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SALMONELLA IN BRAZILIAN AND IMPORTED PET REPTILES

Isabel Valéria Abalem de Sá[1]; Claude André Solari[1,2]*

[1]Departamento de Bacteriologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil.
[2]Departamento de Patologia e Apoio Clínico, Escola de Medicina e Cirurgia, UNIRIO, Rio de Janeiro, RJ, Brasil

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ABSTRACT

The presence of salmonellae in fecal samples or cloacal swabs of 97 pet reptiles (15 snakes, 24 lizards and 58 chelonians) was investigated. Thirty seven animals had national origin and 60 were imported. *Salmonella* spp was detected in 39.1% of the reptiles, being 62.5% in lizards, 53.3% in snakes and 25.8% in chelonians. Strains belonged to subspecies I (44.7%), II (10.5%), IIIa (5.2%), IIIb (21.0%) and IV (18.5%) of the *enterica* species, with predominance (55.3%) of subspecies usually found in cold-blooded animals (II to IV). In the subspecies I, the serovars Albany, Enteritidis and Typhimurium predominated. The *Trachemys scripta elegans* imported turtles corresponded to 93.3% (14/15) of the salmonellae-positive chelonians. The national iguanas presented a high rate of colonization (77.7% - 7/9). These animals pose a potencial risk to the human health, demanding sanitary control and more information to the public.

Key words: *Salmonella,* pet reptiles.

INTRODUCTION

The genus *Salmonella* comprises two species: (a) *Salmonella enterica*, which is divided into six subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI); and (b) *Salmonella bongori* (formerly-subspecies V). According to the O (somatic), Vi (capsular) and H (flagellar) antigenic factors these species and subspecies are classified in 2.435 serovars. Usually the 1435 serovars that belong to the *enterica* species subspecies *enterica* (I) colonize the enteric tract of warm-blooded animals, while the other serovars (1000) of the II, IIIa, IIIb, IV and VI subspecies and of the *bongori* species are found in cold-blooded animals and in the environment (16). Although salmonellae had already been isolated from reptiles in 1939 (5), their association to human salmonellosis was only described in the 50s (3). The increasing emergence of exotic *Salmonella* serovars, related to cold-blooded animals and rarely detected in the human being, led to the characterization of pet turtles as an important infection source, capable of causing 280.000 of the 2.000.000 estimated annual human cases (13). Consequently, some states into USA require a *Salmonella*-free certificate for turtles and banned poisonous reptiles and reptiles exceeding a certain size. The consequence was an estimated reduction of 100.000 annual cases of salmonellosis in one to nine years old children (9). The Centers for Disease Control and Prevention (CDC) established recommendations about handling of pet reptiles, seeking mainly the protection of children, pregnant women and immuno-compromised hosts (6). However, despite the warnings, the number of pets kept in captivity and the notifications of human salmonellosis caused by these continued to grow. From 1994 to 1995, thirteen health state agencies in the United States, reported to the CDC human infection cases caused by uncommon *Salmonella* serovars, in which the patients had had direct or indirect contact with reptiles (snakes, lizards and turtles). In most cases, the same *Salmonella* serovar was isolated from the patient and from the reptile. In some cases, the infection resulted in an invasive disease, septicemia and meningitis (6). Mermin, Hoar and Angulo (14) reported 32 cases of salmonellosis caused by the

* Corresponding author. Mailing address: Departamento de Patologia e Apoio Clínico, Escola de Medicina e Cirurgia, UNIRIO. Rua Mariz e Barros, 775. Tijuca, 202270–004, Rio de Janeiro, RJ, Brasil. Fax (+5521) 2569-5787. E-mail: solarica@domain.com.br

serovar Marina associated to iguanas, and observed that 26 (81%) were children less than one year old. Eleven patients (34%) were hospitalized during 2 to 21 days and one patient died. From those patients that reported contact with iguanas, only four (14%) touched directly the animal. CDC also reported that in the period 1996/1998, sixteen states in the United States notified reptile-associated human salmonellosis (7).

In order to contribute with information on the epidemiology of salmonellosis in Brazil, we characterized the presence of salmonellae in pet reptiles, identifying the species, the subspecies and the serovars circulating among these animals.

MATERIALS AND METHODS

Swabs of cloacae or recently-emitted feces from 97 national and imported reptiles (15 snakes, 24 lizards and 58 chelonians), sold either at the formal or at the informal market, were introduced in Cary-Blair transport medium (Difco) and processed in less than 5 days. After pre-enrichment in 10 mL of Buffered Peptone Water (Merck) incubated 16-18 h at 37ºC (11,15), an aliquot of 0.1 mL was transferred to 10 mL of Rappaport-Vassiliadis Broth (Merck) and another one of 1.0 mL to 10 mL of Tetrathionate Broth Base (Difco) (19). After incubation at 37ºC for 18 to 24 h, the broths were streaked onto indicative selective media (Hektoen Enteric Agar and Brilliant-green Phenol-red Lactose Sucrose Agar, Merck). After incubation at 37ºC for 18 to 24 h, 3 to 5 lactose positive and negative colonies, suspected of *Salmonella* spp, were selected and submitted to preliminary biochemical identification using Triple Sugar Iron Agar, Lysine Iron Agar and Urea Broth (Merck). Strains presenting a biochemical profile suggestive of *Salmonella* spp, were submitted to additional biochemical tests (12). The strains confirmed as salmonellae were differentiated in species and subspecies (16). Before performing the antigenic characterization (rapid slide agglutination), each culture was tested for smooth (S) or rough phase (R), done in saline solution at 2%. Cultures that autoagglutinated were considered in the rough phase. Once in the smooth phase, the cultures were serotyped using *Salmonella* OH polyvalent antiserum (Fundação Oswaldo Cruz), somatic (O) and flagellar (H) polyvalent antisera and the respective monovalent antisera - Difco and Sanofi-Pasteur (16).

RESULTS AND DISCUSSION

The presence of *Salmonella* spp was detected in 39.1% (38/97) of the reptiles, being 62.5% (15/24) in lizards, 53.3% (8/15) in snakes and 25.8% (15/58) in chelonians (Table 1). This result agrees with that described by Chiodini and Sundberg (8), who accomplished an extensive revision about this subject. Despite the high proportion of reptiles carrying S*almonella* spp in the enteric tract, there is no clinical manifestation and the elimination can be either continuous or intermittent (2,6). The intermittent excretion in the feces was well demonstrated by Burnham *et al.* (4) who observed that in ten cultures of the feces of the same animal (iguana), only one was positive.

Aiming to improve the technique sensitivity, besides pre-enrichment in Buffered Peptoned Water and double enrichment in Rappaport-Vassiliadis and Tetratrionate broths, both lactose negative and lactose positive colonies were submitted to confirmation tests. Lactose positive colonies correspond to the former *Arizona* group and now belong to subspecies IIIa and IIIb of the *S. enterica* species.

As shown in Table 1, 38 strains were isolated and 17 (44.7%) belonged to subspecies I, more usually associated to warm-blooded animals. The remaining strains belonged to subspecies *salamae* (4 strains, 10.5%), *arizonae* (2 strains, 5.2%), *diarizonae* (8 strains, 21.0%) and *houtenae* (7 strains, 18.5%), which are subspecies more commonly isolated from cold-blooded animals and environment. In the period from 1981 to 1990, the Centers for Disease Control, USA, reported that these four subspecies (II, IIIa, IIIb, and IV) were involved in more than 50% of the reptile-associated human salmonellosis (1).

As most all serovars that belong to the subspecies I (Table 2) have already been characterized in reptiles (1,3,4,8,9,10). Nevertheless, in regards to climatized animals in captivity, it is possible that they contaminated either by the ingestion of water or food containing salmonellae, or through contact with feces of dogs, cats, wild animals (birds) or animals with peri-domicile habits (rodents) which explain the presence of serovars usually associated to warm-blooded animals.

The serovar IIIb 17:z_{10}:e,n,x,z_{15}, found in a snake, has already been identified in turtles and in human patients by Weiss *et al*

Table 1. Frequency of *Salmonella enterica* subspecies isolated from reptiles.

Subspecies	Snakes		Lizards		Chelonians		Total	
	Number	%	Number	%	Number	%	Number	%
enterica (I)	3	37.5	4	26.6	10	66.6	17	44.7
salamae (II)	-	-	4	26.6	-	-	4	10.5
arizonae (IIIa)	1	12.5	1	6.6	-	-	2	5.2
diarizonae (IIIb)	3	37.5	1	6.6	4	26.6	8	21.0
houtenae (IV)	1	12.5	5	33.3	1	6.6	7	18.5
Total	8	100	15	100	15	100	38	100

Table 2. *Salmonella enterica* serovars and subspecies isolated from reptiles.

Subspecies	Serovar	Animal
I	Albany (2)*	Chelonian
I	Enteritidis (2)	Lizard
I	Hadar	Snake
I	Kottbus	Chelonian
I	Litchfield	Chelonian
I	Loanda	Chelonian
I	Montevideo	Snake
I	Newport	Chelonian
I	Orientalis	Lizard
I	Typhimurium (2)	Chelonian
I	4,5:e,h:-	Snake
I	Non-typable ** (2)	Chelonian
I	Rough	Lizard
II	42:g,t:-	Lizard
II	58:l,z_{13},z_{28}:z_6 (2)	Lizard
II	Non-typable	Lizard
IIIa	Non-typable	Snake
IIIa	Non-typable	Lizard
IIIb	17:z_{10}: e,n,x,z_{15}	Snake
IIIb	38: - : -	Snake
IIIb	38: - : -	Lizard
IIIb	60:r:z (4)	Chelonian
IIIb	Non-typable	Snake
IV	16:z_4,z_{32}:-	Lizard
IV	50: - : -	Lizard
IV	50: - : -	Chelonian
IV	Non typable	Snake
IV	Non typable (3)	Lizard

( ) *: Number of isolated strains; Non-typable **: agglutination only with *Salmonella* polyvalent antiserum.

(20). The serovar IIIb 60:r:z, detected in four turtles, had also been observed by Weiss *et al.* in turtles and patients (20). Despite the lack of flagellar identification, the serovar IIIb 38: -: -, could be included in the four antigenic formulas of serogroup O38, associated to snakes and lizards and also detected in humans (20). Weiss *et al*. (20) also reported that the isolation of the IIIb 60:r:z serovar from hemoculture of 11 patients, demonstrating the extraintestinal localization.

With regards to the nine species (5 national and 4 imported) of snakes (Table 3), the risk represented by the constrictor snakes is obvious, particularly *Python regius* (imported) and *Boa constrictor* (national), commonly kept as pets, coiled around the trunk, limbs or neck of their owners. As shown, eight *Salmonella* serovars were isolated from these animals, and three of them belonged to subspecies I and five to subspecies IIIa, IIIb and IV, which are related to cold-blooded animals.

In the eleven lizard species (7 national and 4 imported) studied (Table 4), 15 *Salmonella* strains were isolated. Four belonged to the subspecies I and the others to subspecies II, IIIa, IIIb and IV, usually reptile-associated. The multiplicity of subspecies found in the iguanas stands out, as well as the coincidence of serovar II 58:lz_{13},z_{28}:z_6 in *Lacerta lepida* and in *Phelsuma madagascariensis*, kept in captivity by the same importer, which suggests cross- contamination.

In the ten chelonian species (5 national and 5 imported) studied (Table 5), a diversity of serovars in the subspecies I was observed. However, the presence of the serovar IIIb 60:r:z in four turtles of the same species, kept by the same importer, vigorously suggests a cross-contamination in the lot. However, a possible vertical transmission (transovarian) should also be considered. On the other hand, among *Salmonella* strains isolated from two chelonian species, 93.3% (14/15) were associated to the *Trachemys scripta elegans* turtle.

Table 3. *Salmonella enterica* subspecies and serovars isolated from snakes.

Animal	Origin	*Salmonella enterica*		
		Subspecies	Serovar	Number Positive/Total
Boa constrictor	N	IIIb	Non-typable*	1/1
Boa constrictor amarali	N	-	-	0/1
Chironius quadricarinatus	N	IIIb	17:z_{10}:e,n,x,z_{15}	1/1
Oxyrhopus trigeminus	N	I	4,5:e,h: -	1/1
Gonyosoma oxycephalum	I	I	Hadar	1/1
Phyton curtus	I	-	-	0/1
Python molurus molurus	I	-	-	0/2
Phyton regius	I	I	Montevideo	6/6
		IIIa	Non-typable	
		IV	Non-typable	
Thamnodynastes strigilis	N	IIIb	38: - : -	1/1

N– National; I– Importe; Non-typable *: agglutination only with *Salmonella* polyvalent antiserum.

Table 4. *Salmonella enterica* subspecies and serovars isolated from lizards.

Animal	Origin	*Salmonella enterica*		
		Subspecies	Serovar	Number Positive/Total
Ameiva ameiva	N	IV	16:z_4,z_{32}	1/2
Arthrosaura ocellata	N	IV	50: - : -	1/1
Cnemidophorus lemniscatus	N	I	Enteritidis	1/1
Corucia zebrata	I	I	Orientalis	2/2
		II	42:g,t: -	
Eublepharis macularius	I	-	-	0/1
Kentropyx calcarata	N	-	-	0/1
Iguana iguana	N	I	Rough	7/9
		I	Enteritidis	
		II	Non-typable*	
		IIIa	Non-typable	
		IV	Non-typable (3)**	
Lacerta lepida	I	II	58:lz_{13},z_{28}:z_6	1/1
Ophiodes striatus	N	IIIb	38: - : -	1/1
Phelsuma madagascariensis	I	II	58:lz_{13},z_{28}:z_6	1/3
Uranoscodon superciliosa	N	-	-	0/2

N– National; I– Importe; Non-typable : agglutination only with *Salmonella* polyvalent antiserum; ( )** Number of isolated strains.

Table 5. *Salmonella enterica* subspecies and serovars isolated from chelonians.

Animal	Origin	*Salmonella enterica*		
		Subspecies	Serovar	Number Positive/Total
Chelydra serpentina	I	-	-	0/5
Geochelone carbonaria	N	-	-	0/5
Geochelone denticulata	N	IV	50: - : -	1/5
Giblus grandis	I	-	-	0/1
Graptemys pseudogeographica	I	-	-	0/2
Hydromedusa maximiliani	N	-	-	0/3
Phrynops giblus	N	-	-	0/1
Trachemys scripta dorbigni	N	-	-	0/1
Trachemys picta	I	-	-	0/1
Trachemys scripta elegans	I	I	Albany (2)*	14/34
		I	Kottbus (1)	
		I	Litchfield (1)	
		I	Loanda (1)	
		I	Newport (1)	
		I	Typhimurium (2)	
		I	Non-typable* * (2)	
		IIIb	60:r:z (4)	

N– National; I– Importe; ( )* Number of isolated strains; Non-typable; **: agglutination only with *Salmonella* polyvalent antiserum.

Among the isolated *Salmonella* strains, 57.9% could be completely serotyped, 13.1% were partially characterized, 26.3% were non-typable and 2.7% were in the rough phase (R). These results can hardly be compared to those of other authors, mainly because very few studies include results on rough forms (20) or untypable strains (1,4,10). These data are available in our study because the Brazilian *Salmonella* reference laboratories (Instituto Oswaldo Cruz and Instituto

Adolfo Lutz) include this information in their routine testing reports (17,18).

The antigenic characterization of salmonellae is a powerful epidemiological tool and should be better used in laboratorial diagnosis of infection processes, leading to a better knowledge of the circulation dynamics of this pathogen.

In conclusion, pet reptiles represent a potential risk to the human health, being fundamental that the authorities exert sanitary control over them (*Salmonella*-free certificate) and proceed explanation campaigns to the public.

RESUMO

Salmonella em répteis de estimação nacionais e importados

A presença de salmonelas em amostras de fezes ou *swabs* cloacais de 97 répteis de estimação (15 cobras, 24 lagartos e 58 quelônios), dos quais 37 eram de origem nacional e 60 importados, foi investigada. *Salmonella* spp foi detectada em 39,1% dos répteis estudados, sendo 62,5% em lagartos, 53,3% em cobras e 25,8% em quelônios. Foram caracterizadas as subespécies I (44,7%), II (10,5%), IIIa (5,2%), IIIb (21,0%) e IV (18,5%) da espécie *enterica*, com predominância (55,3%) das subespécies habitualmente encontradas em animais de sangue frio (II a IV). Na subespécie I, houve maior freqüência dos sorovares Albany, Enteritidis e Typhimurium. As tartarugas importadas *Trachemys scripta elegans* corresponderam a 93,3% dos quelônios portadores de salmonelas. Os iguanas nacionais apresentaram elevado índice de colonização (77,7% - 7/9). Conclui-se que esses animais apresentam risco potencial à saúde humana, havendo necessidade de controle sanitário e maior esclarecimento ao público.

Palavras-chave: *Salmonella*, répteis de estimação.

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