

Originally published as:

Weiss, B., Rabsch, W., Prager, R., Tietze, E., Koch, J., Mutschmann, F., Roggentin, P., Frank, C. Babies and bearded dragons: Sudden increase in reptile-associated Salmonella enterica serovar tennessee infections, Germany 2008 (2011) Vector-Borne and Zoonotic Diseases, 11 (9), pp. 1299-1301.

DOI: 10.1089/vbz.2010.0239

This is an author manuscript. The definitive version is available at: <u>http://online.liebertpub.com</u>

Babies and Bearded Dragons: Sudden Increase in Reptile-Associated *Salmonella* enterica Serovar Tennessee Infections, Germany 2008

Bettina Weiss¹, Wolfgang Rabsch², Rita Prager², Erhard Tietze², Judith Koch¹, Frank Mutschmann³, Peter Roggentin⁴, and Christina Frank^{1,5}

¹Department of Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany. ²National Reference Centre for *Salmonella* and Other Pathogens, Robert Koch Institute, Wernigerode, Germany.

³Exomed, Berlin, Germany.

⁴Department of Medical Microbiology, Institute for Hygiene and the Environment, Hamburg, Germany. ⁵Institute for Hygiene and Public Health, University of Bonn, Bonn, Germany.

Abstract

Introduction: In 2008 a marked increase in *Salmonella* enterica serovar Tennessee infections in infants occurred in Germany. In March and April 2008, eight cases were notified compared to a median of 0–1 cases in 2001–2006.

<u>Materials and Methods:</u> We carried out an investigation including a case–control study to identify the source of infection. A patient was a child < 3 years of age with *Salmonella* Tennessee isolated from stool from September 1, 2007, through December 31, 2008, identified through the national surveillance system. A control was a child with a notified rotavirus infection in the matching district, frequency matched by age group. We conducted telephone interviews on feeding, herbal infusions, and animal contact. Matched odds ratios (mOR) were calculated using exact conditional logistic regression. For *Salmonella* Tennessee isolates, pulsed-field gel electrophoresis and multiple-locus variable number tandem repeat analysis were performed. Further cloacal swab samples of reptiles kept in case households were investigated.

<u>Results:</u> We identified 18 cases < 3 years. Ten children were male; median age was 3 months (1–32 months). In 8 of 16 case households reptiles were kept. Direct contact between child and reptile was denied. Other forms of reptile contact were reported in four of the remaining eight households. Ten case- and 21 control-patients were included in the study. Only keeping of a reptile and "any reptile contact" were associated with *Salmonella* Tennessee infection (mOR 29.0; 95% CI 3.1 ± ∞ and mOR 119.5; 95% CI 11.7 ± ∞). Identical *Salmonella* Tennessee strains of child and reptile kept in the same household could be shown in 2 cases.

<u>Discussion</u>: Reptiles were the apparent source of *Salmonella* Tennessee infection in these infants. Indirect contact between infants and reptiles seems to be sufficient to cause infection and should therefore be avoided.

Introduction

Wih an annual incidence of 147/100,000 infants, enteric *Salmonella* are the main etiologic agents of bacterial gastroenteritis notified in this age group in Germany. Infections can lead to potentially fatal conditions like sepsis and meningitis.

In May 2008 the Robert Koch Institute was informed about an apparent cluster of *Salmonella* enterica serovar Tennessee infections in infants. Data from the national surveillance system showed 8 cases in March/April 2008 compared to a median of 0–1 cases in March/April of 2001–2006. We conducted an outbreak investigation. Explorative interviews soon pointed toward reptile contact as the hypothesized exposure.

Materials and Methods

Cases were identified from the national mandatory laboratory-based surveillance system. A case was defined as a child < 3 years of age with *Salmonella* Tennessee isolated from stool from September 1, 2007, through December 31, 2008. For the case-control study, we included cases notified through May 31, 2008 (first case per household). We aimed for three controls per case. A control was a child with a notified rotavirus infection in the matching district, frequency matched by age group (p6 and > 6 months).

We conducted telephone interviews using a questionnaire on breast feeding, infant formula, herbal infusions, and animal contact during a 7-day period before detection of *Salmonella* Tennessee (cases) or interview (controls). In statistical analysis, matched odds ratios (mOR) were calculated with exact conditional logistic regression (STATA 10; StataCorp).

Salmonella Tennessee isolates from children were sent to the National Reference Centre for Salmonella and other Enteric Pathogens for serotyping (White-Kauffmann-Le Minor scheme) (Grimont and Weill 2007), followed by pulsed-field gel electrophoresis (PFGE; Xbal digestion, PulseNet standards) (Hunter et al. 2005), and a cluster analysis (BioNumerics, version 6.0, Applied Maths). For a limited number of samples multiple-locus variable number tandem repeat (VNTR) analysis (MLVA) including 11 VNTR loci was performed.

Cloacal swab samples of reptiles were pre-enriched in selenite broth and cultured on McConkey agar, and any *Salmonella* Tennessee isolate was further processed as described above.

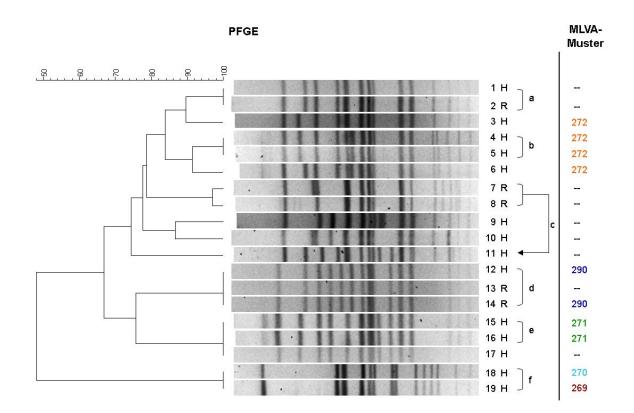


Figure 1: Cluster analysis of pulsed-field gel electrophoresis (PFGE) patterns and corresponding multiple-locus variable number tandem repeat (VNTR) analysis (MLVA) patterns of *Salmonella* Tennessee strains from children and reptiles. H, human isolate; R, reptile isolate. a, c, and d: human isolate and corresponding reptile isolate from the same household; b: isolates from twins; e: isolates from nonrelated children living in the same household; f: isolates from elder children (23 and 30 months of age) without reptile contact or contact to each other. For cluster analysis the unweighted pair group method with arithmetic mean was used. Positional tolerance settings: optimization 1%; tolerance 1%.

Results

From September 1, 2007, through December 31, 2008, 18 cases < 3 years were identified. Case-patients lived in 16 households in 10 federal states, 10 were male, and median age was 3 months (1–32 months). Among 13 children with known symptoms, 11 had diarrhea, 5 bloody stools, and 7 fever. Two children were asymptomatic. Four children were admitted to hospital, but no complications were reported.

In 8 of 16 case households reptiles were kept: bearded dragons (*Pogona vitticeps*) in 7 (in 1 household together with a chameleon), and a snake (*Boa constrictor*) in 1 household. All parents denied direct contact between child and reptile. In four of the remaining eight households, "other reptile contact" was reported. This included in one household each: (1) reptiles kept until 5 weeks before detection of *Salmonella* Tennessee in the child, (2) a visit by a friend who kept a reptile, (3) visiting a reptile exhibition with the infant, and (4) the father working in a pet shop with reptiles.

We included 10 case- and 21 control-patients in the study.

In bivariate analysis only keeping of a reptile and "any reptile contact" (combined variable: keeping of a reptile or "other reptile contact") were associated with *Salmonella* Tennessee infection (mOR 29.0; 95% CI 3.1 $\pm \infty$ and mOR 119.5; 95% CI 11.7 $\pm \infty$). None of the controls reported any reptile contact.

The laboratory investigations included 14 *Salmonella* Tennessee patient isolates and revealed a variety of PFGE patterns (Fig. 1). Identical patterns were found in twins, two unrelated children from the same household, and the two eldest children without a known link.

Reptile samples could be obtained from four case households. In three, *Salmonella* Tennessee could be detected in bearded dragons, and from the fourth, a snake excrement, *Salmonella* Monschaui was grown. In two of three households with *Salmonella* Tennessee–positive reptiles, PFGE patterns of the respective children and reptiles were identical. In the third household, in which bearded dragons were bred (offspring not available for testing) the patterns clearly differed. MLVA showed concordant as well as discordant results with PFGE (Fig. 1).

Discussion

The epidemiological and microbiological investigation revealed that keeping a reptile—and reptile contact in general— was a risk factor for *Salmonella* Tennessee infections in infants. These risk factors explain 78% of the 18 notified cases. PFGE results demonstrated identical patterns of human and corresponding reptile isolates in most households.

While reptile-associated salmonellosis has been reported since the 1960s from the United States and Canada (CDC 1995, Woodward 1997), this risk factor has been described for the first time for a larger group of patients in Germany. Most reports from Europe refer to single cases (Editorial Team et al. 2008), but recently a reptile-associated outbreak of *Salmonella* Typhimurium was investigated in the United Kingdom (Harker 2010). Underestimation of the problem seems likely, as information on the source of infection is not collected systematically.

The majority of reptiles involved in our investigations were bearded dragons. Although no exact numbers on the frequency of bearded dragons in Germany exist, an increasing popularity of these comparatively cheap and easy-to-handle reptiles would explain the recent increase in cases.

Molecular subtyping by PFGE and MLVA revealed different strains among the *Salmonella* Tennessee isolates, suggesting several independent rather than one common source of infection (i.e., reptiles from one breeder or contaminated feed) (Fuller 2008).

However, assuming bearded dragons to be a natural reservoir of *Salmonella* Tennessee, they might well harbor distinct strains simultaneously.

This points to a general problem associated with reptile examination.

It has been shown that various reptiles can be colonized by multiple serovars, for example, chameleons (Willis 2002) and adult female snakes that can transmit *Salmonella* during pregnancy and birth to their newborns (Schroeter 2006).

Therefore, the identification of different serovars (or strains) in reptiles and children does not exclude the reptile as the source of infection, especially as some reptiles were sampled months after the infant's infection.

As direct contact between reptiles and infants was denied, indirect contact appears to be sufficient to cause infection. The most likely mode of transmission is contact with surfaces, or hands contaminated with reptile feces. This indirect mode of transmission has already been described (Friedman 1998).

The fact that mostly very young children were affected can be explained by the vulnerability of this age group caused, for example, by gastric hypochlorhydria and insufficient mucosal immunity (Blaser and Newman 1982).

This report demonstrates that reptiles are a potential source for *Salmonella* infections in infants in Europe as well. In accordance with the CDC recommendations (CDC 2007) any direct or indirect contact of reptiles and infants should be avoided. Infants' household members are recommended to practice strict hand hygiene after any reptile contact.

Acknowledgments

The authors thank local public health departments for supporting the case-control study.

Disclosure Statement

No competing financial interests exist.

References

Blaser, MJ, Newman, LS. A review of human salmonellosis: I. Infective dose. Rev Infect Dis 1982; 4:1096–1106.

Centers for Disease Control and Prevention. Reptileassociated salmonellosis—selected states, 1994–1995. MMWR Morb Mortal Wkly Rep 1995; 44:347–350.

Centers for Disease Control and Prevention. Turtle-associated salmonellosis in humans— United States, 2006–2007. MMWR Morb Mortal Wkly Rep 2007; 56:649–652.

Editorial Team, Bertrand, S, Rimhanen-Finne, R, Weill, FX, et al. *Salmonella* infections associated with reptiles: the current situation in Europe. Euro Surveill 2008; 13:pii-18902.

Friedman, CR, Torigian, C, Shillam, PJ, Hoffman, RE, et al. An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. J Pediatr 1998; 132:802–807.

Fuller, CC, Jawahir, SL, Leano, FT, Bidol, SA, et al. A multi-state *Salmonella* Typhimurium outbreak associated with frozen vacuum-packed rodents used to feed snakes. Zoonoses Public Health 2008; 55:481–487.

Grimont, PAD, Weill, FX. Antigenic Formulae of the *Salmonella* Serovars, 9th edition. Paris: WHO Collaborating Centre for Reference and Research on *Salmonella*, 2007. Available from www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/ 01s-000036-089

Harker, KS, Lane, C, De Pinna E, Adak, GK. An outbreak of *Salmonella* Typhimurium DT191a associated with reptile feeder mice. Epidemiol Infect 2010; 14:1–8.

Hunter, SB, Vauterin, P, Lambert-Fair, MA, Van Duyne, MS, et al. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J Clin Microbiol 2005; 43:1045–1050.

Schroeter, M, Speicher, A, Hofmann J, Roggentin, P. Analysis of the transmission of *Salmonella* spp. through generations of pet snakeS. Environ Microbiol 2006; 8:556–559.

Willis, C,Wilson, T, GreenwoodM, Ward, L. Pet reptiles associated with a case of salmonellosis in an infant were carrying multiple strains of *Salmonella*. J Clin Microbiol 2002; 40:4802–4803.

Woodward, DL, Khakhria, R, Johnson, WM. Human salmonellosis associated with exotic pets. J Clin Microbiol 1997; 35:2786–2790.

Address correspondence to:

Bettina Weiss Department of Infectious Disease Epidemiology Robert Koch Institute DGZ-Ring 1 13086 Berlin Germany E-mail: weissb@rki.de