Salmonella carriage and change in serovar distribution in broiler giblets at slaughterhouse level in Turkiye: first report using ISO 6579-1:2017 and ISO 6579-3:2014

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ABSTRACT This study aimed to determine the prevalence and serovar distribution of salmonellae in liver, heart, and spleen (**LHS**) and gizzard (**G**) of slaughtered broilers. For this, a total of 60 sample units, comprised of 30 LHS and 30 G collected from 3 slaughterhouses, were analysed by reference methods for detection and serotyping as revised ISO 6579-1:2017 and ISO 6579-3:2014, respectively. Also, *Salmonella*-specific real-time PCR (**Salm-PCR**) was used for species confirmation, while *Salmonella* Enteritidis (***S.* Enteritidis**) and *Salmonella* Typhimurium (***S.* Typhimurium**) specific real-time PCR (SE/ST-PCR) was evaluated to determine its efficiency for rapid detection of the serovars mandated in current legal regulations compared to standard serotyping. All LHS (100%−30/30) and 90% (27/30) of G samples harbored *Salmonella* with an overall prevalence of 95% (57/60) in samples examined, where all isolates were confirmed as *Salmonella* by Salm-PCR. The most prevalent serovar in broiler giblets was *S.* Virchow (80.70%−46/57) followed by *S.* Enteritidis (19.30%−11/57). SE/ST-PCR (%17.54−10/57) could not detect one G isolate, which was serotyped as *S.* Enteritidis by standard serotyping. High relative accuracy (98.25%), sensitivity (100%) and specificity (100%), and agreement between methods (κ: 0.94) verified SE/ST-PCR's potential to be used as an alternative in rapid detection of *S.* Enteritidis and *S.* Typhimurium. Data on high *Salmonella* prevalence in broiler giblets of slaughterhouse origin, and detection of the pathogen by the implementation of all requirements indicated in the revised ISO 6579-1:2017 standard method, enabling the determination of actual prevalence in the samples with high sensitivity and specificity is of significance for public health. Additionally, identification of *S.* Virchow as the dominant serovar followed by *S.* Enteritidis with a relatively lower prevalence, and absence of *S.* Typhimurium in broiler giblets are important findings for Turkiye. This up to date data, obtained by strict application of ISO 6579-3:2014 procedures, indicated a shift in circulating serovars in the broiler industry. The objective findings in this study would bring awareness to national/international literature, and may be of use in future improvements in legal regulations.

Key words: *Salmonella*, broiler, giblet, ISO 6579-1:2017, ISO 6579-3:2014, real-time PCR

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INTRODUCTION

Poultry meat and products remain as the most important source for foodborne salmonellosis despite ongoing national prevention and control programs (CDC, 2022; EFSA, 2022). Giblets of slaughtered broilers as liver, heart and spleen (**LHS**) and gizzard (**G**) have high demand in developing countries due to their comparably lower cost, and in developed countries for their rich nutritive value (iron, magnesium, essential amino acids, protein, vitamin B12, riboflavin, pantothenic acid, niacin, choline, folate), lower fat content and calories (USDA, 2019).

Cross contamination of *Salmonella* to giblets (particularly liver) occurs as a result of the technology used during slaughtering process. These contaminated giblets can enter the retail chain, and be sold under improper conditions as raw or can receive insufficient thermal applications (sauté, stir-fry, deep-fry cooking, pate). Also, they can post-contaminate other (cooked, ready to

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eat, eaten raw) products due to inappropriate segregation of food utensils or designated spaces for raw and cooked food preparations. All these scenarios could end up as salmonellosis cases/outbreaks depending on the dissemination/consumption of the contaminated product (CDC, 1984; CDC, 2012; Lanier et al., 2018).

In recent studies on the prevalence of *Salmonella* in poultry giblets obtained from slaughterhouse or retail stores; 2.2-59.40% of the livers (Telli et al., 2018; Jung et al., 2019; Procura et al., 2019; Yüksel et al. 2019; Adhikari et al. 2020; Liu et al., 2020; Tohamy et al., 2022; Abdelhamed et al., 2023); 0 to 5% of hearts (Acaröz et al., 2018; Telli et al., 2018); 8.10% of spleen samples (Alcantara et al., 2022); and 0 to 20% of the gizzards (Acaröz et al., 2018; Telli et al., 2018; Raji et al., 2021; Tohamy et al., 2022) were found to harbor the pathogen. When the studies involving serotyping were examined, the most frequently identified *Salmonella* serovars were reported as Enteritidis, Typhimurium, Virchow, Infantis, Schwarzengrund and Kentucky. These data indicate that such giblets pose risk to public health from the aspect of their low hygienic quality and possible *Salmonella* carriage. Additionally, under these situations, the poultry sector faces significant economic losses particularly when restrictions applied in exports.

In Turkiye, current Turkish Food Codex (**TFC**) Regulation on Microbiological Criteria Annex 2 on food safety criteria dictates examination of post-chill poultry carcasses for *Salmonella* spp., and if positive, serotyping of the isolates to determine the presence of *Salmonella* Enteritidis (***S.* Enteritidis**) or *Salmonella* Typhimurium (***S.* Typhimurium**) including monophasic *Salmonella* Typhimurium 1,4,[5],12:i:. However, there is no section regarding sampling/sample processing from giblets to determine the presence of the pathogen. This gap would lead to increases in gastroenteritis cases related to the consumption of a neglected and important source of *Salmonella*, with its increasing consumption rate in our country (TFC, 2018).

In 2017, the ISO 6579:2002 standard for the isolation and identification of *Salmonella* from foods was revised to propagate *Salmonella* growth over competing flora, and to enable unbiased identification of all possible serovars present in the sample (ISO, 2017a). Briefly, inclusion of an option in selecting the secondary selective enrichment medium from a semisolid medium as Modified Semi-solid Rappaport Vassiliadis Agar (**MSSRVA**) or Rappaport-Vassiliadis Soya Peptone Broth (**RVSB**), in addition to the mandated enrichment in Mueller Kauffman Tetrathionate Novobiocin Broth (**MKTTnB**) in the revised standard enhanced the survival chance of the pathogen. Also, selection to utilize of a secondary selective enrichment agar medium, next to the primary isolation on Xylose Lysine Deoxycholate Agar (**XLDA**) made the newest version of ISO 6579 less prescriptive, with the objective to obtain well-isolated colonies compared to XLDA.

Current literature on methods used for *Salmonella* isolation from broiler giblets indicated that this was mostly performed by the 2002 version of ISO 6579 (Telli et al., 2018; Hassan et al., 2019; Hassan et al., 2020; Raji et al., 2021; Abdel-Kader et al., 2022; Alcantara et al., 2022; Tohamy et al., 2022), by USDA-FSIS method (Jung et al., 2019), APHA method (Saleh et al., 2022), and by other isolation and identification approaches (Byomi et al., 2019; Procura et al., 2019; Adhikari et al. 2020). Additionally, rapid and reliable PCR-based *Salmonella* confirmation tests accompanied culture methods (Telli et al., 2018; Jung et al., 2019; Liu et al., 2020; Raji et al., 2021; Abdelhamed et al., 2023). In most of the above studies, *Salmonella* serotyping was performed by conventional methods, while none indicated using ISO 6579-3:2014 (ISO, 2014) standard method for this aim.

Up to our knowledge, there are only 3 studies on broiler giblets, which reported using ISO 6579-1:2017 to determine *Salmonella* from slaughterhouse livers (Abdelhamed et al., 2023), retail giblets (Ndlovu et al., 2023), and retail livers (Yüksel et al. 2019). When methods used in these studies were examined in detail, the flow for *Salmonella* detection indicated that it was not ISO 6579-1:2017, but contrarily was a method similar to ISO 6579:2002. All this reveals the absence of a study in literature, which yet actually applied the requirements indicated in the revised *Salmonella* standards to objectively define the prevalence and serovars in broiler giblets.

Thus, this study mainly aimed to determine the prevalence and confirm *Salmonella* in broiler LHS and G samples by ISO 6579-1:2017 and by *Salmonella*-specific real-time PCR (**Salm-PCR**). Secondly, this study aimed to identify the currently circulating *Salmonella* serovars by standard serotyping, and to evaluate *S.* Enteritidis and *S.* Typhimurium specific real-time PCR (**SE/ST-PCR**)'s verification efficiency to ISO 6579-3:2014.

MATERIALS AND METHODS

Samples

A total of 60 units of LHS and G samples were collected from 3 different (A, B, and C) slaughterhouses located in Bursa and Balıkesir, with average slaughter capacity (broiler/d) of 185.000, 200.000 and 280.000, respectively. These plants, which hold the highest broiler population in Turkiye and received broilers from Marmara and Aegean regions, were visited between August 2021- January 2022 for a period of 6 months.

Standard Strains

Salmonella isolation, serotyping, Salm-PCR and SE/ST-PCR was performed using *S.* Enteritidis 64K (M. Y. Popoff, Institut Pasteur, Paris Cedex 15, France), and *S.* Typhimurium NCTC 12416 (Refik Saydam, Turkish Public Health Agency, Ankara) as positive controls.

Sampling

Each slaughterhouse was visited twice, and 5 units of LHS (1 LHS unit = 9-10 liver, 6-7 heart and 3-5 spleen); and 5 units of G (1 G unit = 20 gizzard) with different lot registration numbers were sampled per visit, totaling 20 sample units per plant (Kokoszyński et al., 2016). All samples were transferred to the laboratory in coolers and examined for the presence of *Salmonella* within 2 h (ISO, 2017b).

Detection of Salmonella by ISO 6579-1:2017

For this, in preenrichment, 1 LHS or G sample unit was homogenized by massaging from outside the sample bag. From this homogenate, approximately 25 to 30 g sample, comprised of 1/2 liver, 1 heart and 1 spleen, was weighed, and homogenized in stomacher after adding 225 mL buffered peptone water (**BPW** - Oxoid, CM1049) and incubated at 37°C for 18 h (ISO, 2017a, ISO, 2017b). For selective enrichment of samples; 1) One milliliter of BPW was transferred to 10 mL Mueller Kauffman Tetrathionate Novobiocin Broth (**MKTTnB** - Oxoid, CM1048), and incubated at 37°C for 24 h. 2) One hundred microliters of BPW was transferred to 10 mL Rappaport-Vassiliadis Soya Peptone Broth (**RVSB** - Oxoid, CM0866), while 3) another 100 μL BPW was inoculated on to Modified Semi-solid Rappaport Vassiliadis Agar (**MSSRVA** - Oxoid, CM1112), the newly added selective enrichment medium in the revised standard, and incubated at 41.5°C for 24 h. Selective enrichment was applied by using 2 solid agar media, where 20 μL from MKTTnB, RVSB and MSSRVA was streaked on to both Xylose Lysine Deoxycholate Agar (**XLDA** - Oxoid, CM0469), and Brilliance Salmonella Agar (**BSA** - Oxoid, CM1092) supplemented with Salmonella Selective Supplement (Oxoid, SR0194), and incubated at 37°C for 24 h. One well-isolated *Salmonella* suspect typical colony on one of the selective plates was selected, and subjected to confirmation by biochemical identification on TSI, LIA, urea, ß-galactosidase and indole tests, and API 20E (Biomerieux, 20100). In case of a negative result, 4 typical colonies from XLDA or BSA were subjected to confirmation tests (ISO, 2017a). Pure isolates were stored at −20°C.

Confirmation by Salm-PCR

Template DNA from biochemically identified *Salmonella* isolate was obtained following the procedure in Foodproof StarPrep One Kit (Biotecon, S400 07, Germany) manual. Briefly, each frozen culture stored in −20°C was thawed, homogenized by vortexing (Stuart, SA8), and centrifuged at 8,000 × *g* for 5 min (Thermo Scientific, MicroCL 17, Waltham, MA). After the supernatant was removed, 200 μL lysis buffer was added to the pellet, vortexed, incubated at 97.5°C in a block heater (Techne, DB-2D-FDB02DD) for 10 min, followed by a final vortexing and centrifugation at 13,000 × *g* for 2 min. Each template DNA with a concentration of 100 ng/μL, and absorbance values between 1.6 and 2.0 (Nanodrop Spectrophotometer, Thermo Scientific, ND-1000, Waltham, MA) was used as template for Salm-PCR analysis (Light Cycler 2.0 Instrument, Roche, 03531414201, Germany), which was performed by using a Foodproof *Salmonella* Detection Kit (Biotecon, R310 27, Germany). The total PCR reaction volume was 20 μL comprised of 5 μL of template DNA added into 15 μL PCR mix (13 μL of Foodproof *Salmonella* Master Mix [ready-to-use primer and hybridization probe mix], 1 μL of Foodproof *Salmonella* Enzyme Solution [FastStart Taq DNA Polymerase and heat labile Uracil-DNA Glycosylase], 1 μL of Foodproof *Salmonella* Internal Control. The Foodproof *Salmonella* Control template DNA and PCR-grade water were used as PCR positive and negative controls, respectively. The amplification protocol (pre-incubation: 2 min at 40°C and 10 min at 95°C; amplification: 45 cycles of 0 s denaturation at 95°C, binding and fluorescence signal readout at the end for 30 s at 59°C, and 5 s elongation at 72°C; and cooling for 30 s at 40°C) and data analysis was performed as indicated in the kit insert, and by the LightCycler software version 4.1, respectively.

Serotyping by ISO 6579-3:2014

Serological identification of *Salmonella* isolates was performed by following the procedures of ISO 6579-3:2014, and with reference to White-Kauffmann-Le Minor Scheme (Grimont and Weill, 2007), to Guibourdenche et al. (2010), and to Issenhuth-Jeanjean et al. (2014) by using commercial O-, H-, and Vi-antisera (Becton-Dickinson). Slide agglutination and tube agglutination tests were applied for the analyses of somatic, capsular, and flagellar phase antigens, respectively. Procedure was carried out using the scheme for serotyping the 5 important *Salmonella* serovars (Enteritidis, Typhimurium, Infantis, Virchow, Hadar) of public health concern specified in Annex D of the standard (ISO, 2014). Also, for confirmatory purposes, selected *Salmonella* isolates were serotyped by the National Enteric Pathogens Reference Laboratory (Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products).

Evaluation by SE/ST-PCR

SE/ST-PCR analysis was performed using the template DNA isolated in "Confirmation by Salm-PCR" section, and Foodproof *Salmonella* Enteritidis & Typhimurium Detection Lyokit (Biotecon Diagnostics, R 602 34, Germany) in LightCycler 480 (Roche Diagnostics, 05015278001, Germany) instrument with FAM, HEX and ROX channels, which were required to detect *S.* Enteritidis, *S.* Typhimurium and Internal

Table 1. Results on determination and confirmation of *Salmonella* including serotyping and SE/ST- PCR.

		ISO (%)			PCR (%)		
			Serovar				
Sample type (n)	Slaughterhouse (n = 10)	*Salmonella*	Enteritidis (1,9,12:g,m:-)	Virchow (6,7,14:r:1,2)	spp.	SE/ST	Non-SE/ST
Liver-Heart-Spleen (30)	A	10	4	6	10	4	6
	B	10	2	8	10	2	8
	C	10	0	10	10	0	10
		30	6 (20.00)	24 (80.00)	30 (100.00)	6 (20.00)	24 (80.00)
Gizzard (30)	A	10	1[1]	9	10	0[1]	10
	B	8	0	8	8	0	8
	C	9	4	5	9	4	5
		27	5 (18.52)	22 (81.48)	27 (90.00)	4 (14.81)	23 (85.19)
Total (60)		**57 (95.00)**	**11 (19.30)**	**46 (80.70)**	**57 (95.00)**	**10 (17.54)**	**47 (82.46)**

[1]One isolate, which was identified as serovar Enteritidis by standard serotyping, was not detected by SE/ST-PCR.

Amplification Control (**IC**), respectively. Specific PCR tube strips for 96 reactions with prefilled lyophilized reagents (primer and hydrolysis probes specific for *S.* Enteritidis and *S.* Typhimurium DNA, IC, Taq DNA Polymerase, heat-labile Uracil-DNA Glycosylase) were placed on a PCR port tube, and 25 μL of template DNA from each sample was placed into microplate wells. Also, 25 μL PCR-grade H_2O, and 25 μL Foodproof *Salmonella* Enteritidis & Typhimurium Detection Control were placed into separate wells for negative and positive control, respectively. Sealed strips were vortexed for 30 s at 200 × *g*, and placed into the instrument. The amplification protocol started with a one cycle pre-incubation with 2 steps (step 1: 4 min at 37°C, step 2: 5 min at 95°C), and followed by a 50-cycle amplification comprised of step 1 for 5 s at 95°C, and step 2 for 60 s 60°C, where fluorescence reading was automatically detected by the instrument. Data analysis was performed as indicated in the kit insert, and by the LightCycler software as comparisons of fluorescence readings from channel FAM for *S.* Enteritidis, channel HEX for *S.* Typhimurium, and channel ROX for IC for each sample, and the results were interpreted accordingly.

Statistical Analyses

Evaluation of SE/ST-PCR results as alternative to reference method of standard serotyping for relative accuracy, sensitivity, and specificity was performed according to ISO 16140-6:2019 (ISO, 2019). The degree of confidence of alternative method to the reference method was determined by Cohen's kappa (κ) test (Landis and Koch, 1977). This enabled determination of SE/ST-PCR's typing efficiency for *S.* Enteritidis and *S.* Typhimurium.

RESULTS

In this study, all LHS samples (30/30, %100.00), and 27 out of 30 G samples collected from 3 slaughterhouses were found to carry *Salmonella*, with an overall prevalence of 95% (57/60), regardless of the sample type. All 57 *Salmonella* isolates were determined as *Salmonella* by Salm-PCR analysis. Standard serotyping results indicated the dominant serovar as *S.* Virchow (6,7,14:r:1,2) (46/57, 80.70%) followed by *S.* Enteritidis (1,9,12:g,m:-) (11/57, 19.30%) regardless of the sampled slaughterhouse. When evaluated by sample type, majority (24/30, 80%) of the LHS isolates from all slaughterhouses were serovar Virchow, while 20% (6/30) were serovar Enteritidis. Similarly, G isolates' serovars were mostly Virchow 81.48% (22/27) followed by Enteritidis 18.52% (5/27), as well (Table 1).

Based on SE/ST-PCR results, 10 out of 57 isolates (17.54%) were *S.* Enteritidis, while remaining 47 isolates (82.46%) were serovars other than *S.* Enteritidis and *S.* Typhimurium. Six out of 30 LHS isolates (20%), and 4 out of 27 G isolates (14.81%) were *S.* Enteritidis, while the remaining 23 G isolates were not detected as *S.* Typhimurium. When standard serotyping and SE/ST-PCR findings were evaluated collectively, 19.30% (11/57) and 17.54% (10/57) of the isolates were *S.* Enteritidis, respectively, while none of them were *S.* Typhimurium (Table 1).

Statistical analysis results revealed that SE/ST-PCR had accuracy, sensitivity and specificity values of 98.25, 100, and 100%, respectively. In comparison to standard serotyping, this indicated an almost perfect (0.81-1.00) agreement between methods with a κ = 0.94 degree of confidence (Table 2).

Table 2. Relative accuracy, sensitivity and specificity results of the SE/ST-PCR.

Reference method ISO		Alternative method SE/ST-PCR					
Positive	Negative	$False_{negative}$	$False_{positive}$	Accuracy (%)	Sensitivity (%)	Specificity (%)	κ
10	46	1	0	98.25	100.00	100.00	0.94

κ: Cohen kappa coefficient.

DISCUSSION

One of the biggest challenges in our study was the obligation to dig through all previous studies covering similar subjects to ours, and to determine whether which ones literally used 2 enrichment media (mandatory selective enrichment in MKTTnB and RVSB or one newly added semisolid medium to the revised standard as MSSRVA), and utilized a secondary selective enrichment agar medium in addition to the primary isolation on XLDA, as mandated in the newest version of ISO 6579. Since we strictly applied the requirements emphasized in the revised ISO 6579-1:2017 (ISO, 2017a) in our work, we wanted to have a fair comparison of our data to studies, which claimed using the same version of ISO 6579 for the detection of *Salmonella*, preferably from broiler giblets of slaughterhouse origin. Thus, when the methods section of a single study by Abdelhamed et al. (2023), where the authors reported a 2.2% *Salmonella* prevalence in slaughterhouse-level chicken giblet samples collected from a small-scale slaughterhouse by using revised ISO 6579-1:2017 was investigated, use of only 1 selective enrichment broth (**RVSB**), and only one type of selective agar (**XLDA**) was determined. In other 2 studies, Ndlovu et al. (2023) and Yüksel et al. (2019) reported that 13% of the chicken giblets (gizzard, heart and liver) and 27% of the liver samples carried the pathogen by using the revised method, respectively. However, in those studies, the samples originated from retail markets opposed to our slaughterhouse samples.

Current studies investigating the presence of *Salmonella* in broiler giblet mostly used ISO 6579:2002, the former version of the standard. Within these, where sampling was done in slaughterhouses, 8.10% of the spleen samples and 9.60% of the spleen, liver, kidney, and heart samples were found positive for *Salmonella enterica* (Alcantara et al., 2022), and *Salmonella* (Hassan et al., 2020), respectively. In studies, where samples were collected from retail points, *Salmonella* prevalence was 0% in livers (Tok et al., 2023), 10.07% in liver and gizzards (Abdel-Kader et al., 2022), 5% in livers and 1.66% in gizzards (Tohamy et al., 2022), 64% in liver, heart and gizzards (Hassan et al., 2019), and 17.50, 20, and 0% in liver, heart and gizzards (Telli et al., 2018), respectively. In a study, where the performance of a culture procedure with a latent one-way primary/pre-enrichment against direct streaking on to a differential medium was evaluated, 4.80% of chicken liver samples collected from different slaughterhouses were found to be contaminated with *Salmonella* (Procura et al., 2019). Fowler et al. (2021) reported that 17.78% of heart, liver and spleen samples of slaughterhouse origin was positive for *Salmonella* spp. In studies, where samples were collected from retail sale points, *Salmonella* detection rate was indicated as 26.67% in giblets by APHA method (Saleh et al., 2022), 10% in ready-to-eat gizzards with a method recommended by WHO (Raji et al., 2021), 9.70% in chicken livers (Liu et al., 2020), 30.50% in livers (Adhikari et al., 2020), 68.42% in liver, gizzard, heart by a modified and the former version of ISO 6579 (Byomi et al., 2019), and 59.40% in chicken livers with BAX PCR applied parallel to USDA-FSIS method (Jung et al., 2019).

Our *Salmonella* prevalence rate of 95% (Table 1) stood much higher than all above studies using giblet samples, with the lowest (0%) and the highest (68.42%) prevalence rates with an average of 30 to 50%. The most important factor for high *Salmonella* prevalence is related to the effective implementation of the country's national control program. All other sub-factors arise from inefficiencies in these types of programs, and can be linked to the broiler (carriage state, stress during transfer, pre-slaughter practices, and cross-contamination during slaughter), to the sample/sampling (type, variety, sampling site - retail or slaughterhouse), to other factors (region, season, period), as well the methodological differences or omission of application of essential requirements indicated particularly in standard methods, which would definitely affect the outcomes of the studies. For example, in our study, we used all 3 selective enrichment media mandated in the revised standard, which both enabled us to evaluate the selectivity performances of these media for our sample types, and also believe that this helped us to obtain an unbiased growth/survival milieu for the *Salmonella* serovars present at the time of sampling, which would otherwise could have left undetected. Thus, we strongly believe that one reason for our high *Salmonella* prevalence rate was related to using MSSRVA in selective enrichment, which has isolation superiority over other 2 enrichment media for our sample type. In detail, when 9 out of 57 (15.79%) positive samples were negative in MKTTnB and RVSB enrichment media, they were positive in MSSRVA (data not shown in the table).

The most prevalent serovar in our work was as *S.* Virchow followed by *S.* Enteritidis (Table 1). In previous studies reporting Virchow in their giblet samples by using conventional serotyping, Abdel-Kader et al. (2022) found *S.* Virchow (15.38%) as the predominant serovar followed by *S.* Enteritidis (7.69%) and *S.* Typhimurium (7.69%) in liver and gizzard samples in Egypt. Another study from the same country, *S.* Virchow (12.5%) prevalence in heart, liver and gizzard samples was lower than *S.* Infantis (50%) and *S.* Kentucky (25%) (Hassan et al., 2019). In Fowler et al. (2021)'s research from Nepal, *S.* Virchow (5%) was listed in the 3 most prevalent serovars identified from liver, heart and spleen samples after *S.* Typhimurium (49%) and *S.* Enteritidis (35%). The prevalence rates of *S.* Virchow in these studies are much lower than our findings (80.70%) (Table 1). In Turkiye, up to our knowledge, despite no up-to-date data on *Salmonella* serovars identified from broiler giblets, a retail chicken meat study indicated *S.* Virchow (31.23%) as the second most dominant serovar in their samples (Tok et al., 2023). *S.* Enteritidis was isolated as the second most prevalent serovar (19.30%) in this study. Within other recent reports, our prevalence rate was parallel to Procura et al. (2019) (18%), and Ndlovu et al. (2023) (23.07%), higher than Abdel-Kader et al. (2022) (7.69%), and lower than Fowler et al.

(2021) (35%), and Byomi et al. (2019) (33.30%). While *S.* Enteritidis and *S.* Typhimurium were reported as the dominant serovars for almost 10 yr in Turkiye (Cufaoglu et al., 2023), our findings nearly opposed to this by a decrease in and an absence of serovar Enteritidis and Typhimurium, respectively. There are also similar reports by several authors (Telli et al., 2018; Adhikari et al., 2020; Raji et al., 2021; Alcantara et al., 2022; Abdelhamed et al., 2023), who reported no identification of *S.* Typhimurium in their samples. In our study, the dominancy of *S.* Virchow over *S.* Enteritidis, and the absence of *S.* Typhimurium in giblet samples is a significant and novel finding for Turkiye. Serovar Virchow has high invasion capability, antimicrobial resistance capacity, and increasing prevalence rate (Na et al., 2020), and has been reported in chicken meat-related outbreaks since 2017 (ECDC-EFSA, 2023). Therefore, it is noteworthy to monitor this serovar in terms of public health and industrial practices.

By our standard serotyping and SE/ST-PCR, 19.30% and 17.54% of the isolates were determined as Enteritidis. This difference arises from one gizzard sample, which was found positive for serovar Enteritidis by standard serotyping, while it was negative in SE/ST-PCR (Table 1). Still, SE/ST-PCR's high agreement to standard serotyping (κ: 0.94) (Table 2) indicates that it can be a good alternative for rapid detection of these 2 serovars, where urgent results for broiler industry settings are required. Up to our knowledge, there is no study using both conventional/standard serotyping and *S.* Enteritidis and *S.* Typhimurium specific PCR, which then evaluated the effectiveness of the rapid method. Therefore, we could not discuss this finding of ours to any other study. Regardless, among studies detecting Enteritidis and Typhimurium serovars only by rapid methods, Hassan et al. (2020) found that giblet samples were 75% and 25% positive for *S.* Enteritidis and *S.* Typhimurium by serovar-specific PCR, while Telli et al. (2018) reported absence of either serovars in liver, heart and gizzard samples by *S.* Enteritidis and *S.* Typhimurium specific duplex PCR.

CONCLUSIONS

Results of this study revealed that inclusion of the mandated requirements while implementing ISO 6579-1:2017 standard method had a significant effect on our high (95%) *Salmonella* prevalence in broiler giblets. This emphasizes the wide consumption of these contaminated giblet products in Turkiye has the potential to pose hazard in public health. Serotyping by ISO 6579-3:2014 standard method indicated a change in circulating serovars in broiler industry in Turkiye by the predominance of *S.* Virchow (80.70%), comparably lower prevalence of *S.* Enteritidis (19.30%), and absence of *S.* Typhimurium. We believe these findings would serve as objective data for national/international literature, and for legal authorities in updating regulations related to tolerance and serovars of *Salmonella*.

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DISCLOSURES

The authors declare no conflicts of interest.

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