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Prevalence and characteristics of *Salmonella* species isolated from captive reptiles in the Czech Republic

Z. Tomastikova[1]*, S. Barazorda Romero[2], Z. Knotek[2], R. Karpiskova[1]

[1]Veterinary Research Institute, Brno, Czech Republic

[2]University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

*Corresponding author: tomastikova@vri.cz

ABSTRACT: This study was aimed at determining the prevalence and characterising the strains of *Salmonella* species in various captive reptiles in the Czech Republic. A total of 211 samples of cloacal swabs from lizards, chelonians and snakes, and 14 swabs from terraria surfaces were collected between November 2014 and July 2015. After isolation according to the reference method (EN ISO), *Salmonella* spp. isolates were characterised using serotyping and macrorestriction analysis followed by pulsed field gel electrophoresis and antimicrobial susceptibility testing. Altogether, 39 isolates were obtained from 29 (19%) reptiles and from terraria surfaces. Among the different reptilian species, *Salmonella* spp. were found in 22 (25.6%) lizards, three (17.6%) snakes and four (8%) chelonians with 31 isolates classified as *Salmonella enterica* subspecies *enterica* and eight isolates classified as *Salmonella enterica* subsp. *salamae.* In total, 14 different serotypes were detected, with the most frequent serotypes being *Salmonella* Oranienburg, *S*. Fluntern, *S*. Tennessee and *S*. Cotham. Resistance to one antimicrobial agent (ampicillin, tetracycline or streptomycin) was detected in five isolates. The results of the macrorestriction analysis within the serotype groups showed varying level of heterogeneity. This study confirms that reptiles kept as pets can be both carriers and reservoirs of *Salmonella* spp., and that they can harbour various serotypes with intermittent excretion of the bacteria in faeces. Half of the detected serotypes have been involved in human reptile-associated salmonellosis cases in the past. *S. enterica* subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5, monophasic *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– and its biphasic form (*S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5) have apparently been isolated from reptiles for the first time in this study.

Keywords: reptile-associated salmonellosis; serotyping; macrorestriction analysis; antimicrobial susceptibility; reptile species

Salmonellosis is a zoonotic disease with a worldwide distribution. Two species of the genus *Salmonella* (*S. enterica* and *S. bongori*) have been described so far. From the perspective of human disease, *S. enterica* subsp. I (*enterica*) is of primary importance. Warm-blooded animals that can harbour *Salmonella* spp. in their intestinal tracts are reservoirs of this pathogen. The other *Salmonella enterica* subspecies (subsp. II, IIIa, IIIb, IV and VI) and *S. bongori* are mainly found in cold-blooded animals and in the environment (Eng et al. 2015). Salmonellosis in humans is typically transmitted via the alimentary route. Another possible route of transmission is contact with infected animals. In addition, pets, including reptiles that are often kept in direct contact with humans, may also constitute a risk group (De Freitas Neto et al. 2010).

Reptiles are known as reservoirs of *Salmonella*. Early studiy reported that up to 90% of these animals may carry this pathogen in their digestive tract

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(Woodward et al. 1997). The range of detected serotypes is wide. Besides common causative agents of human salmonellosis, such as *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S*. Enteritidis) and *Salmonella enterica* subsp. *enterica* serotype Typhimurium (De Sa and Solari 2001; Gay et al. 2014), reptiles may be carriers of a variety of rare serotypes, and one animal can harbour a mixture of serotypes simultaneously. The pathogen is usually shed intermittently in faeces (Chiodini and Sundberg 1981). Clinical manifestations of salmonellosis in reptiles are rare and include, e.g., enteritis, pneumonia, nephritis or oophoritis (Onderka and Finlayson 1985).

All *Salmonella* spp. serotypes have the potential to cause disease in humans, and development of the disease depends on the virulence of the particular serotype (Sarwari et al. 2001). Infection can be contracted by direct contact with reptiles or indirectly from an environment contaminated by the faeces of infected animals. Cases of reptile-associated salmonellosis (RAS) have been observed in humans (e.g., Bertrand et al. 2008; Pees et al. 2013). RAS cases have been reported to be associated with young age and a higher rate of hospitalisation (Murphy and Oshin 2015). The growing popularity of keeping reptiles as pets may be contributing to an increase in the number of such cases (Piasecki et al. 2014).

In this study, we hypothesised that captive reptiles in the Czech Republic may serve as reservoirs of less frequent *Salmonella* spp. serotypes for humans. The prevalence, clinical symptoms and frequency of excretion in reptiles remain unclear.

The first aim of this study was to evaluate *Salmonella* spp. prevalence and serotype diversity in captive reptiles in the Czech Republic and to undertake characterisation of the isolates obtained using selected typing methods (serotyping, macrorestriction analysis, antimicrobial susceptibility testing). The second aim was to compare the results of this study with available literature sources regarding the occurrence of the detected serotypes in reptiles and humans, and especially their role in RAS cases.

Studies dealing with *Salmonella* spp. detection and characterisation in captive reptiles have been carried out in various countries. Some recent surveys have been carried out in Europe (e.g., Ebani et al. 2005; Wikstrom et al. 2014) and to a limited extent also in the Czech Republic (Barazorda Romero et al. 2015). However, in the Czech Republic, no detailed studies have been undertaken to date.

MATERIAL AND METHODS

Animals and sample collection. The collection of samples was carried out from November 2014 to July 2015. Within this period, 211 samples of cloacal swabs from 153 reptiles and 14 swabs from terraria surfaces were analysed. The reptiles originated from 42 sources (breeders No. 1–42). The study included 69 reptiles intended for educational purposes at the Avian and Exotic Animal Clinic at the University of Veterinary and Pharmaceutical Sciences, Brno (breeder No. 1), 52 reptiles kept by the University students (breeders No. 2–12) and 32 patients of the Clinic (breeders No. 13–42).

Anamnestic data were collected for each animal (country of origin, age, type of housing, feed, presence of signs of gastrointestinal disease, handlers and previous antibiotic treatment, if received).

According to diet, the reptiles were divided into carnivorous/insectivorous, omnivorous or herbivorous. They were further divided according to their medical histories into a group of animals with clinical signs of gastrointestinal disease (diarrhoea, lethargy, anorexia, inappetence, malnutrition, flatulence) or without clinical signs.

Cloacal swabs were collected from each animal. After collection, the swabs were placed in Amies transport medium (Copan, Italy) and examined immediately. Most reptiles that were positive for *Salmonella* were sampled repeatedly. Swabs were also taken from terraria surfaces where these animals were kept. Animals that tested negative, but shared the same terrarium with animals that were positive for *Salmonella* or originated from the same source (the same breeder), were also examined repeatedly.

Isolation and serotyping of *Salmonella* spp. The isolation of *Salmonella* spp. was carried out in agreement with the EN ISO 6579 (2002) guideline with some modifications. The swabs were removed from transport medium, transferred into buffered peptone water (Oxoid, United Kingdom), and incubated at 37 °C for 24 h under aerobic conditions. After selective enrichment in Muller-Kauffmann tetrathionate novobiocin broth (Oxoid, United Kingdom), the culture was streaked onto plates of xylose lysine deoxycholate agar (Oxoid, United Kingdom) and Rambach agar (Merck, Germany). Confirmation of suspected colonies was performed serologically. Serotyping of the isolates was carried out with the slide agglutination method using commercial antisera (BioRad, France and Denka Seiken, Japan). The

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identification of serotypes was carried out according to the White-Kauffmann-Le Minor scheme.

Antimicrobial susceptibility testing. The isolates were tested for susceptibility to 15 antimicrobial agents with the disc diffusion method using Mueller-Hinton agar (Oxoid, UK) in compliance with the Clinical and Laboratory Standards Institute guidelines (CLSI 2016). The antibiotic discs (Oxoid, UK) contained the following antibiotics: ampicillin (10 µg), amoxicillin-clavulanic acid (30 µg), cefotaxime (30 µg), meropenem (10 µg), chloramphenicol (30 µg), streptomycin (10 µg), kanamycin (30 µg), gentamicin (10 µg), sulfonamides (300 µg), sulfamethoxazole-trimethoprim (25 µg), trimethoprim (5 µg), tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and aztreonam (30 µg). The results were evaluated according to the CLSI standards (CLSI 2016), and all isolates were classified as sensitive, intermediate or resistant. The E-test was performed in resistant strains using M.I.C.Evaluator™ strips (Oxoid, United Kingdom) to determine the minimum inhibitory concentration (MIC) of the respective antibiotics (tetracycline, ampicillin). The results of the E-tests were evaluated according to CLSI standards (CLSI 2016).

Macrorestriction analysis. Macrorestriction analysis followed by pulsed field gel electrophoresis was carried out according to the PulseNet Europe protocol for strains of the *Enterobacteriaceae* family using the *Xba*I restriction endonuclease (Takara Bio, Japan) and a CHEF-DRIII instrument (BioRad, USA). The obtained results were analysed in Bionumerics software (version 5.1, Applied Maths, Belgium). The parameters of the analysis included coefficient Dice, dendrogram type UPGMA, optimization 1.1% and band position tolerance 1.0%.

Statistical analysis. The prevalence of *Salmonella* spp. in reptiles in relation to their dietary specialisation and to the presence or absence of clinical signs of gastrointestinal disease was statistically evaluated using GraphPad Prism software (version 5.04, GraphPad, USA).

RESULTS

Isolation and serotyping of *Salmonella* spp.

Salmonella spp. were isolated from 29 (18.95%) reptiles; a total of 36 isolates were obtained. Among the different reptilian species, *Salmonella* was found in 22 lizards, three snakes and four chelonians. The remaining three isolates were found in swabs collected from terraria surfaces. We identified a total of 14 serotypes. The following serotypes were found most frequently: *S. enterica* subsp. *enterica* serotype Oranienburg (*S.* Oranienburg) (11 isolates), *S. enterica* subsp. *enterica* serotype Fluntern (*S.* Fluntern) (six isolates), *S. enterica* subsp. *enterica* serotype Tennessee (*S.* Tennessee) (four isolates) and *S. enterica* subsp. *enterica* serotype Cotham (*S.* Cotham) (four isolates; Table 1). Thirty-one isolates belonged to subsp. I (*enterica*) and eight isolates were classified in subsp. II (*salamae*). The most common serotype within subsp. I was *S.* Oranienburg, and the most common within subsp. II was *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:–. From the terraria surfaces, *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:–, *S.* Fluntern and *S. enterica* subsp. *salamae* serotype O:30;H:l,z28;H:z6 were isolated, each from a different terrarium.

Repeated examinations

The examinations were repeated for the following reasons: seven reptiles were positive for *Salmonella* in the first collection, 12 reptiles were negative for *Salmonella* but kept together in a terrarium with positive animals and nine reptiles were negative for *Salmonella* but kept by a breeder with positive animals in another terrarium. Testing was carried out one to three times, based on the availability of the animal for the examination and the owner's approval. Repeated examinations were carried out in 28 animals (19 *Eublepharis macularius*, five *Trachemys scripta elegans*, one *Testudo marginata*, *Lampropeltis triangulum*, *Geochelone pardalis* and *Pantherophis guttatus*) together with three swabs from terraria surfaces.

Antimicrobial susceptibility testing

Most of the isolates (34/39) exhibited sensitivity or intermediate sensitivity to all tested antibiotics. Resistance to ampicillin was detected in one isolate (*S.* Tennessee from a *Eublepharis macularius,* breeder No. 1). One isolate was resistant to tetracycline (*S.* Cotham from a *Pogona vitticeps*, breeder

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Table 1. Overview of the *Salmonella* spp. isolated from the investigated reptile species and their serotypes

Reptile species	No. of animals	No. of samples	No. of positive animals	No. of *S.* isolates	Pos. all	Pos. given	Serotype (No. of isolates)	Full scheme	Source (No. of breeder)
Order Squamata Suborder Sauria (lizards)	86	127	22	28	75.9	25.6			
Physignathus cocincinus	5	5	0	0	0	0			
Pogona vitticeps	13	13	5	5	17.2	5.8	*S. enterica* subsp. *enterica* serotype Cotham (3) *S. enterica* subsp. *enterica* serotype Tennessee (1) *S. enterica* subsp. *enterica* serotype Ago (1)	[I O:28;H:i;H:1,5] [I O:6,7,14;H:z29;H:[1,2,7]] [I O:30;H:z38;H:-]	2, 8 24 37
Eublepharis macularius	24	65*	14	20	48.3	16.3	*S. enterica* subsp. *enterica* serotype Oranienburg (8) *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– (2) *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5 (1) *S. enterica* subsp. *enterica* serotype Fluntern (5) *S. enterica* subsp. *enterica* serotype Tennessee (3) *S. enterica* subsp. *salamae* serotype O:4,12;H:b;H:e,n,x (1)	[I O:6,7,14;H:m,t;H:[z57]] [II O:1,40;H:g,[m],[s],t;H:[1,5]] [II O:1,40;H:g,[m],[s],t;H:[1,5]] [I O:6,14,18;H:b;H:1,5] [I O:6,7,14;H:z29;H:[1,2,7]] [II O:1,4,[5],12,[27];H:b;H:[e,n,x]]	1 1 1 1 1, 12 12
Iguana iguana	19	19	0	0	0	0			
Basiliscus vittatus	4	4	2	2	6.9	2.3	*S. enterica* subsp. *enterica* serotype Oranienburg (2)	[I O:6,7,14;H:m,t;H:[z57]]	8
Pachydactylus bibroni	1	1	0	0	0	0			
Chamaeleo calyptratus	15	15	0	0	0	0			
Rhacodactylus ciliatus	4	4	1	1	3.4	1.2	*S. enterica* subsp. *enterica* serotype Oranienburg (1)	[I O:6,7,14;H:m,t;H:[z57]]	8
Uromastyx acanthinura	1	1	0	0	0	0			
Order Testudines (chelonians)	50	64	4	4	13.8	8			
Testudo horsfieldi	7	7	0	0	0	0			
Trachemys scripta elegans	28	38*	2	2	6.9	4	*S. enterica* subsp. *enterica* serotype Enteritidis (1) *S. enterica* subsp. *enterica* serotype Agona (1)	[I O:1,9,12;H:g,m;H:–] [I O:1,4,[5],12;H:f,g,s;H:[1,2]]	1 27

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Table 1 continued

Reptile species	No. of animals	No. of samples	No. of positive animals	No. of *S.* isolates	Pos. all	Pos. given	Serotype (No. of isolates)	Full scheme	Source (No. of breeder)
Chrysemys picta	1	1	0	0	0	0			
Geochelone pardalis	3	5*	0	0	0	0			
Testudo marginata	2	4*	1	1	3.4	2	*S. enterica* subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5 (1)	[II O:1,13,23;H:z29;H:1,5]	1
Testudo hermanni	5	5	1	1	3.4	2	*S. enterica* subsp. *enterica* serotype Cotham (1)	[I O:28;H:i;H:1,5]	2
Trachemys scripta scripta	2	2	0	0	0	0			
Graptemys pseudogeographica	1	1	0	0	0	0			
Chelus fimbriatus	1	1	0	0	0	0			
Order Squamata Suborder Serpentes (snakes)	17	20	3	4	10.3	17.6			
Python regius	2	2	0	0	0	0			
Pantherophis guttatus	7	9*	2	2	6.9	11.8	*S. enterica* subsp. *enterica* serotype Othmarschen (1) *S. enterica* subsp. *salamae* serotype O:4,12;H:b;H:e,n,x (1)	[I O:6,7,14;H:g,m,[t];H:–] [II O:1,4,[5],12,[27];H:b;H:[e,n,x]]	1 12
Corallus hortulanus	2	2	0	0	0	0			
Bitis gabonica	2	2	0	0	0	0			
Lampropeltis triangulum	4	5*	1	2	3.4	5.9	*S. enterica* subsp. *enterica* serotype Newport (2)	[I O:6,8,20;H:e,h;H:1,2]	4

Full scheme = Full serological scheme of the serotypes according to the White-Kauffmann-Le Minor scheme, Pos. all = positivity from all positive animals (%), Pos. given = positivity from a given reptile group -lizards, chelonians or snakes (%)

*Including repeated examinations

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No. 2) and three isolates were resistant to streptomycin (two isolates of *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– from an *Eublepharis macularius*, breeder No. 1 and one isolate of *S.* Agona from a *Trachemys scripta elegans*, breeder No. 27). Resistance was confirmed by determination of the MIC in strains resistant to tetracycline (MIC: 16 µg/ml) and ampicillin (MIC: 32 µg/ml). The MIC for streptomycin was not determined.

Macrorestriction analysis

Macrorestriction profiles (pulsed profile) were determined in all but one isolate (Figure 1). The pulsed profile of the *S. enterica* subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5 isolate (isolated from *Testudo marginata*, breeder No. 1) was not obtained due to its resistance to the used restriction enzyme.

Statistical analysis

Salmonella were more frequently detected in the group of carnivorous/insectivorous reptiles (63 animals in total/15 positive animals) than in omnivorous (53 animals in total/10 positive animals) and herbivorous reptiles (37 animals in total/2 positive animals). The differences between the first group and the other two groups were not statistically significant ($P = 0.063$, χ^2-test).

More *Salmonella* isolates were isolated from reptiles without clinical signs of gastrointestinal disease (115 animals in total/24 positive animals) than from the reptiles showing clinical manifestations (38 animals in total/3 positive animals). The difference between these groups was not statistically significant ($P = 0.086$, Fisher's exact test).

Analysis of anamnestic data

Most of the reptiles were bred in the Czech Republic (150/153), with the exception of two reptiles from Slovakia (*Trachemys scripta scripta*, breeder No. 5) and one from Uganda (*Bitis gabonica*, breeder No. 20). The age of the tested animals ranged from one month (*Pogona vitticeps*, breeder No. 33) to 27 years (*Trachemys scripta elegans*, breeder No. 27). Most animals were under one year of age (28/153), followed by the age category of 1–2 years (27/153), 9–10 years (17/153) and 14–15 years (23/153). The age of 13 reptiles was not specified. Most animals were kept in aquaria or terraria in households and had no access to outdoor areas. All animals except one (*Pachydactylus bibroni*, breeder No. 8) had regular or irregular contact with humans.

DISCUSSION

The results of this study show a prevalence rate of *Salmonella* spp. of about 19% in the studied reptiles. Available literature sources have reported varying levels of prevalence, namely, 54.1% in Germany and Austria (Geue and Loschner 2002), 32.6% in Poland (Piasecki et al. 2014), 49% in Sweden (Wikstrom et al. 2014), 13% in Croatia (Lukac et al. 2015), 30.9% in Taiwan (Chen et al. 2010) and 13.6% in Italy (Bertelloni et al. 2016). Our results did not differ significantly from those obtained in other countries. Due to the intermittent shedding of *Salmonella* in reptile faeces, repeated examinations were carried out in 18.3% of the animals investigated in this study, which led to the detection of nine positive animals that would otherwise have remained undetected.

The highest *Salmonella* spp. prevalence was observed in lizards (25.6%), followed by snakes (17.6%) and chelonians (8%; Table 1). The prevalence rates in these groups of reptiles are consistent with the findings of other authors (De Sa and Solari 2001; Piasecki et al. 2014; Lukac et al. 2015). The increased diagnostic yield could be affected by the number of lizards as they constituted the largest group in this study (86/153), and many of them were examined repeatedly. The lowest positivity for *Salmonella* spp. in this study was found in chelonians, which is in agreement with the results of other authors (Chen et al. 2010; Lukac et al. 2015). The feed composition could contribute to the low prevalence observed. Due to the fact that most of the chelonians were herbivorous and omnivorous it can be assumed that the majority of them were given feeds containing low levels of *Salmonella* strains (such as feeds derived from plants, vegetables or granulated commercial mixtures).

Animal-based products are considered to be the main source of *Salmonella* spp. (De Freitas Neto et al. 2010); thus, a higher prevalence of this pathogen can be anticipated in reptiles fed with this type

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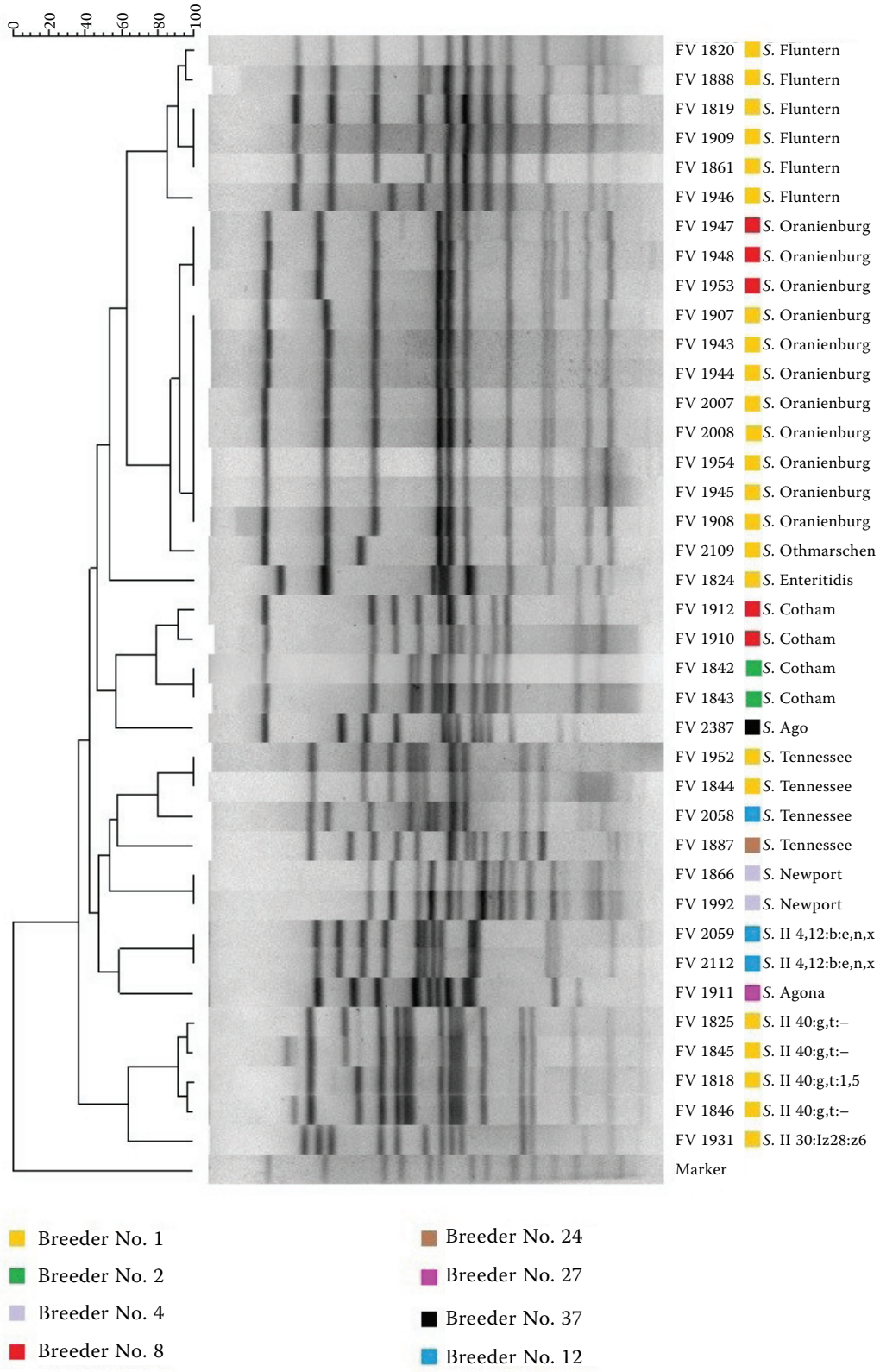

Figure 1. Pulsed profiles of 38 *Salmonella* spp. isolates. The serotypes are abbreviated as follows, *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– (*S.* II 40:g,t:–), *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5 (S. II 40:g,t:1,5), *S. enterica* subsp. *salamae* serotype O:4,12;H:b;H:e,n,x (*S.* II 4,12:b:e,n,x), *S. enterica* subsp. *salamae* serotype O:30:H:l,z28:H:z6 (*S.* II 30:l,z28:z6). *S.* Braenderup H9812 was used as a molecular weight marker

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of feed. This presumption was not confirmed statistically, but our results may have been affected by the low level of contamination in animal-based products fed to the animals, or the unequal animal numbers in the compared groups.

The higher *Salmonella* spp. prevalence observed in animals without clinical signs of gastrointestinal disease (although not statistically significant) is in agreement with the predominance of asymptomatic salmonellosis in reptiles; thus, it is more accurate to refer to colonisation/infestation rather than infection (Baumler et al. 1998).

The contact of reptiles with environments contaminated with the faeces of feral animals (e.g., birds and rodents) or direct contact with these animals may increase the risk of *Salmonella* transmission. Most animals investigated in this study (150/153) were kept in permanent habitats without access to outdoor areas so they had no contact with free-living wild animals. Three of the reptiles did have access to an outdoor area (a garden paddock in the summer), but no *Salmonella* was retrieved from any of them.

Furthermore, the unequal age distributions of the groups of reptiles investigated did not allow us to make any assessments of the correlations between age and *Salmonella* prevalence.

Salmonella transmission from reptiles to humans is dependent on contact with an infected animal. In most cases in this study, contact was reported between the handlers (breeders, family members, caregivers) and reptiles (152/153). *Salmonella* can also contaminate the surfaces of terraria (Wikstrom et al. 2014) as was confirmed by the three isolates found in swabs collected from terraria in this study. In two cases, the serotype detected in the swab from the terrarium surface was the same as the serotype isolated from the reptile bred in this terrarium (*S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– and *S.* Fluntern). In the third case, the serotype was different (*S. enterica* subsp. *salamae* serotype O:30;H:l,z28;H:z6). This phenomenon reflects the ability of reptiles to carry different serotypes simultaneously (Chiodini and Sundberg 1981).

The contribution of all the 14 serotypes detected in this study to RAS cases and human salmonellosis cases not linked to contact with reptiles and their occurrence in specific reptile species in this study and other studies is shown in Table 2.

With respect to epidemiological significance, *S.* Enteritidis is the serotype which is most frequently responsible for human salmonellosis in the Czech Republic (Myskova and Karpiskova 2014). The source of the *S.* Enteritidis in this study could have been raw chicken meat fed to the infected terrapin.

S. enterica subsp. *enterica* serotype Agona (*S.* Agona) is one of the serotypes commonly responsible for salmonellosis in humans (EFSA and ECDC 2015). In this study, the terrapin from which the *S.* Agona was isolated was fed with beef and chicken meat (data on heat treatment is unavailable), which might have caused the colonisation.

One suspected RAS case was described in this study and was caused by *S. enterica* subsp. *enterica* serotype Ago (*S.* Ago) recovered from a bearded dragon; the owner suffered from salmonellosis caused by the same serotype. Although the human strain was not available for detailed analysis, it is highly probable that the bearded dragon was the source of this serotype.

S. enterica subsp. *salamae* serotype O:30; :l,z28; H:z6 was obtained from a swab taken from a terrarium inhabited by reptiles of the *Eublepharis macularius* species. Whereas no *Salmonella* was isolated from the animals occupying the terrarium, the other animals from the same source (breeder No. 1) carried a wide variety of *Salmonella* serotypes (Table 1).

There are no information in the available literature on the occurrence of the remaining isolated serotypes: *S. enterica* subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5, monophasic *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– and its biphasic form (*S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5) in reptiles or in other sources. Thus, this is apparently the first report of the isolation of these serotypes from reptiles.

The obtained data indicate that one half of the isolated serotypes (7/14) has been involved in suspected or confirmed RAS cases in the past. Some of them were found sporadically (e.g., *S.* Fluntern), while others were involved more frequently (e.g., *S.* Oranienburg) and one RAS outbreak also occurred (*S.* Cotham). If these serotypes are causative agents of human disease, it is always appropriate to consider contact with reptiles as a possible source of infection. No RAS cases were reported for *S. enterica* subsp. *salamae* serotype O:4,12;H:b;H:e,n,x and *S.* Agona, but their isolation from reptiles as well as from humans indicates that reptiles can be a possible source of these serotypes. However, it must be taken into consideration that the results are to a large extent influenced by the different systems of data collection in various countries and, therefore,

Table 2. Comparison of the occurrence of serotypes detected in reptiles in this study with other studies, the occurrence of these serotypes in reptile-associated salmonellosis (RAS) cases and in salmonellosis cases not linked to contact with reptiles

Detected serotype	Reptile species (this study)	Reptile species (other studies)	Confirmed/*suspected RAS cases	Human cases not linked to contact with reptiles
S. enterica subsp. *enterica* serotype Ago	*Pogona vitticeps*	*Chamaeleo calyptratus* (Barazorda Romero et al. 2015) unspecified reptiles (Chen et al. 2010)	*this study Bertrand et al. 2008	CDC 2016 Dedicova D: unpublished results (three cases)
S. enterica subsp. *enterica* serotype Agona	*Trachemys scripta elegans*	*Chamaeleo verrucosus* (Ebani et al. 2005) *Ameiva ameiva* (Everard et al. 1979) *Opheodrys vernalis* (Chambers and Hulse 2006)	Data not known	Dedicova D: unpublished results (29 cases) Zaidi et al. 2006 CDC 2008, CDC 2011
S. enterica subsp. *enterica* serotype Cotham	*Pogona vitticeps* *Testudo hermanni*	*Pogona vitticeps* (CDC 2014) *Pogona vitticeps* (Pees et al. 2013)	Pees et al. 2013 CDC 2014	Dedicova D: unpublished results (six cases)
S. enterica subsp. *enterica* serotype Enteritidis	*Trachemys scripta elegans*	*Cnemidophorus lemniscatus*, *Iguana iguana* (De Sa and Solari 2001) *Uromastyx* spp. (Munch et al. 2012) *Elaphe vulpina, Python regius*, *Morelia spilota* (Geue and Loschner 2002)	Stam et al. 2003 Bertrand et. al. 2008	Myskova and Karpiskova 2014
S. enterica subsp. *enterica* serotype Fluntern	*Eublepharis macularius*	*Eublepharis macularius* (Ebani et al. 2005) *Iguana iguana* (Woodward et al. 1997) unspecified reptiles (Wikstrom et al. 2014)	*Makin et al. 1996	CDC 2016 Dedicova D: unpublished results (one case)
S. enterica subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5	*Testudo marginata*	Data not known	Data not known	Data not known
S. enterica subsp. *salamae* serotype O:4,12;H:b;H:e,n,x	*Eublepharis macularius* *Pantherophis guttatus*	*Testudo graeca* (Lapage et al. 1966) unspecified reptile (Aleksic et al. 1996)	Data not known	Schrire et al. 1987 Aleksic et al. 1996
S. enterica subsp. *salamae* serotype O:40;H:g,t;H:-	*Eublepharis macularius*	Data not known	Data not known	Data not known
S. enterica subsp. *salamae* serotype O:40;H:g,t;H:1,5	*Eublepharis macularius*	Data not known	Data not known	Data not known

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Table 2 continued

Detected serotype	Reptile species (this study)	Reptile species (other studies)	Confirmed/*suspected RAS cases	Human cases not linked to contact with reptiles
S. enterica subsp. *enterica* serotype Newport	*Lampropeltis triangulum*	*Trachemys scripta elegans* (De Sa and Solari 2001) *Iguana iguana* (Sylvester et al. 2014) *Lampropeltis* spp., *Elaphe guttata, Morelia viridis, Boa constrictor* (Geue and Loschner 2002) *Elaphe alleghaniensis, Thamnophis sirtalis.* (Chambers and Hulse 2006) *Elaphe* spp., *Lampropeltis* spp. (Nakadai et al. 2005) unspecified reptile (Wikstrom et al. 2014)	Pees et al. 2013	CDC 2016 Angelo et al. 2015 Dedicova D: unpublished results (31 cases)
S. enterica subsp. *enterica* serotype Oranienburg	*Eublepharis macularius* *Rhacodactylus ciliatus* *Basiliscus vittatus*	*Tupinambis teguixin* (Gopee et al. 2000) *Iguana iguana* (Sylvester et al. 2014) *Elaphe obsoleta, Elaphe guttata, Pituophis melanoleucus* (Pfleger et al. 2003) unspecified reptiles (Bauwens et al. 2006)	Aiken et al. 2010 Pees et al. 2013	Dedicova D: unpublished results (25 cases) Yang et al. 2014 Katsuno et al. 2003 Landry et al. 2007 Werber et al. 2005
S. enterica subsp. *enterica* serotype Othmarschen	*Pantherophis guttatus*	*Physignathus lesueurii* (Ebani et al. 2005) *Furcifer pardalis* (Geue and Löschner 2002) unspecified reptile (Chen et al. 2010)	Data not known	CDC 2016 Kim et al. 2007
S. enterica subsp. *enterica* serotype Tennessee	*Eublepharis macularius* *Pogona vitticeps*	unspecified reptile (Wikstrom et al. 2014) *Pogona vitticeps* (Pees et al. 2013)	*Pees et al. 2013 Weiss et al. 2011	Dedicova D: unpublished results (six cases)
S. enterica subsp. *salamae* serotype O:30;H:l,z28;H:z6	*Eublepharis macularius*[#]	*Eublepharis macularius* (Pedersen et al. 2009) *Basiliscus plumifrons* (Pfleger et al. 2003) unspecified reptiles (Bauwens et al. 2006)	Data not known	CDC 2016

[#]Isolate was obtained from the terrarium where the reptile was bred

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it is possible that many cases may have not been recorded. Regarding serotypes frequently involved in human disease (*S.* Enteritidis, *S.* Agona), RAS cases may remain undetected when the infection is traced to its source if attention is focused only on food of animal origin.

Host specificity between a particular reptile species and *Salmonella* serotypes has not yet been established (Briones et al. 2004). This could be due to the high number of reptile species bred in captivity together with the large variety of serotypes isolated from them.

Antimicrobial susceptibility testing revealed a low number of resistant strains. No resistance was detected in *Salmonella* isolates obtained from terraria surfaces. This could be explained by the fact that the reptiles in this study have not been exposed to high levels of antimicrobial therapeutics, which could result in a low prevalence of antibiotic-resistant strains. Courses of treatment with unspecified antibiotics were recorded in only two animals. However, the strains that were isolated from them were sensitive to all tested antibiotics. Another factor may be the low prevalence of resistant strains in the reptile feed. While a high proportion of sensitive strains was also reported by other authors (Geue and Loschner 2002; Gay et al. 2014; Sylvester et al. 2014), there are studies showing an increased prevalence of resistant and multi-drug-resistant strains in reptiles (Ebani et al. 2005; Chen et al. 2010; Bertelloni et al. 2016). The highest level of resistance in this study was observed for streptomycin (7.7%). Resistance to this antibiotic in *Salmonella* strains derived from reptiles was noted by a number of other authors (Seepersadsingh and Adesiyun 2003; Chen et al. 2010; Barazorda Romero et al. 2015; Bertelloni et al. 2016). A low prevalence of tetracycline resistance was detected in this study (2.6%), which is in agreement with published results (Ebani et al. 2005; Sylvester et al. 2014). However, some authors have documented a relatively high prevalence of resistant strains (Gopee et al. 2000; Giacopello et al. 2012). The number of ampicillin-resistant strains in this study was low (2.6%), which is consistent with the results of Gopee et al. (2000), although data showing an increased occurrence of resistant strains have also been published (Ebani et al. 2005; Giacopello et al. 2012).

The results of the macrorestriction analysis showed that *Salmonella* spp. strains formed clusters which were determined by their serotypes (Figure 1). Varying degrees of genetic diversity were observed within serotype groups based on fragment differences in the pulsed profiles. A high level of heterogeneity was observed for *S.* Tennessee strains (60% similarity), which exhibited three different pulsed profiles, each associated with different breeder. A higher similarity could be observed for *S.* Cotham strains (80% similarity) obtained from two breeders and for *S.* Fluntern strains (85.1% similarity) obtained from one breeder. On the other hand, *S.* Oranienburg strains displayed the lowest heterogeneity with two highly similar pulsed profiles (92.4% similarity) despite the strains were isolated from two breeders. Biphasic *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5 exhibited almost the same pulsed profile (96.5% similarity) as its monophasic form (*S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:–). Pulsed profiles that were indistinguishable from each other occurring within a specific serotype group belonged in all cases to isolates obtained from the same breeder.

In conclusion, the results of this study show that the prevalence of *Salmonella* spp. in captive reptiles in the Czech Republic is 19% and is comparable to the prevalence found in other countries. A variety of serotypes was detected, 50% of which have been previously described to be involved in RAS cases. The role of reptiles bred in the Czech Republic in acting as carriers and reservoirs of *Salmonella* spp., a role which is associated with their intermittent excretion of the bacteria, has been confirmed. *S. enterica* subsp. *salamae* serotype O:1,13,23;H:z29;H:1,5, monophasic *S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:– and its biphasic form (*S. enterica* subsp. *salamae* serotype O:40;H:g,t;H:1,5) have apparently been isolated from reptiles for the first time. Even though reptiles are a minor source of *Salmonella* spp. for human disease, the steady rise in the popularity of keeping reptiles as pets may lead to an increase in RAS cases. Accordingly, reptile-associated salmonellosis remains a topical issue that deserves the attention of professionals, breeders and the general public.

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