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PREVALENCE OF *Salmonella* SPP. IN BREEDING FLOCKS, LAYING HENS AND BROILERS IN DIFFERENT REGIONS OF BULGARIA IN 2016

Albena Dimitrova

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Abstract

In 2016 a total number of 297 samples were tested in the frame of *Salmonella* control programmes in Bulgaria including official control ($n = 101$) and control by the business operators ($n = 196$). The samples originated from 3 categories of hen flocks: 48 pcs. samples from breeding flocks, 175 pcs. from laying hens and 74 pcs. from broilers. The samples belonged to 3 different groups: I – fresh feces, boot swabs and fabric socks swabs ($n = 281$), II – dust samples ($n = 10$), and III – egg shells ($n = 6$). The laboratory tests were performed in accordance with EN ISO 6579: 2003/A1:2007. *Salmonella* positives were found in 40% of dust samples ($n = 10$) and 2.09% of fresh feces, boot swabs, fabric socks swabs, and egg shells ($n = 287$). The percentage of *Salmonella* spp. positive samples was 3.37%, including 0.67% of breeding flocks and 2.69% of laying hens. The prevalence of *Salmonella* in breeding flocks was 4.17% and among laying hen herds – 4.57%. There was no *Salmonella* spp. detected in broilers.

The ratio of isolated *Salmonella* serovars was: Enteritidis – 60%, Senftenberg – 20%, and Corvallis and Infantis – 10%. In total 6.93% of the samples tested for official controls ($n = 101$) and 1.53% for business operator control ($n = 196$) were positive for *Salmonella*. It is recommended the replacement of dust samples with another type of sample to be solely as an exception and the control of *Salmonella* spp. in samples from the primary production to be as much as possible under the official control.

Key words: *Salmonella* spp., serovar, prevalence, control, laying hens, breeding flocks, broilers

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Introduction. The control of *Salmonella* spp. in hen flocks requires continuous improvement of the environmental monitoring, pathogen detection methods, and management measures implemented in the poultry farms [1]. In each country, *Salmonella* serotypes change annually as a consequence of globalization, and especially trade of live animals, animal products and feed, as well as human migration. The main sources of *Salmonella* foodborne outbreaks are eggs and poultry meat [2]. Salmonellosis is the second, most commonly reported zoonosis in humans in the European Union (EU) in 2016 after campylobacteriosis. The serovars Enteritidis, Typhimurium, including monophasic Typhimurium, Infantis and Derby are the most commonly identified. The proportion of salmonellosis outbreaks due to *S. enteritidis* continuously increase in 2016. The reported food and animals monitoring data indicate that *Salmonella enteritidis* is highly associated with laying hens, broilers and broiler meat [3].

The increase in confirmed cases of salmonellosis in people in the EU since 2014 evoked a study of the contributing factors and the possibility for control during the poultry production. Revision of the five current targeted serovars in breeding flocks showed that there is a reason to maintain Enteritidis, Typhimurium (including monophasic variants) and Infantis, whereas Virchow and Hadar could be replaced by Kentucky or Heidelberg, Thompson or variable serovar in the national control programmes. Inclusion of all serovars is expected to be more effective as there is wide variation in the detected serovars in breeding flocks across EU Member States (EU MS) and over time [4]. For countries reporting sources of zoonoses and zoonotic agents in 2016, the prevalence of targeted *Salmonella* serovars in breeding hens, broilers and turkeys at the primary production level is reduced or stabilized, compared to previous years, in contrast to the spread of *S. enteritidis* in laying hens, which is increasing significantly [3].

In Bulgaria, National Control Programmes of *Salmonella* in breeding flocks and laying hens of *Gallus gallus* have been in place since 2008 and for *Salmonella* in broilers has been running since 2009 [5–7]. The aim of *Salmonella* control programmes in breeding flocks is to determine the health status to S. Enteritidis, S. Hadar, S. Infantis, S. Typhimurium (including the monophasic S. Typhimurium serotype of antigenic formula 1,4 [5],12:(i), S. Virchow, as well as all other *Salmonella* spp., and to achieve a reduction to 1% or less than 1% of the maximum percentage of *Gallus gallus* positive breeding flocks for target serovars [5]. For laying hens of *Gallus gallus*, the aim of the control programme is to determine the health status for S. Enteritidis and S. Typhimurium and to reduce the percentage of positive flocks for S. Enteritidis and S. Typhimurium in adult laying hens by 10 to 40% depending on the prevalence in the previous year in accordance with Art. 1 of Regulation 517/2011. Alternatively, the maximum percentage of flocks should be reduced to 2% or less [6]. The National *Salmonella* Control Programme of broiler flocks includes determining the status of S. Enteritidis and S. Typhimurium (including the monophasic S. Typhimurium) and achieving of

maximum percentage of flocks that remain positive for these serovars of 1% or less [7].

The purpose of this study was to investigate the prevalence of *Salmonella* in breeding hens, laying hens and broilers in some regions of Bulgaria in 2016.

Materials and methods. The subject of the study were 297 pcs. samples taken for the purposes of official control (OC) and own checks by the food business operators (FBO) in the frame of *Salmonella* national control programmes in Bulgaria. The samples were divided by flock categories: breeding flocks, laying hens and broilers, in 3 groups, as follows: group I – fresh feces, boot swabs, fabric socks swabs – 281 pcs., group II – dust samples – 10 pcs., and group III – egg shells – 6 pcs. (Table 1).

T a b l e 1

Number of samples tested for *Salmonella* spp. isolation from different categories of hen flocks in 2016

Tested categories hen herds	Samples taken at OC*, pcs.			Samples taken at FBO**, pcs.			Samples total
	Fresh feces, boot swabs, fabric socks swabs	Dust samples	Eggs shells	Fresh feces, boot swabs, fabric socks swabs	Dust samples	Eggs shells	
Breeding flocks	8	0	1	34	0	5	48
Laying hens	73	10	0	92	0	0	175
Broilers	9	0	0	65	0	0	74
Total	90	10	1	191	0	5	297

*OC – official control; **FBO – own checks by the food business operators

The microbiological tests were performed at laboratory "Bacterial Diseases of Animals", National Center for Animal Health, National Diagnostic Research Veterinary Medical Institute (NDRVMI), Sofia for the period 01.01.2016 – 31.12.2016.

For the cultivation and isolation of *Salmonella* spp. the following media were used: Buffered Peptone Water; Semisolid Rappaport Vassiliadis Medium Base Modified; Novobiocin supplements; Xylose-Lysine Deoxycholate Agar; Brilliant Green Agar; Nutrient Agar; Brain Heart Infusion Broth; HiSalmonella Identification kit. Reference strain of *Salmonella enterica* subsp. *Enterica*, serovar Enteritidis 13076 was used as positive control. The samples were tested according to EN ISO 6579:2003/A1:2007. The serological confirmation of the isolates was performed using combined agglutinating Anti-Salmonella I (A-E+Vi) test. Serotyping was performed at the National Reference Laboratory (NRL) for *Salmonella*,

Campylobacter, *Staphylococcus* and Antimicrobial Resistance at the National Center of Food Safety, NDRVMI.

Results. Of the 297 pcs. (100%) tested samples, 10 pcs. (3.37%) were found to be positive for *Salmonella* spp., including: 2 pcs. (0.67%) from breeding flocks, 8 pcs. (2.69%) from laying hens, 0 pcs. (0%) from broilers. The positive samples from different herd categories are distributed as follows: breeding flocks – 4.17%; laying hens – 4.57%; broilers 0% (Table 2).

T a b l e 2

Salmonella spp. positive samples tested in 2016

Tested categories hen herds	Tested samples		*Salmonella* spp. positive samples		
			From all tested samples		In the appropriate category
	pcs.	%	pcs.	%	%
Breeding flocks	48	16.16	2	0.67	4.17
Laying hens	175	58.92	8	2.69	4.57
Broilers	74	24.91	0	0.0	0.0
Total	297	100	10	3.37	

All 10 pcs. *Salmonella* spp. positive samples isolated from breeding flocks and laying hens belong to 4 *Salmonella* serovars: Enteritidis serovar (6 samples), Senftenberg (2 samples), Infantis and Corvallis – each from one sample. S. Enteritidis serovar was proved in 6 samples from laying hens originating from 4 different poultry farms in 3 regions of Bulgaria: 2 isolates from boot swabs, 3 pcs. from dust samples and 1 from fresh feces. S. Corvalis serovar was isolated from a dust sample. S. Infantis was detected in one sample of fresh feces from laying hens. Combination of two serovars (Enteritidis and Corvalis) from one flock was isolated and S. Senftenberg serovar was proven in 2 egg shells samples from breeding flocks.

T a b l e 3

Salmonella positive samples by type of control, herd category and sample type

Type of control	Herd category	Region	Type of sample	Isolated serovar	Positive samples, pcs.
OC*	Laying hens	A	Dust	Corvallis	1
OC	Laying hens	A	Boot swabs Dust	Enteritidis	1 1
OC	Laying hens	B	Dust	Enteritidis	1
OC	Laying hens	C	Fresh feces Dust	Enteritidis	1 1
OC	Laying hens	D	Fresh feces	Infantis	1
FBO**	Breeding flocks	E	Egg shells	Senftenberg	2
FBO	Laying hens	A	Boot swabs	Enteritidis	1

Salmonella spp. positive results were confirmed in 2.09% of 287 samples of fresh feces, boot swabs, fabric socks swabs, and egg shells, while *Salmonella* spp. was confirmed in 4 out of 10 (40%) dust samples.

As *Salmonella* spp. positive were found 6.93% of 101 samples tested in the frame of official control and 1.53% were positive of 196 samples tested as own checks by the FBOs. The ratio of *Salmonella* serovars is as follows: Enteritidis – 60%, Senftenberg – 20% and Corvallis and Infantis – 10% for each (Table 3).

Discussion. The results of our study on the *Salmonella* prevalence in poultry flocks in Bulgaria during 2016 revealed that the highest percentage of positive samples (4.57%) were found in laying hens and the most commonly isolated serovar was S. Enteritidis, in 75% of the cases. The tendency of increasing of confirmed positive samples in laying hens flocks in Bulgaria – 0.59% in 2014 [8], 3.93% in 2015 [9] and 4.57% in 2016 is in correlation with EU report showing *Salmonella* in laying hens in 3.71% of herds in 2016 and 2.67% in 2015. The predominant share of both target serovars (Enteritidis and Typhimurium) was 1.44% in 2016 and 1.04% in 2015. Enteritidis was the most commonly reported target serovar in 2016 in Bulgaria and at the EU level [3].

Our findings with regard to the mandatory testing of dust samples, without possibility of replacing them with other type of samples (feces, boot swabs and fabric socks swabs) are in agreement with the opinion of Arnold et al. [10], Schulz et al. [11], Watanabe et al. [12] and Im et al. [1], for more successful isolation of *Salmonella* spp., when investigations include dust samples. To improve the detection of *Salmonella* prevalence in the poultry flock, Schulz et al. [11] recommend a combination of dust (25 g aggregate dust sample from 20 sites) and fecal samples (aggregate 250 g from 5 different sites). The authors report that testing of dust samples is more reliable than fecal samples, because *Salmonella* spp. is able to survive better in dry conditions unlike other members of the family Enterobacteriaceae.

The results of our study show the presence of *Salmonella* spp. in 40% of the dust samples and 2.09% of the fresh feces, and are in line with the finding of Gole et al. [13] stating that *Salmonella* is detected most in dust samples and less in boot swabs and feces. The results revealed by us contradict those of Im et al. [1] to isolate *Salmonella* from laying hens to the highest extent from feces (41.8%) and less from dust (40.3%) and egg shells (17.2%). The dominant presence of Enteritidis serovar (40%) in 175 samples of laying hens tested under the current investigation correlates with the studies of Hulaj et al. [14] who found it as the most commonly isolated serovar in laying hens. The equal percentage of S. Enteritidis positive dust samples (50%) and boot swabs and fresh feces (50%) from laying hens found in our study differs from the results of Hulaj et al. [14] who detected Enteritidis presence in 16.3% of dust and 11% of the fecal samples.

The simultaneous detection of serovar Enteritidis from boot swabs and Corvalis from a dust sample in one flock of laying hens in our study is similar to that

reported by other authors, such as Soria et al. [15], which simultaneously isolated Enteritidis and Kentucky in a laying herd, and Gama et al. [16], who reported the presence of Infantis, Mbandaka, Schwarzengrund and Sandiego serovars in one flock and Enteritidis, Javiana, Mbandaka and Infantis serovars in another flock of laying hens.

The results for *Salmonella* prevalence in breeding flocks in Bulgaria show only one positive flock from 48 samples tested. The isolated serovar was Senftenberg which is not a target serovar for this category. The most commonly reported target serovar in breeding flocks in EU is Enteritidis with a prevalence of 0.3%, followed by S. Typhimurium – 0.16%, S. Infantis – 0.06% and only one flock – positive for S. Virchow and S. Hadar [3].

The comparative analysis of the data for *Salmonella* presence in broiler flocks in Bulgaria in 2016 and previous years reveals no positive samples in 2015 [9] and only 0.8% positive for 2014 [8]. The presence of *Salmonella* spp. in broilers at EU level was 2.6% in 2016 and 2.2% in 2015. The prevalence of both target serovars (Enteritidis and Typhimurium) was 0.21% in 2016 and 0.26% in 2015. Nine EU MS and 2 non-MS reported no flocks infected with S. Enteritidis or S. Typhimurium. In 2016, all reporting MS, with the exception of the Czech Republic and Malta, achieved the EU target of positive herds for S. Enteritidis and/or S. Typhimurium of 1% or less [3].

Under the current study the target serovar – Typhimurium was not detected in the investigated samples in all three poultry categories in 2016 compared to 2015 when it was isolated from laying hens [9]. To some extent it could be explained by the fact that Enteritidis serovar is more often isolated from egg content due to its better adherence to the mucosa of the reproductive tract unlike Typhimurium [17].

The comparative analysis of *Salmonella* positive samples tested under official control (OC) and as own checks by food business operators (FBO) in Bulgaria in the period 2014–2016, shows significant difference in the percentage of *Salmonella* positive samples in favour of official controls – 0.8% (2014), 7.4% (2015), and 6.5% (2016) (for OC), and 0.6% (2014), 0.5% (2015), and 1.5% (2016), respectively, for own checks [8,9]. Similar findings were reported for countries implementing zoonosis control and monitoring programmes as described in the 2016 European Report – 10.5% positive for *Salmonella* samples from official control and 2.4% for own checks by FBO, indicating the need to analyze the reasons for these inconsistencies. According to data provided by the EU Competent Authority (CA) in 2016, 8212 flocks were tested with 10.5% positive for *Salmonella* and only 1.5% positive for targeted serovars. The total number of samples tested by the food business operators was 241 673 with 2.4% positive for *Salmonella* and 0.08% positive for targeted serovars. Different hypotheses may explain the discrepancies between the data reported by the CA and the FBO, for example, the difference in the percentage of *Salmonella* positive samples may partly be related to the fact that CA sampling may be at risk at any time, when the CA

deems it necessary. However, inconsistencies could also be related to differences in sampling techniques and sensitivity of laboratory methods [3].

Conclusion. Of a total of 297 samples tested in 2016 originating from breeding flocks, laying hens and broiler flocks in Bulgaria, 3.37% were found positive for *Salmonella*. In breeding flocks, the percentage of positive for *Salmonella* spp. samples was 4.16%, S. Senftenberg was isolated. In the laying hens, 4.57% positive samples were found, and serovars Enteritidis, Corvallis and Infantis were isolated. Among the broiler flocks, there was a favourable trend of no *Salmonella* positive samples. For a consecutive year, laying hens are the category with most prevalence of *Salmonella* spp. and the largest variety of serovars. Serovar Infantis has been present for the last 3 years – 2014, 2015 and 2016, Enteritidis, has been isolated for the past 2 years, and in 2016 it emerges as dominant over the other serovars – 75%. It is highly recommended the samples originating from the primary production to be taken for *Salmonella* spp. detection under the official control. The replacement of dust samples with another type of sample is recommended only as an exception.

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National Diagnostic and Research Veterinary Medical Institute
15 A, P. Slaveykov Blvd
1606 Sofia, Bulgaria
e-mail: aldimas@abv.bg