The incidence and antibiotic resistance of *Salmonella* species isolated from cloacae of captive veiled chameleons

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Abstract

Salmonella can be present in the intestinal flora of captive reptiles without clinical disease or it can cause life threatening morbidity. The presence of certain species of *Salmonella* in reptiles is consistent with them being the source of contamination in some cases of human disease. Thus, *Salmonella* positive animals can be a potential public health concern even more when strains acquire resistance to antibiotics. The nature and extent of *Salmonella* harboured by different species of reptiles commonly kept in captivity are not known. The aims of this study were to analyse the incidence of *Salmonella* species in cloacae as an indicator of the intestinal flora in a cohort of healthy captive bred female veiled chameleons. A cloacal sample was taken from each of fifteen healthy captive bred, adult female veiled chameleons that were housed at a teaching and research clinic. *Salmonella* isolates were confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and positive cases were serotyped by slide agglutination test. *Salmonella* organisms were detected in 12 chameleons. Eighty percent of chameleons harboured 1 of 4 subspecies and serovars of *Salmonella*. All strains belonged to the species *enterica*, predominantly subspecies *enterica* (91.7 %) and were distributed among 4 different serovars: *S.* Ago (58.3 %), *S.* Blijdorp (16.7 %), *S.* Tennessee (16.7 %) and *S.* IV 45:g,z_{51}:- (8.3 %). Antibiotic resistance to streptomycin was detected in one of 12 *Salmonella* strains: *S.* IV 45:g,z_{51}:-. Our study extended the list of *Salmonella* found in healthy captive animals and included serovars *S.* Tennessee and *S.* IV 45:g,z_{51}:- that have been associated with morbidity in humans.

Reptiles, intestinal flora, public health, MALDI-TOF MS

An investigation of the source of *Salmonella* and the cause of antibiotic resistance is important because of the reports of zoonotic transmission of *Salmonella* associated with captive reptiles, the impact on human health and the ability of *Salmonella* to acquire antibiotic resistance (Frye and Jackson 2013). *Salmonella* is normally found in the intestinal flora of clinically healthy reptiles, and *Salmonella* positive animals may shed a mixture of *Salmonella* serovars in their faeces intermittently without showing any clinical signs (Chiodini and Sundberg 1981; Burnham et al. 1998). However, in stressed and/or immunosuppressed reptiles, *Salmonella* caused bacterial granuloma, septicaemia, pneumonia, nephritis, and death (Onderka and Finlayson 1985). Previous studies have identified *Salmonella* serovars Agona, Ajiobo, Arizonae, Durban, Ebrie, Enteritidis, Gaminara, Houten, Othmarschen, Pomona, Simi, Tel-el-kebir, IV 43:z_4,z_{23}:- and IIIb 61:z_{52}:z_{53} in a few healthy captive individual chameleons (Woodward et al. 1997; Geue and Löscher 2002; Willis et al. 2002; Ebani et al. 2005; Pees et al. 2013). To date it is not known whether *Salmonella* species would be different or more widespread within a collection of healthy captive chameleons. The aims of this study were (1) to analyse the incidence of *Salmonella* species in a homogeneous group of captive healthy veiled chameleons housed in a research and teaching facility, and (2) to evaluate its antimicrobial resistance to a panel of antibiotics.

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Materials and Methods

Animals

A total of 15 female veiled chameleons (*Chamaeleo calyptratus*), aged 18 months old and weighing 163 g ± 35.39 g, were included in this study. All were captive bred and kept in an isolated experimental facility at the Avian and Exotic Animal Clinic, Brno, Czech Republic. The animals were housed and handled in agreement with the Branch Commission for Animal Welfare of the Ministry of Education, Youth and Sports of the Czech Republic. Chameleons were kept in small groups of two to four in five glass terrariums (75 × 88 × 75 cm) equipped with artificial plastic plants, under 12:12 light/dark regime provided by a flood metal halide lamp (Bright Sun FLOOD Jungle 70 Watts, Lucky Reptile, Germany) at a temperature between 24 °C and 35 °C. The air humidity of 60 to 80% was regulated by spraying the plants with fresh tap water. Chameleons were fed three times a week with iceberg lettuce and crickets dusted with a mix of calcium and vitamin D_3 (Nekton MSA, Germany). Fresh water was provided *ad libitum* in clean ceramic bowls. The animals were identified with electronic microchips (Alvic, Alvetra und Werfft GmbH, Austria) administered subcutaneously under a brief alfaxalone anaesthesia (Knotek et al. 2011). The health of all animals was checked daily for 12 months. Two to four months before the study, three chameleons were treated with marbofloxacin (10 mg/kg q24h subcutaneously, for 7 days, Marbocyl 2% inj. ad us. vet. Vétoquinol S.A., France), for the following conditions: chameleon number 11 was hospitalized for 2 weeks due to treatment of a bacterial abscess in the mouth cavity; chameleon number 13 was hospitalized for 3 weeks due to elbow inflammation and chameleon number 15 was treated with marbofloxacin plus ampicillin (given as a bolus of 0.083 grams into the coelom, Ampicilin 0.5 g inj. Biotika a.s., Slovakia) after surgical ovariectomy. The other animals did not have any exposure to antibiotics. Before sample collection all females were clinically examined, including collection of fresh faeces for parasitology, and blood samples for haematology and plasma chemistry analysis. Within the whole period of the study chameleons were clinically healthy.

Sample collection

Each female chameleon was manually restrained and a sterile cotton swab (Copan Italia S.p.A, Italy) was gently inserted and twirled inside the cloaca. Samples were transported to bacteriological laboratory in Amies transport medium (Copan Italia S.p.A., Italy).

Cultivation, identification and serotyping of *Salmonella* strains

Cloacal swabs were placed in a tube with 9 ml of buffered peptone water (Oxoid, Ltd., England) and incubated at 37 °C for 24 h under aerobic conditions. From each tube, 100 µl were inoculated onto the surface of modified semisolid Rappaport Vassiliadis medium (Oxoid, Ltd., England) and incubated at 41.5 °C for 24 to 48 h. Once a grey white turbid zone around the inoculated drop was observed, a loopful of the culture was streaked onto plates of xylose-lysine-deoxycholate agar (Oxoid, Ltd., England) and brilliant green agar (Oxoid, Ltd., England). The plates were incubated at 37 °C for 24 h. Confirmation of suspected *Salmonella* isolates was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI- TOF MS, MALDI Biotyper, Bruker Daltonics, Germany) in accordance with the user (MALDI Biotyper 3.0 User Manual Revision 2, Bruker Daltonics, Germany). Isolates positive for *Salmonella* were serotyped by slide agglutination testing using two commercial O- and H- antisera (BioRad, France; Denka Seiken, Japan). The serovar identification was carried out according to Kauffmann-White-Le Minor scheme (Grimont and Weill 2007).

Antimicrobial sensitivity test

Salmonella strains were tested for their sensitivity to 12 antimicrobial agents by the disc diffusion method on Mueller-Hinton agar (Oxoid, Ltd., England) in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI 2013). The following antibiotics (Oxoid, Ltd., England) and concentrations per disc were used: amoxicillin clavulanate (30 µg), ampicillin (10 ppm), cefalotin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 ppm), nalidixic acid (30 µg), potentiated sulfonamides (25 µg), streptomycin (10 ppm), sulfonamides (300 µg) and tetracycline (30 µg). The zone of inhibition around each disc was measured and *Salmonella* strains were classified as sensitive (S), intermediate (I) or resistant (R) according to CLSI standards (CLSI 2013).

Results

Salmonella organisms were detected in 12 (80%) of the 15 chameleons investigated (Table 1). All strains of *Salmonella* belonged to the species *enterica*. We identified 4 different serovars: *S.* Ago (58.3%), *S.* Blijdorp (16.7%), *S.* Tennessee (16.7%) and *S.* IV 45:g,z_{51}:- (8.3%). All but one (*S. enterica* subspecies *houtenae)* belonged to the subspecies *enterica* (91.7 %). The distribution of serovars varied between animals and terrariums. The most widespread, *Salmonella* Ago, was found in at least one chameleon from each terrarium,

and in two terrariums, two animals were positive. The cloacal swabs of three chameleons were negative for *Salmonella*. These animals were housed in different terrariums, separated from each other, and each with an animal that was *Salmonella* positive. Two of the five terrariums showed a high diversity of serovars, with 3 different serovars present in three different animals.

Table 1. *Salmonella enterica* subspecies and serovars isolated from cloacae of female veiled chameleons.

Terrarium	Female chameleon	Antibiotic treatment before the study	*Salmonella enterica*		Antimicrobial sensitivity to antibiotics*
			Subspecies	Serovar	
A	No. 1		*enterica*	Ago	S
	No. 2		*enterica*	Tennessee	S
	No. 3		*houtenae*	45:g,z_{51}:-	R to streptomycin
B	No. 4		*enterica*	Ago	S
	No. 5		*enterica*	Ago	S
	No. 6		*enterica*	Blijdorp	I to streptomycin
	No. 7		*enterica*	Tennessee	S
C	No. 8		*enterica*	Ago	S
	No. 9		negative finding		
D	No. 10		*enterica*	Ago	S
	No. 11	yes	negative finding		
	No. 12		*enterica*	Ago	S
	No. 13	yes	*enterica*	Blijdorp	I to streptomycin
E	No. 14		*enterica*	Ago	S
	No. 15	yes	negative finding		

*Sensitivity (S)/ Resistance (R)/ Intermediate (I) to: amoxicillin clavulanate, ampicillin, cefalotin, ceftazidime, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid, potentiated sulfonamides, streptomycin, sulfonamides, tetracycline.

Of the 12 strains investigated, only one, *Salmonella enterica* subspecies *houtenae* serovar IV 45:g,z_{51}:-, was found to be resistant to streptomycin. This serovar was found in only one chameleon (number 3), but this animal was not one of those that had received prior treatment with antibiotics. Two chameleons (housed separately from each other) were positive for *Salmonella enterica* subspecies *enterica* serovar Blijdorp. Cloacal cultures from these animals both demonstrated intermediate sensitivity to streptomycin. Only one of these, (number 13), had previously received antibiotic treatment (marbofloxacin). Chameleons numbers 11 and 15, previously treated with antibiotics, were both negative for *Salmonella*. Interestingly, animal number 11 was housed with number 13, the treated animal positive for *Salmonella* Blijdorp, described above. Thus, of the three chameleons having received prior antibiotic treatment, two were negative for *Salmonella* and the third was positive for *Salmonella* Blijdorp with intermediate sensitivity to streptomycin.

Discussion

A large number of different *Salmonella* subspecies and serovars can be found on cloacal swabs of healthy captive chameleons (Woodward et al. 1997; Geue and Löscher 2002; Willis et al. 2002; Ebani et al. 2005; Pees et al. 2013). Our current study of 15 healthy captive female chameleons did not find any of the serovars previously reported, but extended the list found in this species with additional four different *S. enterica* serovars. The different *Salmonella* subspecies and serovars in the current study compared to prior studies likely reflect the isolation of these cohorts from each other. The common finding of *S.* Ago in our research

and teaching facility likely reflects the co-housing of terrariums in the same facility leading to cross contamination.

It has previously been reported that a single reptile or its environment, may simultaneously harbour several different *Salmonella* serovars (Chiodini and Sundberg 1981). The current study found up to three different serovars in the same terrarium, however, only one serovar in each animal. Interestingly, chameleons sharing the same terrarium did not harbour the same *Salmonella* serovars; perhaps this was because reptiles can shed *Salmonella* intermittently and because only one sample was taken from each animal. Alternatively, individual animals may show variable susceptibility or resistance to the broad spectrum of *Salmonella* strains to which they are exposed, leading to opportunistic overgrowth of one particular serovar.

In the current study *S.* Ago was found in one or two chameleons in every terrarium. *Salmonella* Ago has previously been described in captive lizards and turtles in Taiwan (Chen et al. 2010). The initial source of *Salmonella* organisms in our cohort of chameleons is not known. However, we speculate that the high percentage of *Salmonella enterica* subspecies *enterica* in general, could result from colonization of the intestines of our female veiled chameleons after ingestion of food contaminated with *Salmonella* or through carer handling practices. We did not detect the serovars *S.* Telel-kebir and *S.* II 35:g,m,s:- in the current study that were previously found associated in pet chameleons with human cases of salmonellosis (Willis et al. 2002; Bertrand et al. 2008). *Salmonella* Tennessee has been found in humans, in a captive Major skink, Solomon Islands skink and four-lined plated lizard in Belgium, and was reported to be responsible for salmonellosis in humans after contact with a bearded dragon in Germany (Pasmans 2005; Weiss et al. 2011; Pees et al. 2013); and the *Salmonella* IV 45:g,z_{51}:- has been found in humans, in captive green iguanas in Canada, and was reported to be responsible for salmonellosis in humans after contact with a bearded dragon and a gecko in Germany (Woodward et al. 1997; Bertrand et al. 2008). Thus, these *Salmonella* serovars found in our current study could potentially lead to human disease and thus the animals harbouring them pose a public health risk.

Two of the three chameleons previously treated with marbofloxacin +/- ampicillin, were negative for *Salmonella.* These few data are consistent with elimination of *Salmonella* by antibiotic treatment. The low incidence of acquired antibiotic resistance of *Salmonella* strains in the present study might be due to the absence of previous exposure of most of the female veiled chameleons to antibiotics. Our results are in accordance with studies on the acquired resistance of *Salmonella* strains to streptomycin in captive reptiles (Seepersadsingh and Adesiyun 2003; Chen et al. 2010). This cross-sectional observational study was not designed to investigate the development of resistance to antibiotics, and only a small number of animals can contribute to the consideration of this subject.

It is possible that contamination of animals occurred with *S.* Blijdorp that already possessed resistance. The current investigation together with previous studies of captive healthy chameleons shows a high incidence and diversity of *Salmonella* subspecies and serovars in their intestinal flora. Some of the *Salmonella* found are capable of causing morbidity in humans. These findings are of public health concern because of the possible unwitting transmission of *Salmonella* to humans from a reptile that is clinically healthy. The current study utilized chameleons from a research and teaching institute housed in the same facility and exposed to the same personnel and sources such as substrate, furnishings, food, and water. The diversity of *Salmonella* indicates that sources of *Salmonella* in healthy captive chameleons require investigation and control, e.g. of the substrate and crickets used for food. Further research exploring the general applicability of our findings to reptiles in different households and consequently under varied conditions, would better represent the zoonotic potential posed by pet reptiles to their owners and would make an interesting comparison with our isolated collection.

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