Occurrence and distribution of *Salmonella* serovars associated with human infection isolated from irrigation waters and food-producing animals in southern Italy: eleven-year monitoring (2011-2021)

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Abstract

Salmonella is one of the main zoonotic agents causing food-borne diseases in Europe. The main reservoirs of the infection are represented by domestic and wild animals, and the infection occurs by direct contact or following the consumption of contaminated food or water. The study aimed to evaluate the presence of *Salmonella* spp. in food-producing animals and irrigation waters in southern Italy and the serovar distribution. From 2011 to 2021, a total of 473 samples from 6 different animal species (bovine, buffalo, goat, ovine, swine, poultry, and wild boars) and 313 irrigation water samples were collected and analyzed. The overall percentage of positive samples was 56.87% in organs, 50.85% in feces, and 20.45% in irrigation waters. By animal species, the most frequently detected serovar was *Salmonella* Typhimurium in bovine (17.39%), in buffalo (13.10%) and swine (28.21%), and *S.* Kentucky (24.78%) in poultry. The subspecies *diarizonaeIIIb* was frequently detected in goats (40.00%) and ovine (83.33%), while *salamaeII* (14.12%) and *diarizonae*IIIb (11.76%) were frequently isolated in wild boars. In the irrigation water samples, the most frequently detected serovar was *S.* Napoli (25%). Results revealed that, although in Europe, control strategies aimed at preventing the spread of *Salmonella* have been implemented, the prevalence of this pathogen in food-producing animals and irrigation waters is high. Considering the risk to public health associated with the contamination of products or foods, more stringent control interventions are needed at primary production and along the food chain.

Introduction

Salmonella spp. are Gram-negative bacteria belonging to the family of *Enterobacteriaceae* and are comprised of 2 species (*S. enterica* and *S. bongori*). 6 subspecies belong to the species *enterica*: *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *enterica*, *S. enterica* subsp. *houtenae*, *S. enterica* subsp. *indica* and *S. enterica* subsp. *salamae*. The subspecies *enterica* includes more than 2600 serotypes, of which 99% are associated with animal and human infections (Ferrari *et al.*, 2019). *Salmonella* can also be divided into typhoid serovars (*e.g.*, Paratyphi A, B, and C), presenting only a limited range of hosts as reservoirs, and non-typhoid serovars (*e.g.*, *S.* Typhimurium), which are capable of triggering infections in both humans and in a broad range of animals, such as livestock, birds, pets, cold-blooded animals, and wild fauna (La Tela *et al.*, 2021). In animals, *Salmonella* colonizes the intestinal tract, and depending on the pathogen and host factors, the infection can be asymptomatic or involve invasive disease (Stevens and Kingsley, 2021). Healthy or symptomatic animals can shed the pathogen through their feces and contaminate the water, foodstuffs, and environment, surviving for a long period of time (Tegegne, 2019). *Salmonella* may reach humans following direct contact with infected animals or through the consumption of contaminated food and water (Aung *et al.*, 2020). Moreover, water can be a source of microbial contamination of fresh produce and a vehicle for *Salmonella* transmission.

Indeed, in the latest years, the number of foodborne outbreaks due to the consumption of food of non-animal origin, such as fresh fruits and vegetables, has been increasing (Liu *et al*., 2018).

Infection in humans is generally self-limited and usually does not require specific treatment, but in some cases, the infection can be more serious, and the use of antimicrobial agents is essential (Peruzy *et al*., 2020). According to the most recent report of the World Health Organization in 2010, *Salmonella* was regarded as one of the most common agents of foodborne illness, causing over 93.8 million gastrointestinal illnesses per year in the world (Gong *et al*., 2022).

In the European Union in 2021, salmonellosis, mainly due to *S. enterica,* was the second most commonly reported bacterial foodborne zoonose, with 60,050 confirmed cases, and was an important cause of foodborne outbreaks mainly associated with the consumption of eggs and egg products, mixed food, bakery products, pig meat and products thereof, and vegetables (EFSA and ECDC, 2022). The top 5 *Salmonella* serovars involved in human infections were *S.* Enteritidis, *S.*Typhimurium, monophasic *S.* Typhimurium (1,4, [5],12:i:-), *S.* Infantis, and *S.* Derby.

The worldwide epidemiology of *Salmonella* serovars is complex in terms of its distribution and transmission (Ferrari *et al*., 2019), and it is important to monitor the possible contamination routes for humans. At present, long-term studies on the occurrence and serovar distribution of *Salmonella* in food-producing animals and irrigation waters are limited in Europe.

The collection of updated data is essential for the development of regional and serovar-specific intervention and control programs. For this reason, the present work aimed to evaluate the prevalence of *Salmonella* in food-producing animals and irrigation waters and the serovar distribution among the different sources.

Materials and Methods

Sampling

From 2011 to 2021, a total of 473 pooled organs (spleen, liver, and diaphragm pooled in one sample) and 293 feces of food-producing animals (bovine, buffalo, goat, ovine, swine, poultry, and wild boars) from, respectively, 473 and 293 different animals and 313 irrigation water samples were collected in Campania and Calabria regions in southern Italy and analyzed to detect *Salmonella* spp. (Table 1). In particular, except for wild boars, organ and feces samples were collected from the animals at the slaughterhouse and on the farm, respectively. Wild boar samples were collected at different hunters' private houses during the hunting seasons (October-December). Water samples were collected following ISO 19458 (2006) from the irrigation ponds and wells on different farms. Samples were transported at 4°C to the laboratory and processed within 1 hour after sampling.

Detection and serotyping analysis

Salmonella was detected and isolated in animal and irrigation water samples by using the corresponding normalized microbiological methods, ISO 6579-1 (2017) and ISO 19250 (2010).

Afterward, the isolates were serotyped at the Campania Region *Salmonella* Typing Center (Department of Food Microbiology, *Istituto Zooprofilattico Sperimentale del Mezzogiorno*, Portici, NA, Italy) following the Kaufmann-White scheme.

Statistical analysis

The differences in the occurrence of *Salmonella* spp. in the different sources were assessed using the chi-square test. A probability value of less than 0.05 (p<0.05) was defined as statistically significant.

Results

Prevalence of *Salmonella* spp. in different matrices

A total of 1079 samples were analyzed for *Salmonella* spp. from 2011 to 2021. Regardless of the nature of the sample, the overall percentage of positive samples was 44.67% (n=482) (Table 1). By source, the highest percentage of *Salmonella*-positive samples among food-producing animals was reported in the organs (n=269/473, 56.87%). The highest percentage of positive samples was reported in the following species: buffalo (organs: 93.75%; feces: 96.15%), poultry (organs: 86.67%; feces: 78.05%), and swine (organs: 76.77%; feces: 53.33%).

The difference in the isolation of *Salmonella* from organs and feces of bovine origin to all other sources was significant (p<0.05), as was the comparison between pathogen presence in the organs of wild boars and all the other species (p<0.05), except for goats (p>0.05). However, in this latter case, the number of analyzed samples was extremely low. Moreover, concerning the *Salmonella* prevalence in feces, significant differences were found between buffalo, swine, and poultry (p<0.05).

Serovars distribution between different matrices

Strains isolated from food-producing animals belonged mainly to the species *S. enterica* (417/418, 99.76%) and the subspecies: *enterica* (n=373, 89.45%), *diarizonae* (n=23, 5.52%), *salamae* (n=16, 3.84%), *houtenae* (n=3, 0.72%) and *arizonae* (n=2, 0.48 %) (Table 2). Only one strain was assigned to the species *S. bongori* (Table 2). Among the subspecies *enterica*, serotyping identified a total of 62 serovars. No information regarding the serovar was

Table 1. Number of samples collected and number and percentage of *Salmonella*-positive samples grouped by source. Values in columns bearing different lowercase letters are significantly different (p<0.05).

Matrix	Number	Positive number (%)	
Organs	473	269	56.87
Bovine[a]	78	17	21.79
Buffalo[b]	128	120	93.75
Goat[bd]	3	3	100.00
Ovine[b]	11	11	100.00
Swine[b]	30	23	76.67
Poultry[b]	15	13	86.67
Wild boar[cd]	208	82	39.42
Feces	293	149	50.85
Bovine[a]	108	6	5.56
Buffalo[b]	26	25	96.15
Goat[bdf]	2	2	100.00
Ovine[bef]	1	1	100.00
Swine[f]	30	16	53.33
Poultry[cde]	123	96	78.05
Wild boar[edef]	3	3	100.00
Water	313	64	20.45
Total	1079	482	44.67

Table 2. The number of Salmonella species, subspecies, and serovars isolated from 2011 to 2021 from irrigation water, organs, and feces by animal species.

Species	Subspecies	Serovar	Bovine O	Bovine F	Buffalo O	Buffalo F	Goat O	Goat F	Ovine O	Ovine F	Pork O	Pork F	Poultry O	Poultry F	Wild Boar O	Wild Boar F	I.W.
S. enterica	enterica I	Agbeni	1														
		Agona			5	1								3			2
		Albany												1			
		Altona			4												
		Anatum		1	3	2											
		Apeyeme												1			
		Ball													5		
		Bardo			3												
		Blockley												1			
		Bovismorbificans		1	3	1								1			
		Brandenburg			2			1			1			1			
		Bredeney			1							1		1			
		Carno			3	3											
		Cerro												2			
		Coeln			1									2	3		
		Corvallis												1			
		Cremieu			2												
		Dabou												1			
		Derby	3			1					2				4		2
		Duesseldorf													1		
		Enteritidis			2									6	1		
		Essen												1			
		Ferruch			1												
		Fischerhuette												1	4		2
		Give			8							1			5		
		Goldcoast			3												
		Hadar												1			8
		Havana											1				
		Infantis			1						1		8	3			
		Johannesburg			1												
		Kapemba															2
		Kasenyi		1	1	1									8		2
		Kedougou												1			
		Kentucky												27			
		Kottbus			4										1		2
		Lexington												1			
		Livingstone			1								2	11			2
		London			6	1					2	1		1			
		Manhattan					1								2	1	2
		Mbandaka												5			
		Messina													1		
		Mishmarhaemek												2			
		Monophasic S. Typhimurium			9		2			1	3			1			
		Montevideo												1			
		Muenchen	1		11	2						1		1			
		Muenster			5	1											
		Napoli			1			1						2	8	1	12
		Newport			1										2		
		Oranienburg			2												
		Orlando												1			
		Reading															2
		Rissen	3			1					4						2
		Senftenberg												2			
		Stanley			2												
		Stanleyville			4	3									1		
		Szentes				1											2
		Thompson			1									2	2		
		Typhi (bioser)										1					
		Typhimurium	3	1	16	3					5	6	1	4	3	1	4
		Umbilo			4										2		
		Veneziana													2		
		Vuadens			2												
		Waycross			1												
		Winchester												1			
		Worthington															2
		N.I.	5	2	6	2					7		1	4	2		10
	salamae II													2	2		
	arizonae IIIa														12		
	diarizonae IIIb		1			2			9						10		3
	houtenae IV								2						1		
S. bongori															1		3
Total			17	6	120	25	3	2	11	1	23	16	13	96	82	3	64

I.W, irrigation water; O, organs; F, feces; N.I., no information on the serovars.

recorded for 29 isolates (Table 2). *S.* Typhimurium was the most frequently detected (n=43, 10.29%) followed by *S.* Kentucky (n=27, 6.46%). By animal species, the most frequently detected serovars were *S.* Typhimurium (17.39%), *S.* Derby and *S.* Rissen (13.04% both) in bovine, *S.* Typhimurium (13.10%), *S.* Muenchen (8. 97%) and Monophasic *S.* Typhimurium (7.59%) in buffalo, *S.* Typhimurium (28.21%), Monophasic *S.* Typhimurium and *S.* Rissen (10.26% both) in swine; *S.* Kentucky (24.78%), *S.* Livingstone (11.93%) and *S.* Infantis (10.09%) in poultry. Moreover, the subspecies *diarizonaeIIIb* was frequently detected in goats (40.00%) and ovine (83.33%), while *salamaeII* (14.12%) and *diarizonaeIIIb* (11.76%) were frequently isolated in wild boars (Table 2). In the latter, *S.* Napoli (10.59%) was also frequently detected. Among the identified subspecies *enterica, S.* Napoli (25%) was also the most frequently detected serovar in the irrigation water samples, followed by *S.* Hadar (16.67%) and *S.* Typhimurium (8.33%).

Discussion

To our knowledge, this is the first study that provides a complete overview of the occurrence of *Salmonella* spp. in food-producing animals and irrigation waters between 2011 and 2021 in Italy.

The highest percentage of positive samples among animal sources was reported for buffalo (organs: 93.75%; feces: 96.15%), poultry (organs: 86.67%; feces: 78.05%), and swine (organs: 76.77%; feces: 53.33%). In buffalo, ingestion is the main route of infection, and the disease in these animals is characterized by severe gastrointestinal lesions, profuse diarrhea, severe dehydration, and death (D'angelo *et al.*, 2022). There are few studies about the prevalence of *Salmonella* in buffaloes. The occurrence of this pathogen reported in the present study was higher than that of Silva *et al*. (2018) (6.90%) and Sychanh *et al.* (2013) (6.69%). Results are of particular concern since the demand for buffalo meat is increasing due to its nutritive value (Abd-Elghany *et al*., 2022), and the presence of *Salmonella* in it due to improper evisceration can be a risk to public health.

In poultry, the occurrence of this pathogen in the present study was higher than the European average (2.5 %) (EFSA and ECDC, 2022) and than those recorded in other studies conducted outside the European Union: Iran=7.2% (Farahani *et al*., 2022), Burkina Faso=52.42% (Kagambèga *et al*., 2018). At present, most human cases of salmonellosis are attributed to poultry products that may become contaminated during the entire production chain, from the farm to retail. Monitoring the occurrence of *Salmonella* in poultry is the first step in the control of this zoonosis.

A high prevalence of *Salmonella*-positive samples was found in swine and was higher than those reported in other studies conducted in Europe from 2000 to 2020 (Roasto *et al.*, 2023). *Salmonella* in swine can cause clinical disease and frequently contaminate the meat, which, if not properly cooked, can represent a risk for humans (D'Incau *et al*., 2021).

The overall prevalence of *Salmonella* carriers in wild boars was lower than that reported in another study conducted in Italy (46.7%) (Piras *et al*., 2021) but was higher than that of a study conducted in Spain (fecal sample=2.9 %; lymph nodes=5.1%) (Gil Molino *et al.*, 2019). The presence of *salmonella* in these animals is of particular concern because they may excrete the pathogen through their feces, spreading the pathogen into the environment. Moreover, if the evisceration is not properly performed, contami-

nation of wild boar carcasses may occur. Although the consumption of wild boars is still lower than that of other domestic animals, it is increasing worldwide (La Tela *et al*., 2021).

The occurrence of *Salmonella* in the irrigation water analyzed was 20.45%. Results are in contrast with those of Nguyen *et al*. (2021) and Burjaq and Abu-Romman (2020), who reported a prevalence of 57.1% and 32.2%, respectively, and with those of Falardeau *et al*. (2017), who reported a low occurrence of this pathogen in this matrix (2.7%).

In the present study, *S. enterica* subsp. *enterica,* followed by 4 *non-enterica* subspecies (*diarizonae*, *salamae, houtenae,* and *arizonae),* were found. The highest percentage of *non-enterica* subspecies was found in ovines (100%), goats (40%), and wild boars (29.41%). For ovine, although the number of samples was limited, the results of the present work are in line with those of Methner and Moog (2018), who detected *S*. *diarizonae* in 82.2% of their samples. Wild boar results are in line with those of La Tela *et al*. (2021), Peruzy *et al.* (2019), and Peruzy *et al*. (2022), in which all *Salmonella* strains isolated from wild boar killed in the Campania region belonged to the subspecies *enterica.*

During the period under investigation (2011-2021), serotyping identified 62 serovars, and *S.* Typhimurium and *S.* Kentucky were the most frequently detected.

In bovine and buffalo, the most frequently detected serovar was *S.* Typhimurium. *S.* Typhimurium, along with *S.* Enteritidis, is a generalist serovar capable of triggering infections in both humans and a wide range of animals (Ferrari *et al*., 2019). Bovine results are not surprising since *S. typhimurium* is the most prevalent serovar in Europe and Africa (Ferrari *et al*., 2019). For buffalo, results are in line with those reported in a previous Italian study (Borriello *et al.*, 2012), but in contrast with Abd-Elghany *et al*. (2022) in Egypt, where the most prevalent serovar was *S.* Enteritidis. Moreover, in line with the study of Borriello *et al*. (2012), *S.* Muenchen was also frequently detected in buffalo. In our earlier research conducted in the same sampling area, *S.* Typhimurium was the most frequently reported serovar also on buffalo carcasses, while on bovine carcasses, monophasic *S.* Typhimurium was the most frequently found (Peruzy *et al*., 2022).

In swine, the most detected serovars were *S.* Typhimurium, monophasic *S.* Typhimurium, and *S.* Rissen. These serovars are frequently detected in the pig production chain in Italy (Bonardi *et al*., 2016; Chalias *et al*., 2022; Peruzy *et al*., 2022). Pigs are asymptomatic carrier disseminators of this pathogen throughout the production chain (Ferrari *et al*., 2019). Surprisingly, in the present work, *S.* Derby was detected at a very low level; this serovar is known to be associated with pigs and, in recent years, has been increasingly isolated in human cases in Europe (EFSA and ECDC, 2022). Concerning *S.* Rissen, although not frequently isolated in the case of salmonellosis in Europe, it is commonly associated with human infections in the United States of America and Asia (Silveira *et al*., 2019).

Poultry and poultry products are considered important reservoirs and sources of human salmonellosis. In the present study, *S.* Kentucky, *S.* Livingstone, and *S.* Infantis were frequently detected. These serovars are commonly reported in this source in Europe (EFSA and ECDC, 2022), but while *S.* Kentucky and *S.* Livingstone are not commonly associated with human infections, in recent years, *S.* Infantis has become a relevant agent of human salmonellosis (Montone *et al*., 2023). Interestingly, *S.* Enteritidis was isolated at a low level from these animals. This result is in contrast with those reported by several studies, which confirm that this source is strictly related to *S.* Enteritidis (EFSA and ECDC, 2022).

However, the results of the present work reflect those reported in our earlier research (Peruzy *et al*., 2022), in which *S.* Enteritidis was never isolated from broiler sources.

In the irrigation waters, the most frequently reported serovar was *S.* Napoli, which is among the top serovars causing human infections in Italy, even though it is relatively uncommon in other European countries (Leati *et al*., 2021). Irrigation water may contribute to the survival of *Salmonella* in vegetables (Cristiano *et al.*, 2021). Indeed, over the years in Italy, cases of salmonellosis have also been linked to the consumption of fresh vegetables, with *S.* Napoli implicated (Giner-Lamia *et al*., 2019).

Conclusions

A high occurrence of *Salmonella* spp. in food-producing animals and irrigation water was found. Despite the implementation of different strategies against *Salmonella*, the results of the present work revealed that the prevalence in food-producing animals and waters of *Salmonella* serovars implicated in human infection in southern Italy is high. In the present work, *S.* Typhimurium was the most frequently isolated serovar.

S. Typhimurium, along with other serovars isolated in the present work, is among the "top 20" serovars responsible for confirmed cases of salmonellosis in Europe. Considering the risk to public health associated with the contamination of products or foods, more stringent control interventions are needed at primary production and along the food chain.

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