PREVALENCE OF MOBILE SEROVARS OF *SALMONELLA SPP.* ISOLATED FROM BREEDING HENS, LAYING HENS AND BROILER CHICKENS IN 2010

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Abstract

For the control of mobile Salmonella presenting a high zoonotic risk and also involving property damage through mortality, a National Program was implemented in Romania to control mobile Salmonella infections in breeding hens, which includes relevant serovars represented by S. enteritidis, S. infantis, S. hadar, S. typhimurium and S. virchow and relevant serovars represented by S. enteritidis and S. typhimurium for laying hens and broiler chickens.
The faeces samples were collected with a frequency established by the Community legislation while the program aimed at reaching a prevalence of 1% or less of the flocks with a confidence interval (confidence limit) of 95% for breeding hens and broiler chickens and up to 2% for flocks of laying hens.
Following the study conducted, 655 strains of Salmonella enterica subsp. Enterica were isolated.
For the breeding hens, serovar S. enteritidis had the highest incidence - 15 strains were identified, for serovar S. typhimurium only one strain was isolated, while the other relevant serovars represented by S. infantis, S. hadar and S. Virchow were not identified.
For the laying hens, S. enteritidis had the highest incidence - 19 strains were identified, while for serovar S. typhimurium 5 strains were identified.
In farms of broiler chickens S. enteritidis had the highest incidence as well - 38 strains were identified, while serovar S. Typhimurium was not identified.

***Key words*:** *breeding hens, broiler chickens, laying hens, Salmonella.*

INTRODUCTION

In intensive poultry farming there was an increase in the frequency of mobile Salmonella infections, namely paratyphoid infections, associated with the occurrence and circulation of new serovars involved in the etiology of these diseases. Occurrence and evolution of paratyphoid infections cause losses through mortality, implementation of prevention and control measures and restrictions on the trade in poultry (Clep, 2011; Gast, 2008).
Besides the economic issues, mobile Salmonella infections are also important in terms of health, due to the high zoonotic risk of the serovars

involved. Poultry products (eggs, meat and their derivatives) are the major source of human infection with mobile Salmonella (Clep, 2011; Gast, 2008). In breeding hens, laying hens and broiler farms, the folowing measures are taken in order to prevent the mobile Salmomonella spreading: biosecurity, vertical transmission avoidance and usage of barrier flora, the latter one being a new concept based on probiotic products usage and competitive exclusion of flora. The usage of the barrier flora method, is replacing the preventive therapy based on antibiotics and sulphonamides, which is currently forbiden in the EU countries.
Based on the Community legislation in this field, a National Programme for control of mobile Salmonella infections with zoonotic risk for species *Gallus gallus* in breeding, laying and broiler farms was developed in our country (Clep, 2011).
Research that is subject of this paper was conducted in order to analyze epidemiologically the effectiveness of measures within the program performed in 2010.

MATERIALS AND METHODS

In order to prepare this paper, a longitudinal epidemiologic study was conducted throughout 2010. That year was the second year of the National Programme for control of mobile Salmonella infections with zoonotic risk for species *Gallus gallus*. Such longitudinal study was based on primary data collected from holdings with breeding hens, laying hens and broiler chickens nationwide.
This study analysed the prevalence of serovars isolated from farms with breeding hens, laying hens and broiler chickens.
The primary data collected nationwide were presented in tables, processed and presented graphically to be interpreted.
In **holdings with breeding hens** legislation provides for two types of controls:
- self control (at the initiative of farmers);
- official control (performed by the County Sanitary Veterinary and Food Safety Directorate)

Within the self control, samples are taken every two weeks from the hatchery or the holding. Currently, in Romania, the sampling is performed in the farm.
As regards the official control, samples are taken in the farm, three times during the production cycle:

- four weeks following the beginning of laying;
- eight weeks before the end of the laying period;
- in the middle of the laying period.

Samples taken are represented by faeces and disposable footwear (boot swab) made of absorbent material.

The programme aims at reaching prevalence up to 1% of the flocks with a confidence interval (confidence limit) of 95%. Samples are represented by at least 1 g of fresh faeces taken from several points of the housing, directly proportional to the number of poultry of the flock, as it is shown in Table 1.

Table 1

Number of locations from which the samples are prelevated

Number of birds kept in the breeding flock	Number of points that are collected faeces samples to be taken from the breeding hens flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

In **holdings with laying hens** legislation provides for two types of controls in terms of samples taken:

- self control performed at the initiative of farmers;
- official control performed by the state veterinary services.

Production stages to be covered by sampling through the self-control programme of the farmer are as follows:

- one day old chickens;
- pullets two weeks before the beginning of laying or transfer to the layer house;
- every 15 weeks during the laying period (Daneș, 2010);

The state veterinary services take samples at least:

- annually in a poultry flock but in holdings with at least 1 000 poultry;
- when poultry reach the age of 24 weeks (with a margin of plus or minus 2 weeks), in flocks of laying hens kept in houses where the previous poultry flock was infected with *Salmonella.*

The state veterinary services should obligatorily take samples when infections with *S. enteritidis* or *S. typhimurium* are suspected following an epidemiological survey on the foodborne illness outbreaks. It is also necessary to take official samples in all other flocks of laying hens of the holding, where the presence of *S. enteritidis* or *S. typhimurium* is detected in one of the flocks of laying hens of the holding.
The program aims at achieving prevalence up to 2% of the flocks with a confidence interval (confidence limit) of 95%.
In **broiler chicken farms,** legislation provides for two types of controls:
- self control performed at the initiative of farmers;
- official control performed by the state veterinary services.

Sampling within the self-control should take place 3 weeks before slaughtering the broiler chickens.
As regards the official control carried out by the state veterinary services, it must include every year at least one flock with broilers of 10% of the broiler farms with more than 5000 heads.
Faeces samples are taken to reach prevalence up to 1% of the flock with a confidence interval (confidence limit) of 95%.
Designated persons who use disposable footwear (socks, slippers) walk inside the house on a well-established route corresponding to the surface (permanent litter, grids). Footwear used is priory moistened with diluting solutions recommended by the National Reference Laboratory (0.8% sodium chloride, 0.1% peptone, distilled or double-distilled water, pH = 7). The routes used by the designated persons must represent 50% of the housing.
Samples taken were sent under refrigeration conditions to the accredited county laboratories within 24 hours, where they were processed within 48-96 hours from sampling.
Bacteriological examinations are conducted in accordance with ISO 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. This methodology is used by the authorized county sanitary veterinary laboratories.

RESULTS AND DISCUSSIONS

In 2010, following the analysis of samples taken from flocks with breeding hens, laying hens and broiler chickens, 655 strains belonging to the species *Salmonella enterica subsp. Enterica* were identified.

In case of detecting mobile Salmonella in holdings with breeding hens in our country, in 2010, 55 strains of mobile Salmonella belonging to 10 serovars were isolated; the results are presented in Table 2.

Table 2

Serovars of mobile Salmonella isolated from flocks of breeding hens in Romania in 2010

No.	Breeding hens: 55 strains		%
1	S. agora	1	1.8
2	S. amsterdam	2	3.6
3	S. enteritidis	15	27.3
4	S. ifantis	1	1.8
5	S. livingstone	5	9.1
7	S. mbandaka	3	5.5
8	S. montevideo	13	23.6
9	S. thompson	10	18.2
10	S. typhimurium	1	1.8
11	S. uganda	4	7.3

Frequency of serovars and strains isolated from the breeding flocks is variable. Thus, serovar *S. enteritidis,* with 15 strains isolated and identified, had the highest frequency, while serovars *S. typhimurium, S. ifantis* and *S. agora* had the lowest frequency, with one strain each.

Following the analysis of the frequency of the mobile serovars we notice that serovars *S. enteritidis* and *S. typhimurium* were identified out of the five serovars relevant for the breeding hens, namely *S. enteritidis, S. infantis, S. hadar, S. typhimurium* and *S. virchow.*

As regards the mobile Salmonella in the holdings with laying hens in our country, in 2010, 179 strains were isolated, belonging to 15 serovars; the results are presented in Table 3.

Following the analysis of the frequency of the mobile serovars in laying hens we notice that both serovars relevant for flocks of laying hens were

identified, namely serovar *S. enteritidis* within which 19 strains were isolated and identified and *S. typhimurium* within which 5 strains were isolated and identified. Serovar *S. livingstone* – a serovar that is not relevant - had the highest frequency, with 45 strains isolated and identified, while serovars *S. gallinarum*, *S. senftenberg* and *S. agora*, with one strain each, had the lowest frequency.

Table 3

Serovars of mobile Salmonella isolated from flocks of laying hens in Romania in 2010

No.	Laying hens: 179 strains		%
1	S. agora	1	0.6
2	S. bredeney	2	1.1
3	S. enteritidis	19	10.6
4	S. gallinarum	1	0.6
5	S. hadar	3	1.7
7	S. ifantis	7	3.9
8	S. livingstone	45	25.1
9	S. mbandaka	6	3.4
10	S. montevideo	33	18.4
11	S. newport	4	2.2
12	S. senftenberg	1	0.6
13	S. tennessee	5	2.8
14	S. thompson	42	23.4
15	S. typhimurium	5	2.8
16	S. uganda	5	2.8

In case of detecting mobile Salmonella in holdings with broiler chickens in 2010, 421 strains of mobile Salmonella belonging to 18 serovars were isolated; the results are presented in Table 4.

Serovar *S. ifantis*, with 267 strains identified, had the highest incidence in farms with broiler chickens. Out of the two serovars relevant for broiler chickens, namely *S. enteritidis* and *S. typhimurium*, only *S. enteritidis* was identified, with 38 strains identified and isolated.
Serovar *S. virchow* was not identified in breeding hens, laying hens and broiler chickens.
Some serovars considered exotic for Romania, such as *S. senftenberg* and *S. thompson*, occurred due to imports of replacement chickens and day-old chickens from third countries, where frequency of such serovars is increased. Exotic serovars entered free countries, including Romania, due to the epidemiology track of Salmonella, worldwide, determined firstly by the trade in poultry from third countries where legislation is more permissible on the control of mobile *Salmonella* infections.

Table 4

Serovars of mobile Salmonella isolated from flocks of broiler chickens in Romania in 2010

No.	Broiler chickens: 421 strains		%
1.	S. amsterdam	1	0.2
2.	S. bredeney	2	0.5
3.	S. enteritidis	38	9.02
4.	S. glostrup	1	0.2
5.	S. hadar	26	6.2
6.	S. ifantis	267	63.4
7.	S. insangi	3	0.7
8.	S. kentucky	11	2.6
9.	S. kottbus	2	0.47
10.	S. liverpool	2	0.47
11.	S. livingstone	4	0.95
12.	S. mbandaka	1	0.2
13.	S. montevideo	3	0.7
14.	S. orion	2	0.47
15.	S. senftenberg	14	3.3
16.	S. taksony	33	7.8
17.	S. tennessee	7	1.7
18.	S. thompson	4	0.95

Much less serovars were isolated from holdings with breeding hens compared to the farms with broiler chickens because the imported flocks are smaller, biosecurity rules are very strict and control performed through sampling is rigurous.

Frequency of mobile *Salmonella* serovars isolated in our country was variable in recent years, whereas number of serovars and strains isolated increased. The results provided by other authors were influenced largely by the developments of intensive poultry farming and trade in poultry.

Volintir quoted by (Verdeş, 2001) showed in 1975, following a study, that a percentage of 63-93% *S. typhimurium* was isolated from broiler chickens and hens, while a much lower percentage of other serovars was isolated and Sicoe quoted by (Daneş, 2010) showed in 1988 that serovars *S. typhimurium* and *S. enteritidis* had the highest frequency.

Following the liberalization of trade in poultry in our country (Draghia *et al*., 1993) showed that 5% of the breeding hens were carriers of mobile *Salmonella*, while the dominant serotypes were *S. enteritidis* (47,4%) and *S. typhimurium* (18,6%).

During 2001-2005, (Tatu-Chitoiu *et al.*, 2006) studied 2807 mobile *Salmonella* strains, out of which 2402 were isolated from poultry, while *S. enteritidis* was the dominated serovar, with a frequency of 43.3%, and serovar *S. djugu* had the lowest frequency (1.25%). In this study, 57 serovars were identified, out of which 7 were considered new serovars for our country.

Worldwide, frequency of the mobile serovars isolated from breeding hens, laying hens and broiler chickens is variable and changes periodically, depending on many factors. In USA, serovars *S. heidelberg*, *S. kentucky*, *S. enteritidis*, *S. seftenberg* are frequently isolated in *Gallus gallus* species, while 21 mobile serovars were isolated in the EU, out of which 5 were considered relevant serovars for breeding hens due to their high frequency and zoonotic risk (Gast, 2008; Popa *et al*., 2006), and 2 serovars, namely *S. enteritidis* and *S. typhimurium* are considered relevant for broiler chickens and laying hens.

Serovars isolated in 2010 from breeding hens under the National Program are represented by high frequency serovars isolated both in USA and the European Union, but their number and the number of strains isolated is much lower compared to the laying hens and broiler chickens.

Due to the intervention of favorable factors, the mobile *Salmonella* track is complex and promotes the circulation of certain serovars. Thus, in 2010, in

our country, out of the relevant serovars, serovar *S. enteritidis* was dominant in breeding hens, laying hens and broiler chickens.

CONCLUSIONS

Pathological material samples were collected in accordance with the legislative provisions for holdings with breeding hens, laying hens and broiler chickens.
Bacteriological examinations are conducted in accordance with the legislative provisions in authorized county laboratories and National Salmonella Reference Laboratory within IDAH Bucharest where serotyping is performed as well.
In 2010, 655 strains of mobile Salmonella were isolated from breding hens, laying hens and broiler chickens. For the breeding hens, relevant serovar *S. enteritidis* had the highest incidence - 15 strains were identified, for relevant serovar *S. typhimurium* only one strain was isolated, while the other relevant serovars represented by *S. infantis*, *S. hadar* and *S. Virchow* were not identified. For the laying hens, out of the two relevant serovars, *S. enteritidis* had the highest incidence - 19 strains were identified, and 5 strains were identified for serovar *S. typhimurium*. In farms of broiler chickens *S. enteritidis* had the highest incidence as well - 38 strains were identified, while serovar *S. Typhimurium* was not identified.
Following the analysis of data, we notice that serovar *S. enteritidis* was identified in breeding hens, laying hens and broiler chickens. As regards serovar *S. virchow,* it was not identified in any category of poultry. Also, serovars considered exotic for Romania, such as *S. senftenberg* and *S. thompson* were isolated.
Most serovars isolated in 2010 from breeding hens, laying hens and broiler chickens are often isolated in EU or non-EU countries.

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