

THE PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *Salmonella* SPECIES ISOLATED FROM CAPTIVE REPTILES AT LJUBLJANA ZOO

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Summary: Cloacal swabs from 74 healthy reptiles at Ljubljana Zoo were examined for the presence of salmonellae. Thirty nine reptiles underwent at least one antimicrobial treatment 24 - 48 months before sample collection. The identification of salmonellae was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and positive strains were serotyped. Salmonellae were found in 29.7% of all reptiles investigated, in 55.6% of reptiles kept with regularly direct contact with zoo visitors, and in 26.2% of reptiles kept strictly in terraria. The isolation prevalence was 38.6%, 18.2% and 12.5% in snakes, lizards and chelonians, respectively. *Salmonella enterica* subspecies *enterica* was the most common (63.6%) followed by subspecies *diarizonae* (31.8%) and subspecies *arizonae* (4.5%). The *Salmonella enterica* subsp. *enterica* serotypes Infantis 6,7,14:r:1,5 and Uzaramo 1,6,14,25:z₄,z₂₄- were detected in 27.3% and 36.4% of *Salmonella* positive samples, respectively. Resistance to antimicrobial agents was found in 9% of strains. A high percentage (63.6%) of *Salmonella* positive reptiles at Ljubljana Zoo shed serotypes that are known to be causative agents of human salmonellosis. This is the first documented isolation of *Salmonella enterica* subsp. *diarizonae*, serotype IIIb 57:k:e,n,x,z₁₅ from captive reptiles.

Key words: captive reptiles; salmonellosis; antibiotic resistance; MALDI-TOF MS

Introduction

As a source of educational programs many zoos and rescue centres traditionally provide opportunities to visitors for direct contact and handling with certain captive reptiles. Reptiles are important reservoirs of salmonellae as they harbour these bacteria without showing any clinical signs (1, 2). Carriage rates of *Salmonella* spp. in captive reptiles vary between 50% and 86% (3, 4, 5). Factors associated with poor husbandry, systemic

viral infections and intestinal parasitism in reptile collections may lead to reactivation of clinical salmonellosis and bacterial shedding (6, 7, 8). Cases of human salmonellosis associated with pet reptiles are known and have been well documented (9, 10, 11). The infection can be transmitted to humans by direct contact or through an environment contaminated with salmonellae (2, 12).

Salmonellae are often resistant to ampicillin, tetracycline, colistin sulphate, streptomycin, sulfamethoxazole/trimethoprim and nalidixic acid (5, 13, 14). For that reason care must be taken with the use of antibiotics as it may result in the development of antibiotic resistance (6).

The purpose of this study was to investigate the prevalence and antimicrobial resistance of salmonellae isolated from captive reptiles at Ljubljana Zoo.

Materials and methods

Animals

Cloacal swabs from 74 clinically healthy reptiles at Ljubljana Zoo, Slovenia, were examined for the presence of salmonellae. Sixty-seven reptiles (19 lizards, 42 snakes, six chelonians) were captive bred, and seven reptiles (three lizards, two snakes, two chelonians) were rescued from the wild. In the period of sample collection all reptiles were kept at the Ljubljana Zoo for more than one year. From the 74 reptiles, 39 underwent at least one five-day fluoroquinolone treatment against salmonellae 24 - 48 months before the study. Nine reptiles have been used for teaching purposes and have had contact with visitors, whereas the remaining 65 reptiles did not have any contact with visitors.

Sample collection

Each animal was manually restrained and a sterile cotton swab (Copan Italia S.p.A, Italy) was gently inserted and turned inside the reptile's cloaca. Samples were transported in Amies transport medium (Copan Italia S.p.A, Italy) and kept in 5 °C until processed, 48–120 hours after collection.

Cultivation, identification and serotyping of salmonellae strains

Cloacal swabs were placed into 5 ml of buffered peptone water (BPW) (Oxoid Ltd., UK) and incubated overnight at 37 °C. Upon incubation, 0.1 ml of BPW was dropped onto modified semisolid Rappaport-Vassiliadis agar (Oxoid Ltd., UK), incubated at 41.5 °C and inspected for typical growth (halo zone around the inoculated drop) at 24 and 48 hrs. In parallel, Xylose lysine deoxycholate agar (Oxoid Ltd., UK) was inoculated with the positive samples and incubated at 37 °C overnight to select *Salmonella* isolates. Suspect *Salmonella* colonies were cultured on blood agar, incubated at 37 °C for 24 hours, and then confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Microflex LT,

Bruker Daltonics, Germany). *Salmonella* isolates were serotyped by slide agglutination assay using commercial O and H antisera (BioRad, France; Denka Seiken, Japan). The identification of serotypes was carried out according to Kauffmann-White-Le Minor scheme (15).

Antimicrobial susceptibility test

Salmonella isolates were tested for susceptibilities to amoxicillin/clavulanic acid (30 µg), ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), colistin sulphate (10 µg), streptomycin (10 µg), neomycin (30 µg), gentamicin (10 µg), sulfonamide compounds (300 µg), sulfamethoxazole/trimethoprim (25 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg) and marbofloxacin (5 µg) (Oxoid Ltd., UK), using antibiotic disk diffusion method (16).

Results

Salmonellae were found in 29.7% of all reptiles investigated (Table 1), in 55.6% of reptiles kept with regularly direct contact with zoo visitors, and in 26.2% of reptiles kept without direct contact with visitors. The prevalence of salmonellae in reptiles with previous antimicrobial treatment and without antimicrobial treatment was 35.9% and 62.9% respectively. Snakes had the highest prevalence (38.6%) of salmonellae. *Salmonella enterica* subspecies *enterica* was the most common (63.6%), followed by the subspecies *diarizonae* (31.8%) and subspecies *arizonae* (4.5%). In each positive individual one serotype was isolated. The *Salmonella enterica* subsp. *enterica* serotypes Infantis 6,7,14:r:1,5 and Uzaramo 1,6,14,25:z₄,z₂₄- were detected in 27.3% and 36.4% of *Salmonella* positive samples, respectively.

Resistance to antimicrobial agents was found in 9% of *Salmonella* strains. Resistance to ampicillin and amoxicillin/clavulanic acid was detected in one strain of *Salmonella enterica* subsp. *enterica* serotype Infantis 6,7,14:r:1,5 isolated from a *Zamenis longissimus*, and resistance to streptomycin was detected in one strain of *Salmonella enterica* subsp. *diarizonae* serotype IIIb 57:k:e,n,x,z₁₅ isolated from a *Vipera ammodytes*. Both snakes were previously treated with fluoroquinolones. Strains of *Salmonella enterica* subsp. *enterica* serotype Infantis 6,7,14:r:1,5,

Table 1: The prevalence of *Salmonella* species and serotypes isolated from reptiles at Ljubljana Zoo

Reptile species	Number of samples collected	Samples positive for <i>Salmonella</i> spp. (%)	<i>Salmonella</i> serotypes
Lizards	22	4 (18.2%)	
<i>Pogona vitticeps</i>	5	0	
<i>Chlamydosaurus kingii</i>	2	0	
<i>Lacerta viridis</i>	1	0	
<i>Lacerta agilis</i>	7	2	Infantis 6,7,14:r:1,5
<i>Furcifer pardalis</i>	2	0	
<i>Iguana iguana</i>	1	1	IIIb 65:z ₁₀ :e,n,x,z ₁₅
<i>Ophisaurus apodus</i>	4	1	IIIb 57:k:e,n,x,z ₁₅
Snakes	44	17 (38.6%)	
<i>Python regius</i>	2	0	
<i>Python bivittatus</i>	2	0	
<i>Pantherophis guttatus</i>	10	4	Infantis 6,7,14:r:1,5*, IIIb 53:z ₁₀ :z ₃₅ , IIIa 53:z ₄ :z ₂₃ -
<i>Elaphe quatuorlineata</i>	3	1	IIIb 53:z ₁₀ :z ₃₅
<i>Zamenis longissimus</i>	3	2	Infantis 6,7,14:r:1,5, Uzaramo 1,6,14,25:z ₄ :z ₂₄ -
<i>Pantherophis emoryi</i>	6	3	Uzaramo 1,6,14,25:z ₄ :z ₂₄ *, IIIb 53:z ₁₀ :z ₃₅
<i>Lampropeltis triangulum</i>	1	0	
<i>Thamnophis sirtalis</i>	6	3	Uzaramo 1,6,14,25:z ₄ :z ₂₄ -
<i>Natrix tessellata</i>	2	0	
<i>Natrix natrix</i>	4	1	Infantis 6,7,14:r:1,5
<i>Vipera berus bosniensis</i>	2	1	IIIb 53:z ₁₀ :z ₃₅
<i>Vipera ammodytes</i>	2	2	IIIb 57:k:e,n,x,z ₁₅ , Uzaramo 1,6,14,25:z ₄ :z ₂₄ -
<i>Vipera aspis francisciredi</i>	1	0	
Chelonians	8	1 (12.5%)	
<i>Geochelone sulcata</i>	2	0	
<i>Emys orbicularis hellenica</i>	1	0	
<i>Emys orbicularis orbicularis</i>	4	0	
<i>Trachemys scripta scripta</i>	1	1	Uzaramo 1,6,14,25:z ₄ :z ₂₄ *,
Total	74	22 (29.7%)	

* Serotypes isolated from reptiles with direct contact with zoo visitors

Salmonella enterica subsp. *enterica* serotype Uzaramo 1,6,14,25:z₄,z₂₄⁻, *Salmonella enterica* subsp. *diarizonae* serotype IIIb 53:z₁₀:z₃₅ and *Salmonella enterica* subsp. *diarizonae* serotype IIIb 57:k:e,n,x,z₁₅ isolated from a *Pantherophis guttatus*, *Zamenis longissimus*, *Pantherophis emoryi* and *Ophisaurus apodus* respectively, were intermediate susceptible to streptomycin.

Discussion

Despite modern zoos make efforts to provide suitable conditions for captive reptiles, the enclosure design would hardly replicate the reptile's natural habitat. Keeping reptiles in groups leads to intense competition for basking spots and food items, sexual frustration and increased exposure to faeces (17). In comparison with the results of Martínez Barreda et al. (3), Geue and Löschner (4) and Corrente et al. (5) who assumed shedding rates up to 50% in captive reptiles, a relatively low prevalence of *Salmonella* spp. (29.7%) was found in our study. This difference may be due to the fact that the animals in the present study were only sampled once, and the excretion of *Salmonella* spp. is intermittent (18, 19). The higher frequency of salmonellae isolation in snakes than in lizards and chelonians is in agreement with previously published data (4, 14, 20).

In the present study *Salmonella* subspecies *enterica* was the most common (63.6%) followed by the subspecies *diarizonae* (31.8%) that was isolated mainly in snakes with a history of previous antimicrobial treatment.

Mitchell and Shane (6) and Johnson-Delaney (8) remarked that antimicrobials may merely suppress the excretion of salmonellae without their complete elimination, and antimicrobial treatment in reptiles without any clinical symptoms of salmonellosis can promote the emergence of antimicrobial resistant strains. This statement is accordance with our finding because more than one third of reptiles treated with fluoroquinolones were found positive for salmonellae. Generalized use of antimicrobial agents in reptiles or in their environment is not recommended (21).

A high percentage (63.6%) of *Salmonella* spp. positive reptiles at Ljubljana Zoo shed serotypes that are known to be causative agents of clinical salmonellosis. *Salmonella enterica* subsp. *enterica* serotype Infantis 6,7,14:r:1,5 belongs to the top ten

Salmonella serotypes that may cause gastroenteritis in humans (22), and multidrug-resistant clones of this serotype have been already detected (23). *Salmonella enterica* subsp. *enterica* serotype Uzaramo 1,6,14,25:z₄,z₂₄⁻ has been related to a case of reptile-associated human salmonellosis (24). To our knowledge this study is the first to document isolation of *Salmonella enterica* subsp. *diarizonae* serotype IIIb 57:k:e,n,x,z₁₅ from captive reptiles.

Results of this study could help to improve knowledge about the prevalence of *Salmonella* spp. in zoo reptilian collections. It is clear that proper hygiene practices are needed in order to minimize the infectious risk for zoo personnel and visitors. Recommendations for reducing the risk of transmission of *Salmonella* to humans from reptiles include the following: (i) Veterinarians and pet store owners should provide information to clients about the risk of acquiring salmonellosis from reptiles. (ii) People with immature or weakened immune system, including children, pregnant women, elderly people and immunocompromised persons should avoid contact with reptiles. (iii) People should wash their hands after handling these animals or their environments. (iv) Kitchen sinks should not be used to bath reptiles or to wash their dishes, cages or aquariums. (v) Reptiles should not be allowed to roam freely throughout a home. (vi) Other household pets, such as dogs and cats, should not be in contact with reptiles, their cages, faeces or feed to reduce spread of transmission of salmonellae. (vii) Zoos and exhibits should be equipped with adequate hand-washing facilities, and food and drinking should not be allowed in animal-contact areas. (viii) Good care of household reptiles should be given to reduce stress, which may cause the excretion of salmonellae (25).

Future studies will be extended to identify sources of infection and transmission routes of salmonellae in reptiles at Ljubljana Zoo.

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POJAVLJANJE VRST *Salmonella* IN NJIHOVA ODPORNOST PROTI PROTIMIKROBNIM SREDSTVOM V IZOLATIH PLAZILCEV V ZOO LJUBLJANA

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Povzetek: Kloakalni brisi 74 zdravih plazilcev so bili v ljubljanskem živalskem vrtu pregledani na prisotnost salmonel. Devetintrideset plazilcev je bilo zdravljenih z vsaj enim protimikrobnim zdravilom 24 - 48 mesecev pred zbiranjem vzorcev. Določanje salmonel je bilo opravljeno s pomočjo masne spektrometrije MALDI (ionizacija v matriksu z lasersko desorpcijo) ter s pomočjo serotipizacije pozitivnih sevov. Salmonele so bile ugotovljene v 29,7 % vseh preiskanih plazilcev, in sicer pri 55,6 % plazilcev, ki so v rednem neposrednem stiku z obiskovalci živalskega vrta, in pri 26,2 % plazilcev, ki so nastanjeni izključno v terarijih. Okuženih je bilo 38,6 % kač, 18,2 % kuščarjev in 12,5 % želv. Najpogosteje je bila izolirana *Salmonella enterica*, podvrsta *enterica* (63,6 %), sledita pa podvrsti *diarizonae* (31,8 %) in *arizonae* (4,5 %). *Salmonella enterica*, subsp. *enterica* serotipa Infantis 6,7,14:r:1,5 je bila odkrita pri 27,3 %, serotipa Uzaramo 1,6,14,25:z4,z24- pa v 36,4 %. Odpornost proti protimikrobnim sredstvom je bila ugotovljena pri 9 % sevov. Visok odstotek (63,6 %) na salmonelo pozitivnih plazilcev v ljubljanskem živalskem vrtu je bil okužen z enim izmed serotipov, ki so znani povzročitelji salmoneloze pri ljudeh. To je prva dokumentirana izolacija *Salmonella enterica* subsp. *diarizonae*, serotip IIIb 57:K: E, n, x, Z15 pri plazilcih v ujetništvu.

Ključne besede: plazilci v ujetništvu; salmoneloza; odpornost proti antibiotikom; MALDI-TOF MS