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Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium

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Abstract

During a period of about three years the faeces of five species of amphibians (35 individuals) and of 23 species of reptiles (103 individuals) living in one vivarium with terrariums imitating different types of ecosystems were examined for salmonellae. From 54 out of 376 faecal samples *Salmonella* spp. was isolated (=14%). Twenty-one different *Salmonella* strains were found. Salmonellae could be isolated about twice as often from animals kept under arid or mesic conditions than from animals living in humid or aquatic environments although this was not statistically significant. Statistically significant for the rate of *Salmonella* excretion was the animals' diet and the class the animals are belonging to. Animals feeding on mice ($p = 0.04$) and reptiles in general ($p = 0.04$) were more commonly excreting *Salmonella*. Duration of stay was also a significant factor ($p = 0.0005$), whereby the relative risk for *Salmonella* excretion increased with the factor 2.91 per year during the investigation period. *Salmonella* strains were not necessarily transferred among animals living in the same terrarium or among the inhabitants of different terrariums. The pattern of *Salmonella* excretion was generally fragmentary. The outsides as well as the insides of the walls of the terrariums were also tested for salmonellae several times, but salmonellae have never been isolated.

Key words: *Salmonella* – amphibians – reptiles – herpetology – health risk – transmission route

Introduction

Salmonella enterica is a major cause of gastroenteritis in humans and also affects many animals (Woodward et al., 1997). It is well known that amphibians and reptiles harbour different strains of salmonellae without showing any symptoms of illness. The frequency of *Salmonella* isolation has been found to be higher in reptiles than in mammals or birds (Gopee et al., 2000). There have been increased numbers of case reports of human infection (CDC, 1992a, b, 1995; Gerson, 1996; Johnson-Delaney, 1996; Mermin et al., 1997; Sanyal et al., 1997; Woodward et al., 1997), and fatal cases of human salmonellosis associated with exotic pets are rapidly emerging (CDC, 1999). It is estimated that three to five percent of all cases of human salmonellosis are associated with direct or indirect contact

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Table 1. List of all animal species tested including the climate they are living in.

species		class	climate
Latin name	English term		
Xenopus clivii	Clawed Frog	amphibia	aquatic
Chelus fimbriatus	Matamata Turtle	reptiles	aquatic
Emydura subglobosa	Red-Bellied Short-Necked Turtle	reptiles	aquatic
Clamydosaurus kingii	Frilled Lizard	reptiles	arid
Oplurus cuvieri	Madagascar Swift	reptiles	arid
Pogona vitticeps	Bearded Dragon	reptiles	arid
Tiliqua scincoides	Eastern Blue-Tongued Skink	reptiles	arid
Zonosaurus madagascariensis	Madagascar Girdled Lizard	reptiles	arid
Ceratophrys cranwelli	Horned Frog	amphibia	humid
Dyscophus guineti	Tomato Frog	amphibia	humid
Dendrobates histrionicus	Harlequin Poison Dart Frog	amphibia	humid
Phyllobates bicolor	Black-Legged Poison Dart Frog	amphibia	humid
Corallus caninus	Emerald Tree Boa	reptiles	humid
Thamnophis radix	Plains Garter Snake	reptiles	humid
Basiliscus plumifrons	Green Basilisk	reptiles	humid
Furcifer oustaleti	Oustalet's Chameleon	reptiles	humid
Furcifer pardalis	Panther Chameleon	reptiles	humid
Iguana iguana	Green Iguana	reptiles	humid
Physignathus cocincinus	Thai Water Dragon	reptiles	humid
Varanus prasinus	Green Tree Monitor	reptiles	humid
Elaphe guttata	Corn Snake	reptiles	mesic
Elaphe obsoleta	Black Rat Snake	reptiles	mesic
Pituophis melanoleucus	Bull Snake	reptiles	mesic
Agama atricollis	Blue-Headed Tree Agama	reptiles	mesic
Aristelliger lar	Hispaniolan Giant Gecko	reptiles	mesic
Cordylus warreni	Warren's Girdle-Tailed Lizard	reptiles	mesic
Mabuya quinquetaeniata	Rainbow Rock Skink	reptiles	mesic
Platysaurus imperator	Emperor Flat Lizard	reptiles	mesic

with exotic pets (Bartlett et al., 1977; Woodward et al., 1997).

The aim of this study was to determine the occurrence, distribution and strain characteristics of salmonellae excreted by captive amphibians and reptiles (Table 1) living under controlled conditions in one vivarium within terrariums of distinct climates.

Anamnestic and ecological data are combined with data of frequency and distribution of salmonellae to obtain a risk assessment. Therefore we examined the faeces of amphibians (5 species; 35 individuals) and reptiles (23 species; 103 individuals) during a period of about three years for the occurrence of salmonellae and for the distribution of this bacterium regarding species and serotypes.

Materials and methods

During the investigation period 75 faecal samples from 35 specimens of amphibians (belonging to five species) and 301 faecal samples from 103 specimens of reptiles (belonging to 23 species) were tested for salmonellae. The investigation was performed immediately after obtaining the animals and afterwards in intervals of 49 days on an average. All animals investigated were kept in the vivarium of the Museum of Natural History Vienna. During the investigation period all tested animals were free of symptoms connected with the presence of salmonellae. The faecal samples were picked up immediately after defecation, trying to avoid any contamination with environmental material, put into sterile faeces tubes and processed within 12 hours. The samples were suspended in sterile sodium chloride peptone broth (buffered) pH 7.0 (Merck KgaA Darmstadt, D) and incubated for 24 ± 4 hours at 30 °C. Afterwards 1 ml of this solution was put into 9 ml Rappaport-Vassiliadis broth (Merck KgaA Darmstadt, D) and incubated for 2–5 days at 30 °C. Presence of salmonellae in this selective enrichment broth was tested by cultivation on SMID agar (bioMerieux GesmbH Vienna, A). Typical colonies grown on SMID agar were agglutinated (Oxoid Limited Basingstoke, GB) and biochemically identified by api20E system (bioMerieux GesmbH Vienna, A). Furthermore the isolated strains were investigated for their serovar at the National Salmonella Reference Laboratory according the Kauffmann White Scheme, specific isolates were further characterised by phage typing (BBSUA Graz, A).

Additionally five faecal samples of mice, which were assigned as feed, and 20 swab samples of the walls of the terrariums were investigated for salmonellae in the same way as stated above.

Demographic data (climate, feed, suborderial position) were analysed for risk characterization by a multivariate logistic regression analysis (Statistica 5.5.) giving rounded p values of < 0.05 for significance.

Results

Salmonella was detected in 54 out of 376 faecal samples (overall colonization rate 14%). Table 2 presents the *Salmonella* serotypes isolated from all animals tested.

Fourteen of the 28 animal species tested excreted *Salmonella* at least once. The faeces of only one of five amphibian species, namely *Ceratophrys cranwelli,* contained *Salmonella* (4% of the amphibian faecal samples) whereas it was detected in the faeces of 13 out of 23 reptile species. Snakes had the highest infection rate (24%), saurians had an intermediate rate (17%), turtles had the lowest one (3%).

Although salmonellae could be isolated from samples of animals living in terrariums with arid or mesic climate two times more frequently than in terrariums with humid or aquatic climate (Table 3), the climatic condition of a terrarium had no statistically significant influence on *Salmonella* excretion. Reptiles in general ($p = 0.04$) and animals feeding on mice ($p = 0.016$) had a significantly higher risk of excreting *Salmonella*. However, in this study *Salmonella* was never isolated from mice. During the three years of investigation the relative risk of *Salmonella* excretion increased with the factor 2.91. Therefore time was a significant factor, too ($p = 0.0005$).

Figure 1 gives the distribution of salmonellae over the time for all tested animal species. *Salmonella* of the subspecies III was only isolated four times (7%) whereas *Salmonella* of the subspecies I was found 45 times (83%). Other *Salmonella* strains included a subspecies II, a pili-less F-group *Salmonella* and four rough *Salmonella* types, for which the antigenic formula was undeterminable. No *Salmonella* strains of the subspecies IV, V and VI were isolated.

Regarding the time distribution pattern – with only one exception (*Ceratophrys cranwelli*) – salmonellae were not detected in the first faeces samples of newly introduced animals. Different distributions of *Salmonella* occurrence were found: only a single isolation, intermittent excretion over a long or a short period of time, only one type of *Salmonella* or different types of *Salmonella* within one host individual. Permanent excretion of salmonellae was never observed. Three times the same type of *Salmonella* could be isolated simultaneously from different hosts within one to 37 days (Fig. 1): *S. oranienburg* was found in all three species of one

Table 2. List of *Salmonella* serovares isolated (in bracket the antigen formula) including the animals they were found in (in bracket the number of isolations per animal).

Salmonella serovar (antigen formula)	animal (No of isolations)
pililess type of the F-group (11 : – : –)	*Pogona vitticeps* (1)
rough *Salmonella* type (not determinable)	*Pogona vitticeps* (1); *Tiliqua scincoides* (2); *Pituophis melanoleucus* (1)
Salmonella Abidjan (39 : b : 1,w)	*Ceratophrys cranwelli* (2)
Salmonella blockley (6,8 : k : 1,5)	*Mabuya quinquetaeniata* (3)
Salmonella fresno (9,46 : z38 : –)	*Tiliqua scincoides* (2)
Salmonella gaminara (16 :d : 1,7)	*Furcifer oustaleti* (3); *Pogona vitticeps* (2); *Varanus prasinus* (1); *Elaphe guttata* (1)
Salmonella gatuni (6,8b : e, n,x)	*Pogona vitticeps* (1); *Aristelliger lar* (2)
Salmonella hvittingvoss (16 : b :e, n,x)	*Furcifer oustaleti* (1)
Salmonella II (30 : 1,z28 : z6)	*Basiliscus plumifrons* (1)
Salmonella IIIa (arizonae) (44 : z4,z23 : –)	*Elaphe obsoleta* (1)
Salmonella IIIa (arizonae) (40 : z4,z23 : –)	*Pituophis melanoleucus* (2)
Salmonella IIIb (arizonae) (47 :î :z53)	*Pituophis melanoleucus* (1)
Salmonella montevideo (6,7 : g, m,s : –)	*Pogona vitticeps* (3)
Salmonella newport (6,8 : e, h : 1,2)	*Varanus prasinus* (1); *Elaphe guttata* (1)
Salmonella nima (28 :y : 1,5)	*Thamnophis radix* (1); *Pituophis melanoleucus* (1)
Salmonella oranienburg (6,7 : m, t : –)	*Elaphe obsoleta* (1); *Elaphe guttata* (3); *Pituophis melanoleucus* (1)
Salmonella ramonby (13,23 :z4z24 : –)	*Emydura subglobosa* (1)
Salmonella schwarzengrund (1,4,12,27 :d : 1,7)	*Pogona vitticeps* (2); *Tiliqua scincoides* (1)
Salmonella tenessee (6,7 :z29 : –)	*Basiliscus plumifrons* (4)
Salmonella tornow (45 : g, m(s),(t) : –)	*Furcifer oustaleti* (1); *Pogona vitticeps* (1); *Iguana iguana* (1); *Pituophis melanoleucus* (1)
Salmonella wandsworth (39 : b : 1,2)	*Pogona vitticeps* (2); *Ceratophrys cranwelli* (1)

Table 3. Comparison of *Salmonella* isolation concerning animal classes, the climates they are living in and the feed.

class/climate/feed	No species	No species with *Salmonella*	% of species with *Salmonella*	No animals	No samples	No of samples with *Salmonella*	% of samples with *Salmonella*
amphibians	5	1	20	35	75	3	4
amphibians/aquatic/insects	1	0	0	3	6	0	0
amphibians/humid/insects	3	0	0	27	51	0	0
amphibians/humid/insects, mouse	1	1	100	5	18	3	17
reptiles	23	13	57	79	301	51	17
reptiles/aquatic/fish	1	0	0	6	18	0	0
reptiles/aquatic/insects, mouse, plants	1	1	100	4	14	1	7
reptiles/humid/insects	3	2	67	12	37	10	27
reptiles/humid/insects, mouse	1	1	100	4	10	2	20
reptiles/humid/insects, mouse, plants	1	0	0	4	11	0	0
reptiles/humid/plants	1	1	100	4	11	1	9
reptiles/arid/insects	3	0	0	9	23	0	0
reptiles/arid/insects, mouse, plants	2	2	100	8	64	18	28
reptiles/mesic/insects	5	2	40	28	51	5	10
reptiles/mesic/mouse	3	3	100	17	44	14	32
amphibians and reptiles	28	14	50	138	376	54	14
reptiles/humid/fish	1	1	100	5	11	1	9
reptiles/humid/mouse	1	0	0	2	7	0	0
amphibians and reptiles/aquatic, humid, mesic and arid/mouse	10	8	80	44	168	38	23
amphibians and reptiles/aquatic and humid	15	7	47	76	194	18	9
reptiles/arid and mesic	13	7	54	62	182	36	20
reptiles/aquatic and humid	10	6	60	41	69	15	22
reptiles/aquatic	2	1	50	10	32	1	3
reptiles/humid	8	5	63	31	87	14	16
reptiles/arid	5	2	40	17	87	18	21
reptiles/mesic	8	5	63	45	95	18	19
amphibians and reptiles	28	14	50	138	376	54	14

terrarium, whereas *S. gaminara* was isolated from different species living in different terrariums, and *Salmonella* of a rough type was excreted by two species of one terrarium and by one species of another terrarium.

In most cases even animals living together in one terrarium excreted salmonellae of different serotypes. Some animals never showed a *Salmonella* infestation although they were living together with *Salmonella* excreting animals.

Furthermore we found the rare strains *S. gatuni* (6,8 : b : e, n,x), *S. nima* (28 : y : 1,5), and *S. romanby* (13,23 : z4z24 : –).

When testing the inside and the outside of the terrarium walls salmonellae could never be isolated.

Discussion

Salmonellosis in humans due to direct or indirect contact with exotic pets is well known (Woodward et al., 1997). The number of human infections with rare and with amphibians and reptiles associated *Salmonella* serotypes has been increasing over the last twenty years (CDC, 1999).

It is well documented that amphibians and reptiles are often symptomless carriers of salmonellae (Chiodini and Sundberg, 1981; Hird et al., 1983; Madsen et al., 1998; Onderka and Finlayson, 1985). All the animals investigated in this study were asymptomatic, although 20% of the amphibian and 57% of the reptile species excreted *Salmonella*, which is in good agreement with previously published data (Bartlett et al., 1977; Friedman et al., 1998).

The relative risk of excreting *Salmonella* was for reptiles 5.23 times higher in comparison with amphibians, which proved to be statistically significant ($p = 0.04$). This can be interpreted as an indicator for phylogenetically originated similarities of the anatomy and physiology influencing the reaction towards *Salmonella* infections. Considering the climate of the terrariums we isolated salmonellae twice as often from animals in terrariums with arid or mesic climate than from animals in humid or aquatic milieu, which is in agreement with previous investigations (Appelt et al., 2000). But

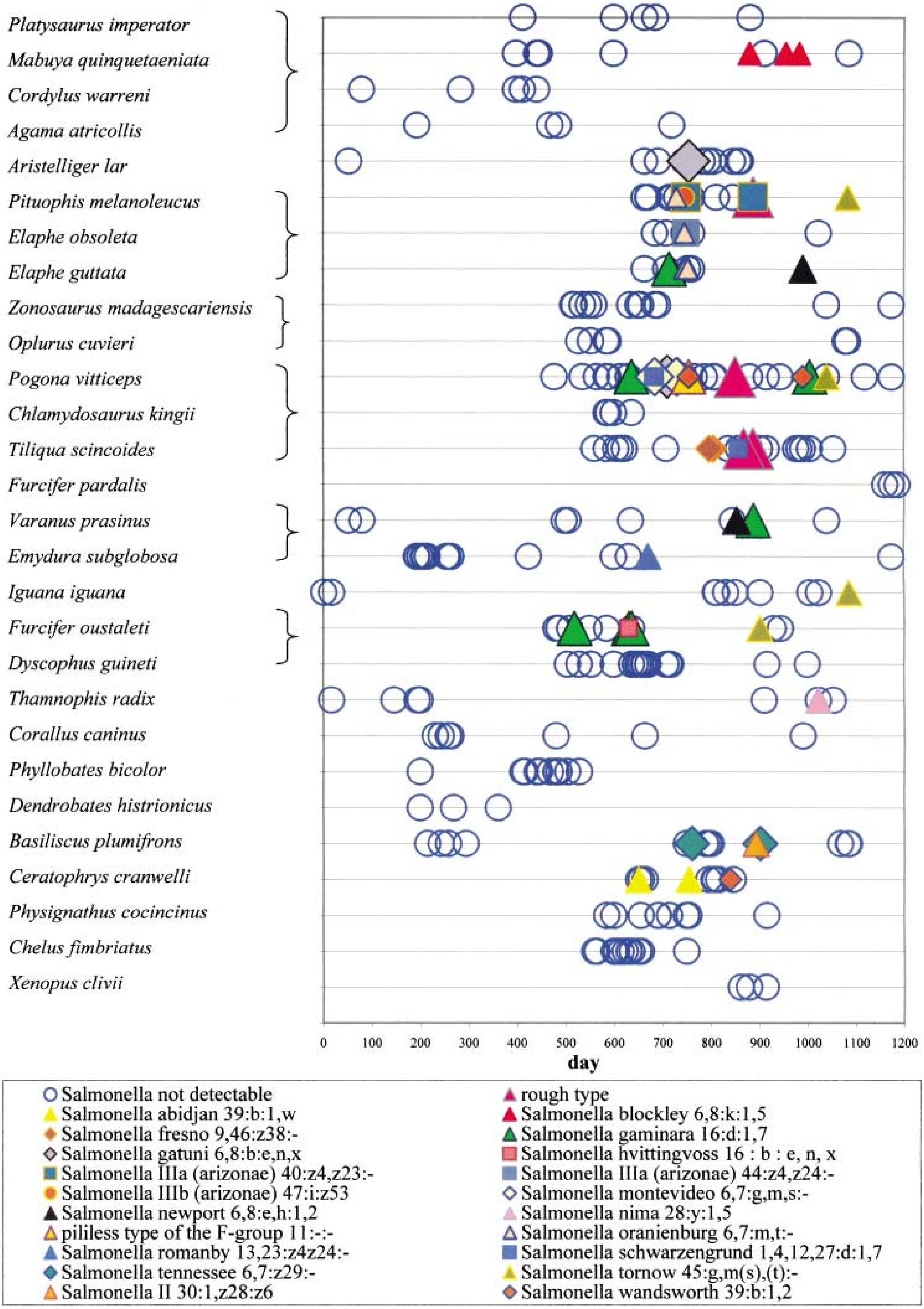

Fig. 1. *Salmonella* investigation of the faeces of amphibians and reptiles living together in one vivarium during a period of about three years. Brackets indicate animals living together in one terrarium.

statistical analysis of our data gave no significance concerning the climate of the terrariums. Our data indicate that these results are also mainly due to the animals feed (namely mice, $p = 0.016$), although salmonellae could never be isolated from the mice or the people handling the animals (unpublished data). The fact that *Salmonella* isolation was obviously not randomly connected with the feeding of mice gives a good explanation for isolating mainly salmonellae of the subspecies I, which are typical for mammals. The present data are in agreement with the findings of Wokatsch and Rohde (1979), who stated that saurians excrete mainly salmonellae of the subspecies I. To our knowledge we are the first isolating *S. gatuni* (6,8 : b : e, n,x), *S. nima* (28 : y :1,5), and *S. romanby* (13,23 : z4z24 : –) from reptiles. Only 7% of our isolates belonged to subspecies III, which is said to be typical for snakes and other reptiles (Sanyal et al., 1997). Other *Salmonella* subspecies were detected in minimal numbers. Although cold-blooded animals are regarded as usual carriers for *Salmonella* subspecies IV (Aleksic et al., 1996; Cyriac and Wozniak, 2000; Woodward et al., 1997), surprisingly no such strain was found in our study.

The reason for the occurrence of so many different *Salmonella* strains remains unclear. Perhaps it is due to multi-infections with different *Salmonella* strains at the same time or due to a permanent incoming from extern.

In general the isolation of salmonellae occurred less often than expected, only 14% of all samples gave positive results. This detection rate seems to be rather unusually low considering the fact that other authors assume shedding rates up to 90% in reptiles (CDC, 1992b; Chiodini and Sundberg, 1981; Ward, 2000; Woodward et al., 1997). But it has to be kept in mind that the excretion of these bacteria is intermittent (Minette, 1984; Sanyal et al., 1997; Ward, 2000). This fact as well as the use of different methods and different collectives of host animals are considered to be the reason for the variable detection rates found by different authors (Mathewson, 1979). The significance of the factor time ($p = 0.0005$) considering *Salmonella* excretion is remarkable, although the time of the investigation was not long enough to see where this trend of 2.91 fold increase per year is finally going to.

Once in our study a dual infection with two different *Salmonella* strains in one individual was detected – a *Pituophis melanoleucus* shed a rough *Salmonella* type and *Salmonella* IIIa (arizonae) (40 : z4,z23 : –) at the same time. The isolation of different *Salmonella* types on successive days might be an indication for a more frequent occurrence of multi-infections. Other authors, who tested free living animals, privately kept animals and animals of public vivariums, also found an occurrence of many different *Salmonella* strains and an intermittent *Salmonella* excretion (Cambre et al., 1980; Wokatsch and Rohde, 1979). In contrast to other authors (Cambre et al., 1980; MacNeill and Dorward, 1986) testing reptile collections, with one exception our animals never showed *Salmonella* infestations in the first investigated faeces after their arrival in the vivarium of the Museum of Natural History Vienna.

An important finding for the vivaristic community is that there seems to be no epidemic transfer of salmonellae from one terrarium to the next and from one animal to another even if the animals are living in the same terrarium. This confirms previous findings (Chiodini and Sundberg, 1981) and the thesis is supported by the fact that salmonellae were never isolated from the inside as well as from the outside of the terrarium walls. Nevertheless we have to consider that the recovery of salmonellae from the surfaces might be poor.

Due to the reasons stated above amphibians and reptiles can never be regarded as *Salmonella* free. Moreover it has been stated that all reptiles carry *Salmonella* because these bacteria are part of their normal flora (Meehan, 1996), even if they do not shed them all the time.

We advice especially children, pregnant women and immunocompromised persons to avoid direct or indirect contact with exotic pets or to follow at least the recommendations for reducing the risk of transmission of salmonellae by washing hands after handling the animals or their cages, keeping the animals out of kitchens and other food-preparation areas, not bathing the animals and not washing their dishes, cages or aquariums in kitchen sinks, and cleaning and disinfection of bathtubs, if used for bathing or washing the animals or their stuff (CDC, 1995, 1999; Friedman et al., 1998; Ward, 2000). Moreover, all people coming in direct or indirect contact with such exotic animals should be aware of the risks and behave accordingly.

The risk to people visiting a public vivarium to get a *Salmonella* infection seems to be low according to the fact that it was not possible to isolate salmonellae from the walls (inside as well as outside) of the terrariums.

Since we found a fragmentary pattern of *Salmonella* excretion the occurrence of a regular spreading of *Salmonella* strains in the host animal pool is unlikely.

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