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PREVALENCE OF *SALMONELLA* IN CAPTIVE REPTILES FROM CROATIA

Maja Lukac, D.V.M., Ph.D., Karl Pedersen, D.V.M., and Estella Prukner-Radovic, D.V.M.

Abstract: Salmonellosis transmitted by pet reptiles is an increasing public health issue worldwide. The aim of this study was to investigate the prevalence of *Salmonella* strains from captive reptiles in Croatia. From November 2009 to November 2011 a total of 292 skin, pharyngeal, cloacal, and fecal samples from 200 apparently healthy reptiles were tested for *Salmonella* excretions by bacteriologic culture and serotyping. These 200 individual reptiles included 31 lizards, 79 chelonians, and 90 snakes belonging to private owners or housed at the Zagreb Zoo, Croatia. *Salmonella* was detected in a total of 13% of the animals, among them 48.4% lizards, 8.9% snakes, and 3.8% turtles. Representatives of five of the six *Salmonella enterica* subspecies were identified with the following proportions in the total number of isolates: *Salmonella enterica enterica* 34.6%, *Salmonella enterica houtenae* 23.1%, *Salmonella enterica arizonae* 23.1%, *Salmonella enterica diarizonae* 15.4%, and *Salmonella enterica salamae* 3.8%. The 14 different serovars isolated included several rarely occurring serovars such as *Salmonella* Apapa, *Salmonella* Halle, *Salmonella* Kisarawe, and *Salmonella* Potengi. These findings confirm that the prevalence of *Salmonella* is considerable in captive reptiles in Croatia, indicating that these animals may harbor serovars not commonly seen in veterinary or human microbiologic practice. This should be addressed in the prevention and diagnostics of human reptile-transmitted infections.

Key words: *Salmonella*, bacteriology, chelonians, lizards, snakes.

INTRODUCTION

Wild and captive reptiles are considered reservoirs of numerous zoonoses, the most important of which is salmonellosis.^{14,15,28,39} The number of pet reptiles has increased worldwide in recent years. The most popular reptiles are chelonians, though there are also a large number of lizards and snakes kept as pets.³⁹ Reptiles are mostly asymptomatic carriers and natural reservoirs of *Salmonella*, though disease is rarely reported.³⁷ Although the proportion of worldwide infections in humans transmitted by reptiles is low, this increases with the increasing number of reptiles kept as pets, which has been recognized as a significant public health issue in Europe and the United States.^{13,21}

Humans acquire salmonellosis most frequently via infected animal feces, and the bacterium is commonly transmitted by handling of animals or equipment.^{17,20} Infection can also be transmitted via human clothing that was in contact with reptiles or via animal bites and scratches.^{23,42}

Infections in humans are most-frequently presented with mild gastroenteritis, and a large number of cases are likely resolved undetected.^{25,43} However, severe complications such as septicemia, meningitis, or arthritis may arise, especially in children and immunocompromised individuals.^{6,12,19,29,32,35,41} In the United States, it was estimated that reptile exposure contributes to approximately 6% of all sporadic human cases of salmonellosis each year.³⁶

Many countries have recognized the risk of the increasing number and popularity of pet reptiles. In the United States, regular case reports of reptile-associated salmonellosis have been available since 1994.^{7–13} Cases of *Salmonella* infection associated with pet reptiles have also been reported in a number of European countries.^{1,4,16,19,21,35,41,43} In an effort to reduce the number of pet reptile-associated infections in humans, some European countries have introduced systemic monitoring of the prevalence of *Salmonella* serovars from captive reptiles.¹⁸

The European Union is the largest global importer of reptiles.²² It is therefore imperative that more information be obtained on the colonization and excretion of zoonotic pathogens in these animals. There are several reports regarding the prevalence of *Salmonella* in captive reptiles in northern and central European countries, specifically in Austria,²⁵ Belgium,² Denmark,⁴⁰ Germany,^{25,31,34,50} Italy,^{17,20} Poland,⁵⁵ Spain,²⁸ and

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Sweden.⁵² To our knowledge, there have been no investigations of the prevalence of *Salmonella* in pet reptiles in Croatia or the broader region. This report presents the results of *Salmonella* isolation in samples collected from pet reptiles kept by private owners or at the Zagreb Zoo, Croatia, during a 2-yr period.

MATERIALS AND METHODS

Samples

During the period from November 2009 to November 2011, a total of 292 samples were collected from 200 animals. These samples were collected from 31 lizards (suborder *Sauria*), 79 chelonians (order *Chelonia*), and 90 snakes (suborder *Serpentes*) kept by private owners or the Zagreb Zoo. Skin ($n = 16$), pharyngeal ($n = 104$) or cloacal ($n = 131$) swabs, and fecal samples ($n = 41$) were collected after manual restraint. Each animal was sampled only once. All the sampled pet reptiles were brought to the outpatient clinic for routine examination or due to various health problems not related to salmonellosis. Only one snake showed clinical symptoms of the disease in the form of caseous plaques in the pharynx and difficulty breathing. All samples were analyzed at the Department of Poultry Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb.

Bacteriology

Skin, pharyngeal, and cloacal swabs from snakes and lizards were plated directly on brilliant green agar (BGA) (Oxoid Ltd., Basingstoke, Hampshire RG24 8PW, England) and xylose lysine deoxycholate agar (XLD) (Merck, KgaA, 64293 Darmstadt, Germany), incubated at 37°C for 24 hr, and then for 24 hr at room temperature. Fecal samples were enriched in selenite cystine broth (Becton-Dickinson and Company, 38801 Le Pont de Claix Cedex, France), incubated for 24 hr at 37°C, then plated on BGA and XLD agars, incubated for 24 hr at 37°C, and then for 24 hr at room temperature.

Up to five *Salmonella* suspect colonies were further evaluated by streaking on a triple sugar iron agar (TSI, bioMérieux SA, F-69280 Marcy-l'Étoile, France) and incubated at 37°C for 24 hr to facilitate identification and agglutination. Pure colonies were agglutinated with polyvalent antisera (Antiserum *Salmonella* Omnivalent Omni-O, Bio-Rad, 92430 Marnes-la-Coquette, France), and identification of *Salmonella* to the subspecies

level was carried out by biochemical tests as outlined by Grimont and Weill.²⁶

In addition to the procedure described above, skin, pharyngeal, and cloacal samples of chelonians were first placed into selenite broth and incubated at 37°C for 24 hr. The samples were then plated on BGA and XLD agars and incubated at 37°C for 24 hr and then for 24 hr at room temperature. In this manner, the highest *Salmonella* isolation rate was expected for chelonian samples, in line with our former laboratory experience with *Salmonella* isolation from reptile samples.

Serotyping was performed by slide agglutination method using specific polyclonal antisera against O- and H-antigens (Statens Serum Institut, DK 2300 Copenhagen, Denmark). The agglutination reactions were interpreted in accordance with the White-Kauffmann-le Minor protocol.²⁶

RESULTS

The distribution of *Salmonella* is summarized in Table 1. Of the 292 samples collected, 8.9% (26/292) tested positive for *Salmonella*. Of these positive findings, 24.4% (10/41) were in fecal, 10.7% (14/131) in cloacal, 6.0% (1/16) in skin, and 1.0% (1/104) in pharyngeal samples. The samples that were most-frequently positive were fecal and cloacal samples from lizards, followed by fecal samples from snakes and cloacal samples from snakes and chelonians. Most samples from skin and pharynx were negative. *Salmonella* was detected in 13% (26/200) of animals, though there were significant differences between lizards, snakes, and chelonians. The highest prevalence of 48.4% (15/31 positive animals) was found in lizards followed by 8.9% (8/90) in snakes and 3.8% (3/79) in chelonians (Table 1).

All of the subspecies isolated belonged to the species *Salmonella enterica* (Table 2). The most represented was *S. enterica enterica* (34.6%), followed by *S. e. houtenae* and *S. e. arizonae* (23.1% each), *S. e. diarizonae* (15.4%), and *S. e. salamae* (3.8%). The most-represented serotypes of *S. e. enterica* were *Salmonella* Halle, *Salmonella* Kisarawe, and S.IV 45:g, z51:- (Table 2). Six subsp. *S. e. arizonae* isolates from snakes were not serotyped but only identified biochemically to the subspecies level.

The species distribution of *Salmonella* in snakes, lizards, and chelonians is shown in Table 3. Different subspecies of *Salmonella* were represented, including *S. e. enterica* (I), *S. e. salamae* (II), *S. e. arizonae* (IIIa), *S. e. diarizonae* (IIIb), and

Table 1. Skin, pharyngeal, cloacal, and fecal samples stratified by reptile group, site of sampling, and *Salmonella* findings.

Animals	Number of animals ^a	Samples				
		Skin	Pharynx	Cloaca	Feces	Total
Snakes	8/90 (8.9%)	0/0 (0.0)	1/80 (1.3)	4/56 (7.1)	3/10 (30.0)	8/146 (5.5)
Lizards	15/31 (48.4%)	1/8 (13.0)	0/9 (0.0)	7/19 (36.8)	7/15 (46.7)	15/51 (29.4)
Chelonians	3/79 (3.8%)	0/8 (0.0)	0/15 (0.0)	3/56 (5.4)	0/16 (0.0)	3/95 (3.1)
Total	26/200 (13.0%)	1/16 (6.0)	1/104 (1.0)	14/131 (10.7)	10/41 (24.4)	26/292 (8.9)

^a Number positive/total (% positive).

S. e. houtenae (IV). *Salmonella enterica houtenae* was found in 6 of 15 (40%) positive lizards, including all five green iguanas (*Iguana iguana*) (Table 3). Other *S. enterica* serovars-subspecies detected in lizards were *Salmonella* Kisarawe in three bearded dragons (*Pogona vitticeps*), *Salmonella* Apapa in one bearded dragon, *Salmonella* Senftenberg in one bearded dragon, *Salmonella* Potengi in one Cuban rock iguana (*Cyclura nubilata*), *S. e. diarizonae* in one common chameleon (*Chamaeleo chamaeleon*) and in one Cuban rock iguana, and one *S. e. salamae* in one bearded leaf chameleon (*Rieppeleon brevicaudatus*). *Salmonella enterica arizonae* was isolated in six of eight (75%) positive snakes while the remaining two animals had *S. e. diarizonae*. In all three positive Hermann's tortoises (*Testudo hermanni*), *Salmonella* Halle was detected (Table 3).

DISCUSSION

Representatives of five of six subspecies of *S. enterica*, belonging to 14 different serovars, were isolated from captive reptiles in this study. Although the proportion of subspecies I serovars was the highest (34.6%), not one of them belonged to those most-frequently seen in humans in the European Union, where *Salmonella* Enteritidis, monophasic *Salmonella* Typhimurium, *Salmonella* Typhimurium, *Salmonella* Infantis, and *Salmonella* Stanley were the top five serovars associated with human illness in 2012.³⁰ In another recent investigation,⁵⁵ where reptile isolates belonging to the top 15 serovars found in humans were characterized, the presence of serovars common in poultry, such as *Salmonella* Kentucky, was reported. This might be a result of feeding contaminated raw meat to carnivore reptiles in their study. The results of the present study do not indicate this manner of *Salmonella* transmission in captive reptiles in Croatia. Moreover, some rarely occurring *S. enterica* serovars were detected in our study such as *Salmonella* Apapa, *Salmonella* Halle, *Salmonella* Potengi, and

Salmonella Kisarawe. *Salmonella* Apapa has previously been reported as a pathogen in pet reptile-associated human infections.^{16,21,41} Pees et al.⁴¹ recently demonstrated that most of the reptile-associated infections in children in Germany during 2010–2011 were not associated with the *Salmonella* serovars commonly related to human salmonellosis. Those authors concluded that, currently, no specific serovar seemed to be of particular zoonotic importance and, accordingly, that all *Salmonella* strains found in reptiles should be considered potentially infectious to children. We would add that, in this context, the rarely occurring serovars should not be overlooked. In addition to the *S. e. enterica* subspecies, *S. e. houtenae*, *S. e. arizonae*, *S. e. diarizonae*, and *S. e. salamae* have also been described as causative agents in human infections associated with direct or indirect contact with pet reptiles^{1,16,19,21,48} Interestingly, in a Swiss study, Overesch et al.³⁸ found that none of the isolates from snakes belonged to *S. e. enterica* and that most of the isolates from

Table 2. The number/proportion of isolates by *Salmonella* subspecies.

<i>Salmonella enterica</i> subspecies	n (%) isolates	Serovar (n isolates)
<i>S. e. enterica</i> (I)	9 (34.6)	Apapa (1)
		Halle (3)
		Kisarawe (3)
		Potengi (1)
		Senftenberg (1)
<i>S. e. houtenae</i> (IV)	6 (23.1)	11:z4,z23:- (1)
		16:z4,z32:- (1)
		44:z4z23:- (1)
		45:g,z51:- (3)
<i>S. e. arizonae</i> (IIIa)	6 (23.1)	ND ^a (6)
<i>S. e. diarizonae</i> (IIIb)	4 (15.4)	14:z10:z:z56 (1)
		47:z52:z (1)
		47:z10:z35 (1)
		61:l,v:1,5,7 (1)
<i>S. e. salamae</i> (II)	1 (3.8)	21:z10:- (1)

^a ND indicates not determined.

Table 3. *Salmonella* isolates by reptile species.

Reptile species	Number of positive animals	<i>Salmonella</i> serotype
Snakes		
<i>Python regius</i> , royal python	3	IIIa ^a
	1	IIIb 47:z10:z35
<i>Boa constrictor</i>	1	IIIa ^a
<i>Pantherophis</i> <i>guttatus</i> , corn snake	1	IIIb 14:z10:z:z56
	1	IIIa ^a
<i>Morelia viridis</i>	1	IIIa ^a
<i>Lamprophis</i> <i>fuliginosus</i> , African house snake	1	IIIa ^a
	1	IIIa ^a
Lizards		
<i>Iguana iguana</i>	3	IV 45:g,z51:-
	1	IV 11:z4,z23:-
	1	IV 16:z4,z32:-
<i>Pogona vitticeps</i>	3	I Kisarawe
	1	I Apapa
	1	I Senftenberg
	1	IV 44:z4z23:-
<i>Cyclura nubila</i>	1	IIIb 47:z52:z
	1	I Potengi
<i>Rieppoleon</i> <i>brevicaudatus</i>	1	II 21:z10:-
<i>Chamaeleo</i> <i>chameleon</i>	1	IIIb 61:l,v:1,5,7
Chelonians		
<i>Testudo hermanni</i>	3	I Halle

^a Serovar not determined.

lizards also belonged to subspecies other than *S. e. enterica*, while most isolates from chelonians did belong to *S. e. enterica*. This is similar to the patterns found in the present study.

This study showed that *Salmonella* was present in a total of 13.0% of animals tested. The range in the literature is from 5.0 to 86.0%.^{17,20,25,28,37,38,40,50,55} This variability likely reflects the fact that *Salmonella* in reptiles is shed intermittently,^{5,14} so that a negative finding does not necessarily mean that the animal is free of *Salmonella*. Intermittent shedding of *Salmonella* in reptiles and the wide array of collection and sampling techniques have been proposed to be the main reasons for the variability in detection rates.⁴⁶ This could also mean that the 13% prevalence found in the present study may be underestimating the true prevalence, although several sampling sites, such as skin, pharynx, and cloaca in addition to feces, were used for *Salmonella* detection.

The highest proportion of positive animals, 48.4%, was among the lizards. *Salmonella enterica*

houtenae was isolated from all the positive green iguanas, and all of these animals were held by private owners. This may indicate the issue of *Salmonella* dissemination during the breeding and transport of animals from farms to stores as well as the transmission of infection from one animal to another during their stay at pet shops. *Salmonella* shedding from the gastrointestinal tract is also facilitated by stress due to transport, overcrowding at pet shops, or incorrect, inadequate housing.⁴⁹ Green iguanas are among the most commonly kept lizards in Croatia and, therefore, it is necessary to emphasize the risk of *Salmonella* infections transmitted by those pet animals. Moreover, recent reports indicate that lizards, not chelonians as suggested by earlier reports,¹⁴ may be the most-common source of *Salmonella* in human reptile-associated salmonellosis.^{27,41} In particular, bearded dragons have been suspected as a source of infection for young children.^{41,51,54} The finding that all the bearded dragons tested in our study were *Salmonella*-positive supports this assumption.

Among the snakes, the most-commonly infected were royal pythons (*Python regius*). This finding may be explained by the fact that these are predominantly ground-dwelling terrestrial animals and therefore are more prone to *Salmonella* infections. When kept in terrariums, ground-dwelling snakes may be exposed to an increased risk of contact with contaminated feces, as previously hypothesized by Shröter et al.⁴⁸ Because of their docility, royal pythons are also very popular pet reptiles in Croatia. *Salmonella enterica arizonae* and *S. e. diarizonae* were isolated from those animals, and these subspecies have previously been described as human pathogens transmitted by snakes.^{3,23,47} Two arboreal snakes (green tree python, *Morelia viridis*) were also positive. This may be explained by the fact that those animals were purchased from pet sellers, not from breeders. It was reported that reptile collections with purchased animals had a significantly higher prevalence of *Salmonella* than did collections from pure breeders.²⁵ Both *Salmonella* subspecies that were predominantly isolated from lizards and snakes, *S. e. arizonae* and *S. e. houtenae*, have already been described as causes of infections in children and immunocompromised adults.^{16,47,48} It should be noted that all the samples but one in this study were taken from pet reptiles that were brought to the outpatient clinic for routine examinations or for various health problems unrelated to salmonellosis. Therefore, all the

animals, with one exception, were asymptomatic carriers.

Reptile-transmitted salmonellosis in humans has long been well known in North and South America owing to the large number of pet reptiles, particularly turtle farms in the United States, and also to alternative medicine in South America where people become infected through the ingestion of dried snake meat products as a medicine.^{3,33,45,53} Although infections related to exposure to reptiles and other exotic pets represent only a small proportion of all human salmonellosis cases, it is likely an underestimated and growing problem in Europe, particularly due to the increasing number of reptiles kept as pets.^{4,16,19,21,44,47} If the reptiles are already present at home, health professionals should instruct their patients, in detail, how to prevent potential infection. The same attention should be paid to reptile salmonellosis in zoologic gardens and at all exhibitions where people, particularly small children, come into direct contact with reptiles.²⁴ Some countries, such as the United States and Sweden, regulate the reptile trade and have consequently reduced the number of infections.^{15,18} Therefore, systemic monitoring of salmonellosis prevalence in pet reptiles and the education of pet owners, pet traders, veterinarians, and health professionals seem to be necessary to reduce the risk of *Salmonella* transmission from reptiles to humans.

In conclusion, the results of this study, to our knowledge the first of this type in Croatia, indicate that captive reptiles harbor a significant number of *Salmonella* strains, some of which are rare and exotic. It can be anticipated that with an increasing number of pet reptiles, the risk of reptile-transmitted infections will also increase. With this in mind, the less-commonly occurring serovars identified in this study should also be considered in daily practice.

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