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THE PREVALENCE OF *SALMONELLA* SEROTYPES IN DOMESTIC AND WILD ANIMALS AND THEIR SPATIAL DISTRIBUTION IN ROMANIA, BETWEEN 2015 AND 2016

PREVALENȚA SEROTIPURILOR *SALMONELLA* LA ANIMALE DOMESTICE ȘI SĂLBATICE ȘI DISTRIBUȚIA LOR SPAȚIALĂ ÎN ROMÂNIA, ÎNTRE 2015 ȘI 2016

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ABSTRACT | REZUMAT

Salmonella are major worldwide zoonotic pathogens infecting a wide range of animal species as well as humans. This study investigates the prevalence of circulating *Salmonella* serotypes in domestic and wild animal species in Romania and the spatial distribution of *Salmonella* isolates at county level in the period 2015-2016. There were collected 2551 samples for the isolation and serotyping of *Salmonella* according to ISO 6579:2002/Amd 1:2007 protocol. The findings of this study show that 36 *Salmonella* serotypes were isolated in 1357 *Salmonella*-positive samples in 2015, and 28 serotypes in 1188 *Salmonella*-positive samples in 2016. One sample from pigeon and one from hen were negative in 2015, and one sample from pigeon and three samples from hen were negative in 2016. In 2015, the most frequently isolated serotypes were *S.* Infantis (50.92%), *S.* Enteritidis (9.87), *S.* Senftenberg (4.72%), *S.* Tennessee (4.72), and *S.* Kentuky (3.83%). In 2016, the most frequently isolated serotypes were *S.* Infantis (48.15%), *S.* Agona (7.83%), *S.* Enteritidis (7.66%), *S.* Tennessee (7.66%), *S.* Liverpool (4.29%), and *S.* Senftenberg (4.29%).

In order to reduce the prevalence of serotypes with major zoonotic potential, we recommend continuing collaboration of all the professionals involved in the security of the food chain.

Key words: *Salmonella, serotypes, distribution, Romania, animals*

Salmonelele sunt agenți patogeni cu potențial zoonotic major, răspândiți in toata lumea, capabili să infecteze numeroase specii de animale și omul. Acest studiu investighează prevalența serotipurilor de *Salmonella* circulante în diferite specii de animale din România precum și distribuția spațială a izolatelor *Salmonella*, la nivel județean, în perioada 2015-2016. Au fost colectate 2551 de probe și analizate în vederea izolării și serotipizării *Salmonella* conform standardului ISO 6579: 2002/Amd 1:2007. Rezultatele acestui studiu arată că în anul 2015 au fost izolate 36 de serotipuri în cele 1357 de probe *Salmonella* pozitive și 28 de serotipuri în cele 1188 de probe *Salmonella* pozitive în 2016. O probă de la porumbei și una de la găină a fost negative în 2015 și o probă de la porumbei și trei probe de găină au fost negative în 2016. În 2015, cele mai frecvente serotipuri izolate au fost *S.*Infantis (50,92%), *S.*Enteritidis (9,87) *S.*Senftenberg (4,72%), *S.*Tennessee (4,72%) și *S.* Kentuky (3,83%). În 2016, cele mai frecvente serotipuri izolate au fost *S.* Infantis (48,15%), *S.* Agona (7,83%), *S.* Enteritidis (7,66%), *S.* Tennessee (7,66%) *S.* Liverpool (4,29%) și *S.* Senftenberg (4,29%).

In scopul reducerii prevalenţei serotipurilor cu potenţial zoonotic major, recomandăm colaborarea continuă a tuturor celor implicați în securitatea lanțului alimentar.

Cuvinte cheie: *Salmonella, serotipuri, distribuţie, România, animale*

Salmonella is a diverse bacterial species comprising more than 2600 serotypes (4). This pathogen commonly colonizes a range of animals such as mammals, amphibians, reptiles, birds and insects (5).

Diseases caused by *Salmonella* represent a very important public health problem among the bacterial foodborne pathogens (7, 9). It is estimated that 93.8 million cases and 155,000 deaths are associated with gastroenteritis due to *Salmonella* species each year worldwide (8). Human salmonellosis has been associated with contaminated food products, mainly those of animal origin such as poultry, beef, pork and dairy products and direct contact with infected animals, respectively (1, 2, 3).

The aim of this study was to determine the prevalence of *Salmonella* serotypes circulating in different animal species in Romania and the spatial distribution

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of *Salmonella* isolates at county level, between 2015 and 2016.

MATERIAL AND METHODS

Sampling

As part of the official activities concerning the *Salmonella* serotypes surveillance, in 2015 a number of 1361 samples were analyzed and in 2016, 1195 samples. The samples were from 13 animal species (table 1), domestic and wild, located all over the country in farms, backyards or zoo gardens.

Table 1

Samples used for *Salmonella spp.* isolation, between 2015 and 2016, in Romania

No.	Species	Year	
		2015	2016
1.	Cattle	4	4
2.	Goat	1	4
3.	Turkey	2	1
4.	Hen	1298	1119
5.	Sheep	24	45
6.	Pigeon	6	4
7.	Quail	2	3
8.	Duck	5	0
9.	Swine	15	6
10.	Mink	1	0
11.	Chinchilla	0	1
12.	Fox	0	1
13.	Lion	0	1

Method of isolation

All samples were analyzed according to ISO 6579: 2002/Amd 1:2007 protocol for the isolation of *Salmonella* (6). The data generated were entered into a Microsoft Excel 2007 software. The frequency of each *Salmonella* serotype isolated as well as the frequency of *Salmonella* serotype from different matrices and county were determined.

RESULTS AND DISCUSSIONS

In 2015, 36 *Salmonella* serotypes were isolated in 1357 positive samples (serotyping in 4 samples was not possible), while in 2016, only 28 *Salmonella* serotypes were isolated in 1189 positive samples. The serotypes isolated in 2015 were obtained from cattle (4 strains), goat (1 strain), turkey (2 strains), hen (1298 strains), sheep (24 strains), pigeon (5 strains), quail (2 strains), duck (5 strains), swine (15 strains)~~,~~ and mink (1 strain).

All serotypes isolated in 2015 from cattle (4 isolates), goat (1 isolate), pigeon (5 isolates) and mink (1 isolate) were *S*. Typhimurium. Similar situations were recorded for turkey, quail and sheep, where one serotype was isolated in each species: *S.* Gallinarum in turkey (2 isolates), *S*. Kentuky in quail (2 isolates), and *S*. Abortus ovis in sheep (24 isolates). From duck samples, three serotypes were isolated: *S.* Derby (40.00% 2/5), *S.*Regent (20.00%,1/5),and *S.*Anatum (40.00% 2/5), while in swine five serotypes: *S*. Typhimurium (60.00%, 9/15), *S.* Derby (13.33%, 2/15), *S.* Rissen (13.33%, 2/15), *S.* Infantis (6.66%, 1/15), and *S.* Bredeney (6.66%, 1/15). As expected, most serotypes (86.11%, 31/36) were typed in the hens, from which most samples were analysed. The following serotypes were isolated in hen samples: *S*. Typhimurium (2.31%, 30/1298), *S.* Gallinarum (1.31%, 17/1298), *S.* Kentuky (3.85%, 50/1298), *S.* Infantis (53.16%, 690/1298), *S.* Bredeney (0.62%, 8/1298), *S.* Abony (0.14%, 2/1298), *S.* Agona (1.69%, 22/1298), *S.* Albany (0.23%,3/1298),*S.*Amsterdam (0.23%,3/1298) *S.*Borbeck (0.23%, 3/1298), *S.*Brandemburg (0.15%, 2/1298), *S.* Chester (0.07%, 1/1298), *S.* Cubana (0.07%, 1/1298), *S.* Enteritidis (10.32%, 134/1298), *S.* Fyris (0.07%, 1/1298), *S.* Glostrup (0.38%, 5/ 1298), *S.* Hadar (0.60%, 7/1298), *S.* Havana (0.38 %, 5/1298), *S.* Kottbus (1.93%, 25/1298), *S.* Liver-pool (3.00%, 39/1298), *S.* Livingstone (1.15%, 15/ 1298), *S.* Mbandaka (1.39%, 18/1298), *S.* Montevi-deo (1.08%, 14/1298), *S.* Newport (1.31%, 17/1298) *S.* Ohio (0.07%, 1/1298), *S.* Orion (0.07%, 1/1298), *S.* Senftenberg (4.93%,64/1298), *S.*Taksony (0.85%, 11/1298), *S.*Tennessee (4.93%,64/1298), *S.* Uganda (0.15%, 2/1298), *S.* Thomson (3.31%, 43/ 1298).

Serotypes isolated in 2016 were obtained from cattle (4 strains), goat (4 strains), turkey (1 strain), hen (1119 strains), sheep (45 strains), pigeon (3 strains), quail (3 strains), swine (6 strains), chinchilla (1 strain), fox (1 strain), and lion (1 strain).

All serotypes isolated in 2016 from pigeon (3 isolates), swine (6 isolates), chinchilla (1 isolate), fox (1 isolate) and lion (1 isolate) were *S*. Typhimurium. Only one serotype was isolated from goat samples - *S*. Abortus ovis (4 isolates) and turkey - *S*. Gallinarum (1 isolate).

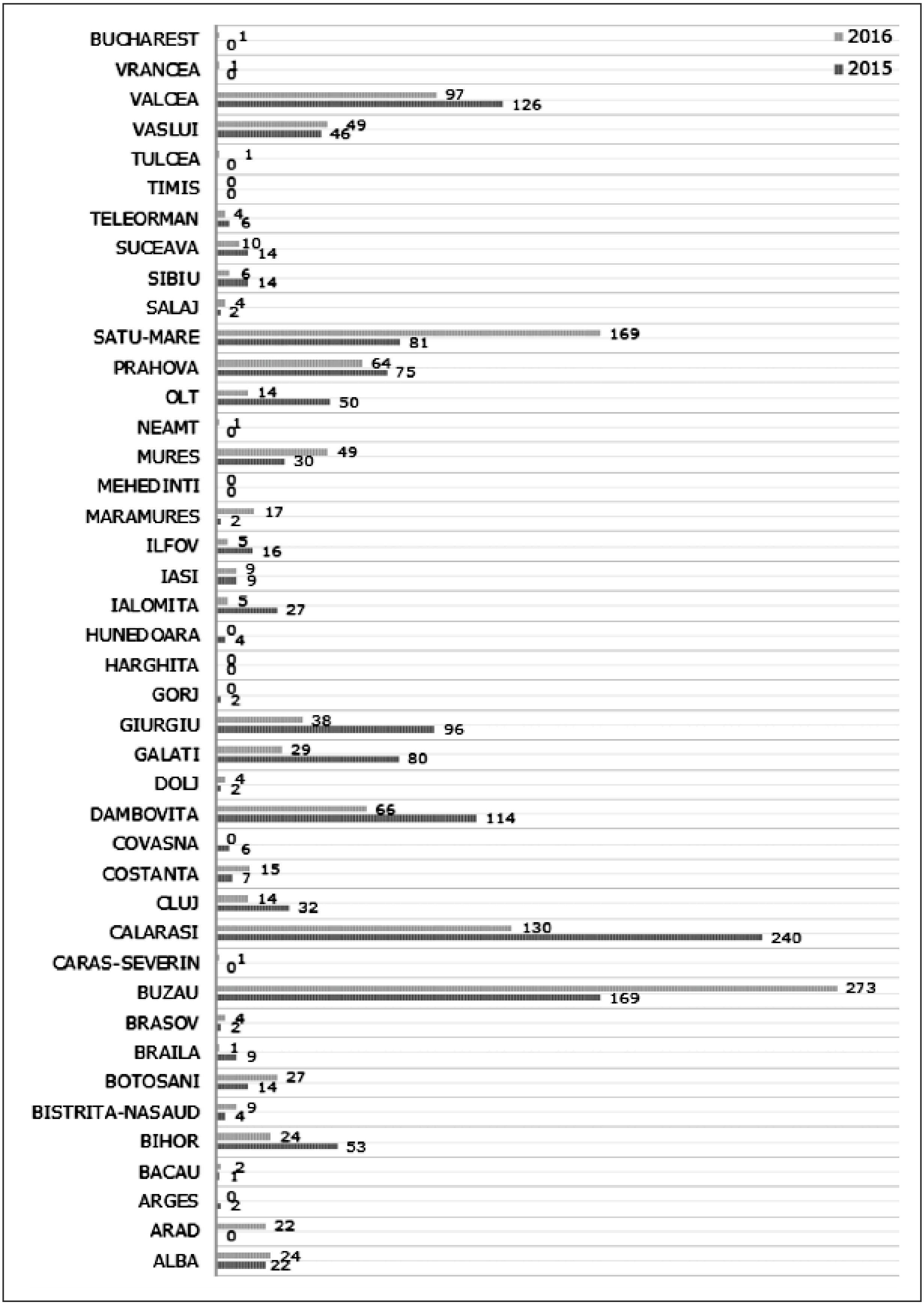

Fig. 1. *Salmonella* serotypes distributions in Romanian counties, between 2015 and 2016

From cattle samples, three serotypes were isolated: *S.* Typhimurium (25.00%, 1/4), *S.* Enteritidis (25.00%,1/4), and *S.* Montevideo 50.00%,2/4), while from sheep two serotypes were isolated: *S.* Abortus ovis (93.33%, 42/45) and *S.* Enteritidis (6.66%, 3/45) and from quail samples two serotypes: *S.* Typhimurium (66.66%, 2/3) and *S.* Hadar (33.33%, 1/3).

From hen samples were isolated 92.86% (26/28) of all serotypes recorded in 2016, as follows: *S.* Typhimurium (0.45%, 5/1119), *S.* Gallinarum (0.98%, 11/1119), *S.* Kentuky (4.29%, 48/1119), *S.* Infantis (51.12%, 572/1119), *S.* Bredeney (0.27%, 3/1119), *S.* Agona (8.31%, 93/1119), *S.* Albany (0.09%, 1/1119), *S.* Bovis modificans (0.09%, 1/1119), *S.* Chester (0.09%, 1/1119), *S.* Enteritidis (7.77%, 87/1119), *S.* Glostrup (0.09%, 1/1119), *S.* Gloucester (0.09%, 1/1119), *S.* Havana (0.63%, 7/1119), *S.* Kottbus (0.29%, 3/1119), *S.* Liverpool (4.56%, 51/1119), *S.* Livingstone (0.89%, 10/1119), *S.* Mbandaka (1.70%, 19/1119), *S.* Montevideo (0.18 %, 2/1119), *S.* Muenster (0.98%, 11/1119), *S.* Newport (0.71%, 8/1119), *S.* Orion (0.36%, 4/1119), *S.* Senftenberg (4.56%, 51/1119), *S.* Taksony (2.86%, 32/1119), *S.* Tennessee (8.13%, 91/1119), *S.* Thomson (0.36%, 4/1119), *S.* Virchof (0.18%, 2/1119).

It is noted that in 2015 the most frequent isolates belonged to the serotypes *S.* Infantis, *S.* Enteritidis, *S.* Senftenberg, *S.* Tennessee and *S.* Kentuky, and in 2016 they belonged to the serotypes *S.* Infantis, *S.* Agona, *S.* Enteritidis, *S.* Tennessee, *S.* Liverpool and *S.* Senftenberg. It is noticed that over the two years, the dominant serotypes persisted. However, comparatively with previous years, there were some changes in the prevalence of the circulating serotypes, at least in the hen farms (7, 10, 11).

The county distribution of *Salmonella* isolates (Fig. 1) was high in Buzău (442 isolates), Călăraşi (370 isolates), Satu-Mare (250 isolates), Vâlcea (223 isolates), Dâmboviţa (180 isolates), Prahova (139 isolates), Giurgiu (134 isolates), Galaţi (109 isolates), Vaslui (95 isolates), and Bihor (77 isolates). During the researched period, *Salmonella* was not identified in the counties Harghita, Mehedinţi and Timiş.

Spatial analyses of *Salmonella* isolates in 2015 (Fig. 2) revealed two poles of high incidence, in the southeast and northwest Romanian counties, with the highest values recorded in Călărași, Buzău, Vâlcea, Dâmbovița, Giurgiu and Satu Mare counties (>120 isolates) and lowest (no isolates) in Bucharest, Arad, Caraș Severin, Harghita, Mehedinți, Neamț, Timiș, Tulcea and Vrancea counties.

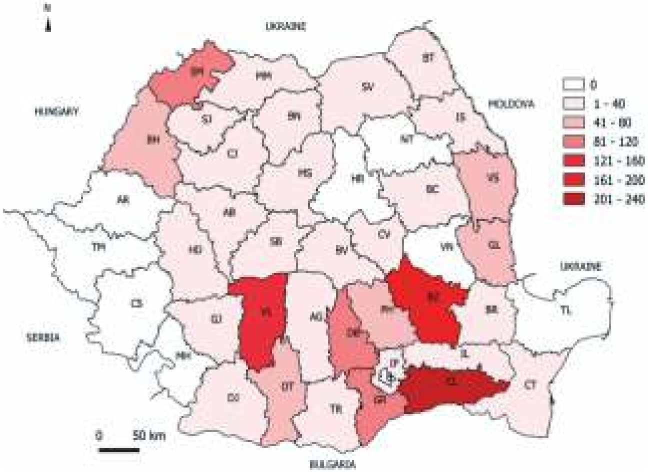

Fig. 2. Spatial distribution of *Salmonella* isolates in Romanian counties during the year 2015

During 2016, spatial analyses of *Salmonella* isolates (Fig. 3) showed the maintenance of the two poles with high prevalence (southeast and northwest), with the highest values recorded in Buzău, Călărași, Satu Mare, and Vâlcea (>118 isolates) and lowest (no isolates) in Argeş, Covasna, Gorj, Harghita, Hunedoara, Mehedinți and Timiș counties.

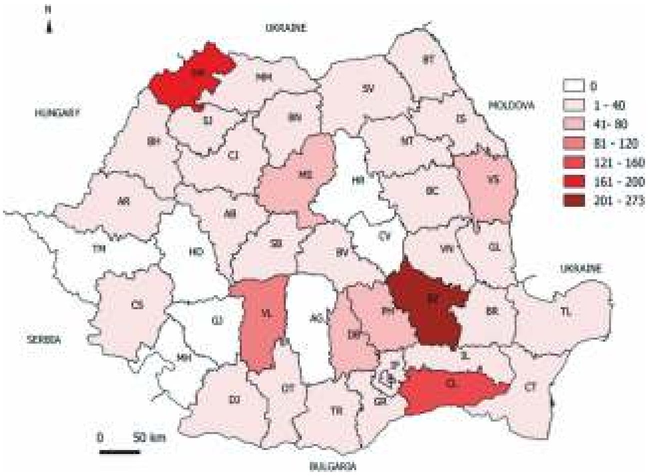

Fig. 3. Spatial distribution of *Salmonella* isolates in Romanian counties during the year 2016

CONCLUSIONS

In 2015, the most frequently isolated serotypes were *S.* Infantis (50.92%, 691/1357), *S.* Enteritidis (9.87, 134/1357), *S.* Senftenberg (4.72%, 64/1357), *S.* Tennessee (4.72%, 64/1357), and *S.* Kentuky (3.83%, 52/1357). In 2016, the most frequently isolated serotypes were *S.* Infantis (48.15%, 572/1188), *S.* Agona (7.83%, 93/1188), *S.* Enteritidis (7.66%, 91/1188), *S.* Tennessee (7.66%, 91/1188), *S.* Liverpool (4.29%, 51/1188), and *S.* Senftenberg (4.29%, 51/1188). The serotype *Salmonella* Typhimurium was isolated from mink, lion, fox and chinchilla.

The counties with the highest prevalence of *Salmonella* isolates in the year 2015 were Călăraşi (17.69%, 240/1357), Buzău (12.45%, 169/1357), Vâlcea (9.28%, 126/1357), Dâmboviţa (8.40%, 114/1357), Giurgiu (7.07%, 96/1357), and Satu-Mare (5.97%, 81/1357). In 2016, the highest prevalence of *Salmonella* isolates was in: Buzău (22.98%, 273/1188), Satu-Mare (14.23%, 169/1188), Călăraşi (10.94%, 130/1188), Vâlcea (8.16%, 97/1188), Dâmboviţa (5.55%, 66/1188), and Prahova (5.39%, 64/1188).

During the researched period, *Salmonella* was not identified in Harghita, Mehedinţi and Timiş counties.

In order to reduce the prevalence of serotypes with major zoonotic potential, we recommend continuing collaboration of all the professionals involved in the security of the food chain. Also, the surveillance and monitoring should be increased in the entire country.

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