http://dx.doi.org/10.1590/1678-4162-9010

Arq. Bras. Med. Vet. Zootec., v.69, n.2, p.415-421, 2017

Gross and histologic features of gastritis due to *Ophidascaris arndti* in tropical rattlesnakes (*Crotalus durissus*)

[*Alterações macro e microscópicas da gastrite associadas com* Ophidascaris arndti *em cascavéis (*Crotalus durissus*)*]

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ABSTRACT

The tropical rattlesnake (*Crotalus durissus*) is a snake of great importance for biomedical industry since its poison is used for the production of antiophidic serum and researches. Several conditions related to animal health, such as diseases and parasites, which can promote the reduction of poison production by these snakes should be investigated. Accordingly, the aim of this study was to characterize the gross and microscopic lesions related to the presence of *Ophidascaris arndti* in stomachs of tropical rattlesnakes. The gastrointestinal tract of thirty-five South American rattlesnakes captured in Southeastern region of Brazil were analyzed and nineteen animals showed infestation by the parasites, found in the small intestine and, especially, in the stomach of the hosts. Grossly, lesions were characterized by mucosal ulcers occasionally associated with hemorrhage. Microscopic alterations included histiocytic granulomas, fibrosis, necrosis, and hemorrhage. Based on these findings, the diagnosis of a parasitic granulomatous disease was made. The lesions may be related to the cause of death in captivity snakes, since the lesions can promote secondary infections by opportunistic bacteria. Moreover, the intense inflammatory response accompanied by *fibrosis* may be related to poor functioning of the gastric snakes, which it may exhibit frequent regurgitation of the food.

Keywords: snake, parasitic granuloma, parasitic gastritis, Nematoda

RESUMO

A cascavel (Crotalus durissus) *é uma serpente de grande importância para a indústria biomédica, uma vez que seu veneno é utilizado para a produção de soro antiofídico e para pesquisas. Condições diversas relacionadas ao estado de saúde do animal, como doenças e parasitoses, que possam promover a diminuição da produção de veneno por essas serpentes, devem ser investigadas. O objetivo deste estudo foi caracterizar os achados macro e microscópicos relacionados à presença de nematódeos* Ophidascaris arndti *nos estômagos de cascavéis. O trato gastrointestinal de 35 cascavéis capturadas na região Sudeste do Brasil foi avaliado Dessas, 19 apresentavam infestação pelo parasito, que foi encontrado predominantemente no estômago e no intestino delgado dos hospedeiros. Macroscopicamente, as lesões foram caracterizadas por úlceras nas camadas mucosas, ocasionalmente associadas com hemorragia. As alterações microscópicas apresentaram granulomas histiocíticos, fibrose, necrose e hemorragia. Baseado em tais achados, o diagnóstico de doença granulomatosa parasitária foi realizado. As lesões encontradas podem estar relacionadas à causa das mortes das serpentes em cativeiro, uma vez que podem favorecer infecções secundárias por bactérias oportunistas. Além disso, a intensa resposta inflamatória e a fibrose podem estar relacionadas ao mau funcionamento gástrico das serpentes, que podem apresentar frequente regurgitação do alimento.*

Palavras-chave: serpente, granuloma parasitário, gastrite parasitária, Nematoda

Recebido em 25 de julho de 2016
Aceito em 27 de julho de 2016
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INTRODUCTION

Crotalus durissus (Squamata, Viperidae) is a pit viper species, known as South American rattlesnake or tropical rattlesnake, that poses as a serious problem in medicine and veterinary medicine. Specimens kept in captivity are commonly used for production of immunobiologics and provision of samples (e.g., venom, tissues, and excreta) for research in different areas. A common problem for maintenance of such animals in captivity is the infestation by helminths which can interfere in venom production. Since quality and quantity of venom produced by snakes is related with health status, parasitic diseases can influence directly in venom extraction.

In Brazil, the nematode *Ophidascaris* (Ascaridida, Ascarididae) is commonly observed in reptiles, especially in snakes (Pinto *et al*., 2010). This parasite is generally encountered within the stomach, where they often embed deep in the submucosa and may be seen projecting from a single focal ulcerating lesion (Jacobson, 2007). Such parasites can cause mechanic obstruction, regurgitation, and perforations of viscera. Additionally, they can act as spoliators and promote an intense inflammatory response at fixation sites (Wilson and Carpenter, 1996; Jacobson, 2007).

Free-ranging reptiles are parasitized with a variety of endoparasites, but few reports associate parasitism with morbidity or mortality and there is a lack of histopathological data on injury caused by helminths in rattlesnakes. Accordingly, the aim of this study was to describe, macro and microscopically, the gastric lesions associated with the presence of *Ophidascaris* in rattlesnakes from Brazil.

MATERIALS AND METHODS

A total of 35 tropical rattlesnakes stranded in Minas Gerais State, Southeast Brazil, rescued by Ezequiel Dias Foundation – FUNED, Brazil, were donated from July 2011 to January 2012 to the Veterinary Parasitology Laboratory, Institute of Biological Sciences, Federal University of Minas Gerais – UFMG, Brazil, and euthanatized using 0.4 to 3.5mL of sodic pentobarbital intracelomically. Then, the snakes were submitted to necropsy. Animals which presented endoparasites with nematode-like appearance fixed at the mucosal surface of the stomach had their stomachs separated. One portion of parasitized areas in each animal was immersed in a 0.85% NaCl solution with subsequent storage at 4° C in order to allow removal of nematodes from the mucosal surface for subsequent evaluation. Then, the parasites were fixed in 10% neutral buffered formalin at 80°C. After fixation, six couples of parasites, of each rattlesnake, were immersed in Amman's lactophenol solution for seven days for clarification. This study was approved by the Ethics Committee in Animal Experimentation (CEUA/UFMG) under the protocol number 176/2012.

Parasites were then observed under light microscopy using a stereoscope and photographed using a digital camera. Morphometric analysis was performed and results were compared with previous descriptions of the species in order allow its characterization. Morphologically, specimens collected were identified as *Ophidascaris arndti* considering the presence of mouth with three square lips, interlabia present, claviform esophagus, esophageal ventriculus and intestinal cecum absent, two uterine branches, vulva more or less in the middle of the body, gubernaculum absent, equal or subequal and long spicules, 36 pairs (medium) of pre-cloacal papillae, one pair of ad-cloacal and four to six pairs of post-cloacal papillae (Freitas, 1968; Pinto *et al*., 2010).

Other parasitized portions of the stomach were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Then, 4μm sections were obtained and stained using hematoxylin and eosin and Gomori's trichrome. Subsequently, slides were evaluated under light microscopy.

RESULTS

From the 35 received rattlesnakes 19 presented endoparasites characterized as *O. arndti* (Figure 1). At necropsy, *Ophidascaris* specimens were prominently found within the stomach embedded at gastric mucosa (Figure 2A). Elevated ulcerated round lesions measuring 1-2 mm in diameter were detected in the mucosal surface (Figure 2B) after removal of parasites. On cut surfaces, the lesions were firm and extended to the submucosa. The anterior portion of the parasites could be frequently observed on the external serosal surface of the stomach (Figure 3). Additionally, worms were freely observed in the stomach cavity.

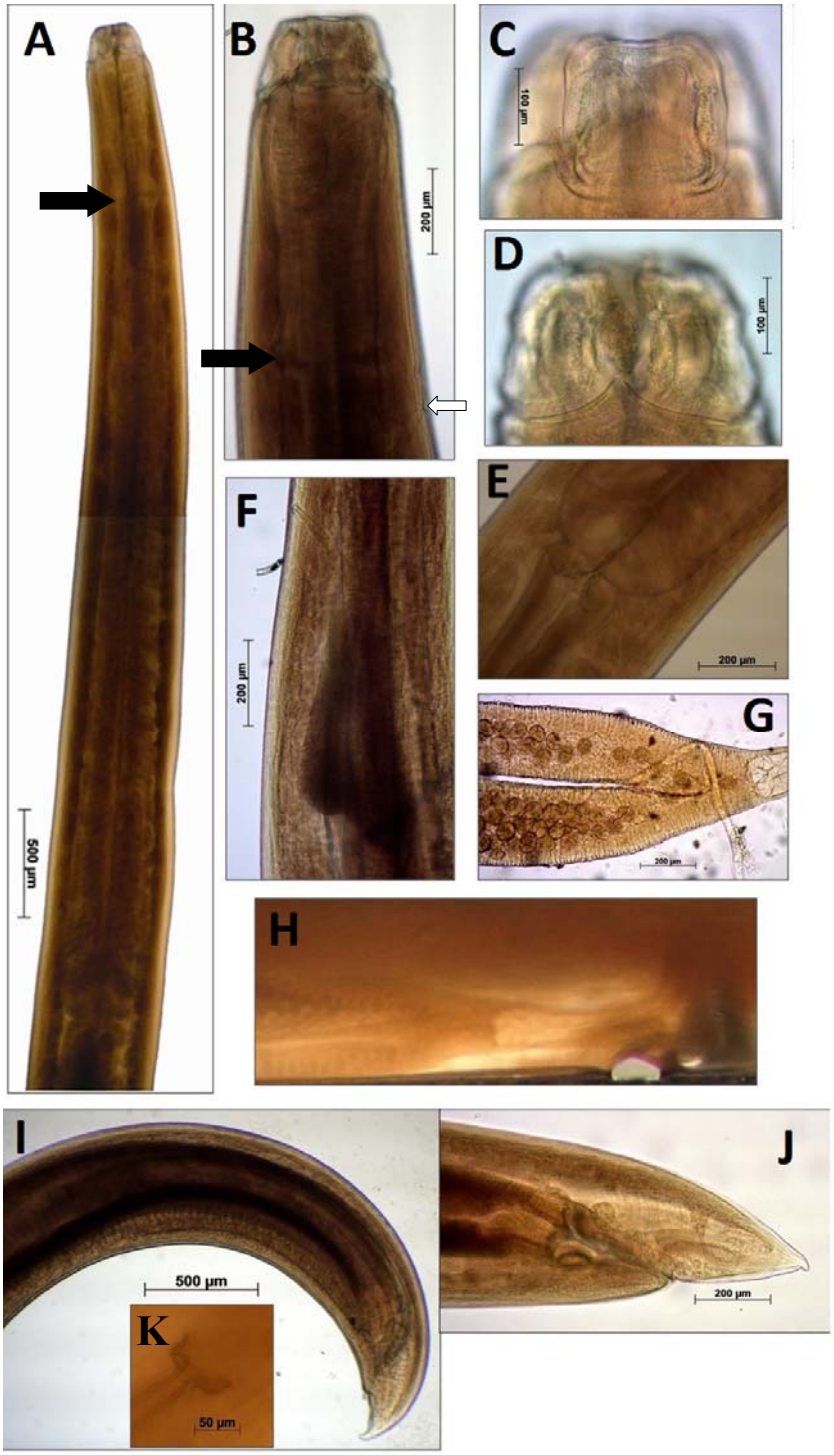

Figure 1. Morphologic aspects of *Ophidascaris arndti*. (A) Claviform esophagus and nerve ring (arrow); (B) nerve ring (black arrow) and cervical gland opening (white arrow); (C) dorsal lip; (D) interlabial groove; (E) esophago-intestinal junction; (F) cervical glands (G) uterine branches (H) ovejector; (I) blunt conical tail in male; (J) tale of a female; (K) spicule.

Figure 2A. *Ophidascaris arndti* embedded in gastric mucosa.

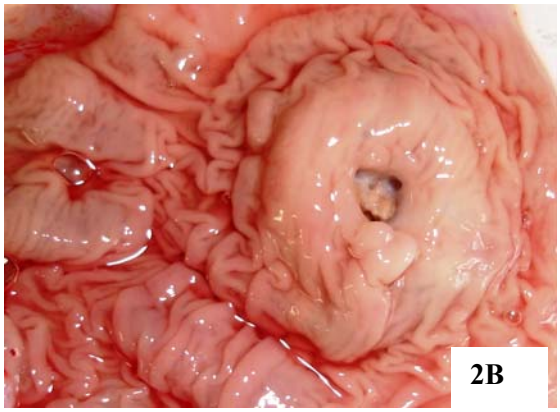

Figure 2B. Ulcers could be detected after removal of worms at areas of parasite embedding.

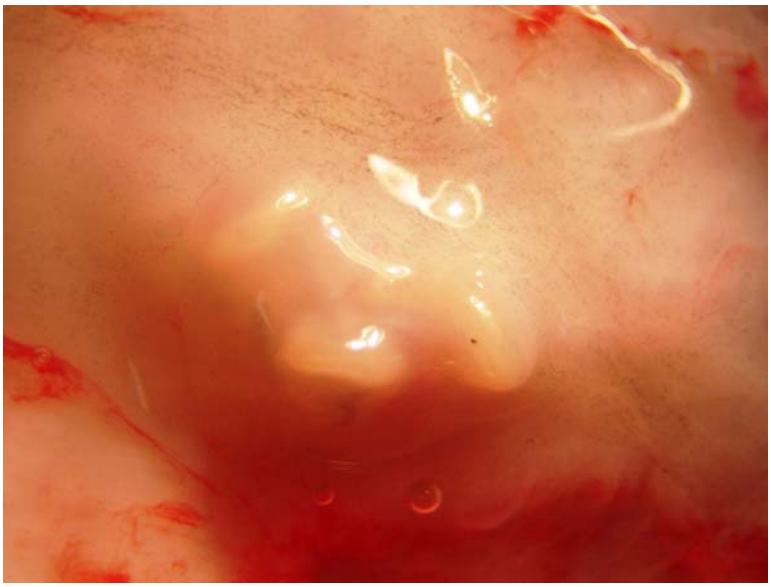

Figure 3. Stomach of a tropical rattlesnake parasitized by *Ophidascaris arndti*. Areas where the anterior portion of worms were detectable from the external surface of the organ.

All parasitized snakes presented a thickening of the gastric wall around insertion areas. Parasites usually were fixed in a focal region of the organ but two or more locations of fixation with fewer nematodes were occasionally detected. Animals with a higher parasite load showed a higher increase in thickening of the gastric wall.

Microscopically, multifocal inflammatory lesions composed of lymphocytes, macrophages, plasma cells, and epithelioid cells were observed in mucosal, submucosal, and muscular layers of the stomach (Figure 4), as well as fibrotic changes (Figure 5). Based on these findings, a diagnosis of multifocal granulomatous gastritis was made.

Few parasites were observed within the small intestine, particularly at the initial portion. Most of the nematodes were not fixed or only slightly fixed to the intestinal mucosa. No gross lesions were observed in the intestinal tissue.

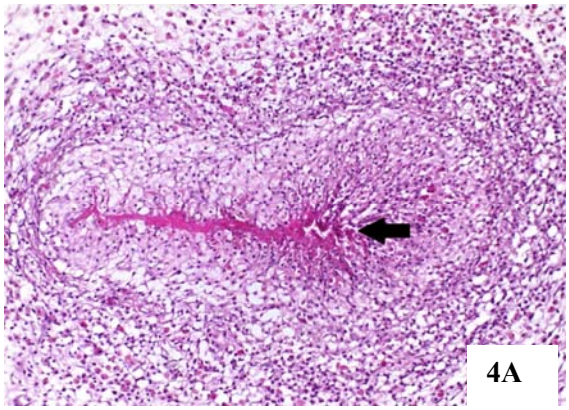

Figure 4A. Gastric histiocytic granuloma due to *Ophidascaris arndti* in a tropical rattlesnake. A) Round lesions with necrotic foci at the center (arrow) could be observed.

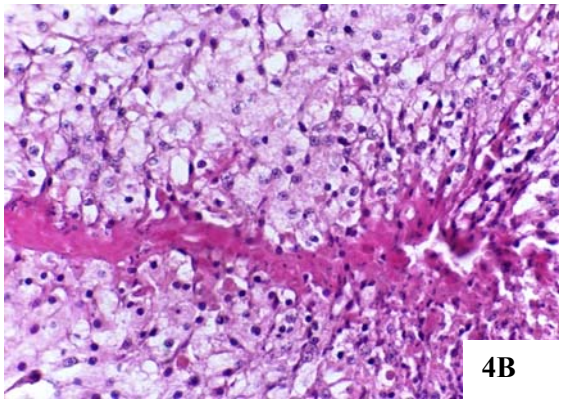

Figure 4B. Many epithelioid macrophages could be observed surrounding the necrotic foci. Hematoxylin and eosin (A = x200; B = x600).

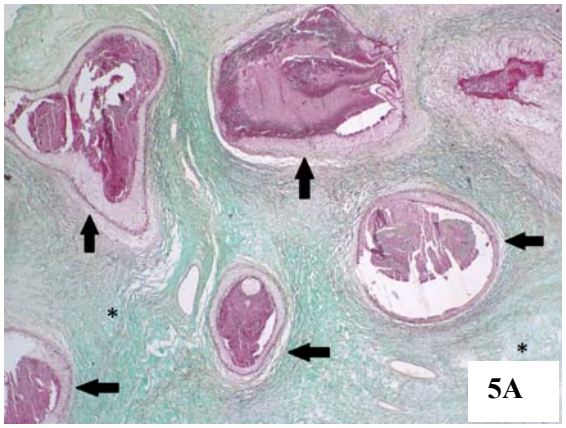

Figure 5A. Stomach of a tropical rattlesnake parasitized by *Ophidascaris arndti*. A and B) Several parasites can be observed (arrow) in association with severe fibrotic change (asterisk);

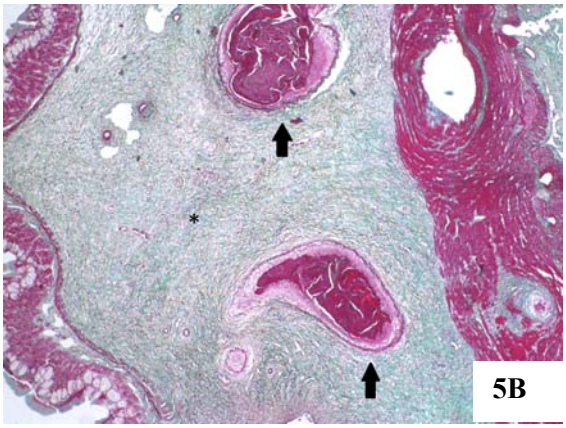

Figure 5B. Stomach of a tropical rattlesnake parasitized by *Ophidascaris arndti*. A and B) Several parasites can be observed (arrow) in association with severe fibrotic change (asterisk);

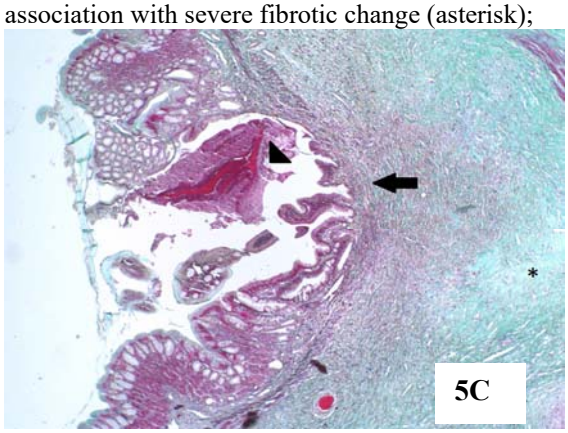

Figure 5C. Focal loss of gastric mucosa covering and mucus-producing glands can be observed (arrow) in association with fibrotic changes (asterisk). The anterior portion of the nematode can be visualized (arrowhead).

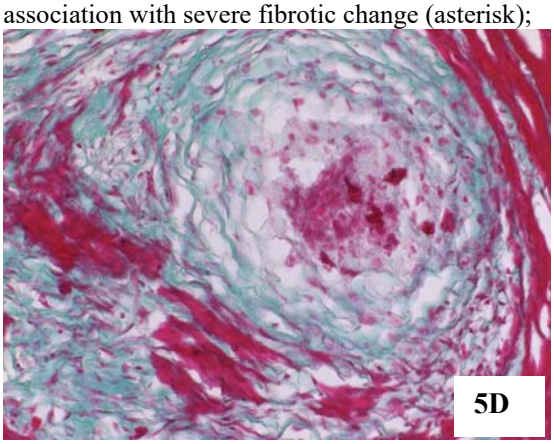

Figure 5D. Destruction of the muscular layer associated with inflammation. Gomori's trichrome stain (A, B, and C = x40; D, x400).

DISCUSSION

Wildlife parasitology is rarely approached from the veterinary medical point of view and reports of clinical parasitism in wild animals are scarce. Consequently, there is limited understanding of the pathogenic effects on the health of such animals. A useful method for assessing the negative effect of parasites on the health of wildlife or captive animals is the description of lesions, particularly at its microscopic level.

It is known that captive snakes are frequently distressed and develop different diseases in consequence, including parasitic conditions. Some authors describe the 'pooradaptation syndrome' related to captivity as the most important cause of mortality in snakes in the first year of breeding (Braz *et al*., 2012). This is an important feature considering that snakes develop remarkable susceptibility to pathogenic agents during this period.

Lesions caused by other *Ophidascaris* species in snakes are usually characterized as necro-ulcerative gastroenteritis (Elbihari and Hussein, 1973; Brar *et al*., 1990; Wilson and Carpenter, 1996, Anderson, 2000), in concordance with the changes observed in this study, in which prominent gross and microscopic alterations were detected in stomachs of the snakes since fixation of worms produced a direct damage to the gastric mucosa.

Reptile gastric mucosa produces a wide variety of enzymes and hydrochloric acid for digestion which is occurs by chemical and mechanical means. Additionally, there are several mucus-producing cells that are important for protection of the mucosa which is also very elastic, allowing distension of the organ and consequent storage of large volumes of food (Mitchell and Diaz-Figueroa, 2005).

It is possible that parasitism by *O. arndti* can interfere in gastric physiology because worm fixation to the mucosa can lead to destruction of glands, consequently leading to a decrease in mucus production and a higher susceptibility to ulcer formation and infection by opportunistic bacteria. This suggestion is reinforced by the microscopic evaluation of stomachs, which revealed histiocytic granulomas in submucosa and serosa of parasitized snakes. Histiocytic granulomas are typically differentiated from heterophilic granulomas by the composition of their necrotic centers and are usually induced by intracellular bacteria while heterophilic granulomas are usually related with extracellular organisms (Montalli, 1988). In addition to changing mucus secretions, a thickening of the gastric wall can be related to food regurgitation because the fibrosis could distort the muscular contraction capacity of the stomach.

The genus *Ophidascaris* have an indirect life cycle: the eggs are eliminated in environment through snake feces. The intermediary hosts (in general, rodents and amphibians) ingest the eggs containing the L2 larvae. The snakes eat the intermediary hosts with L2 larvae on the muscles or viscera, these will hatch, perforate the intestine of the definitive host and migrate to the lungs, where they will molt to L3 larvae. Then, these larvae migrate up the pharynx where they are swallowed along with bronchial secretions. In the stomach, molting occurs to L4 larvae and L5 (adults), which will penetrate firmly into the stomach wall, copulate and eliminate eggs to the environment again (Araujo and Machado, 1980; Anderson, 2000).

There are several different antiparasitics used in treating nematode infestation in snakes, as Fenbendazole (25 to 50mg/kg orally once a day for 5 days and repeated in 10 days or 50-100mg/kg orally, repeated in 14 days, except in Ball pythons), Ivermectin (0.2mg/kg intramuscularly or subcutaneously and repeated in 2 weeks) and Albendazole (50mg/kg orally once) (Wilson and Carpenter, 1996; Mitchell and Diaz-Figueroa, 2005).

CONCLUSION

The findings indicate that the impact of parasitism by *Ophidascaris arndti* in free-ranging tropical rattlesnakes may be severe, inducing prominent gross and histopathologic lesions. Consequently, diagnosis and treatment of such individuals may be suggested in order to reduce distress and the mortality of snakes in captivities.

ACKNOWLEDGEMENTS

This study was supported in part, by the Fundação de Amparo a Pesquisa de Minas

Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

ANDERSON, R.C. *Nematode parasites of vertebrates:* their development and transmission. 2.ed. Wallingford, England: CAB International, 2000. 650p.

ARAUJO, P.; MACHADO, M.I. Data on the biological cycle of the ascaridoid *Ophidascaris trichuriformis*, parasite of snakes. *Ann. Parasitol. Hum. Comp*., v.55, p.333-346, 1980.

BRAR, R.S.; GUPTA, P.P.; MISRI, J. Observation on the post-mortem lesions in two pythons. *Indian Vet. J.,* v.67, p.171-173, 1990.

BRAZ, H.B.; ROCHA, M.M.T.; FURTADO, M.F.D. Maintaining rear-fanged snakes for venom production: an evaluation of mortality and survival rates for *Philodryas olfersii* and *P. patagoniensis* in captivity. *J. Venom. Anim. Toxins incl. Trop. Dis*., v.18, p.164-172, 2012.

ELBIHARI, S.; HUSSEIN, M.F. *Ophidascaris filaria* (Dujardin, 1845) from the African Rock Python, *Python sebae*, in the Sudan, with a note on associated gastric lesions. *J. Wildl. Dis*., v.9, p.171-173, 1973.

FREITAS, J.F.T. Revisão do gênero *Ophidascaris* Baylis, 1921 (Nematoda, Ascaridoidea). *Mem. Inst. Oswaldo Cruz*, v.66, 129p., 1968.

JACOBSON, E.R. Parasites and parasitic diseases of reptiles. In: _____ (Ed.). *Infectious diseases and pathology of reptiles, color atlas and text*. Boca Raton: CRC Press, 2007. p.571-665.

MITCHELL, M.A.; DIAZ-FIGUEROA, O. Clinical reptile gastroenterology. *Vet. Clin. Exot. Anim. Pract.,* v.8, p.277-298, 2005.

MONTALI, R.J. Comparative pathology of inflammation in the higher vertebrates (reptiles, birds and mammals). *J. Comp. Pathol*., v.99, p.1-26, 1988.

PINTO, R.M.; MUNIZ-PEREIRA, L.C.; PANIZZUTTI, M.M.H. *et al*. Nematode parasites of the neotropical rattlesnake, *Crotalus durissus* Linnaeus, 1758 (Ophidia, viperidae) from Brazil: new records and general aspects. *Neotrop. Helminthol*., v.4, p.137-147, 2010.

WILSON, S.C.; CARPENTER, J.W. Endoparasitic diseases of reptiles. *Semin. Avian Exot. Pet. Med*., v.5, p.64-74, 1996.