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**BERTIELLOSIS IN BRAZILIAN NON-HUMAN PRIMATES:  
NATURAL INFECTION IN *Alouatta guariba clamitans*  
(CABRERA, 1940) (PRIMATES: ATELIDAE)  
IN SANTA CATARINA STATE, BRAZIL**

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ABSTRACT

The first case of parasitism by *Bertiella mucronata* (Cestoda: Anoplocephalidae) in Brazilian non-human primates, *Alouatta guariba clamitans* (Cabrera 1940) (Primates: Atelidae), from Santa Catarina State, is here reported. Free-ranging monkeys from three different cities of the Itajaí-Açu River Valley region were found to be infected. Prevalence and daily elimination of proglottids in 28 captive howler monkeys are described. We warn about the possibility of human bertiellosis in this region. This information should be utilized in sanitary evaluations of *A. clamitans* specimens submitted to translocation or reintroduction programs.

KEY WORDS: *Bertiella mucronata*. *Alouatta guariba clamitans*. Natural environment. Captivity. Zoonotic potential.

INTRODUCTION

The genus *Bertiella* (Stiles & Hassal 1902) has high heterogeneity and includes cestodes which are parasites of Marsupialia, Dermoptera, Rodentia and Primates in Africa, Asia, South America and Australia (15).

This genus belongs to the family Anoplocephalidae, and comprises heteroxenic parasites that require oribatid mites, important members of the soil fauna with a worldwide distribution, as intermediate hosts. Denegri listed oribatid

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mites which serve as intermediate hosts of anoplocephalid tapeworms. The accidental ingestion of infected mites, found in habitats associated with monkeys, may result in human parasitism (14).

Twenty-nine species were reported by Schmidt (32), of which *Bertiella studeri* and *Bertiella mucronata* are known to infect man. Denegri & Perez-Serrano (15) carried out a comprehensive review of all human cases of bertiellosis reported in the literature, describing a higher prevalence of human infection caused by *B. studeri* when compared to *B. mucronata*.

The geographical distribution of *B. studeri* is limited to the Eastern hemisphere, the only exceptions being those cases cited by Cameron (4), in African Primates introduced onto St. Kitts Island, and by Stunkard et al. (34), in a child in Minnesota, the latter considered to be the first autochthonous case in the United States of America. Recently, Galan-Puchades et al. (19) reported the first case of human bertiellosis in Spain. The natural hosts in Africa and Asia for *B. studeri* have been reviewed by Denegri (12).

The geographical distribution of *B. mucronata* is in South America, with three human cases reported in Argentina (1, 18, 20), and one case in Cuba (7) and Paraguay (9). In Brazil, the first human case was described by Pessoa (28) in São Paulo, the second report being described by Costa et al. (6) in Formiga (Minas Gerais), and the third by Paçô et al. (27) in Goiânia (Goiás).

In South America the non-human primates hosts of *B. mucronata* are *Callicebus personatus nigrifrons*, *Cebus apella fatuellus*, *Cebus capucinus*, *Callithrix sagui* and *Alouatta caraya* (16).

The genus *Alouatta* (Lacépède, 1799) is the most widely distributed of the New World monkeys, ranging from Mexico (17) to Argentina (3). Meyner (25) identified for the first time *Taenia (Bertia) mucronata* in a black howler monkey (*Alouatta caraya*) from Paraguay. Pope (29) found a 7% (n = 84) and Santa Cruz et al. (31) detected a 29.4% (n = 74) rate of infection in *A. caraya* from Argentina.

Cabrera (2) recognized two subspecies of *Alouatta guariba*: the northern brown howler monkey (*Alouatta guariba guariba*) which ranges from Bahia to Minas Gerais, and the southern brown howler (*Alouatta guariba clamitans*) classified as 'quite vulnerable' on the Red List of Threatened Species (22), ranging from Rio de Janeiro to São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (33), and stretching further westwards into the Misiones territory of Argentina (8).

The main objective of this paper is to report on the first case of bertiellosis in a Brazilian neotropical non-human primates, the natural definitive hosts of the genus *Bertiella*.

## MATERIALS AND METHODS

Fecal samples of 27 specimens of southern brown howler monkeys (*Alouatta guariba clamitans*), 18 males and 9 females, and 1 male of black and

golden howler monkey (*Alouatta caraya*) were collected between April and December 2005. These animals were received or maintained in the scientific captivity of the Biological Research Center of Indaial – CEPESBI (n° 1/42/98/000708-90) (21).

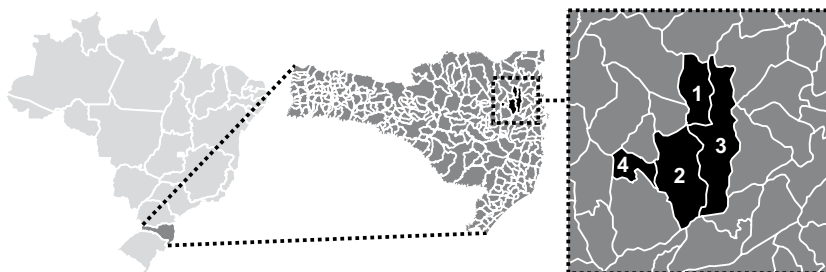
In January 2006, daily samples of 9 positive individuals, 7 males and 2 females, were collected for 26 consecutive days, to check for proglottid emissions.

The monkeys were kept in cages measuring 3.0 x 5.0 x 2.6 m (width x length x height). Ropes, tree trunks and food platforms served as environmental enrichment devices at the enclosures. The cages were cleaned and disinfected daily, and people who managed the animals used safety devices. The monkeys received four daily meals, composed of fruits, vegetables, canine ration and *Cercropia glazioui* and *Sechium edule* leaves.

Fecal samples were collected in the morning, soon after defecation, and were preserved in sodium acetate - acetic acid - formaldehyde and analyzed at the Laboratory of Parasitology of the University of Blumenau (FURB). The Ritchie method (11) was used to analyze for eggs and cysts.

Several types of proglottids were manually separated from fecal samples, fixed in AFA, stained with clorhydric carmine, dehydrated in a graded alcohol series, cleared with xylene and mounted in Canada balsam. Paraffin-embedded proglottids longitudinal sections were also stained with hematoxylin and eosin and examined microscopically (24).

In the period between April 2005 and April 2006, post mortem examinations were carried out on seven brown howler monkeys – four females and three males, which came from forest fragments in four municipalities of the Itajaí-Açu River Valley region: Blumenau, Ascurra, Indaial and Pomerode (Figure 1). These monkeys had been found dead and brought to the Santa Catarina State Environmental Police.



*Figure 1.* Map of the State of Santa Catarina showing the municipalities where the animals submitted to post mortem examinations came from. 1 - Pomerode, 2 - Indaial, 3 - Blumenau, 4 - Ascurra.

Adult parasites were collected from the small intestines of the monkeys during the necropsies. Material handling and identification was carried out in the

same way as described for fecal proglottids. Proglottids and eggs were measured and photographed with the aid of a micrometer coupled to the ocular lens of an Olympus CX31 microscope.

The presence of oribatid mites in leaves of *Cercropia glazioui* used to feed the captive animals was investigated. Taxonomic identification of mites was based on Subías and Balogh (35).

All specimens were treated with a single oral dose of 20 mg/kg of praziquantel (Cestox®) after the collection of fecal samples.

This research was previously approved by the FURB Ethics Committee on Animal Experimentation (protocol no. 033/04-A).

## RESULTS

From 28 captive animals studied, 50% showed gravid proglottids in at least one fecal sample. Daily monitoring of nine animals for 26 days demonstrated that proglottid emissions in the specimens varied from 1 to 24 counts (n = 9), with intervals between emissions ranging from 1 to 9 days (Figure 2).

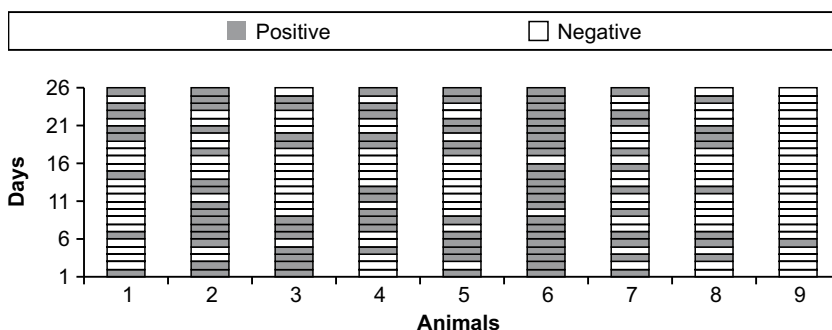


Figure 2. Daily variation, in a 26 days period, on gravid proglottids emission in fecal samples of nine brown howler monkeys (*Alouatta guariba clamitans*) kept in captivity.

None of the 382 fecal samples analyzed by Ritchie methods showed positive results for cestode eggs. However, all animals that had proglottids in their feces gave positive results for other intestinal parasites: *Giardia* spp. - 100% (14/14), *Entamoeba* spp. - 50% (7/14), *Endolimax* spp. - 57% (8/14), *Iodamoeba* spp. - 28.5% (4/14) and *Trypanoxyuris* spp. - 57% (8/14).

Of the seven free-ranging animals submitted to necropsy, three showed a single adult cestode in the small intestine (Figure 3). These monkeys lived in forest fragments in Blumenau, Indaial and Ascurra. A single juvenile male kept in captivity died during the research period, and bertiellosis was diagnosed in the post mortem

examination, with a single parasite in the small intestine. Despite the parasite being 85 cm long, the animal did not show any clinical signs besides proglottids in its feces.

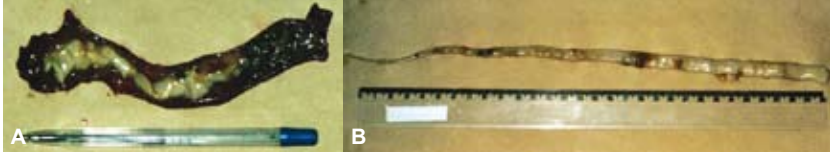


Figure 3. A - Presence of *B. mucronata* in a small intestine segment. B - Adult parasite, around 30 cm long.

The number of gravid proglottids by segment ranged from 8 to 72 (n = 22). They had dimensions varying between 6.5 - 7 mm x 0.32 - 0.75 mm (n = 10). The eggs were typical, having 35-40  $\mu\text{m}$  (n = 7) in diameter, with the presence of a bifurcated pyriform apparatus in the central region of 12.5  $\mu\text{m}$  (n = 7).

Longitudinal histological sections showed the presence of a single transverse uterus, extending in tubular stage across the middle of the proglottid, posterior to the testes, and in some proglottids surpassing the level of the osmoregulatory canals (Figure 4).

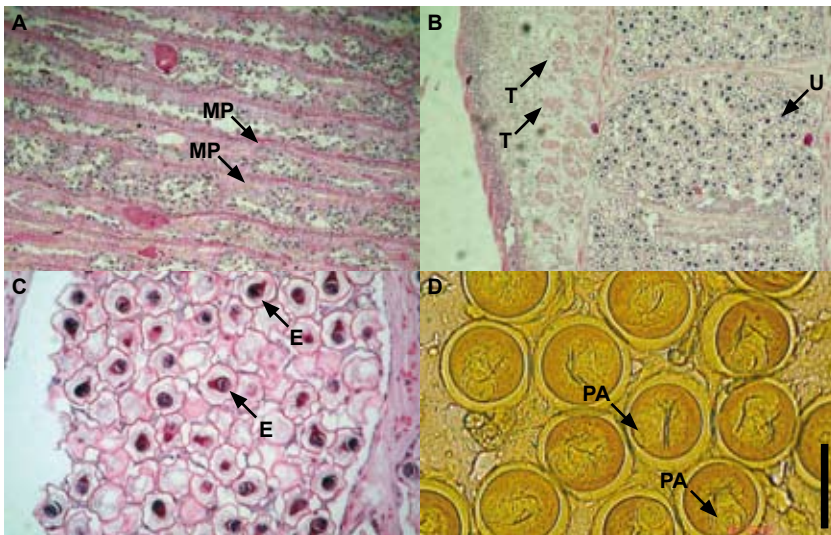


Figure 4. Nonhuman *Bertiella mucronata*. A (40x), B (100x), C (400x) – hematoxiline and eosine histological section of mature proglottids in longitudinal position. D – Eggs of *Bertiella mucronata*, Optical photomicrography (400 x). Abbreviations: MP – mature proglottids; T – testes; U – uterus; E – eggs; PA – pyriform apparatus. Scale bars: 35  $\mu\text{m}$ .

A specimen of *Aeroppia* sp. (Oribatida: Oppidae) was found in a leaf of *Cecropia glazioui* used to feed the captive animals. Mite species present in the CEPESBI soil were not investigated.

After oral treatment with praziquantel (Cestox®), in a single dose of 20 mg/kg, captive animals no longer showed proglottid emission in their feces.

## DISCUSSION

The diagnosis of *B. mucronata* is based on the identification of gravid proglottids, egg morphology and geographic distribution. Gravid proglottids are several times wider than long, and are shed in groups of about 2 dozen at a time. Free eggs from gravid segments or stool are 40-46 µm long and 36-40 µm wide in *B. mucronata*, and 48-60 µm long and 40-60 µm wide in *B. studeri* (12).

This is the first report of infection by *Bertiella mucronata* in *Alouatta guariba clamitans*. The occurrence of this cestode in *Alouatta caraya* in the natural environment has been reported in Argentina (29, 31, 30) and in Paraguay (9).

According to Dunn (16) the infection is related to herbivory, through accidental ingestion of oribatid mites bearing cysticercoids. Therefore, two hypotheses are presented to explain the infection source of the captive animals: leaves supplied in captivity that came from areas which free-ranging populations have access may have harbored infected mites; and soil contamination in captivity by proglottids with subsequent infection of mites.

The oribatid mite found in the leaves fed to the monkeys in captivity is not present on the list of intermediary hosts for cestodes of the family Anoplocephalidae given by Denegri (14). Previously, Denegri (13) has demonstrated under experimental conditions the development of cysticercoids of *B. mucronata* from a person in the oribatid species *Dometorina suramericana* and *Schelorbates atahualpensis*.

Cestodes generally cause discrete pathogenesis in the definitive host other than utilizing host's nutrients and vitamins. There is no indication that natural infection poses a serious health hazard for wild animals (23).

There has been little information published on cestode proglottids or egg emission frequency in neotropical primates. Pacheco et al. (26) found no signs of sequential grouping of positive results in close samples, showing a daily variation in positivity for *Hymenolepis* spp. eggs in *Callicebus nigrifrons*.

Negative periods of *B. mucronata* proglottid emission in feces during a one month period can hinder research on this cestode in the natural environment. However, there is a surprisingly limited amount of parasitological information regarding non-human primates kept in captivity, resulting in a possible distortion of infection characteristics when compared to natural conditions (26).

Endoparasitosis indexes are directly related to the area and degree of fragmentation of the forests available to the howlers (31). Furthermore, the authors highlight the importance of understanding the effects of parasitism in promoting

better management of a species and the potential danger for translocated or reintroduced populations. *B. mucronata* parasitosis should be considered in disease screening of species such as *A. guariba clamitans* and *A. caraya* when submitted to management programs.

The distribution and population biology of multi-host pathogens is becoming increasingly important for wildlife conservation and human health (10). Pope (29), Coppo et al. (5) and Santa Cruz et al. (31), have reported the alarming epidemiological finding that parasitosis produced by *B. mucronata* in howler monkeys has increased 420% in only 30 years.

There has also been a constant increase in the number of human cases, not only in tropical and subtropical regions, but at nearly all latitudes, suggesting the importance of the zoonosis. Most cases described come from individual records given by physicians, and have not undergone proper epidemiological studies (15).

This study advances the hypothesis of the occurrence of human bertiellosis in the State of Santa Catarina, Brazil. In agreement with Denegri and Perez-Serrano (15), we believe that the available data do not provide a true picture of this type of parasitosis in humans and suggest that further epidemiological investigation should be carried out to test this hypothesis.

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#### RESUMO

Bertielose em primata não humano brasileiro: infecção natural em *Alouatta guariba clamitans* (Cabrera, 1940) (Primates: Atelidae) do estado de Santa Catarina – Brasil

Este artigo descreve o primeiro relato de parasitismo por *Bertiella mucronata* (Cestoda-Anoplocephalidae) em primata não humano brasileiro, da subespécie *Alouatta guariba clamitans* (Cabrera, 1940) (Primates: Atelidae), no estado de Santa Catarina. Animais de ambiente natural, provindos de três municípios diferentes da região do Vale do Rio Itajaí-Açu, estavam parasitados. São também descritas a prevalência e a eliminação diária de proglotes nas fezes de 28 bugios ruivos cativos. Alerta-se para a possibilidade de ocorrer bertielose humana na região. Para evitar a introdução deste parasito em áreas indenes, a investigação desta parasitose deve ser realizada sempre em avaliações sanitárias de espécimes de bugios ruivos suscetíveis de serem submetidos a processos de translocação e reintrodução.

DESCRITORES: *Bertiella mucronata*. *Alouatta guariba clamitans*. Ambiente natural. Cativeiro. Zoonose.

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