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Case report

GRANULOMATOUS PROVENTRICULITIS ASSOCIATED WITH *MICROTETRAMERES* (NEMATODA: TETRAMERIDAE) INFECTION IN THE CATTLE EGRET (*BUBULCUS IBIS*) FROM RIO DE JANEIRO, BRAZIL

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Summary

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Host-parasite interactions can change and be rediscovered, exhibiting shifts or new pathogenies, which are mainly described for those poorly-studied species. The infection by *Microtetrameres* was firstly described in *Bubulcus ibis* in 1991, in which the reports of this infection have been related to mild lesions and low pathogenicity, mainly resulting in proventricular gland atrophy. In the two cases described in the present work, we report severe lesions, represented by serious inflammation associated with proventricular gland disruption. This fact may favour opportunistic pathogens, thus highlighting the importance of *Microtetrameres* infections in *B. ibis*.

Key words: *Bubulcus ibis, Microtetrameres* infection, severe proventriculitis

The family *Ardeidae* currently includes 70 nominal species, of which five are extinct and 46 have suffered population decline. The ardeid *Bubulcus ibis* (Cattle egret) is a non-threatened species, although it is hunted and persecuted when it nests in urban areas, and its natural habitats have been reduced. *Bubulcus ibis* has an expanding global population and can be found in Oceania, the Americas, Europe and Africa (IUCN, 2024).

Microtetrameres is a genus of spirurid nematodes, commonly found parasitising the proventriculus of birds. These parasites are peculiar among the nematodes, because their sexual dimorphism is highly marked, in which females show a globular aspect whereas males are much smaller and filiform (Anderson *et al.*, 2009). Species of *Microtetrameres* have an indirect life cycle, using invertebrates as intermediate hosts and trophic transmission to the final host, where females infect the gastric glands (Anderson, 2000).

Currently, there are several reports of *B. ibis* infected by *Microtetrameres*, most of them from Europe (Nogues-Ervola *et al.*, 2002; Navarro *et al.*, 2005; Eldein *et al.*, 2015; Santoro *et al.*, 2016; Drago *et al.*, 2020). In Brazil, there are no reports of these host-parasite associations. Moreover, the histopathology caused by *Microtetrameres* spp. in *B. ibis* has never been described. Therefore, the aim of the present study was to report and describe the histopathology caused by *Microtetrameres* sp. infecting *B. ibis*, in the State of Rio de Janeiro, Brazil.

Case description

In July 2019, two adult *B. ibis* males (weight 200 g and 190 g, respectively) were found dead at the Superporto do Açu (21°49'19.4"S, 41°02'00.8"W), State of Rio de Janeiro, Brazil. During necropsies, nematodes were found in the proventriculus, washed in saline, fixed in 4% formaldehyde and preserved in 70% ethanol. Tissue samples, from which nematodes were recovered, were fixed in 10% buffered formaldehyde. For histopathological analysis, samples were dehydrated in an ethanol series (70%, 90%, 100%), embedded in paraffin wax, sectioned (5 µm), stained with haematoxylin and eosin, and mounted in permanent slides with Canada balsam. For morphological study of parasites, specimens were cleared in glycerine as follows: a drop of glycerine-water solution in a ratio of 1:20 was put on a glass slide, the parasite was placed on it and covered by a coverslip, the slide was transferred to a heating plate at 56 °C, and as the water evaporated, a drop of more concentrated solution was added to the edge of the coverslip, in the concentrations 1:10, 1:5, and 1:2 until only glycerine was left. Taxonomic identification of parasites was according to Anderson *et al.* (2009). Microscopic analyses were performed using a Nikon Eclipse 80i microscope (Kurobane Nikon, Otawara, Tochigi, Japão) and the software NIS-Elements BR.

Nematodes found in the gastric glands were all gravid females (Fig. 1A–C). The body was highly swollen and coiled; only the anterior and posterior ends were filiform as in a typical nematode (Figs. 1A–C). The body had four loops (Fig. 1A). At the anterior end, the stoma was expanded and the pharynx smooth, both sclerotised (Fig. 1B). At the posterior end, the tail was conical, the cuticle transversely striated and reflected over about its mid-length, and supported by a sclerotised apparatus (Fig. 1C). The uterus occupied most of the body and was full of embryonated eggs, containing first-stage larvae (Fig. 1D).

The macroscopic analysis of the tissue revealed a slight increase in the ostium of the proventricular gland and multifocal caseous plaques. Microscopically, the proventricular glands were atrophied and compressed by the parasites, which were associated with an intense focal inflammatory process. The proventricular alveoli that were associated with the parasites were intensely compressed and suffered from severe numeric atrophic loss; glands

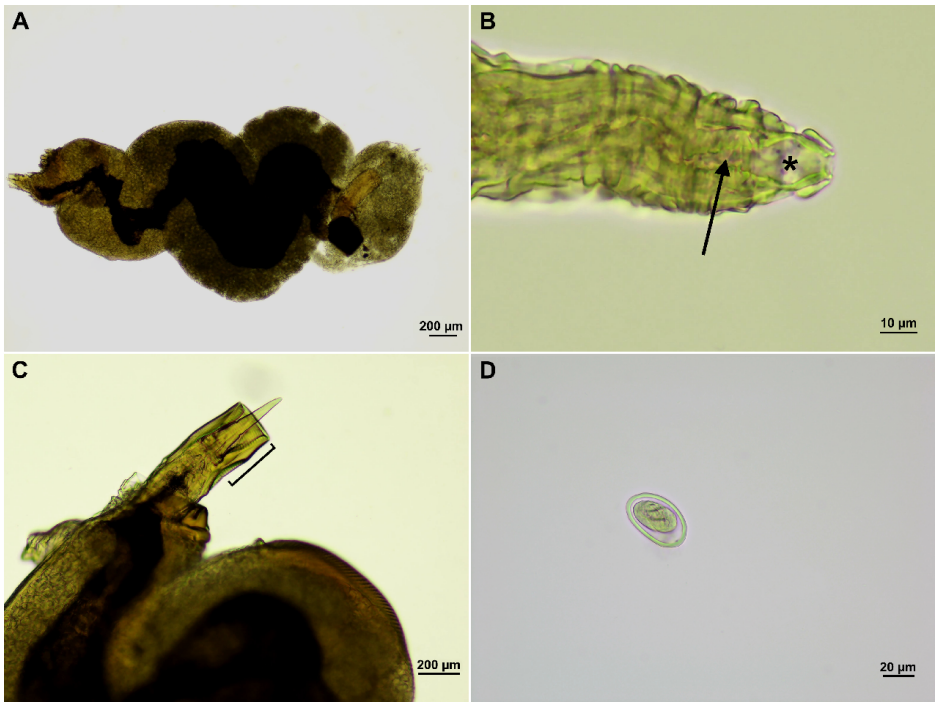

Fig. 1. *Microtetrameres* sp. collected in the proventriculus of *Bubulcus ibis* from the State of Rio de Janeiro, Brazil. **A.** Whole body of the female, the anterior part at the right, is broken. **B.** Anterior end of female showing the scleritized stoma and pharynx (black arrow). **C.** Posterior end of female showing a conical tail with the cuticle reflected over it. **D.** Egg extracted from the uterus, containing first stage larva.

adjacent to the parasites were slightly compressed (Fig. 2A). The parasitised glands were surrounded by reactive and moderately proliferative stroma, compatible with fibrosis. The caseous plaque consisted of proventricular gland atrophied and ruptured, and *Microtetrameres* surrounded by a well-delimited inflammatory process with high cellularity (Fig. 2B–C). The inflammation consisted of focally extensive inflammatory infiltrate, composed of a high amount of heterophils in the centre, and macrophages and giant multinucleated cells peripherally (Fig. 2B). Eosinophils and lymphocytes were rare, which characterised an ulcerative proventriculitis and heterophilic granuloma.

The present parasites were assigned to the genus *Microtetrameres* mainly because the females had a swollen and coiled body, and the pharynx was smooth (Anderson *et al.*, 2009). *Tetrameres* is the most closely related genus to *Microtetrameres*; the body of females is not coiled in the first (Anderson *et al.*, 2009). The most common *Microtetrameres* species reported in *B. ibis* is *M. spiralis* (Nogues-Ervola *et al.*, 2002; Navarro *et al.*, 2005; Eldein *et al.*, 2015; Santoro *et al.*, 2016).

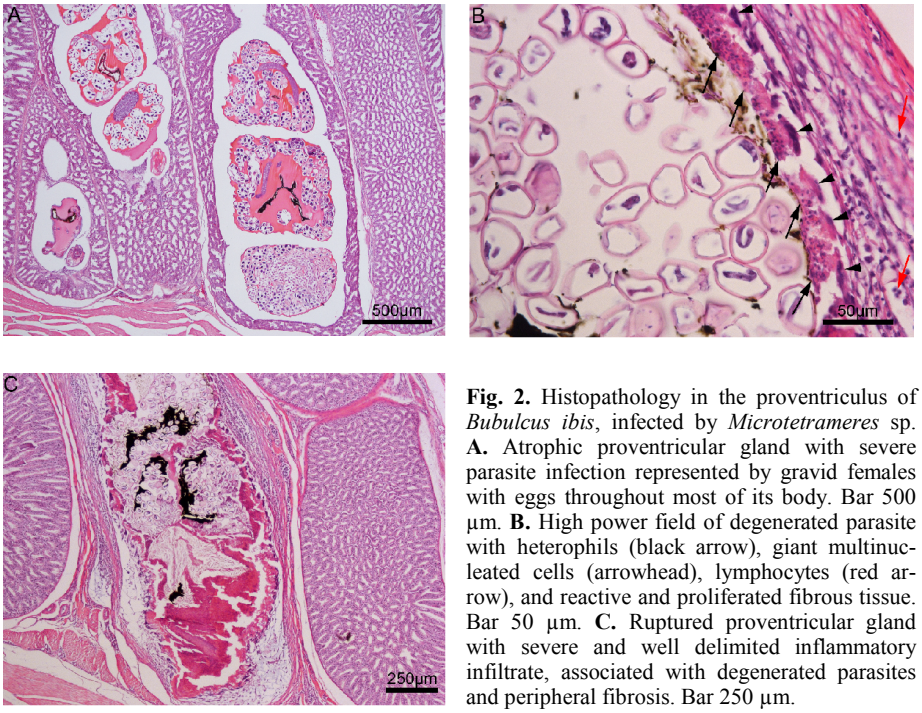

Fig. 2. Histopathology in the proventriculus of *Bubulcus ibis*, infected by *Microtetrameres* sp. **A.** Atrophic proventricular gland with severe parasite infection represented by gravid females with eggs throughout most of its body. Bar 500 µm. **B.** High power field of degenerated parasite with heterophils (black arrow), giant multinucleated cells (arrowhead), lymphocytes (red arrow), and reactive and proliferated fibrous tissue. Bar 50 µm. **C.** Ruptured proventricular gland with severe and well delimited inflammatory infiltrate, associated with degenerated parasites and peripheral fibrosis. Bar 250 µm.

In South America, there is a report from Argentina of *M. canadensis argentinensis* parasitising *B. ibis* (Labriola & Suriano, 1996). However, in the present study, we could not identify the parasites to the species level, since males are crucial for the diagnosis and were not found during necropsy. Moreover, the specimens were not adequately preserved for DNA isolation and genetic studies.

The infections by *Microtetrameres* in *B. ibis* have been investigated; however, previous studies reported only focal inflammation with no severity (Al-Mayah *et al.*, 1991) or no lesion associated with the parasite (Hassan & Abdel-Aal, 1999; Abou Shafeey, 2019). Here, in the proventriculus of *B. ibis*, as a consequence of the infection, we reported for the first time a multifocal locally extensive inflammation, with severe inflammatory infiltrate, consisting of a high amount of heterophils, macrophages, and multinucleate giant cells, which characterised a heterophilic granuloma in addition to glandular atrophy and a more compatible compression adjacent to the parasites.

Glandular atrophy in the proventriculus has been also detected in other bird species infected by *Microtetrameres*, but a marked inflammatory response was absent, although female parasites were found with blood cells in the intestine (Ellis, 1970; Clark *et al.*, 1979). A similar atrophy in proventricular glands was observed in the present study. However, rupture of the proventricular glands associated with *Microtetrameres* infection was described for the first time in this report.

Lesions that cause destruction of the basement membrane, in organs with bacterial flora, can pose a serious risk to animal health, since such lesions tend to favour secondary infections which can progress to blood tissue infection and septic shock. (Stanton & Zachary, 2022). In this sense, the rupture of the proventricular glands described in this case, became a gateway for other microbial infectious agents, which can cause the death of the animal. This process may be occurring in more cases of *Microtetrameres* infection in *B. ibis* and other species.

Our study revealed that the infection by *Microtetrameres* in *B. ibis*, previously known to cause only mild glandular inflammation and atrophy, is also capable of causing a more severe proventriculitis and tissue compression, which can result in serious illness to the animal. However, such identification will require further efforts to find male specimens and material suitable for genetic sequencing.

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