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Pet Snakes as a Reservoir for *Salmonella enterica* subsp. *diarizonae* (Serogroup IIIb): a Prospective Study

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Reptile-associated *Salmonella* infections are an increasing problem for humans. We have prospectively screened two breeding groups of 16 pet snakes for colonization with *Salmonella* species. Various serovars of *S. enterica* subsp. *diarizonae* were found in 81% of the snakes. To avoid transmission, strict hygienic precautions should be applied when reptiles are handled.

Exotic reptiles have enjoyed increasing popularity as pets during the last few years. This increase in popularity has led to an increase in the number of reptile-associated salmonella infections which occur every year in the United States (presently estimated at 93,000) (4; J. Mermin, L. Hutwagner, D. Vugia, P. Kirley, J. Bender, J. Koehler, T. McGivern, R. Marcus, F. Angulo, and the FoodNet Working Group, 36th Annu. Meet. Infect. Dis. Soc. Am., 1998 [http://www.cdc.gov/foodnet/pub/idsa/1998/mermin_j.htm]). Children under the age of 10 years and immunocompromised people seem to be especially prone to infections with reptile-associated S*almonella* spp. and often experience severe clinical courses, including fatalities due to septicemia and meningitis (2, 4). For this reason, in 1975, the U.S. government banned the trade of turtles with carapaces smaller than 4 in. from front to back. This measure led to a 77% reduction in turtle-associated salmonellosis (5). However, due to the increasing popularity of exotic reptiles as pets, the incidence of reptile-associated salmonellosis is still increasing. For example, the isolation of *Salmonella enterica* subsp. *houtenae* serovar Marina from humans increased from 2 cases in 1989 to 47 cases in 1998, and the number of *S. enterica* serovar Poona cases increased from 199 in 1989 to 341 in 1998 (3). The main serotypes isolated from patients with reptile-associated salmonellosis include *S. enterica* subsp. *diarizonae* (IIIb) serovars, the *S. enterica* subsp. *houtenae* (IV) serovars Chameleon and Marina, and the *S. enterica* subsp. *enterica* (I) serovars Java, Stanley, and Poona (1). Very frequently, turtles and iguanas, which are often allowed to roam freely in keepers' homes and serve as pets for children, have been identified as sources of human infection. Moreover, corn snakes, pythons, and boas have also been identified as sources of *Salmonella* infections (1, 4, 5). Dried rattlesnake preparations used in folk medicine have led to severe *Salmonella* infections with fatalities (7, 11).

Although various reptiles, such as iguanas, turtles, and snakes, have been described retrospectively as sources of human salmonellosis (5, 8, 11), it is unclear how frequently certain species of reptiles are colonized by *Salmonella* species. Therefore, we have prospectively screened fecal samples from two breeding groups of snakes for the presence of *Salmonella*.

To assess the prevalence of *Salmonella*, fresh stool samples were collected from 10 rhinoceros-horned vipers (*Bitis nasicornis*, Shaw 1802) and 6 eyelash vipers (*Bothriechis schlegelii*, Berthold 1846) with a swap and immediately processed according to a standard protocol for the detection of enteric pathogens. This protocol follows the recommendations of the German Society for Hygiene and Microbiology and includes the use of nonselective blood agar, MacConkey agar, a Selenite enrichment broth, and the *Salmonella*-selective agars salmonella-shigella agar and xylose-lysine-deoxycholate agar. All agar plates were incubated for 48 h at 37°C. After 24 and 48 h, the agar plates were inspected for the growth of *Salmonella*. The enrichment broth was incubated for 18 h at 37°C and then transferred to salmonella-shigella agar and xylose-lysine-deoxycholate agar, which were incubated at 37°C for another 24 h. Identification was achieved by using API E identification systems (BioMerieux, Lyon, France). Serotyping was performed by direct agglutination on microslides (6) at the National Reference Centre for Enteric Pathogens (Institut für Hygiene und Umwelt, Hamburg, Germany). Generation of the antisera was performed by immunization of New Zealand rabbits according to World Health Organization guidelines (9). For pulsed-field gel electrophoresis (PFGE), genomic DNA was digested with *Xba*I. The resulting fragments were resolved in a 1% agarose gel with a CHEF DR III system (Bio-Rad Laboratories, Richmond, Calif.). Ramped pulse times ranged from 5 to 35 s over 32 h at 6 V and 14°C. Gels were stained with ethidium bromide, destained in distilled water, and photographed under UV illumination. PFGE profiles were analyzed according to established criteria outlined by Tenover and coworkers (10).

Salmonella strains of serogroup IIIb were isolated from the fecal samples of all *B. nasicornis* snakes and from three of the six *B. schlegelii* snakes. An overview of the serological typing results is given in Table 1. Altogether, 12 serovars of *Salmonella* serogroup IIIb were isolated, with 48:i:z being the only

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TABLE 1. Results of serotyping of *Salmonella* serogroup IIIb strains isolated from 13 snakes[a]

Snake	Age (yr)	O antigen	H1 antigen	H2 antigen
B. nasicornis				
1	3	50	r	z53
2	3	17	l,v	z35
		48	i	z
3	4	48	i	z
4	0.5	59	z10	z53
5	0.5	61	i	z
		61	i	z
6	5	18	l,v	z
7	5	50	r	z
8	3	38	k	z
		50	z52	z35
9	3	61	c	z35
		47	z52	e,n,x,z15
		47	z52	e,n,x,z15
10	3	48	i	z
B. schlegelii				
1	3	48	i	z
2	2	50	k	z
3	2	48	i	z

[a] Longitudinal samples were examined for *B. nasicornis* snakes 2, 5, 8, and 9. Follow-up samples were collected after 1 month (snake 2), after 11 months (snake 5), after 15 months (snake 8), and after 11 and 22 months (snake 9).

serovar to be isolated from different snakes. Follow-up samples were obtained from four animals over a period of 1 to 22 months. All of these snakes were permanently colonized by *Salmonella* serogroup IIIb strains. Serotyping revealed identical strains in *B. nasicornis* snake 5 over the entire follow-up period (Table 1). The clonal identity of these strains was demonstrated by PFGE (Fig. 1). The other three snakes showed changes of colonizing *Salmonella* serovars during follow-up (Table 1). *B. nasicornis* snake 2 showed a change from *Salmonella* serotype 17:l,v:z35 to *Salmonella* serotype 48:i:z within 1 month. This strain was identical to that isolated from *B. nasicornis* snake 3, as shown by PFGE (Fig. 1). Identical clones of *Salmonella* serotype 48:i:z, which were different from those isolated from the *B. nasicornis* snakes, were also found in two *B. schlegelii* snakes (Fig. 1). This result may be due to the fact that the two *B. nasicornis* snakes, as well as the two *B. schlegelii* snakes, with this serovar had been kept together prior to this study for breeding purposes. All other animals examined in this study were always kept alone in separate cages. PFGE analysis of the *Salmonella* serotype 48:i:z strain isolated from *B. nasicornis* snake 10 showed a different banding pattern.

Antibiotic testing was performed by the disk diffusion method and revealed the susceptibilities of all *Salmonella* isolates to penicillins, cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones.

It is unclear whether the snakes acquired *Salmonella* in utero, perinatally, by ingestion of contaminated prey, or by contact with the contaminated feces of other reptiles. In our study group of 16 pet snakes, *Salmonella* serogroup IIIb exhibited a high prevalence (81%). The numbers of examined snakes are too small for a statistical analysis, but 100% of the *B. nasicornis* snakes were colonized, while *Salmonella* serogroup IIIb was detectable in only 50% of the *B. schlegelii* snakes. This finding may be due to the different behaviors of the snake species. *B. nasicornis* is a ground-dwelling snake, whereas *B. schlegelii* inhabits bushes and trees. Especially when these snakes are kept in terrariums, *B. nasicornis* has an ele-

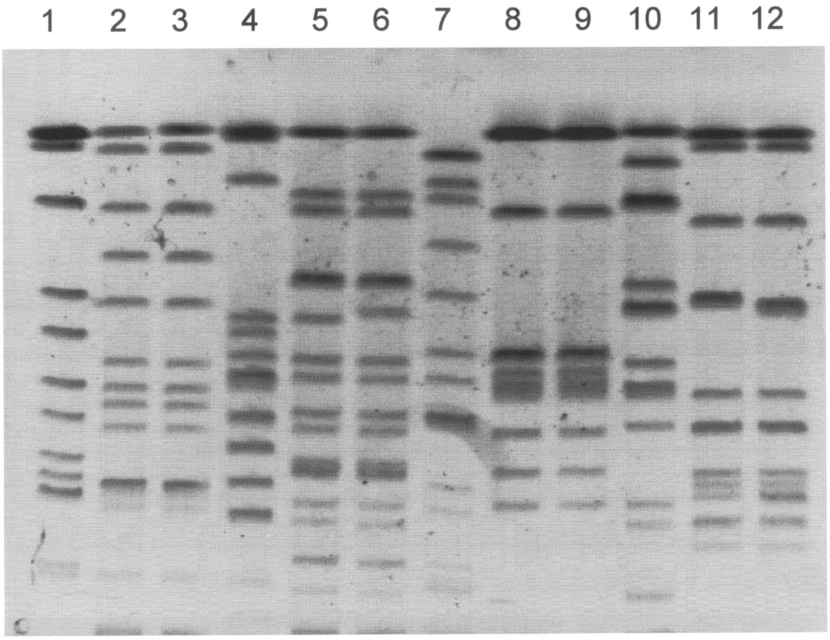

FIG. 1. PFGE of 12 *Salmonella* serogroup IIIb isolates. Lane 1, isolate of serovar 50:r:z53 from *B. nasicornis* snake 1; lanes 2 to 6, isolates of serovar 48:i:z from *B. nasicornis* snakes 2 and 3 (lanes 2 and 3, respectively), from *B. nasicornis* snake 10 (lane 4), and from *B. schlegelii* snakes 1 and 3 (lanes 5 and 6, respectively); lane 7, isolate of serovar 59:z10:z53 from *B. nasicornis* snake 4; lanes 8 and 9, isolates of serovar 61:i:z from *B. nasicornis* snake 5; lanes 10 to 12, isolates of serovars 61:c:z35 (lane 10) and 47:z52:e,n,x,z15 (lanes 11 and 12) from *B. nasicornis* snake 9.

vated risk of coming into contact with contaminated feces. The observation that those snakes that were kept together for a while exhibited identical *Salmonella* serogroup IIIb isolates supports the assumption that colonization occurs mainly by contact with contaminated feces. Moreover, the *B. nasicornis* group was fed exclusively with rodents from a supplier whose surveillance cultures for the animals were regularly negative for enteric pathogens, including *Salmonella* spp. Therefore, transmission by contaminated prey in this breeding group of snakes is highly unlikely.

Our study is the first prospective investigation of the prevalence of *Salmonella* serogroup IIIb serovars in two breeding groups of pet snakes. Our results indicate that very high percentages of snakes are colonized with S*almonella* spp. This finding obviously applies especially to ground-dwelling snakes. To prevent reptile-associated *Salmonella* infections, the Centers for Disease Control and Prevention have issued recommendations for the handling of reptiles. Besides the standard recommendations, like that for thorough hand washing with soap and water after handling reptiles or reptile cages, these recommendations stipulate that reptiles (i) should not be kept in child care centers, (ii) should be kept out of households with children younger than 1 year of age and immunocompromised persons, and (iii) should not be allowed to roam freely throughout the home or living area (4).

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