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Case report

Pathological and parasitological findings in a Brazilian hoary fox (*Lycalopex vetulus*, Lund, 1842) infected by *Oslerus osleri* (Cobbold, 1876) (Nematoda: Filaroididae)

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Abstract

Oslerus osleri (*O. osleri*) is a filaroid nematode of the respiratory tract of domestic and wild canids that leads to parasitic tracheobronchitis. The aim of this study was to report the first occurrence of *O. osleri* in hoary fox in Minas Gerais State, Brazil. The animal was found struck by vehicle in a periurban area and died before any clinical procedures were attempted. At necropsy the animal showed multiple bone fractures. The mucosa of the middle and distal part of the trachea, and primary bronchi were irregular and moderately thickened, containing numerous cylindrical parasites forming nests, firmly adhered to the mucosa. Histopathological examination revealed diffuse inflammation of the mucosa and numerous parasites embedded in the lamina propria with nematode characteristics. The parasitological evaluation including morphological structures and the size of parasites confirmed the occurrence of *O. osleri* in the Brazilian hoary fox. The conclusion of this report is that Brazilian hoary fox is naturally infected by *O. osleri* and this finding is important to know the distribution and epidemiology of this little known parasite in Brazil.
Key Words: wild canid, hoary fox, Filaroididae, *Oslerus osleri*, tracheobronchitis, histopathology.

Introduction

The Brazilian hoary fox (*Lycalopex vetulus*) (Lund 1842) (Carnivora: Canidae), also called raposinha-do-campo (Portuguese) is native to Brazil. It occurs primarily in the south-central cerrado habitats (mosaic of grasslands and xerophytic vegetation) at open and low elevations, and is rarely observed in densely wooded cerrado, floodplains, and dry or gallery forests. This animal is mainly active at night and is commonly found alone. Natural diet consists of insects, fruits, small mammals and birds. It has adapted well to insect-rich livestock pastures and areas of agriculture with soybean, rice, corn and eucalyptus (5, 6, 13, 9). The hoary fox is a small canid weighing three to four kilograms with a short muzzle and big ears. The length of head and body ranges from 49 to 71 cm and the tail measures 25-38 cm (5, 7). Upper parts of the body are pale gray and lower parts are cream-colored. The lower jaw is nearly black as well as the tip and base of the tail (7). The species is currently listed as Least Concern on the Brazilian Red List of Threatened Species and classified as Near Threatened in the Minas Gerais State List of Threatened Species (8).

Oslerus osleri (Cobbold, 1876), previously classified as *Filaroides osleri* (*F. osleri*), is a nematode of the superfamily Metastrongyloidea, family Filaroididae, with worldwide distribution causing respiratory disease in

domestic and wild canids (11). The adult nematode resides in tracheobronchial nodules in the lamina propria, often concentrated around the tracheobronchial bifurcation (27). Maternal grooming is assumed to be the main transmission route in domestic dogs, whereas regurgitated feedings between adults and their pups is the route of transmission in wild canids (3, 4). This study reports pathological and parasitological findings in a hoary fox naturally infected with *O. osleri*.

Case report

On May 6th, 2013, a young female specimen of hoary fox (*Lycalopex vetulus*), weighting 4 kg was found struck by vehicle on BR-040 near the city of Sarzedo at 20º02'07" S and 44º08'41" W. The animal was referred to the Centro de Triagem de Animais Silvestres (CETAS-IBAMA) in the city of Belo Horizonte (Figure 1). At clinical presentation, the animal was determined to have multiple bone fractures and neurogenic shock. The animal died before supportive therapy could be initiated. The necropsy was performed at the Pathology sector, School of Veterinary Medicine, Universidade Federal de Minas Gerais (UFMG).

Figure 1. Female of Brazilian hoary fox (*L. vetulus*) specimen referred to the CETAS of Belo Horizonte city after struck by vehicle.

Grossly, the animal showed diffuse and intense areas of hemorrhage in the subcutaneous and skeletal muscles of the hind limbs, and inguinal and sacral areas. In addition, there were complete fractures of the femur, ischium and pubis. Bladder was ruptured.

The mucosa of the middle and distal parts of the trachea and primary bronchi were irregular and moderately thickened. Two red-whitish elevated areas measuring 0.2 cm in diameter, on the distal trachea were observed and, in the tracheal bifurcation, there was another area measuring 0.7 cm in diameter. These areas were associated with numerous cylindrical parasites forming nests, firmly adhered to the mucosa (Figure 2). The lungs were pale-reddish, emphysematous and had multiple foci of mildly firm, whitish granularity on the cut surface. Pieces of the trachea, lungs, intestine, kidneys, liver, heart, lymph nodes, brain and bone marrow were collected and fixed in 10% neutral-buffered formalin, routinely processed and embedded in paraffin. Four micrometer sections were stained with hematoxylin and eosin (HE). Contents of small and large intestine were collected during necropsy and Baermann technique was used for the detection of parasites.

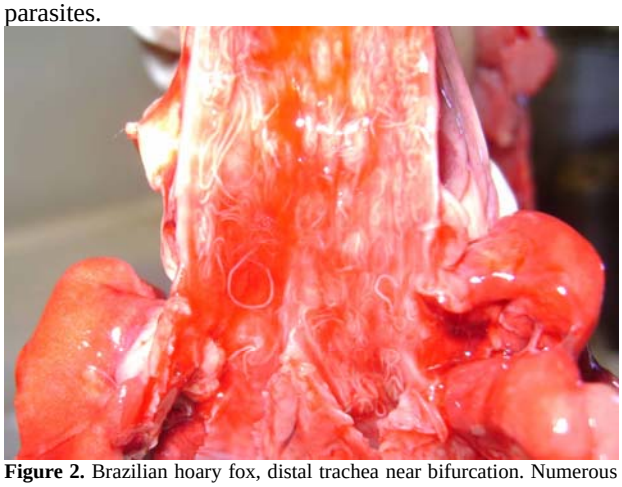

Figure 2. Brazilian hoary fox, distal trachea near bifurcation. Numerous cylindrical parasites adhered to the thickened and irregular tracheal and bronchial mucosa.

Parts of the middle and distal parts of the trachea containing numerous adhered cylindrical parasites were dissected in Petri dishes containing 0.85% saline. The nematodes were collected under a stereomicroscope and fixed in 10% formalin heated at 80ºC. Subsequently, a sample of 14 specimens (nine males and five females) was clarified in Solution of Amann Lactophenol for microscopic identification.

Histopathology revealed diffuse irregular thickening of the mucosa due to intense hyperemia, edema, inflammation and numerous parasites embedded in the lamina propria (Figure 3). These parasites were surrounded by a thin layer of fibroblasts and collagen, in addition to intense infiltration of lymphocytes, plasma cells, and histiocytes. Adjacent to parasites was also observed mild vascular proliferation and some remaining glands. Areas with loss of surface epithelium and deposition of fibrin and red blood cells were also present. Groups of parasites were observed in transverse and longitudinal sections. There were some places where these parasites were also found inserted in the adventitia of the trachea and in the submucosa of the primary bronchi. Cross-sections of the parasites were also observed in the subcapsular sinus of an adjacent lymph node.

Histologically, these parasites had nematode characteristics, including presence of a body cavity, thin outer wall, delicate cuticle, and coelomyarian muscles. Large intestine with few multinucleated cells and uterus with numerous embryonated eggs (Figure 4) could also be seen. In the lungs there were multifocal atelectasis, hemorrhage and moderate multifocal to coalescing interstitial histiocytic pneumonia. In addition, several megakaryocytes were present.

The length of the male specimens was 7,2 ± 0,73 mm and for females, the length was 13,2 ± 1,22 mm.

Both presented buccal vestigial cavity, a protuberant rostrum and cuticular collar located in the cervical region, 13.1 ± 0,71 µm posterior to the buccal cavity of males and 16.1 ± 0.71 µm of females.

Figure 3. Brazilian hoary fox, trachea. Diffuse irregular thickening of the mucosa containing numerous parasites embedded in the lamina propria surrounded by inflammatory cells. Hematoxylin and eosin.

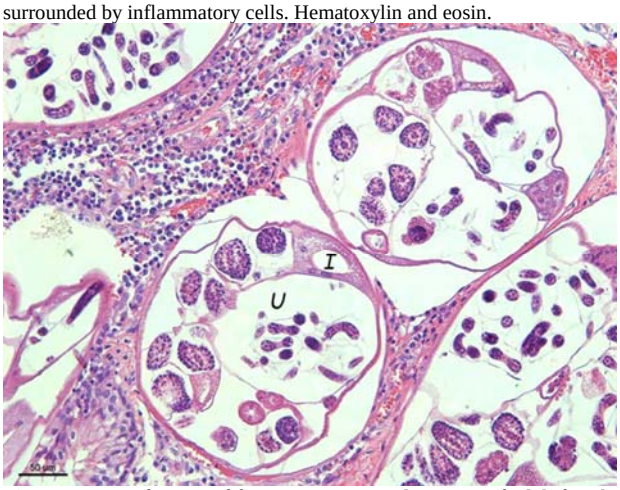

Figure 4. Magnification of figure 3. Transversal sections of adult female parasites surrounded by a thin layer of connective tissue, and infiltration of lymphocytes and plasma cells. *O. osleri*: I: intestine with few multinucleated cells. U: uterus with several embryonated eggs.

The excretory system presented three vesicles that opened into a common duct next to the buccal cavity. The esophagus was flask-shaped measuring 216.7 ± 14.56 µm in male and 238.7 ± 14.89 µm in the female. The posterior end of the worm was non-bursate, the gubernaculum, 37, 2 ± 1.22 µm in length, was present and small equal spicules 100,9 ± 4.80 µm long (Figure 5). The distance between the opening cloaca from the posterior end was 26,4 ± 1.90 µm. The ovoviviparous females contained uterus with larvae, extended far forward, almost to the esophageal-intestinal junction. The posterior end of the anus and vulva opening were close together in the cleft and had no vaginal sphincter (Figure 6). The morphological structures and the size of parasites, both males and females, were consistent with *O. osleri* as previously described (21, 24).

Figure 5. Posterior end of *O. osleri* male with spicule and gubernaculum. Lateral view.

Figure 6. Posterior end of *O. osleri* female containg several embryonated eggs. Lateral view.

Discussion

This is the first report of *Oslerus osleri* infection in a Brazilian hoary fox. There are only two reports of *O. osleri* in Brazilian wild canids, both described in maned wolves (*Chrysocyon brachyurus*) (10, 26).

Grossly, infections are usually characterized by prominent nodules in the tracheal mucosa near the bifurcation; however, when heavy infections occur, many nodules could be seen extending to the secondary bronchi (17). Prominent nodules were not evident in the present case as reported in domestic and wild canids (11, 25, 27). In the animal of this report, the lesions indicated an acute to subacute process, with not sufficient cellular infiltrate to form prominent nodules. Within two to three months, the parasites would stimulate a granulomatous response in the host, producing a nodular lesion (11). In the herein described animal, the parasites were present in the lamina propria of trachea and bronchi, surrounded by moderate mononuclear infiltrate, without marked tissue reaction.

Other studies have reported intense eosinophilic infiltrate associated with mononuclear cells involving parasites (18, 25, 27), furthermore, there is a study

describing suppurative tracheitis with mucosal metaplasia (20), features not detected in our case.

Animals infected with *O. osleri* may present a sporadic chronic non-productive cough, dyspnea and inspiratory tracheal noises that intensify when they perform physical efforts (4, 18, 27). Nevertheless, some animals do not show previous respiratory signs and, the onset of acute respiratory distress may occur when prominent tissue inflammation, containing these nematodes near the bifurcation of the trachea, creates stenosis of the lumen (20). The animal of the present report did not survive long enough to allow a clinical examination characterizing any respiratory compromise, although some irritation of the trachea and bronchi induced by the inflammation and heavy infestation could be suspected.

The presence of this parasite in domestic and wild canids is underdiagnosed for two reasons: studies of parasites in wild canids are focused on the gastrointestinal tract and few parasitological exams in the respiratory tract are made (25). Most parasitological studies using fecal analysis are not adequate to detect low numbers of larvae of *O. osleri* eliminated with the faeces (27). In this animal, a parasitological exam was made using the fecal Baermann technique, with negative results. The more efficient parasitological test for this parasite using a fecal sample is floating in a solution of zinc sulphate (22).

Clinical diagnosis of infection by *O. osleri* can be obtained by biopsy using a bronchoscope followed by histological analysis (18, 27). Canids with clinical signs of sporadic cough with the cough reflex positive during clinical examination should be considered suspicious for the present condition. *O. osleri* infections should be included as a differential diagnosis in dogs showing clinical signs of tracheobronchitis, particularly in young dogs under two years of age (27). Concerning gross and histopathological findings, *Crenosoma vulpis* (*C. vulpis*) should be considered as a differential diagnosis, but differently from *O. osleri*, most *C. vulpis* specimens are found only in the lumen of bronchi (23).

Following the diagnosis, medication can be effective. Several classes of anthelmintics, especially benzimidazoles and macrocyclic lactones have been used to eliminate *O. osleri* infection (27).

Transmission of *O. osleri* occurs by direct contact and/or ingestion of the first stage larvae. Intermediate host and intrauterine infection does not occur (4, 11). The eggs and first-stage larvae are carried by the mucociliary escalator to the bifurcation of the trachea and then to the oropharynx where they are swallowed and eliminated in the faeces or remain in the saliva (4, 15). Most dogs become infected due to the social behavior of cleaning the puppies or by regurgitation of food (11, 15). After uptake of first-stage larvae, these cross the intestinal wall and via lymphatic or via hepatic venous circulation reach the right atrium of the heart. Subsequently, the larvae travel to the pulmonary capillaries, penetrate the alveoli and migrate into the tracheobronchial tissue. The five stages of the parasite larvae develop in the lung. After a period of 10-21 weeks, the adult females lay fertilized eggs of the parasite in the lumen of the trachea (11, 15). A study of *O. osleri* in naturally and experimentally infected foxes in Australia suggests that this is an accidental host, infecting themselves by eating larvae of *O. osleri* on food regurgitated by other canids (12).

The effect of urban expansion on biodiversity conservation is significant (16). Urban sprawl leads to the loss of habitat for wildlife species and these are forced to occupy and to adapt to environments with different degrees of anthropogenic changes. The plasticity and adaptability of individuals in the peridomestic environment has been demonstrated through studies of the impact of urban areas on the richness and distribution of various carnivorous species (14, 19). However, the response of each species to urbanization processes varies widely (1, 2). This environment may act as reservoirs and source of infection for humans and domestic animals.

These animals can also suffer epizootic infections and, consequent population declines related to the transmission of infectious and parasitic diseases by domestic animals. The animal in this study was found in a periurban area and its contact with areas occupied by domestic infected dogs was the possible source of infection.

Knowledge of helminths that affect wild canids becomes important to assess the possible damage to that population and their possible zoonotic potential. It is not known if the parasites found in the Brazilian hoary fox could directly affect their ecology and, epidemiological studies would be needed in the population of foxes in Brazilian Cerrado Biome.

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