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Prevalence of Enterobacteriaceae in *Tupinambis merianae* (Squamata: Teiidae) from a captive facility in Central Brazil, with a profile of antimicrobial drug resistance in *Salmonella enterica*

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Abstract

Prevalence of Enterobacteriaceae in *Tupinambis merianae* (Squamata: Teiidae) from a captive facility in central Brazil, with a profile of antimicrobial drug resistance in *Salmonella enterica*. The present study reports the presence of Enterobacteriaceae in tegu lizards (*Tupinambis merianae*) from a captive facility in Central Brazil. From a total of 30 animals, 10 juveniles and 20 adults (10 females, 10 males), 60 samples were collected, in two periods separated by 15 days. The samples were cultivated in Xylose-lysine-deoxycholate agar (XLT4) and MacConkey agar. The *Salmonella enterica* isolates were tested for antimicrobial susceptibility. A total of 78 bacteria was isolated, of which 27 were from juveniles of *T. merianae*, 30 from adult males, and 21 from adult females. *Salmonella enterica* was the most frequent bacteria, followed by *Citrobacter freundii*, *Escherichia coli*, *Enterobacter sakasakii*, *Kluivera* sp., *Citrobacter amalonaticus*, *Serratia marcescens*, *Citrobacter diversus*, *Yersinia frederiksenii*, *Serratia odorifera*, and *Serratia liquefaciens*. *Salmonella enterica* subsp. *diarizonae* and *houtenae* showed resistance to cotrimoxazole, and serum *Salmonella enterica* Worthington showed resistance to tetracycline and gentamicin. *Salmonella enterica* Panama and *S. enterica* subsp. *diarizonae* showed intermediate sensitivity to cotrimoxazole. In addition to Enterobacteriaceae in the tegu lizard, pathogenic serotypes of *S. enterica* also occur, and their antimicrobial resistance was confirmed.

Keywords: Antibiotic sensitivity, enteric bacteria, reptiles, salmonellosis, serovars.

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Resumo

Prevalência de Enterobacteriaceae em *Tupinambis merianae* (Squamata: Teiidae) em cativeiro no Brasil Central, com o perfil de resistência a drogas antimicrobianas em *Salmonella enterica*. O presente estudo relata a presença de Enterobacteriaceae em lagartos teiús (*Tupinambis merianae*) de cativeiro no Brasil Central. De um total de 30 animais, 10 jovens e 20 adultos (metade de cada sexo), 60 amostras foram coletadas, em dois períodos separados por 15 dias. As amostras foram cultivadas no meio xilose-lisina-desoxicolato e ágar MacConkey. Os isolados de *Salmonella enterica* foram testados quanto à sensibilidade aos antimicrobianos. Um total de 78 isolados de bactérias foi encontrado, sendo 27 de indivíduos jovens de *T. merianae*, 30 de machos adultos e 21 de fêmeas adultas. A bactéria *Salmonella enterica* foi encontrada com maior frequência, seguida de *Citrobacter freundii*, *Escherichia coli*, *Enterobacter sakasakii*, *Kluivera* sp., *Citrobacter amalonaticus*, *Serratia marcescens*, *Citrobacter diversus*, *Yersinia frederiksenii*, *Serratia odorifera* e *Serratia liquefaciens*. *Salmonella enterica* subsp. *diarizonae* e *houtenae* apresentaram resistência ao cotrimoxazol, enquanto *S. enterica* Worthington foi resistente a tetraciclina e gentamicina. *Salmonella enterica* Panamá e *S. enterica* subsp. *diarizonae* apresentaram resistência intemediária ao cotrimoxazol. Além de Enterobacteriaceae no lagarto teiú, sorovares patogênicos de *S. enterica* foram isolados e sua resistência antimicrobiana foi confirmada.

Palavras-chave: Bactéria entérica, répteis, salmonelose, sensibilidade a antibióticos, sorovares.

Introduction

The genus *Tupinambis* includes the largest lizards of South America, as well as the largest members of Teiidae, reaching more than 60 cm snout-vent length. There are six species: *T. duseni*, *T. longilineus*, *T. merianae*, *T. quadrilineatus*, *T. rufescens*, and *T. teguixin* (Péres Jr. and Colli 2004), which occur only in South America, east of the Andes, from Amazon to northern Patagonia (Péres Jr. and Colli 2004).

These lizards can carry *Salmonella* found in intermittent excretion (Jong *et al.* 2005), and any change in intestinal ecology can trigger or increase the agent elimination in feces. The main source of infection is through direct or indirect contact with feces of another reptile, which often occurs between juveniles and adults (Mitchell and Shane 2001). Chiodini (1982) suggested transovarian transmission in reptiles, as a result of isolation of *Salmonella* in fetuses and in internal tissues of females.

Salmonella are intracellular organisms. The two species (*S. bogori* and *S. enterica*) have cosmopolitan distributions (Grimont and François-Xavier 2007). Six subspecies of *S. enterica* have been isolated from the environment and animals—viz., *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (Popoff and Le Minor 2001). *Salmonella e. enterica* frequently is found in wild and captive reptiles (Mermin *et al.* 2004). There are more than 2500 serovars of *Salmonella*, approximately 1000 of which have been isolated in reptiles, and it is not rare to find more than one serovar in the same individual (Mitchell 2006).

Outbreaks of human salmonellosis have been reported in several countries, mainly associated with the ingestion of contaminated water and food, such as raw eggs, beef, pork, and poultry. In addition, direct contact between pets and their owners also causes infections. Because reptiles eliminate *Salmonella* through digestive tract with no apparent clinical symptoms, they are considered potential source of infection for humans, especially among children and immune-compromised adults. They also may be responsible for transmitting the pathogen to pets and into environment (Shinohara *et al.* 2008).

Other enterobacteria also cause illness in reptiles and can be isolated from healthy animals (Mitchell and Shane 2001). In Brazil, Moreno *et*

al. (1973) isolated enterobacterias of amphibians and reptiles from the wild, demonstrating a high occurrence of these bacteria in these groups. Mathewson (1979) identified 14 species of Enterobacteriaceae from the intestines of lizards. Enterobacteria often are found as secondary agents in diseases, worsening the clinical condition of the hosts. Species of Enterobacteriaceae also may act as primary agents, affecting mainly debilitated animals (Mitchell 2006). Possibly, the importance given to *Salmonella*, the most studied bacteria, contributes to the shortage of scientific articles addressing other genera of the family Enterobacteriaceae (Mathewson 1979).

Today, antimicrobial resistance is a major concern worldwide, owing to the rise of multiresistant bacteria, and to the failure in the treatment of many diseases in human and animal medicine. It is thought that indiscriminate use, particularly in livestock or captive animals, has contributed to the presence of resistant bacteria in humans (Freitas Neto *et al.* 2010).

Our goal was to isolate and identify bacteria of the family Enterobacteriaceae in *Tupinambis merianae* and to determine the profile of antimicrobial drug resistance in *Salmonella* isolates.

Materials and Methods

We collected samples at a rural captive facility for *Tupinambis merianae* in Brasília, Distrito Federal. We analyzed bacteriological samples at Laboratório de Bacteriologia, Escola de Veterinária e Zootecnia of the Universidade Federal de Goiás (EVZ/UFG). We collected a total of 60 samples representing 30 animals—10 juveniles (8 mo old) and 20 adults (10 females, 10 males)—in two collecting events separated by 15 days.

After capturing the animals with a dip net, restraining them with their venter up, and cleaning the area around the cloaca with physiological saline and alcohol (70%), we conducted a cloacal lavage with the use of an urethral tube, attached to a 10-ml syringe containing peptone saline water (0.1%). The water was introduced in each individual through the cloaca (8 ml in adults and 3 ml in juveniles) and was immediately vacuumed. We transferred the contents to an individual test tube with screw cap, and stored it in ice in an isothermal box before transportating the samples to the laboratory.

Processing of Bacteriological Samples

We processed bacteriological samples using the protocol established by Georgia Poultry Laboratory (1997). We used Selenite Cystine and Rappaport-Vissiliadis broths, as well as solid Xylose-lysine-deoxycholate agar (XLT4) and MacConkey agar. Three to five colony-forming units (CFUs), with the same morphological characteristics, were transferred to triple sugar iron agar (TSI) and submitted to these biochemical tests: urea broth, methyl red broth, Simmons citrate, sulfide-indole-motility (SIM), malonate broth, phenylalanine deaminase, lysine decarboxylase, and glucose and lactose fermentation.

Reading and interpretation of biochemical tests were performed according to Bergey's Manual of Determinative Bacteriology (Holt 1994). We submitted isolates with biochemical reactions compatible with *Salmonella* to serological test, with antiserum polyvalent somatic O. For serological typing, these samples were sent in nutrient Agar, to the Laboratório de Enterobactérias of Fundação Oswaldo Cruz (FIOCRUZ).

To determine differential occurrences of *Salmonella* in juvenile and adult *Tupinambis merianae*, we calculated and compared frequencies using the Fisher's Exact Test, adopting a 5% significance level, through software BioStat 5.0.

Test of Resistance to Antimicrobials

We tested for resistance, isolates with phenotypic profile of *Salmonella*, and classified

them as resistant, sensible, or intermediate resistant, according to Kirby-Bauer technique (Bauer *et al*. 1966). We used the following antibiotics: chloramphenicol (30 mg), norfloxacin (10 mg), trimethoprim-sulfamethoxazole (25 mg), cefotaxime (30 mg), gentamicin (10 mg), ciprofloxacin (30 mg), tetracycline (30 mg), and amikacin (30 mg).

Five CFUs, with similar morphological characteristics, were moved with an inoculating loop to 5 ml of Casoy broth. We incubated the inoculated broth until its turbidity reached 0.5 on Macfarland scale. In sequence, we moistened the swab in the broth, removed the excess, and rubbed it in various directions over a Mueller-Hinton plate, until a uniform and homogeneous layer of the inoculum was formed. After an interval of 10–15 min necessary for diffusion of the broth in the agar, the discs were deposited with a tweezers on the inoculated surface. We pressed the disks for a better grip with the surface, and maintained a distance of about 3 cm between the disks. The plates were then incubated in an inverted position for 18–24 hours, at a temperature of 35–37°C, after which, we measured the inhibition zones with a ruler.

Results

We isolated and identified seven genera and eleven species of Enterobacteriaceae, with a total of 78 isolates. We obtained 27 isolates from juveniles of *Tupinambis merianae*, 30 from adult males and 21 from adult females. All species were detected in adults, corresponding to 65.4% (51/78) of the isolates, whereas in juveniles, only seven species were isolated, with 34.6% (27/78) of isolates (Table 1).

We obtained 43 isolates from the initial samples, and 35 from the samples collected 15 days later. *Serratia odorifera, Serratia liquefaciens, Yersinia frederiksenii,* and *Kluivera* sp. were isolated only in the second sample collection, and *Citrobacter diversus* was the only bacteria found exclusively in the first. We found different occurrences of *Salmonella* in juveniles (10 and 0) and in adult males (6 and 1) between the two periods of collection (Table 1).

The most common Enterobacteriaceae was *Salmonella enterica*, present in 26.9% (21/78) of the isolates, followed by *Citrobacter freundii* 15.4% (12/78), *Escherichia coli* 11.5% (09/78), *Enterobacter sakasakii* 10.2% (08/78), *Kluivera* sp. 9.0% (07/78), *Citrobacter amalonaticus* 9.0% (07/78), *Serratia marcescens* 5.1% (04/78), *Citrobacter diversus* 3.8% (03/78), *Yersinia frederiksenii* 3.8% (03/78), *Serratia odorifera* 2.6% (02/78), and *Serratia liquefaciens* 2.6% (02/78) (Figure 1).

The frequencies of *Salmonella enterica* in juveniles and adult tegu lizards differed significantly according to Fisher's exact test ($p < 0.05$), with a 100% of positive samples in juveniles (10 samples), and 55% in adults (20 samples). We identified three subspecies of *Salmonella enterica* in 21 isolates and four serovars in 14 isolates of tegu lizards (*Tupinambis merianae*) (Table 2).

We performed the tests for antimicrobial sensitivity on 21 samples of *Salmonella enterica* isolated from tegu lizards, and resistance to the drugs tested was observed in four samples. Trimethoprim-sulfamethoxazole was the least effective antibiotic tested, responsible for 50% of the antimicrobial resistance observed. Tetracycline and gentamicin were responsible for 25% of antimicrobial resistance each. *Salmonella enterica diarizonae* and *S. e. houtenae* showed resistance to cotrimoxazole in two samples, whereas serovar *Salmonella enterica* Worthington showed resistance to tetracycline and gentamicin. *Salmonella enterica* Panama and *S. e. diarizonae* showed intermediate sensitivity to cotrimoxazole (two samples; Table 3).

Discussion

The presence of *Salmonella* in reptiles was first described by Caldwell and Ryerson (1939), who isolated the bacteria in the lizards *Phrynosoma solare*, *Heloderma suspectum* and

Table 1. Number of isolates obtained for the family Enterobacteriaceae among juvenile and adult male and female tegu lizards, in two different periods of samples collection (P1: Isolates obtained from samples collected on the first period; P2: Isolates obtained from samples collected on the second period).

	Juveniles		Females		Males		Total		TOTAL
Enterobacteriaceae	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	**P1**	**P2**	
Salmonella enterica	10	–	2	2	6	1	**18**	**3**	**21**
Escherichia coli	1	1	–	1	3	3	**4**	**5**	**9**
Citrobacter amalonaticus	1	2	1	1	1	1	**3**	**4**	**7**
C. freundii	2	2	6	–	–	2	**8**	**4**	**12**
C. diversus	–	–	1	–	2	–	**3**	**0**	**3**
Enterobacter sakasakii	1	1	1	1	2	2	**4**	**4**	**8**
Kluivera sp.	–	3	–	2	–	2	**0**	**7**	**7**
Serratia odorifera	–	v	–	–	–	2	**0**	**2**	**2**
Serratia marcescens	1	1	–	2	–	–	**1**	**3**	**4**
Serratia liquefaciens	–	–	–	–	2	–	**0**	**2**	**2**
Yersinia frederiksenii	–	1	–	1	–	1	**0**	**3**	**3**
Total	**16**	**11**	**11**	**10**	**16**	**14**	**43**	**35**	**78**

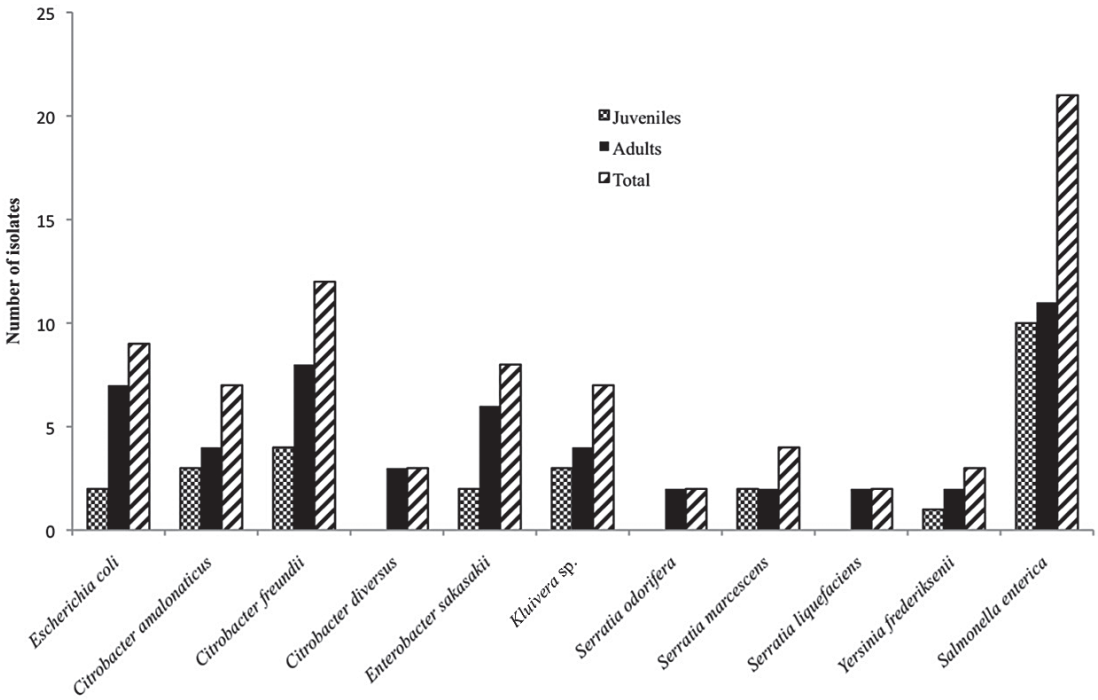

Figure 1. Isolates frequency of Enterobacteriaceae species found in adults and juveniles *Tupinambis merianae*.

Table 2. Subspecies and serovars of *Salmonella enterica* isolated from samples of *Tupinambis merianae*.

Subspecies and serovars	Number of isolates
S. enterica subsp. *houtenae*	6
S. enterica subsp. *enterica*	2
S. enterica subsp. *diarizonae*	13
TOTAL	**21**
S. enterica Worthington	3
S. enterica Panama	8
S. enterica Adelaide	2
S. enterica Typhimurium	1
TOTAL	**14**

Ater sauromalas. Since then, other isolates of the bacteria were obtained from different reptilian species, as demonstrated by Iveson *et al*. (1969), Chiodini (1982), and Shane *et al.* (1990). These reptile species were also pointed as reservoirs of specific serovars and of the ones pathogenic to humans (Meervenne *et al.* 2009).

We found a higher frequency of *Salmonella* isolates in juvenile tegu lizards (10/10) than in adults (11/20). In most species, the bacterial population of gastrointestinal tract usually is established in adults, owing to contact between the animal and its environment, as well as management activities (Mitchell 2006). According to Mitchell and Shane (2001), juvenile reptiles are more susceptible to infection, because they are undergoing the enteric colonization process. Moreover, Scott and Foster (1997) found no difference between isolates of *Salmonella* in these two age groups in alligators

Table 3. Antimicrobial resistance of subspecies and serovars of *Salmonella enterica* isolated from samples of *Tupinambis merianae*. R - resistance; I - intermediate sensitivity.

ISOLATES	Cloranfenicol	Amicacina	Gentamicina	Ciprofloxacina	Cotrimoxazole	Tetraciclina	Norfloxacina	Cefotaxima
Salmonella enterica Worthington	–	–	R	–	–	R	–	–
Salmonella enterica Panama	–	–	–	–	I	–	–	–
S. enterica subsp. *houtenae*	–	–	–	–	R	–	–	–
S. enterica subsp. *diarizonae*	–	–	–	–	R/I	–	–	–
Isolates with resistance (%)	**0**	**0**	**4,76**	**0**	**9,52**	**4,76**	**0**	**0**
Isolates with intermediate sensitivity (%)	**0**	**–**	**0**	**0**	**9,52**	**0**	**0**	**0**

(*Alligator mississippiensis*). Fluctuations on the presence of *Salmonella* in juveniles and in adult males from the initial and second collecting events are consistent with the knowledge that reptiles present intermittent secretion of these bacteria (Mitchell 2006).

Salmonella enterica, detected in 26.9% of samples from *Tupinambis merianae* in this study, was also reported by Sá and Solari (2001), who obtained 39.1% of positive isolates in exotic and wild reptiles in Brazil. Pfleger *et al.* (2003) isolated *Salmonella* in 14.4% of the samples analyzed from captive amphibians and reptiles. *Salmonella* was most abundant in reptiles. Nakadai *et al*. (2005) detected a higher frequency (74.1%) of positive samples in fecal material of pet reptiles in Japan.

Similarly, in a study conducted in Panama, Kourany and Telford (1981) obtained 29.4% positive isolates for *Salmonella* from fecal samples of 447 lizards, representing 27 species of seven families. Mathewson (1979) and Bastos *et al.* (2008) investigated *Salmonella* and other Enterobacteriaceae in reptiles and obtained isolation indexes of 27.3 and 47.8%, respectively. Smith *et al.* (2012) obtained 88 isolates (80%) of *Salmonella* in 110 samples of *Gekko gecko* imported from Indonesia to the United States. Moreover, in New Zealand, Kikillus *et al.* (2011) analyzed 378 cloacal swabs of captive exotic reptiles, and only 43 (11.4%) were positive for *Salmonella* sp. Nowakiewicz *et al.* (2012) detected *Salmonella* in 15 samples (18.75%), from 80 Russian tortoises (*Agrionemys horsfieldii*) examined in Russia. We conclude that the frequency of positive isolates for *Salmonella* found in the present study is similar to those reported by most other studies conducted worldwide.

The subspecies of *Salmonella* that we isolated in this work from the tegu lizard (*Salmonella enterica* subsp. *enterica*, *S. enterica* subsp. *diarizonae*, and *S. enterica* subsp. *houtenae*) occur in red-footed tortoises (*Chelonoidis carbonaria;* Nunes *et al.* 2010), jararaca (*Bothropoides jararaca;* Bastos *et al.* 2008), and Galapagos land iguanas (*Conolophus subcristatus;* Franco *et al.* 2011). *Salmonella e. enterica* also was found by Nowakiewicz *et al.* (2012) in Russian tortoises (*Agrionemys horsfieldii*); these authors also isolated *S. enterica salamae*, a taxon not found at the present study.

The serovar *Salmonella enterica* Panama was first described in *Tupinambis merianae* by Maciel *et al.* (2010). They also found the serovars *S. enterica* Rubislaw, *S. enterica* Kids, *S. enterica* Carrau, *S. enterica* Agona, *S. enterica* Saintpaul, *S. enterica* Brandenburg, which we did not identify. Therefore, *T. merianae* can carry and harbor different serovars of *Salmonella enterica.*

The detection of *Salmonella enterica* Typhimurium in this study is of great importance, because it is a relevant serovar of domestic animals and to humans; it is related to infections resulting from ingestion of contaminated foods (Freitas Neto *et al.* 2010). This serovar was also described in other reptiles and amphibians—e.g., the Eastern Kingsnake (*Lampropeltis getulus;* Cambre *et al*. 1980), Saltwater Crocodiles (*Crocodylus porosus;* Thomas *et al.* 2001), and salamanders (*Desmognathus fuscus fuscus*, *Gyrinophilus porphyriticus porphyriticus* and *Hemidactylium cutatum;* Chambers and Hulse 2006).

The serovar *Salmonella enterica* Adelaide detected in the present study, also was reported by Bauwens *et al.* (2006) in lizards. This record is significant, because this serovar is responsible for clinical disease occurring in humans as a result of their contact with reptiles, especially snakes (Jong *et al.* 2005).

It is noteworthy, that the serovar *Salmonella enterica* Worthington has not been reported in reptiles, but we isolated it in tegu lizards. This serovar is also responsible for infections in humans (mainly in newborns in hospital outbreaks) owing to close contact among humans and their low immunity of newborns (Muley *et al.* 2004).

The presence of these latter three serovars of *Salmonella enterica* (Typhimurium, Adelaide, and Worthington) in tegu lizards, reveals that

reptiles transmit pathogenic serovars to humans and other animals, and that the serovars are typical of those of other animals, such as birds.

Citrobacter is another member of Enterobacteriaceae detected in *Tupinambis* samples, with 15.4% of positive isolates. The frequency is similar to that found by Morais *et al.* (2011) for *Podocnemis expansa* (11.1%), but higher than for *Podocnemis unifilis* (6.6%). Moreno *et al.* (1973) showed a higher frequency for *Citrobacter* in reptiles and amphibians, with 35.3% of positive isolates.

We detected *Escherichia* in two juveniles and in seven adults of *Tupinambis merianae*, with a total frequency of 11.4% of positive samples. Our result resembles that of Mathewson (1979), who identified *Escherichia* in 19.4% of the samples and noted that this pathogen was the third most common of iguanid lizards. In jararaca snakes, Bastos *et al.* (2008) isolated this genus in 12.3% of total samples. Research with Amazon turtles (*Podocnemis* sp.) revealed the presence of *Escherichia* in 14 cloacal swabs (14/64) and in one oral cavity (1/45) (Morais *et al.* 2011). Goppe *et al.* (2000) isolated *Escherichia coli* in cloacal and fecal swabs of mammals, birds and reptiles from Trinidad. The frequency of positive isolates was significantly higher in mammals than in birds and reptiles. In reptiles, *E. coli* was only found in 90 of 173 samples (52%). Differences in diet and enviromental factors between mammals and reptiles account for the higher frequence of *E. coli* in mammals.

We found only one species of *Enterobacter* (*E. sakasakii*) in *Tupinambis*. Other species of the genus have been found in iguanid lizards—e.g., *E. aerogenes* (3.0%) and *E. cloacae* (41.8%) (Mathewson 1979). The latter species also was isolated in 23.5% of jararaca samples (Bastos *et al.* 2008). Santoro *et al.* (2006) isolated *E. agglomerans* (31.4%) and *E. cloacae* (2.8%) from green turtles. The species we isolated in the tegu lizard, *E. sakasakii*, was not reported in any of these studies.

Bastos *et al.* (2008) reported one positive sample for *Kluivera* sp. in jararaca, whereas we isolated the genus in three juvenile, two male, and two female tegu lizards, corresponding to a total of 10.25% samples.

In tegu lizards, *Serratia* species were less frequent (5.12%), corroborating the results of other studies on reptiles, such as Mathewson (1979) (5.88% in iguanids), Bastos *et al.* (2008) (1.5% in jararaca), and Morais *et al.* (2011) (3.12% in *Podocnemis unifilis*). However, Santoro *et al.* (2006) isolated *Serratia marcescens* in 14.2% of green turtles samples, which suggests the possible presence of this genus in the gastrointestinal tract of healthy reptiles.

The enterobacterium *Yersinia frederiksenii* causes clinical disease in humans, especially in children and teenagers (Chomel 1992). This species was found in 3.84% of tegu lizard samples. The zoonotic character of the genus *Yersinia* is related mainly to pet rodents, and the species *Y. enterocolitica* and *Y. pseudotuberculosis* are the most involved with infections, especially in cases of low immunity (Chomel 1992). Shayegani *et al.* (1986) reported this genus in fecal samples of wild mammals, birds, and reptiles. The only species found in reptiles. Among reptiles, *Yersinia intermedia* was found only in the turtle *Chelydra serpentina* (25% of the samples were positive). Our results, as well as those of Shayegani *et al.* (1986), demonstrated positive samples obtained from healthy animals. Although reptiles are not a common source of infection, they can harbor *Yersinia* in their gastrointestinal tract, and thus, may represent a risk factor for humans and other animals.

The intestine contains an heterogeneous mix of different bacterial populations, and the cloacal lavage method used in this study allowed the collection of uncontaminated bacterial populations. Our results are similar to those of studies that used other methods to collect Enterobacteriaceae in reptiles (Moreno *et al.* 1973, Mathewson 1979, Santoro *et al.* 2006, Bastos *et al.* 2008), indicating that the technique used was appropriate.

Another goal of this study was to determine the resistance profile of the isolates of *Salmonella enterica*. We found that *S. enterica* Worthington

was resistant to gentamicin. Enterobacteriaceae and other Gram-negative pathogens, such as *Pseudomonas* and *Aeromonas*, are the main cause of illness in reptiles (Mitchell 2006). The use of gentamicin is widespread in reptiles, and among the aminoglycosides, it is perhaps the most studied drug for these animals. It is one of the few groups of antibiotics that have treatment protocols developed by pharmacokinetic studies (Mitchell 2006).

The same serovar, *Salmonella* Worthington, also is resistant to tetracycline. Neither Bastos *et al.* (2008) nor Nowakiewicz *et al.* (2012), found any resistance to this antibiotic in their isolates from snakes and lizards, respectively. However, Madsen *et al.* (1998) observed intermediate sensitivity for tetracycline in isolates of *S. enterica*, from the skin of Nile crocodiles (*Crocodylus niloticus*). Wheeler *et al.* (2012), who studied *Salmonella* and other Enterobacteriaceae in reptiles from the Galapagos Islands, found similar results.

The use of tetracyclines in veterinary and human medicine is common because they are low-cost antibiotics that are less toxic. The erroneous and indiscriminate use of this antibiotic is responsible for the formation of resistant strains of bacteria (Maia-Pereira *et al.* 2010). A great part of the dissemination of resistant strains is a result of the use of antibiotics in animal food, as an antimicrobial growing promoter (Van den Bogaard and Stobberingh 2000).

In recent studies, isolates from reptiles did not show *Salmonella enterica* with resistance to the drug association trimethoprim-sulfamethoxazole (Madsen *et al.* 1998, Bastos *et al.* 2008, Nowakiewicz *et al.* 2012, Wheeler *et al.* 2012). However, in isolates of *Salmonella enterica* from the tegu lizard, *S. e. houtenae* and *S. e. diarizonae* were resistant to this drug. Additionally, a sample of *S. e. diarizonae* and one of the serovar *S. enterica* Panama showed intermediate sensitivity to the drug. Therefore, in the present study, the association trimethoprim-sulfamethoxazole was the antibiotic less effective *in vitro* against *Salmonella* in tegu lizards.

Total elimination of *Salmonella* in reptiles is not feasible. Use of antibiotics seems not to be successful in eliminating the microorganism, because shortly after being administered, the animals excrete it in feces (Mitchell 2006). In addition, the misuse of these drugs can lead to proliferation of strains resistant to antibiotics, increasing the virulence of these bacteria (Cambre *et al.* 1980). Thus, it is important to consider research on alternate treatments for salmonellosis—treatments that do not affect the normal microbiota of these animals (Mitchell 2006).

According to the antimicrobial susceptibility test carried out on isolates from the tegu lizard, antibiotics and chemotherapeutics that are common and have a widespread use, showed a profile of increased resistance when compared with newer drugs, such as ciprofloxacin, norfloxacin and cefotaxime. This may be a consequence of the fact that resistant strains are selected and scattered by the indiscriminate use of antibiotics (Van den Bogaard and Stobberingh 2000).

Because *Salmonella* strains isolated from reptiles are naturally pathogenic for humans (Shinohara *et al.* 2008), the use of antibiotics in reptiles may contribute to the proliferation of a more severe strain of the bacteria, increasing risks to humans. Therefore, great caution is needed when recommending a treatment for salmonellose or other enterobacteria in reptiles. The use of antibiotic, when applicable, must be done for a given period of time, and the drug has to be selected based on microbiologic culture and antimicrobial susceptibility test, considering also physiological aspects during treatment, such as ectothermy.

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