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Molossinema wimsatti infection in the brain of Pallas's mastiff bats (*Molossus molossus*)

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Short Communication

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Abstract

The present report describes two cases of infection by *Molossinema wimsatti* in the brain of Pallas's mastiff bats (*Molossus molossus*). The first bat was captured and killed by a domestic cat in a suburban area of the municipality of Patos, Paraiba, northeastern Brazil. The second bat was found crawling on the ground in the same area before dying. No gross lesions were found at necropsy. Histology of the central nervous system revealed filarioid nematodes in the brain ventricles and cerebellum. There were adults, subadults and eggs, the latter sometimes containing microfilariae. No inflammatory response was observed in bat 1, while bat 2 presented a mild lymphoplasmacytic meningoencephalitis. Three nematodes were recovered and submitted for parasitological examination. The diagnosis of *M. wimsatti* infection was based on the histomorphological and parasitological characteristics of the agent and its location in the brain ventricular system of insectivorous bats. The infection likely occurs in other insectivorous bats from South American and Caribbean countries but may be overlooked.

Introduction

Nematode parasitism of the central nervous system is rare in bats. However, there are reports of the infection by *Angiostrongylus cantonensis* and *Molossinema wimsatti* (Farina *et al.*, 2018).

Angiostrongylus cantonensis resides in the pulmonary arteries of rats (definitive host), hence the sobriquet 'rat lungworm' ascribed to the parasite (Farina *et al.*, 2018). The complex life cycle of this nematode includes intermediate, paratenic and accidental hosts (Spratt, 2015). The infection has been reported in a variety of domestic animals, mammalian and avian wildlife, and humans (Spratt, 2015). Neurological disease has been reported in black and grey-headed flying foxes (Reddacliff *et al.*, 1999; Barrett *et al.*, 2002).

The filarioid nematode *M. wimsatti* was reported in the central nervous system of insectivorous bats. These nematodes reside particularly in the ventricular system of the brain (Nguyen & Myers, 1987). Clinical signs are minimal and rarely reported (Lichtenfels *et al.*, 1981; Nguyen & Myers, 1987). The life cycle of this parasite remains unknown (Nguyen & Myers, 1987).

The present report describes two cases of *M. wimsatti* infection in Pallas's mastiff bats (*Molossus molossus*), which, to the best of our knowledge, is the first report of the condition in Brazil.

Results

Case 1

An adult female Pallas's mastiff bat (*M. molossus*) was captured and killed by a domestic cat in a suburban area of the municipality of Patos, Paraiba, north-eastern Brazil (7.0192°S, 37.2751°W).

Case 2

The second case was in an adult male Pallas's mastiff bat (*M. molossus*) that was found at dusk crawling on the ground in the same area. The bat was placed in a metal cage for observation and died a day later.

Pathological findings

At post-mortem examination, both bats were in good body condition and had no gross lesions other than small bite pounds on the wings (case 1). No search for microfilaria in the peripheral blood was performed.

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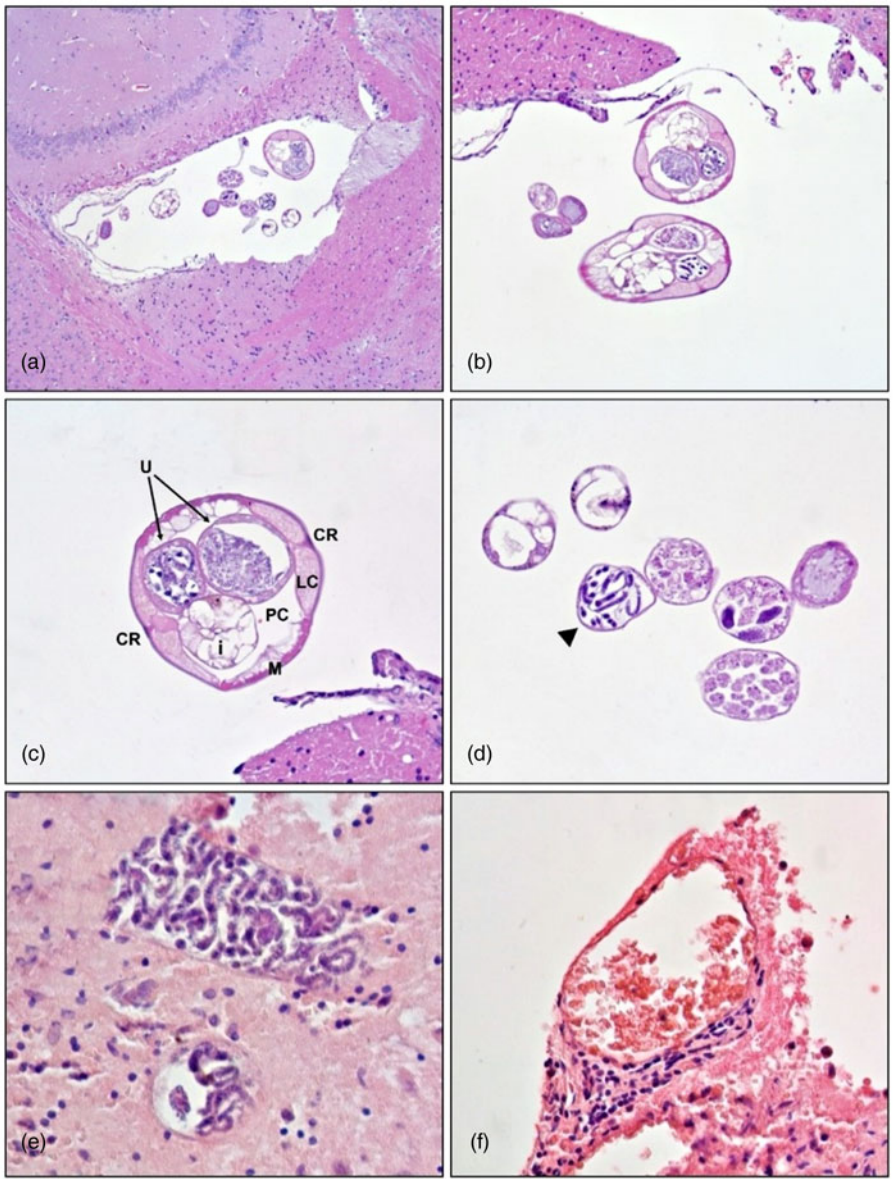

Fig. 1. *Molossinema wimsatti* in the brain of insectivorous bats. (A) Brain, right lateral ventricle. Filarioid nematodes: adult, subadult and eggs. Haematoxylin and eosin (HE). Objective 5×. (B) Brain, left lateral ventricle. Cross-section of two adults and three subadults (arrow). Filarioid nematodes. HE. Obj. 20×. (C) Cross-section of an adult female. Filarioid nematode showing coelomyarian–polymyarian musculature (M), lateral chords (LC), internal cuticular ridge (CR), pseudocoelom (PC), two uteri (U) and intestine (i). HE. Obj. 40×. (D) Cross-section of subadult forms (arrow) and eggs, one of which containing microfilariae (arrowhead). HE. Obj. 40×. (E) Cerebellar neuroparenchyma. Cyst-like structures (presumably eggs) containing numerous microfilariae. HE. Obj. 40×. (F) Cerebellum, leptomeninges. Perivascular cuffing composed of lymphocytes, plasma cells and rare macrophages. HE. Obj. 40×.

Samples from the skin, central nervous system and organs from the thoracic and abdominal cavities were fixed in 10% neutral buffered formalin, processed routinely for histopathology, embedded in paraffin, cut into 3 µm sections and stained with haematoxylin and eosin.

One hemisphere of each brain was frozen as a laboratory standard protocol. Upon the histological finding of nematodes in the central nervous system, the brains' frozen halves were reassessed. Nematodes were recovered using stereomicroscopy, fixed in 10% neutral buffered formalin and maintained in alcohol–formalin–acetic acid. They were then cleared with lactophenol and morphologically analysed by light microscopy.

Histopathological examination of the central nervous system revealed filarioid nematodes in the lateral (case 1), third and fourth ventricles of the brain, and cerebellar neuroparenchyma (case 2). There were adults, subadults and eggs, sometimes containing microfilariae (fig. 1a, b).

The adult forms, seen in transverse sections, were round, had thin coelomyarian–polymyarian musculature, and were divided into dorsal and ventral fields by lateral cords with internal cuticular ridges. In the pseudocoelom there was a slender intestine and, if female specimens, two uteri (fig. 1c). The diameter of the adult nematodes ranged from 130 to 150 µm. These morphological characteristics are consistent with that of *M. wimsatti*.

The subadult forms had similar shapes to that of adults but were smaller (up to 70 µm in diameter) and lacked well-defined internal structures. Also, round thin-shelled eggs, measuring ± 50 µm in diameter, and sometimes containing microfilariae, appeared in ventricles (fig. 1d). In case 2, multifocally within the white and grey matter of the cerebellum there were numerous microfilariae within cyst-like structures (presumably eggs) (fig. 1e).

There was no evidence of tissue damage or inflammatory response in the nervous system of bat 1, while bat 2 presented a mild non-suppurative meningoencephalitis characterized by perivascular cuffs composed of lymphocytes, plasma cells and rare macrophages (fig. 1f).

Three filarioid nematodes (two females and a male) were recovered from the brain ventricular system of bat 2 (fig. 2a),

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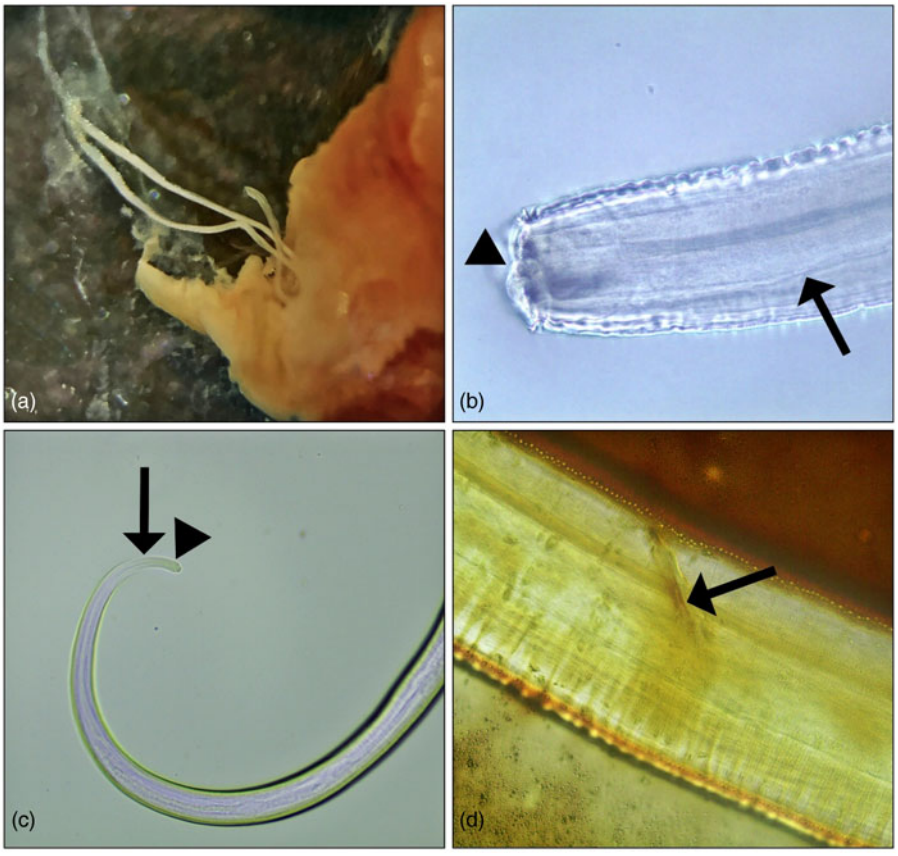

Fig. 2. *Molossinema wimsatti* in the brain of insectivorous bats. (A) Adult filarioid nematodes recovered from the brain ventricular system. Stereomicroscopy (SM). Objective 2×. (B) Anterior body portion. Inconspicuous buccal cavity (arrowhead) and a long slender cylindrical oesophagus (arrow). Light microscopy (LM). Obj. 40×. (C) Female, posterior body portion. Small cylindrical tail with phasmids that surmount small hemispherical bases flanking the tip of the tail (arrow). Note the anus almost at the tip of the tail (arrowhead). LM. Obj. 10×. (D) Male, posterior body portion. Arcuate spicule (arrow). Contralateral spicule is not visible due to overlap. LM. Obj. 40×.

and were identified as *M. wimsatti* according to the morphological features previously described (Georgi *et al.*, 1987).

Adult nematodes were characterized by an inconspicuous buccal cavity and a long slender cylindrical oesophagus, not divided into muscular and glandular portions (fig. 2b). The females measure approximately 51 mm in total body length and have a small cylindrical tail with phasmids that surmount small hemispherical bases flanking the tail's tip (fig. 2c). The anus is present almost at the tip of the tail (sub-terminal). The male measure approximately 20 mm in total body length. The tail presented two arcuate spicules, unequal in size and shape (fig. 2d). The anus is present at the tip of the tail (terminal).

Discussion

The diagnosis of *M. wimsatti* infection was based on the histomorphological and parasitological characteristics of the agent and its location in the brain ventricular system of insectivorous bats.

Infections by *M. wimsatti* have been identified in bats from South America and the Caribbean – in most cases, as an incidental finding. Infection occurred in a supposedly healthy bat from El Colegio, Colombia (Lichtenfels *et al.*, 1981), and in an outbreak of bat mortality in the island of Trinidad, Republic of Trinidad and Tobago, when more than 60 bats were parasitized; however, the nematodes did not contribute to the death of the bats (Georgi *et al.*, 1987; Nguyen & Myers, 1987).

Pallas' mastiff bats are insectivores with a diet consisting of mosquitoes and other airborne insects (Esberard & Bergallo, 2010). In the previously reported cases of *M. wimsatti* infection, the affected bats were also insectivores (Lichtenfels *et al.*, 1981; Nguyen & Myers, 1987). Despite that, the life cycle of this parasite remains unknown but presumably involves an arthropod.

Interestingly, no microfilariae have been found in the blood or tissues of any of the bats previously affected (Nguyen & Myers, 1987), making it even more challenging to determine the life cycle of the nematode.

Although clinical signs are rare in bats with brain parasitism by *M. wimsatti*, bat 2 had neurologic disorders, consistent with ataxia and inability to fly. Similarly, ataxia was present in a previously reported case (Nguyen & Myers, 1987). It is fair to assume that in massive infections bats may manifest neurological signs, such as prostration, inability to fly or flying inappropriately low, conditions that may even favour their capture by natural predators.

In these cases, the nematodes were seen mainly in the brain ventricular system. They can be found in the lateral, third and fourth ventricles, and in the subdural space of the cervical and thoracic spinal cord (Nguyen & Myers, 1987). In some cases, dilation of the affected ventricle may occur but injury to the periventricular parenchymal tissue was observed only in one case, in which the animal had symptoms (Nguyen & Myers, 1987; Farina *et al.*, 2018). In bat 2, numerous microfilariae were deposited within the cerebellar neuroparenchyma, which may have incited the inflammatory response and the clinical signs.

Georgi *et al.* (1987) provide a detailed description of *M. wimsatti* morphological features in scanning electron microscopic, histology and art illustrations, and data that provided sufficient features to identify *M. wimsatti* and differentiate it from other species of bat nematodes by light microscopic examination.

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Differential diagnosis should include infections caused by *A. cantonensis*, *Litomosoides* and *Litosoma* spp.

Angiostrongylus cantonensis infections cause eosinophilic and granulomatous meningoencephalitis, usually associated with focal areas of malacia and nematodes in the inflamed meninges and brain parenchyma (larval migration) (Reddacliff *et al.*, 1999; Barrett *et al.*, 2002). In some rare cases, inflammation may be absent (Barrett *et al.*, 2002). In these cases, the histomorphological features and location of the agent can assist the identification.

Litomosoides and *Litosoma* spp. have morphological characteristics that might resemble *M. wimsatti* but have only been described in the thoracic cavity of bats without affecting the central or peripheral nervous system (Farina *et al.*, 2018).

In conclusion, infection by *M. wimsatti* occurs as an incidental finding or as a cause of neurological disorders in Pallas's mastiff bats from north-eastern Brazil. The infection likely occurs in other insectivorous bats from South American and Caribbean countries but maybe overlooked.

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Conflicts of interest. None.

Ethical standards. We declare that the present paper followed all the ethical procedures present in the International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences. In addition, the Laboratory of Animal Pathology of the Federal University of Campina Grande did not carry out animal experiments, it only received tissue samples or performed necropsies.

References

Barrett JL, Carlisle MS and Prociv P (2002) Neuro-angiostrongylosis. in wild black and grey-headed flying foxes (*Pteropus* spp). *Australian Veterinary Journal* **80**, 554–558.

Esberard CEL and Bergallo HG (2010) Foraging activity of the free-tailed bat *Molossus molossus* (Chiroptera; Molossidae) in southeastern Brazil. *Brazilian Journal of Biology* **70**, 1011–1014.

Farina LL, Lankton JS and Terio KA (2018) Chiroptera. pp. 607–633 *in* McAloose D and St. Leger J (*Eds*) *Pathology of wildlife and Zoo animals*. San Diego, Elsevier.

Georgi JR, Georgi ME, Jiang J and Frongillo M (1987) Molossinema wimsatti gen. et sp. n. (Nematoda: Onchocercinae) from the brain of *Molossus ater* (Chiroptera: Molossidae). *Proceedings of the Helminthological Society of Washington* **54**, 225–230.

Lichtenfels JR, Bhatnagar KP, Whittaker FH and Frahm HD (1981) Filaroid nematodes in olfactory mucosa, olfactory bulb, and brain ventricular system of bats. *Transactions of the American Microscopical Society* **100**, 216–219.

Nguyen HT and Myers DD (1987) Filaroid nematodes in the central nervous system of free-tailed bats (*Molossus ater*). *Laboratory Animal Science* **37**, 672–674.

Reddacliff LA, Bellamy TA and Hartley WJ (1999) *Angiostrongylus cantonensis* infection in grey-headed fruit bats (*Pteropus poliocephalus*). *Australian Veterinary Journal* **77**, 466–468.

Spratt DM (2015) Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: a review. *International Journal for Parasitology, Parasites and Wildlife* **4**, 178–189.

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