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ScienceDirect
Veterinary Parasitology 151 (2008) 227–232
veterinary parasitology
www.elsevier.com/locate/vetpar

Mixed infection by *Libyostrongylus douglassii* and *L. dentatus* (Nematoda: Trichostrongylidae) in *Struthio camelus* (Ratites: Struthioniformes) from Brazil with further morphological characterization of adults

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Received 18 July 2007; received in revised form 4 November 2007; accepted 7 November 2007

Abstract

The genus *Libyostrongylus* includes three species, *L. douglassii*, *L. dentatus* and *L. magnus* that occur as parasites in the proventriculus of *Struthio camelus*. We confirmed a mixed infection by *L. douglassii* and *L. dentatus* in farmed ostriches from the southeast of Brazil for the first time, and provided new information on some morphological characters that differentiate these species. Adult nematodes collected from the proventriculus of ostriches were observed by light and scanning electron microscopy. Morphologic characterization and morphometric analysis of the nematodes enabled the distinction of both species and corroborated results of prior studies. Specimens of *L. dentatus* have a buccal capsule with a prominent esophageal tooth. Furthermore, males and females of *L. dentatus* were larger (4954 and 9347 μm) than those of *L. douglassii* (3411 and 4229 μm), but measurements for most characters in both species were smaller then those previously reported. Besides, the cephalic structure based on scanning electron microscopy differs, and *L. dentatus* has thick lips with round papillae, whereas *L. douglassii* has fine lips with lengthened papillae. The confirmation of both species in South America strongly suggests that the mixed infection may be common in farmed ostriches.
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Keywords: *Libyostrongylus douglassii*; *Libyostrongylus dentatus*; *Struthio camelus*; Trichostrongylidae; Nematodes

1. Introduction

The genus *Libyostrongylus* Lane, 1923 contains three species, *L. douglassii* (Cobbold, 1882) Lane, 1923, *L. dentatus* Hoberg, Lloyd and Omar, 1995 and *L. magnus* Gilbert 1937, all of which are parasites in the proventriculus of *Struthio camelus* Linnaeus 1783 (ostriches) (Hoberg et al., 1995). Of these, *L. douglassii* is considered the most pathogenic, causing mortality above 50% in juvenile and occasionally in adult ostriches (Reinecke, 1983). This species has been found in Australia (Barton and Seward, 1993), the United States of America (Hoberg et al., 1995) and

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doi:10.1016/j.vetpar.2007.11.009

Europe (Ponce Gordo et al., 2002), but appears to have been introduced from Africa coincidental with the translocation of infected hosts. Hoberg et al. (1995) redescribed *L. douglassii* and described *L. dentatus* based on specimens collected from the southern United States. This study showed that mixed infections are common in regions of the United States where ostriches have been introduced for farming purposes.

Ostriches have been raised in Brazil since the early 1990s with birds imported from the United States and Europe. Brazilian flocks are composed of about 200 thousands birds, mainly in the Southeast region of the country where they have shown good adaptation to the tropical climate (ACAB, 2007). Parasites are incompletely documented for Brazilian ostriches, although the occurrence of nematodes identified as *Libyostrongylus* was reported in the north region of Rio de Janeiro State where the parasite was present in birds in five of the six farms studied (Bonadiman et al., 2006).

Although the systematics and taxonomy of this genus have been explored, little is known about the ultrastructure of some characters. In this study, light and scanning electron microscopy were used to identify and define new morphological characters for those species of *Libyostrongylus* based on specimens found in ostriches from the northern region of Rio de Janeiro State, Brazil.

2. Materials and methods

2.1. Nematodes

Three adult ostriches were necropsied and the proventriculus, ventriculus and isthmus from each bird was examined. In one of the birds, many adult specimens of *Libyostrongylus* were found and collected. All animals came from the same farm in the northern region of Rio de Janeiro State, Brazil (21°19′23″S, 41°19′40″W), an area of about 20–30 m of altitude, and characterized by the Aw Köppen climate type. This farm had a high prevalence of the parasite as examined by OPG (Bonadiman et al., 2006); all the birds on this particular farm had been imported from the United States and Spain. Following collection from the proventriculus, the nematodes were washed in phosphate buffered saline and fixed in AFA or glutaraldehyde as specified below.

Representative specimens were deposited in the United States National Parasite Collection (USNPC), USDA, ARS, Beltsville, MD, USA (USNPC No. 96442 for *L. dentatus* and 96443 for *L. douglassii*) and in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC No. 35413 and 35414, respectively).

Specimens used for comparative purposes are as follows: (1) *L. dentatus*, paratypes of males and females, USNPC 83825 and voucher specimens, USNPC 83826, all in *S. camelus*; and (2) *L. douglassii*, voucher specimens, USNPC 83439, in *S. camelus*.

2.2. Light microscopy and measurements of nematodes

The nematodes were fixed in AFA (70 °C) overnight, transferred to 70% ethanol and 5% glycerin, cleared with pure glycerin, mounted on slides and observed with an Axioplan (Zeiss) light microscope equipped with differential interference contrast. Measurements were based on 13 males and 11 females for *L. douglassii* and 2 males and 3 females for *L. dentatus* from Brazil. Eggs were measured *in utero* and include 50 for *L. douglassii* and 30 for *L. dentatus*.

2.3. Scanning electron microscopy

The nematodes were fixed for 2 h in 2.5% glutaraldehyde, 4% freshly prepared formaldehyde, 5 mM calcium chloride in 0.1 M cacodylate buffer, pH 7.2, post-fixed in 1% osmium tetroxide, 5 mM calcium chloride and 0.8 potassium ferrocyanide in cacodylate buffer. The samples were dehydrated in an acetone series, critical point dried with CO_2, mounted in stubs, sputter-coated with gold, and examined in a Zeiss 962 scanning electron microscope operating at 15 KV.

3. Results

The morphologic and morphometric analysis of adult nematodes allowed the distinction between two species: *L. douglassii* (Cobbold, 1882) Lane, 1923 and *L. dentatus* Hoberg, Lloyd and Omar, 1995 (Tables 1 and 2). *L. douglassii* has no esophageal tooth at the cephalic extremity (Fig. 1A). Females have a relative short ovejector (Fig. 1B) and a tail with no cuticular inflation and rounded tip (Fig. 1C). In males, spicules have a main shaft ending in a point (Fig. 1D). *L. dentatus* has a prominent esophageal tooth at the cephalic extremity (Fig. 1E). Females have a long ovejector (Fig. 1F), a tail with prominent cuticular swelling at the anus and a digitate tip (Fig. 1G). The main shaft of the spicules terminates in a rounded point with a hyaline sheath (Fig. 1H).

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