Detection of *Salmonella enterica* in a sand lizard (*Lacerta agilis*, Linnaeus, 1758) city population

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ABSTRACT

Salmonellosis is one of the most urgent public health problems across the world. Reptiles are a known reservoir of *Salmonella* spp. and in some regions they are also associated with human salmonellosis. This concerns especially popular pet reptiles, e.g. turtles or bearded dragons; however, there is also a need for studies regarding wild reptiles as a pathogen source. In this study, sand lizards (*Lacerta agilis*) were investigated as a potential reservoir of *Salmonella* spp. in Poznań, Poland, using cloacal swabs and faecal samples. Moreover, clonal analysis of the isolates was conducted using ERIC-PCR fingerprinting. Thirty eight lizards were investigated, nine of which (24%) proved positive for *S. enterica* subsp. *houtenae*. The prevalence level was lower than previously observed in exotic species (up to above 40%). Two clones were present in several lizards. Specimens with similar clones were captured at the same location and time, suggesting horizontal transfer of bacterial strains between lizards. Because the isolated subspecies of *Salmonella* is very rarely reported as a causative agent of human salmonellosis, sand lizards seem to pose little or no threat for public health.

KEYWORDS

infectious disease, environmental microbiology, clonal analysis, reptile-associated salmonellosis

Salmonella spp. is a regular component of the microbial flora of the digestive tract in reptiles (Mitchell & Shane, 2001, see also Benskin et al., 2009 for birds). In mammals their presence leads to salmonellosis (Buxton, 1957) and reptiles have therefore been linked to salmonellosis oubreaks in humans (Warwick et al., 2001; Hassl & Benyr, 2003; Mermin et al., 2004; Bauwens et al., 2006; Bertrand et al., 2008). *Salmonella enterica* subsp. *enterica* is the most pathogenic subspecies, and transmission from pet reptiles to humans has previously been reported (Woodward et al., 1997; Hidalgo-Vila et al., 2007; Pedersen et al., 2009; Chen et al., 2010). Prevalence of *Salmonella* spp. in reptiles is often high (e.g. Geue & Löschner, 2002: 54.1%; Briones et al., 2004: 41.5%), and transovarial transfer may occur from mother to clutch (Chiodini, 1982). Other studies however also report that *Salmonella* spp. is absent (Geue & Löschner, 2002).

Due to their close contact with humans, studies which link human salmonellosis with reptiles have previously large focused on pet reptiles (e.g. *Trachemys scripta;* Warwick et al., 2001; Nagano et al., 2006; CDC, 2008), and our knowledge on the occurrence of *Salmonella* spp. in wild European reptiles is still very incomplete (e.g. *Emys orbicularis:* Hidalgo-Vila et al., 2007, *Natrix natrix:* Wuthe et al., 1979; Rostami et al., 2009, *Vipera berus:* Wuthe et al., 1979). The sand lizard (*Lacerta agilis*) is the most widespread reptile species in Europe (Bischoff, 1984), but our knowledge about the presence of *Salmonella* spp. is restricted to a single individual investigated which was tested positively for *Salmonella sofia* (currently *S. enterica* subsp. *salamae*) several decades ago (Koopman & Janssen, 1973). The aim of this study was to document the occurrence of *Salmonella* in a Polish population of *L. agilis*.

64 The study was carried out in May - June 2014 in two localities in Poznań,
65 Poland (near Rusałka Lake, 52°25’38.86N, 16°52’14.63E and on the edge of Morasko
66 forest, 52°28’03.03N, 16°55’50.54E) in habitats which are strongly effected by
67 anthropogenic activities. *Lacerta agilis* is a short-legged, ground-dwelling diurnal lizard
68 with about 66 - 73mm snout-vent-length (SVL, Ekner et al., 2008).
69 Lizards were captured using nets or by hand. SVL of caught lizards were
70 measured to classify them as adult (> 45 mm SVL), sub-adults (35 – 45 mm SVL), or
71 juveniles (< 35 mm, Gvozdik 2000; Dudek et al. 2015). Juveniles were lizards in their
72 first year of life, subadults were in their second year of life, and adults were at least
73 three years old. Individuals were sexed based on the presence of femoral pores and the
74 expanded gonadal area in the tail base in males. During the mating season males also
75 have green body colouration.
76 After taking cloacal swabs, all lizards were placed overnight in separate sterile
77 boxes. On the next day, the faecal samples were collected using Amies Agar Gel
78 transport swabs (Oxoid) and processed within four hours. To avoid pseudoreplication,
79 the lizards were marked using medical cautery units (following Ekner et al., 2011), and
80 each individual was used only once. After sample collecting, all individuals were
81 released at the place of their capture. Cloacal swabs and stool specimens were
82 inoculated into Rappaport-Vassiliadis Enrichment Broth (Oxoid) and incubated 48
83 hours at 42°C. If bacterial growth was observed, the culture was inoculated onto
84 Brilliant Green Agar (Oxoid) and incubated 18-24 hours at 35°C. Lactose-negative
85 isolates were identified using API 20E kits (bioMérieux) and complementary tests for
86 utilization of malonate, mucate and salicine (Popoff & Le Minor, 2005).

Isolates were genotyped using ERIC-PCRs, with primers complementary to enterobacterial repetitive intergenic consensus sequences. Genomic DNA was isolated using the Genomic Mini Kit (A&A Biotechnology). PCR reactions with primers ERIC1R and ERIC 2 were conducted in a C1000 thermal cycler (Bio-Rad) following Versalovic et al. (1991). Amplicons were separated on 2% agarose gels, and banding patterns were analyzed using GelCompar II 3.5 software (Applied Maths) using the Dice Similarity Coefficient and UPGMA clustering. Isolates sharing DNA fingerprinting patterns above 95% similarity were considered clones. The experiments were done in duplicate. Prevalence and confidence limits (95 % CL) for binary, presence-absence, data were calculated in Microsoft Excel 2013 (Microsoft).

A total of 38 individuals (13 females, 20 males, and 5 juveniles) were captured. Specimens taken from 10 lizards yielded bacterial growth in Rappaport-Vassiliadis Enrichment Broth. Nine of these cultures grew as typical red-coloured lactose-negative colonies on Brilliant Green Agar, and were identified with API 20E as *S. enterica*. Complementary tests revealed that all nine isolates were malonate- and mucate-negative and salicine-positive, which allowed identifying them as *S. enterica* subsp. *houtenae*. Prevalence was estimated as 0.237 (95% CL = 0.102 - 372).

The ERIC fingerprints consisted of 8 - 18 bands ranging from 180 bp to 6300 bp in size. The dendrogram showed the presence of two clusters with 100% similarity (Fig. 1). The first cluster comprised isolates no. J10 and J11, cultured from lizards collected on 04 July 2014; the second one consisted of isolates no J33 and J36, cultured from lizards collected on 10 July 2014. With less than 90% similarity, the remaining five isolates were genetically unrelated.

Nine out of 38 *L. agilis* individuals were positive for *S. enterica* in faecal samples. Such a prevalence rate is below most previously reported values (e. g. Geue & Löschner, 2002 on captive individuals: 47.4% for lizards, distributed across Agamidae 60%, Chamaeleonidae 71.4%, Iguanidae 62.1%, Phrynosomatidae and Scinidae 33.3%, Gekkonidae 16.7%, Crotaphytidae, and Poychrotidae 0%; Colubridae 96.2%, Boidae 56.1%). All salmonellae isolates cultured from stool and cloacal swabs were identified as *S. enterica* subsp. *houtenae*. Strains of this subspecies are mostly isolated from cold-blooded animals and their environment (Geue & Löschner, 2002; Popoff & Le Minor, 2005; Bauwens et al., 2006; Bertrand et al., 2008; Pedersen et al., 2008; Hydeskov et al., 2013; Gay et al., 2014), and have also been found in intestines of wild boars (Chiari et al., 2013; Zottola et al., 2013) and birds (Millán et al., 2004). Overall in reptiles, *S. enterica* subsp. *houtenae* is the third most commonly found *Salmonella* taxon after *S. enterica* subsp. *enterica* and *S. enterica* subsp. *Diarizonae*, ahead of *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *a*nd *S. bongori* (Bertrand et al., 2008).

Clonal analysis showed the presence of two clusters consisting of isolates with identical ERIC fingerprintings, i.e. clones. These clones were cultured from lizards captured at the same day and location (Morasko and Rusałka Lake, respectively). This suggests that strains of *Salmonella* spp. can be transmitted horizontally between lizards. There is a need to investigate whether transovarial transfer of *Salmonella* spp. can occur in sand lizards, as is the case for snakes (Chiodini, 1982).

The frequent occurrence of *S. enterica* subsp. *houtenae* likely poses little or no threat for public health, as this subspecies is only very rarely reported in pet reptiles and as a causative agent of human salmonellosis (Bertrand et al., 2008; Hoszowski et al.,

2000, 2012; Sadkowska-Todys & Czarkowski, 2013). There is however a need for further studies on other common reptile species as potential reservoirs for *Salmonella*.

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FIGURES

Fig. 1. Dendrogram showing genetic relatedness of bacterial isolates determined by ERIC-PCR typing. Cluster 1 comprises isolates J10 and J11; cluster 2 comprises isolates J33 and J36.