Herpetology Notes, volume 3: 263-265 (2010) (published online on 30 August 2010)

Note on the intestinal bacterial populations of free living snakes in Italy

Ernesto Filippi[1]*, Gian Lorenzo D'Alterio[2], Alberto Brozzi[3], Michela Micci[3], Pietro Politi[4] and Diego Mantero[4]

As part of a survey of the herpetofauna in the "Riserva Naturale Regionale Selva del Lamone" protected area (Lazio Region, Central Italy), a bacteriological pilot study was carried out between March 23rd and June 10th 2008. Thereby, eleven snake specimens were captured and screened for cloacal pathogens.

The study area is located in Northern Lazio, at the border with Tuscany, and is characterized by a rich and diverse herpetofauna (Filippi et al., 2008). Distributed over a plain area (350 m a.s.l.) the dominant vegetation type consists of Turkey oak (*Quercus cerris*) with other deciduous trees, shrubs, arable land and pasture. The study area was surveyed for snakes along standardised routes, covering various micro-habitats. Snakes were captured by hand, and several parameters were recorded: a) date, site (GPS coordinates) and micro-habitat; b) species; c) sex; d) snout-vent length and tail length.

Out of 77 captured snakes (see Table 1) eleven specimens belonging to three snake species were sampled by cloacal swab for bacteriological analysis: 7 Asp Vipers (*Vipera aspis)*, 3 Western whip snakes (*Hierophis viridiflavus*), 1 Aesculapian snake (*Zamenis longissimus*).

Briefly, swabs were inoculated onto Brain Heart Infusion broth and incubated at 30 +/- 1 C° and 37 +/- 1 C° for 24 hours. Broths were then transferred onto blood and agar incubated as above. Colonies were identified by Gram's staining oxidase test, by inoculation onto Triple Sugar Iron and by API 20E system (Bio-Meurieux). Colonies identified as *Salmonella* spp. were then typified by the autoagglutination saline test, the polyvalent agglutination antisera test (A-O67) and by serological testing following the Kauffmann and White scheme (Carter and Cole, 1990; Holt et al., 1994; OIE, 2008).

The results of the bacteriological examination of the cloacal swabs and other data are reported in Table 2. All sampled snakes tested positive for a mixed Gram-negative bacterial flora. However, three *V. aspis* and one *Hierophis viridiflavus* yielded positive results for *Salmonella enterica diarizonae IIIb*.

Many reptiles harbor a mixed Gram-negative bacterial flora in their cloaca, and this presence is difficult to interpret. Bacteria, such as *Pseudomonas* spp., *Klebsiella* spp., *Serratia* spp. and *Providencia* spp., all of which have been isolated in this study, are all

1 Piazza Capri 20, 00141 Rome, Italy;
e-mail: ernesto.filippi@fastwebnet.it
2 Ambulatorio Veterinario Farnese, Via Circonvallazione snc 01010 Farnese (Viterbo), Italy;
e-mail: gianlorenzod@yahoo.it
3 Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Strada Terme 01100 Viterbo, Italy;
e-mail: alberto.brozzi@izslt.it; michela_micci@yahoo.it
4 Riserva Naturale Regionale Selva del Lamone, Corso Vittorio Emanuele III 395 - 01010 Farnese (Viterbo), Italy;
e-mail: lamone2005@libero.it
*Corresponding author

Table 1. Sampled snake population divided by species.

Species	***Hierophis viridiflavus***	***Zamenis longissimus***	***Elaphe quatuorlineata***	***Natrix natrix***	***Natrix tessellata***	***Vipera aspis***
Number of specimens	40	6	2	4	2	23

Table 2. Date, species, sex and size (Svl: snout-vent length (cm); Tl: tail length (cm)) and results of bacteriological analysis of cloacal swab

Date	Species	Sex	Svl	Tl	Isolates
23 March	*V. aspis*	M	50	62	*Hafnia alvei; Salmonella enterica* sub. *diarizonae*
30 April	*H. viridiflavus*	F	90	122	*Serratia liquefaciens; Citrobacter* spp.*; Morganella morganii; Buttiaux agrestis*
30 April	*H. viridiflavus*	F	71	99	*Serratia liquefaciens; Citrobacter* spp.*; Morganella morganii; Buttiaux agrestis; Lactococcus lactis*
30 April	*V. aspis*	F	40	46	*Serratia liquefaciens; Citrobacter* spp.*; Morganella morganii; Buttiaux agrestis*
30 April	*V. aspis*	M	54	63	*Pseudomonas fluorescens; Serratia liquefaciens; Citrobacter* spp.*; Morganella morganii; Buttiaux agrestis*
07 May	*V. aspis*	M	43	47.5	*Klebsiella pneumoniae; Serratia liquefaciens; Citrobacter* spp.
07 May	*V. aspis*	F	57	--	*Serratia liquefaciens; Citrobacter* spp.
07 May	*Z. longissimus*	M	94	117	*Serratia liquefaciens; Citrobacter* spp.
10 June	*V. aspis*	F	46	51.5	*Escherichia coli; Corynebacterium* sp.*; Providencia rettgeri; Salmonella enterica sub. diarizonae*
10 June	*V. aspis*	M	49	56	*Salmonella enterica* sub. *diarizonae; Providencia rettgeri;*
10 June	*H. viridiflavus*	F	84	111	*Salmonella enterica* sub. *diarizoane; Providencia rettgeri; Corynebacterium* spp.*; Pasteurella pneumotropica*

considered opportunistic pathogens in reptiles, and their presence in association with overt clinical signs should be considered as significant (Rosenthal and Mader, 1996).

Salmonella enterica has been previously isolated from free-living snakes (Malmqvist et al., 1995; Geue and Löschner, 2002; Briones et al., 2004; Köbölkuti et al., 2009), captive snakes (Kennedy, 1973; Chiodini and Sundberg, 1981; Geue and Löschner, 2002; Schroter et al., 2004) and their environment in captivity (Bauwens et al., 2006), including pet snakes in Italy (Ebani et al., 2005).

However, to the best of the authors' knowledge these are the first reported *Salmonella* isolates from wild specimens of *Vipera aspis* and *Hierophis viridiflavus*.

Salmonella species are potential pathogens for reptiles in general (Funk, 1996), and *Salmonella enterica diarizonae* is a rarely reported zoonotic agent (Bruins et al., 2006).

The present study, which was carried out by conservation biologists and veterinary health professionals, highlighted a potential biological threat to the snakes under survey. Additionally, the role of wild snakes as a reservoir of *Salmonella* species was

confirmed, hence the need to apply strict hygienic precautions when handling them.

Considering the decline of many snake populations (Reading et al., 2010) and that the spreading of diseases is considered to be one of the causes for the decline in reptiles in general (Gibbons et al., 2000), it is important to remember that "today's commensal may be tomorrow's pathogen" (Cooper, 2000).

Acknowledgements. We thank the whole staff of Riserva Naturale Regionale Selva del Lamone for their assistance in the field. M. Capula critically reviewed an earlier version of this paper. The work of one of the authors (EF) was supported by Riserva Naturale Regionale Selva del Lamone- Regione Lazio (projects No 295-384/2007). No animals were damaged or killed during the present project, and all were handled according to the international scientific standards.

References

Bauwens, L., Vercammen, F., Bertrand, S., Collard, J.M., De Ceuster, S. (2006): Isolation of *Salmonella* from environmental samples collected in the reptile department of Antwerp zoo using different selective methods. J. Appl. Microbiol. **101**: 284-9.

Briones, V., Téllez, S., Goyache, J., Ballesteros, C., Pilar Lanzarot, M., Domínguez, L., Fernández-Garayzábal, J. F. (2004): *Salmonella* diversity associated with wild reptiles and amphibians in Spain. Envir. Microbiol. **6**: 868-871.

Bruins, M.J., de Boer, A.M., Ruijs, G.J. (2006): Gastroenteritis caused by *Salmonella* from pet snakes. Ned. Tij. Gen. **150**: 2266-9.

Carter, G. R., Cole G. R (Eds) (1990): Diagnostic Procedures in Veterinary Bacteriology and Mycology. San Diego, California, Academic Press Inc.

Chiodini, R.J., Sundberg, J.P., 1981. Salmonellosis in reptiles: a review. Am. J. Epidemiol. **113**: 494–499.

Cooper, J.E. (2000): Reptilian microbiology. In: Laboratory Medicine: avian and exoticpets, p. 223-228. Fudge, A.M., Ed., Philadelphia, W.B. Saunders Company.

Ebani, V.V., Cerri, D., Fratini, F., Meilli, N., Valentini, P., Andreani, E. (2005): *Salmonella enterica* isolates from faeces of domestic reptiles and a study of their antimicrobial in vitro sensitivity. Res. Vet. Sci. **78**: 117-21.

Filippi, E, Baragliu, G.A., Biselli, F., Ceccarini, A., Ciavatta, D., De Parri, I., Fapperdue,G., Mantero, D., Marchionni, F., Politi, P., Sschiavano, A., Terazzi, A. (2008): Check-list of the herpetofauna inhabiting the Riserva Naturale Regionale Selva del Lamone (Viterbo-Latium): new important records in central Italy. In:Herp. Sard., p. 270-273. Corti, C., Ed., Latina, Ed. Belvedere.

Funk, R.S. (1996): Diarrhea. In: Reptile Medicine and Surgery, p. 322-325. Mader, D.R., Ed., Philadelphia, W.B. Saunders Company.

Geue, L., Löschner, U. (2002): *Salmonella enterica* in reptiles of German and Austrian origin. Veterinary Microbiology. 84: 79-91.

Gibbons JW, Scott, D.E, Ryan, T.J., Buhlmann, K.A., Tuberville, T.D., Metts, B.S., Greene, J.L., Mills, T., Leiden, Y., Poppy, S., Winne, C.T. (2000): The global decline of reptiles, déjà vu amphibians. BioScience **50**: 653-666.

Holt J. G., Krieg N. R., Sneath P. H. A., Staley J. T., Williams S. T. (Eds). Bergey's Manual of Determinative Bacteriology, 9th Edition, Baltimore, USA Williams & Wilkins.

Kennedy, M.E. (1973): *Salmonella* isolations from snakes and other reptiles. Can. J. comp. Med. **37**: 325-326.

Köbölkuti, L., Spinu, M., Cadar, G., Czirjak,G., Kiss, T. (2009): Classical and molecular monitoring of the prevalence of *Salmonella* spp. carriage in freeliving and captive native snakes in Romania. Lucrari Stiinifice Med. Vet. **XLII**: 294-297.

Malmqvist, M., Jacobsson, K.G., Häggblom, P., Cerenius, F., Sjöland, L., Gunnarsson, A. (1995): *Salmonella* isolated from animals and feedstuffs in Sweden during 1988-1992. Acta Vet. Scand. **36**: 21-39.

OIE (2008): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th Edition, Paris, OIE - World Organisation for Animal Health.

Reading, C. J., Luiselli, L.M., Akani, G.C., Bonnet, X, Amori, G., Ballouard, J.M., Filippi, E., Naulleau, G., Pearson, D., Rugiero, L. (2010): Are snake populations in widespread decline? Biology Letters: in press.

Rosenthal, K.L., Mader, D.R. (1996): Microbiology. In: Reptile Medicine and Surgery, p. 117-125. Mader, D.R., Ed., Philadelphia, W.B. Saunders Company.

Schröter, M., Roggentin, P., Hofmann, J., Speicher, A., Laufs, R., Mack, D. (2004): Pet Snakes as a Reservoir for *Salmonella enterica* subsp. *diarizonae* (Serogroup IIIb): a Prospective Study. Appl. Environ. Microbiol. **70**: 613–615.

Accepted by ***Zoltán T. Nagy***