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Salmonella control in primary poultry production in breeding flocks, laying hens and broilers for the period 2020-2022

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Abstract

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Salmonellosis is the second most frequently reported zoonosis in the EU after campylobacteriosis. According to monitoring programs for the control of *Salmonella* spp. for the period 2020-2022, were tested 492 samples, of which 434 pcs. fresh feces, fabric socks, boot swabs; 13 pcs. eggshells and 45 pcs. dust samples. The samples originate from 3 categories of flocks: 45 pcs. from breeding flocks, 251 pcs. of laying hens and 196 pcs. from broilers. Diagnostic materials are tested according to ISO 6579-1:2017.

From the 492 examined samples, 18 pcs. of them were positive for *Salmonella* spp. (3.65%), incl. 2.22% from breeding flocks, 6.37% from laying hens and 0.51% from broilers. Their relative proportion by type of samples is: fresh feces – 83.33%, dust samples -11.11% and fabric swabs – 5.55%. For the last three years are presented data on current *Salmonella* serovars. The seven serovars of *Salmonella* were typed with the following proportion: Enteritidis – 27.8%, Infantis – 27.8%, Senftenberg – 16.7%, Bovismorbificans – 11.1%, Typhimurium – 5.5%, Corvalis – 5.5%, Stanby – 5.5%.

It is saving the trend of previous years to demonstrate the highest percentage of positive *Salmonella* spp. samples from the category of laying hens. The lower percentage of positive samples in the three categories of birds from the primary production is proof of the effectiveness and the need to implement the monitoring programs for Salmonella control and their importance for protecting the health of birds and people, respectively.

Keywords: programs; control; *Salmonella* spp.; serovar; breeding flocks; laying hens; broilers

Introduction

The presence of *Salmonella* spp. in poultry populations is considered a major risk factor for the presence of *Salmonella* spp. in eggs and poultry meat. To control *Salmonella* in the poultry production chain and limit the risk of contamination of poultry products, National Salmonella Control Programs (NCP) have been introduced in European Union (EU) countries, in accordance with EU legislation (Regulation (EU) 2160/2003).

In Bulgaria, the national programs for control of *Salmonella* in breeding flocks of poultry (SCP-breeding flocks) and in laying hens of the species *Gallus gallus* (SCP-laying hens) have been implemented since 2008, and the one for the control of *Salmonella* in broilers (SCP-broilers) has been implemented since 2009.

The first and second most frequently reported human zoonoses in the EU, in 2021 are campylobacteriosis and salmonellosis, respectively. Campylobacteriosis and salmonellosis cases increased compared to 2020, but decreased compared to previous years. *S. enteritidis* remains the most frequently reported cause of foodborne outbreaks (EFSA, 2022).

Risk factors for *Salmonella* infection of poultry flocks in primary poultry production can be humans (manure or dust particles on clothes, under shoes, in the farmer's hair, hands, etc.), rodents (but also cats, dogs and insects, *Salmonella*

Table 1. Number of samples tested for isolation of *Salmonella* spp. in 2020–2022

Tested samples in 2020–2022	Fresh feces, fabric socks, boot swabs	Dust samples	Eggsshels	Total
OC	190	44	11	245
FBOp	244	1	2	247
Total	434	45	13	492

carriers or mechanical carriers), contaminated feed and insufficiently cleaned and/or disinfected equipment and utensils (tools, egg trays, crates and containers) (Costa, 2021).

Limiting the spread of *Salmonella* spp. in poultry flocks requires continuous improvement of environmental monitoring, pathogen detection methods, and management measures in poultry farming (Im et al., 2015).

In each country *Salmonella* serotypes change annually as a result of globalization and especially trade in animals, animal products and feed, as well as international travel and human migration (Barbour et al., 2015). Setting a target that includes all serovars is expected to be more effective, as the most current *Salmonella* serovars in breeding flocks vary across EU Member States and over time (Koutsoumanis et al., 2019).

The present study aims to investigate and compare the prevalence of *Salmonella* in poultry facilities in breeding flocks of hens, laying hens and broilers, both according to the type of control and according to the type of samples examined, and to investigate the presence of the different serovars in the flocks, for the period 2020-2022.

Materials and Methods

The object of the study were 492 samples, taken for the purposes of official control (OC) – 245 pcs. and food business operation (FBOp) – 247 pcs., in connection with the implementation of Monitoring Programs for *Salmonella* control, distributed in 3 groups: I group – fresh feces, fabric socks and boot swabs – 434 pcs., II group – dust samples – 45 pcs. and III gr. – eggshells – 13 pcs. samples for the period 01.01.2020 – 31.12.2022 (Table 1).

Table 2. Number of samples tested for *Salmonella* according to type of control and category of the flocks

Category of the flock	Type of control		Samples examined, number
	OC	FBOp	
Breeding	36	9	45
Laying hens	205	46	251
Broilers	4	192	196
Total	245	247	492

A total of 492 samples examined originate from 3 categories of flocks: breeding flocks – 45 pieces, laying hens – 251 pieces and broilers – 196 samples (Table 2).

In total, for the three-year period of the study, a total of 103 poultry farms (PF) were examined, which were registered and respond to all requirements for identification, biosecurity and human treatment, used intensive breeding technologies, fed with permitted and certified feed and applied modern immunoprophylaxis schemes, prevention and control of infectious diseases. The study included 24 PF for breeding flocks of hens, 18 tested by OC and 6 by FBOp, 73 PF for raising laying hens, 60 examined by OC and 13 by FBOp and 6 PF for broilers, 2 examined by OC and 4 by FBOp (Table 3).

To isolate *Salmonella* spp., we used the following media: Buffered Peptone Water Granulated; Semisolid Rappaport Vassiliadis Medium Base Modified; Novobiocin supplement; Xylose-Lysine Deoxycholate Agar; Brilliant Green Agar Base Phosphates; Nutrient Agar; Brain Heart Infusion Broth; kit for biochemical identification HiSalmonella. As a positive control, we used a reference strain of *Salmonella enterica* subsp. *enterica*, serovar *Typhimurium* and *Enteritidis.* We developed the samples according to the ISO 6579-1:2017. The serological confirmation of the isolates was carried out with common agglutinating Anti-Salmonella serum I (A-E+Vi), and the serotyping was carried out at the National reference laboratory „Salmonella, Campylobacter, Staphylococcus and Antimicrobial Resistance“, at the National Center for Food Safety at National Diagnostic Research Veterinary

Table 3 Examined poultry farms (PF) for *Salmonella*, according to the category of flocks (breeding, laying hens and broilers) and type of control (OC and FBOp)

Flocks	2020			2021			2022			2020–2022		
	OC	FBOp	Total	OC	FBOp	Total	OC	FBOp	Total	OC	FBOp	Total
Breeding	7	4	11	7	2	9	4	0	4	18	6	24
L. hens	17	7	24	22	3	25	21	3	24	60	13	73
Broilers	1	1	2	1	1	2	0	2	2	2	4	6
Total	25	12	37	30	6	36	25	5	30	80	23	103

en by OC (12 pieces) is 2 times higher than those found by

Table 4. Positive for *Salmonella* spp. samples depending on the category of flocks

Category of the flock	OC n = 245			FBOp n = 247			Tested sam-ples	Positive samples	
	fresh feces, fabric socks, boot swabs	Dust	Egg-shells	fresh feces, fabric socks, boot swabs	Dust	Egg-shells		Pcs.	%
Breeding	23	2	11	7	0	2	45	1	2.22
L. hens	164	41	0	45	1	0	251	16	6.37
Broilers	3	1	0	192	0	0	196	1	0.51
Total	190	44	11	244	1	2	492	18	3.65

Institute (NDRVI). The research was conducted in the lab. „Bacterial diseases of animals and nutrition media", at the National Center for Animal Health at the NDRVI.

Results

According to the *Salmonella* control programs in primary poultry production a total of 492 samples which were examined, positive for *Salmonella* spp. are 3.65%, in the category of breeding flocks – 2.22%, for laying hens – 6.37% and in broiler flocks – 0.51% (Table 4).

Of the 434 samples of fresh feces, fabric socks and boot swabs tested, positive for *Salmonella* spp. are 3.68%. Of the tested 45 pcs. dust samples, 4.44% were positive, and from 13 pcs. eggshells no positive for *Salmonella* samples have been proven (Table 5).

From the samples originating from breeding flocks, *S. enteritidis* was isolated – from one sample of fresh feces. From the category of laying hens, 16 positive samples for *Salmonella* were isolated: *S. enteritidis* – from 3 pcs. samples of fresh feces, *S. typhimurium* – from one sample of fresh feces, *S. enteritidis* – from one dust sample, *S. bovismorbificans* – from a dust sample and from fresh feces, *S. infantis* – from 5 pcs. samples of fresh feces, *S. corvalis* – from one sample of fresh feces, *S. senftenberg* -from 3 pcs. samples of fresh feces. From the category of broilers: *S. stanby*, one positive sample from fabric socks (Table 6).

Of the proven 18 positive samples for *Salmonella* spp. 7 serovars of Salmonella were serotyped. Two serovars dominate – *Enteritidis* and *Infantis*, with an equal share distribution (27.8%), followed by *Senftenberg* (16.7%), *Bovismorbificans* (11.1%) and *Typhimurium, Corvalis* and *Stanby* with an equal share of 5.5% (Table 7).

Of the 245 samples tested by OC, positive for *Salmonella* spp. are 4.89%, and of the tested 247 samples according to FBOp, 2.42% are positive (Table 8).

The highest percentage of positive samples for *Salmonella* was proven in the category of laying hens (6.37%), lower in breeding flocks (2.22%) and the lowest in broilers (0.51%). The number of *Salmonella* positive samples, prov-

Table 5. Positive for *Salmonella* spp. samples depending on the type of samples

Type of examined samples	Samples, pcs.	Positive samples	
		Pcs.	%
Fresh feces, fabric socks, boot swabs	434	16	3.68
Dust	45	2	4.44
Eggshells	13	0	0
Total	492	18	3.65

Table 6. Isolated *Salmonella* serovars by flocks category and type of sample

Flocks	Type of samples, pcs.	Serovar *Salmonella*
Breeding	Fresh feces– 1	Enteritidis – 1
Laying hens	Fresh feces – 3 Fresh feces – 1 Dust – 1 Fresh feces – 1 Dust – 1 Fresh feces – 5 Fresh feces – 1 Fresh feces – 3	Enteritidis – 3 Typhimurium – 1 Enteritidis – 1 Bovismorbificans – 1 Bovismorbificans – 1 Infantis – 5 Corvalis – 1 Senftenberg – 3
Broilers	Fabric socks – 1	Stanby – 1

Table 7. Relative part of isolated serovars *Salmonella* for the period 2020-2022

Serovar	Pcs.	%
Enteritidis	5	27.8
Infantis	5	27.8
Senftenberg	3	16.7
Bovismorbificans	2	11.1
Typhimurium	1	5.5
Corvalis	1	5.5
Stanby	1	5.5

Table 8. Positive for *Salmonella* spp. samples, depending on the type of control

Type control	Samples examined, Pcs.	Positive for *Salmonella* spp. samples	
		Pcs.	%
OC	245	12	4.89
FBOp	247	6	2.42

Table 9. Positive for *Salmonella* samples, depending on the category of the flock

Category of the flock	Positive for *Salmonella* samples			
	OC	FBOp	Positive / examined, pcs.	Positive, %
Breeding	0	1	1 / 45	2.22
Laying hens	11	5	16 / 251	6.37
Broilers	1	0	1 / 196	0.51
Positive /examined, Pcs	12 / 245	6 / 247	18 / 492	3.65

Table 10. Positive for *Salmonella* spp. poultry farms (PF)

Year	Tested PF	Positive PF tested by:		Positive for *Salmonella* spp. PF	
		OC	FBOp	Num. PF /Category flocks	%
2020	37	3	2	5 /4 Laying hens + 1 Breeding /	13.5
2021	36	2	0	2 /1 Laying hens + 1 Broilers /	5.5
2022	30	3	1	4 Laying hens	13.3
Total	103	8 pcs. – 72.7%	3 pcs. – 27.3%	11–10.6%	

FBOp (6 pieces) with almost the same number of examined samples (Table 9).

Out of a total of 103 poultry farms (PF) examined for the period 2020-2022, 11 (10.6%) were positive for *Salmonella,* 8 PF (72.7%) were proven during the examination of samples by OC, and 3 PF (27.2%) were proven by sampling for the needs of FBOp, distributed as follows: 9 PF are intended for breeding laying hens and by one PF, each for breeding flocks and broilers (Table 10).

The relative share of positive *Salmonella* spp. PF (n=11), tested over a period of 3 years is as follows: laying hens – 81.81%, breeding flocks and broiler flocks – 9.09% each of them. (Table 11).

Of the examined 341 pcs. flocks positive for Salmonella are 3.23%. The highest percentage of positive flocks is found in laying hens (7.56%), followed by breeding flocks (3.57%) and broilers (0.52%) (Table 12).

Table 11. Relative part of positive for *Salmonella* spp. PF for the period 2020-2022

Category of the flock	Positive for *Salmonella* spp. PF /n = 11/	
	Num.	%
Breeding	1	9.09
Laying hens	9	81.81
Broilers	1	9.09

Discussion

Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in humans after Campylobacteriosis and is a major cause of foodborne epidemics in EU and non-EU Member States. The top 5 Salmonella serovars involved in human infections in general are distributed as follows: *S. enteritidis* (54.6%), *S. typhimurium* (11.4%), monophasic *S. typhimurium* (1,4, [5], 12:i:) (8.8%), *S. infantis* (2.0%) and *S. derby* (0.93%) (EFSA, 2022).

Zoonoses that are present at stage of the primary production must be adequately controlled to protect the health of the final consumer. The purpose of EU Regulation (EC) No 2160/2003 is to ensure that appropriate and effective measures are taken to detect and control *Salmonella* at all stages of production, processing and distribution, especially at primary production stage, including in food for animals in order to reduce the risk of *Salmonella* for public health by implementing specific control programs by Member States and economic operators in the production and trade of food for human and animals.(Regulation (EC) No 2160/2003).

Table 12. Positive for *Salmonella* flocks for the period 2020-2022

Year	Breeding			Laying hens			Broilers		
	Num. examinated	Positive		Num. examinated	Positive		Num. examinated	Positive	
		num	%		num	%		num	%
2020	16	1	6.26	53	4	7.55	49	0	0
2021	7	0	0	46	1	2.17	73	1	1.37
2022	5	0	0	20	4	20	72	0	0
Total	28	1	3.57	119	9	7.56	194	1	0.52

Salmonella can spread in poultry farms through vertical and horizontal transmission. Potential sources for the spread of *Salmonella* in the poultry production chain are breeders, hatcheries, chickens, the environment, feed, rodents and wild birds. Management practices at the breeder flock stage have a deep effect on the transmission of *Salmonella* within an integrated production system (Liljebjelke et al., 2005) and *Salmonella* control should start from the breeder flocks, having in mind their potential for spread to the rest of the farm from the poultry production chain (Rajan et al., 2016).

For breeding flocks, the aim of the program is to determine the health status of poultry of the hen species in the Republic of Bulgaria with regard to *Salmonella enteritidis, S. hadar, S. infantis, S. typhimurium,* (including monophasic *S. typhimurium* serotypes with antigenic formula 1,4,/5/,1 2 : i), S. Virchow as well as all other Salmonella species and a reduction to 1% or less of the maximum percentage of adult breeding flocks of the species *Gallus gallus* positive for *S. enteritidis, S. infantis, S. hadar, S. typimurium* serotypes with antigenic formula 1,4,/5/,1 2 : i) (SCP – breeding flocks).

Of those examined in 2020 – 2022, 492 samples, 3.65% positive for *Salmonella* spp. samples were found, compared to 2017 – 2019, when 6.31% of 744 samples were proven positive (Dimitrova et al., 2022). Of the breeding flocks investigated in the current study, 3.57% were positive flocks for *Salmonella* spp., and 2.22% were the positive samples, and one of the five target serovars – serovar *Enteritidis* – was proven, in contrast to our previous studies in 2014 (the positive samples were 0.55%, serovar *Corvalis* was isolated from one breeding flock) (Dimitrova et al., 2016), in 2015 (1.52% of tested samples and 9.09% of tested flocks were positive, Infantis was isolated from one flock) (Dimitrova & Savova-Lalkovska, 2019), in 2016 (4.17% of samples were positive, Senftenberg was proven from one flock) (Dimitrova, 2022) and in 2017-2019 (9.61% of samples were positive, isolated *infantis, enteritidis, typhimurium* and *derby*) (Dimitrova et al., 2022). In breeding flocks of *Gallus gallus* in the EU in 2021, *Salmonella* was detected in 2.5% of flocks tested, compared to 2.0% and 2.3% for 2020 and 2019 respectively. Prevalence of flocks , which were positive for any of the five target serovars, was 0.58% in 2021, 0.52% in 2020 and 0.62% in 2019. All reporting countries, except Austria, Belgium, Greece, Ireland and Poland have achieved target for spreading of *Salmonella* in the flocks of a maximum of 1%. The most commonly reported target serovar is *S. enteritidis*. The total number of breeding flocks positive for *S. enteritidis* (55) increased compared to 2020 (29 positive flocks) and 2019 (53 positive flocks). *S. typhimurium* (including the monophasic variant) was the second most frequently reported target serovar (with 15 positive flocks). The third most commonly reported serovar was *S. infantis* (with 6 positive herds). Regarding the other target serovars, 3 flocks were positive for *S. virchow* (0.02%) and 2 flocks for *S. hadar* (0.01%) (EFSA, 2022).

For laying hens, the aim is to determine the health status of the flocks with regard to *S. enteritidis* and *S. typhimurium* (including the monophasic *S. typhimurium* serotype with antigenic formula 1,4,/5/,1 2 : i) and to reach the Union target of reducing the percentage of positive flocks of *S. enteritidis* and *S. typhimurium* (including the monophasic *S. typhimurium* serotype with antigenic formula 1,4,/5/,1 2 : i) in adult laying hens of the species *Gallus gallus,* in compliance with Art. 1 of Regulation 517/2011 (SCP – laying hens).

For the flocks of laying hens in the present study, it was found that 7.56% of the examined flocks were positive, the positive samples were 6.37%, 6 serovars were isolated: *enteritidis, typhimurium, bovismorbificans, infantis, corvalis, senftenberg* in contrast to 2014 (0.59% of the samples were positive, 2 serovars *isangi* and *infantis* were isolated from 2 flocks) (Dimitrova et al., 2016), in 2015 (15.38% of the examined flocks were positive, positive samples were 3.93%, isolated are 5 serovars *enteritidis, typhimurium, infantis, corvalis* and *thompson* from 4 flocks) (Dimitrova & Savova-Lalkovska, 2019), in 2016 (4.57% of the tested samples were positive, 3 serovars – *enteritidis, infantis* and *corvalis* were isolated from 6 different flocks) (Dimitrova, 2022) and in 2017 – 2019 (8.62% of samples from laying hens were positive, 7 serovars were isolated – *enteritidis, infantis, thompson, mbandaka, cottbus, djugo* and *agona*) (Dimitrova et al., 2022). In the EU, Salmonella was detected in 3.3% in 2021, compared to 4.0% in 2020. The EU prevalence of laying hen flocks positive for either of the two target serovars was 1.3%, which was stable compared to 2020, when 1.3% of tested flocks were positive for the target serovars. Seven Member States (Belgium, Cyprus, Czech Republic, France, Malta, Poland and Spain) have not met the target of reducing positive flocks by 2% or less. The most frequently reported target serovar was *S. enteritidis* (EU herd prevalence of 1.0%), with 81.3% of 407 *S. enteritidis* – positive flocks reported from 6 Member States. For *S. typhimurium* (including the monophasic variant) there is a prevalence in EU flocks of 0.32% (EFSA and ECDC, 2022).

Perilli et al. (2022) studied the most prevalent Salmonella serotypes, between 2015 and 2020, within the Italian National Salmonella Control Program in Poultry. The samples are represented by feces and dust from flock of laying hens, broilers and breeding flocks. Feces detected through fabric socks accounted for the largest number of positive samples. *S.* Infantis is the predominant serotype in broiler flocks. *S. typhimurium* and *S. enteriditis* were found at low levels in

laying hens. In our study, *S. infantis* and *S. enteriditis* were the most frequently isolated serovars, also from fresh faecal samples, but from flocks of laying hens.

For broilers, the aim is to determine the health status with regard to *S. enteritidis* and *S. typhimurium* (including monophasic *S. typhimurium* serotype with antigenic formula 1,4/5/,1 2:i). The reduction of *S. enteritidis* and *S. typhimurium* in broilers must represent the maximum percentage of broiler flocks that remain positive for *S. enteritidis* and *S. typhimurium* to 1% or less (SCP – broilers).

In the broiler flocks in 2020-2022, *Salmonella* spp. was detected in 0.52% of the examined flocks, 0.51% of the samples were positive, one serovar was isolated – Stanby, in contrast to what was found in previous studies in 2014 (positive samples were 0.8% , serovar Infantis was isolated) (Dimitrova et al., 2016), in 2015 and 2016 (0% *Salmonella* positive broiler flocks) (Dimitrova & Savova-Lalkovska, 2019; Dimitrova, 2022), and 2017 – 2019. (0% *Salmonella* positive flocks) (Dimitrova et al., 2022). In the EU, *Salmonella* was found in 3.8% of flocks tested in 2021, compared to 3.9% in 2020 and 3.6% in 2019. EU prevalence of broiler flocks was positive for some of the two target *Salmonella* serovars -0.28%, which is similar to the prevalence in previous years (0.25% in 2020 and 0.20% in 2019). Three Member States (Czech Republic, Luxembourg and Malta) did not achieve the target of 1% or less of broiler flocks positive for *S. enteritidis* and/or *S. typhimurium*. In 2021, *S. enteritidis* represented 51.4% of flocks positive for the target serovars, while *S. typhimurium* represented 48.6% (EFSA , 2022).

Salmonella spp. is recognized worldwide as one of the leading causes of acute bacterial gastroenteritis in humans resulting from consumption of animal products. *Salmonella enteritidis, S. typhimurium* and its monophasic variant are the main serovars responsible for human disease. Serovar *Infantis* has emerged as the fourth most common serovar associated with human disease. A total of 95% isolates of *infantis* serovars originate from broilers and their derived products (Montoro-Dasi et al., 2023). Considering the ranking of the 20 most common Salmonella serovars in the EU in food of animal origin, focusing on those most frequently isolated from humans, *S. enteritidis* is the most frequently reported serovar in laying hens and the second most – often reported in broilers and *S. infantis* from broilers (one of 2 *Salmonella* isolates from broilers belongs to this serovar) (EFSA, 2022; 2023).

To optimize the detection of *Salmonella* prevalence in the flock, Schulz et al. (2011) recommended the combination of dust (25 g pooled dust sample from 20 locations) and faecal samples (250 g pooled from 5 different locations). The scientists report that the examination of dust samples has a greater sensitivity compared to the other types of samples, because, *Salmonella* spp. unlike other members of the Enterobacteriaceae family, it is able to survive better in a dry environment. The detection of *Salmonella* spp. in 4.44% of dust samples and 3.68% of fresh feces in 2020 – 2022 is in unison with what was found by Gole et al. (2017), to isolate Salmonella to a greater extent in dust samples and to a lesser extent from boot swabs and fresh feces.

When the aim of the study is to detect infected flocks, environmental samples, especially boot swabs, would be the recommended samples, as they are more sensitive than those from individual hens and are need in fewer numbers. Based on a relative comparison in cell-free culture systems, pooled faeces appear better than dust samples. For caged systems, dust samples give better results. However, as the EU plans to eliminate caged birds, this may be less relevant in the future. The use of pooled faecal samples is recommended for cell-free systems if samples different than or in addition to boot swabs are to be collected (Pacholewicz et al., 2023).

Our understanding of testing dust samples in parallel with other types of samples (faecal, boot swabs, or fabric socks) to more successfully isolate *Salmonella* spp. and detect Salmonella-positive poultry farms when investigations also include dust samples is consistent with Arnold et al. (2011) opinion, Schulz et al. (2011), Watanabe et al. (2012), and Im et al. (2015) and Dimitrova et al. (2022). Dust present in poultry farm can contain high concentrations of microorganisms and serve to transmit pathogens from the bedding to the birds. The results of an *in vitro* study by Pal et al. (2021) showed that higher levels of Salmonella in bedding increased the probability of detecting *Salmonella* in dust and recommended the development of methods to control dust contamination due to its role in *Salmonella* transmission.

Regulation (EC) No. 200/2012 requires MS to report the results obtained by the OC separately from those obtained by the food chain business operators (FCOs). The results of the present study showed that out of 492 examined primary production samples, out of 245 samples tested by OC, 4.89% were positive and out of 247 samples tested by FBOp, 2.42% were positive, which is in line with our previous studies in 2017-2019, when it was found that 9.80% of the samples tested by OC were positive, compared to 1.84% tested by FBOp (Dimitrova et al., 2022), and in 2016 (6.93% positive samples out of 101 samples tested by OC, and 1.53% positive from 196 samples, by FBOp) to find differences between the results of OC and FBOp (Dimitrova, 2022). The prevalence of target Salmonella serovars in competent authority (CA) samples was consistently higher than in FBOp samples for both broilers and fattening turkeys. Discovery of the causes of discrepancies between sampling results, by the CA

and FCOs, should be encouraged as an essential condition to ensure data reliability (EFSA and ECDC, 2022; 2023).

We share the understanding of Baptista et al. (2023) on control of Salmonella in the poultry production chain, combined with biosecurity measures, as an important tool to maintain and ensure the sanitary status of laying hen flocks and breeding flocks to protect human health.

Conclusion

For the period 2020 – 2022, the highest percentage of positive *Salmonella* spp. samples were proven in the category of laying hens – 6.37%, with the presence of the two most pathogenic serovars for humans – *enteritidis* and *typhimurium,* dominance of serovar *infantis* – 31.25%, followed by *enteritidis* – 25%, *senftenberg* – 18.75%, *bovismorbificans* – 12.5% and *typhimurium* and *corvalis* – 6.25% each. Of the examined 434 pcs. samples of fresh feces, boot swabs and fabric socks positive for *Salmonella* spp. were 16 (3.68%) and of the 45 dust samples tested, 2 (4.44%) were positive. It was found that 72.7% of positive for *Salmonella s*pp. poultry farms were proven as a result of OC and 27.2% of poultry farms – FBOp. The positive samples taken under OC (4.89%) are twice as many as those examined from FBOp (2.42%).

The trend established over the previous 9 years for the lowest percentage of *Salmonella* positive samples or no samples among broiler flocks, in contrast to breeding flocks and laying hens, is maintained. The decrease in the percentage of positive samples over the years in the three categories birds of primary production stage is the evidence of the effectiveness of implementing monitoring programs to control *Salmonella.*

The results of the present study will help to determine in which category of birds there is the greatest need for increased control measures. The overall control of *Salmonella* along the food chain will contribute to reducing the spread of the pathogen in primary production, the detection of *Salmonella* in food products of animal origin and the protection of human health.

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