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HELMINTHS OF TOAD *RHINELLA ICTERICA* (BUFONIDAE), FROM THE MUNICIPALITY OF BOTUCATU, SÃO PAULO STATE, BRAZIL

HELMINTOS DEL SAPO *RHINELLA ICTERICA* (BUFONIDAE), DE LA MUNICIPALIDAD DE BOTUCATU, ESTADO DE SÃO PAULO, BRAZIL

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Resumen

Quince ejemplares del sapo bufonido *Rhinella icterica* (Spix, 1824), 7 machos y 8 hembras, colectados en el municipio de Botucatu, Estado de São Paulo, Brasil, desde marzo del 2002 hasta enero del 2003, fueron analizados en busca de helmintos parásitos. Todos los sapos estaban infectadas con al menos un parásito (prevalencia global = 100%). Cinco especies de helmintos fueron encontradas, entre ellas cuatro especies de nematodos, *Rhabdias fuelleborni* Travassos, 1926, *Oswaldocruzia subauricularis* (Rudolphi, 1819), gen. sp. no identificada Cosmocercidae Travassos, 1925 y larvas de Physaloptera Rudolphi, 1819, y una especie acantocéfalo, *Acanthocephalus saopaulensis* Smales, 2007. *Rhabdias fuelleborni* y *A. saopaulensis* fueron las especies más abundantes. A excepción de las larvas de Physalopteridae, todos los helmintos presentaron una alta prevalencia (> 70%). Todas las especies de helmintos presentaron una tendencia hacia un patrón más agregado de distribución en los hospederos muestreados.

Palabras clave: Acanthocephala – Amphibia - Fauna helmíntica - Índice de discrepancia –Nematoda - *Rhinella icterica*.

Abstract

Fifteen specimens of bufonid toad *Rhinella icterica* (Spix, 1824), 7 males and 8 females, collected in the municipality of Botucatu, São Paulo State, Brazil, from March 2002 to January 2003, were examined for helminth parasites. All toads were infected with at least one parasite species (overall prevalence = 100%). Five helminth species were found, including four nematode species, *Rhabdias fuelleborni* Travassos, 1926, *Oswaldocruzia subauricularis* (Rudolphi, 1819), gen. sp. no identified Cosmocercidae Travassos, 1925 and larvae of Physaloptera Rudolphi, 1819, and one acanthocephalan species, *Acanthocephalus saopaulensis* Smales, 2007. *Rhabdias fuelleborni* and *A. saopaulensis* were the most abundant species. Except for Physalopteridae larvae, all helminths occur in high prevalence (> 70%). All helminth species presented a distribution toward to more aggregated pattern in these host samples.

Key words: Acanthocephala - Amphibian - Discrepancy index - Helminth fauna - Nematoda - *Rhinella icterica*.

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INTRODUCTION

The Neotropical region harbors the highest amphibian biodiversity (Frost, 1985; Izecksohn & Carvalho-Silva, 2001) and Brazil has a great number of amphibian species of the world (IUCN, 2009; SBH, 2009). The knowledge of the helminth amphibian parasites is lower and the majority of studies dealing with their parasites are taxonomic descriptions and only few ones have been focused parasite populations and community structure (Goldberg *et al*., 1995; Linzey *et al.*, 1998; Puga & Torres, 1999; Boquimpani-Freitas *et al*., 2001; Bursey *et al*., 2001; Goldberg & Bursey, 2003; Luque *et al*., 2005; Holmes *et al*., 2008; Campião *et al.*, 2009).

The Cururu toad, *Rhinella icterica* (Spix, 1824), is largely distributed in the South American, inhabits forest regions, open vegetal formations and eventuality urban ambient (Izechsohn & Carvalho-Silva, 2001; Silvano *et al*., 2004). Despite of little knowledge on amphibian parasites, the helminth fauna of this species have been well studied (Kloss, 1971; Faria, 1978; Rodrigues *et al*., 1978, 1982; Vicente *et al*., 1990; Martins, 2004, Luque *et al*., 2005), however, only Luque *et al*. (2005) focused on the ecological data of helminths of *R. icterica* from Rio de Janeiro State, Brazil.

The aim of this study was to evaluate the helminth fauna of the Cururu toad *R. icterica* in the municipality of Botucatu, São Paulo State, Brazil.

MATERIALS AND METHODS

A total of 15 animals comprising 7 males and 8 females were collected from March 2002 to January 2003 in the municipality of Botucatu, São Paulo State, Brazil. The toads were captured in a ruderal field around ponds and streams. The toads were euthanatized with sodium thiopental solution and after examined for helminth parasites. In the laboratory, toads were necropsied and body cavity, lungs and the gastrointestinal tract were surveyed for endoparasites. The helminths were fixed in alcohol-formaldehyde-acetic acid (AFA) and stored in 70% ethanol.

For identification, acanthocephalans were stained with carmine and cleared with eugenol. Nematodes were cleared with lactophenol. Mean intensity of infection, mean abundance and prevalence was calculated according to Bush *et al*. (1997).

The discrepancy index (*D*) was calculated as suggested by Poulin (1993). The index has a minimum value of zero ($D = 0$), when all hosts harbor the same number of parasites. When all parasites are found in a single host, aggregation is maximum ($D = 1$). This index was calculated with software Quantitative Parasitology 3.0 (Rózsa *et al*., 2000). Voucher helminth specimens were deposited in the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB) at the Universidade Estadual Paulista, São Paulo State, Brazil.

RESULTS

All toads were infected with at least one parasite species (overall prevalence = 100%). Five helminth species were found, including four nematode species, *Rhabdias fuelleborni* Travassos, 1926, *Oswaldocruzia subauricularis* (Rudolphi, 1819), Cosmocercidae gen. sp. no identified Travassos, 1925 and larvae of *Physaloptera* Rudolphi, 1819, and one acanthocephalan species, *Acanthocephalus saopaulensis* Smales, 2007 (Table 1).

Regarding the infection sites, lungs harbored only *R. fuelleborni*, at stomachs only larvae of *Physaloptera* were found; on the small and large intestines were found *A. saopaulensis* and *O. subauricularis*, and further the unidentified Cosmocercidae species were found at large intestine (Table 1). Except for *Physaloptera* larvae, all helminths occurred in high prevalence (> 70%).

Rhabdias fuelleborni and *A. saopaulensis* were the most abundant species. With relation to intensity of infection, *A. saopaulensis* and *R. fuelleborni* presented the higher mean intensities of infection (Table 1).

All helminths species presented a distribution trend to aggregate in this host sample (Table I). The majority of infected amphibian harbored four helminth species (47%, n = 7), with a smaller percentage harboring three (33%, n = 5) or two species (20%, n = 3) (Figure 1).

DISCUSSION

Nematodes are the main helminth parasites found in the toad *R. icterica* (Luque *et al*., 2005), with few record of acanthocephalan infecting this anuran species (Smales, 2007). However, in this study *A. saopaulensis* was the more prevalent (86.7%) and abundant (723 specimens) parasite species. *Acanthocephalus saopaulensis* was recently described by Smales (2007) as new species of toad *R. icterica*, with low prevalence (33.3 %), mean intensity (22), and abundance (7.3). The present study found higher infection pattern than the data presented by Smales (2007) (Table 2), suggesting a different composition in the helminth parasite community associated with geographical variation. Also, the helminth richness found infecting *R. icterica* in São Paulo State was smaller than from Rio de Janeiro State (Luque *et al*., 2005), reinforcing the environment component in the establishment of the helminth fauna of this anuran species.

Rhabdias fuelleborni and species of the genus *Cosmocerca* Diesing, 1861 infect several anurans species and occurs in many countries in South America (Baker & Vaucher, 1984; González & Hamann, 2004; Goldberg *et al*., 2007). *Rhabdias fuelleborni* was registered in host population from *R. icterica* (Vicente *et al.*, 1990; Luque *et al*., 2005), *R. marina* and *R. schneideri* (Vicente *et al.*, 1990). All infection parameters, such as prevalence, mean intensity of infection and mean abundance, were higher on toads from Botucatu municipality (São Paulo State) in contrast to toads from Miguel Pereira municipality (Rio de Janeiro State) which has available data on literature (Luque *et al*., 2005).

Rhabdias fuelleborni has been reported for *R. marina*, with high prevalence, intensity and abundance means (Ragoo & Omah-Maharaj, 2003). Regarding the genus *Cosmocerca*, Vicente *et al.* (1990) reported several helminth parasite species for *R. icterica*.

Oswaldocruzia subauricularis were recorded harboring intestine of two populations of *R. icterica*, one from Curitiba municipality (Paraná State) (Vicente *et al.*, 1990) and other from Miguel Pereira municipality (Luque *et al*., 2005). In comparison with last population, all infection parameters were higher on toads from Botucatu municipality (Table II).

According Aho (1990), the higher richness helminth fauna in amphibians host are related to time that hosts spend in water during larval stages and breeding season. In this period the anurans are exposed to acanthocephalans and other helminths that are typically transmitted through the ingestion of aquatic invertebrates. Further, during terrestrial life-cycle hosts are exposed to transcutaneous penetration by nematode larvae (e.g. *Rhabdias* spp. and some Cosmocercidae) and ingestion of eggs of nematodes (such as Trichostrongylidae Leiper, 1912 and Cosmocercidae) (Aho, 1990). Moreover, this toad species presents a wide feeding spectrum that include 27 food items (Sabagh & Carvalho-e-Silva, 2008), increasing the possibility of infection by a large number of species of parasites.

The higher prevalence, mean intensity of infection and abundance, mainly for *A. saopaulensis*, found in this *R. icterica* population could be related to differences in local composition of helminth species (component community, *sensu* Bush *et al*., 1997) or host ecological characteristics such as aggregation of the host population and differential consumption of dietary items. However, additional studies on other *R. icterica* population in a wide geographical distribution could improve the knowledge of this host-parasites relationship.

A high infection rate by cosmocercid nematodes (prevalence = 80%; mean intensity of infection = 16 ± 5.5, range 1-68) was observed in the studied sample, however, the species was not identified because only females were found in all anuran hosts. The absence of males and the high number of eggs containing larvae may suggest a parthenogenetic reproduction mechanism in this helminth population, similar to other nematodes such as *Strongyloides* spp. and *Rhabdias* spp. (Anderson, 2000). We have no information on the occurrence of parthenogenesis in Cosmocercidae and so future studies will be conducted to better understanding this curious case.

Table 1. Prevalence, mean abundance (± standard error), mean intensity (± standard error), discrepancy index (D), and site of infection of helminths from *Rhinella icterica* (n = 15) from Botucatu, São Paulo State, Brazil. MA - mean abundance. MII - mean intensity of infection. CHIBB - Coleção Helmintológica do Instituto de Biociências de Botucatu.

Helminth	Prevalence (%)	MA ± SE	MII ± SE (range)	*D*	Site of infection
Nematoda					
Cosmocercidae CHIBB 289, 293, 297, 301, 303, 309, 314, 321, 326, 330, 334, 344, 350	80	12.8 ± 4.7 (0-68)	16 ± 5.5 (1-68)	0.595	Large intestine
Rhabdias fuelleborni CHIBB 286, 290, 294, 298, 310, 315, 318, 322, 327, 331, 335, 338, 341	80	30.3 ± 10.1 (0-105)	37.8 ± 11.6 (3-105)	0.596	Lung
Oswaldocruzia subauricularis CHIBB 288, 292, 296, 300, 304, 305, 306, 313, 324, 325, 333, 336, 337, 339, 343, 345, 348	73.3	5.6 ± 1.4 (0-20)	7.6 ± 1.5 (2-20)	0.476	Small and large intestine
Physalopteridae larvae CHIBB 332	6.7	0.4	6	-	stomach
Acanthocephala					
Acanthocephalus saopaulensis CHIBB 287, 291, 295, 299, 302, 307, 308, 311, 312, 316, 317, 319, 320, 323, 328, 329, 340, 342, 346, 347, 349	86.7	48,2 ± 12,8	55,6 ± 13,6 (1-190)	0.471	Small and large intestine

Table 2. Comparison between prevalence, mean intensity (± standard error), mean abundance (± standard error) and infection site of three population of toad *Rhinella icterica*. I = intestine; SI = small intestine; LI = large intestine; L = lung.

PARASITE	Prevalence (%)	Nº sampled	Range	Mean Intensity ± SE	Mean Abundance ± SE	Infection Site	Reference
Acanthocephalus saopaulensis	33.3 86.7	3 15	1-190	22 55.6 ± 13.6	7.3 48.2 ± 12.8	I SI, LI	Smales (2007) present study
Oswaldocruzia subauricularis	12.5 73.3	32 15	1 - 9 2 - 20	4.5 ± 0.7 7.6 ± 1.5	0.5 ± 0,3 5.6 ± 1.4	SI SI, LI	Luque *et al*. (2005) present study
Rhabdias fuelleborni	53.1 80.0	32 15	1 - 31 3 - 105	8 ± 1.6 37.8 ± 11.6	4.2 ± 1.3 30.3 ± 10.1	L L	Luque *et al*. (2005) present study

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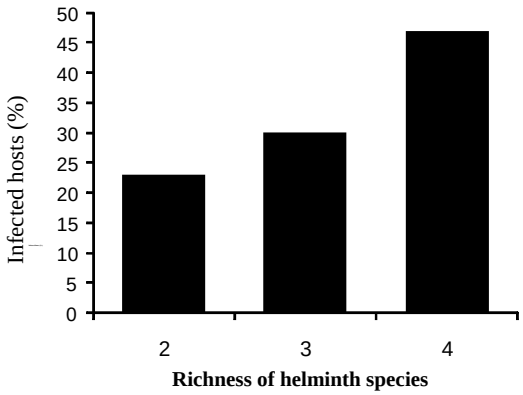
Figure 1. Percentage of *Rhinella icterica* (n = 15) infecting with two, three or four helminth species in the municipality of Botucatu, São Paulo State, Brazil.



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