Preprints.org

Communication

Food Safety Monitoring of *Salmonella* spp. in Northern Italy 2019-2021

Daniela Manila Bianchi [*] , Paola Barzanti , Daniela Adriano , Francesca Martucci , Monica Pitti , Carla Ferraris , Irene Floris , Roberta La Brasca , Carmela Ligotti , Sara Morello , Giulia Scardino , Noemi Musolino , Clara Tramuta , Cristiana Maurella , Lucia Decastelli

Communication

Food safety monitoring of *Salmonella* spp. in northern Italy 2019-2021

Daniela Manila Bianchi¹*, Paola Barzanti², Daniela Adriano¹,³, Francesca Martucci¹, Monica Pitti¹,³, Carla Ferraris¹, Irene Floris¹, Roberta Labrasca¹, Carmela Ligotti¹, Sara Morello¹, Giulia Scardino¹, Noemi Musolino¹, Clara Tramuta¹, Cristiana Maurella², Lucia Decastelli¹,³

1   SC Sicurezza e Qualità degli Alimenti - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy;

2   SC Epidemiologia e Analisi del Rischio - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Torino, Italy;

3   Centro di Riferimento per la Tipizzazione delle Salmonelle, CeRTiS, Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Via Bologna, 148, 10154 Turin, Italy

*   Correspondence: manila.bianchi@izsto.it; Tel.: +39 0112686465; Via Bologna 148, 10154 Torino, Italy;

Abstract: Salmonella is the second most frequent bacterial pathogen involved in human gastrointestinal outbreaks in the European Union; it can enter the food production chain from animal or environmental sources or from asymptomatic food operators. European food legislation has established microbiological criteria to ensure consumer protection. *Salmonella* is listed under both process hygiene criteria and food safety criteria. Each Member State designates an agency to organize or perform controls and other official activities. This paper describes the official control plans performed by competent authorities in northern Italy in the three-year period 2019-2021. A total of 4413 food samples were delivered to the IZS Food Safety laboratories for *Salmonella* detection and 36 (0.8 %) tested positive. *Salmonella* was most frequently detected in poultry meat samples (25/36 positive samples) followed by other meat products and pork products. The official controls for the protection of consumer health apply the EU's from farm-to-fork approach: the samples were collected during production (food production plants) and on the market and from collective catering (restaurants, cafeterias, canteens). This manuscript will provide information about the presence of *Salmonella* in foodstuffs and can help competent authorities to set control plans based on a risk assessment.

Keywords: *Salmonella* spp; food safety; food official control

1. Introduction

Salmonella is a gram-negative, facultative anaerobe bacterium that inhabits the intestines of humans and animals [1]. Wild birds can be reservoirs as can domestic animals [2]. *Salmonella* can also be found in water, the environment, and contaminated food products [1].

The genus *Salmonella* is divided into two species: *S. enterica* and S. *bongori*. *S. enterica* has six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) [3]. More than 2600 serovars of *S. enterica* are known [1]. *Salmonella* can grow in a solution of 0.4-4% sodium chloride and within temperatures from 5 to 47° C (optimum range, 32-35° C), is sensitive to heat (70° C), and can grow in a pH from 4 to 9 (optimum range, 6.5-7.5 pH). While it can survive in dried food products, optimum water activity is between 0.99 and 0.94. *Salmonella* spp. is inhibited at pH <3.8, temperature <7° C, and water activity below 0.94 [1].

Salmonella is the second most frequent bacterial pathogen involved in human gastrointestinal outbreaks in the European Union, particularly *S.* Enteritidis and *S.* . Typhimurium [4]. According to the European Food Safety Agency (EFSA) [5], salmonellosis was the second most often reported zoonosis in Europe in 2020 and the most frequently reported causative agent of foodborne outbreaks.

Salmonellosis is characterized by gastroenteritis accompanied by nausea, vomiting, abdominal cramps, bloody diarrhea, headache, feverish conditions, and myalgia. Infants and the elderly are at greater risk of dehydration. While generally a self-limiting illness, salmonellosis-related deaths have been recorded in the very young, the elderly, and the immunocompromised [6].

Salmonella strains can enter the food production chain from animal or environmental sources or from asymptomatic food operators. According to the annual report on zoonoses in the European Union (EU) published by the EFSA and the European Center for Disease Prevention and Control (ECDC), most of the foodborne outbreaks in 2021 (733, 19.3% of the total) were caused by *Salmonella*, mainly after the consumption of eggs and egg products, bakery products, meats and products thereof [7]. Pork meat is frequently associated with cases of salmonellosis [8]. Furthermore, *Salmonella* serovars involved in human infection were *S.* Enteritidis (54.6%), *S.* Typhimurium (11.4%), monophasic *S.* Typhimurium (8.8%), *S.* Infantis (2.0%), and *S.* Derby (0.93%) [7].

Salmonella enterica, for example, is responsible for 1.4 million cases of foodborne salmonellosis annually in the United States alone. Infection can occur after eating undercooked meat, poultry and eggs, and contaminated ready-to-eat products [9]. Various kinds of food can be contaminated by *Salmonella*. In 2022, a foodborne outbreak caused by multidrug-resistant monophasic *S.* Typhimurium linked to chocolate products [10] involved 150 cases reported across ten European countries and predominantly affected young children [11]. Another salmonellosis outbreak in Europe was linked to sesame-based products imported from Syria [12].

European food legislation has established microbiological criteria to ensure consumer protection. *Salmonella* is listed under both process hygiene criteria (indicating acceptable functioning of food production) and food safety criteria, which define the acceptability of a single or a batch of food products entering the market [13]. As concerns food safety criteria, European Commission regulation EC 2073/2005 requires the absence of *Salmonella* in 25 g or mL in five sampling units of a wide range of foods: meat, milk and milk products, eggs and egg products, live bivalve mollusks and live echinoderms, tunicates and gastropods, and ready-to-eat foods. The absence of *Salmonella* in food production areas tested under process hygiene criteria is determined on the carcasses of cattle, sheep, goats, horses and pigs; the absence in 25 g of a pooled sample of neck skin is checked in poultry carcasses of broilers and turkeys.

According to Regulation (EU) 625/2017 [14] each Member State designates an agency to organize or perform controls and other official activities. In Italy, food safety agencies designated by the Ministry of Health, the regional health departments, and the local health services are mandated to set and carry out annual regional food safety plans [15]. Based on risk assessment studies, the plans state the number of samples to be collected and analyzed and by what criteria, the type of food matrix to be investigated, and the point in the food chain to be tested (under the responsibility of food business operators or on the market).

Food safety agencies can make use of official laboratories for accredited analysis of food samples. In Italy, the Istituti Zooprofilattici Sperimentali (IZS) form the national network of laboratories that provide scientific support and chemical and microbiological analyses for food safety agencies and other control bodies.

This article describes the work of the food safety laboratory of the IZS, Turin, and reports the results of *Salmonella* detection analysis of food products collected during official monitoring of the food chain of animal and vegetable origin for the three-year period   2019-2021.

2. Materials and Methods

2.1. Sampling

Food samples were collected in the context of official food safety monitoring by local health services (Piedmont, Liguria,, Valle d'Aosta). All samples were collected using sterile instruments according to instructions for obtaining samples and for transport to the laboratory, as described in international standard protocols [16]. The food samples were delivered to the IZS laboratories at the

temperature reported on the label by food business operators for correct conservation of foodstuff and analyzed within 24 hours after arrival at the lab.

2.2. Rapid Screening Methods

Detection of *Salmonella* spp. was performed by rapid screening methods validated according to ISO 16140: 2021 [17] and accredited (Table 1).

Table 1. Rapid and alternative methods validated and accredited for *Salmonella* spp. detection in food.

Method	Approach	Manufacturer Certification
ELFA	Immunoenzymatic	VIDAS-AFNOR BIO 12/32-10/11
PCR	Molecular biology	Bio-Rad -IQ Check Prep -AFNOR BRD 07/6-07/04[1]
		Applied Biosystem -AFNOR ABI 29/01-09/07

2.3. Microbiological Methods

Isolation and identification of *Salmonella* spp. was performed according to ISO6579 [18] using medium described in international standard protocols (Table 2).

Table 2. Culture media and incubation time and temperature for *Salmonella* spp. isolation .

	Medium	Incubation
BPW	Buffered peptone water	18 ± 2 h; $37 \pm 1°$ C
RVS	Rappaport-Vassiliadis soja broth	24 ± 3 h; $41.5 \pm 1°$ C
MKTTn	Muller-Kauffmann tetrathionate novobiocin	24 ± 3 h; $37 \pm 1°$ C
XLD	Xylose lysinedesoxycholate agar	24 ± 3 h; $37 \pm 1°$ C
BGA	Brilliant green agar	24 ± 3 h; $37 \pm 1°$ C

Five suspected colonies from each Petri dish were streaked on Columbia blood agar (CBA) (Becton&Dickinson, Franklin Lakes, NJ, USA) and incubated at 35° C for 24±2 h. One isolated colony from each CBA plate was analyzed with a MALDI Biotyper® Sirius System mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) by the extended direct transfer (eDT) procedure. One loop of biomass was transferred to a MBT96 polished steel BC target plate (Bruker Daltonik GmbH) spot. The air-dried sample spot was overlaid with 1 µL of a formic acid water solution (70% v/v) and, after air-drying, with 1 µL of matrix HCCA (α-cyano-4-hydroxycinnamic acid) solution (Bruker Daltonik GmbH). Target plates of the samples were analyzed and spectra were acquired in positive ion mode in the m/z range 2000–20000. As an alternative, one isolated colony from each CBA plate was streaked on triple sugar iron agar (TSI) (Biolife Italiana S.r.l., Milan, Italy). Positive TSI colonies were confirmed by biochemical analysis using API 20E galleries (bioMérieux, Marcy l'Étoile, France).

2.4. Serotyping

The strains confirmed *S. enterica* were subcultured on Columbia blood agar (Becton&Dickinson) at 37° C for 24 h and then serotyped according to the Kaufmann-White and Le Minor scheme [19] using O and H antisera (Statens Serum Institut, Artillerivej, Denmark).

2.5. Statistical Analysis

After cleaning and preparing the data, descriptive statistical analysis was conducted to obtain information about the distribution of *Salmonella* detection analyses in food matrices, criteria, and sampling sites monitored during the three-year period and to investigate for a possible seasonal effect. Prevalence and confidence intervals were calculated using Stata Special Edition, release 17.1 (StataCorp, College Station, TX, USA).

3. Results

3.1. Samples Delivered to the Laboratories

A total of 4413 food samples were delivered to the IZS Food Safety laboratories for *Salmonella* detection during the three-year period 2019-2021. The main sampling plans were: 1) official monitoring of national food products (n=4222); 2) official monitoring of imported/exported food products (n=126); 3) official monitoring of imported/exported food products after previous reports of non-compliance (n=65).

In 2019, 1548 samples were collected and analyzed, 1404 in 2020 and 1461 in 2021. Table x presents the number of samples collected by year and region: Piedmont, 1222 in 2019, 1080 in 2020, 1152 in 2021; Liguria, 302 in 2019, 304 in 2020, 279 in 2021; Valle d'Aosta, 24 in 2019, 20 in 2020, 30 in 2021 (Table 3).

Table 3. Number of food samples collected by year and region.

	2019	**2020**	**2021**	**Total**
Piedmont	1222	1080	1152	**3454**
Liguria	302	304	279	**885**
Valle d'Aosta	24	20	30	**74**
Total	**1548**	**1404**	**1461**	**4413**

The entire food production and serving chain undergoes official monitoring by the competent agency. Sampling is conducted on foodstuff still under the responsibility of the food business operator (in production plants) or on foodstuffs for sale on the market or at collective serving (Table 4).

Table 4. Number of samples collected per year at food production plants and serving food chains.

	2019	**2020**	**2021**	**Total**
Market	1192	1064	1072	3328
Food production	341	317	372	1.030
Food serving/catering	15	23	17	55
Total	**1548**	**1404**	**1461**	**4413**

Food matrices were collected according to the regional plans for food safety based on risk assessment studies of food categories (Table 5).

Table 5. Number of samples collected by year and food category.

Food matrix	**2019**	**2020**	**2021**	**Total**
Non-poultry meat	427	373	421	1221
Dairy products	299	247	258	804
Fish and fish products	185	247	183	615
Vegetable and fruits	128	108	111	347
Meat products	118	89	127	334
Mixed food	96	68	92	256
Poultry meat	110	105	73	288
Confectionery	59	62	72	193
Cereals and seeds	46	36	49	131
Eggs and egg products	35	35	42	112
Spices	22	22	19	63
Pasta	23	12	14	49
Total	**1548**	**1404**	**1461**	**4413**

3.1. Detection of Salmonella spp.

Of the total of 4413 food samples collected and analyzed, 36 (0.8 %) tested positive for *Salmonella* spp. Of the total of 3328 samples collected on the market, 33 (0.99%) tested positive and 3 of the total of 1030 (0.29%) collected at a production plant tested positive.

Thirty food samples collected during monitoring of national foodstuff products (n=4222) tested positive (30/4222; 0.71%). The rate of positive samples collected during official monitoring of imported/exported food products was 1/126 (0.79%) and 5/65 (7.69%), respectively, of food products collected for testing after previous results were non-compliant.

Salmonella spp. was most frequently detected in poultry meat samples (25/36 positive samples) followed by other meat products and pork products. The remaining *Salmonella* strains were found in fishery samples, a raw-milk cheese sample, and in bulk cereals from a third country(Table 6). The percentage of positive samples by year ranged between 0.45% (2019) and 1.64% (2020) (Table 7).

Table 6. Number and percent of *Salmonella*-positive samples by food category and year.

Food matrix	Total	2019	2020	2021	Total	%
Non-poultry meat	1221	4/427	1/373	2/421	7	0.57
Dairy products	804	0/299	1/247	0/258	1	0.12
Fish and fish products	615	2/185	0/247	0/183	2	0.33
Poultry meat	288	0/110	21/105	4/73	25	8.7
Cereals and seeds	131	1/46	0/36	0/49	1	0.77
Total	4413	7/1548	23/1404	6/1461	36	0.81

Table 7. Number and percent of *Salmonella*-positive samples by year.

Year	Total	Number	%
2019	1548	7	0.45
2020	1404	23	1.64
2021	1461	6	0.41
Total	4413	36	0.81

3.2. Salmonella Serovars

Table 8 presents the *Salmonella* serovars according to the Kauffman-White scheme. *Salmonella* Infantis was the most frequently isolated and identified serovar (n=19), followed by *S*. Derby (n=3), *S*. Enteritidis (n=3), and the monophasic variant *S*. Typhimurium 4,5,12;i; -(n=3). *S*. Infantis and *S*. Enteritidis were most often isolated from poultry meat, while *S*. Derby and *S*. Typhimurium were most often isolated from pork products. Other serovars (Table X) were identified in one sample; one strain isolated from a fish product (*Gallus provincialis*) was classified as group *O:11; H: e,h,x* and could not be assigned to a specific serovar.

Table 8. *Salmonella* serovars by food category.

Serovar	No.	Poultry meat	Pork meat	Dairy	Cereals	Fishery
S. Infantis	19	17	2			
S. Derby	3	1	2			
S. Enteritidis	3	3				
S. Typhimurium*	3		3			
S. Agona	1	1				
S. Anatum	1	1				
S. Brandenburg	1			1		
S. Bredeney	1	1				

S. Minnesota	1			1	
S. Rissen	1				1
S. Thompsons	1	1			
S. enterica**	1				1
Total	**36**	**25**	**7**	**1**	**1**	**2**

*4,5,12;i;-   ** O:11; H: e,h,x.

4. Discussion

According to the online EFSA dashboard [20], between 2017 and 2021 sampling units from five food categories were tested for *Salmonella* spp. from 37 countries (29 EU member states). The food category with the highest number of positive units was meat and meat products (38,853 positive units of a total of 997,615 units tested during 2021), followed by egg and egg products (77 positive units of a total of 14817 units tested during 2021), and fish and fishery products (63 positive units of a total of 15,259 units tested during 2021). The categories fruit, vegetables, and juices (7 positive units of a total of 12,485 units tested) and milk and dairy products (28 positive units of a total of 45,182 units tested) had the lowest number of positive units during 2021.

The high number of *S.* Infantis detected in poultry meat is shared by the 2019 report published by the National Reference Center for Salmonellosis. In 2019 *S.* Infantis was the most often detected serotype *Gallus gallus* frams and 44% of *S.* Infantis strains was detected in broilers [21]. *S.* Infantis was massively reported for broiler matrices in the EU in 2019, from animals (36.3% of all serotyped isolates), and from other food matrices (49.1%). More than 50% of the *S.* Infantis isolated in 2019 from broilers was reported for Italy. In addition, *S.* Infantis and *S.* Derby isolates were most often reported for Italy in 2020, which accounted for 43% and 38.3% of the isolates positive for these serovars, respectively [5]. Broiler meat is a common source of *Salmonella* and the contamination of broiler farms has been increasingly associated with persistent serovars, such as *S.* Infantis. [22]

A certain seasonality for *Salmonella* detection has been suggested. Salmonellosis may be caused by the convergence of different factors, including human behavior, prevalence in animal reservoirs, consumption patterns, and bacterial environmental survival. Variation in seasonal prevalence seems to be greater during warmer months and lower during colder months [23]. The multivariable Poisson regression model we used to investigate for a time effect revealed that the covariates representing the season in which the sample was collected and analyzed were not statistically significant. The observation period is quite short and other variables related to the COVID-19 pandemic might have made it difficult to identify any expected seasonal trend. The only significant time-related covariate was the difference in rates recorded for 2020 and 2019: the PR reflects the high proportion of *Salmonella* spp. positive samples in 2020 versus 2019 (3.62 times higher). Nontyphoidal salmonellosis, such as *S.* enteritidis, *S.* Newport and *S.* Typhimurium serotypes, are frequently associated with foodborne disease outbreaks from contaminated eggs, meat, milk products, and poultry [24].

The safety monitoring plans for foods of animal and vegetable origin are carried out at the local level and based on the number of food producing plants located in the area and the resident population. Our Institute covers all of northwestern Italy with Piedmont (about 4.3 million inhabitants), Liguria (about 1.5 million inhabitants), and Valle d'Aosta (about 125,000 inhabitants). Considering the differences in regional population density, the number of samples collected per region appears to be consistent. Furthermore, the number of analyses carried out in the three regions was constant during the three-year period for the total number of samples collected and for the type of matrix delivered to the laboratory.

Analytical tests are planned at all stages of the production chain: the official controls for the protection of consumer health apply the EU's from farm-to-fork approach: the samples were collected during production (food production plants) and on the market (for sale to the final consumer and in business-to-business sales). In addition, a small percentage of samples was also collected from collective catering (restaurants, cafeterias, canteens) to monitor good hygienic practices.

The IZS laboratories use rapid screening methods to detect food-related pathogens, with negative results obtained in less than 24 h from the start of analysis (less than 48 h after collection): this is essential in the monitoring of fresh and perishable food. If the lab test result is non-compliant with established legal limits, food health officers undertake procedures to withdraw and recall food products considered unsafe according to Regulation (EU) 178/2002 art. 14 [25].

Monitoring of food imports from third countries entails a series of random samplings. The three-region positivity rates for *Salmonella* spp. were similar to the national rates in official controls; however, following reports of non-compliance at the time of importation into Europe through an Italian border point, subsequent analytical checks are performed to protect consumer health in Member States and to prevent entry of potentially harmful food products into the EU.

In the context of official monitoring, controls following previous non-compliance reports play a vital in protecting consumer health. Detection of unhygienic practices at food plants or in certain food products is followed up with subsequent checks, as confirmed by these data. The food safety agencies perform serial controls to investigate the source of contamination and to determine whether corrective actions implemented by food business operators are truly effective.

Our data show a higher percentage of *Salmonella* positivity in food samples recorded for 2020. Food safety monitoring and sampling continued to protect consumer health during the COVID-19 pandemic. The relatively higher percentage of positives is not statistically significant and there are currently no published data to compare similar trends for other EU countries or other food pathogens. The economic losses and difficulties in operations management food business operators experienced because of the pandemic restrictions may have affected food safety standards in supply chains and production plants. This issue is awaiting consideration by other working groups and laboratories involved in food safety monitoring.

References

1. Jajere, S. M. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet World* **2019**, *12*, 504-52.
2. Ehuwa, O.; Jaiswal, A.K.; Jaiswal, S. Salmonella, Food Safety and Food Handling Practices. *Foods* **2021,** *10*, 907.
3. Chattaway, M. A.; Langridge, G. C.; Wain, J. Salmonella nomenclature in the genomic era: a time for change. *Sci Rep* **2021**, *11*, 7494.
4. Paniel, N.; Noguer, T. Detection of Salmonella in food matrices, from conventional methods to recent aptamer-sensing technologies. *Foods* **2019**, *8*, 371.
5. European Food Safety Authority; European Centre for Disease Prevention and Control.; The European Union one health 2020 zoonoses report. *EFSA journal* 2021, *19*:6971, 1-324
6. Turgeon, P.; Ng, V.; Murray, R.; Nesbitt, A. Forecasting the incidence of salmonellosis in seniors in Canada: A trend analysis and the potential impact of the demographic shift. *PLoS One* **2018**, *13*, e0208124.
7. European Food Safety Authority ;European Centre for Disease Prevention and Control, The European Union One Health 2021 Zoonoses Report. EFSA Journal **2022**, 20:7666, 1-273 doi:10.2903/j.efsa.
8. Chalias, A.; Grispoldi, L.; Cenci Goga, B. A risk assessment model for Salmonella spp. in swine carcasses. *EFSA Journal* **2022,** *20*, e200405.

9. Brown, E. W.; Bell, R.; Zhang, G.; Timme, R.; Zheng, J.; Hammack, T. S.; Allard, M. W. Salmonella genomics in public health and food safety. *EcoSal Plus* **2021,** *9*, eESP-0008.

10. European Centre for Disease Prevention and Control, European Food Safety Authority.; Multi-country outbreak of monophasic Salmonella Typhimurium sequence type 34 linked to chocolate products–first update–18 May 2022. *EFSA Supporting Publications* **2022**, *19*, 7352E, 1-25.

11. Larkin, L.; Pardos de la Gandara, M.; Hoban, A.; Pulford, C.; Jourdan-Da Silva, N.; de Valk, H.; Browning, L.; Falkenhorst, G.; Simon, S.; Lachmann, R.; Dryselius, R.; Karamehmedovic, N.; Börjesson, S.; van Cauteren, D.; Laisnez, V.; Mattheus, W.; Pijnacker, R.; van den Beld, M.; Mossong, J.; Ragimbeau, C.; Vergison, A.; Thorstensen Brandal, L.; Lange, H.; Garvey, P.; Nielsen, C. S.; Herrera León, S.; Varela, C.; Chattaway, M.; Weill, F. X.; Brown, D.; McKeown, P. Investigation of an international outbreak of multidrug-resistant monophasic Salmonella Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022. *Eurosurveillance* **2022**, *27*, 2200314.

12. European Centre for Disease Prevention and Control, European Food Safety Authority.; Multi-country outbreak of multiple Salmonella enterica serotypes linked to imported sesame-based products - 14 October 2021.. *EFSA Supporting Publications* **2021**, *18*, 6922E, 1-25.

13. EU. COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* 2005, 2073.

14. EU. COMMISSION REGULATION (EU) 2017/625 of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Off. J. Eur. Union* 2017, 625

15. Piedmont Region Competent Authority. Piano Regionale Integrato dei controlli di Sicurezza Alimentare (PRISA 2022) Bollettino Ufficiale n. 14 del 7 / 04 / 2022. Available on line: http://www.regione.piemonte.it/governo/bollettino/abbonati/2022/14/attach/dda1400000507_10100.pdf (accessed on 30 May 2023)

16. ISO SO/TS 17728:2015 Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples. Available online: https://www.iso.org/standard/60322.html (accessed on 30 May 2023)

17. ISO 16140-3:2021 Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory Available online: https://store.uni.com/en/uni-en-iso-16140-3-2021 (accessed on 30 May 2023)

18. UNI EN ISO 6579-1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. Available online: https://store.uni.com/en/uni-en-iso-6579-1-2020 (accessed on 20 May 2023).

19. Kauffmann-White and Le Minor. Antigenic formulae of the Salmonella serovars. Bergey's Man. Syst. Bacteriol. 1984, 1, 429–445. Available online: https://www.pasteur.fr/sites/default/files/veng_0.pdf (accessed on 6 March 2023).

20. European Food Safety Authority (EFSA) Salmonella Dashboard. Available on line: https://www.efsa.europa.eu/en/microstrategy/salmonella-dashboard (Accessed on 30 May 2023)

21. National Reference Centre for Salmonellosis. Annual Report. Available on line: https://www.izsvenezie.it/documenti/temi/salmonellosi/enter-vet/entervet-report-2019.pdf (Accessed on 30 May 2023)

22. European Food Safety Authority; European Centre for Disease Prevention and Control.; The European Union one health 2021 zoonoses report. EFSA *Journal* 2022; 20 (12): 7666

23. Magossi, G., Bai, J., Cernicchiaro, N., Jones, C., Porter, E., Trinetta, V. Seasonal Presence of Salmonella spp., Salmonella Typhimurium and Its Monophasic Variant Serotype I 4,[5],12:i:-, in Selected United States Swine Feed Mills. Foodborne Pathogens and Disease. Apr 2019.276-281.

24. Qamar, F.N., Wajid, H., Qureshi S.. Salmonellosis including enteric fever. Pediatric Clinics 69.1 (2022): 65-77.

25. EU. COMMISSION REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Off. J. Eur. Union 2002, 178.*