LETTER TO THE EDITOR

UDC: 579.62::616.993
DOI: 10.2298/VSP160809222B

Neglected zoonosis – The Prevalence of *Salmonella* spp. in pet reptiles in Serbia

Zanemarene zoonoze – prevalencija *Salmonella* spp. kod reptila, kućnih ljubimaca, u Srbiji

To the Editor:

Non-typhoidal *Salmonella* represents an important human and animal pathogen worldwide. Most human salmonellosis cases are foodborne, but each year infections are also acquired through direct or indirect animal contact [1]. Contact with reptiles represents an important source of this zoonotic agent, and recent increases in the popularity of exotic pets have resulted in an increase in the number of cases of reptile-associated salmonellosis and rapidly emerging public health problems. Reptile associated *Salmonella* infections in humans tend to be more likely associated with systemic disease than with foodborne infections. Especially among children, the elderly or pregnant women, septicemia, meningitis, arthritis, soft-tissue abscesses, osteomyelitis, pericarditis, myocarditis, peritonitis and urinary tract infections have been repeatedly described, leading to severe disease and comparably high mortality rates. Most reports of reptile-associated salmonellosis concern babies (under one year of age) and young children (up to six-year-olds) [2].

Reptiles have become popular pets and, for example, approximately 3% of households in the USA own one or more reptiles as pets, resulting in a total of approximately 7.3 million reptiles. The number of pet turtles has doubled in recent years, and approximately 2 million turtles are now kept in over 1 million households, and more than 400,000 USA households keep snakes and in excess of 700, 000 households own lizards [3]. In the European Union, exotic reptiles have been enjoying increasing popularity as pets during the last few years. In 2007, more than 500,000 reptiles were imported to Germany only *via* the Frankfurt/Main Airport. This increase in popularity has led to an increase in the number of reptile-associated salmonella infections which occur every year [4].

In the 1970s, it was estimated that each year 280,000 out of 2,000,000 registered cases of human salmonellosis in the United States were associated with turtles, annually contributing an estimated 14–23% of salmonellosis cases among children [5]. One study, conducted in 2004, estimated that in the USA reptile exposure contributes to approximately 74,000 human cases each year [6]. This represents 6% of all sporadic human cases, and reptile-associated cases are estimated to contribute 11% of sporadic human cases in the population < 21 years of age. In the European Union, apparent prevalence estimates vary considerably among member states and over time, ranging from 1% in the UK and the Netherlands up to 5% in Sweden [7].

To the best of our knowledge, the occurrence and prevalence of species, subspecies and serovars of *Salmonella* in reptiles kept as pets has not been investigated in the Republic of Serbia.

From September 2015 to May 2016 cloacal swabs and faeces were taken from snakes, lizards and turtles kept as pets. Isolation and identification of *Salmonella* spp. was performed according to EN ISO 6579: 2008, Annex D - Horizontal method for the detection of *Salmonella* spp., using prescribed bacterial culture media. Identification of suspected *Salmonella* isolates was confirmed by commercial biochemical test set BBL™ Crystal™ Enteric/Nonfermenter ID and then reconfirmed by matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS, Vitek MS, bioMérieux) according to the manufacturer's instructions. All *Salmonella* isolates were typed to the serovar level in the National Reference Laboratory for *Salmonella*, *Shigella*, *Vibrio cholerae* and *Yersinia enterocolitica*. Serotyping was performed by slide agglutination and the detection of the presence of *Salmonella* O- and H- antigens with the Institute of Public Health of Serbia and the Statens Serum Institut from Copenhagen antisera in accordance with the Kauffman White Le Minor scheme.

In the period observed, the presence of *Salmonella* spp. was examined in 34 snakes, 47 lizards and 7 turtles, representing 10 reptile species as shown in Table 1. In total, *Salmonella* was isolated from 19 out of 88 reptiles (21.59%). All isolates belonged to species *Salmonella enterica,* and two different subspecies of *S. enterica* subsp. *enterica* and *S. enterica* subsp. *diarizonae*. The presence of *Salmonella* spp. was detected in 6 out of 10 reptilian species: royal python – *Python regius*, milk snake – *Lampropeltis triangulum,* bearded dragon – *Pogona vittice,* leopard gecko – *Eublepharis*

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Table 1

List of all tested reptiles and isolated *Salmonella enterica* serovars from reptiles

Name (Latin, English)	Number of animals tested	*Salmonella enterica* subsp. *enterica* (*Salmonella enterica* subsp. *diarizonae*)	Number of isolates
Snakes			
Python regius (Royal python)	19	Serovar *S.* Apapa 45 : m,t : -	1
		Serovar *S.* Benin 9,46 : y : 1,7	2
Lampropeltis triangulum (Milk snake)	5	Serovar *S.* Benin 9,46 : y : 1,7	2
Pantherophis guttatus (Corn snake)	4	/	/
Heterodon nasicus (Western hognose snake)	3	/	/
Boa constrictor (Red-tailed boa)	2	/	/
Euprepiophis mandarinus (Mandarin rat snake)	1	/	/
Lizards			
Eublepharis macularius (Leopard gecko)	30	Serovar *S.* Ago 30 : z38: -	3
		Serovar *S.* Apapa 45 : m,t : -	2
		Serovar *S.* Hadar 6,8 : z10 : e,n,x	1
		(Serovar *S.* IIIb 50 : r : z)	1
Pogona vittice (Bearded dragon)	10	Serovar *S.* Ago 30 : z38: -	2
Hemitheconyx caudicinctus (African fat-tailed gecko)	7	(Serovar *S.* IIIb 47 : - : z35)	1
Turtles			
Trachemys scripta elegans (Red-eared slider)	7	Serovar *S.* Thompson 6,7 : k : 1,5	3
		Serovar *S.* Umbilo 28 : z10 : e,n,x	1
Total		All subspecies	19
		Salmonella enterica subsp. *enterica*	17
		Salmonella enterica subsp. *diarizonae*	2

macularius, african fat-tailed gecko – *Hemitheconyx caudicinctus,* and red-eared slider *Trachemys scripta elegans. Salmonella* was isolated from 5 out of 34 snakes (14.71%), from 10 out of 47 lizards (21.27%) and from 4 out of 7 turtles (56.14%). In total, 8 different serovars of *Salmonella enterica* were isolated from reptiles as shown in Table 1. In this study, five out of eight *Salmonella* serovars were found for the first time in Serbia: *S.* Ago, *S.* Apapa, *S.* Benin, *S* IIIb 47 : - : z35 i *S* IIIb IIIb 50 : r : z.

Salmonella is frequently isolated from reptiles kept in private homes, a study from Italy showed that 24% of reptiles carried *Salmonella* [8], and in Austria and Germany *Salmonella* was isolated from 54.1% of reptiles sampled in their home environment [9]. *Salmonella* infections are highly prevalent in captive lizards in Belgium, where different serovars were isolated from 75.8% of cloacal and 59.5% of faecal samples from captive lizards respectively [10]. In our study, *Salmonella* was isolated from 21.59% of the reptiles, and the prevalence rate was lower than in many other studies and surveys.

The presence of two serovars S. Thompson and S. Hadar was detected in examined turtles, whereas the said serovars have been occasionally isolated from human clinical material in Serbia. The largest outbreak of salmonellosis ever recorded in the Netherlands, with 1,149 confirmed cases, was caused by *S.* Thompson [11]. Cases of human salmonellosis, due to S. Thompson among other serovars, have been reported in Japan following consumption of raw blood, viscera and raw meat as well as cooked meat of turtle *Trionyx sinensis japonicas* [12]. In Europe, S. Hadar is the 4th most common serovar isolated from humans.

Serovar S. Apapa, which belongs to Reptile Exotic Pet Associated Salmonellosis (REPAS), was isolated from lizards and snakes. Zoonotic potencial of *S.* Apapa associated with reptiles have been confirmed in Germany and the United States [13].

Findings of this study confirm that the prevalence of *Salmonella* spp. is considerable in pet reptiles in Serbia, which as potential source of this zoonotic pathogen poses a threat to human health. Although the Republic of Serbia defined national legislation governing import, export and trade of these animals, including the mandatory registration of pet shops and pet kennels, there are no available data on the number of reptiles and their health status.

In order to improve animal health and welfare, as well as to prevent the occurrence and spread of infectious animal diseases, including zoonoses, that can be transmitted from animals to humans, it is necessary to establish an adequate veterinary control of keeping, breeding

and trade of exotic animals. This, together with the training programme of pet reptile owners and public awareness campaign, would contribute to the reduction of risk of contagious diseases that could compromise animal or human health.

Acknowledgements

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 31087).

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