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1 *Salmonella* serotypes in reptiles and humans, French Guiana

2

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16

17 Abstract

18 In French Guiana, a French overseas territory located in the South American northern coast,

19 nearly 50% of *Salmonella* serotypes isolated from human infections belong to serotypes rarely

20 encountered in Metropolitan France. A reptilian source of contamination has been

21 investigated. Between April and June 2011, in the area around Cayenne, 151 reptiles were

22 collected: 38 lizards, 37 snakes, 32 turtles, 23 green iguanas and 21 caimans. Cloacal swab

23 samples were collected and cultured. Isolated *Salmonella* strains were identified

24 biochemically and serotyped. The overall carriage frequency of carriage was 23.2% (95%

25 confidence interval: 16.7-30.4) with 23 serotyped strains. The frequency of *Salmonella*

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

30

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

32

33 **1. Introduction**

34 *Salmonella* is a major foodborne pathogen found worldwide. Non-typhoidal *Salmonella*
35 causes 93.8 million human cases of gastroenteritis and 155,000 deaths annually worldwide
36 (Majowicz, et al., 2010). The *Salmonella* genus includes two species: *S. enterica* and *S.*
37 *bongori*. *S. enterica* is subdivided into six subspecies: *enterica* (former subgenera I), *salamae*
38 (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI). Over 2,600 serotypes
39 were identified according to the White-Kauffmann-Le Minor scheme (Grimont and Weill,
40 2007). The digestive tract of warm-blooded animals is the main reservoir of *Salmonella*
41 subspecies *enterica* (Grimont and Weill, 2007), while other subspecies are mainly found in
42 cold-blooded animals. Reptiles can harbour multiple subspecies and serotypes simultaneously
43 (Pedersen et al., 2009). Most human salmonellosis is associated with eating contaminated raw
44 or undercooked food of animal origin. Other transmission routes exist: human-to-human
45 contacts, consuming contaminated vegetables, water (Wegener et al., 2003) and direct contact
46 with infected animals, including pets. In western countries serotypes from reptiles are rarely
47 encountered in humans (Woodward et al., 1997; O'Byrne and Mahon, 2008). Within the
48 European Union, it is estimated that <1% of human salmonellosis are associated with reptiles
49 (Bertrand et al., 2008). Furthermore, serotypes from reptiles seem to be responsible for more
50 severe complications and hospitalizations (Cieslak et al., 1994).

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

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

58

59 **2. Materials and Methods**

60 **2.1. Surveillance of human *Salmonella* infections.**

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

67

68 **2.2. Frequency of *Salmonella* carriage in reptiles.**

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

75 Cloacal swab samples were collected and kept at room temperature ($26 \pm 4^\circ\text{C}$) for a
76 maximum of 48 hr. Samples were incubated in 9 ml of buffered peptone water for 16-20 hr at
77 37°C . Three drops of the pre-enrichment broth were inoculated onto specific medium
78 (Hektoen agar, BioMérieux, Marcy L'Etoile, France) and incubated at 37°C for 21-27 hr.
79 Lactose-negative colonies were subjected to the urease test and incubated at 37°C for 21-27
80 hr. When urease was negative (suspected *Salmonella*), biochemical identification of an
81 isolated colony was pursued with the API 20E Kit (BioMérieux). Isolates were sent to the
82 FNRC-Salm (except subspecies *arizonae* determined by the API 20E kit) for serotyping
83 according to the White–Kauffmann–Le Minor scheme. All strains underwent antimicrobial-
84 susceptibility testing with the disk-diffusion method on Mueller–Hinton agar (Bio-Rad,
85 Marnes-La-Coquette, France) against 16 antimicrobials (amoxicillin, streptomycin,
86 spectinomycin, azithromycin, kanamycin, netilmicin, amikacin, tobramycin, gentamicin,
87 nalidixic acid, ofloxacin, ciprofloxacin, chloramphenicol, sulfonamides, trimethoprim and
88 tetracycline), according to the guidelines of the French Society for Microbiology (available at:
89 <http://www.sfm-microbiologie.org/>).

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

93

94 **3. Results**

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

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

99 30.4), ranging between 14.3% and 32.4%, depending on the type of reptile (Table 1), but did
100 not differ significantly ($p = 0.5$). Habitat was significantly associated with *Salmonella*
101 carriage ($p < 0.001$), with frequencies of 5.8%-39.6%. The frequency of carriage was higher
102 for reptiles captured in forests and caged/contained in or around private homes.

103 *S. enterica* subspecies represented 47.2% (17/36) of all isolates (Table 2). Serotyped strains
104 (23/36) belonged to both subspecies *enterica* (17/23, 73.9%) and *houtenae* (6/23, 26.1%) and
105 were susceptible to all antibiotics tested. One lizard was co-infected with two *enterica*
106 subspecies serotypes, Newport and Infantis. Fourteen different serotypes were isolated. The
107 most common serotypes were Anatum (3/23, 13.0%), Rubislaw (13.0%) and subspecies
108 *houtenae* serotype 50:g,z₅₁:– (13.0%). At least three different serotypes were observed for
109 each reptile class except for caimans, which hosted only one.

110 In 2011, the FNRC-Salm received 154 human *Salmonella* acquired in French Guiana (87
111 isolates from Cayenne, 35 from Saint-Laurent-du-Maroni area, 30 from Kourou and two
112 additional from Metropolitan France and Martinique Island, French West Indies). Of the 154,
113 139 (90.3%), 14 (9.1%) and 1 (0.6%) belonged, respectively, to *enterica*, *houtenae* and
114 *diarizonae* subspecies (Table 3). Frequently isolated serotypes among *enterica* subspecies
115 were Panama (18/139, 12.9%), Oranienburg (11/139, 7.9%), Saintpaul (11/139, 7.9%) and
116 Uganda (10/139, 7.2%) and the *houtenae* subspecies 50:g,z₅₁:– serotype (10/14, 71.4%).

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

119

120 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

146 be sources of stress, inducing the onset of symptoms and *Salmonella* excretion (Friendship et
147 al., 2009; Maciel et al., 2010). *Salmonella* frequency of carriage by reptiles caged/contained
148 in or around private homes was high at 38.1% which tends to confirm higher frequency of
149 contamination in wild reptiles. All of the latter had been caught in their natural environment
150 and were captive for < 6 months. A limitation in our study is that each reptile type was not
151 sampled in all habitats, e.g., green iguanas were sampled only at the zoo. However,
152 considering all reptiles sampled, the forest dwellers were, on the whole, fairly well-
153 represented (31.8%), in contrast to most of studies that focused on captives (Sá and Solari,
154 2001; Pedersen et al., 2009).

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

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

168 We observed high serotype diversity among reptile samples obtained around Cayenne, with
169 identification of 14 different serotypes among the 23 isolates sent to the FNRC-Salm. At least
170 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.

187

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.

195

196 **Conflict of interest statement**

197 We declare that we have no conflicts of interest.

198 **Authors contributions**

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

202

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210

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290

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 2Distribution 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.