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Original Scientific Paper

Detection of *Salmonella spp.* in clinical and food samples from poultry (2020–2025): a broiler farm outbreak case study - highlighting biosecurity and economic implications

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ABSTRACT

This study analyzed the positive rate of *Salmonella* spp. in chicken primary production and food samples examined at the Scientific Institute of Veterinary Medicine of Serbia between 2020 and 2025 and evaluated biosecurity measures on a broiler farm during an outbreak. A total of 2,728 primary production samples and 6,289 food samples were tested. *Salmonella* was detected in 3.23% of the primary output and 9.76% of food samples, with S. Enteritidis being the most common serovar. A detailed biosecurity assessment of the affected farm revealed an overall score of 90%, surpassing national and global averages, yet it identified critical gaps in broiler depopulation, carcass removal, and cleaning protocols. An economic analysis showed a loss exceeding 58.2% per production cycle of 10,000 broilers due to market restrictions following *Salmonella* detection. These findings highlight the need for continuous biosecurity improvements and rigorous surveillance to mitigate *Salmonella* risk and reduce financial impacts in poultry production.

1. Introduction

Salmonella spp. is globally recognized as a significant zoonotic foodborne pathogen, posing major public health concerns and causing substantial economic losses in both animal production and human healthcare systems (*Majowicz et al*., 2010). Foodborne illness remains a pressing issue in Serbia. In 2023, salmonellosis was the most frequently reported bacterial enteric disease in humans in Serbia, with 1,217 confirmed cases. Food was identified as the transmission route in 14 of the 21 recorded outbreaks that year (*Institute of Public Health of Serbia "Dr Milan Jovanović Batut"*, 2024). However, this represents the number of reported cases, while the actual number is assumed to be significantly higher due to underreporting. In response, both veterinary and human health sectors are actively working to control and reduce the presence of *Salmonella* along the food production chain. Collaboration between these two sectors is of particular importance in addressing the issue of antimicrobial resistance. A particularly serious concern is the rise of multidrug-resistant *Salmonella* strains (*Petrović et al*., 2015; *Nikolić et al*., 2017; *Kureljušić et al*., 2017; *Kureljušić et al*., 2021). Emerging antimicrobial-resistant variants continue to pose a significant threat to both animal and public health (*Wigley*, 2024). In the Serbian poultry industry, the predominant serovars are *Salmonella* Enteritidis, *S.* Infantis, and *S.* Typhimurium (*Velhner et*

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al., 2011; *Spalević et al*., 2023). Poultry is considered a primary source of human salmonellosis, primarily transmitted through the consumption of contaminated meat and eggs (*Shaji et al*., 2023). Birds can become infected during production through various routes, including contaminated feed, litter, water, aerosols, and contact with vectors such as rodents, insects, or other animals (*O'Bryan et al*., 2022). Complete eradication of *Salmonella* is deemed unrealistic. Therefore, effective control strategies rely on vaccination—particularly with multivalent vaccines—combined with strict biosecurity measures and robust surveillance systems capable of identifying production risks, antimicrobial-resistant strains, and serovars not covered by vaccines (*Wigley*, 2024). To reduce the incidence of human salmonellosis, veterinary authorities must implement control measures at all stages—from poultry rearing and production to slaughter and processing, as well as consumer education (*Velhner et al*., 2005; *Official Gazette of RS*, 2024).

This study aimed to analyze the positive rate of *Salmonella* spp. and its serotypes in primary production and food samples collected between 2020 and 2025, to investigate potential transmission pathways on broiler farms, and to assess the associated financial impact in the event of an outbreak.

2. Materials and methods

At the Scientific Institute of Veterinary Medicine of Serbia, a total of 2,728 chicken primary production samples were analyzed for *Salmonella* over five and a half years. These samples were chicken droppings from broiler, laying hens, and breeder flocks; incubated eggs (both unhatched eggs at the end of the hatching process and hatched eggshells); transport crates and carcasses from the transport and the first three days of life. Primary production specimen sampling was conducted per local regulations (*Official Gazette RS*, 2018; *Official Gazette RS*, 2024).

Additionally, 6,289 food samples from broiler slaughterhouses—broiler meat, meat products, neck skin, and environmental swabs from food-processing surfaces (such as working tables, knives, cutting boards, and equipment in contact with raw poultry)—were included in the study. The *Salmonella* spp. detection, isolation, and serotyping were performed according to respective parts of *ISO 6579* (2017).

To explore potential transmission pathways of *Salmonella* on broiler farms and the associated financial costs, a case study was conducted on a farm that experienced an outbreak in the early months of the year. This farm houses 10,000 broiler chickens in a single production unit and completes approximately 4.5 production cycles annually, introducing new birds every 11 to 12 weeks. Broilers are typically slaughtered between 42 and 56 days of age, resulting in a downtime of at least 14 days between cycles. The pathogen was detected in fecal samples collected within three weeks before slaughter. Farm observations and assessments of biosecurity measures were conducted using a standardized broiler farm checklist. The Biocheck.UGent risk-based scoring system (http://www.biocheck.ugent.be) was employed to evaluate broiler farm biosecurity. The overall score was calculated as the average of the internal and external biosecurity scores. The veterinarian and farm owner were informed of the study's purpose and process before filling out the questionnaire. The farm owner provided written informed consent for data collection, sharing, and publication. During the visit, their responses were compared with actual on-farm observations, and the correct answers were recorded accordingly.

To assess the financial impact of the *Salmonella*-positive finding, a partial budget analysis (*Cai et al*., 2024) was conducted based on actual production costs and market prices. The farm raised 10,000 broilers in a single cycle using its facilities (i.e., no rental costs). Costs included the purchase of one-day-old chicks (0.42 €/chick), electricity (593 €), water (150 €), heating (590 €), vaccines and vitamins (491 €), laboratory analyses (200 €), labor for night shifts (1,200 €), labor for bird loading (700 €), and feed (18,870 €). An average mortality rate of 4% was assumed based on previous flock data. At the time of the *Salmonella* detection (boot swab sampling), the birds had reached an average body weight of 3,270 g. Due to the positive result, broilers could only be sold to slaughterhouses authorized to process meat for thermal treatment, at a significantly reduced price of 0.64 €/kg, compared to the regular market price of 1.10 €/kg. The economic loss was calculated as the difference between potential and realized income, adjusted for actual mortality.

3. Results

Detection rates of Salmonella spp. (2020-2025)

During the examined period (2020-2025), out of a total of 2,728 chicken primary production samples, *Salmonella* spp. was found in 88 (3.23%)

samples (Table 1). The highest positive rate was recorded in 2022 (10.29%), while the lowest was in 2023 (0.75%). Serotyping revealed 5 *Salmonella* serovars. The most frequently isolated serovar was *S.* Enteritidis (69.31%), followed by *S.* Typhimurium (21.59%), *S.* Infantis (4.54%), *S.* Mbandaka (2.27%), and *S.* Senftenberg (2.27%), as shown in Table 1.

The highest number of positive samples, serovar *S.* Enteritidis, was detected in the samples originating from broiler flocks (Table 2).

Concerning the broiler flock, besides *S.* Enteritidis, *S.* Typhimurium, and *S.* Infantis were also isolated (Fig. 1).

Table 1. Positive rate of *Salmonella* spp. and *Salmonella* serovars in primary production samples in the period 2020-2025.

	2020	2021	2022	2023	2024	2025	Total
Number of analyzed samples	856	624	379	266	405	181	2,728
Number of positive samples	20	19	39	2	4	4	88
Positive rate (%)	2.33	3.04	10.29	0.75	0.99	2.21	3.23
S. Enteritidis	16	4	37	-	-	4	61
S. Typhimurium	2	11	-	2	4	-	19
S. Infantis	-	4	-	-		-	4
S. Mbandaka	2	-	-	-	-	-	2
S. Senftenberg	-	-	2	-	-	-	2

Table 2. Number of S. Enteritidis within the flocks in the period 2020-2025.

Category	2020	2021	2022	2023	2024	2025
Parent flock	4	0	0	0	0	0
Layers	0	0	2	0	0	2
Broilers	12	4	35	0	0	2

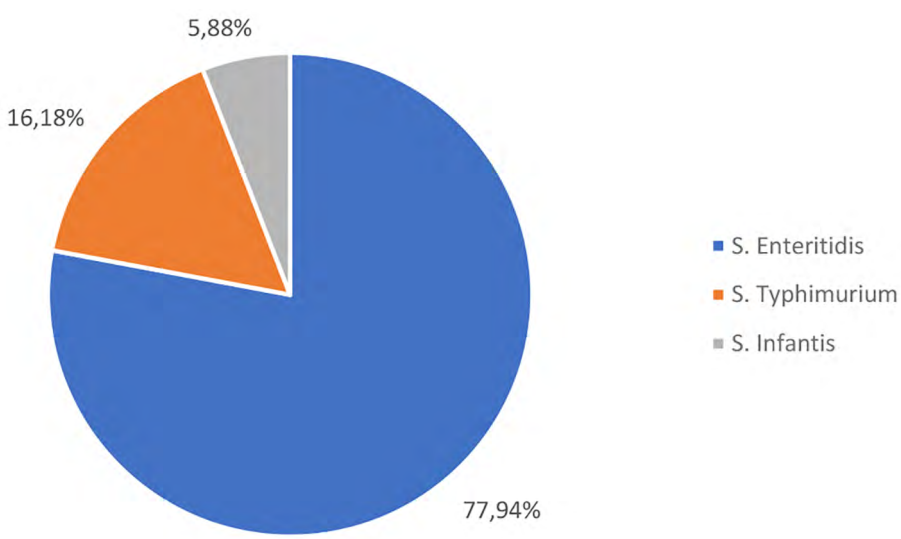

Figure 1. *Salmonella* serovars identified in the samples originating from broiler flocks 2020–2025

Table 3. Positive rate of *Salmonella* spp. in food samples from broiler slaughterhouses in the period 2020-2025.

	2020	2021	2022	2023	2024	2025	Total
Number of analyzed samples	848	1151	1323	1225	1330	412	6289
Number of positive samples	65	145	92	178	90	44	614
Positive rate (%)	7.66	12.60	6.95	14.53	6.77	10.69	9.76

During the period 2020-2025, out of a total of 6,289 food samples, *Salmonellae* spp. were detected in 614 (9.76%) samples (Table 3). The highest positive rate was recorded in 2023 (14.53%), and the lowest in 2024 (6.77%).

Biosecurity assessment result (case farm)

During the farm visit, it was noted that the farm had a biosecurity plan in place, with defined measures related to both external and internal biosecurity. Summarized results of the biosecurity evaluation is presented in Table 4.

The overall biosecurity score was 90%, which was higher than the national and global average. The national average presents the biosecurity scores that were taken from the Biochek.UGent database, and were obtained by the completion of 46 questionnaires.

The external biosecurity score and internal biosecurity score for the farm with *Salmonella* contamination were both 90%, with the following specific deficits. Notably, broiler depopulation, manure, and carcasses are subcategories within the category of external biosecurity, and had the lowest mean score for this farm compared to other categories. Usually, depopulation of flocks is performed in three to four steps. Farmworkers and part-time, paid workers from outside who are involved in the poultry depopulation process were not provided with specific clothing, shoes, or gloves—so no measures were in place at the beginning of the process. The loading truck arrived empty, cleaned, and disinfected; however, the catching process was carried out by a large group of workers who failed to adhere to biosecurity protocols during the operation. The carcass storage facility was enclosed and equipped with refrigeration; however, it is not situated within a designated dirty area of the farm and lacks adequate separation from the farm's production units. Also, we noticed there was no explicit signage or formal notice restricting access to the poultry houses for individuals (employees or visitors) without prior registration. While such a rule was assumed, it was neither visibly communicated nor consistently enforced.

Table 4. Biosecurity evaluation results on the observed farm

Biosecurity assessment	Farm (%)	National Average* (%)	Global Average (%)
One-day-old chick purchasing	90	62	67
Broiler depopulation	69	48	65
Feed and water supply	92	60	62
Manure and carcass removal	88	39	67
Farm workers and visitors	100	73	76
Material supply	100	81	70
Infrastructure and biological vectors	89	81	82
Farm location	100	73	68
External biosecurity score	90	65	70
Disease management	88	77	80
Cleaning and disinfection	87	54	71
Measures between compartments	100	71	75
Internal biosecurity score	90	66	75
Overall biosecurity score	90	65	72

*National Average - taken from the Biochek.UGent database, average obtained by completing 46 questionnaires.

Concerning internal biosecurity, the lowest score was obtained for the cleaning and disinfection (87%) subcategory. The farm implemented its own cleaning, disinfection, and rodent control procedures without engaging a professional service provider. While a specific protocol was followed after each production cycle, the effectiveness of these measures was not verified—no microbiological testing of surface swabs or water and feed lines was conducted. At the beginning of each production cycle, before one-day-old chicks were housed, the farm routinely introduced and then disinfected the bedding material. However, to maintain litter quality, the farm periodically added straw during the cycle. In such cases, the supplemental straw was introduced into the production unit without prior disinfection.

The total production cost for the cycle was estimated at 26,994 €. Assuming a 4% mortality (9,600 marketable birds), the expected live weight yield was approximately 31,392 kg (9,600 birds × 3.27 kg). Under normal conditions, the expected gross income from the sale would have been approximately 34,531.20 € (31,392 kg × 1.10 €/kg). Despite an expected net profit of approximately 7,537 € (≈27.9%) under normal market conditions, the confirmed detection of *Salmonella* and subsequent market restrictions led to a net financial loss of around 6,903 € for the production cycle. This corresponded to a total negative financial impact of approximately 14,440 € (≈58.2%) compared to normal conditions, underscoring the critical economic importance of effective biosecurity measures and preventive strategies in broiler production (Table 5).

Table 5. Partial budget analysis of a 10,000-bird broiler flock affected by *Salmonella*: Estimated costs and revenue losses

Category	Value (EUR)
Total production cost	26994
Marketable birds*	9600
Total live weight (kg)	31392
Expected income (normal market)	34531.2
Actual income (Salmonella restriction)	20090.88
Income loss due to Salmonella	14440.32
Net result (normal conditions)	7537.2
Net result (Salmonella case)	-6903.12

*Assumed mortality rate is 4%, meaning that 4% of the birds did not survive or were not marketable: 10,000 × (1 - 0.04) = 10,000 × 0.96 = 9,600 birds

4. Discussion

This study revealed a dynamic pattern of *Salmonella* positivity rates in the broiler production chain from 2020 to 2025. Notably, in 2021, a significant discrepancy was observed between primary production and food chain contamination: while the *Salmonella*-positive rate in samples from broiler farms was elevated, the highest incidence throughout the entire study period was found in food samples. In contrast, in 2022, the situation reversed, with a higher positive rate at the farm level compared to food products. The data also indicate that in 2022, the positive rate of *Salmonella* in food samples was low, while the highest positive rate in primary production samples—originating from broiler farms—was recorded in the same year. Notably, in 2023 and 2024, *Salmonella* was not detected in any samples from broiler farms. However, in 2023, food samples collected from broiler slaughterhouses showed the highest *Salmonella* positive rate recorded during the entire 2020–2025 period.

These fluctuations could have partly arisen from the effects of the COVID-19 pandemic. Concerns about the transmission of infection through the food system led to measures such as surface sanitation, improved hygiene in workplaces, safe food handling and distribution practices, and physical distancing protocols (*Rizou et al*., 2020). According to *FAO* (2020), consistent implementation of hygiene and food safety practices—regardless of the production system—can significantly reduce the risk of contamination with harmful pathogens. Strengthening such measures, as outlined in Codex guidelines (*FAO*, 2020), likely contributed to the reduced

contamination levels in food products observed in 2022, while also alleviating pressure on public health systems during the pandemic. Additionally, the observed discrepancy could stem from differences in sample origin—clinical samples were predominantly collected from the Belgrade area, whereas food samples originated from a broader geographic region across Serbia, which could influence the representativeness and comparability of the data.

Concerning the results from the case farm and established biosecurity protocols, all materials entering a poultry house during the production cycle—including bedding—should undergo proper hygiene measures to minimize the risk of introducing pathogens. The introduction of non-disinfected straw during the cycle, as observed on the farm, created a potential biosecurity gap. Best practices recommend that any additional litter material be stored in a clean, protected area and disinfected before entry, or that litter management strategies be designed to avoid the need for mid-cycle additions (*Ivanov*, 2001; *Voss-Rech et al*., 2017; *Course et al.*, 2021). Additionally, the farm should verify the efficacy of the applied protocol through methods such as bacteriological testing, as visual inspection alone is not a reliable indicator of hygiene status in broiler houses (*Luyckx et al*., 2015; *Maletić et al*., 2023).

A partial budget analysis demonstrated the severe economic consequences that *Salmonella* outbreaks can have on broiler production. These findings underscore the crucial importance of implementing and maintaining effective biosecurity measures. Preventive actions protect animal health and preserve the farm's economic viability by minimizing the risk of costly disease outbreaks (*Maletić et al*., 2023; 2025).

5. Conclusion

This study confirmed the continued presence of *Salmonella* spp. in broiler production and food samples in the samples received between 2020 and 2025, with *S.* Enteritidis as the dominant serotype. Although the assessed farm had a high overall biosecurity score, critical weaknesses were identified. The substantial financial losses observed underline the importance of continuous surveillance and strict adherence to biosecurity protocols as essential strategies for reducing *Salmonella*-related risks in poultry farming.

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