International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(2): 323-325

www.veterinarypaper.com
Received: 09-12-2023
Accepted: 27-02-2024

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Morphological identification of *Murshidia indica* from an Asian elephant: A case report

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Abstract
Murshidia species are gastrointestinal nematodes belonging to the family Trichonematidae which colonises the caecum of elephants. *Murshidia* infection are commonly reported in elephant populations concurrently with other gastrointestinal nematodes. Prevalence of this parasite has been found in both captive and wild Asian elephants. In spite of the common occurance of this small Strongyle, there are relatively less information available on the molecular and morphological identification of various species belonging to these genera. The present case report provides photomicrographs for morphological identification of *Murshidia* received on the necropsy of a captive Asian elephant from Kerala, India. Identification by microscopy and comparison with available photomicrographs data of various species revealed typical features of *Murshidia indica.* Although further identification using molecular techniques is further required, morphological identification has its importance.

Keywords: morphology, *Murshidia*, Strongyle, Asian elephant

1. Introduction
The Asian elephants are widespread across India, and exists in fragmented populations in south, north, central, and northeast of the country [1]. With a history spanning approximately 4500 years in the domestication of elephants, India currently oversees the management of 3400-3600 elephants in captivity [2, 3]. These captive elephants have been employed for various purposes in India, including historical roles in warfare, logging, cultural and religious ceremonies, as well as modern applications like in zoo, circus, wildlife tourism, and safeguarding sanctuaries and national parks. In the present scenario, wild elephants are primarily found in the forested hilly regions of four different areas: (i) the foothills of the Himalayas in the north, (ii) the northeastern states, (iii) the forests of east-central India, and (iv) the forested hilly tracts of the Western and Eastern Ghats in southern India [4]. Displaying habitat adaptability, these mega-herbivores thrive in various environments from wet tropical evergreen forests to semi-arid thorn and scrub forests. However, the highest elephant densities are observed in tropical deciduous forests. Given their need for extensive territories rich in food and water, elephants play a crucial role in maintaining biodiversity and ecological balance [5]. *Elephas maximus* is placed in Schedule I and Part I of Indian Wildlife Protection Act (1972). Indians have maintained cultural and religious connections with captive elephants, making them symbolic and fostering public support for conservation.
Gastrointestinal nematodes, found abundantly in the digestive tract of Asian elephants include various species such as Strongyles, roundworms, and threadworms. During times of limited resources, gastrointestinal parasites can adversely affect the well-being of elephants. Infestations of gastrointestinal nematodes in elephants can lead to symptoms such as weight loss, lethargy, diarrhoea, and abdominal discomfort. Young elephants may be more susceptible to these parasites, and severe infestations can potentially pose a threat to their well-being [6].
Asian elephants have been documented to host various genera of Strongyles, including *Murshidia*, *Equinurbia*, *Choniangium*, *Quilonia*, *Bunostomum*, *Grammocephalus*, and others [7]. The superfamily *Strongyloidea* exhibits distinctive features, notably a well-developed buccal capsule devoid of teeth or cutting plates. This capsule is typically topped with one or more leaf crowns, known as corona radiata, found in both male and female individuals.

In males, there is the presence of a copulatory bursa. Furthermore, the family *Strongylidae* is characterized by the presence of six branches in the dorsal ray. Among these strongyles, *Murshidia* is the most common. Genus *Murshidia* belongs to the subfamily Cyathostominae and family *Strongylidae*. One of the characteristics of Cyathostominae is that there is external or internal corona radiata with several elements that emerge from the base of the buccal capsule. They are measuring 20-30 mm in length; these slender worms exhibit a forward-facing mouth characterized by a prominent mouth-collar. The buccal capsule, cylindrical in shape, may or may not have teeth and are commonly found in the caecum of the Indian elephant [8].

The free-living environmental stages of gastrointestinal nematodes are significantly influenced by climatic conditions. Extreme temperatures can hinder their development and survival. Adequate moisture is essential for the larvae to progress from the soil to the pasture. Therefore, factors such as rainfall and vegetation availability can limit transmission and impact the variability in infection patterns [9].The larvae of these strongyles emerge from eggs found in elephant dung and achieve infectivity following a developmental period spanning several weeks [10, 11]. Subsequently, the infective larvae migrate to the surrounding soil, the bases of plants, and notably, to nearby water sources. Elephants, while feeding or drinking, may ingest these larvae [10]. Upon ingestion, adult worms of the *Strongylidae* family establish themselves in the intestines. The adult worms attach to the intestinal lining, causing tissue damage, inflammation, and ulcers. Continuous blood-feeding by the worms results in blood loss, leading to anaemia. The host's immune system responds to the presence of the worms, causing inflammation and immune-mediated effects. Within these locations, they lay eggs that are then excreted in dung, thus initiating a new cycle of life. The clinical signs observed included anorexia, mud-eating tendency, colic, reduced intake of food and water, paleness of visible mucous membranes, foetid diarrhoea, anaemia and eosinophilia.

2. Materials and Methods

2.1. Sample collection

Fresh worms containing faecal sample from elephant were received to the helminthology lab, Division of Parasitology, ICAR-IVRI, Bareilly packed in icebox. The worms were retrieved by thoroughly washing with tap water.

2.2. Morphological identification

Both male and female worms were separated and length of the worms were measured. The worms were later kept in lactophenol (approx. up to 4 h) for clearing. Anterior and posterior end of the worms were dissected, processed and observed under light microscope for morphological examination.

3. Results and discussion

A total of 48 males and 56 female worms were received. Average total length of the male and female was 15-17 mm 20-25 mm respectively. On microscopic examination, *Murshidia indica* could be identified. Identification was based on buccal capsule, oesophagus, bursal ray arrangement in males and vulva in females.

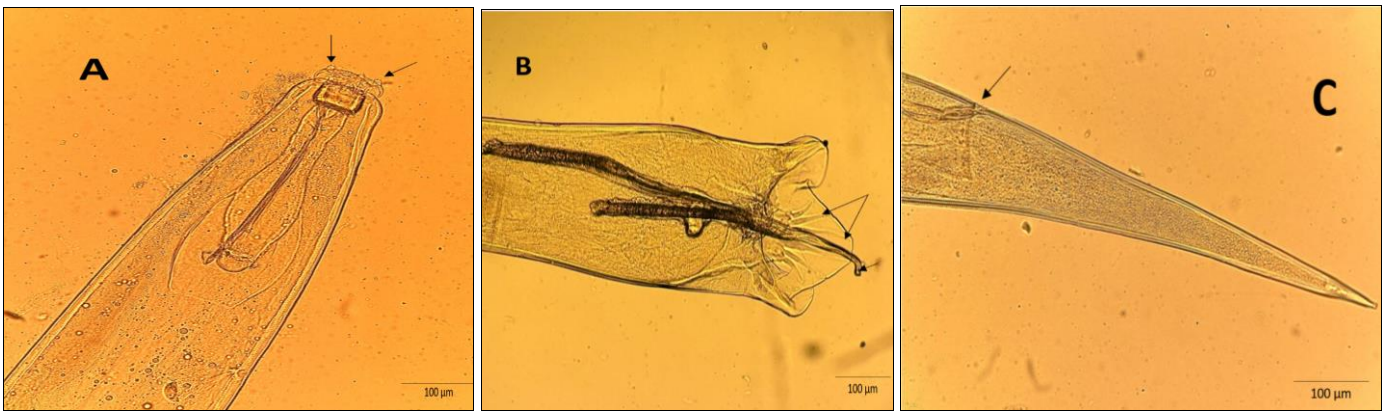

Fig 1: Photomicrographs of *Murshidia indica*. A, anterior end of *Murshidia indica*, showing two lateral lips of mouth collar with prominent head papillae (arrows); B, posterior end of a male showing dorsal rays of copulatory bursa (large arrows) and spicule (small arrow); C, posterior end of a female showing anus (arrow)

4. Conclusion

Here we have isolated the worm from faecal sample and then identified the worm as *Murshidia indica* based on the morphological findings. Both anterior and posterior end were examined after clearing the worm in lactophenol. The photomicrographs clearly shows the morphological characteristics specific to species like lateral lips, mouth collar, head papillae at the anterior end and showing dorsal rays of copulatory bursa and spicules in males and pointed tail end of female.

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