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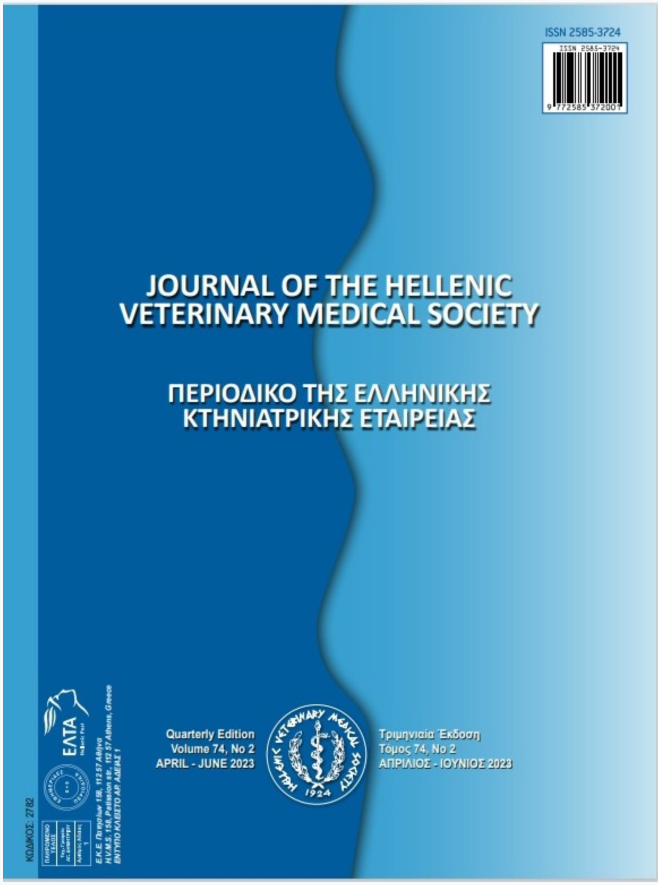

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A retrospective study on the isolated strains of *Salmonella* in poultry

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ABSTRACT: Poultry represents a significant global reservoir of *Salmonella* spp. Poultry salmonellosis results in significant economic losses, and some serovars also have zoonotic potential. Some poultry can be infected with *Salmonella* species without showing clinical signs of the disease, but it is a significant source of the spread of the infection. Clinical symptoms range from digestive problems (white diarrhea, malabsorption) to bacteremia and death. This study aimed to retrospectively show the presence of bacteria *Salmonella enterica* subspecies *enterica* of various serovars in poultry in the Belgrade epizootiological area in a period of six years (2014-2019). A total of 4580 samples were examined, including incubated eggs, dead chickens, broiler feces, and laying hens with 207 positive samples. *Salmonella* was isolated by standard microbiological methods followed by serological typing. In the examined period, the prevalence of poultry salmonella was 4.52%. The highest number of examined samples was recorded in 2017 (879) and positive in 2018 (65), while the lowest prevalence was recorded during the year 2016 with 7 positive samples. Of all isolated and serotyped *Salmonella*, the most commonly isolated serovar was S. Enteritidis (65.28%), followed by S. Infantis (21.30%), S. Mbandaka (6.02%), S. Senftenberg (3.24%), S. Typhimurium (1.85%), *S.* Agona (0.93%), *S.* Taksony (0.93%), and the least common is *S.* Tshiongwe (0.46%). The first report of serovars: *S.* Agona, *S.* Taksony and *S.* Tshiongwe have been during 2018. The highest number of positive specimens was found in laying hens faces (116 of 921 tested), broiler faces (73 of 1147), chickens carcasses (12 of 1443), and incubated eggs (6 of 1069). Complete eradication of *Salmonella* from production is a challenging goal because of a heterogeneous serovars pool and various sources of infection. Prevention is the best tool for controlling *Salmonella*: hygiene, biosecurity, and where applicable - vaccination. It is a great responsibility of the poultry farmers to apply the existing standards and to improve the new ones.

***Keywords*:** Enterica, microbiology, prevalence, Serbia

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INTRODUCTION

Bacteria from the genus *Salmonella* are usually an enterological pathogen that can infect almost any animal. Their presence in poultry poses a risk for human health via contaminated meat and eggs used in nutrition. *Salmonella* infection in poultry flocks can manifest as an acute or chronic disease, and infected poultry is a reservoir of different *Salmonella* serovars.

Monitoring and controlling *Salmonella* in domestic poultry has become a priority in recent years, as most human cases of salmonellosis are thought to be related to the consumption of eggs and poultry products (Whiley and Ross, 2015). *Salmonella* has been declared the most common and important zoonosis by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) since 1950 (Mouttotou et al., 2017). For this reason, it is necessary to apply harmonized control regulations throughout the poultry production chain.

In the Republic of Serbia, poultry *Salmonella* issues are endorsed in bylaw the Rulebook on determining measures for early detection, diagnosis, prevention of spread, control, and eradication of poultry infection with certain *Salmonella* serovars (Official Gazette of RS No.36/18, 2018) which is almost identical as respective EU regulations. The goal is to reduce the prevalence of *Salmonella* in primary production, including testing materials from the environment and poultry. This study aims to inform readers of the presence of poultry *Salmonella* in a Belgrade epizootiological area and to present the most prevalent serovars.

MATERIAL AND METHODS

For a period of six years, samples of incubated eggs, chicks' carcasses, and feces of both broilers and laying hens were examined, and results for Belgrade epidemiological area are presented. Samples were analyzed for the presence of *Salmonella* using relevant 6579 ISO standard (ISO, 2007; ISO, 2017) and OIE methods (OIE, 2016, 2018), including posthoc serological typing in National Reference Laboratory for animal Salmonellosis. Monophasic variants of *S.* Typhimurium (1,4,[5],12:i:-.) are for this epidemiological survey presented as *S.* Typhimurium. The samples of incubated eggs (unhatched eggs at the end of the hatching process and hatched eggshells) were from broiler breeders flocks, laying hens and broilers carcasses from transport and first three days of life, broilers faces were sampled from commercial farms, three weeks before slaughter and laying hen feces from cage system. The results were processed by descriptive statistics and presented in percentages.

RESULTS

In the examined period, 4580 samples of poultry were examined for the presence of *Salmonella* consisting of 1069 samples of incubated eggs, 1443 carcasses chickens samples, 921 feces samples originating from laying hens, and 1147 from broilers. The percentage of submitted samples by year is shown in the table (Table 1).

Serotyping identified 8 *Salmonella* serovars. The most commonly isolated serovar was *S.* Enteritidis (68.10%), followed by *S.* Infantis (17.87%), *S.* Mbandaka (6.28%), *S.* Senftenberg (3.39%), *S.* Typhimurium (1.93%), *S.* Agona (0.97%), *S.* Taksony (0.96%) and finally *S.* Tshiongwe (0.48%) which is shown in the tables by years (Table 2).

Most positive samples were found in laying hens feces (66.31%), followed by broiler feces (32.64%), dead chickens (6.89%), and least in incubated eggs (3.09%) (Table 3). The most positive samples (31.39%) were recorded in 2018, and the least in 2016 (3.38%).

In the examined period, the prevalence of *Salmonella* was 4.52%, shown by year (Table 4). The highest prevalence was recorded in 2018 (8.03%), and the lowest in 2016 (0.83%).

Table 1: Distribution of samples for testing for salmonella in the period 2014-2019.

Samples share (%)	Incubated eggs	Dead chickens	Laying hen feces	Broiler feces
2014	28.74	28.00	19.41	23.85
2015	30.67	20.76	22.86	25.71
2016	33.88	27.51	18.18	20.43
2017	28.33	30.15	20.02	21.50
2018	10.38	38.57	17.80	33.25
2019	11.12	39.64	23.20	26.04

Table 2: Prevalence of *Salmonella* serovars in the period 2014-2019 to the total number of isolates (%)

Year/ Serovar	*S.* Enteritidis	*S.* Infantis	*S.* Mbandaka	*S.* Senftenberg	*S.* Typhimurium	*S.* Agona	*S.* Taksony	*S.* Tshiongwe	Total
2014	24.15	0	0	0	1.45	0	0	0	25.60
2015	5.31	0	0	0	0	0	0	0	5.31
2016	1.93	1.45	0	0	0	0	0	0	3.38
2017	12.56	3.86	0	0.97	0	0	0	0	17.39
2018	17.39	11.59	0	0.97	0.48	0	0.48	0.48	31.39
2019	6.76	0.97	6.28	1.45	0	0.97	0.48	0	16.91
∑	68.10	17.87	6.28	3.39	1.93	0.97	0.96	0.48	100

Table 3: Percentage of positive samples in relation to the number of examined by years

Year	Incubated eggs	Dead chickens	Laying hen feces	Broiler feces	Total
2014	0	0	38.17	1.76	39.93
2015	0	4.59	1.67	2.96	9.22
2016	0.70	0	1.95	1.16	3.81
2017	1.20	0.38	0.11	6.35	8.04
2018	1.19	1.92	13.19	14.50	30.80
2019	0	0	11.22	5.91	17.13
∑	3.09	6.89	66.31	32.64	100

Table 4: *Salmonella* prevalence by years

Year	2014	2015	2016	2017	2018	2019	∑
Total number of samples	675	525	847	879	809	845	4580
Number of positive samples	53	11	7	36	65	35	207
Prevalence (%)	7.85	2.10	0.83	4.10	8.03	4.14	4.52

DISCUSSION

This paper aims to establish the causes of the formation and spread of *Salmonella* spp. among poultry, as well as the identification of serovars. In the last six years, the prevalence of the disease has grown from 0.83% in 2016 to 8.03% in 2018, to reach an overall 4.52% at the end of the study period, which is below expectations because European regulations stipulate that the prevalence of *Salmonella* drops below 1%. Other authors report results similar to ours. Previous findings in Serbia report a prevalence of 7.3%. during 2010-2014 in the parts of the Pannonia plain region (Pajić et al., 2015). The largest number of examined samples was of dead chickens (1443), with 6.89% positive. Broiler feces participated with 32.64% in the total number of examined samples, with 66.31% positive with the most frequently isolated *S.* Enteritidis and *S.* Infantis but also with the appearance of some serovars in 2018-19 that we did not isolate in previous years: *S.* Agona*, S.* Taksony*, S.* Tshiongwe. The lowest prevalence of isolated *Salmonella* was in incubated eggs (0.70%), although they participated in the total number of examined samples with a share of 23.34%. In this case, too, *S.* Enteritidis and *S.* Infantis were most often isolated. The feces of laying hens with a share of 20.11% in the total number of samples showed 15.61% of positive samples. Serovars *S.* Enteritidis and *S.* Infantis were the most often isolated, but in 2018-19 the occurrence of *S.* Mbandaka and *S.* Senftenberg was also detected. In some geographical regions, only a few *Salmonella* serovars of epidemiological significance are usually present. The prevalence of serovars varies and changes over time, but some serovars are constantly found with greater frequency than others. This study shows the presence of 8 different *Salmonella* serovars in poultry. In our study, the most common serovars were *S.* Enteritidis (68.10%) and *S.* Infantis (17.87%). The previous local study reports the most often isolate *S.* Infantis (50.3%) and *S.* Enteritidis (48.2%) (Pajić et al., 2015). The European Food Safety Authority states that serovars that are most often isolated in poultry flocks are: *S.* Enteritidis*, S.* Typhimurium*,* and *S.* Infantis (EFSA, 2019). In our epizootiological area*, S.* Enteritidis*, S.* Infantis and *S.* Mbandaka were the most represented serovars in the examined period. In our study, the serovar *S.* Enteritidis was represented by 68.10%, and some authors report that it is represented with 59-62% in Europe (Carrique Mas and Davies, 2008). However, dominant serovars change over time and differ depending on the country and region (Vidanović et al., 2008). *S.* Infantis was represented in our trials by 17.87%, mostly in broiler chickens. In the EU in 2017, *S.* Infantis was represented with 46.5-50.6% in broiler chickens and broiler meat (EFSA, 2019). Resistant strains can be spread from animals to humans through the food chain, which is a public health concern (Puvača and de Llanos Frutos, 2021) . Slovenia reports that the number of positive flocks of *S.* Infantis has increased by more than 100% in just two years since 2010 and that *S.* Infantis was subsequently detected on some farms despite the application of sanitary measures, proving its high resistance due to the formation of a biofilm that increases microbial tolerance to chemical, physical and biological agents (Pate et al., 2019). This indicates that this serovar can spread throughout the chain production of broiler and can survive in the environment. The spreading of *S.* Mbandaka has been noted throughout the world, it was classified as one of the ten most common serovars responsible for cases of salmonellosis in humans in the EU (EFSA, 2013). There are reports of the prevalence of serovar *S.* Mbandaka on laying farms as high as 54.40% (Gole et al., 2014). It is one of the most widespread serovars found in the dust of laying hen farms in Japan (Iwabuchi et al., 2010). Since 1996 *S.* Mbandaka has been continuously isolated in Poland in various veterinary sources, including poultry (Hoszowski et al., 2016), and during 2010, it was responsible for 32% of all cases of salmonellosis in Poland (Wałga et al., 2010). In 2010, the Austrian *Salmonella* Reference Center reported an increase in the number of salmonelloses caused by *S.* Mbandaka in the eggs of a flock of laying hens fed contaminated by commercial food that was responsible for the epidemic (Allerberger, 2012). *Salmonella* is one of the most important foodborne zoonotic pathogens, with significant health and economic impact (Tasić, 2018) in both humans and animals. The control of *Salmonella* in animal feedstuffs is important, principally to protect the human food chain from contamination by *Salmonella* derived from infected animals. *Salmonella* can reach into the animal feed by multiple ways and during all production stages (Tomicic et al., 2019). In our surveys, it was represented by 6.02% and only in year 2019. One of the most resistant salmonella serovars is *S.* Senftenberg. It is more resistant than the most isolated serovars to heating, drying, low pH (data not shown). It is most commonly found in raw poultry feed ingredients, feed mills, incubators, farms (Wiedemann et al., 2015; Maury and Pulido-Landínez, 2017). In our study, it was represented by 3.39% with increased prevalence since 2017. Diseases caused by the *S.* Typhimurium are of particular importance to public health because they are associated with food poisoning in humans, primarily concerning eggs and egg products. In our study, it was represented with 1.93%. It is encouraging that in 2019, the appearance of this serovar was not recorded. Other isolated serovars: *S.* Agona*, S.* Taksony and *S.* Tshiongwe are represented in a smaller prevalence (0.48-0.97%) but it should be taken into account that they have first appeared in 2018.

CONCLUSION

Complete eradication of *Salmonella* spp. in industrial poultry is doubtful, and even a prevalence limit of 1% presents a laudable goal, but a combination of proper management, biosecurity, vaccination protocols, along with meticulous laboratory testing and timely diagnostics, can achieve it. The prevalence of infection and the type of serovar in the poultry changes over time. There are emerging new contemporary serovars, some are reappearing, and there are common, persistent findings. Therefore there is a necessity for detection and sampling, which is again left to the owner. Vaccination of laying hens and breeders should be a part of the control strategy. One of the small scale alternatives in the prevention program is probiotics and organic acids, which has proven to be a valuable tool in the fight against *Salmonella* infection.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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