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Case Report

Angiostrongylus spp. in the nasal mucosa of *Didelphis* sp

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Abstract

Nematodes of the genus *Angiostrongylus* are parasites capable of inhabiting the pulmonary arteries, heart, bronchioles, and mesenteric veins of various hosts. Opossum species of the genus *Didelphis* are considered synanthropic animals, cohabiting with humans and domestic animals and potentially disseminating zoonotic pathogens. This study aimed to report the changes caused by *Angiostrongylus* sp. nasal infection in *Didelphis aurita* and *D. albiventris.* Five animals were admitted to the Veterinary Hospital of the Federal University of Paraná, Curitiba, PR, Brazil, due to traumatic accidents. Three animals exhibited neurological clinical signs and, due to the severity of their condition, were euthanized or died. The necropsy of deceased animals revealed numerous adult worms in the nasal sinuses of all five individuals. Tissue sections, including nasal sinuses, were collected and submitted for histological evaluation. Adult parasites were recovered for generic morphological identification. Histopathological evaluation showed fibrinosuppurative and necrotic rhinitis along with sinusitis rhinitis and sinusitis with intralesional nematodes. Morphological identification demonstrated that the parasites belong to the genus *Angiostrongylus* (Metastrongylidae). This study is the first report of the genus *Angiostrongylus* sp. infecting the nasal mucosa of free-living opossums (*Didelphis aurita* and *Didelphis albiventris*).

Keywords: nasal mucosa, nematoda, *Didelphis*, wild opossums.

Introduction

Nematodes of the genus *Angiostrongylus* are parasites capable of infecting a wide variety of hosts and tissues. Such parasitic infections occur naturally in numerous birds, rodents, carnivorous mammals, and marsupial species, as well as in humans, as definitive hosts, and in various snail species (Gastropoda) as intermediate hosts (2, 16, 21, 3, 9, 10). These nematodes inhabit pulmonary arteries, the heart, bronchioles, and mesenteric veins (22, 1, 18). Larval stages of *A. cantonensis* develop in the central nervous system, migrating to pulmonary arteries to mature and mate (11). In accidental hosts, the development of *A. cantonensis* to the 4th and 5th larval stages in neural tissue can lead to neurological signs, such as ataxia, stupor, and limb paresis/paralysis, due to the induction of a granulomatous and eosinophilic response in brain tissue (21, 18).

Two species of nematodes, *Didelphostrongylus hayesi* (Prestwood, 1976) and *Heterostrongylus heterostrongylus* (Travassos, 1925), that infect the cardiovascular and respiratory systems of marsupials, have been reported parasitizing two opossum species, *Didelphis marsupialis* and *D. aurita*, respectively (9).

Animals of the genus *Didelphis* are well adapted to human environments and are often found on house roofs, in tree cavities, and in other shelters in urban and peripheral areas. They have crepuscular and nocturnal habits and are considered synanthropic animals (4). *Didelphis* sp. cohabit with humans and domestic animals in rural and urban environments, making them potential disseminators of zoonotic pathogens (17).

Parasites found in *Didelphis* sp. are highly diverse, including arthropods, helminths, and protozoa. Many of these parasites impact the health of their hosts, causing disease or even death. Some are significant for public health, as they can act as vectors of zoonotic pathogens or be the etiological agent of human diseases (6).

Therefore, the objective of this study was to report the changes caused by *Angiostrongylus* sp. nasal infection in *Didelphis aurita* and *Didelphis albiventris.*

Case description

Between September and October 2022, five opossums (*Didelphis* sp.) were rescued by environmental agencies due to traumatic accidents and referred to the Veterinary Hospital of the Federal University of Paraná, Curitiba, PR, Brazil. Three animals exhibited neurological clinical signs such as apathy, ataxia, nystagmus, hind limb paresis, and motor incoordination. Due to the severity of their condition, these animals were euthanized or died. The other two animals died as a result of their traumatic injuries. All dead animals were necropsied.

Macroscopic evaluation

The necropsy revealed multiple perforating and cutting lesions, laceration of parenchymatous organs, extensive skull and spine fractures, and multiple large focal hematomas, indicating the traumatic nature of death. All necropsied animals had a wide variety of parasites, primarily found in the gastric mucosa, such as *Turgida turgida* and Physalopteroidea, in the cecum. Four individuals had nodules ranging from 0.1–0.5 cm, which were slightly exophytic, yellowish, multifocal to coalescent on the pleural surface. Small parasitic structures, cylindrical and whitish, were found in the lumen of bronchi and bronchioles.

All five necropsied animals had numerous morphologically elongated adult nematodes (Fig. 1), which were white with a blackened spiraled filament in the middle nasal conchae, ethmoidal, and nasal sinuses. The nasal mucosa appeared reddish to brownish with moderate to severe mucopurulent exudate draining from the cribriform plate.

Histopathological evaluation

During the necropsy, tissue fragments were collected, fixed in 10% buffered formalin for 48 hours, processed routinely, and submitted for histopathological evaluation. The nasal sinuses were carefully dissected, and sequential sections were made and kept in a decalcifying solution for 24 hours before tissue processing and histological evaluation.

Microscopic examination of the lungs revealed a moderate to marked neutrophilic, eosinophilic, and histiocytic interstitial and peribronchial inflammatory infiltrate, associated with adult and larval stages morphologically compatible with nematodes of the genus *Angiostrongylus.* In the bronchial lumen, the parasitic structures were surrounded by moderate infiltration of eosinophils, epithelioid cells, and foamy macrophages. Additionally, there was moderate hypertrophy of the adventitial tunica of pulmonary arterioles.

Within the lamina propria of the nasal mucosa, multiple fibrinous and necrotic foci with marked neutrophilic, eosinophilic, and histiocytic inflammatory infiltrates were observed (Fig. 2c). Desquamated epithelial cells and numerous adult and larval stages of *Angiostrongylus* sp. were also present in the nasal sinuses (Fig. 2a,b).

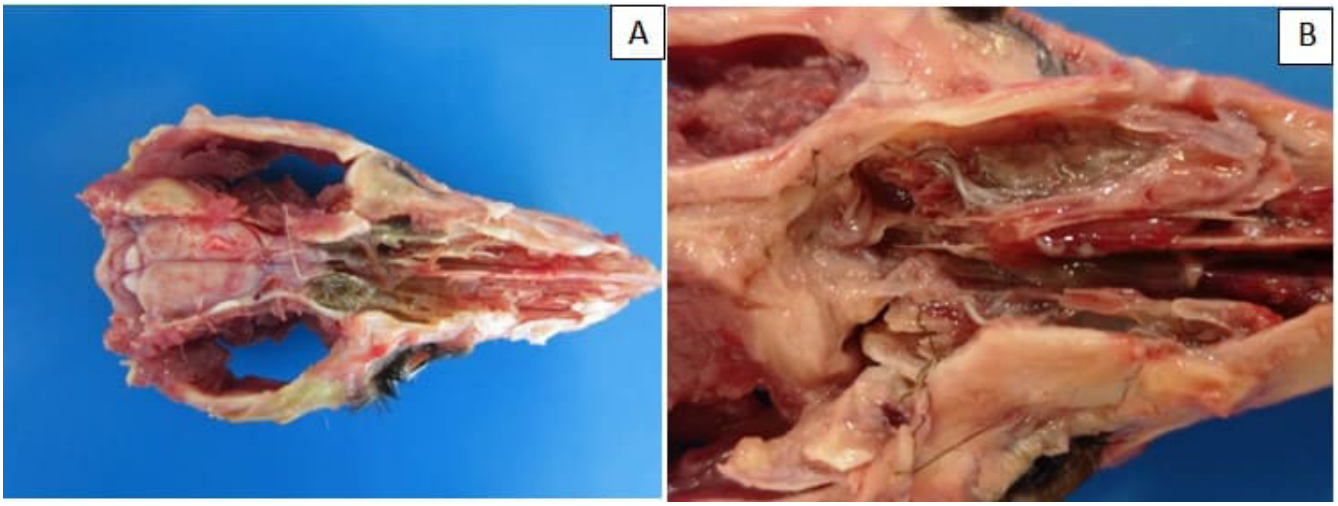

Figure 1. A. Skull of male adult *Didelphis albiventris* infected with *Angiostrongylus* sp.
B. Nasal conchae containing multiple *Angiostrongylus* sp. parasites.

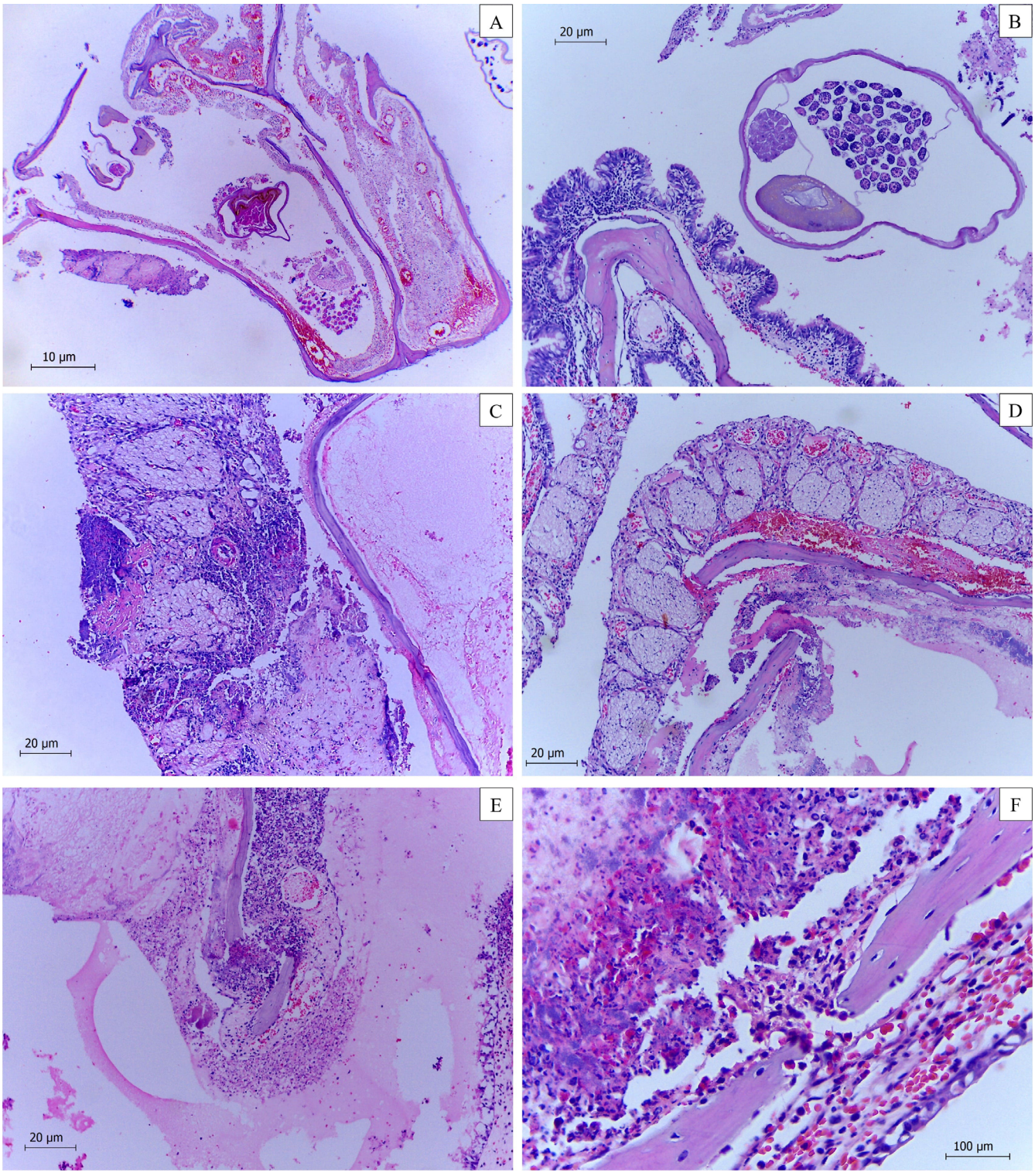

Figure 2. Sections of nasal conchae of *Didelphis* sp., subjected to hematoxylin and Eosin (HE) staining. A. Tangential sections showing adult parasites in the lumen of the nasal cavity (4x). B. Tangential section displaying the uterine portion of a female parasite containing numerous developing eggs. Tr - reproductive tube, td - digestive tube (10x). C. Inflammatory cells infiltrating the mucosa and submucosa, accompanied by fibrin deposition and areas of necrosis (10x). D. Focally extensive moderate hemorrhage of the mucosa and submucosa (10x). E. Focus demonstrating bone lysis of the nasal conchae associated with marked neutrophilic, eosinophilic, and histiocytic inflammatory infiltrate (10x). F. Focus of bone lysis (40x) associated with necrosis, moderate neutrophilic, eosinophilic, and histiocytic inflammatory infiltrate, with intralesional bacterial colonies.

Histologically, female nematodes can be identified by a thin cuticle, coelomic musculature, a body cavity containing two reproductive tubes in different stages of maturation (larvae or embryonated eggs), and an intestinal tract lined by a few multinucleated cells (Fig. 2b). Lateral cords can also be observed in this section.

A moderate number of bacterial colonies were observed in the lamina propria and nasal lumen. Notably, multiple foci exhibited bone lysis (Fig. 2e,f). Furthermore, there was moderate nasal mucosa hyperplasia accompanied by multifocal to focal extensive, moderate mucosal, and submucosal hemorrhage (Fig. 2d).

Morphological identification

Adult parasites were recovered by dissecting the nasal turbinates, measured, fixed in 10% buffered formalin, and kept in a 70% ethyl alcohol solution for morphological identification. Morphological identification and sex differentiation were performed using a Zeiss Stemi 2000-C loupe and a Leica DM500 optical microscope. Approximate measurements in micrometers and photomicrographs were recorded using Leica Application Suite software. The posterior section of the female parasites was macerated to obtain eggs in the final stage of development. The identification and classification of the samples at the genus level were performed according to Anderson (2).

For morphological identification, five male and five female nematode specimens were recovered. Both sexes had a filiform body and exhibited pronounced sexual dimorphism, with females being larger than males. However, the anterior portion of either sex did not display dimorphism: both sexes possessed a mouth with small or rudimentary lips, a small buccal cavity, and a rudimentary or absent capsule. The esophagus was claviform (Fig. 3a), and the excretory pore was located approximately 30 µm from the oral opening. The males were approximately 2.3–3.4 cm in length. The posterior portion of males consisted of a copulatory bursa with four pairs of lateral rays (latero-lateral, anterolateral, mediolateral, and posterolateral) and a dorsal ray (Fig. 3b). These rays shared a common stem and a bifurcation at the distal half of the tip, with the apices forming rounded structures. The dorsal rays were prominent and exhibited a "Y" shape. Additionally, a pair of spicules projected through the cloacal opening in three males. These spicules were long, striated, and had pointed and serrated distal ends (Fig. 3c,d). The gubernaculum was not observed. Conversely, the females were approximately 6.4 cm in length. Their reproductive system consisted of two uterine tubes that spiraled around the intestine, giving rise to the characteristic "barber pole" appearance typical of the genus (Fig. 3e,f). The female tail was digitiform, with the vulva located approximately 35 µm from the caudal end (Fig. 3g). The eggs were elliptical, translucent, and in the terminal phase, larvated. L1 larvae were present, characterized by a rabditiform esophagus (Fig. 3h).

Discussion

Infections of the nasal cavities by nematode parasites are rare occurrences, typically restricted to a few parasitic genera (12). For example, the genus *Anatrichosoma*, comprises two species, *A. cutaneum* and *A. cynamolgi*, which infect the nasal mucosa of Asian and African monkeys (7, 8) and the oral mucosa of marsupials in Australia (19). Notably, these infections have been associated with minimal inflammation in their respective hosts (19). Another example is *Trichosomoides nasalis*, which has been reported to infect the nasal epithelium of murids in Senegal (12).

Subsequently, an experimental study by Fall *et al.* (13) demonstrated that *T. nasalis* larvae develop in striated muscle fibers and, in their adult stages, migrate from the abdomen and thorax to the nasal mucosa, infecting the host's pseudostratified epithelium. Under natural conditions, the inflammation of the nasal mucosa induced by these parasites can reduce the competitiveness of infected rodents when foraging or seeking mates (13). Adult *Skrjabingylus nasicola* nematodes were found in the nasal sinuses of mustelids, and a massive infestation can cause considerable distortion of the frontal bones (15, 14). *Skrjabingylus nasicola* parasitism is characterized by bony and pigmentation changes in the sinus region, including swelling, thinning, or perforation of the nasal sinuses and conchae (20).

Besides the observed damage to the nasal sinus bones and adjacent tissues associated with larval migration, the extent of other pathophysiological changes induced by *Skrjabingylus* sp. infection remains unknown (20). In all five necropsied *Didelphis* sp. in this study, the lesions caused by *Angiostrongylus* sp. partially resembled those caused by *Skrjabingylus nasicola,* particularly in the alterations observed in the nasal cavity bone structure.

The macroscopic and microscopic findings and morphological characteristics of adult parasites in the nasal sinuses indicate fibrinosuppurative and necrotic rhinitis and sinusitis. Although similar parasitic structures were observed in the pulmonary parenchyma, the macroscopic differences in the parasites' size and morphology indicate distinct species co-infecting the hosts. The characteristic lesions and number of affected individuals suggest a new habitat for parasites of this genus. The identification of male and female adults, production of mature eggs, and presence of first-stage larvae in the affected tissue indicate that the nasal mucosa is a site of replication in opossum species (*Didelphis aurita* and *Didelphis albiventris*). The adaptation of parasitic nematodes to replicate in opossum nasal mucosa may represent a significant cause of morbidity in these animals, ruling out the possibility of erratic migration of the parasites to the nasal site.

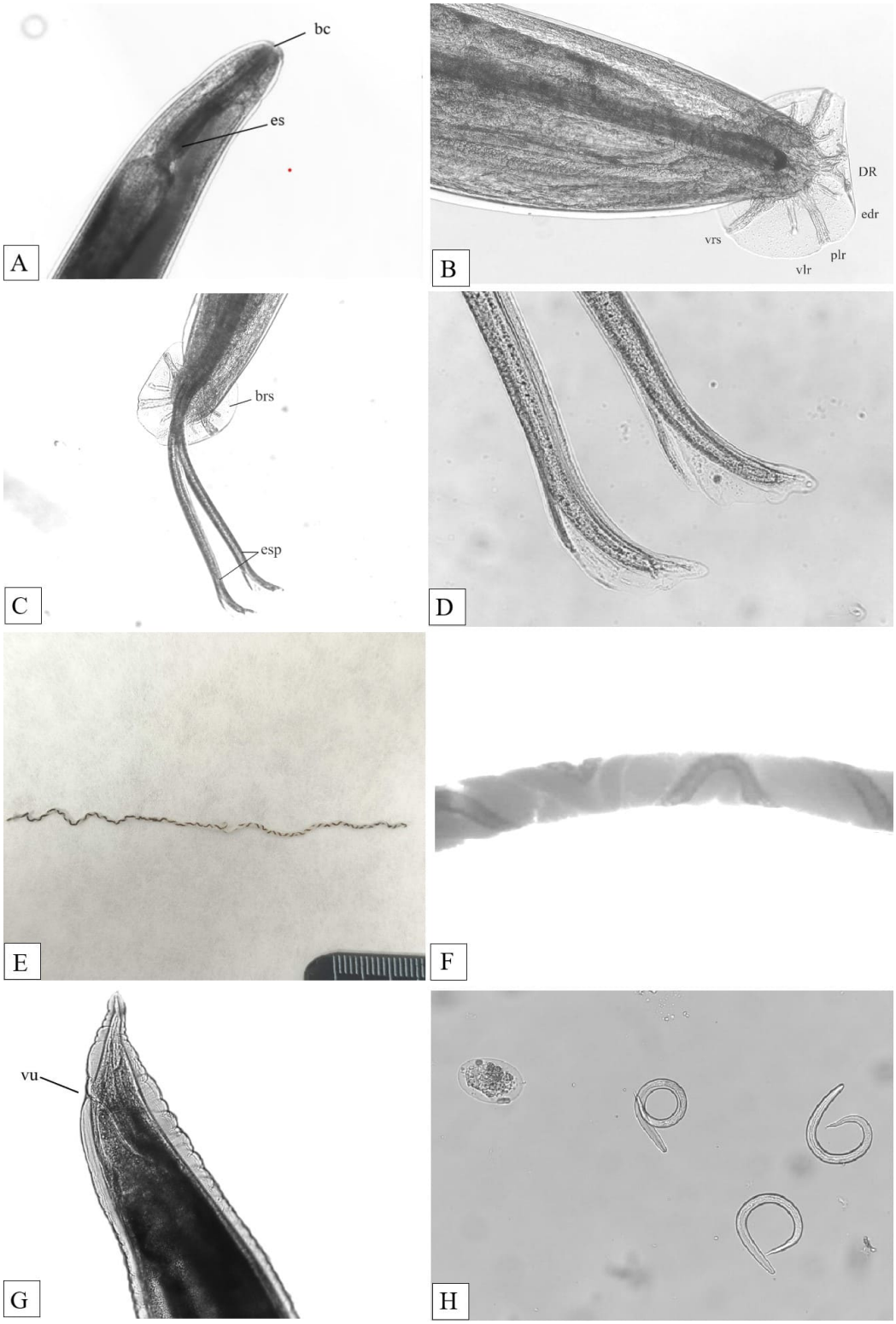

Figure 3. Morphological identification of *Angiostrongylus* specimens. A. Anterior portion. bc - oral opening, es - esophagus. B. Posterior portion of the male showcasing the copulatory bursa with symmetrical digitiform rays. Vrs - ventral, vlr - ventrolateral, plr - posterolateral, dr - dorsal. C. Posterior portion of the male showcasing the copulatory bursa with a pair of externalized spicules. D. The spicules are striated, with a pointed and serrated tip (40x). E. Adult female displaying the characteristic "barber pole" appearance. F. Adult female with two tubular uteri spiraled around the intestine, forming the "barber pole" appearance. G. Posterior portion of the female. Vu - vulva. H. Embryonic eggs and L1 larvae recovered from macerated mature females.

Additionally, opossums are primarily nocturnal and forage, mainly because of their sense of smell. As frugivorous/omnivorous animals, they rely on this sense to locate and consume fruits, small rodents, birds, lizards, snakes, amphibians, and various invertebrates, including gastropods (5). Therefore, a nasal cavity infection can significantly impair their foraging capabilities.

Considering the parasitic life cycle of *Angiostrongylus* sp., humid urban areas likely harbor gastropods infected with the parasite. These areas may serve as significant sites of infection for opossums, domestic animals, and humans. Thus, further investigations are needed to determine the impact of *Angiostrongylus* on public health.

Although histopathological lesions were not observed in the central nervous system, the neurological signs reported in the animals' clinical history may be partly associated with parasitic infection. Diagne *et al.* (12) suggest that infections in the nasal sinuses can alter the host's olfactory function and behavior.

Marsupial species rely on chemical communication through characteristic behaviors such as urinating and rubbing their chest and anal region on objects and substrates for territory marking (5). Similar to rodents (12), it is plausible that *Angiostrongylus* sp. infection in the nasal sinuses may impair olfaction, thereby modifying the host's behavior and food-seeking activities.

Regarding the parasite load, experimental infections in natural and aberrant definitive hosts with *A. dujardini* indicate a correlation between high doses of infective larvae and the onset of clinical signs or death, a characteristic likely common among *Angiostrongylus* sp. (21).

Since all five opossums were free-living and lacked prior medical histories, it is unclear whether these animals exhibited clinical signs resulting from the nasal infection before rescue or if the neurological signs developed due to the traumatic events.

Conclusion

To the authors' best knowledge, this study represents the first report of the genus *Angiostrongylus* infecting the nasal mucosa of free-living opossums (*Didelphis aurita* and *Didelphis albiventris*). The parasitic infection may have compromised the health of these animals, making them more susceptible to threats such as vehicular collisions, animal attacks, and blunt traumas. Further genome sequencing studies are necessary to precisely identify the specimens encountered. Moreover, given this parasite's unknown natural life cycle, it is imperative to assess the risk of transmission to both domestic and wild animals.

Conflict of Interest

The authors declare no competing interests.

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