

## ARTÍCULOS ORIGINALES/ORIGINAL ARTICLES

### GROSS LESIONS INDUCED BY NEMATODES OF *BOTHROPS JARARACA* AND *BOTHROPS ALTERNATUS* IN BRAZIL WITH TWO NEW RECORDS

### MACRO LESIONES INDUCIDAS POR NEMATODOS DE *BOTHROPS JARARACA* Y *BOTHROPS ALTERNATUS* EN BRASIL CON DOS NUEVOS REGISTROS

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#### Resumen

Resultados de exámenes de heces han sido comparados a los obtenidos durante necropsias de serpientes infectadas con helmintos. Muestras de heces de ejemplares de *Bothrops jararaca* e *B. alternatus* fueron investigadas y tres animales fueron positivos para huevos durante los exámenes: un caso representando parásitos autóctonos de serpientes, otro relativo a especies introducidas accidentalmente junto con ratones naturalmente infectados que son ofrecidos como alimentación para las serpientes y otro con la asociación de ambos. Resultados de las necropsias de los mismos ejemplares indican la presencia de los nematodos *Ophidascaris travassosi* Vaz, 1938, *Kalicephalus costatus* (Rudolphi, 1819), *K. inermis* Molin, 1861, *Kalicephalus* sp. y *Acanthorhabdias acanthorhabdias* Pereira, 1927. Las especies *Bothrops jararaca* es un nuevo registro de huésped para *Ophidascaris travassosi* y *Bothrops alternatus* para *A. acanthorhabdias*; los resultados con relación a lesiones macroscópicas inducidas por los nematodos son presentados.

**Palabras clave:** *Bothrops* spp. - Brasil - Nematodos - Patología - Serpientes.

#### Abstract

Results of fecal examinations have been compared with those obtained during necropsies of snakes infected with helminths. Stool samples from specimens of *Bothrops jararaca* and *B. alternatus* were investigated and three animals were positive for eggs during coprological screenings: one case representing autochthonous snake parasites, other related to species accidentally introduced together with naturally infected mice snakes fed on, and another with the association of both. Necropsies of those same specimens, showed the presence of the nematodes *Ophidascaris travassosi* Vaz, 1938, *Kalicephalus costatus* (Rudolphi, 1819), *K. inermis* Molin, 1861, *Kalicephalus* sp. and *Acanthorhabdias acanthorhabdias* Pereira, 1927. *Bothrops jararaca* is a new host record for *O. travassosi* and *Bothrops alternatus* for *A. acanthorhabdias*; the gross pathological lesions induced by the nematodes are reported.

**Key words:** *Bothrops* spp. - Brazil - Nematodes - Pathology - Snakes.

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## INTRODUCTION

Species included in the genus *Bothrops* Wagler, 1824, occur in a great number, with a large geographical distribution in the Neotropical region, appearing from Mexico to the North of Argentina and are absent only in Chile (Gomes & Puerto, 1993), inducing several cases of snake poisoning in humans and animals (Souza, 2000).

In the extensive survey of nematodes of Brazilian reptiles (Vicente *et al.*, 1993), the data on parasites recovered from captive snakes are very few, in despite of their importance for the knowledge of the helminth fauna infecting those hosts aiming at the improvement of maintenance conditions to the further extraction and production of antiophidic serum.

The present investigation evaluated the prevalence of infection by helminths in captive specimens of *Bothrops jararaca* and *B. alternatus*, previously obtained in wild environments, with data on new host records for two of the nematode species recovered during necropsies, together with comments on the macroscopic lesions induced by the parasites, taking into account the sensibility and adaptation of the hosts maintained under laboratory conditions.

## MATERIALS AND METHODS

Nematodes were obtained from five specimens of *Bothrops jararaca* (Wied, 1824) and one of *B. alternatus* (Duméril, Bibron & Duméril, 1854) maintained in the serpentarium of the Army Biology Institute (IBEx), Rio de Janeiro, RJ, Brazil. Animals were captured in the wild and further donated by volunteers from the State of Rio de Janeiro (two specimens of *B. jararaca* [one male, one female], necropsies LRS002, LRS004, respectively, from the municipality of Resende - 22°28'08"S, 44°26'48"W, three [one male, two females], necropsies LRS003, LRS001, LRS006, respectively, from the municipality of Petrópolis - 22°30'18"S, 43°10'43"W, and from the State of Minas Gerais (one female specimen of *B. alternatus*) necropsy LRS005 from the municipality of Três Corações - 21°41'49"S, 45°15'12"W. When in captivity, animals were

submitted to the same conditions of temperature, feeding and luminosity. The maintenance procedures adopted in the serpentarium of the IBEx are intensive and animals are kept individually in special plastic cages. Snakes are fed once a week, on mice (*Mus musculus* L., 1758) raised in the IBEx animal house, with a food amount corresponding to nearly 10% of the weight of the snake. Wild specimens maintained in the serpentarium are kept under quarantine for adaptation and also to avoid the transmission of pathogens to those already screened for ecto and endoparasites. The specimens studied here were investigated during the quarantine period.

Stool samples were recovered and processed for analysis in accordance with the method of Gordon & Whitlock (Mattos Jr & Motta, 1996).

Necropsies were performed from September to December, 2001, according to the technique of Gomes & Puerto (1993) and helminthological procedures followed Amato *et al.* (1991). Briefly, organs were dissected in Petri dishes containing a 0.85% NaCl solution.

Nematodes were collected and fixed in hot AFA solution [(acetic acid (2%) + formaldehyde (3%) + 70% ethanol (93%)] and processed for study according to Muniz-Pereira & Amato (1995, 1998).

The identification of the nematodes was based on data after Yamaguti (1961), Schad (1962), Freitas (1968) and Vicente *et al.* (1993).

A couple of nematode specimens, just after their identification were preserved as whole mounts (Canada balsam + beechwood creosote) and other amounts, kept as wet material in the fixative solution.

In the present investigation, the designation "introduced species" refers to those helminths that commonly infect mice and accidentally contaminated the snakes when they fed on naturally parasitized mice and also for this reason, eggs of introduced species were detected during coprological examination. Representative specimens of each studied nematode species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, RJ, Brazil. NHR refers to New Host Record.

## RESULTS

Three out of the six coprological screenings were positive for helminth eggs. In two, eggs of nematodes that usually infect reptilian hosts, were observed, with one case of infection with specimens of *Kalicephalus* sp. appearing alone (LRS 004) and one with *Kalicephalus* sp. associated to *Ophidascaris* sp. together with eggs of the accidentally introduced species, related to the nematodes *Syphacia* sp., *Aspicularis* sp. and the cestode *Rodentolepis* sp. [= *Hymenolepis* = *Vampirolepis*] (LRS006); in the other positive case, the introduced species were the only to appear associated (LRS005); eggs of Rhabdiasidae nematodes were absent. (Table 1). In the snakes with mixed infections, haemorrhagic foci in the intestinal mucosa, pulmonary and tracheal exudate and stomatitis were observed.

Gross lesions observed in the stomach and intestine of the parasitized snakes appeared as ulcers associated to the penetration sites of the nematodes that invaded the mucosa and submucosa of the organ and the ulcers were either active or healed. Lesions induced in the intestine of the infected hosts by specimens of the genus *Kalicephalus* consisted of haemorrhagic foci and alterations of the mucosa in the sites of parasite attachment. Some snakes presented a cyst in the gall bladder, pneumonia and stomatitis. As for the changes observed in snakes by account of infections with *Acanthorhabdias acanthorhabdias*, they appeared as a mild mucous pulmonary exudate; snakes parasitized with these nematodes had been in captivity for a short period. New host records for two species, already well described and figured, are considered in taxonomic summaries.

Nematoda, Diaphanocephaloidea,  
Diaphanocephalidae

*Kalicephalus costatus* (Rudolphi, 1819)  
Taxonomic summary: host: *Bothrops jararaca*; site of infection: stomach; locality: municipality of Resende, State of Rio de Janeiro, RJ, Brazil; size of parasite load: 03 males, 03 females; deposited specimens: CHIOC no.35.228 (wet material).

*Kalicephalus inermis* Molin, 1861  
Taxonomic summary: host: *Bothrops jararaca*;

site of infection: intestine; locality: municipality of Petrópolis, State of Rio de Janeiro, RJ, Brazil; size of parasite load: 03 males, 07 females; deposited specimens: CHIOC no. 35.227 (wet material).

*Kalicephalus* sp.

Taxonomic summary: host: *Bothrops alternatus*; site of infection: intestine; locality: municipality of Três Corações, State of Minas Gerais, MG, Brazil; size of parasite load: 01 male, one female; specimens deposited: CHIOC no. 36.233 a-b (whole mounts).

Nematoda, Ascaridoidea, Ascarididae

*Ophidascaris travassosi* Vaz, 1938

Taxonomic summary: host: *Bothrops jararaca* (NHR) [Pit viper]; other host: *Crotalus durissus terrificus* Laurenti, 1768 [South American rattlesnake]; site of infection: esophagus; locality: municipality of Petrópolis, State of Rio de Janeiro, RJ, Brazil; size of parasite load: 02 males, 04 females; deposited specimens: CHIOC no. 35.225 (wet material).

Nematoda, Rhabditoidea, Rhabdiasidae

*Acanthorhabdias acanthorhabdias* Pereira, 1927 (= *Rhabdias labiata*)

Taxonomic summary: host: *Bothrops alternatus* [Crossed pit viper] (NHR); other host: *Waglerophis merremii* (Wagler, 1824) (= *Rhadnea merremii*) [Wagler's snake]; site of infection: lungs; locality: municipality of Três Corações, State of Minas Gerais, MG, Brazil; size of parasite load: 09 females; deposited specimens: CHIOC: no. 35.230 (wet material). *Rhabdias labiata* Pereira, 1927 was proposed as a junior synonym of *A. acanthorhabdias* Pereira, 1927, by Baker (1987).

## DISCUSSION

Periodical fecal screenings from captive snakes, although considered as an important step for the detection and control of worm burdens, is, in fact, only an accessory tool, since a the reliable helminth identification only occurs by means of the necropsies. The present obtained results related to coproscopy were not statistically considered, since they differ from those obtained during the recovery of worm burdens, regarding the prevalence of infection with adult worms, since three, out of the six, fecal samples obtained from

wild snakes were positive for helminth eggs, and of which two showed the presence of nematodes commonly found in snakes, namely *Kalicephalus* sp. and *Ophidascaris* sp. (Table 1). The presence of eggs representing species of the genera *Aspicularis*, *Syphacia* and *Rodentolepis* appearing alone or associated to other autochthonous nematodes of snakes, clearly indicates that those helminths, in fact, were accidentally introduced, since they naturally infect the mice snakes were fed on. Conversely, necropsies confirmed that snakes that appeared to be negative for autochthonous helminths, on the basis of fecal examination presented adult worm burdens, as it was observed in the specimen of *B. alternatus*, LRS005, only positive for introduced species and that was further found to be infected, during necropsy, with males and females of *Ophidascaris travassosi* and *Kalicephalus* sp. Similarly, the specimen of *B. jararaca*, LRS001 was negative either for autochthonous helminths or introduced species by fecal examination and further showed to be harboring adult *Kalicephalus* sp. worms in the intestine. Nevertheless, either unauthorized or unnecessary necropsies are to be avoided in serpentariums where snakes are maintained for venom extraction procedures, only. In the present case, the aim of the investigation was to supply data on the helminth fauna of wild specimens of *Bothrops* spp. to further support the evaluation of anti-helminthic drugs to be administered in order to prevent the spreading of massive infections to the colony.

Specimens of the genus *Kalicephalus*, due to their active percutaneous via of infection, present a maximized pathogenic action, even in artificial environments and thus, parasitized snakes with these nematodes appeared with severe cases of stomatitis. Significant intestinal lesions were not observed except for discrete haemorrhagic foci near the sites nematodes were attached.

The present data are in agreement with those of Grego *et al.* (2004) that reported to the finding of acute and caseous enteritis in specimens of *B. jararaca* infected with *K. inermis*.

According to Fowler (1986) and Frye (1991) species of *Rhabdias* have a life cycle with alternating homogonic and heterogonic phases, also with percutaneous infections, and thus with the increasing probability to infect captive snakes. In despite of the low prevalence, snakes parasitized

with specimens of *Acanthorhabdias*, were more sensible to adaptation, also presenting more severe lesions, characterized by necrosis in the sites of parasite attachment. Rubião (1995) and Grego *et al.* (2004) referred to cases of mucous pneumonia in specimens of *B. jararaca* infected with species of *Rhabdias* and these alterations caused by the parasitism with *A. acanthorhabdias* were also observed during this investigation. Santos *et al.* (2008), reported to similar changes in specimens of the South American rattlesnake *Crotalus durissus terrificus* parasitized with *Rhabdias* spp. Granulous and caseous gastritis due to the presence of *Ophidascaris* sp. worms in *B. jararaca* (Grego *et al.*, 2004) were not presently observed in snakes harboring the ascaridid *O. travassosi* that appeared with a low parasitic burden.

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Table 1. Coprological results for the presence of helminth eggs in specimens of *Bothrops* spp.: *Kalicephalus* sp. (*Kal.*), *Ophidascaaris* sp. (*Ophi.*), *Rhabdiasidae* (*Rha.*), *Rodentolepis* sp. (*Rod.*), *Syphacia* sp. (*Sypha.*), *Aspiculuris* sp. (*Aspic.*), N = Negative, P = Positive, M = Male, F = Female

Specimen (no.)	Sex	Results	Autochthonous nematodes		Introduced species (accidental infections)			
			<i>Kal.</i>	<i>Oph.</i>	<i>Rha.</i>	<i>Rod.</i>	<i>Sypha.</i>	<i>Aspic.</i>
LRS001	F	N	-	-	-	-	-	-
LRS002	M	N	-	-	-	-	-	-
LRS003	M	N	-	-	-	-	-	-
LRS004	F	P	+	-	-	-	-	-
LRS005	F	P	-	-	-	+	+	+
LRS006	F	P	+	+	-	+	+	+

