ISSN 1517-6770

Scientific Note

Blood samples from *Liophis typhlus* (Linnaeus, 1758) (Serpentes, Dipsadinae) by cardiac puncture without surgical procedures

Alyssa Rossi Borges[1]; Celso Henrique Varela Rios[1,2]; Moara Lemos[3]; Usha Vashist[4]; Bernadete Maria de Sousa[1,5] & Marta D'Agosto[1,6]

[1]*Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, 36036-900, Juiz de Fora, Minas Gerais, Brasil. E-mail: alyssaborges@gmail.com*

[2]*E-mail: celso-rios@bol.com.br*

[3]*Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Campus Ilha do Fundão, 21941-590, Rio de Janeiro, Brasil. E-mail: moara.lemos@gmail.com*

[4]*Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas, 37890-000, Muzambinho, Minas Gerais, Brasil. E-mail: usha.vashist@eafmuz.gov.br*

[5]*E-mail: bernadete.sousa@ufjf.edu.br*

[6]*E-mail: marta.dagosto@ufjf.edu.br*

Abstract. The challenge for wildlife researchers is the appropriate choice of blood sampling methods that do not cause large stress or harm to animals. The most frequent blood sampling method in snakes is caudal venipuncture, but it has disadvantages, like the risk of vein collapse of small specimens and the risk of traumatizing the cloacal musk glands of females and the hemipenis of males, causing prolapse of these organs. The blind ground snake *Liophis typhlus* is poorly documented regarding anatomic, haematological and parasitological aspects. The aim of this study is to present a new protocol to sample blood from *L. typhlus* that is easy to perform, reliable in terms of quantity, and allows the animal to survive without causing undue damage or stress.

Keywords: Anatomy, blood, Brazil, snake, wild animals.

Resumo. Amostras de sangue de Liophis typhlus (Linnaeus, 1758) (Serpentes, Dipsadinae) por punção cardíaca, sem procedimentos cirúrgicos. O desafio para os pesquisadores que trabalham com animais silvestres é a escolha apropriada do método de coleta sanguínea que não cause grande estresse ou danos aos animais. O método de coleta mais utilizado para serpentes é a punção da veia caudal, mas ele possui desvantagens, como o risco de colabamento da veia de animais de pequeno porte e o risco de traumatizar a glândula caudal de fêmeas e o hemipenis de machos, ocasionando o prolapso desses órgãos. *Liophis typhlus* é pobremente documentada com relação aos aspectos anatômicos, hematológicos e parasitológicos. O objetivo deste trabalho é apresentar um novo protocolo para coletar sangue de *L. typhlus* que é fácil de realizar, fornece uma quantidade suficiente de sangue e permite a sobrevivência do animal sem causar danos ou estresse desnecessários.

Palavras-chave: Anatomia, sangue, Brasil, serpente, animais selvagens.

There is no better way to make a diagnosis than laboratory evaluation of the blood (Mader & Rudloff, 2005). Therefore, the challenge for wildlife researchers is the appropriate choice of blood sampling methods that do not cause large stress or harm to animals (Mader & Rudloff, 2005; Neves Júnior *et al.*, 2006). The execution of studies which require knowledge about reptiles' anatomy is hindered mainly by the lack of appropriate literature and the fact that most of the illustrative texts published cover

European and North American species (Gomes *et al.*, 1989).

The most frequent blood sampling in midsize and large snakes is caudal venipuncture (Almosny & Monteiro, 2007), but like other sampling methods, it has disadvantages. Among the difficulties found in caudal vein venipuncture is the risk of vein collapse of small specimens due to the difficulty of finding small range needles and syringes which do not cause too large a vacuum for the vein range and difficulties for large animal containment, since snakes need to be totally immobilized for success of the procedure (Neves Júnior *et al.*, 2006). In addition, this technique does not allow repetitive sampling, there is a risk of traumatizing the cloacal musk glands of females and the hemipenis of males, causing prolapse of these organs (Bush & Smeller, 1978; Hernandez-Divers, 2005). Additionally, some species, such as *Liophis typhlus* (Linnaeus, 1758) (Colubridae), produce cloacal discharge when handled (Marques *et al.*, 2005), which could cause sample contamination.

Cardiac puncture is an effective method to take blood samples from these reptiles, since the tolerance of snakes to cardiac puncture allows several repeated samplings in the tissue (Bush & Smeller, 1978; Mayer & Martin, 2004; Kolesnikovas *et al.*, 2007), provides a larger quantity of blood (Bush & Smeller, 1978), presents a lower risk of sample contamination, and might be applied in snakes of all sizes (Hernandez-Divers, 2005).

The blind ground snake *Liophis typhlus* is a small South American snake, up to 50 cm of snout-vent length and 100 g of body mass (Marques *et al.*, 2005), that is distributed from Colombia and Venezuela to northern Argentine (Forlani *et al.*, 2010). Although this species has wide distribution in the Brazilian Atlantic Rainforest and Cerrado (Sousa *et al.*, 2010), it is rarely captured in field traps (Marques *et al.*, 2005) and is poorly documented regarding anatomic, haematological and parasitological aspects.

The aim of this communication is to present a new protocol to sample blood from *L. typhlus* that is easy to perform, reliable in terms of quantity, and allows the animal to survive without causing undue damage or stress.

The *Liophis typhlus* specimens (N = 4), two males and two females, used to develop this protocol were collected by pitfall and funnel traps in the Serra de São José Environmental Protection Area, in the municipality of Tiradentes, Minas Gerais state, Brazil. The snakes were kept in individual glass aquariums (60 x 35 x 45 cm) and were fed with two species of toads: *Proceratophrys boiei* (Wied-Neuwied, 1825) (Cycloramphidae) and *Odontophrynus cultripes* Reinhardt and Lütken 1861"1862" (Cycloramphidae), with water supplied *ad libitum*. The measures of snout-vent length (from rostral scale up to cloacal opening) were made with millimetre tape and were averaged. Two specimens, a male and a female, were deposited in the collection of the Herpetology Laboratory of Juiz de Fora Federal University. The first specimen (snake 1) was deposited in the collection and used to check the anatomic position of the heart and the other three specimens were subjected to heart puncture.

The heart position and the relation establishment between heart position and the number of ventral shields (topographical anatomy) were determined according to Gomes & Puorto (1993). A longitudinal ventral incision was performed on the body of Snake 1, going from the cloacal opening up to the cranial region of the heart, to determine the anatomic position of the heart. Heart position in live specimens was identified by pulse detection (Mayer & Martin, 2004) and ventral shield counting.

The asepsis of the puncture site was made with 100% ethyl alcohol and disposable syringes (1 ml) with hypodermic needle (13 x 4.5) were used to perform the cardiac puncture. The snakes were observed daily for 30 days after blood sampling for possible reactions arising from heart puncture.

The studied specimens had snout-vent length averaging 46.75 ± 4.89 cm and one of the female specimens (snake 4) was ovate. The heart was located on the left side of the ventral shields, from 22 up to 25. The highest intensity pulse occurred at ventral shields 24 and 25. Among living specimens, only snake 4 showed variation in pulse detection, presenting slight movement of shield 26. Snakes 2 and 3 showed pulse from ventral shields 22 up to 25, as expected by the anatomical study of the necropsied specimen (snake 1).

To sample the blood, the needle was superficially inserted between ventral shields 24 and 25, in the opposite direction to them (Fig. 1). It was possible to draw 100 µl of blood from each specimen, which is enough for parasitological, haematological and molecular studies.

All three snakes presented satisfactory recovery, without indicating reactions of cardiac tamponade, and fed normally in the subsequent days. Stress signs, such as intense movement in terrariums, were observed before and after cardiac puncture. Snake 4 was ovate at the moment of heart puncture and laid five eggs normally.

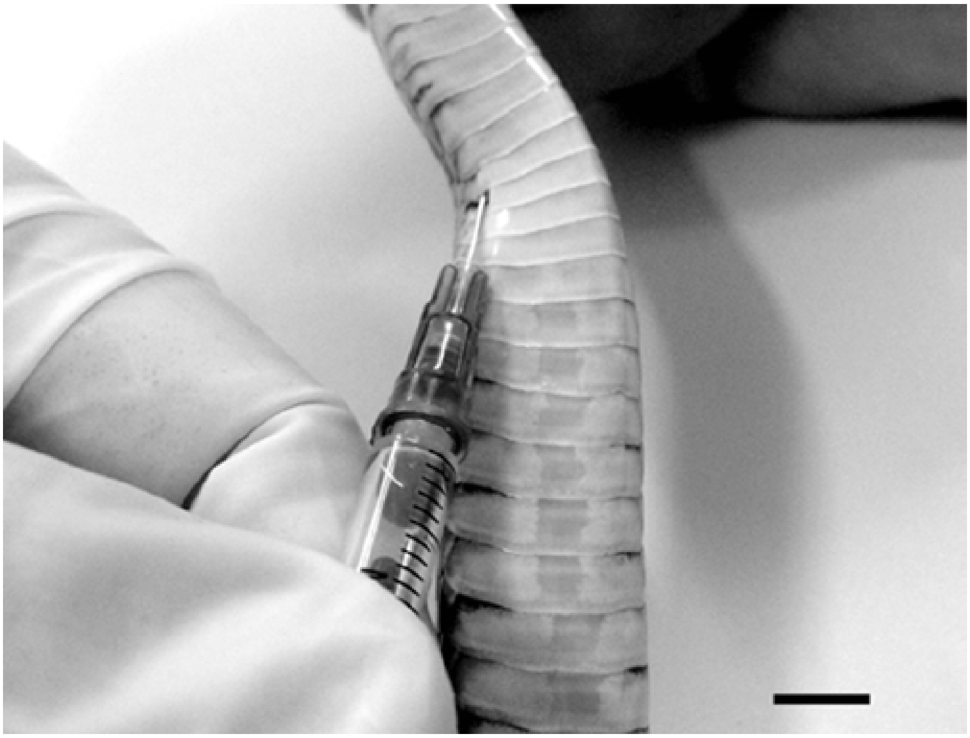

Figura 1. Position of needle insertion to draw blood samples by cardiac puncture from *Lyophis typhlus*. Scale bar = 10 mm.

Significant variations on the anatomy of snakes only occur among different habits and distinct phylogenetic positions (Funk, 2005). In this study, we verified variations in pulse detection in one snake, possibly attributed to the differences in body length, which was also verified in *Bothropoides jararaca* (Wied, 1824) (Viperidae) by Gomes & Puorto (1993). According to Bush & Smeller (1978), cardiac puncture is a technique that demands more time than caudal vein venipuncture because it requires surgery. Nevertheless, this protocol shows that it is possible to perform the technique without surgical procedures.

The fact the snakes did not show change in behaviour in terrariums before the cardiac puncture and the snake 4 laid viable eggs suggests that this method, although invasive, did not stress or cause substantial damage to the animals. Therefore, this method is effective and quick for improved blood sampling of *L. typhlus*. This will enable the performance of haematological, serological and parasitological studies on these animals and might be applied for other *Liophis* species.

Acknowledgments

This work was performed according to the Brazilian College of Animal Experimentation – COBEA (protocol number 010/2008) and with the permission of the Brazilian Institute of the Environment and Natural Resources – IBAMA/SISBIO (number 17917-1) and the State Institute of Forests – IEF (number 118/09). We thank CNPq for support for this work and Waldyr Imbroisi Rocha and Karlijn Willems for reviewing this manuscript.

References

Almosny, N. R. P. & Monteiro, A. O. 2007. Patologia clínica, pp. 939-966. *In*: Cubas, Z. S.; Silva, J. C. R. & Catão-Dias, J. L. (eds.). **Tratado de animais selvagens**. São Paulo, Roca. 1376p.

Bush, M., & J. Smeller. 1978. Blood collection and injection techniques in snakes. **Veterinary Medicine and Small Animal Clinician 73** (2): 211-214.

Forlani, M. C.; Bernardo, P. H.; Haddad, C. F. B. & Zaher, H. 2010. Herpetofauna do Parque Estadual Carlos Botelho, São Paulo, Brasil. **Biota neotropica 10** (3): 265-309.

Funk, R. S. 2005. Snakes, pp. 42-58. *In*: Mader, D. R. (ed.). **Reptile medicine and surgery**. Saint Louis, Saunders Elsevier. 1264p.

Gomes, N.; Puorto, G.; Buononato, M. A. & Ribeiro, M. F. M. 1989. Atlas anatômico de *Boa constrictor* Linnaeus, 1758 (Serpentes, Boidae). **Monografias do Instituto Butantan 2**: 1-59.

Gomes, N., & Puorto, G. 1993. Atlas anatômico de *Bothrops jararaca* Wied, 1824 (Serpentes: Viperidae). **Memórias do Instituto Butantan 55** (1): 69-100.

Hernandez-Divers, S. J. 2005. Diagnostic techniques, pp. 490-532. *In*: Mader, D. R. (ed.). **Reptile medicine and surgery**. Saint Louis, Saunders Elsevier. 1264p.

Kolesnikovas, C. K. M.; Grego, K. F. & Albuquerque, L. C. R. 2007. Ordem Squamata – subordem Ophidia (Serpente), pp. 68-85. *In*: Cubas, Z. S.; Silva, J. C. R. & Catão-Dias, J. L. (eds.). **Tratado de animais selvagens**. São Paulo, Roca. 1376p.

Mader, D. R. & Rudloff, E. 2005. Emergency and critical care, pp. 533-548. *In*: Mader, D. R. (ed.). **Reptile medicine and surgery**. Saint Louis, Saunders Elsevier. 1264p.

Marques, O. A. V.; Eterovic, A.; Strüssmann, C. & Sazima, I. 2005. **Serpentes do Pantanal - guia ilustrado**. Ribeirão Preto, Holos, I + 179p.

Mayer, J., & Martin, J. C. 2004. Reptile care in the animal shelter, pp. 177-188. *In*: Miller, L. & Zawistowski, S. (eds.). **Shelter medicine for veterinarians and staff**. Arnes, Blackwell Publishing. 744p.

Neves Júnior, J. M.; Machado, R. R. C.; Pires, J. R.; Lauriggio, A. J.; Passos, A. A. M. S. & Couto, A. L. G.. 2006. Descrição de uma nova técnica para punção venosa em serpentes, através do estudo em Jibóias (*Boa constrictor*). **Revista da Universidade Rural, Série Ciências da vida 26** (Supl.): 37-38.

Sousa, B. M.; Nascimento, A. E. R.; Gomides, S. C.; Varela Rios, C. H.; Hudson, A. H. & Novelli, I. A. 2010. Répteis em fragmentos de Cerrado e Mata Atlântica do Campo das Vertentes, Estado de Minas Gerais, Sudeste do Brasil. **Biota neotropica 10** (2):129-138.

Recebido: 02/01/2013
Revisado: 01/11/2013
Aceito: 05/12/2013