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Morphological Characterization of *Dictyocaulus viviparous* (Nematoda: Trichostrongyloidea) Infecting the Domestic Cattle, *Bos taurus* in Al-Baha Area, Saudi Arabia

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Abstract: The lungworm *Dictyocaulus viviparous* is a parasitic nematode that affects cattle and other ruminants worldwide. In this research, we studied the morphological ultrastructure of *D. viviparous*, which was found in the lungs of infected cattle (*Bos taurus*) from Al Baha area. The morphological characters of the adult worm were examined by using light and scanning electron microscopy (SEM). The results indicated that the *D. viviparous* worm has a slender, thread-like morphology. It has a buccal capsule at the anterior end. The study discussed the differences between male and female worms focusing on the structure of the male copulatory bursa and female reproductive system. This study will help in the clear identification of this species. The findings will help researchers, veterinarians, and livestock farmers in their efforts to prevent and control lungworm infection in domestic animals.

Key words: Morphology - Ultrastructure - Lungworm - *Dictyocaulus viviparous* - Cattle - Saudi Arabia

INTRODUCTION

Helminth infections are common parasites in domestic animals, where, they are susceptible to various types of nematode infection. Nematode infections in domestic animals can lead to a range of animal health problems, including poor growth, weight loss, diarrhea, and anemia [1]. Good practices such as regular deworming and good hygiene practices, such as proper disposal of feces and maintaining clean living environments, are crucial in preventing and controlling worm infections in domestic animals [2, 3]. These play a vital role in Saudi Arabia's economy, contributing to various sectors such as agriculture, food production, and the overall livelihood of its population. Animals including camels, sheep, goats, and cattle, hold a significant importance in the country. *Dictyocaulus viviparus* is commonly known as the lungworm of cattle and causes parasitic pneumonia and bronchiolitis in adult cattle and calves. This lungworm species is found worldwide and can cause significant economic losses in the livestock industry [4, 5]. The parasite has a direct life cycle so infection merely requires management factors that allow the parasite to accumulate in the environment and the infective larvae to be ingested by naive cattle. Mature worms inhabit the bronchi and bronchioles of the host's respiratory system, where they lay eggs [6, 7]. These eggs hatch into larvae in the external environment after being coughed up, ingested, and passed out in feces. The larvae are then ingested by grazing animals [7]. The larvae then pass through the intestinal wall and into the lungs via the circulatory system. In the lungs, they develop into mature worms and mate, continuing the life cycle. The presence of adult worms in the lungs can cause respiratory problems such as coughing, and decreased weight gain in infected animals [8]. *D. viviparus* infection is more common in

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young animals, as they have not yet developed immunity to the parasite [9]. The disease is often seen in areas with high rainfall and where animals graze on contaminated pastures. Overcrowding and poor management practices can also contribute to the spread of infection. Lungworm infection can be diagnosed based on clinical signs, fecal examination for the presence of eggs or larvae, and sometimes lung tissue examination [10]. The treatment for this condition involves using anthelmintic medication to kill adult worms and reduce the number of larvae in the lungs. If the situation becomes severe, it may be necessary to provide supportive care to manage any respiratory distress. Prevention and control of *D. viviparus* infection involve implementing good pasture management practices. This includes avoiding overcrowding, rotational grazing, and minimizing exposure to contaminated pastures. Regular deworming of animals, especially young ones, can also help prevent infection. The present study aimed to study the morphological ultrastructure of the pathogenic nematode isolated from the domestic cattle, *Bos taurus*, inhabiting Al-Baha area, Saudi Arabia.

MATERIALS AND METHODS

Ten digestive tracts from the domestic cattle, *Bos taurus* (Family: Bovidae) were obtained from slaughterhouses in the Al-Baha area of Saudi Arabia in 2022. These specimens were transported to the Parasitology lab located in the Biology Department, College of Science and Arts at Almikhwah, Al-Baha University. The worms were rinsed in normal saline after being extracted from infected animals to eliminate mucus. The samples were placed in plastic jars with 0.85% saline solution for helminth parasite examination. They were then fixed in hot 70% ethyl alcohol, preserved in glycerin alcohol, and mounted in glycerin and glycerin jelly. Photomicrographs were taken with a Zeiss research microscope equipped with a Canon Digital Camera. The morphology of the posterior parts of male and female worms were evaluated according to Stevenson [11] methodology. Species identification involved cutting the tails of adult male worms before the bursa and mounting them in lactophenol for examination under a microscope to observe the spicules clearly. The specimens were prepared for SEM by fixing them in 3% buffered glutaraldehyde, washing them in cacodylate buffer, dehydrating them in a series of ethanol alcohol, and finally processing them in a critical point drier called "Bomer - 900" with freon 13. They were coated with gold-palladium using a Technics Hummer V and examined at 20 KV using an Etec Autoscan in Joel SEM.

RESULTS

Out of the ten gastrointestinal tracts examined, six were infected with nematode worms, with an average of 10-17 worms per tract. These worms were thin and thread-like, measured about 3 to 8 cm in length. They have a mouth leading to a small buccal capsule. Similar to other roundworms, their bodies are covered with a flexible but tough cuticle. Worms have a tubular digestive system with a mouth and an anus, a nervous system, but no excretory or circulatory organs like blood vessels or a heart. The recovered worms were adult, slender, and medium-sized, reaching up to 8 cm in length (Figs. A-I). Male worms have a copulatory bursa that contains two short, thick spicules used for attachment during copulation (Figs. B, C, F-H). The males measure around 4.0 - 5.5 cm in length, with a triangular-shaped buccal ring and fused posterolateral and mediolateral rays, except at their extremities. Spicules were short, stout, and boot-shaped. Females were approximately one-third longer than males, with a whitish to grayish color and a pointed end terminated in an anal opening (Figs. D, I). The female worms have well-developed ovaries and their uteri terminate in a posterior opening known as the vulva, positioned slightly behind the middle of the worm.

DISCUSSION

Dictyocaulus viviparus has been found in tropical, subtropical, and temperate countries, and affects dairy herds, sheep, buffaloes, and cattle [12-15]. The existence of infectious nematode larvae in vibrant green meadows is facilitated by heavy rainfall during hot and humid periods, increasing the chances of ingestion by these animals. *D. viviparus* has been reported in many tropical and subtropical countries as India [16], Brazil [17], Turkey [18], and Malaysia [19]. It also recorded in Ireland [20], Germany [21], Sweden [22], and the Netherlands [23]. In addition to domestic animals, lung worm infection caused by *D. viviparus* has been observed in wildlife, including roe deer [24] and wild cervids [25]. The larvae of the parasite are ingested and then mature into adult worms by penetrating the intestine, traveling through the lymph

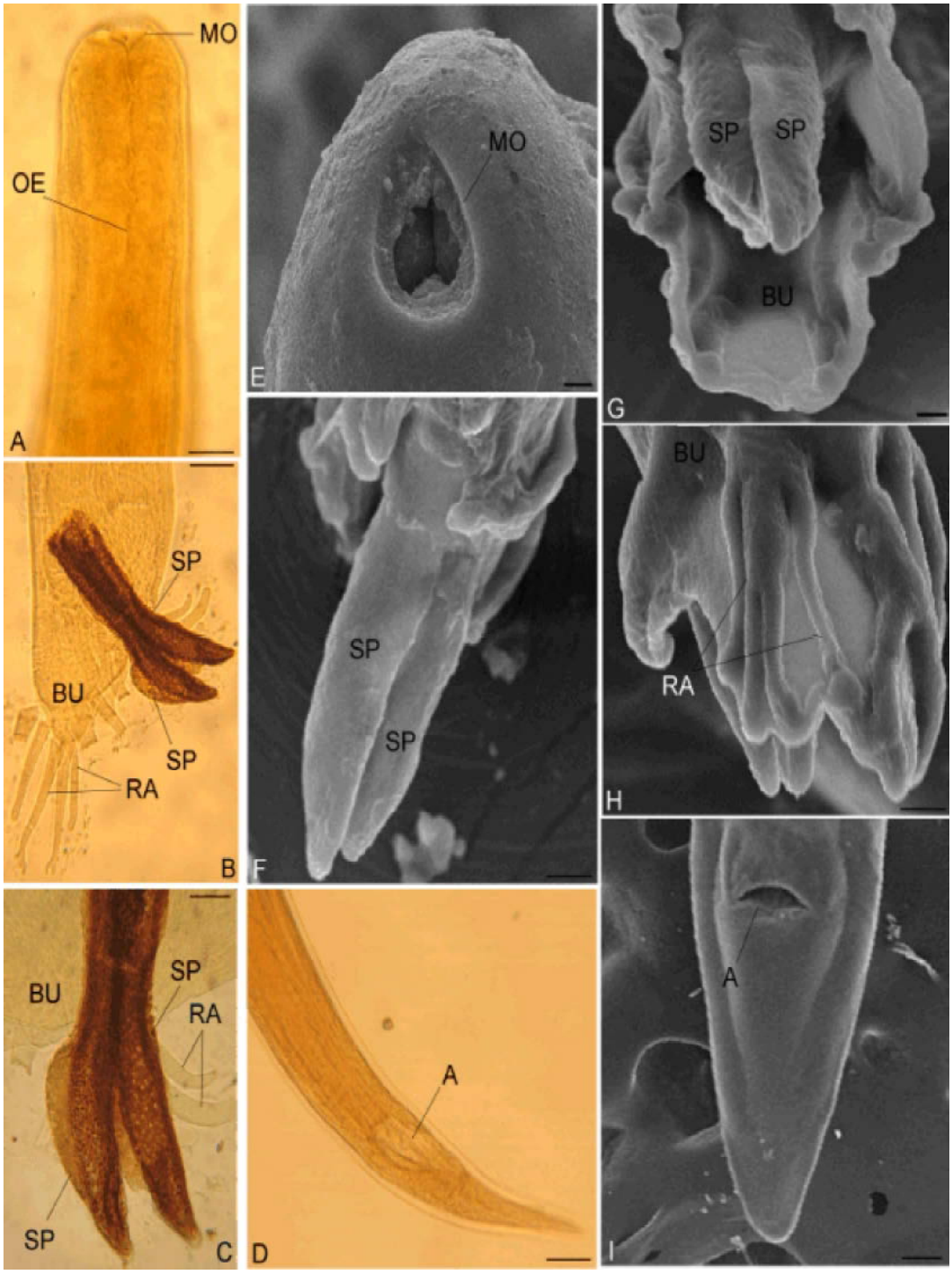

Fig. A-D: Photomicrographs of *Dictyocaulus viviparous* showing: (A) anterior end of adult worm showing esophagus (OE), mouth opening (MO). (B, C) the posterior end of an adult male terminated at a copulatory bursa (BU) supported by rays (RA) and two boot-shaped spicules (SP), (D) posterior end of an adult female with anal opening (A).

Fig. E-I: Scanning electron micrographs showing: (E) the anterior end with mouth opening (MO), (F-H) the posterior end of an adult male with two extruded spicules (SP) from the copulatory bursa (BU) supported by rays (RA), (I) The posterior end of adult female with an anal opening (A).

nodes and bloodstream to reach the lungs [25]. In the lungs, the presence of eosinophils and mast cells leads to pathological changes, causing airway restriction, edema, emphysema, and collapse of the alveoli. Same respiratory alterations were also observed in Rocky Mountain elk [27], red deer calves [28], hangul (*Cervus elaphus hanglu*), cattle [21], and sheep, goats [29], due to *D. viviparus* infection. Small ruminants, such as sheep and goats, are economically important animals and serve as a source of immediate cash for smallholder families. Lungworm infections are common among small ruminants with a significant impact on these economically valuable animals. The lungworms that cause common parasitic diseases in sheep and goats belong to the super families Trichostrongyloidea or Metastrongyloidea. *Protostrongylus* and *Dictyocaulus* are the main causes of lungworm infections. *Dictyocaulus* filaria, *Muellerius capillaris*, and *P. rufescens* are the most commonly affecting species of respiratory nematodes in livestock animals. *Dictyocaulus* filaria has a direct life cycle, while *Muellerius* and *Protostrongylus* have an indirect life cycle [30]. Morphologically, all species of *Dictyocaulus* are similar and can only be differentiated by the shape of their mouth and the thickness of their body cuticle wall (BCW). Divina *et al.* [24], Durette-Desset *et al.* [6], and Gibbons and Khalil [8] identified that triangular-shaped BCW indicates *D. viviparus*, while kidney or bean-shaped BCW indicates *D. eckerti*. Nevertheless, these traits have limited value in distinguishing the nematode parasite to species level, as their measurements seem to be influenced by both species of the host and the overall body size of the worms [24]. The nematodes found in the bronchi and bronchioles of artiodactylids were classified under the genus *Dictyocaulus* by Railliet and Henry [31]. A historical and taxonomic overview of the genus was provided by Skrjabin *et al.* [32] based on the characteristics of the cephalic region, the reproductive system of female and spicules, and the bursa of male. Gibbons and Khalil [8] revised the genus *Dictyocaulus* in the superfamily Trichostrongyloidea. They recognized six valid species: *D. filarial* [33], *D. arnfieldi* [31], *D. africanus* [8], *D. eckerti* [32], and *D. viviparous* [31], *D. cameli* [32]. Durette-Desset et al. [6] provided a new description and reinstated *D. noerneri* from *C. capreolus* in France. They suggested that *D. eckerti* should be considered a synonym if it was morphologically identical to *D. noerneri* and proposed maintaining *D. noerneri* as a separate species for lungworms found in European cervids if they were morphologically distinct. Previously, Skrjabin *et al.* [32] considered *D. noerneri* to be a species inquired due to the lack of a complete description. Jansen and Borgsteede [9] proposed *D. noerneri* as a species that needed further investigation due to insufficient information to confirm its identity. They thereby verify that the large lungworm found in cervids is indeed *D. eckerti*. *Dictyocaulus* was established by Railliet & Henry [32] for four species found in the bronchi of herbivores, including *D. noerneri* from roe a new species deer AS. However, they did not provide a location or description of type specimens for *D. noerneri*, making its classification uncertain. There are few morphological differences between *Dictyocaulus* species. Buccal capsule thickness is used to differentiate species, as demonstrated in the studies conducted by Durette-Desset *et al.* [6] and Jansen and Borgsteede [9]. Divina *et al.* [24] reviewed the work of others who also utilized the characteristics of the buccal capsule in the identification of large lungworms in cattle, moose, and roe deer. Divina *et al.* [24] found that the width and shape of the buccal capsule wall, along with molecular investigation (ITS-2), supported the differentiation of the specimens described as *D. viviparus* and *D. eckerti*, suggesting that they may belong to a new species. Examinations of the morphology unveiled a prolonged mouth opening resembling that of *D. eckerti* and *D. africanus*, whereas all other species within the genus, including *D. viviparus*, possess a circular or oval mouth opening. It is worth noting that lungworm infections are endemic in various countries. For instance, in Brazil, a tropical country, the morbidity rate and mortality rate were 7.1% and 13.3% respectively [17]. In Tanzania, a prevalence of lungworm infections ranging from 8% to 28% was observed in dairy cattle through fecal examination from farms in the tropical highlands [34]. In Tanzania, imported dairy breeds were identified as the primary cause of these infections, suggesting that the infection may be due to imported animals infected with *D. viviparus* [34]. Similarly, in Malaysia, *D. viviparus* infection has been reported in Nelore cattle that were imported from Brazil in 1991 [17]. Occurrences of lungworm infection have been previously recorded in the Nelore beef cattle breeding center in Malaysia by Lat-Lat *et al.* [19]. Lungworms are regarded as disease-causing in controlled or enclosed environments in temperate regions [35, 36]. To prevent and control the spread of this parasite in livestock populations, it is crucial to understand its life cycle, clinical signs, and implement appropriate management practices. The parasite identified in this investigation exhibited all of the features of the previously described *D. viviparus* and it is recorded for the first time from this region, future studies should be done to increase the knowledge about the pathogenic effect of these parasites.

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