

## FREQUENCY OF SALMONELLA SPP. MOBILE SEROVARS ISOLATED DURING 2009-2012 FROM BREEDING HENS FLOCKS

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### Abstract

*In order to control paratyphoid infections having a high zoonotic risk and involving significant economic damage, a National Control Programme for mobile Salmonella infections in breeding hens, including relevant serovars represented by S. enteritidis, S. infantis, S. hadar, S. typhimurium and S. virchow, has been implemented in Romania. During 2009-2012, an epidemiological study was conducted based on primary data collected from breeding hen holdings. Samples represented by faeces were taken at a frequency established by the Community legislation while the programme aims at obtaining a prevalence of 1% or less of the flocks with 95% confidence limit for breeding hens. Following the study, 149 strains belonging to the species Salmonella enterica subsp. enterica were isolated. The results obtained show that serotyped mobile Salmonella strains classified serologically into 16 serovars circulated within the breeding hen holdings. 19% of the isolated serovars belonged to S. enteritidis, 5% belonged to S. infantis, 2.6% belonged to S. typhimurium while the incidence of other serovars was much lower.*

**Keywords:** breeding hens, Salmonella, sampling, serovars

### INTRODUCTION

In intensive poultry farming there has been an increase in the frequency of mobile Salmonella infections and paratyphoid infections, respectively, associated with the development and circulation of new serovars involved in the etiology of these diseases. The occurrence and development of paratyphoid infections cause losses through mortality, prevention and control measures and restrictions on the trade in materials of poultry origin (Clep, 2011; Gast, 2008).

Along with the economic significance, mobile Salmonella infections also have health significance due to the particular zoonotic risk of the serovars involved. Poultry products (eggs, meat, and their derivatives) are the major source of mobile Salmonella infection to human (Clep, 2011; Gast, 2008).

In breeding hen holdings defensive measures are applied, such as biosecurity, avoidance of vertical transmission and use of barrier flora, which is a new concept based on the use of probiotics and competitive exclusion flora. Use of the barrier flora replaces preventive therapy with sulfonamides and antibiotics which is currently banned in EU countries.

On the basis of the Community legislation in this field, the National Programme for Control of mobile Salmonella infections with zoonotic risk in Gallus gallus species was

developed in our country, in breeding holdings (Clep, 2011).

Research covered by this scientific paper were conducted in order to analyze epidemiologically the effectiveness of measures within this program conducted in 2009-2012.

### MATERIALS AND METHODS

For the preparation of this paper, an epidemiological study was carried out over a period of four years, namely during 2009-2012. This study was based on primary data collected from breeding hen holdings nationally while prevalence of serovars isolated from breeding hen holdings was observed.

Primary data collected nationally were presented in tables, processed and presented graphically in order to be interpreted.

Legislation stipulates two types of checks in the breeding hen holdings:

- own check (at farmer's initiative);
- official control (conducted by the county sanitary veterinary and food safety directorates).

Within the own check, samples are taken every two weeks, in the hatchery or the holding. In Romania sampling currently takes place in the holding.

As regards the official control, samples are taken in the holding three times during the production cycle:

- four weeks after the onset of laying;
- eight weeks before the end of the laying period;
- in the middle of the laying period.

Samples are represented by faeces, dust and disposable footwear (socks, slippers) made of absorbable material. The programme aims at identifying a prevalence of up to 1% with 95% confidence limit. Samples are represented by at least 1 g of fresh faeces taken from several places in the housing facility, directly proportional to the number of poultry of the flock, as presented in Table 1.

Table 1. Number of locations from which the samples are taken

Number of poultry kept in the breeding flock	Number of places where faeces samples are taken from the breeding hens flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

When the sampling is performed by using the disposable footwear method (socks, slippers), the designated person walks through the housing facility on a well-established route corresponding to the related area (permanent litter, grids). The footwear used is first moistened with diluted solution recommended by the National Reference Laboratory (0.8% sodium chloride, 0.1% peptone, distilled or double distilled water, pH = 7). The routes on which the persons appointed walk shall represent 20% of the housing for each couple of covers of the five pairs to be collected and subsequently grouped into at least two composite samples

The samples taken were sent refrigerated within 24 hours to the county accredited laboratories where they were processed within 48-96 hours from sampling.

Bacteriological examinations are performed in accordance with ISO Standard 6579-2002/Amendment 1:2007 – Horizontal method for detection of *Salmonella* spp. developed by the Community Reference

Laboratory for *Salmonella* spp. isolated from poultry in Bilthoven, the Netherlands. Such methodology is used in the county authorised sanitary veterinary laboratories.

## RESULTS AND DISCUSSIONS

The results obtained following the processing of samples taken from breeding hen flocks during 2009-2012 showed that 149 strains belonging to the species *Salmonella enterica* subsp. *enterica* classified serologically into 16 serovars were identified and results are presented in Table 2.

Frequency of serovars and strains isolated from breeding flocks varies. Thus, serovar *S. enteritidis* had the highest frequency, with 29 strains isolated and identified, while serovar *S. glostrup* had the lowest frequency, with one strain isolated; 2 strains were isolated for each of the serovars *S. liverpool* and *S. taksony*.

The analyse of frequency of the mobile serovars shows that serovars *S. enteritidis*, *S. infantis* and *S. typhimurium* were identified of the 5 relevant serovars for breeding hens represented by *S. enteritidis*, *S. infantis*, *S. hadar*, *S. typhimurium* and *S. virchow*.

Relevant serovars *S. virchow* and *S. hadar* were not identified in breeding hen flocks.

Table 2. Mobile Salmonella serovars isolated from breeding hen flocks during 2009-2012

Position	Broilers: strains isolated during 2009-2012				%	
	Serovar/ year	2009	2010	2011		2012
1	<i>S. agora</i>		1	1	2	2.6
2	<i>S. amsterdam</i>		2	4	1	4.6
3	<i>S. enteritidis</i>	7	15	7		19.4
4	<i>S.glostrup</i>			1		0.6
5	<i>S. infantis</i>		1	3	4	5.3
6	<i>S. kentucky</i>			4	2	4.02
7	<i>S.liverpool</i>				2	1.3
8	<i>S. livingstone</i>		5			3.3
9	<i>S. mbandaka</i>	5	3	8		10.7
10	<i>S. montevideo</i>	3	13		2	12.08
11	<i>S. senftenberg</i>	4		3	5	8.05
12	<i>S.taksony</i>				2	1.3
13	<i>S.tennessee</i>			2	4	4.02
14	<i>S. thompson</i>	2	10	7		12.7
15	<i>S. typhimurium</i>		1	2	1	2.6
16	<i>S. uganda</i>		4		6	6.7
	<b>Total strains</b>	21	55	42	31	
	<b>149</b>	strains	strains	strains	strains	

Several serovars considered as exotic for Romania, such as *S. senftenberg* and *S. thompson* occurred due to imports of replacement youth and one day old chickens from non-EU countries where frequency of these serovars is higher. Several exotic serovars entered free countries, including Romania, due to epidemiologic route of Salmonella, worldwide, produced mainly by trade in materials of poultry origin from non-Eu countries where legislation is not very strict as regards the control of mobile Salmonella infections.

Far fewer serovars were isolated from breeding hen holdings as compared to broilers since flocks imported are smaller, biosecurity rules are very strict and control performed through sampling is rigorous.

Frequency of mobile Salmonella serovars isolated in our country has been variable in recent years. The results provided by other authors were influenced largely by the development of intensive poultry farming and trade in material of poultry origin.

Volintir (1975) cited by Verdes (2001), shows, following a study, that *S. typhimurium* was isolated in proportion of 63-93% from broilers and hens, while the proportion of other serovars was much lower, and Sicoe (1988) cited by Danes (2010) showed that serovars *S. typhimurium* and *S. enteritidis* had the highest frequency.

Following the liberalization of trade in material of poultry origin in our country, Draghia *et al.* (1993) showed that 5% of the breeding hens were mobile Salmonella carriers, while *S. enteritidis* (47.4%) and *S. typhimurium* (18.6%) were dominant serotypes.

During 2001-2005, Tatu-Chitioiu *et al.* (2006) studied 2007 strains of mobile Salmonella out of which 2402 were isolated from poultry, *S. enteritidis* was the dominant serovar, with a frequency of 43.3%, and serovar *S. djugu* (1.25%) had the lowest frequency. Within this study, 57 serovars were identified out of which 7 serovars were considered new for our country.

Frequency of mobile serovars worldwide isolated from breeding hens varies and changes periodically depending on a lot of factors. In USA, serovars *S. heidelberg*, *S.*

*kentucky*, *S. enteritidis*, *S. seftenberg* are frequently isolated from species *Gallus gallus*, and 21 mobile serovars were isolated in the EU, out of which 5 were considered relevant serovars for breeding hens due to the high frequency and zoonotic risk (Gast, 2008; Popa *et al.*, 2006).

Serovars isolated during 2009-2012 from breeding hens within the National Programme are among serovars with high frequency isolated both in USA and EU, but their number and number of isolated strains is much lower than in laying hens and broilers.

Due to the intervention of predisposing factors, the circuit of mobile Salmonella is complex and favours circulation of certain serovars. Thus, during 2009-2012, in our country serovar *S. enteritidis* was dominant of the relevant serovars for breeding hen flocks while relevant serovars *S. virchow* and *S. hadar* were not identified.

## CONCLUSIONS

During 2009-2012, 149 mobile Salmonella strains were isolated in breeding hens.

In breeding hens, the relevant serovar *S. enteritidis* had the highest incidence whereas 29 strains were identified, 8 strains were isolated for *S. infantis* and 4 strains were isolated for *S. typhimurium*.

The other relevant serovars represented by *S. hadar* and *S. virchow* were not identified.

Serovars considered as exotic for Romania, such as *S. senftenberg* and *S. thompson* were also identified.

Most of the serovars isolated during 2009-2012 in breeding hens are frequently isolated in EU or non-Eu countries.

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