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Control of *Salmonella* spp. in Birds in 2015

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Abstract

For the control of *Salmonella* spp. a total of 571 samples from birds were tested in 2015. The samples (fresh feces and shoe pads - 303 pcs.; dust and socks - 244 pcs. and internal organs and feces of exotic birds - 24 pcs.) originating from 56 poultry sites, were classified in 4 categories: breeding flocks - 11 pcs.; laying hens - 26 pcs.; broiler chickens - 18 pcs. and exotic birds - 1. The diagnostic materials were tested according to BDS EN/ISO 6579, Amendment 1: Annex D.

Of all the 56 sites tested (100%), 6 (10.71%) were positive for *Salmonella* spp.: breeding flocks - 1.78%; laying hens - 7.15%; broiler chickens - 0.0%, and exotic birds - 1.78%. *Salmonella* spp.-positive sites were distributed by category as follows: breeding flocks - 9.09%; laying hens - 15.38; broiler chickens - 0.0%, and exotic birds - 100%.

Of the 571 samples studied (100%), the 12 samples (2.1%) found positive for *Salmonella* spp. included: breeding flocks - 0.35%; laying hens - 1.58%; broiler chickens - 0.0%, and exotic birds - 0.17%. The *Salmonella* spp.-positive samples were distributed by category as follows: breeding flocks - 1.52%; laying hens - 3.93%; broiler chickens - 0.0%, and exotic birds - 4.17%.

Five *Salmonella* serovars were typified distributed as follows: Enteritidis - 25% Infantis - 25%; Corvallis - 25%, Typhimurium - 16,67 % and Thompson-8,33%.

In 2015, the most common confirmed human pathogenic serovars of *Salmonella* were: Typhimurium, isolated from laying hens and parrots, and *Enteritidis* from laying hens.

The tendency to isolate *S.* Infantis and *S.* Corvallis from breeding flocks and laying hens remained unchanged.. In 2015, Salmonella spp. was not isolated in the broiler chickens category in contrast to 2014, when S. Infantis was found in 0.8% of the samples.

Keywords: birds, control, serovar, *Salmonella* spp.

Резюме

За контрол на *Salmonella* spp. през 2015 г. са изследвани 571 бр. проби от птици (свежи фекалии и обувни тампони – 303 бр.; прахови проби и марлени чорапи - 244 бр. от кокошки и вътрешни органи и фецес от екзотични птици – 24 бр.), произхождащи от 56 птицевъдни обекти, класифицирани в 4 категории, в т. ч.: развъдни стада – 11 бр.; кокошки носачки – 26 бр.; пилета бройлери – 18 бр. и екзотични птици – 1 бр. Диагностичните материали са изследвани съгласно БДС EN/ISO 6579, Изменение 1, Приложение D.

От изследваните всичко 56 обекта (100%), 6 обекта (10,71%) са положителни за *Salmonella* spp., в т.ч.: развъдни стада – 1,78 %; кокошки носачки – 7,15%; пилета бройлери – 0,0 % и екзотични птици – 1,78%. Положителните за *Salmonella* spp. обекти по категории се разпределят както следва: развъдни стада – 9,09%; кокошки носачки – 15,38; пилета бройлери – 0,0% и екзотични птици – 100%.

От изследваните 571 бр. проби (100%) 12 бр. (2,1%) са положителни за *Salmonella* spp., в т.ч.: развъдни стада – 0,35%; кокошки носачки – 1,58%; пилета бройлери – 0,0 % и екзотични птици – 0,17%. Положителните за *Salmonella* spp. проби по категории птици се разпределят както следва:

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развъдни стада – 1,52%; кокошки носачки – 3,93%; пилета бройлери – 0,0% и екзотични птици – 4,17%.

Типизирани са 5 серовара *Salmonella* със следното дялово разпределение: Enteritidis – 25%; Infantis – 25%; Corvalis – 25%, Typhimurium - 16,67% и Thompson – 8,33%.

През 2015 г. са доказани най-патогенните за човека серовари на *Salmonella*: Typhimurium – от кокошки носачки и папагали и Enteritidis – от кокошки носачки. Запазва се тенденцията за установяване на *S.* Infantis и *S.* Corvalis от развъдни стада и кокошки носачки. От категория пилета бройлери през 2015 г. не е изолирана *Salmonella* spp., за разлика от 2014 г., когато в 0,8% от пробите е доказана *S.* Infantis.

Introduction

The European Union summary reports on the trends and sources of zoonotic agents and foodborne outbreaks indicate that salmonella infections in birds are a public health risk. The two *Salmonella* serotypes with the highest zooanthropogenic significance are Enteritidis and Typhimurium (CR-517, 2011; EUSR-TSZ, 2012; CR-200, 2012; ARZ-D, 2013; Albuqerque *et al*., 2014; Barbour *et al*., 2015). Although *S.* Enteritidis and *S.* Typhimurium are the most common causes of human foodborne outbreaks, the remaining *Salmonella* serovars should also be subject to control (Nelson and Sheppard, 2009). Carrique-Mas and Davies (2008) define *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Hadar and *S.* Virchow as serovars of great public health importance.

ARZ-D (2013) and EUSR-TSZ (2012) related to the establishment and significance of zoonotic pathogens in food of animal origin for the period 2010-2012 showed that the dominant pathogens were *Campylobacter coli/jejuni* - 67.1%, followed by *Salmonella* spp. - 20.3%, *Yersinia enterocolitica* - 6.2%, and Verotoxigenic *E. coli* VTEC - (3.3%).

Mueller-Doblies (2014) cited an EFSA report on the incidence of human infections reported in 2012 due to the consumption of food products of animal origin. The salmonellosis ranked second after campylobacteriosis; *S. typhimurium* - 22.1%, monophasic *S.* Typhimurium serovars with antigenic formula 1, 4 [5], 12: I - 7.2%, *S.* Infantis - 2.5%, *S.* Stanley - 1.4%, *S.* Thompson - 1.3%, serovars Newport, Derby, Panama - 0.9%, *S.* Kentucky - 0.8% and other serovars - 20.7%, i.e. more than 70% of the cases were caused by target serovars.

Salmonella serotypes change annually across different countries, which is a consequence of globalization and especially of the trade with animals, their products, feed and international travel and human migration. The two foods that are a major cause of foodborne outbreaks due to *Salmonella* spp. are eggs and poultry meat (Barbour *et al.,* 2015). In 67.8% of the samples tested, the most predominant serovars proved to be: Infantis - 70.9%, Typhimurium - 18.5%, Derby - 14.56%, Virchow - 4.9% and Enteritidis - 0.97%. More than 50% of the salmonella infections isolated in humans in the EU countries were caused by 5 serovars: Typhimurium, Enteritidis, Newport, Haidelberg and Javianu (CR 517, 2011; Habrun *et al.*, 2012 ARZ-D, 2013).

As the EU works as a single market, the European Commission has played an important role in harmonizing and coordinating the Salmonella control programs in the EU aimed at reducing the prevalence of *Salmonella*, partly based on a reduction in *Salmonella* prevalence at the primary production level.

In the Republic of Bulgaria, the national *Salmonella* control programs for breeding flocks and laying hens have been in operation since 2008 (SCP-BF, 2016; SCP-LH, 2016) and the *Salmonella* control program for broilers has been implemented since 2009 (SCP-B, 2016). The programs aim at establishing the health status of breeding flocks, laying hens of the genus Gallus Gallus and broilers with regard to *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Hadar, *S.* Virchow, and all other *Salmonella* species, and reducing the percentage of *Salmonella*-positive flocks to 1% or less (SCP-BF, 2016; SCP-LH, 2016; SCP-B, 2016) positive for target serovars.

The purpose of this study is to investigate the prevalence of *Salmonella* spp. in breeding flocks, laying hens and broilers as a result of the implementation of the *Salmonella* control programs for poultry in 2015.

Materials and Methods

In 2015, we surveyed a total of 571 samples of birds, including: fresh feces and shoe pads - 303; powder samples and gilt socks - 244 pcs.; internal organs and feces from exotic birds - 24 pcs. Samples originated from 56 sites classified into 4 categories, including breeding flocks (parents) - 11; flocks of laying hens - 26; broiler flocks - 18 pcs. and exotic birds – 1.

Bacteriological research was carried out at

the Laboratory of Bacterial Diseases of Animals at the National Diagnostic Research Veterinary Medical Institute (NDRVMI), Sofia. For the cultivation and isolation of *Salmonella* spp. we used: buffered peptone water, semi-solid Rapaport-Vasiliadis agar, brilliant grunt lactose sucrose agar, xylose lysine deoxycholate agar, nutrient broth and nutrient agar. Diagnostic materials were developed according to Standard BSS EN/ISO 6579 - Horizontal Method for Isolation of Salmonella spp. Amendment 1: Annex D, 2007: Isolation of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage, a method recommended by the Community Reference Laboratory for *Salmonella* in Bilthoven, the Netherlands.

We performed the serological confirmation of the isolates with pooled agglutinating serum A-E serum, and serotyping was performed in the National Reference Laboratory (NRL) for *Salmonella*, *Campylobacter*, Antimicrobial Resistance and *Staphylococcus aureus* at the National Center for Food Safety (NCFS), NDRVMI, according to the Kauffmann-White-Le Minor scheme. For positive control, we used a reference strain of *S.* Enteritidis ATTC 13076.

Results

Of all 56 sites surveyed (100%), 6 (10.71%) were positive for *Salmonella* spp., including: breeding flocks - 1.78%; laying hens - 7.15%; chicken broilers - 0.0%, and exotic birds - 1.78%. *Salmonella* spp.-positive samples were distributed by category as follows: breeding flocks - 9.09%; laying hens – 15.38; chicken broilers - 0.0%, and exotic birds - 100% (Table 1).

Of the 571 samples examined (100%), 12 samples (2.1%) were positive for *Salmonella* spp., including: breeding flocks - 0.35%; laying hens - 1.58%; chicken broilers - 0.0%, and exotic birds - 0.17%. *Salmonella* spp.-positive samples by bird category were distributed as follows: breeding flocks - 1.52%; laying hens - 3.93%; chicken broilers - 0.0% and exotic birds - 4.17% (Table 2).

In the study of 571 samples from the four bird categories taken from a total of 56 sites, we isolated 5 *Salmonella* serovars from 12 samples. *S.* Enteritidis was proved in 3 samples of laying hens from one site (2 fresh feces and 1 powder sample). We isolated *S.* Typhimurium in 2 samples - one of the fresh feces from laying hens and one of parrot feces. *S.* Corvallis was confirmed in 1 sample of fresh feces, one powder sample and one in shoe pad. *S.* Infantis was found in a sample of fresh feces of laying hens and in a sample of gilt socks of breeding flocks, and in one flock we isolated two *Salmonella* serotypes - Typhimurium and Thompson (Table 3).

Table 1. Results for *Salmonella* spp. detected in different categories of poultry sites (n = 56) in 2015

Site category No.		Examined sites		*Salmonella* spp.-positive sites of total studied sites		*Salmonella* spp.-positive sites in the relevant category
		%	No.	%	%	
1.	Breeding flocks	11	19.64	1	1.78	9.09
2.	Laying hens	26	46.43	4	7.15	15.38
3.	Broilers	18	32.15	0	0.0	0.0
4.	Exotic birds	1	1,78	1	1.78	100
	Total	56	100	6	10.71	

Table 2. Results from examination of flocks (n = 571) for *Salmonella* spp. in 2015

Site category No.		Examined samples		Positive for *Salmonella* spp. samples of all examined samples		Positive for *Salmonella* spp. in the relevant category
		%	No.	%	%	
1.	Breeding flocks	132	23.1	2	0.35	1.52
2.	Laying hens	229	40.1	9	1.58	3.93
3.	Broilers	186	32.6	0	0.0	0.0
4.	Exotic birds	24	4.2	1	0.17	4.17
	Total:	571	100	12	2.10	

Table 3. Affiliation of *Salmonella* serovars by flock category

No	Bird category	Flocks of birds	Isolated serovars
		Examined (no.)/ Positive (no.)	
1.	Breeding flocks	11 / 1	*S.* Infantis
2.	Laying hens	26 / 4	*S.* Enteritidis, *S.* Typhimurium *S.* Corvallis, *S.* Thompson, *S.* Infantis
3.	Broilers	18 / 0	-
4.	Exotic birds	1 / 1	*S.* Typhimurium

The spectrum and percentage of *Salmonella* serovars in the 3 *Salmonella* spp.-positive categories - breeding flocks, laying hens and exotic birds was: *S.* Enteritidis, *S.* Infantis and *S.* Corvallis - 25%, *S.* Typhimurium-16.67% and *S.* Thompson-8.33%, and the *Salmonella* serotypes flocks found in the flocks of laying hens consisted of Enteritidis, Typhimurium, Infantis, Corvallis Corvallis and S. Thompson, each serovar accounting for 20%.

Discussion

The prevalence of target serovars (Enteritidis and Typhimurium) for the period 2008 - 2014 in breeding flocks is as follows: 2008 - 0%; 2009 - 3.49%; 2010 - 5.19%; 2011 - 0.82%; 2012 - 0.48%; 2013 - 1.03% and 2014 - 0.84% (SCP-BF, 2016). In 2015, the prevalence is 0%, which means that the program goal has been exceeded by over 1% and is in line with the results from 2008 to 2010. Bulgaria ranked eighth after Cyprus, Switzerland, Poland, Austria, Hungary, The Czech Republic and Greece of prevalence of Enteritidis, Typhimurium, Infantis, Virchow and Hadar serovars in 2012 (Mueller-Doblies, 2014). For serovars different from the targeted in 2015, the prevalence is 9.09%, well above the previous six years. The isolation of *S.* Infantis is not surprising, due to the tendency recorded for this serovar to be present every year and to dominate over the others.

Sivaramalingamet *et al.* (2013) investigated the *Salmonella* spectrum and prevalence in breeding flocks in 2002-2012 and showed that in broiler breeding flocks the prevalence was 47.4% and the dominant serovars in descending order were: Heidelberg, Kentucky, Hadar and Typhimurium. In breeding flocks of laying hens, they recorded –a prevalence of 25.7%, and serovars Heidelberg, Brandenburg, Thompson and Typhimurium. Serovar Enteritidis was not a particular problem for breeding flocks in Canada, unlike in Europe.

In the flocks of laying hens, the prevalence of target serovars was: 2008 - 0.89%; 2009 - 0.82%; 2010 - 0.8%; 2011 - 1.75%; 2012 - 0.67%; for 2013 and 2014 - 0% (SCP-BF, 2016). In 2012, Bulgaria, together with Hungary, ranked seventh place in prevalence of the target serovars (Mueller-Doblies, 2014). In 2015, the prevalence of *Salmonella* spp. was the highest since the start of the program - 15.38%. Positive targets for the target serovars were 7.69 % of the surveyed sites. For the other serovars, the prevalence was 11.53%. In one of the 26 flocks of laying hens we tested a mixed infection of two serovars - Typhimurium and Thompson. Before 2015, Typhimurium serovars were established only once in 2012, and Serovar Thompson was not recorded among the laying flocks. Gradel *et al.* (2002) isolated mixed salmonellosis infection caused by *S.* Indiana - *S.* Typhimurium in a broiler flock, and *S.* Infantis-*S.* Typhimurium in another. Frietas *et. al.* (2013) have demonstrated 4 pairs of serovar combinations in 4flocks: *S.* Lexington - *S.* Typhimurium; *S.* Corvallis - *S.* Lexington; *S.* Typhimurium - *S.* Corvallis; *S.* Minnesota - *S.* Lexington. Serovar Enteritidis appeared to be the dominant serovar in the flocks for 2015, followed by *S.* Infantis, *S.* Corvallis and others. By 2011, the number of serovars and the number of flocks affected by salmonella increased, from 2012 to 2014 it decreased from 6 to 2, and from 15 to 2 (SCP-LH, 2016), in 2013, 2014, respectively, and 2015 there was permanent presence of *S.* Infantis in laying flocks. Habrun *et al.* (2012) isolated 158 isolates of *Salmonella* spp. from laying hens in Croatia in 2012 and established the highest prevalence for serovars Enteritidis - 34%, Mbandaka - 31% and Infantis - 12%. while a considerably lower prevalence was observed for Typhimurium - 5.7%, Agona - 2.5%, Seftenberg - 1.9%, Montevideo - 1.3%, Virchow, Sentleyvile, Isangi and Lomita - 0.6%. In our studies conducted in laying flocks in 2015, *Salmonella*-positive results were found in 3.93% of the samples of the laying flocks, and in

15.38% of the sites. Of the 26 sites studied, 4 were positive for *Salmonella*, and the isolated serovars were 5, each with a prevalence of 20%.

In Europe, *Salmonella* infections in laying hens are most commonly caused by *S.* Enteritidis, in most cases being isolated from eggs due to *S.* Enteritidis - specific tropism and a mechanism of infection of eggs in the formation. In Australia, where *S.* Enteritidis is not endemic, eggs are most commonly infected with *S.* Typhimurium (Wales and Davies, 2011). A major way for infecting eggs in laying hens with *S.* typhimurium is the horizontal infection via contaminated feces (Pande *et al.,* 2016). *S.* Enteritidis shows affinity to the internal organs of the birds and, in particular to the reproductive organs, in the case of laying hens, as a result of which the eggs are already infected in the ovaries, however, secondary contamination with *S.* enteritidis in the external environment is possible after laying. In the European countries, the presence of *S.* Enteritidis in laying hens is greater than infection with *S.* Typhimurium, a serovar more closely related to infected pigs and calves, contaminated feed or rodents (Carrique-Mas and Davies, 2008a).

The *Salmonella* control program for broilers in Bulgaria started in 2009, with prevalence of target serovars as follows: 2009 - 0.37%; 2010 - 0.1%; 2011 and 2012 - 0%; 2013 - 0.11% and 2014 - 0.29% (3). In 2015, none of the 18 flocks studied were positive for *Salmonella* spp.. The comparison of the results for 2015 in this category with previous ones has reinforced the broiler flocks as a category in which the target serovars are below 1% for the seventh consecutive year (Mueller-Doblies, 2014; SCP-B, 2016). More than five-fold reductions in *Salmonella* serovars and their distribution in broiler flocks in 2014 and the prognosis for a favorable trend in limiting *Salmonella* infections in them in the following years in 2015 were justified (Dimitrova *et al.,* 2016).

Ibrahim *et al.* (2013) conducted a sero-epidemiological study on *Salmonella* infections in poultry and their significance for human health, resulting in the fact that, unlike European countries, in Egypt the biggest problem was the broiler category - with a 16.7% prevalence, an intermediate position occupied by the breeding flocks - with 10% overrun and the smallest for laying hens -2%. *S.* Kentucky has been shown to have the highest degree.

When comparing the results of 2014 and 2015, there are overall differences in three directions: the prevalence of *Salmonella* spp. in the relevant category, the presence or absence of the target serovars and the proof of new serovars for the respective category or the formation of permanent presence. In 2014, *Salmonella* spp. was found in each of the studied categories: breeding flocks, laying hens, broilers and exotic birds, but with a low passage of 0.55%, 0.59%, 0.80% and 37.5%, respectively, and without isolation of the two target serovars (Dimitrova *et al.,* 2016), in contrast to 2015, in which *Salmonella* spp. was not isolated from broiler flocks, and the drift in cattle, laying and exotic cattle categories was high at 9.09%, 15.38% and 100%, respectively. With the inclusion of serovar Thompson in the category of laying hens in 2015, the range of serovars expanded and our attention became more focused, as in 2012 it ranked third after the two target serovars (Mueller-Doblies, 2014).

Unlike Breitsma (2014) and Tauxe *et al.* (2014), who found that over 50% of *Salmonella* infections were caused by *S.* Haidelberg and *S.* Javianu, our results found serovar Infantis to exhibit the highest survivability - 33.3%, followed by Enteritidis, Typhimurium, Corvallis, Thompson - with a 16.7% decrease. The results obtained by us reveal serovar Infantis as a dominant serovar, and are in agreement with the results found by Rotaru *et al.* (2012), who surveyed 7 poultry farms in Romania in 2009-2010, and recorded the highest prevalence for serovar Infantis - 85.4% in 2009 and 76.5% in 2010 out of a total of 833 samples tested.

Unlike Prodanov *et al.* (2012), who investigated *Salmonella* serotypes in laying hens in North Macedonia and found that of 61 *Salmonella* strains *S.* Enteritidis were 35, *S.* Typhimurium - 17, *S.* Infantis - 3, *S.* Derby - 2 and one strain *S.* Heidelberg, *S.* Livingstone, *S.* Typhi and non-typing, we isolated serovars Infantis and Isangi in 2014 from laying hens and serovars Infantis, Enteritidis, Typhimurium, Thompson and Corvallis in 2015.

Pet birds are considered to be a rare source of *Salmonella* for humans, but children, the elderly and those with immunosuppressive diseases may become infected (Gerlach, 2013). Ward *et al.* (2003) examined the cause of mortality of 22% of decorative birds visiting a zoological exhibition and isolated *S.* typhimurium and non-typable Salmonella group B from internal organs of parrots, and Piccirillo *et al.* (2010) describe 2 cases of *Salmonella* infection caused by *S.* Typhimurium and underlined the need to control *Salmonella* spp. in decorative and exotic birds for company, exhibitions, zoos and import and export. In 2015, we isolated S. typhimurium from internal organs and feces from parrots

during their quarantine period after being imported into Bulgaria, unlike in 2014, when we also found parasites of *S.* Sentleyvile, which confirmed the results of other researchers (Ward *et al.,* 2003; Piccirillo *et al.,* 2010; Gerlach, 2013).

Conclusions

The highest percentage of *Salmonella*-positive flocks of laying hens is 15.38 %, with the largest variety of serovars: Enteritidis, Typhimurium, Infantis, Corvallis and Thompson, two of which are pathogenic to humans.

For the first time in 2015 *Salmonella* spp. was not isolated from flocks of broilers, unlike the previous 6 years, when at least 3 serovars were confirmed per year.

In the category of breeding flocks, the tendency of the previous 6 years with isolated *S.* Infantis and *S.* Corvallis and lack of target serovars Enteritidis and Typhimurium remained unchanged. It is recommended that decorative and exotic birds be tested for *Salmonella* spp.

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