# First report of *Strongyloides* sp. (Nematoda, Strongyloididae) in *Lutreolina crassicaudata* (Didelphimorphia: Didelphidae)

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#### **Abstract**

The present study reports the first case of the presence of the intestinal nematode *Strongyloides* sp. in fecal examinations of a male *Lutreolina crassicaudata*, considered a synanthropic marsupial species with zoonotic potential. The Willis technique was used for the diagnosis. Presence of typical eggs of *Strongyloides* species in feces was detected. A fecal culture was performed to obtain larval stages, free-living adults and infective third stage larvae (L3) of this nematode after seven days, which was morphologically identified as Strongyloidiae. This is the first report of infection by *Strongyloides* sp. in a tick-tailed opossum from Brazil.

Keywords: opossum, helminth, Nematoda, Strongyloides.

# Primeiro registro de *Strongyloides* sp. (Nematoda, Strongyloididae) em *Lutreolina crassicaudata* (Didelphimorphia: Didelphidae)

#### Resumo

O presente estudo relata o primeiro registro da presença do nematódeo intestinal *Strongyloides* sp. em exames de fezes de um macho de *Lutreolina crassicaudata*, considerado um marsupial sinantrópico com potencial zoonótico. A técnica de Willis foi empregada no diagnóstico. Presença de ovos típicos de *Strongyloides* spp. foi detectada nas fezes. Uma coprocultura foi realizada para obtenção de estágios larvais, adultos de vida livre e larvas infectantes de terceiro estágio (L3) deste nematódeo após sete dias, os quais foram identificados morfologicamente como Strongyloidiae. Este é o primeiro relato de infecção por *Strongyloides* sp. em uma cuíca de cauda grossa do Brasil.

Palavras-chave: cuica, helminto, Nematoda, Strongyloides.

### 1. Introduction

Lutreolina crassicaudata popularly known as thick-tailed opossum is a small marsupial mammal belonging to the Order Didelphimorphia and Family Didelphidae (Gardner, 2005). These marsupials are seeking food and shelter in urban areas, due the destruction of their habitat and ecosystem, thus compounding the synanthropic fauna of cities (Rossi et al., 2006). This species is poorly studied, therefore the information on the helminth fauna of these mammals are scarce. In South America there are only two reports of the presence of gastrointestinal nematodes in L. crassicaudata.

The only records of these parasites were performed by Navone et al. (1991) in Argentina, which were identified for the first time, the nematodes *Travassostrongylus yungaensis* Navone et al. (1991) and *Hoineffia simplicispicula* Navone et al. (1991) (Trichostrongylidae), present in the small intestine. Also, Valente et al. (2001) in Brazil (Pelotas, Rio Grande do Sul State) reported *Capillaria* sp. (Trichuridae) and *Aspidodera raillieti* Travassos, 1913 (Aspidoderidae) in the small and large intestine of *L. crassicaudata*, respectively.

Strongyloides (Nematoda, Strongyloididae) has more than 60 species, which mostly parasitize the small intestine,

especially the region of the duodenum of several mammals, including man and domestic species such as dogs, cats, cattle, sheep, goats, horses and pigs (Grove, 1989).

About 10 species of *Strongyloides* have been identified in mammals Brazilians (Vicente et al., 1997). However, in Brazilian marsupials there are few records of these nematodes. The only report in these animals was described by Froes (1976) in *Didelphis aurita* from Rio Grande do Sul, however in this study the morphological and biometric data from specimens found were insufficient for identification.

The species of *Strongyloides* presents peculiar life cycle, with two distinct stages: a free life with the presence of males and females and other parasite with only parthenogenetic females, which are inserted in the intestinal mucosa of the duodenum (Harvey and Viney, 2001), causing gastrointestinal disorders to hosts as cramping and diarrhea (Taylor et al., 2010). Infection with these nematodes occurs principally by active penetration of infective larvae on the skin, presents in the environment (Bowman, 2006).

Due to the lack of information regarding helminth fauna of these marsupials, the present work aims, report the first occurrence of *Strongyloides* sp. in *L. crassicaudata*.

#### 2. Material and Methods

A male of *L. crassicaudata* was captured and cared for at the Centro de Medicina e Pesquisa em Animais Selvagens belonging to the Faculdade de Medicina Veterinária e Zootecnia – UNESP, in the municipality of Botucatu, State of São Paulo, after being attacked by a domestic dog.

To perform the coproparasitological examinations, a fecal sample, recently eliminated by *L. crassicaudata* specimen was collected and stored in sterile collection bottle. Immediately after collection, the technique of Willis (Ueno and Gonçalves, 1998) was performed.

Presence of typical embryonated eggs of *Strongyloides* spp. in its feces was detected. A fecal culture was performed to obtain larval stages and free-living adults and presence of infective third stage larvae (L3) with a notched tail, which is a characteristic of the *Strongyloides* species, was detected after seven days.

To obtain morphological and biometric data in order to identify the *Strongyloides* species involved in this parasitism from parthenogenetic females, artificial infection by subcutaneous inoculation, with insulin syringe and needle, of approximately 50 infective L3, taken from the same fecal culture by the Baermann technique (Ueno and Gonçalves, 1998) was performed in a male Wistar rat (*Rattus norvegicus*) with one month of age, created in conditions free of prior infection with other nematodes. After the first day of infection, the rat was subjected to daily coproparasitological analysis and euthanized at the end of the 12th day post-infection. The intestinal content was collected and analyzed in an attempt to recover parthenogenetic females.

#### 3. Results

The Willis technique revealed eggs (Figure 1a) with typical morphological characteristics of the Strongyloides species, as thin and transparent shell with the presence of larvae inside. The fecal culture revealed different typical evolutionary stages of Strongyloides species as the first stage larvae (L1) and second stage larvae (L2), infective L3, plus free-living males and females. The L1 and L2 analyzed showed rhabditoid type esophagus, with body, isthmus distinct and clear bulb and tapered tail. However, the L2, different from that observed in the L1, it was observed elongated esophagus, with the division between the bulb and isthmus less evident and the presence of genital primordium. The infective L3 (Figure 1b) had elongated filarioid type esophagus and tapered tail end with incised (Figure 1c). Free-living females (Figure 1d) had the short body, slightly thick cuticle, rhabditoid type esophagus, didelphic and anfidelphic reproductive system, vulva close to half of the body, with prominent lips, uterus with a single row of eggs and tapered tail (Figure 1e). The free-living males (Figure 1f) had cylindrical body, rhabditoid type esophagus and two equal and symmetrical spicules, visible gubernaculum and pre-anal papillae and tapered tail.

The bioassay was not isolated *Strongyloides* species, therefore the presence of eggs in the feces or intestinal parthenogenetic female was not detected.

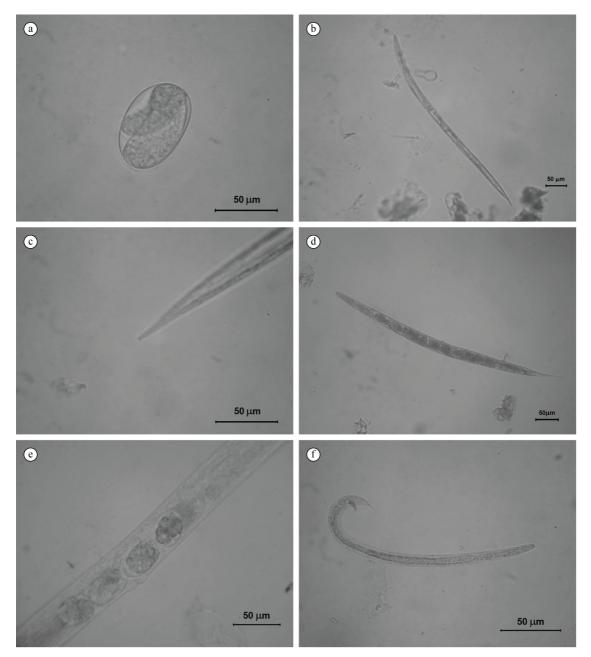
## 4. Discussion

This study reported the first case of *Strongyloides* sp. in *L. crassicaudata*. Several cases of parasitism by species of this genus of gastrointestinal nematode has been reported in Brazil, both in wild and domestic mammals (Vicente et al., 1997). However, records of these parasites were practically absent in marsupial species in Brazil and as in all of South America. Experimental infection in *R. norvegicus* was performed in this study aimed to the isolation and identification of species of the *Strongyloides* genus involved in the parasitism of this South American marsupial.

However, no parthenogenetic female could be recovered from this host, since the thick tailed opossum was reintroduced to the environment after appropriate veterinary treatment, aiming to contribute to the preservation of this species in nature.

To determine the species would be essential to obtain parthenogenetic females, however the isolation of this species of *Strongyloides* in Wistar rat may not have occurred probably as a consequence of the high specificity of the parasitic nematode with *L. crassicaudata*, or possibly the amount of larvae was not enough for the establishment of infection in the rat.

Thus, this marsupial is a new host recorded for *Strongyloides* sp. These finding raises the hypothesis that this kind of *Strongyloides* sp. can also be a zoonotic risk, since its host is already part of the synanthropic fauna of cities, and that some species of this nematode,



**Figure 1.** Egg of *Strongyloides* sp. recovered by the technique of Willis in fecal sample of *Lutreolina crassicaudata* (a); Infective third stage larvae (L3) of *Strongyloides* sp. (b); Notched posterior end of infective L3 of *Strongyloides* sp. (c); Free-living female of *Strongyloides* sp. (d); Uteri with a single row of eggs and prominent vulvar lips in free-living female of *Strongyloides* sp. (e); Free-living male of *Strongyloides* sp. (f).

such as *S. stercolaris* (Neves et al., 2005) are known to be transmitted from animals to the human population. This study contributes towards knowledge of the helminth fauna of sinantropic marsupials in Brazil.

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