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Salmonella serotypes in reptiles and humans, French Guiana

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Abstract

In French Guiana, a French overseas territory located in the South American northern coast, nearly 50% of *Salmonella* serotypes isolated from human infections belong to serotypes rarely encountered in Metropolitan France. A reptilian source of contamination has been investigated. Between April and June 2011, in the area around Cayenne, 151 reptiles were collected: 38 lizards, 37 snakes, 32 turtles, 23 green iguanas and 21 caimans. Cloacal swab samples were collected and cultured. Isolated *Salmonella* strains were identified biochemically and serotyped. The overall carriage frequency of carriage was 23.2% (95% confidence interval: 16.7-30.4) with 23 serotyped strains. The frequency of *Salmonella*

carriage was significantly higher for wild reptiles. Near two-thirds of the *Salmonella* serotypes isolated from reptiles were also isolated from patients in French Guiana. Our results highlight the risk associated with the handling and consumption of reptiles and their role in the spread of *Salmonella* in the environment.

Key words: *Salmonella*; reptiles; humans; serotypes; zoonosis; French Guiana

1. Introduction

Salmonella is a major foodborne pathogen found worldwide. Non-typhoidal *Salmonella* causes 93.8 million human cases of gastroenteritis and 155,000 deaths annually worldwide (Majowicz, et al., 2010). The *Salmonella* genus includes two species: *S. enterica* and *S. bongori*. *S. enterica* is subdivided into six subspecies: *enterica* (former subgenera I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI). Over 2,600 serotypes were identified according to the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). The digestive tract of warm-blooded animals is the main reservoir of *Salmonella* subspecies *enterica* (Grimont and Weill, 2007), while other subspecies are mainly found in cold-blooded animals. Reptiles can harbour multiple subspecies and serotypes simultaneously (Pedersen et al., 2009). Most human salmonellosis is associated with eating contaminated raw or undercooked food of animal origin. Other transmission routes exist: human-to-human contacts, consuming contaminated vegetables, water (Wegener et al., 2003) and direct contact with infected animals, including pets. In western countries serotypes from reptiles are rarely encountered in humans (Woodward et al., 1997; O'Byrne and Mahon, 2008). Within the European Union, it is estimated that <1% of human salmonellosis are associated with reptiles (Bertrand et al., 2008). Furthermore, serotypes from reptiles seem to be responsible for more severe complications and hospitalizations (Cieslak et al., 1994).

In French Guiana, a French overseas territory (around 85,000 km²) located in South America, nearly 50% of *Salmonella* isolates from human belong to serotypes rarely encountered in Metropolitan France (Le Hello and Weill, unpublished data).

The objectives of this study conducted around Cayenne, the main city of French Guiana, were to assess *Salmonella* carriage by different reptile species living in different ecosystems (zoo, forest, commensal environment or caged/contained in or around human dwellings) and to compare reptilian and human serotype distributions.

2. Materials and Methods

2.1. Surveillance of human *Salmonella* infections.

In France, human *Salmonella* surveillance is a voluntary laboratory-based network headed by the French National Reference Center for *Salmonella* (FNRC-Salm). Participating laboratories annually send 8,000-10,000 *Salmonella* isolates to the FNRC-Salm, which serotypes them and runs weekly outbreak-detection algorithms (David et al., 2011). In 2011, all the medical laboratories from French Guiana (8 private biomedical laboratories and 2 public hospitals) participated to the present study.

2.2. Frequency of *Salmonella* carriage in reptiles.

From 5 April to 6 June 2011, reptiles were captured from more-or-less human-occupied environments around Cayenne. Four living conditions were defined: zoo, for captive animals in the zoo; forest, for reptiles captured there; commensal environment, for reptiles living in or around human lodgings (e.g. *Hemidactylus mabouia*); reptiles caged (e.g., snake terrarium) or contained in a space from which there is no egress (e.g., turtles within a garden) in or around a private home.

Cloacal swab samples were collected and kept at room temperature ($26 \pm 4^{\circ}\text{C}$) for a maximum of 48 hr. Samples were incubated in 9 ml of buffered peptone water for 16-20 hr at 37°C . Three drops of the pre-enrichment broth were inoculated onto specific medium (Hektoen agar, BioMérieux, Marcy L'Etoile, France) and incubated at 37°C for 21-27 hr. Lactose-negative colonies were subjected to the urease test and incubated at 37°C for 21-27 hr. When urease was negative (suspected *Salmonella*), biochemical identification of an isolated colony was pursued with the API 20E Kit (BioMérieux). Isolates were sent to the FNRC-Salm (except subspecies *arizonae* determined by the API 20E kit) for serotyping according to the White–Kauffmann–Le Minor scheme. All strains underwent antimicrobial-susceptibility testing with the disk-diffusion method on Mueller–Hinton agar (Bio-Rad, Marnes-La-Coquette, France) against 16 antimicrobials (amoxicillin, streptomycin, spectinomycin, azithromycin, kanamycin, netilmicin, amikacin, tobramycin, gentamicin, nalidixic acid, ofloxacin, ciprofloxacin, chloramphenicol, sulfonamides, trimethoprim and tetracycline), according to the guidelines of the French Society for Microbiology (available at: <http://www.sfm-microbiologie.org/>).

Reptile species were grouped as: caimans, snakes, green iguanas (*Iguana iguana*), other lizards and turtles. Statistical analyses were computed with Stata® v9.0 Stata Corp; χ^2 or Fisher's exact tests were used.

3. Results

During the study, 151 reptiles (Table 1) were collected. They were distributed in habitats as follows: 34.4% (52/151) from zoo, 31.8% (48/151) from forest, 19.9% (30/151) in commensal environment and 13.9% (21/151) caged/contained in or around a private home.

The frequency of *Salmonella* carriage in cloacal samples was 23.2% (35/151, 95% CI: 16.7-

30.4), ranging between 14.3% and 32.4%, depending on the type of reptile (Table 1), but did not differ significantly ($p = 0.5$). Habitat was significantly associated with *Salmonella* carriage ($p < 0.001$), with frequencies of 5.8%-39.6%. The frequency of carriage was higher for reptiles captured in forests and caged/contained in or around private homes.

S. enterica subspecies represented 47.2% (17/36) of all isolates (Table 2). Serotyped strains (23/36) belonged to both subspecies *enterica* (17/23, 73.9%) and *houtenae* (6/23, 26.1%) and were susceptible to all antibiotics tested. One lizard was co-infected with two *enterica* subspecies serotypes, Newport and Infantis. Fourteen different serotypes were isolated. The most common serotypes were Anatum (3/23, 13.0%), Rubislaw (13.0%) and subspecies *houtenae* serotype 50:g,z₅₁:– (13.0%). At least three different serotypes were observed for each reptile class except for caimans, which hosted only one.

In 2011, the FNRC-Salm received 154 human *Salmonella* acquired in French Guiana (87 isolates from Cayenne, 35 from Saint-Laurent-du-Maroni area, 30 from Kourou and two additional from Metropolitan France and Martinique Island, French West Indies). Of the 154, 139 (90.3%), 14 (9.1%) and 1 (0.6%) belonged, respectively, to *enterica*, *houtenae* and *diarizonae* subspecies (Table 3). Frequently isolated serotypes among *enterica* subspecies were Panama (18/139, 12.9%), Oranienburg (11/139, 7.9%), Saintpaul (11/139, 7.9%) and Uganda (10/139, 7.2%) and the *houtenae* subspecies 50:g,z₅₁:– serotype (10/14, 71.4%).

Among the 14 serotypes isolated from reptiles, 64.3% were also isolated from humans: Panama, Saintpaul, Uganda, Javiana, Miami, Newport, Rubislaw, Infantis and IV. 50:g,z₅₁:–.

4. Discussion

121 The global frequency of *Salmonella* carriage by reptiles in the Cayenne area was 23.2%.
122 *Salmonella* carriage frequency did not differ significantly among groups (caimans, green
123 iguanas, other lizards, snakes and turtles), the highest was documented in snake (32.4%) and
124 the lowest in Caiman (14.3%). However, each group is composed of various species; the
125 frequency of carriage might differ depending on the species (Geue and Löschner, 2002).
126 Overall carriage was lower than data obtained from literature. For instance, snake carriage
127 was 32.4% compared to 45%-98% (Sá and Solari, 2001; Geue and Löschner, 2002; Briones et
128 al., 2004; Chen et al., 2010) and green iguana carriage (26.1%) was lower than 55%-90%
129 reported (Mitchell and Shane, 2000; Wheeler et al., 2011). For other lizards, 21.1% harboured
130 *Salmonella*, compared to 38–63% described elsewhere (Sá and Solari, 2001; Geue and
131 Löschner, 2002; Briones et al., 2004; Chen et al., 2010). Difference might be due to the
132 composition of the lizard group, mainly *Hemidactylus mabouia* (Geue and Löschner, 2002).
133 Turtle carriage (18.8%) was close to results observed in literature that ranged from 25% (Sá
134 and Solari, 2001; Briones et al., 2004; Chen et al., 2010) to 80% (Pasmans et al., 2000;
135 Hidalgo-Vila et al., 2007). Finally, 14.3% of sampled caimans harboured *Salmonella*. To our
136 knowledge, only Uhart et al. (2011) reported infection in caimans with fluctuating results
137 (0%-77%) from one year to another.

138 Carriage frequency also differed according to the reptile's habitat. Reptiles captured in the
139 forest had the highest rate (39.6%) and zoo dwellers the lowest (5.8%). Those observations
140 seem to contradict literature data, as some authors reported higher frequencies (47%-88.9%)
141 for zoo-housed reptiles (turtle, snake and lizard) versus 14%-58.8% for their wild counterparts
142 (Geue and Löschner, 2002; Scheelings et al., 2011). That low frequency of carriage among
143 sampled zoo reptiles is difficult to explain as no antibiotics had been administered during the
144 months preceding the study. Indeed, captive conditions should favor inter-individual and
145 interspecies transmission; their captivity and exposure to the general public (e.g. noise) could

be sources of stress, inducing the onset of symptoms and *Salmonella* excretion (Friendship et al., 2009; Maciel et al., 2010). *Salmonella* frequency of carriage by reptiles caged/contained in or around private homes was high at 38.1% which tends to confirm higher frequency of contamination in wild reptiles. All of the latter had been caught in their natural environment and were captive for < 6 months. A limitation in our study is that each reptile type was not sampled in all habitats, e.g., green iguanas were sampled only at the zoo. However, considering all reptiles sampled, the forest dwellers were, on the whole, fairly well-represented (31.8%), in contrast to most of studies that focused on captives (Sá and Solari, 2001; Pedersen et al., 2009).

Comparing our data to those in the literature was difficult as no standard methodology exists for *Salmonella* isolation from reptiles but globally we could have underestimated frequency of carriage. Cloacal swabbing without enrichment step and use of simplified culture methods mainly target *enterica* subspecies, the major source of human and animal clinical infections, might underestimate carriage frequencies. We might also have underestimated *arizonae* and *diarizonae* subspecies, because respectively, 25% and 75% of these subspecies ferment lactose (Grimont and Weill, 2007).

Moreover, due to intermittent *Salmonella* shedding in feces (Bauwens et al., 2006), cloacal swabbing is less sensitive than using digestive tracts from sacrificed animals. However, regulations protecting reptiles restrict it. In addition, only one colony was tested for each animal, consequently coinfection was unlikely. Only one reptile specimen showed multiple infections while reptiles can harbour several *Salmonella* serotypes simultaneously (Chiodini and Sundberg, 1981).

We observed high serotype diversity among reptile samples obtained around Cayenne, with identification of 14 different serotypes among the 23 isolates sent to the FNRC-Salm. At least three different serotypes were isolated from each reptile type (except for caimans which

171 harboured only one *Salmonella* serotyped). Lizards exhibited a wide *Salmonella* diversity,
172 with seven different serotypes found. No patterns of host-specific serotype association were
173 observed, as Briones et al. (2004) claimed. In addition, almost half (47.2%) of *Salmonella*
174 isolates belonged to the subspecies *enterica*, thereby confirming that reptiles serve as
175 reservoirs of this subspecies normally associated with the digestive tract of warm-blooded
176 animals (Geue and Löschner, 2002).

177 In our study, 64.3% of *Salmonella* serotypes isolated from reptiles were also isolated from
178 patients in French Guiana. In contrast only 13% of serotypes isolated were also found in
179 inspected farm chickens (Food, Agriculture and Forestry of French Guiana, unpublished data),
180 it might indicate low environmental contaminations by reptiles. However, no veterinary
181 controls are performed on poultry units producing less than 250 chickens and products are
182 sold directly to consumers. The distribution of *Salmonella* serotypes could differ in such small
183 poultry farming, as facilities (livestock buildings, granary) are less efficient and interactions
184 with reptiles possibly higher.

185 Thus, reptiles might be the direct or indirect source of a high percentage of human *Salmonella*
186 infections in French Guiana.

188 **5. Conclusion**

189 Reptiles are *Salmonella* reservoirs and might represent public health risk, especially in French
190 Guiana, where a huge diversity of reptile species is observed and some in close contact with
191 humans (*Hemidactylus mabouia* having ready access into homes...) and probably livestock.
192 Furthermore, they represent food sources, particularly green iguanas. Therefore, hygiene
193 measures concerning the handling of reptiles, cooking their meat and cleaning kitchen utensils
194 should be promoted.

Conflict of interest statement

We declare that we have no conflicts of interest.

Authors contributions

FB conceived and designed the experiments. NG and BDT collected data. NG performed the experiments. SLH, FXW provided data from humans. NG, FB analyzed the data. NG wrote the paper. SLH, FXW and BDT reviewed, criticised and offered comments on the text.

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TABLES

Table 1

Reptile *Salmonella* carriage according to reptile type or living conditions

Parameter	N (%)	<i>Salmonella</i> frequency		p	OR [95% CI]
		n	% (95% CI)		
Reptile				0.5	
Caimans	21 (13.9)	3	14.3 (3.0–36.3)		1.0
Turtles	32 (21.2)	6	18.8 (7.2–36.4)		1.4 [0.3–6.3]
Green Iguanas	23 (15.2)	6	26.1 (10.2–48.4)		2.1 [0.5–9.8]
Other lizards	38 (25.2)	8	21.1 (9.6–37.3)		1.6 [0.4–6.8]
Snakes	37 (24.5)	12	32.4 (18.0–49.8)		2.9 [0.7–11.7]
Total	151 (100)	35	23.2 (16.7–30.4)		
Living Conditions				< 0.001	
Zoo	52 (34.4)	3	5.8 (1.2–15.9)		1.0
Commensal environment	30 (19.9)	5	16.7 (5.6–34.7)		3.3 [0.7–15.1]
Caged/Contained	21 (13.9)	8	38.1 (18.1–61.6)		10.3 [2.4–44.2]
Forest	48 (31.8)	19	39.6 (25.8–54.7)		11.3 [3.1–41.6]
Total	151 (100)	35	23.2 (16.7–30.4)		

TABLE 2

Distribution of *S. enterica* subspecies among sampled reptiles

Subspecies	Isolates N (%)	<i>Salmonella</i> serotyped	% (95% CI)
<i>arizonae</i> (IIIa)	13 (36.1)	0	36.1 (20.8–53.8)
<i>enterica</i> * (I)	17 (47.2)	17	47.2 (30.4–64.5)
<i>houtenae</i> (IV)	6 (16.7)	6	16.7 (6.4–32.8)
Total	36 (100)	23	

*Two serotypes in one reptile sample.

TABLE 3

Salmonella serotypes isolated from humans in French Guiana in 2011

Serotype	French Guiana (n = 154)*			Metropolitan France (n = 7,521)	
	n	%	Cumulated %	n	%
<i>enterica</i> (I) subspecies	139	90.3		7,401	98.4
Panama	18	12.9	12.9	36	0.5
Oranienburg	11	7.9	20.8	23	0.3
Saintpaul	11	7.9	28.7	61	0.8
Uganda	10	7.2	35.9	3	0.04
Javiana	8	5.8	41.7	8	0.1
Miami	8	5.8	47.4	5	0.07
Newport	8	5.8	53.2	110	1.5
Enteritidis	7	5.0	58.2	1,075	14.5
Poona	7	5.0	63.3	35	0.5
Rubislaw	7	5.0	68.3	4	0.05
Typhi	4	2.9	71.2	43	0.6
Arechavaleta	4	2.9	74.1	2	0.03
1,4,[5],12:i:-	3	2.2	76.2	2,084	28.2
Morehead	3	2.2	78.4	0	0
9,12:-:1,5	2	1.4	79.8	2	0.03
Belem	2	1.4	81.2	0	0
Chester	2	1.4	82.7	22	0.3

Coeln	2	1.4	84.1	13	0.2
Infantis	2	1.4	85.6	150	2.0
Itami	2	1.4	87.0	1	0.01
Jukestown	2	1.4	88.4	0	0
Kentucky	2	1.4	89.9	115	1.6
Typhimurium	2	1.4	91.3	1,843	24.9
Weltevreden	2	1.4	92.8	20	0.3
Rough	1	0.7	93.5	18	0.2
Agona	1	0.7	94.2	43	0.6
Claibornei	1	0.7	94.9	1	0.01
Manhattan	1	0.7	95.6	6	0.08
Oslo	1	0.7	96.4	0	0
Paratyphi B dT+ (biotype Java)	1	0.7	97.1	20	0.3
Sandiego	1	0.7	97.8	3	0.04
Senftenberg	1	0.7	98.5	16	0.2
Telelkebir	1	0.7	99.2	5	0.07
Thompson	1	0.7	100.0	11	0.1
<i>diarizonae</i> (IIIb) subspecies	1	0.6		33	0.4
17:z10:e,n,x,z15	1	100.0	100.0	0	0
<i>houtenae</i> (IV) subspecies	14	9.1		22	0.3
50:g,z51:-	10	71.4	71.4	4	18.2
44:z4,z24:-	2	14.3	85.7	0	0
11:g,z51:-	1	7.1	92.9	0	0
Rough	1	7.1	100.0	0	0

*One per patient.