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CLASSICAL AND MOLECULAR MONITORING OF THE PREVALENCE OF *SALMONELLA* SPP. CARRIAGE IN FREE-LIVING AND CAPTIVE NATIVE VIPERA SNAKES IN ROMANIA

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Summary

Reptile-associated salmonellosis in humans is an increasing public health issue. Apparently, bacteria from the genus *Salmonella* are component of the normal intestinal microbial community for most of the snakes, emerging snakes as a significant source of human salmonellae infections. We screened 16 free-living and 10 captive native *Vipera* snakes for presence of *Salmonella*. Fresh cloacal samples were collected from the individuals with a sterile cotton swab and immediately processed according to a standard protocol for the detection of *Salmonella* (according the OIE). Salmonella was isolated from 5 captive and from 10 free-living snakes (50% and 62.5%, respectively), obtaining a total number of 19 isolates. Concurrent shedding of multiple strains of the bacteria was detected in four free-living snakes. We observed significant differences between the species, the European adder having higher prevalence than the other two species (87.5, 44.44% and 44.44% respectively). 15 isolates out of 19 belonged to *Salmonella* Arizonae, while the other 4 strains were identified as *Salmonella* spp. with our methodology.

Key words: *Salmonella* spp., vipera snakes

To correctly appreciate the public health importance of the reptiles it is important to know the microbiological characteristics and the infectious diseases of the reptiles (1, 2). Infections with micro-organisms belonging to genus *Salmonella* spp. were described in many occasions in reptiles (6), but with the same frequency it is related that these animals appear to be asymptomatic carriers of *Salmonella spp.,* representing a major source of zoonotic transfer. In parallel with the spread of the turtles as companion animals it has gradually been observed a significant amplification in the number of the human salmonelosis with “unusual” and undescribed serotypes for humans (3, 4, 5).

Apparently, bacteria from the genus *Salmonella* are component of the normal intestinal microbial community for most of the snakes, emerging snakes as a significant source of human salmonellae infections. The aim of this study was to determine the role of the Romanian autochthon *Vipera* species in epidemiology of salmonellosis.

Materials and methods

We screened 16 free-living and 10 captive native *Vipera* snakes for presence of *Salmonella*. The studied snake species where the following: common European adder (*Vipera berus*) (captive n=5; free-living n=3), nose horned viper (*Vipera ammodytes ammodytes*) (captive n=5; free-living n=4) and the critically endangered *Vipera ursini rakosiensis* (free-living n=9). Fresh cloacal samples were collected from the individuals with a sterile cotton swab and immediately processed according to a standard protocol for the detection of *Salmonella* (according the OIE). Identification of the isolates was realized by using API20E identification systems (BioMerieux, Lyon, France). The molecular characterization was realized by simplex PCR (ST 11-15 primer pair distinctive for genus *Salmonella* spp.).

Results and discussions

Salmonella was isolated from 5 captive and from 10 free-living snakes (50% and 62.5%, respectively), obtaining a total number of 19 isolates. Concurrent shedding of multiple strains of the bacteria was detected in four free-living snakes. We observed significant differences between the species, the European adder having higher prevalence than the other two species (87.5, 44.44% and 44.44% respectively). 15 isolates out of 19 belonged to *Salmonella* Arizonae, while the other 4 strains were identified as *Salmonella* spp. with our methodology. *Salmonella enterica* serovar Arizonae (*S. enterica* subspecies IIIa) is common *Salmonella* isolate from reptiles and can cause serious systemic disease in humans.

The results obtained after the classical microbiological investigation are presented in tables 1-12.

From the 26 studied vipers we isolated 22 strains of *Salmonella spp.*, 18 were *Salmonella* Arizonae (81.81% of total salmonella) and four strains were identified only at the genus level (*Salmonella spp.*).

Table 1

Prevalence of *Salmonella* Arizonae strains in captive common adders

Lot/Group B.1. – V. berus captive	**Nr. animal**				
Vipera berus, sampling 1.	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5
Salmonella Arizonae	**+**	**+**	**-**	**+**	**+**
***Vipera berus*, sampling 2.**	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5
Salmonella Arizonae	**+**	**+**	**+**	**+**	**-**

A high prevalence (80%) of *Salmonella spp.* was observed in the common adder (*V. berus*), but we couldn't identify *Salmonella spp.* in nose-horned vipers. These results suggested a carrier estate for *Salmonella spp.* in adder, but not in nose horned vipers (see Table 2). From *V. berus* we identified *Salmonella arizonae* (III a) in each case (see Table 1).

Table 2

Prevalence of *Salmonella* Arizonae strains in captive nose horned vipers

Group A.1. – V. ammodytes captive	**Nr. animal**				
***Vipera ammodytes*, sampling 1.**	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5
Salmonella Arizonae	**-**	**-**	**-**	**-**	**-**
***Vipera ammodytes*, sampling 2.**	Nr. 1	Nr. 2	Nr. 3	Nr. 4	Nr. 5
Salmonella Arizonae	**-**	**-**	**-**	**-**	**-**

From the four *V. ammodytes* captured in the wild (group A.M.) we identified six strains of *Salmonella spp.*, four *S.* Arizonae and two unidentified. In table 3 we can observe that in two vipers there is a “double salmonella carriage” phenomenon, an aspect that we can notice also in a common adder belonging to group B.M. (see Table 4). From this group B.M. (common adders captured from wild biotop) there have been identified three strains of *Salmonella spp.*, two *S.* Arizonae and a strain of *Salmonella spp*. unidentifiable by the used protocol.

Table 3

Incidence of *Salmonella* Arizonae and *Salmonella spp*. strains in wild *V. ammodytes*

Group A.M. Vipera ammodytes wild	**Nr.1**	**Nr. 2**	**Nr. 3**	**Nr.4.**
***Salmonella* Arizoanae**	**+**	**+**	**+**	**+**
Salmonella spp.	**+**	**+**	**-**	**-**

Table 4

Incidence of *Salmonella* Arizonae and *Salmonella spp*. strains in wild *V. berus*

Group B.M. Vipera berus wild	**Nr.1**	**Nr.2**	**Nr. 3**
***Salmonella* Arizoanae**	**+**	**+**	**-**
Salmonella spp.	**-**	**+**	**-**

The incidence of the Salmonella spp. carriage in the population of the Hungarian meadow viper (*V. ursinii rakosiensis*) was 44,4%, 5 different strains of *Salmonella spp*. were identified, 4 *Salmonella* Arizonae, two with different aspect on the XLD agar originated from the snake with identification number 103 (see Table 5) and one unidentifiable strain with the used API20E system.

Table 5

Incidence of *Salmonella* Arizonae and *Salmonella spp*. strains in wild *V. u. rakosiensis*

Vipera ursini rakosiensis **Identification number**	**Salmonella**
100	**NO**
101	**NO**
102	**NO**
103	***Salmonella* Arizonae *strain 1*** ***Salmonella* Arizonae *strain 2***
104	**NO**
105	***Salmonella spp.***
106	**NO**
107	***Salmonella* Arizonae**
108	***Salmonella* Arizonae**

Figures 1-2 presents the amplicons obtained with ST 11-15 primer pairs. The ST 11-15 primer pair is distinctive for genus *Salmonella sp.*

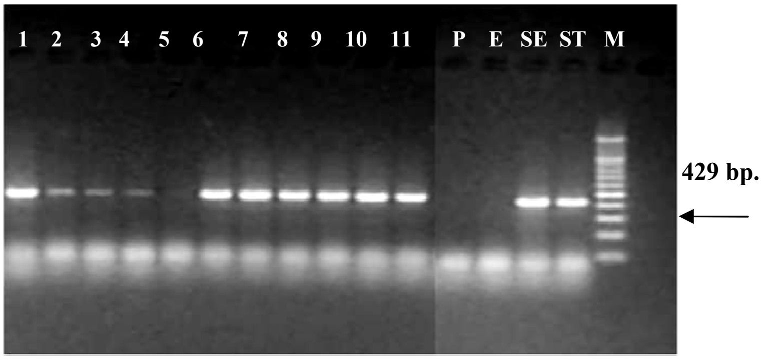

Fig.1. DNA amplicon profiles (429 bp) from 11 strains of *Salmonella spp*. isolated from captive *V. berus* (1-8)
P – Pseudomonas, E – Eschericia are negative controls and SE – Salmonella Enteritidis, ST – Salmonella Typhymurium are positive controls

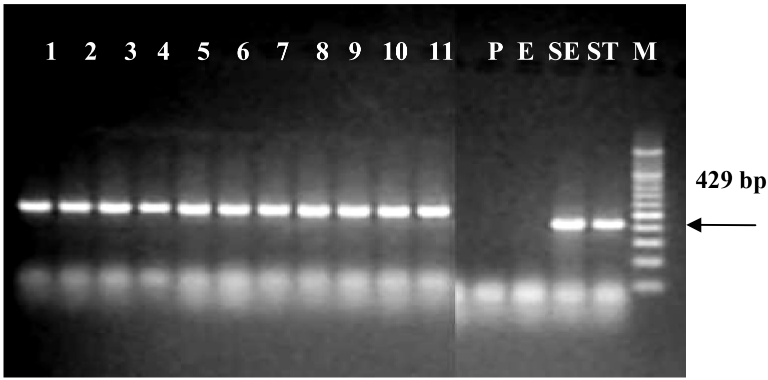

Fig.2. DNA amplicon profiles (429 bp) from 11 strains of *Salmonella spp*. isolated from *V. u. rakosiensis* (R.M.) (1-5) and *V. ammodytes* (A.M.) (6-11), negative control (P – Pseudomonas, E – Eschericia) and positive control (SE – Salmonella Enteritidis, ST – Salmonella Typhymurium).

Conclusions

Our results confirm the risk of transmission of *Salmonella* from free-living and captive *Vipera* snakes to humans.

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