

Distribution of *Salmonella* serovars of animal origin in Türkiye between 2015 and 2020

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Abstract: *Salmonella* is an important zoonotic microorganism and the most isolated among food-borne infections across the world, including Türkiye. The detection and identification of common serovars circulating in Türkiye can present very useful data in the fight against *Salmonella*, which threatens both animal and human health. This study aimed to determine the distribution and diversity of *Salmonella* serovars isolated from the samples sent to Bacteriological Diagnosis Laboratory of the Veterinary Control Central Research Institute. The serotyping results of a total of 1,047 *Salmonella* spp. strains isolated between 2015 and 2020 were retrospectively evaluated. After confirming that the samples isolated were *Salmonella* spp. according to ISO 6579, identification at the species level was carried out by serotyping with the slide agglutination test. A total of 19 serogroups and 75 *Salmonella* serovars were detected. The most commonly isolated *Salmonella* serovar was *Salmonella* Infantis (40.5%), followed by *Salmonella* Liverpool (2.4%), and other serovars (31.3%). The most commonly identified serogroups were C1 (48.2%), D1 (14.4%), B (12.4%), C3 (7.8%), and E4 (4.2%). According to animal species, the most common serovar was *Salmonella* Infantis in chickens, *Salmonella* Montevideo in calves, *Salmonella* Darle in tortoises, *Salmonella* Typhimurium in lamb and *Salmonella* Hessarek in wild birds.

Keywords: Animals, Salmonella serovars, Türkiye.

Türkiye'de 2015-2020 yılları arasında hayvansal orijinli *Salmonella* serovarlarının dağılımı

Özet: Salmonella, Türkiye'de ve dünyada, insan ve hayvan sağlığını tehdit eden, gıda orijinli infeksiyonlar arasında en çok izole edilen zoonoz karakterli, önemli bir mikroorganizmadır. Özellikle ülkemizde sirküle olan yaygın serovarların tespiti ve izole edilen serovarların belirlenmesi hem hayvan sağlığı hem de insan sağlığını tehdit eden *Salmonella*'larla mücadelede oldukça faydalı verilere ulaşmamızı sağlayacaktır. Bu çalışmada Veteriner Kontrol Merkez Araştırma Enstitüsü Bakteriyolojik Teşhis Loboratuvarı'na gönderilen izolat veya örneklerden identifiye edilen *Salmonella* serovarlarının dağılımı ve çeşitliliğini belirlemek amaçlandı. 2015-2020 yılları arasında izole edilip doğrulama ve serotiplendirme amacıyla 1,047 *Salmonella* spp. suşunun serotiplendirilme sonuçları retrospektif olarak değerlendirilmiştir. Gönderilen örnekler ISO 6579'a göre; izolasyonu yapılan kültürlerin *Salmonella* spp. olduğu doğrulandıktan sonra tür düzeyinde identifikasyonu lam aglütinasyon testle serotiplendirilerek gerçekleştirildi. Çalışmada 19 serogrup, 75 *Salmonella* serovarı tespit edildi. En yaygın izole edilen *Salmonella* serovarları sırasıyla *Salmonella* Infantis (40.5%), *Salmonella* Enteritidis (12.9%), *Salmonella* Abony (4.3%), *Salmonella* Kentucky (4.2%), *Salmonella* Typhimurium (4%), *Salmonella* Liverpool (2.4%) ve diğer serovarları (31.3%) olduğu belirlendi. Tespit edilen en yaygın serogruplar ise sırasıyla grup C1 (48.2%), D1 (14.4%), B (12.4%), C3 (7.8%) ve E4 (4.2%)'tü. Tavuklarda en yaygın serovarlar *Salmonella* Infantis, buzağılarda *Salmonella* Montevideo, kaplumbağada *Salmonella* Darle, kuzuda *Salmonella* Typhimurium ve yaban kuşlarında *Salmonella* Hessarek idi.

Anahtar kelimeler: Hayvanlar, Salmonella serovarları, Türkiye.

Introduction

Salmonella agents are facultatively anaerobic, intracellular, Gram-negative bacteria belonging to the Enterobacteriaceae family. The Salmonella genus consists of two species: Salmonella enterica and Salmonella bongori. S. enterica species containing many pathogens are divided into six subspecies, namely Salmonella enterica subsp. I (enterica), II (salamae), Illa (*arizonae*), IIIb (*diarizonae*), IV (*houtenae*), and VI (*indica*) (Brenner et al. 2000; Ke et al. 2014). In the identification of *S. enterica*, the somatic (O), flagella (H) and capsular (Vi) antigenic structures of the species have been very well studied, and to date, more than 2,600 serovars have been defined in this highly diverse species (Cohen et al. 2021). *Salmonella* serovars can cause general and local infectious dis-

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eases in humans and animals (Izgür 2006). Globally, an average of 93,8 million cases of gastroenteritis and 155,000 food-related deaths are annually reported to be caused by Salmonella spp. (Majowicz et al. 2010). The incidence of diseases caused by non-typhoidal Salmonella serovars varies from one country to another, being reported as 690 cases per 100,000 population in Europe and around 100 cases per 100,000 in Israel on an annual basis (Heredia and Garcia 2018). It is estimated that food poisoning caused by salmonellosis in the United States of America results in 1.4 million cases per year, and this number is gradually increasing. Many foods, including poultry products, pork and beef, play an important role in the transmission of salmonellosis infections to humans (Messens et al. 2013; Heredia and Garcia 2018; Yang et al. 2018; Santos et al. 2020). Salmonella is also recognized as an important microbial hazard in animal feeds containing feed raw materials and components (Yang et al. 2018).

According to the 2020 data of the Turkish Statistical Institute, the amount of animal production of Türkiye is as follows: 18 million 158 thousand bovine animals, 54 million 113 thousand ovine animals, 2 million 136 thousand 263 tons of chicken meat, 19 billion 788 thousand 63 chicken eggs, and 58 thousand 212 tons turkey meat (TUIK 2020). In Türkiye, people meet most of their daily protein needs from animal products. The increase in the consumption of animal products increases the risk of transmission of Salmonella through contaminated foods (Foley et al. 2011; Messens et al. 2013). Salmonella enterica subsp. enterica serovars are known to constitute one of the important public health problems in Türkiye, with S. Enteritidis and S. Typhimurium being commonly detected (NSCP 2018).

Salmonella infections, as in various outbreaks, threaten the livestock sector by causing low productivity and economic losses (NSCP 2018). Therefore, the determination of serovar diversity is critical in the development of strategies for the prevention, control and eradication of Salmonella infections. In Türkiye, studies on Salmonella infections have mostly focused on poultry breeding, especially broiler breeding. These studies have been mostly carried out on an enterprise and/or regional basis (Goncagül and Carlı 1999; Akan 2008; Arkali and Cetinkaya 2020). In this study, we aimed to detect common serovars circulating in farms, environments, and animals in Türkiye and evaluate the distribution of isolated serovars. It is considered that the data obtained will contribute to the fight against

Salmonella, which threatens both animal and human health.

Materials and Methods

Sample collection: Between 2015 and 2020, a total of 1,047 *Salmonella* isolates were tested at the laboratory of Bacteriological Diagnosis Laboratory of the Veterinary Control Central Research Institute. The *Salmonella* strains were either submitted to the laboratory by farms or regional (institute) and private veterinary laboratories for confirmatory testing or isolated at Bacteriological Diagnosis Laboratory of the Veterinary Control Central Research Institute. The isolates were obtained during the provision of routine commercial services, animal health surveillance, or laboratory diagnostic testing. A total of 1,047 strains originating from farm animals, their environments, and other sources, including fertilizers were serotyped (Table 1).

Table 1. Number of Salmonella isolates according to thesampling categories and their percentage of the total

Sample Category	n	%
Chicken	980	93.6
Calves	34	3.2
Lamb	2	0.19
Goose	5	0.4
Turkey	1	0.1
Starling	4	0.3
Gull	1	0.1
Parrot	1	0.1
Cow	5	0.4
Tortoise	7	0.6
Quail	4	0.3
Sheep	1	0.1
Fertilizer	2	0.19

Culture, isolation and serotyping: The fecal, drag swap, environmental, dust and organic fertilizer samples were inoculated into pre-enriching buffered peptone water (Oxoid, Basingtoke, Hampshire, UK) at 37 °C for 18-24 hours. Then, the enriched samples were inoculated into the Rappaport-Vassiliadis (RV) (Oxoid, UK) and Mueller-Kaufmann tetrathionate (MKTTn) (Oxoid, UK) broths at 0.1 and 1 ml volumes, respectively. The selectively enriched RV and MKTTn broths were incubated at 41.5 °C and 37 °C, respectively for 24 hours. The cultures from MKTTn and RV were simultaneously plated onto the Brilliance Green Agar (BGA) (Oxoid, UK) and Xylose Lysine Deoxycholate (XLD) (Oxoid, UK) media. The plates were incubated at 37 °C for 18-24 hours, and then examined for the presence of *Salmonella* colonies, which are typically observed purple center in BGA and black-centered in XLD. The internal organ (liver and spleen) samples were inoculated onto 5% blood agar (Oxoid, United Kingdom), nutrient agar (Oxoid, United Kingdom), and MacConkey agar (Oxoid, United Kingdom), and incubated aerobically at 37°C. A colony from the growth medium was selected and tested. (ISO 6579).

The confirmation of the typical *Salmonella* colonies was performed with biochemical tests such as gas (+), sucrose (-), lactose (-), glucose (+), urea hydrolysis (-), indole formation (-), hydrogen sulfide (H_2S) production (+), Voges Proskauer-VP (-), b-galactosidase (ONPG) (-), and lysine decarboxylase (+). After the biochemical tests, *Salmonella*-suspected colonies were identified with the agglutination test using the polyvalent *Salmonella* antiserum (ISO 6579).

Salmonella spp. isolates were serotyped with monovalent and polyvalent antisera against the somatic O and flagella H antigens using the lam agglutination and serum neutralization tests according to the White–Kaufmann–LeMinor scheme (Statens Serum Institute, Copenhagen, Denmark and Denka Seiken, Tokyo, Japan) (Grimont and Weill 2007).

Data collection and statistical analysis: Data were analyzed using the statistical software package IBM SPSS Statistics Standard Concurrent User v. 26 (IBM Corp., Armonk, New York, USA). Relationships between categorical variables were analyzed with the chi-square test. The Bonferroni method, one of the post-hoc tests, was used to examine the differences between variables. In cases where the expected frequencies were less than 20%, the evaluation was made with the Monte Carlo simulation method to include these frequencies in the analysis. A p level of <0.05 was considered statistically significant.

Results

The distribution of the *Salmonella* serovars isolated from different host species from various regions of Türkiye and/or identified from isolates was examined. The collected isolates were serologically classified into 75 serovars under 19 serogroups. Twelve *Salmonella* serovars were identified at the serogroup level and one at the *Salmonella* spp. level. Of the serotyped isolates, 1,045 were identified as *S. enterica* subsp. *enterica*, and two as *S. enterica* subsp. diarizonae and S. enterica subsp. arizonae. Of the 75 identified serovars (including 13 unidentified isolates), only six were dominant in 68.7% (719 isolates), while the remaining serovars were sporadically detected in 31.3% (328 isolates). The six most common serovars were S. Infantis (40.5%), S. Enteritidis (12.9%), S. Abony (4.3%), S. Kentucky (4.2%), S. Typhimurium (4%), and S. Liverpool (2.4%). During the study period, only 33 isolates (Salmonella Aberdeen, Salmonella Adeoya, Salmonella Aequatoria, Salmonella Albany, Salmonella Bardo, Salmonella Bareilly, Salmonella Bignona, Salmonella Bonn, Salmonella Braenderup, Salmonella Cubana, Salmonella Ferruch, Salmonella Halle, Salmonella Indiana, Salmonella Lindenburg, Salmonella Mantopeni, Salmonella Mons, Salmonella Nashua, Salmonella Richmond, Salmonella Rubislaw, Salmonella Stanley, Salmonella Teddington, Salmonella Tennessee, Salmonella Umbilo, Salmonella Veneziana, Salmonella Willemstad, Salmonella II (9,12:z₂₉:1,5), Salmonella II (4,12:z:1,7), Salmonella II (42:z:1,5), Salmonella IIIb (50:k:z₃₅), Salmonella IV (6,14:z₄,z₂₃: -), Salmonella II (6,7:m,t: -), Salmonella diarizonae, and Salmonella arizonae) were isolated once each.

For the examined period, the distribution of the 1,047 isolates according to their origin was as follows: 996 (95.1%) poultry and other birds, 34 (3.2%) calves, two (0.19%) lamb, one (0.1%) sheep, five (0.4%) cows, seven (0.6%) tortoises, and two (0.19%) organic fertilizers (poultry origin) (Table 1).

The rates of isolation according to the sampling method were as follows: 42.5% drag swabs, 25.1% dust swabs, 21.8% environmental samples, 4.1% organ, 4% fecal, 2.2% slaughterhouse and 0.1% organic fertilizer. There was a statistically significant difference in the isolation frequency of the serovars according to the sampling method (p < 0.05). The S. Abony serovars were found to be isolated from drag swab samples at a significantly higher rate than the remaining serovars. The sampling methods used for the isolation of the S. Infantis, S. Enteritidis and other serovars were found to be statistically similar. For environmental samples, the 'other serovars' category was observed to constitute a higher percentage than S. Infantis, S. Enteritidis, and S. Typhimurium. In the dust swab samples group, S. Infantis was isolated at a higher rate than the other serovars. The results of S. Enteritidis and other serovars were statistically similar. The S. Montevideo and S. Dublin serovars were found to be isolated from fecal samples at a similar rate, which was higher compared to the remaining serovars. For organ samples, a higher rate of S. Enteritidis serovars was isolated than the remaining serovars. Lastly, in the slaughterhouse group, the rate of *S*. Infantis isolation was significantly higher compared to the other serovars.

Of the detected serovars, 64 identified serovars and 13 unidentified isolates originated from poultry. The diversity and number of serovars detected in the poultry samples were found to be significantly higher compared to the other animal species. The most common serovars detected in poultry and other birds were *S.* Infantis (42.6%), *S.* Enteritidis (13.4%), *S.* Abony (4.6%), *S.* Kentucky (4.2%), and *S.* Typhimurium (3.6%). The total rate of these five serovars was 68.6%, while the remaining serovars were isolated at a rate of 31.4%.

There was a significant difference between the serovars detected in poultry according to the years (p < 0.05). The frequency of *S*. Infantis serovars iso-

lated in poultry was similar in 2020 and 2017 and higher than in 2019 and 2016. S. Enteritidis had a similar isolation frequency in 2020 and 2016, but this was determined to be lower compared to the remaining years. The isolation frequency of S. Corvallis was similar between 2020 and 2016, and it was observed to be higher than from the remaining years. The lowest isolation frequency for S. Typhimurium was seen in 2015. For S. Poana, the isolation frequency was significantly higher in 2020 compared to the remaining years. For S. Virchow, the years 2020, 2018 and 2015 presented with similar isolation frequencies, which were significantly higher than the remaining years. The isolation frequency of S. Kentucky was similar between 2019 and 2018 and indicated lower values compared to the remaining years (Table 2).

Table 2. Frequency of Salmonella serovars in avian, bovine, ovine and tortoise samples in Türkiye from 2015 to 2020.

Sample		2020			2019			2018			2017			2016			2015		
Source	Rank	Serovar	n	%	Serovar	n	%												
Avian	1	Infantis	63	50.4	Infantis	69	36.9	Infantis	73	46.2	Infantis	76	48.7	Infantis	71	37.5	Infantis	73	40.1
	2	Enteritidis	12	9.6	Enteritidis	25	13.3	Enteritidis	31	19.6	Enteritidis	24	15.3	Abony	35	18.5	Enteritidis	25	13.7
	3	Corvallis	7	5.6	Liverpool	21	11.2	Tomegbe	14	8.8	Kottbus	6	3.8	Enteritidis	17	8.9	Kentucky	22	12.0
	4	Typhimurium	6	4.8	Typhimurium	12	6.4	Typhimurium	10	6.3	Anatum	5	3.2	Kentucky	13	6.8	Virchow	9	4.9
	5	Poona	4	3.2	Anatum	9	4.8	Abony	5	3.1	Abony	4	2.5	Havana	9	4.7	Mbandaka	6	3.3
	6	Virchow	4	3.2	Senftenberg	5	2.6	Virchow	4	2.5	Salford	4	2.5	Corvallis	5	2.6	Molade	6	3.3
	7	Kentucky	3	2.4	Mbandaka	3	1.6	Corvallis	3	1.9	Typhimurium	3	1.9	Newport	4	2.1	Kottbus	4	2.2
	8	Saintpaul	3	2.4	Thompson	3	1.6	Mbandaka	3	1.9	Havana	3	1.9	Typhimurium	3	1.5	Cannstatt	4	2.2
	9	Others	23	18.4	Others	40	21.3	Others	16	10	Others	31	19.8	Others	32	16.9	Others	33	18.1
	10	Total	125		Total	187		Total	159		Total	156		Total	189		Total	159)
Bovine	1	Salford	1	100	Typhimurium	3	100	Montevideo	1	100	Dublin	5	71.4	Montevideo	17	70.8	Kottbus	2	66.6
	2	Others	0		Others	0		Others	0		Typhimurium	2	28.5	Others	7	29.1	Linden- burg	1	33.3
	3	Total	1		Total	3		Total	1		Total	7		Total	24		Total	3	

n represents the number of isolates related to each serovar, and % represents the relative occurrence of serovars for each sample category.

Pearson chi-square test (x^2); avian p < 0.001, bovine p < 0.001, ovine p < 0.083

S. Typhimurium was found to be the serovar with the widest host range (chicken, n = 33; calves, n = 4; lamb, n = 2; goose, n = 1; parrot, n = 1, and gull, n = 1).

The most common serovars in calves were S. Montevideo (52.9%), S. Dublin (17.6%), S. Typhimurium (11.7%), S. Kentucky (5.8%), and S. Enteritidis (5.8%). There was a significant difference between the serovars detected in bovine animals according to the years (p < 0.05). The isolation frequency of the S. Typhimurium serovar was lower in 2016 than in the remaining years. The years 2016 and 2017 presented with lower isolation frequencies for *S*. Kottbus compared to the remaining years. There was no significant difference between the serovars detected in ovine animals by year (p = 0.083). The *Salmonella* serovars isolated from the sheep and lamb were only observed in 2018 and 2015.

The serovars isolated from the tortoises were S. Bareilly, S. Bonn, S. Daarle, S. Halle, S. Newport, and S. Richmond. S. Corvallis and S. Richmond were isolated from the organic fertilizer samples.

The most common serogroups detected were C1 (n = 504, 48.2%), D1 (n = 151, 14.4%), B (n = 130, 12.4%), C3 (n = 82, 7.8%), E4 (n = 44, 4.2%), and C2 (n = 33, 3.1%) in that order. According to the season, the most common serogroups were C1, D1 and B, which were isolated in all seasons.

The distribution of the *Salmonella* serovars isolated according to the season was 31.6% (n = 332) for autumn, 26.7% (n = 279) for spring, 24.7% (n = 259) for winter, and 16.8% (n = 177) for summer (Table 4). There was a significant difference between the serovars in the winter season according to the years (p < 0.05). For *S*. Infantis, the values obtained from 2017, 2016 and 2015 statistically significantly differed from the remaining years, and the highest value was found in 2017. Both *S*. Abony and *S*. Montevideo had statistically significantly higher isolation rates in 2016 compared to the remaining years. There was also a significant difference between the

isolation rates of the serovars for the spring season according to the years (p < 0.05). The observation value for S. Enteritidis in 2018 was higher in 2018 than in the remaining years. For S. Typhimurium, the isolation frequency was statistically similar in all years. A significant difference was also observed between the serovars in the summer season according to the years (p < 0.05). For S. Bredeney, the isolation frequency was statistically similar in all years. For S. Tomegbe, the years 2019 and 2018 presented with statistically significant isolation rates, with the highest value being observed in 2018. For S. Salford, 2017 showed the highest rate of isolation, which was at a significant level. Lastly, a significant difference was found between the serovars in the autumn season according to the years (p < 0.05). S. Liverpool had a significantly higher rate of isolation in 2019 compared to the remaining years.

Table 3. Seasonal frequency of Salmonella serovars in Türkiye from 2015 to 2020.

Seasons	2020	n	%	2019	n	%	2018	n	%	2017	n	%	2016	n	%	2015	n	%
Winter	Infantis	10	33.3	Infantis	18	43.9	Infantis	19	45.2	Infantis	31	67.3	Abony	26	43.3	Enteritidis	13	32.5
	Enteritidis	6	20	Enteritidis	7	17	Enteritidis	16	38.1	Enteritidis	4	8.7	Infantis	17	28.3	Kentucky	7	20.5
	Others	14	46.6	Others	16	39	Others	7	16.6	Others	11	23.9	Others	17	28.3	Others	20	50
	Total	30		Total	41		Total	42		Total	46		Total	60		Total	40	
Spring	Infantis	33	67.3	Infantis	17	30.9	Enteritidis	13	40.6	Infantis	14	56	Infantis	18	36.7	Infantis	34	49.2
	Enteritidis	3	6.1	Enteritidis	12	21.8	Infantis	8	25	Typhimurium	3	12	Montevideo	17	34.6	Enteritidis	7	10.1
	Others	13	26.5	Others	26	47.2	Others	11	34.3	Others	8	32	Others	14	28.5	Others	25	36.2
	Total	49		Total	55		Total	32		Total	25		Total	49		Total	69	
	Infantis	13	54.1	Infantis	18	34.6	Infantis	19	44.1	Infantis	4	26.6	Enteritidis	7	29.1	Infantis	2	10.5
Summor	Bredeney	2	8.3	Enteritidis	4	7.6	Tomegbe	10	23.2	Salford	3	20	Infantis	5	20.8	Enteritidis	2	10.5
Summer	Others	9	37.5	Others	30	57.6	Others	14	32.5	Others	8	53.3	Others	12	50	Others	15	78.9
	Total	24		Total	52		Total	43		Total	15		Total	24		Total	19	
Autumn	Infantis	7	30.4	Infantis	16	38.1	Infantis	27	60	Infantis	27	35	Infantis	31	38.7	Infantis	31	47.6
	Typhimurium	3	13	Liverpool	8	19	Typhimurium	6	13.3	Enteritidis	20	25.9	Abony	8	10	Kentucky	9	13.8
	Others	13	56.5	Others	18	42.8	Others	12	26.6	Others	30	38.9	Others	41	51.2	Others	25	38.4
	Total	23		Total	42		Total	45		Total	77		Total	80		Total	65	

n represents the number of isolates related to each serovar, and % represents the relative occurrence of serogroups for each season. *Pearson chi-square test* (x^2); winter, spring, summer and autumn p < 0,001

Discussion and Conclusion

Salmonella is an important bacterial pathogen that causes foodborne infections worldwide (EFSA 2015; Heredia and Garcia 2018; Aung et al. 2020; Santos et al. 2020). The source of human *Salmonella* outbreaks has been associated with the consumption of contaminated poultry, pork, beef, milk, and eggs (Lapuz et al. 2008; Foley et al. 2011; Milazzo et al. 2016; Fagbamila et al. 2017; Shah et al. 2019; Aung et al. 2020). In Türkiye, studies on *Salmonella* infections have mostly focused on poultry, especially broiler breeding. These studies have been mostly undertaken on an enterprise and/or regional basis (Goncagül and Carlı 1999; Akan 2008; Arkali and Cetinkaya 2020). Determining the epidemiology of *Salmonella* serovars is important not only to protect public health but also to control and monitor the prevalence of *Salmonella* in animals. This is the most comprehensive study conducted in Türkiye to investigate the distribution, diversity, and different host reservoirs of *Salmonella* serovars.

The sampling method is a parameter that plays an important role in Salmonella isolation. In this study, the Salmonella serovars detected in poultry were found to have been most frequently isolated from drag swab, dust and environmental samples, and this was at a statistically significant level (p<0.05). These results are consistent with previous studies using drag, dust, environmental samples but indicate higher isolation rates compared to those using other types of samples (Goncagül and Carlı 1999; EFSA 2007; Fagbamila et al. 2017; Arkali and Cetinkaya 2020). Salmonella serovars isolated from feces show the presence of current infection, while environmental and dust samples show that the pathogen continues to survive in the enterprise after Salmonella has been transmitted into the environment (Fagbamila et al. 2017). Salmonella serovars isolated from bovine animals have been commonly detected in fecal samples (Hadimli et al. 2017; Cakin et al. 2020). In the current study, the most common serovars according to the sample category were S. Enteritidis for organ samples, S. Infantis for drag swabs, S. Infantis for dust swabs, others serovars for environmental samples, and S. Montevideo and S. Dublin for fecal samples. S. Infantis was found to be the most common serovar in drag, dust and environmental samples. These results revealed that S. Infantis contaminated the environment, as well as poultry animal products. The common prevalence of S. Enteritidis in organ samples supports previous studies reporting that this pathogen has more invasive properties than the other Salmonella serovars (Arkali and Cetinkaya 2020).

S. Enteritidis has been reported as the most common serovar in poultry in Asia, Latin America, Europe, and Africa, while S. Kentucky, S. Typhimurium, and S. Sofia are the most prevalent in North America and Oceania (Ferrari et al. 2019). When studies conducted in Türkiye are evaluated in general, it is observed that the most common serovars in poultry are S. Infantis, S. Enteritidis, and S. Typhimurium (NSCP 2018; Arkali and Cetinkaya 2020). In the current study, we identified the most common serovar in poultry as S. Infantis (43.1%), followed by S. Enteritidis (13.5%), S. Abony (4.6%), S. Kentucky (4.2%), and S. Typhimurium (3.3%). These differences may be associated with regional characteristics, flock management styles, poor infrastructure, rearing conditions (cage/ground), inadequate

protection/control and biosecurity measures, sample type (feces, dust, organ, litter, cloacal swab, etc.), and imported poultry.

In the current study, the diversity and number of serovars detected in the poultry samples (64 serovars) were significantly higher compared to the remaining animal species. These results support the idea that poultry can often be asymptomatically infected with different Salmonella serovars (Foley et al. 2011; Ferrari et al. 2019). Different factors related to the environment, transport, imported animals, feed, rodents, humans, and vectorial transmission result in the diversity of Salmonella serovars in poultry and play an important role in the transmission. The higher prevalence of Salmonella in poultry compared to the remaining animal species suggests that poultry plays an important role in the transmission of Salmonella infections to humans (Ferrari et al. 2019; Aung et al. 2020).

In this study, S. Infantis was found to be the most commonly isolated serovar in poultry. This finding is in keeping with the results of previous studies conducted in Türkiye and abroad (Aviv et al. 2014; NSCP 2018; Alba et al. 2020; Arkali and Cetinkaya 2020, Mejia et al. 2020). S. Infantis being the predominant serovar in poultry has been associated with a reduction in S. Enteritidis as a result of implemented control programs (Foley et al. 2011; Antunes et al. 2016). Aviv et al. (2014) revealed that the clone of S. Infantis had a megaplasmid, which increases the virulence properties via adhesion to and invasion of host cells and provides proliferation in host cells. It has been reported that cleaning and disinfection methods are ineffective against these emerging special clones (Garcia-Soto et al. 2020). When studies conducted to explain why S. Infantis is the predominant serovar are evaluated, it is observed that reorganizations in the genes of this serovar allow it to overcome the adaptation period both in the environment and in the host (poultry/ human). In this adaptation period, genes encoding virulence factors in microorganisms are very important. As a result of reorganization in genes during this period, adaptation becomes much easier, which increases survival time (Arda 2006). In the current study, the second most commonly isolated serovar was determined as S. Enteritidis. The isolation frequency of S. Enteritidis in 2020 and 2016 was similar and lower compared to the remaining years. For S. Typhimurium, the lowest isolation frequency was found in 2015. The isolation frequency of S. Kentucky in 2019 and 2018 was similar and lower compared to the remaining years. There was a statistically significant difference in the frequency of serovars detected in poultry according to the years (p < 0.05). As a result of the fluctuations in the isolation rates of these important serovars, there were increases in the ratio of different serovars in poultry. In light of these findings, it can be suggested that control programs targeting certain serovars posing a risk for public health (*S.* Enteritidis and *S.* Typhimurium) have led an increase in other serovars (Foley et al. 2011; Antunes et al. 2016). These results indicate that implemented control measures are not equally effective against all *Salmonella* serovars. Therefore, *Salmonella* control programs should be updated to include other epidemiologically important serovars (Skarzynska et al. 2017).

S. Typhimurium was determined to be the serovar with the widest host range. This serovar has also been previously reported to have the widest host range since it causes diseases in a wide variety of host species, including livestock and poultry, rodents, and birds (Gelaw et al. 2018; Ferrari et al. 2019). These results show that *S.* Typhimurium can be transmitted to humans through different routes.

In Türkiye, there are only few studies investigating *Salmonella* serovars in bovine animals (Hadimli et al. 2017; Cakin et al. 2020). Hadimli et al. (2017) reported that the most common serovars in calves were *S*. Kentucky, *S*. Muenchen, and *S*. Anatum. In the current study, the most common serovars in calves were identified as *S*. Montevideo (52.9%), *S*. Dublin (17.6%), and *S*. Typhimurium (11.7%). These differences may occur depending on regional characteristics and sampling methods. Further studies are needed to determine the epidemiology of *Salmonella* in calves in Türkiye.

Domestic reptiles, which continue to increase in population in recent years, have an important zoonotic potential and play an important role in the transmission of *Salmonella* infections to humans through direct and indirect contact (Wang et al. 2020). In our study, due to the low number of *Salmonella* serovars (0.6%) isolated from tortoises during the study period, we were not able to obtain significant results. However, it should not be forgotten that wild animals and domestic reptiles are *Salmonella* reservoirs.

Foodborne pathogens, such as *Salmonella* can also be found in animal fertilizers. Therefore, fruit and vegetables can play an important role in the transmission of pathogens found in fertilizers to humans (Shah et al. 2019). In the current study, due to the low number of *Salmonella* strains isolated from fertilizers, general data could not be obtained.

The most common serogroups were observed to be C1 (n = 504, 48.2%), D1 (n = 151, 14.4%), B (n = 130, 12.4%), and C2-C3 (n = 115, 11%). These are also known to be the most common serogroups associated with animal and human infections (Messens et al. 2013; Ke at al. 2014; Fuche et al. 2016; Fagbamila et al. 2017). The development of polyvalent vaccines targeting these serogroups may be effective in controlling *Salmonella*.

It has been suggest that *Salmonella* cases increase due to the increase in temperature (Milazzo et al. 2016). In contrast, according to the seasonal evaluation undertaken in the current study, the least number of *Salmonella* strains were isolated in summer (16.8%). This result is consistent with previous studies reporting that increased temperature does not affect *Salmonella* isolation (Traub-Dargatz et al. 2006; Gole et al. 2017).

This is the most comprehensive study conducted in Türkiye to investigate the distribution, diversity and different host reservoirs of *Salmonella* serovars of animal origin. *Salmonella* serovars were mostly detected in poultry. *S.* Montevideo was the most commonly isolated serovar in calves with diarrhea. Further studies are needed to investigate the role of *Salmonella* in diarrheic calves. It is considered that the findings obtained from this study will contribute to future control programs to be implemented against *Salmonella*, which threatens both animal and human health.

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Ethical Statement: In this study, data obtained from the routine bacteriological diagnosis activities of Bacteriological Diagnostic Laboratory of Veterinary Control Central Research Institute were retrospectively evaluated. Ethical approval was not required for the study. The study was submitted to the General Directorate of Food Control of the Ministry of Agriculture and Forestry of the Republic of Türkiye and necessary permissions were obtained (22.04.2021/E-71037622-824-01.03-1276449).

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