PREVALENCE OF SALMONELLA IN CAPTIVE REPTILES FROM CROATIA

Author(s): Maja Lukac, D.V.M., Ph.D., Karl Pedersen, D.V.M., and Estella Prukner-Radovcic, D.V.M.
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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. 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. Reptiles are mostly asymptomatic carriers and natural reservoirs of Salmonella, though disease is rarely reported. 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.

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

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

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. Cases of Salmonella infection associated with pet reptiles have also been reported in a number of European countries. 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.

The European Union is the largest global importer of reptiles. 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, Belgium, Denmark, Germany, Italy, Poland, Spain, and...
Swedish. 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 at 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 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 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’Etoile, 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. 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 antiserum 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.

**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...
Salmonella enterica houtenae was found in 6 of 15 (40%) positive lizards, including all five green iguanas (Iguana iguana) (Table 3). Other Salmonella 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 nubila), S. e. diarizonae in one common chameleon (Chamaeleo chameleon) 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. In another recent investigation, 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. Pees et al. 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. Interestingly, in a Swiss study, Overesch et al. found that none of the isolates from snakes belonged to S. e. enterica and that most of the isolates from

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

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number of animals&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Skin</th>
<th>Pharynx</th>
<th>Cloaca</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snakes</td>
<td>8/90 (8.9%)</td>
<td>0/0</td>
<td>1/80</td>
<td>4/56</td>
<td>3/10</td>
<td>8/146 (5.5)</td>
</tr>
<tr>
<td>Lizards</td>
<td>15/31 (48.4%)</td>
<td>1/8</td>
<td>0/9</td>
<td>7/19</td>
<td>7/15</td>
<td>15/51 (29.4)</td>
</tr>
<tr>
<td>Chelonians</td>
<td>3/79 (3.8%)</td>
<td>0/8</td>
<td>0/15</td>
<td>3/56</td>
<td>0/16</td>
<td>3/95 (3.1)</td>
</tr>
<tr>
<td>Total</td>
<td>26/200 (13.0%)</td>
<td>1/16</td>
<td>1/104</td>
<td>14/131</td>
<td>10/41</td>
<td>26/292 (8.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number positive/total (% positive).

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

<table>
<thead>
<tr>
<th>Salmonella enterica subspecies</th>
<th>n (%) isolates</th>
<th>Serovar (n isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. e. enterica (I)</td>
<td>9 (34.6)</td>
<td>Apapa (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Halle (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kisarawe (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potengi (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Senftenberg (1)</td>
</tr>
<tr>
<td>S. e. houtenae (IV)</td>
<td>6 (23.1)</td>
<td>11:z4,223:::- (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16:z4,232:::- (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44:z4z23:::- (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45:g.z51:::- (3)</td>
</tr>
<tr>
<td>S. e. arizonae (IIIA)</td>
<td>6 (23.1)</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt; (6)</td>
</tr>
<tr>
<td>S. e. diarizonae (IIIB)</td>
<td>4 (15.4)</td>
<td>14:z10:zz56 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47:zz52:z (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47:zz10:zz35 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61:lv:1,5,7 (1)</td>
</tr>
<tr>
<td>S. e. salamae (II)</td>
<td>1 (3.8)</td>
<td>21:zz10:::- (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND indicates 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 salmonelloses.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

<table>
<thead>
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<th>Table 3. <em>Salmonella</em> isolates by reptile species.</th>
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<tr>
<td><strong>Reptile species</strong></td>
</tr>
<tr>
<td><strong>Snakes</strong></td>
</tr>
<tr>
<td><em>Python regius</em>, royal python</td>
</tr>
<tr>
<td><em>Boa constrictor</em></td>
</tr>
<tr>
<td><em>Pantherophis guttatus</em>, corn snake</td>
</tr>
<tr>
<td><em>Morelia viridis</em></td>
</tr>
<tr>
<td><em>Lamprophis fuliginosus</em>, African house snake</td>
</tr>
<tr>
<td><strong>Lizards</strong></td>
</tr>
<tr>
<td><em>Iguana iguana</em></td>
</tr>
<tr>
<td><em>Pogona vitticeps</em></td>
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<tr>
<td><em>Cyclura nubila</em></td>
</tr>
<tr>
<td><em>Rieppeleon brevicaudatus</em></td>
</tr>
<tr>
<td><em>Chamaeleo chameleon</em></td>
</tr>
<tr>
<td><strong>Chelonians</strong></td>
</tr>
<tr>
<td><em>Testudo hermanni</em></td>
</tr>
<tr>
<td><em>Testudo hermanni</em></td>
</tr>
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</table>

* Serovar not determined.
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. 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. 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. Some countries, such as the United States and Sweden, regulate the reptile trade and have consequently reduced the number of infections. 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 the 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.

LITERATURE CITED


