmonella_seqdef) starting from assemblies. Core-genome Multi-Locus Sequence Types (cgMLST) analysis were achieved through Bionumerics Software ver. 7.6.3 (Applied-Maths, Biomerieux) according to the Enterobase cgMLST scheme (Achtman et al., 2021; Alikhan et al., 2018) using single-linkage clustering. The cluster-defining threshold value for allelic distance (AD) was fixed at AD = 5, as proposed by the European Center for Disease Prevention and Control and the European Food Safety Agency (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2021, 2022). Antibiotic resistence genes were *in-silico* determined using Resfinder database via Staramr ver. 0.7.2 (Bharat et al., 2022; Zankari et al., 2012).

2.5. Laboratory surveillance system for salmonellosis

The One-Health laboratory surveillance system for salmonellosis in Emilia-Romagna, which is an administrative region with a population of about 4.5 million residents located in Northern Italy [15], is based on a routine system of WGS typing through cgMLST of: (*i*) all the clinical isolates recovered weekly from the regional network of hospitals; and (*ii*) all the isolates originated from the regional animal health and food safety surveillance activities, which includes testing of official samples, private diagnostic activity on animals and part of the own-check testing by food business operators. To promptly identify potential outbreaks, the surveillance system issues alert for possible outbreaks of salmonellosis to the CA when WGS clusters of isolates (of human and food/animal origin) emerge from the surveillance.

2.6. Sequence accession numbers

Raw reads of the three newly sequenced isolates of the study were deposited at EBI under Project number PRJEB61681.

3. Results

3.1. Salmonella prevalence, serotyping and WGS typing

Three out of 152 (2.0 %; 95%CI: 0.7–5.7) caecal samples tested positive for *Salmonella*. All carcass samples ($N = 152$) were negative. The faecal carriers were three horses (3/146; 2.1 %) while the donkeys ($n = 5$) and the mule were negative. The three isolates belong to the serovar Enteritidis (sequence type ST11), Stanleyville (ST97), and Typhimurium (ST19).

The phenotypic tests on antimicrobial resistance (AMR) performed on the *S. Enteritidis* and *S. Stanleyville* isolates showed that they were sensitive to all the antimicrobials tested. Conversely, the phenotypic tests performed on the *S. Typhimurium* isolate showed resistance to ampicillin, chloramphenicol, sulfamethoxazole and tetracycline (AMR profile: AmCStxT). The *in-silico* assessment of the antimicrobial resistance of the three isolates performed on the assembled genomes confirmed the results obtained phenotypically. Specifically, we did not find the presence of any AMR gene in the *S. Enteritidis* and *S. Stanleyville* isolates, while we found genes of resistance to ampicillin ($bla_{\text{CARB-2}}$), chloramphenicol (*floR*), sulfamethoxazole (*sul1*) and tetracycline (*tet* (G)) in the *S. Typhimurium* isolate.

Salmonella isolates were detected in two restricted temporal slots, i.e., in week 2 (21st June 2021) and week 10 (27th September 2021). In week 2, nine horses were tested and two (EQ-16 and EQ-18) caecal samples tested positive for *S. Enteriditis* and *S. Stanleyville*. The carrier animals came from Hungary (EQ-16; *S. Enteriditis*) and Italy (EQ-18; *S. Stanleyville*). In week 10, seven equids were tested and the positive one (EQ-85), originating from Italy, carried *S. Typhimurium*.

3.2. WGS surveillance and alert system

On 7 July 2021, the WGS-based surveillance of Emilia-Romagna signaled a potential outbreak by *S. Enteritidis* including 9 isolates (each corresponding to a different patient) across three provinces of the region (Piacenza, Parma, and Reggio-Emilia). The first isolates included in the alert dated back to 12 May 2021. In the following months the surveillance system detected further cases belonging to the genomic cluster. Overall, 17 human isolates belonging to the cluster were recovered from the Emilia-Romagna territory between 12 May and 16 August. Within the cluster, the pairwise differences between isolates ranged from zero to three alleles (median value: zero alleles), while the closest non-cluster isolate in the WGS-surveillance database at the time differed by 33 alleles from the cluster.

Furthermore, the WGS-based surveillance signaled that the *S. Enteritidis* isolate (named EQ-16 *S. Enteritidis* isolate) recovered in June 2021 within the monitoring activities at the equids slaughter belonged to the same genomic cluster of the 17 human isolates. The results of the genomic analysis are shown in Fig. 1, where the minimum spanning tree (MST) obtained from cgMLST data is displayed. In the MST, the blue nodes represent isolates from human cases, and the red node represents the EQ-16 *S. Enteritidis* isolate. The MST shows that maximum allelic distance between nodes in the cluster is 3 alleles, suggesting a high level of clonality among the human and horse isolates.

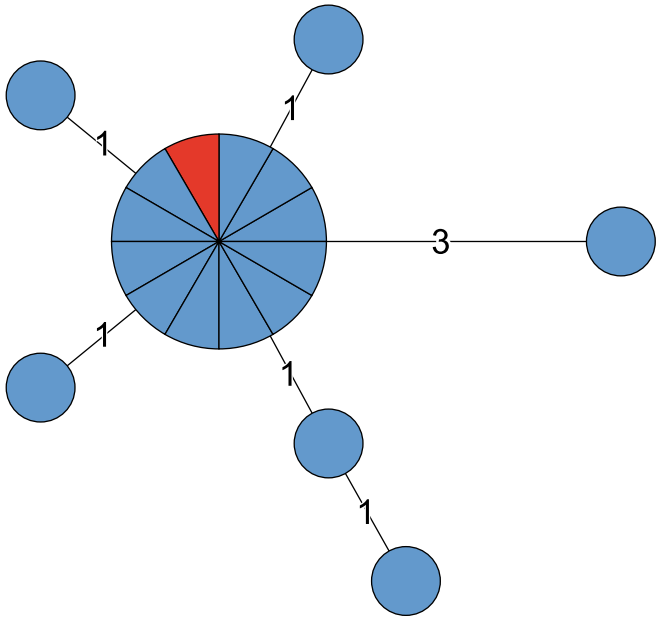

Fig. 1. Minimum Spanning Tree (MST) obtained from cgMLST data for the *S. Enteritidis* isolates belonging to the cluster. Blue nodes represent isolates from human cases, the red node represent the EQ-16 isolate from the horse at slaughter. The numeric labels on the edges represent the pairwise distances between isolates computed as number of allelic differences in cgMLST.

3.3. Epidemiological investigations

Patient interviews were available for 11 cases, 10 confirmed through WGS and 1 probable (since involved in the same household infection event with a WGS confirmed case). However, only eight of the 11 available interviews included information on food consumption in the weeks preceding the onset of the symptoms. Five of the eight cases (four confirmed and one probable) reported the consumption of uncooked horse meat: two confirmed cases purchased the horse meat at the same butchery (B1), one confirmed and one probable case were from the same household and they had purchased horse meat from a different butchery (B2), and the last confirmed case reported the consumption of horse meat in a restaurant (R1).

The trace-back activities of the CA are summarized in Fig. 2. The investigations found that the horse meat served at restaurant R1 had been purchased from butchery B1. Further investigations on the origin of the meat sold in B1 traced it back to the equids slaughter (S1). Specifically, butchery B1 purchased two different batches of meat (which included several cuts) from S1 on 4 and 7 May 2021. Also, the horse meat sold at butchery B2 originated from slaughter S1. In particular, butchery B2 bought one batch of meat from S1 on 7 June 2021 and all the meat derived from a single horse coming from France.

Following the positivity for *Salmonella* found in the caecal samples of equids, an official visit at the slaughter plant pointed out that hygiene at lairage was largely disattended. For this reason, improvement of hygiene measures both at lairage and along the slaughter line at S1 was prescribed. In addition, the S1 Food Business Operator (FBO) increased the number of carcasses routinely tested for surface contamination by *Salmonella* according to Regulation (CE) 2073/2005. Since then, no samples collected at the slaughter from the caecum and carcasses of the equids by the FBO, the competent authorities, and within this study were found positive for *S. Enteritidis* belonging to the genomic cluster associated to the outbreak.

4. Discussion

Previous studies on horses at slaughter found low prevalence of *Salmonella* or no detection at all (Mann et al., 1964; Collobert et al., 2001). Nevertheless, an outbreak of salmonellosis (*S.* Newport) linked to horse meat consumption was reported in France in 2003 (Espié et al., 2005). In this study, the prevalence of *Salmonella* caecal carriers in equids was 2.0 %, in line with the 2.9 % overall prevalence in solipeds reported by seven EU member states in 2021 and lower than those reported for cattle (3.5 %), small ruminants (9.0 %) and pigs (2.9 %) in the EU countries (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022). *Salmonella* was not found on horse carcasses, confirming the low prevalence reported in the EU, ranging from 0.19 % in official controls to 0.15 % in own-checks by FBOs (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022). These values were lower than those reported for the other food-producing animals, i.e., broilers (14.0 % in official controls - 3.2 % in FBO own-checks), turkeys (7.4 % in official controls - 3.2 % in FBO own-checks), pigs (1.7 % in official controls - 1.4 % in FBO own-checks), sheep (1.2 % in official controls - 0.49 % in FBO own-checks) and goats (0.51 % in official controls - 2.1 % in FBO own-checks) (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022).

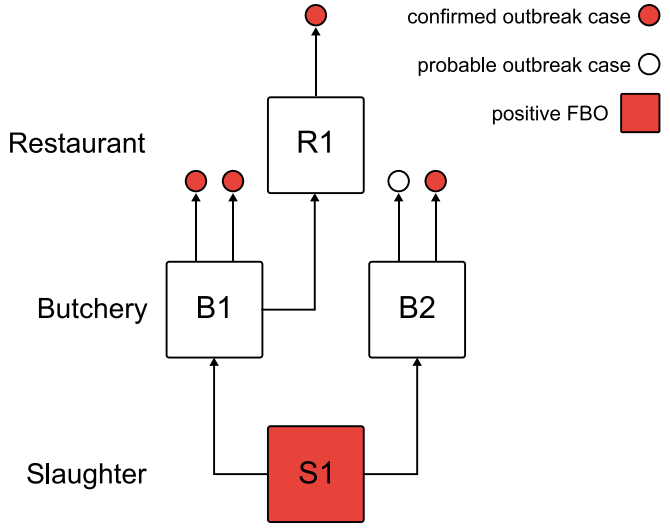

Fig. 2. Graphical representation of horse meat traceability and testing information on human cases and food business operators (FBO) involved in the outbreak investigation. Dots represented the human cases who reported the consumption of horse meat, squares represented the FBOs involved in the investigation. Red square represents the FBO positive to *S. Enteritidis*.

Despite the low prevalence on horse carcasses, this contamination represents a risk to consumers considering the consumption of raw horse meat in some areas. This is the case of some Italian regions where minced horse meat (called “pesto di cavallo”) as well as dried and smoked strips (called “sfilacci di cavallo”) consumed raw represent traditional plates. In particular, the raw minced horse meat plate called “pesto di cavallo” is commonly eaten in the area of the outbreak. Indeed, the risk turned into actual damage in the timeframe of our study when a prolonged outbreak of human salmonellosis occurred and it was eventually traced to different batches of horse meat released from the same slaughterhouse weeks apart from each other. The serovar involved was *S. Enteritidis*, unexpected from solipeds, usually associated with table eggs and poultry meat instead (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2022; Havelaar et al., 2013). Based on the epidemiological and traceability investigations, as well as on the detection of the same clone of *S. Enteritidis* in a horse slaughtered in the plant that released the meat eaten by some of the patients, our report suggests a strong association between *S. Enteritidis* of horse origin and the disease in human cases. Although uncommon, this pathogen-source combination in an outbreak deserves attention from the sanitary perspective for some reasons. Firstly, during investigation of salmonellosis cases in humans due to *S. Enteritidis*, it warns not to forget horses/horse meat as a possible source of this serovar. Secondly, it reminds that, although rarely, raw horse meat can transmit highly pathogenic serovars like Enteritidis, therefore, the risk posed by this food could need a review.

The outbreak reported in the study was identified by the standard regional surveillance for human salmonellosis based on WGS typing. The source of the outbreak was traced to horse meat, in particular to the slaughterhouse of the study, through three types of evidence, (i) genomic matching of the human cases with the isolate from a positive horse, (ii) some of the food histories of the cases who reported consumption of the horse meat, and (iii) the tracing back of the meat eaten by the cases. The generated triple evidence strongly indicates the slaughterhouse as the source of the outbreak, although the outbreak strain was not detected on any of the sampled carcasses, but only in the caecal content of a single animal. In this case, the alert for a possible outbreak was issued by the genomic surveillance system on the basis of the genetic similarity among the *S. Enteritidis* isolates in human cases and the horse at slaughter. Given this initial evidence, the traceability investigation intended to link the food consumed by the patients to the contaminated site in the food chain (in this case the equid slaughter) represents the route of investigation providing the strongest evidence on the source of infection. This is especially true in outbreaks widespread in space and time with a limited number of cases (as in the study context), where the statistical power of classical epidemiological tools, such as case-control studies, is very limited due to the small number of cases, the uncertainties of their food consumption histories and the difficulty to

find suitable controls for the analyses.

Another inconsistency seems to be that the horse meat associated with cases and traced back to the slaughterhouse was produced on 4 and 7 May 2021 and on 7 June 2021 while the horse carrying the outbreak strain of *S. Enteritidis* was slaughtered weeks later, on 21 June 2021. Evidently, the meat from that animal could not have infected those cases. Consequently, we hypothesize that the outbreak strain of *Salmonella* was already present in the slaughterhouse environment before the arrival of the horse from Hungary and that its environmental persistence could have caused the contamination of the horse. In support of this hypothesis there is also the finding that the last batch of horse meat associated with human cases was derived from a horse coming from France and slaughtered two weeks before the Hungarian animal, on 7 June 2021. It appears extremely unlikely that two horses originating from different countries and slaughtered two weeks apart from each other carried the same WGS type of *Salmonella*. Conversely, it seems plausible that both animals and or their meat got contaminated from the slaughterhouse environment. That environment could have been contaminated long before June and the contamination could have involved the meat produced in early May. The long lasting lairage period of the equids at the slaughterhouse, where they arrived daily but were slaughtered only twice a week, supports this hypothesis, in particular considering that the lairage area was completely inadequate to host animals because of abundant manure on the floor, dirty troughs and presence of pests. Notably, *S. Enteritidis* is known for its ability to persist in environments (Marin et al., 2022).

Therefore, a key lesson from the study concerns the need to maintain a high level of hygiene during the entire slaughtering process, which does not start with stunning and exsanguinating of the animals. Indeed, hygiene at lairage is crucial to avoid cross-contamination between animals and the meat thereof. For this reason, lairage conditions should be critically evaluated and biosecurity measures must be properly implemented. In dirty and overcrowded pens, *Salmonella* may rapidly colonize the gut of horses without clinical signs of infection prior to slaughter (Burgess, 2023) and no possibility to recognize the infection at the antemortem inspection by the official veterinarians is given. Possible accidental rupture of the gut during evisceration can cause the contamination of carcasses and equipment by *Salmonella*, enhancing cross-contamination during meat processing.

With regard to the other two serovars identified in horse caecal contents in the present study, *S. Typhimurium* is the most common in horses presenting clinical salmonellosis (Arnold et al., 2021; van Duijkeren et al., 1994, 1995, 2002; Morse et al., 1976; Smith et al., 1978; Vo et al., 2007), while detection of *S. Stanleyville* from horses is a very rare event (Animal and Plant Health Agency, 2022). The multi-drug resistance profile of the *S. Typhimurium* isolate from our study (AmCStxT) is consistent with the diffusion of AMR in *S. Typhimurium* strains of equine origin (Soza-Ossandón et al., 2020). Besides the investigation on the *S. Enteritidis* outbreak strain, data on the occurrence of both common and rare *Salmonella* serovars in horses at slaughter are of concern and highlight the need of monitoring the horse meat supply chain.

Ethical statement

The planning, conduction, and reporting of the study was in line with the Declaration of Helsinki, as revised in 2013. Ethical review and approval and patient consent were waived for this study as it was carried out as part of the salmonellosis surveillance, performed by law in accordance with the Ministry of Health's Decree of 7 March 2022 (https://www.gazzettaufficiale.it/eli/id/2022/04/07/22A02179/sg accessed on 26 June 2023).

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Declaration of competing interest

None.

Data availability

Raw reads of the sequenced isolates of the study were deposited at EBI under Project number PRJEB61681.

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