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PREVALENT *SALMONELLA* SEROTYPES IN SOME ROMANIAN POULTRY FARMS

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Abstract

The European Centre for Disease Prevention and Control and the European Food Safety Authority devote considerable resources to develop tools and recommendations to improve epidemiological investigation of food-borne outbreaks. One of the most important food-borne agents monitored are Salmonella spp. and in order to reduce risk to public health, several policy actions were designed to control salmonella infections. Present study aimed to investigate the prevalence of Salmonella serotypes in seven poultry holdings located in Giurgiu County, Romania. More than three thousand biological samples have been submitted to bacteriological exam. Briefly, samples were pre-enriched in buffered peptone water followed by enrichment in Modified Rappaport-Vassiliadis, Enrichment broth and Selenite Cystine Broth. Resulting cultures have been plated onto XLD, Istrate Meitert and Wilson-Blair agar plates, incubated at 37°C for 24h. Presumptive Salmonella isolates have been biochemically and serologically confirmed. Between 2008 and 2010 were identified and characterized Salmonella serotypes (525 isolates) as follow: S. Virchow (182 strains), S. Enteritidis (10 strains), S. Tallahassee (3 strains), S. Infantis (273 strains), S. Tennessee (9 strains), S. Mbandaka (8 strains), S. Newport (9 strains), S. Amsterdam (2 strains), S. Salamae (1 strain), S. Kottbus (2 strains), S. Glostrup (1 strain), S. Livingstone (2 strains), S. Isangi (1 strain), S. Hadar (19 strains), and S. Thompson (3 strains). The highest prevalence in 2008 registered S. Virchow (182/199) and in 2009-2010 S. Infantis (140/164 and 124/162 respectively). These results show the heterogeneity of Salmonella strains circulating in poultry farms and the competition of serotypes in different years. Also, the high number of isolates with zoonotic potential (264 strains S. Infantis, 10 strains S. Enteritidis) requires increased attention in food security.

Key words**:** *poultry salmonellosis, salmonella incidence, salmonella surveillance*

INTRODUCTION

In 1880, Eberth and Koch revealed in people died of typhoid fever a bacillus which they considered the etiologic agent of this disease [11]. In 1885, Theobald Smith discover the type bacterium (*Salmonella enterica* var. *Choleraesuis*), and denominate the genus and bacteria after Daniel Elmer Salmon, an American veterinary pathologist. The genus *Salmonella* was finally formally adopted in 1900 by J. Lignières [10].

The genus *Salmonella* contains two species (*S. enterica* and *S. bongori*) and several subspecies biochemical and genomic differentiated [6].

The prevalence and involvement in human infections of specific *Salmonella* serotypes differs from a geographical region to other. In *Salmonella* food poisoning are mainly involved *Salmonella* Enteritidis and *Salmonella* Typhimurium, and more rarelly *Salmonella* Infantis, *Salmonella* Virchow and *Salmonella* Hadar [12]. The dominant *Salmonella* serotypes in different Romanian regions vary from year to year and continuous monitoring of serotypes can supply valuable data in *Salmonella* control.

In accordance with Regulation (EC) No 2160/2003 *Salmonella* control programmes aim at reaching the *Salmonella* reduction target set by Regulations (EC) No 1003/2005, No 1168/2006 and No 646/2007 covering in breeding flocks the serotypes *S.* Enteritidis*, S.* Typhimurium*, S.* Infantis*, S.* Virchow and *S.* Hadar, and in laying hen flocks, chickens and

turkeys the serotypes *S.* Enteritidis and *S.* Typhimurium [8].
In the Annual Report on Zoonoses and Food-borne Outbreaks in the European Union for 2010, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) shows that *Salmonella* cases in humans fell by almost 9%. In European Union, the most frequently reported causes of the food-borne outbreaks were *Salmonella* (31% of all outbreaks), Norwalk-like viruses (15%) and *Campylobacter* (9%). The most important food sources in the outbreaks were eggs and egg products, mixed and buffet meals and vegetables and derived products [9].
In poultry, paratyphoid *Salmonellas* rarely produce clinical signs, usually chickens are asymptomatic carriers of bacteria. Clinical signs are usually seen in poultry less than two weeks of age, are non-specific (e.g. diarrhoea, listless, fluffed up feathers) and similar for all serotypes. The bacteria colonise the intestinal tract and occasionally the reproductive tract of carrier fowls. This situation can cause contamination of carcasses and eggs designed for human consumption [6].
The present study aimed to investigate the prevalence of *Salmonella* serotypes in seven poultry holdings located in Giurgiu County, Romania, in the period 2008 - 2010.

MATERIAL AND METHOD

From seven layer and broiler holdings located in Giurgiu County were collected: 1,532 samples in 2008, 833 samples in 2009 and 741 samples in 2010.
Isolation, identification and serotyping of *Salmonella* spp. has been carried out using standardized methods [1, 2, 3, 4, 5, 12]. Briefly, samples were pre-enriched in buffered peptone water followed by enrichment in Modified Rappaport-Vassiliadis, Enrichment broth and Selenite Cystine Broth. Resulting cultures have been plated onto XLD, Istrate Meitert and Wilson-Blair agar plates, incubated at 37°C for 24h. Presumptive *Salmonella* isolates have been biochemically and serologically confirmed.
The populations of layers and broilers in all investigated holding were 8,860,612 subjects in 2008, 8,852,478 subjects in 2009, and 7,104,428 subjects in 2010.

RESULTS AND DISCUSSIONS

In 2008, the positive samples were 12.88% (199/1532), but almost all strains were isolate in 3 holdings (192/199). One holding was *Salmonella*-free in 2008, and in two holding were isolated only one strain.
In 2009, the number of positive samples was lower than in 2008, but the proportion of positive samples per total samples collected increased to 19.69% (164/833). Three holdings were *Salmonella*-free in 2009; all of them with a low prevalence of *Salmonella* in 2008, but the holding *Salmonella*-free in 2008 have been 18.48% (7/18) of samples positive.
A similar situation was noticed in 2010, when 21.86% (162/741) of samples were positive. One holding maintained the *Salmonella*-free status from 2009 to 2010. Two negative holdings in 2009 have been re-contaminated, but the proportion of positive samples was still low (table 1).
In our study the paratyphoid *Salmonella* contamination covered 85.72% holdings in 2008, 57.12% holdings in 2009 and 85.72% holdings in 2010. These data are higher than those reported in the whole Romania or in EU. In Romania the prevalence of *Salmonella* in breeding flocks of *Gallus gallus* (all types of breeding flocks, flock-based data) has been 2.9% in 2008, 1.5% in 2009 and 12.8% in 2010. In EU the prevalence has been 1.8 in 2008, 2.7 in 2009 and 2.0 in 2010 [7, 13, 14].
Despite the established prevention programs, the holdings with high level of contamination (holdings 1, 2 and 7) maintained their increased level of contamination during the entire period of study (fig. 1).

Table 1. Proportion of *Salmonella* spp. positive samples in seven poultry holdings during the period 2008-2010, in Giurgiu County

Poultry holding	2008			2009			2010		
	No. samples collected	Positive samples		No. samples collected	Positive samples		No. samples collected	Positive samples	
		No.	%		No.	%		No.	%
1	448	101	22.54	206	67	32.52	149	48	32.21
2	204	61	29.90	124	55	44.35	130	95	73.08
3	195	1	0.51	127	0	0.00	109	3	2.75
4	241	1	0.41	179	0	0.00	117	6	5.13
5	269	5	1.86	98	0	0.00	141	0	0.00
6	58	0	0.00	38	7	18.42	90	8	8.89
7	117	30	25.64	61	35	57.38	5	2	40.00
Total	**1532**	**199**	**12.99**	**833**	**164**	**19.69**	**741**	**162**	**21.86**

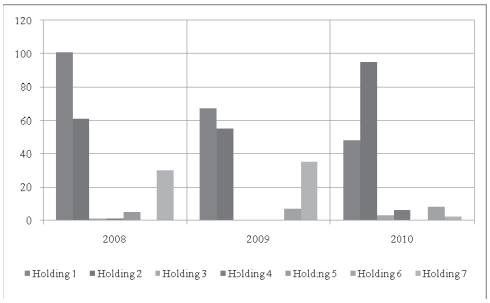

Fig.1.Evolution of *Salmonella* spp. positive samples in seven poultry holdings during the period 2008-2010, in Giurgiu County

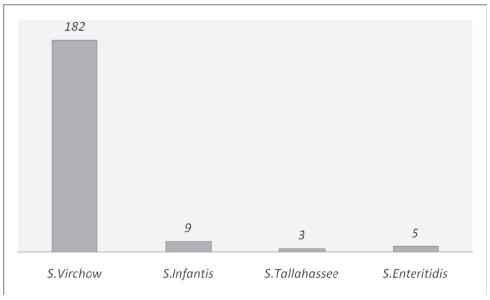

Fig. 2. Number of *Salmonella* serotypes isolated in seven poultry holdings in 2008, in Giurgiu County

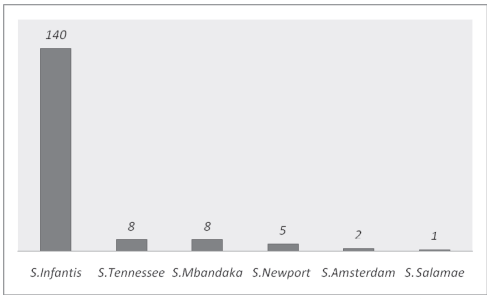

Fig. 3. Number of *Salmonella* serotypes isolated in seven poultry holdings in 2009, in Giurgiu County

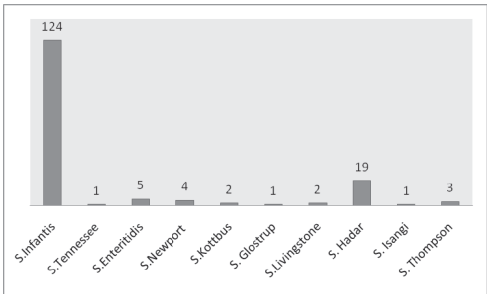

Fig. 4. Number of *Salmonella* serotypes isolated in seven poultry holdings in 2010, in Giurgiu County

Between 2008 and 2010 were identified and characterized *Salmonella* serotypes (525 isolates) as follow:

- *S.* Virchow: 182 strains;
- *S.* Enteritidis: 10 strains;
- *S.* Tallahassee: 3 strains;
- *S.* Infantis: 273 strains;
- *S.* Tennessee: 9 strains;
- *S.* Mbandaka: 8 strains;
- *S.* Newport: 9 strains;
- *S.* Amsterdam: 2 strains;
- *S.* Salamae: 1 strain;
- *S.* Kottbus: 2 strains;
- *S.* Glostrup: 1 strain;
- *S.* Livingstone: 2 strains;
- *S.* Isangi: 1 strain;
- *S.* Hadar: 19 strains;
- *S.* Thompson: 3 strains.

The highest prevalence in 2008 was registered *S.* Virchow (182/199) and in 2009-2010 *S.* Infantis (140/164 and 124/162 respectively (figures 2-4).

In EU, the prevalence of the serovars *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar in adult breeding flocks was 1.3% in 2008, 1.2% in 2009, and 0.7% in 2010[13]. In our study, these serotypes have the following prevalence: 12.92 (196/1532) in 2008, 16.80% (140/833) in 2009, and 19.97% (148/741) in 2010.

An interesting situation was noticed in 2010, when Romania reported the highest prevalence (12.5%) of positive flocks with serovars other than *S.* Enteritidis, *S.* Typhimurium, *S.* Infantis, *S.* Virchow and *S.* Hadar. This has been correlated with data from Giurgiu County, when the serotypes *S.* Tennessee, *S.* Newport, *S.* Kottbus, *S.* Glostrup, *S.* Livingstone, *S.* Isangi, *S.* Thompson covered 8.64% (14/162) of the isolated in 2010. Furthermore, *S.* Typhimurium and *S.* Virchow were not isolated, and *S.* Enteritidis had a prevalence of 3.09% (5/162). In EU, the most common of the targeted serovars in breeding flocks was *S.* Enteritidis [13].

CONCLUSION

The heterogeneity of *Salmonella* strains circulating in poultry farms and the competition of serotypes in different years are high. The high number of isolates with zoonotic potential (264 strains *S.* Infantis, 10 strains *S.* Enteritidis) requires increased attention in food security.

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