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First Record of *Muellerius Capillaris* (Protostrongylidae) and Their Pathologic Changes in Libya

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Abstract

Lungworm infection is a widespread disease in the sheep population worldwide, and the reflection of this disease causes significant health problems and low animals productivity for the local community. Sheep are the most popular red meat sources for Libyan consumers and these animals play an important socioeconomic role with high demand in cultural and religious festivals as it contributes rabidly in national economy. Lungs of (n = 726) sheep carcasses were collected from a local slaughterhouse in El-Beida city / Al-Jabal Al-Akhdar governor/ Libya to investigate the types of lungworms helminths infection and evaluate their lesions. 27 out of 342 (7.89%) samples were infected with small lungworms, as all of them were identified as *Muellerius capillaris* (Nematoda: Protostrongylidae). These lungworms were predominately associated with nodular lesions. Nevertheless, to our knowledge, lung worms of livestock have been poorly studied in local regions of El-Beida city / Aljabal Alakhder area, including all around Libya. Therefore, this study aims to provide an overview of the classification and the pathomorphological lesions of Protostrongylids infection in domestic small ruminants, in specific sheep, in El-Beida city / Libya.

Key words: Lungworms, Al-Jabal Al-Akhdar, El-Beida city, Libya, *Muellerius capillaris*, Pathological alterations

Introduction

North-eastern Libya is home to more than 6 million small ruminants, which are raised under a traditional grazing system. Lamb consumption is part of a long-standing custom among the people of El-Beida city in the Al-Jabal Al-Akhdar municipality (northern east Libya), along with other animal meats such as goats, cattle, camels, and poultry (Ministry of Health and Livestock, 2022). These animals play a significant role in the local livestock industry because they produce milk, wool, skins, and manure, which are in greater demand from traditional and modern industries. In recent years, multiple factors have contributed to the low animal production system and raised concerns about animal health care, and respiratory disease is one of the essential

reasons affecting all ages of sheep breeds in the local area (Mohammed and Ibrahim, 2022, 2024; Mohammed et al., 2022; Ali and Mohammed, 2023).

Several factors contribute to respiratory diseases in sheep including verminous pneumonia which is an inflammatory disease of the lower respiratory tract caused by a variety of types of lungworms. Parasitic bronchitis is the most prevalent respiratory disease afflicting small ruminants of all ages in the Mediterranean region and Africa. Animal production is reduced as a result of this inflammatory disease's varied clinical symptoms, which might include coughing, dyspnoea, nasal discharge, and weight loss. In particular, young animals are more sensitive to the disease leading to death in severe cases (Habte and Simeneh, 2019; Macedo et al. 2022).

Ovine verminous pneumonia occurs because of the infection by genera of the family Protostrongylidae, which belong to parasitic nematodes. *Muellerius capillaris* (*M. capillaris*), also known as hair worms, is one of the members of this family, and they are slender and greyish worms. These types of lungworms have an indirect life cycle (Kaufmann, 2013; Eberhard, 2014). The worms' life cycle begins when females deposit oval-shaped, thin-shelled eggs in the lung, where they develop and hatch into first-stage larvae (L1), also known as the diagnostic stage. These L1 are coughed up, swallowed, and passed into the external environment through faeces. In order to reach the infective stage (L3), the uninfected larvae (L1) search for an intermediate host, such as slugs. Small ruminants become infected by consuming these contaminated slugs. Subsequently, L3 migrates to lung tissues via blood and lymphatic circulation, growing into adult worms that produce unembryonated eggs.

All of these stages are responsible for the inflammatory responses that finally form granulomatous nodules. *M. capillaris* is a microscopic species that forms deeply grey nodules inside the lung tissues of sheep and goats, making it difficult to dissect complete specimens of these worms. As a result, L1 plays a substantial role in the distinguishing between the lungworm species, which are identified based on the observation of the posterior end of recovered larvae from the faeces (Suarez et al., 2014; Taylor et al., 2015; Fesseha and Mathewos, 2021).

In the Africa, *M. capillaris* has been reported in Egypt (Ali et al., 2018), in Tunisia (Elati et al., 2018) and Ethiopia (Habte and Simeneh, 2019) with no record in Libya to date. The current study aimed to identify the species of lungworms that infected sheep in El-Beida city (Al-Jabal Al-Akhdar, northern east Libya) and establish the pathological changes caused by these nematodes.

Materials and Methods

A. Study Area

The municipality of El-Beida, situated at latitude 32°45'N and longitude 21°44'E, has an average sheep population of 408,782 according to the Ministry of Health and Livestock (2022). Data collection took place in El-Beida from October 2020 to March 2023, encompassing different seasons. The study area's characteristics, geographical coordinates, and specifics have been recorded in earlier reports (Mohammed et al., 2022; Mohammed and Ibrahim, 2022; Ali and Mohammed, 2023)

B. Study Animals

The examination of 726 sheep carcasses took place in certain abattoirs of the city, and the examined animals were local breeds, the majority of them were male, older than four months, and all were submitted for routine slaughter. All sheep are managed under a pastoral production system and sheep are usually mixed with other types of livestock (goats, cows, dogs and camels in some areas).

C. Sample Collection

After the sheep were slaughtered, their lungs were thoroughly examined. Subsequently, the lesions were documented and sent to the parasitology and pathology labs at the College of Veterinary Medicine, Omar Al-Mukhtar University, for further assessment.

D. Parasite identification

The samples received at the parasitology lab were examined and dissected. The trachea, bronchi and bronchioles were assessed using the naked eye to determine the presence of grey nodules and other worm species. L1 was collected using the Baermann Apparatus, and the morphological features of L1 were enhanced for species identification by staining them with Lugol solution (Hansen and Perry, 1994).

The sexual structure of males and females was observed by boiling lesions (2 cm^3 pieces) in 40% lactic acid in a water bath for approximately one hour (Panayotova-Pencheva and Alexandrov, 2010). Also, adult worms were recovered directly from nodules when it is possible. Recovered worms (adults and L1) from the sedimentation were examined using the light microscope (CX21FS1-Olympus Optical Co., Ltd., Tokyo, Japan) to identify the species according to morphological basis as previously described by (Gerichter, 1951; Van Wyk et al., 2013). The presence of parasite stages (adults, larvae and eggs) and the occupied changes of lung tissues were studied by the pathologic examination in the pathology Lab.

F. Pathological Investigation

From the positive cases, 1 cm^3 tissue samples were taken from 10% neutral buffered formalin-fixed tissue and dehydrated in graded ethanol before embedding in paraffin. Sections measuring 5 cm in thickness were cut using a rotary microtome (Leica, Germany). They were then stained with Harri's haematoxylin and eosin (Luna, 1968; Bancroft et al., 1996) and examined by an ordinary light microscope. Finally, the stained slides were examined systematically at 4X, 10X, 40X and 100X magnifications for the presence of adults, larvae, eggs of the lung parasites, and the occupied lesions using an ordinary light microscope.

Results and Discussion

Lung lesions were found in 47.1% (n = 342/726) of all sheep examined, and 27 out of 342 (7.89%) samples were infected with lungworms, as all of them were identified as *M. capillaris*. Adult worms are characterized by a small buccal cavity at the anterior end, and the oral opening is triangular in the center with no lips surrounding it. These worms have a cylindrical oesophagus (Figures 1A & B), and its ratio to the body length is 1:70. Figure.1C & D (D1, D2 and D3) present the essential morphological characteristics of the sexual structures of male *M. capillaris* specimens. The copulatory bursa of these worms is small and well-developed and has short rays (Figure 1C). The tail is a spiral and very coiled, and it has separate spicules stem (Figure 1D). Each spicule is divided into two branches, which are connected at a point known as the spicule joint (Figure1 D2). One of the branches is shorter and thinner than the other, which is long and thin (Figure1 D3). Females have longer body structures than males, and their genital tubes occupy almost all of the interior body, while the uterus contains non-embryonic eggs (Figure1 E1). The vulva is located on the posterior end of the body near the anus (Figures1 E2 & E4), and the teguminal sheath expands at the vulva level (Figure1 E3). The tail has a pointed tip.

The first-stage larva (L1) has a rhabditoid oesophagus (Figure1 F1) which is about 50% of the body length. L1 of *M. capillaris* are easily identified by the presence of a curved tail (Figure1 F2) and a dorsal spine (Figure1 F2 black *arrow*) at the posterior extremity of the body. Females lay immature eggs (oviparous) (undeveloped embryo) with a large yolk mass (Figure1 G), which embryonated in few days (Figure1 H), before hatching and developing into L1 (Figure1 F), that migrate from the nodules to the airways.

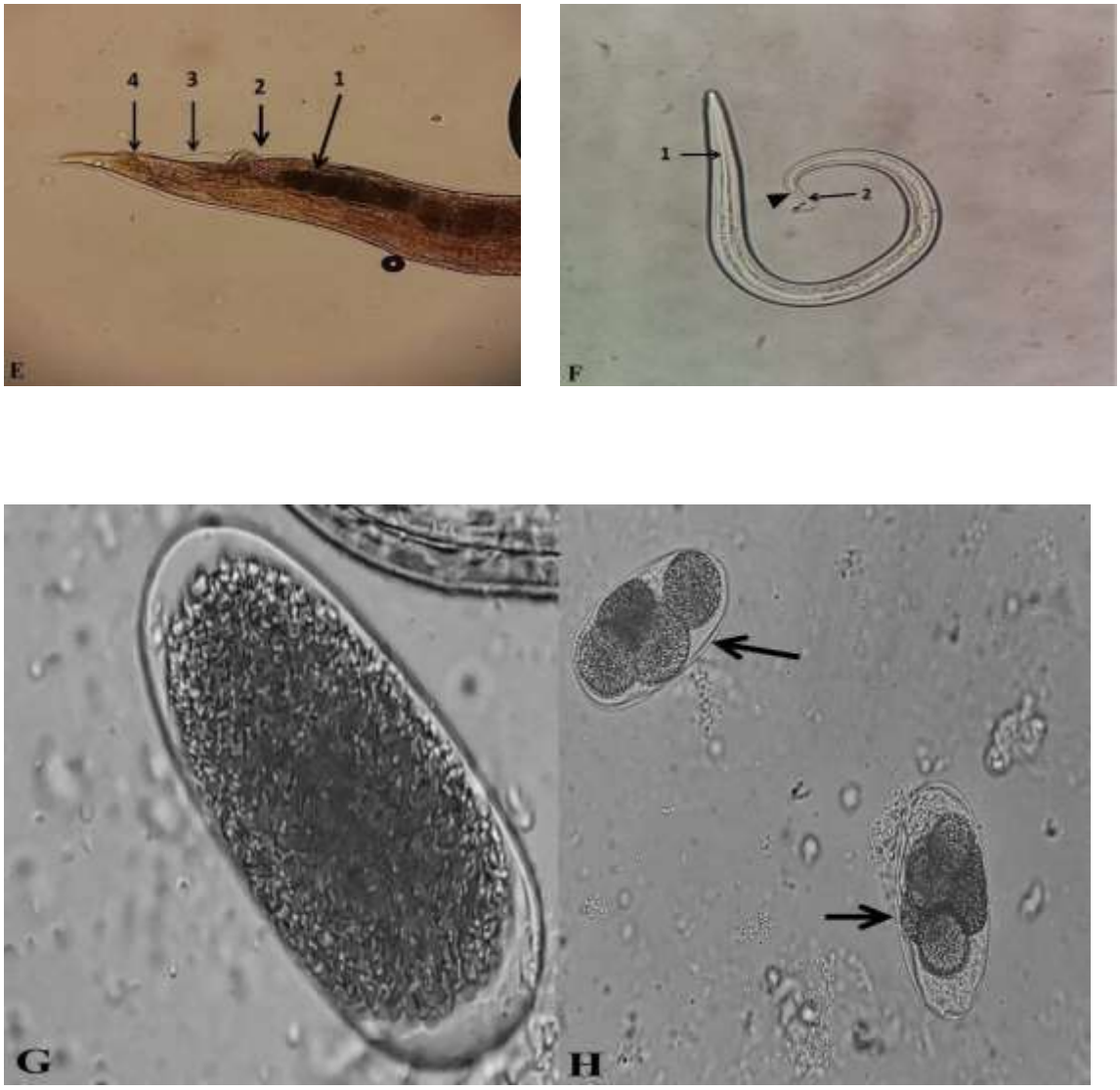

Figure 1. *M. capillaris* (Protostrongylidae) found in local sheep from northern east Libya; original picture: Anterior extremity of adult lung worms (A & B): A: no oral capsule with no papillae (black arrow), B: cylindroid oesophagus (black arrow). Sexual structures of *M. capillaris* adults (C, D & E): C: the distal end of an adult male; dorso-lateral view, short bursa (black arrow), long, thick, dark-brown spicules (black arrowhead), D: Caudal twisted end (arrow 1) of male *M. capillaris*, spicule joints (arrow 2), large branch of the spicule stem (arrow 3), E: posterior end of female, uterus filled with undeveloped eggs (arrow 1), vulva (arrow 2), cuticular vulva (arrow 3), anus (arrow 4). F: First-stage larvae (L1) of *M. capillaris*, oesophagus (arrow 1) has a curved, sharply pointed tail (tail spike) (arrow 2), with a short, dorsal spine (black arrowhead) (X10). G: non embryonic egg (X40), H: embryonic eggs (*M. capillaris*) (black arrows) (X10).

Gross Findings:

The vast majority of lungs affected with worms were grossly diagnosed as granulomatous pneumonia. Most of granulomas were disseminated mainly within in the dorsal-caudal part of the diaphragmatic lung lobe (Figure 2A). However, in two cases the granulomas were located within in the ventral surfaces in addition to dorsal surface of caudal part of the diaphragmatic lung lobe. The number of granuloma varied from one to twelve in one lung (Figure 2B).

The lung granulomas in sheep infected with *M. capillaris* were several, firm, different sizes of white or grayish-yellow or tan nodules (0.1-8 cm) and were also well- delineated and raised from the caudal pleura region and did not extend deep into the pulmonary parenchyma (Figure 2C &

D). These nodules occurred individually or clustered in groups and most of them contained internal cavities filled with a caseous substance (Figure 2E & F).

In some cases (5 out of 24) that affected with *M. capillaris,* lesions were not formed as nodules but as firm, grey to black areas affecting a large part of the lung surfaces (Figure 3A). The affecting part was well differentiated from the surrounding tissue. In addition, infected parts of lungs were consolidated and in some areas were rubbery in texture with diffuse congestion (Figure 3A). There were focal and diffuse emphysema in lung surface (Figure 3B).

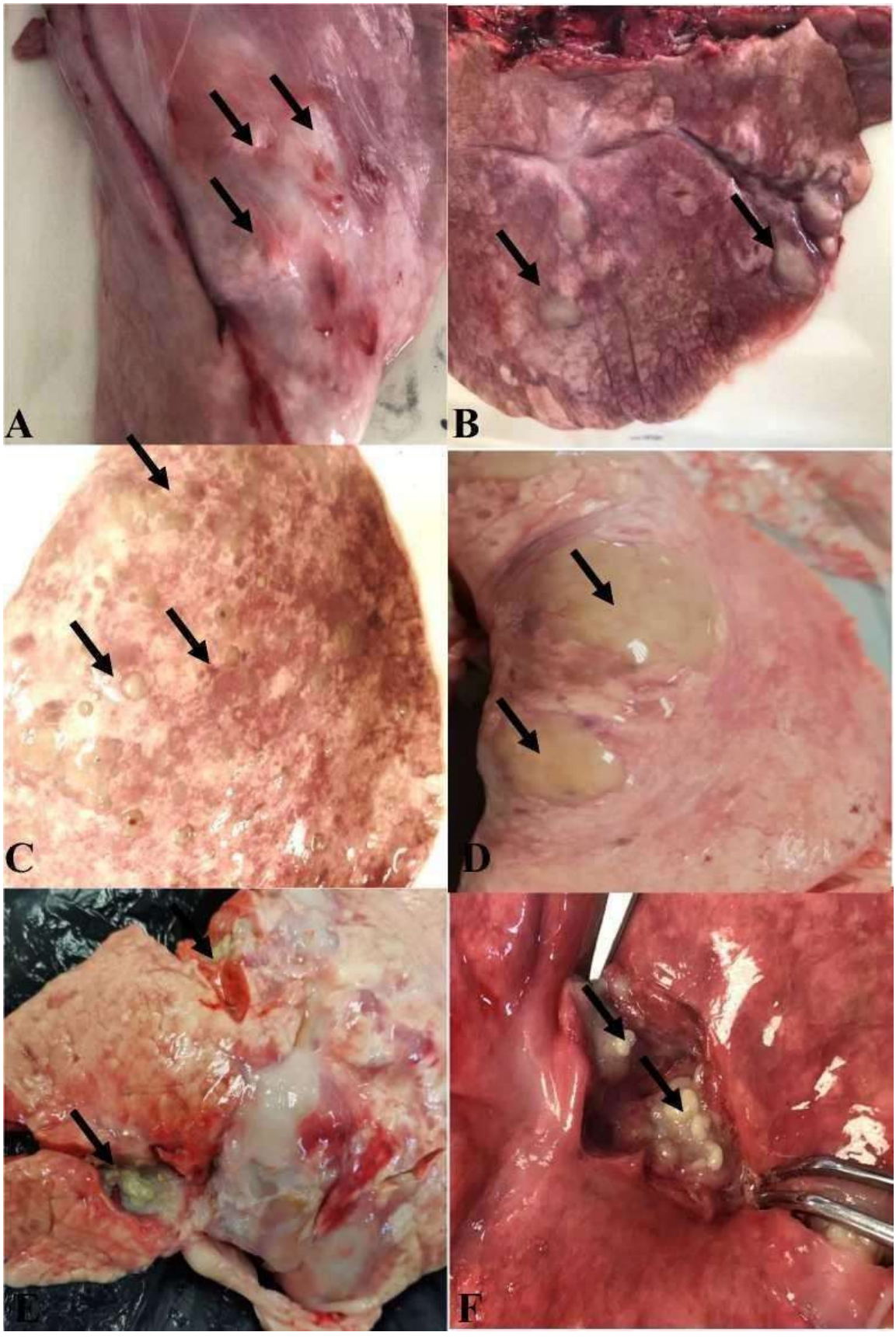
A
B
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Figure. 2. Gross picture of lung granulomas caused by *M. capillaris* : Affected lung had areas of white or greyish nodules on dorsal-caudal part of the diaphragmatic lung lobe (A) (black arrows) and within the ventral surface (B) (black arrows). Clustered small (C) (black arrows) and individual big (D) (black arrows) tan nodules were also well-delineated and raised from the caudal pleura region. Nodules contained internal cavities filled with a caseous substance (E&F) (black arrows).

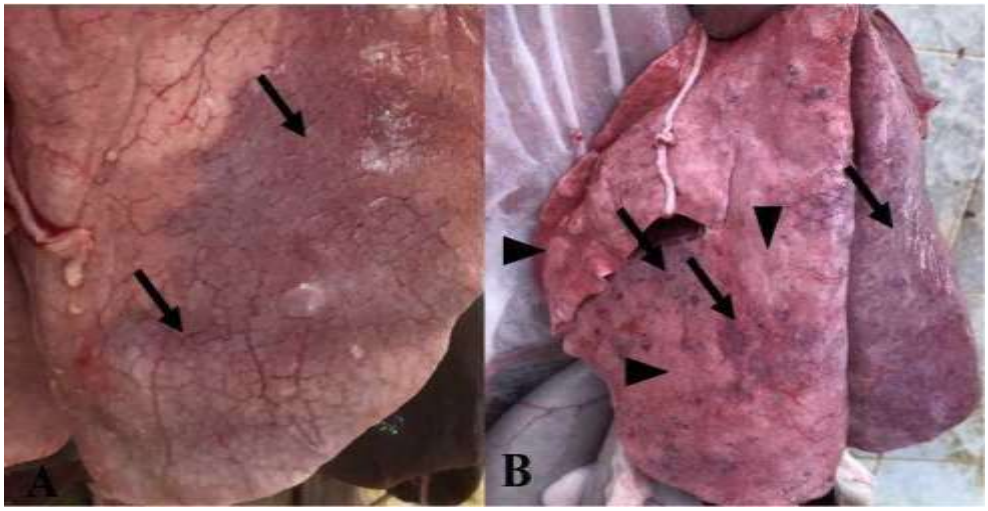

Figure. 3. A) Gross picture of lung with worm (*M. capillaris)* showed firm, dark large area (black arrows). B) Infected lung showed diffuse congestion (black arrows) emphysema (black arrowheads).

Histopathologic findings

The results of the histological investigations of the affected lungs revealed a wide variety of changes involving bronchi, bronchioles, alveoli, and alveolar septa as well as the progress of specific changes in the lung parenchyma around the adult worms, eggs, and larvae of the parasites.

The bronchi and bronchioles contained parasite forms in different stages. Adult nematodes and larvae which were present in cross and longitudinal sections and both were surrounded by mucus (Figures 4A). In addition to parasite stages bronchi and bronchioles enclosed inflammatory cells and necrotic debris admixed with desquamated cells. Cellular infiltrations because of parasite involved large amounts of eosinophils and to a lesser extent neutrophils, macrophages and lymphocytes (Figures 4B).

In some instances, bronchial and bronchiolar mucosa exhibited goblet cell hyperplasia (up to 6 cell layers) and mild to moderate focal segmental squamous metaplasia are occasionally noted in larger bronchi lymphocytes (Figures 4B). The lamina propria contained conspicuous infiltrates of mononuclear cells particularly eosinophils, lymphocytes and plasma cells. Some bronchial glands were moderately enlarged. Hypertrophy and hyperplasia of bronchial and bronchiolar smooth muscle also were noted (Figures 4C).

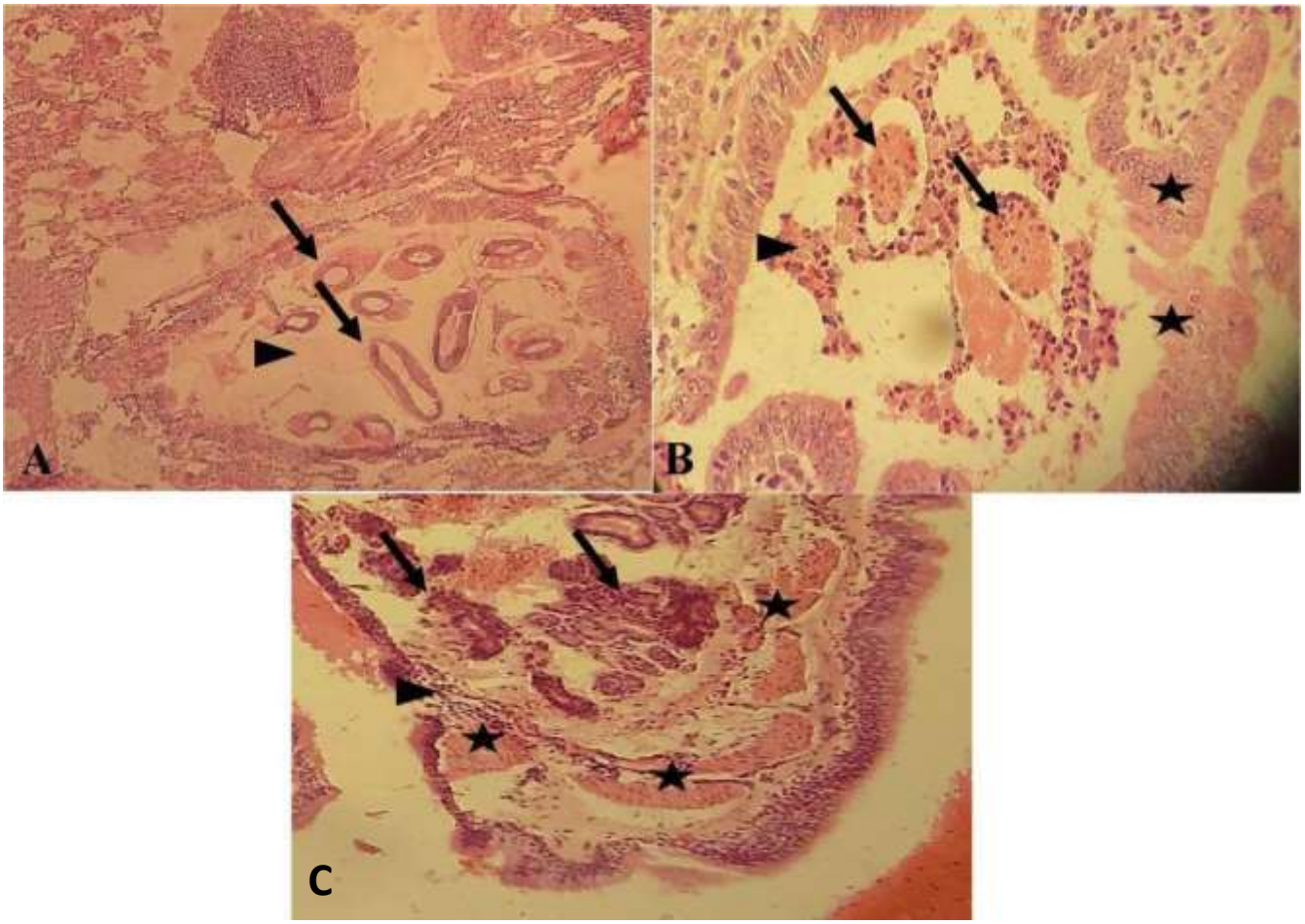

Figure. 4: Histological picture of affected bronchi and bronchioles: (**A)**: The bronchi and bronchioles contained parasite forms in different stages (black arrows). Adult nematodes and larvae which were present in cross and longitudinal sections and both were surrounded by mucus (black arrowheads). (**B)**: The bronchi and bronchioles contained parasite eggs (black arrows), inflammatory cells and necrotic debris admixed with desquamated cells (black arrowheads). Goblet cell hyperplasia and focal segmental squamous metaplasia were noted (black stars). (**C)**: Mononuclear cells infiltration (black arrowhead) was seen. Hypertrophy and hyperplasia of bronchial glands (black arrows) and smooth muscle (black stars) were noted (X10).

Many alveoli also contained a great number of parasite forms (eggs and first stage larvae) surrounded by large numbers of granulocytes, including eosinophils, neutrophils, few macrophages and giant cells (Figure.5A, B & C). Moreover, there was mild exudation of fibrin (amounts of fibrillary to finely granular material and pale eosinophilic proteinaceous fluid (fibrin, cellular debris and edema). In addition to all these changes, sporadic micronodules composed almost exclusively of lymphocytes and plasma cells. A mild hyperplasia of alveolar histiocytes was also observed (Figure.6 D). Hyperplasia of the connective tissue and smooth muscle cells was regularly observed as a thickening of the alveolar septa. (In many cases the alveolar septa were ruptured causing focal or big areas of coalescence of alveolar spaces (emphysema) (Figure 6A). Also, large numbers of fibroblasts leading to increased amounts of collagen (interstitial fibrosis) were noted in the alveolar interstitium) as well as congestion of the alveolar septa (Figure 6B).

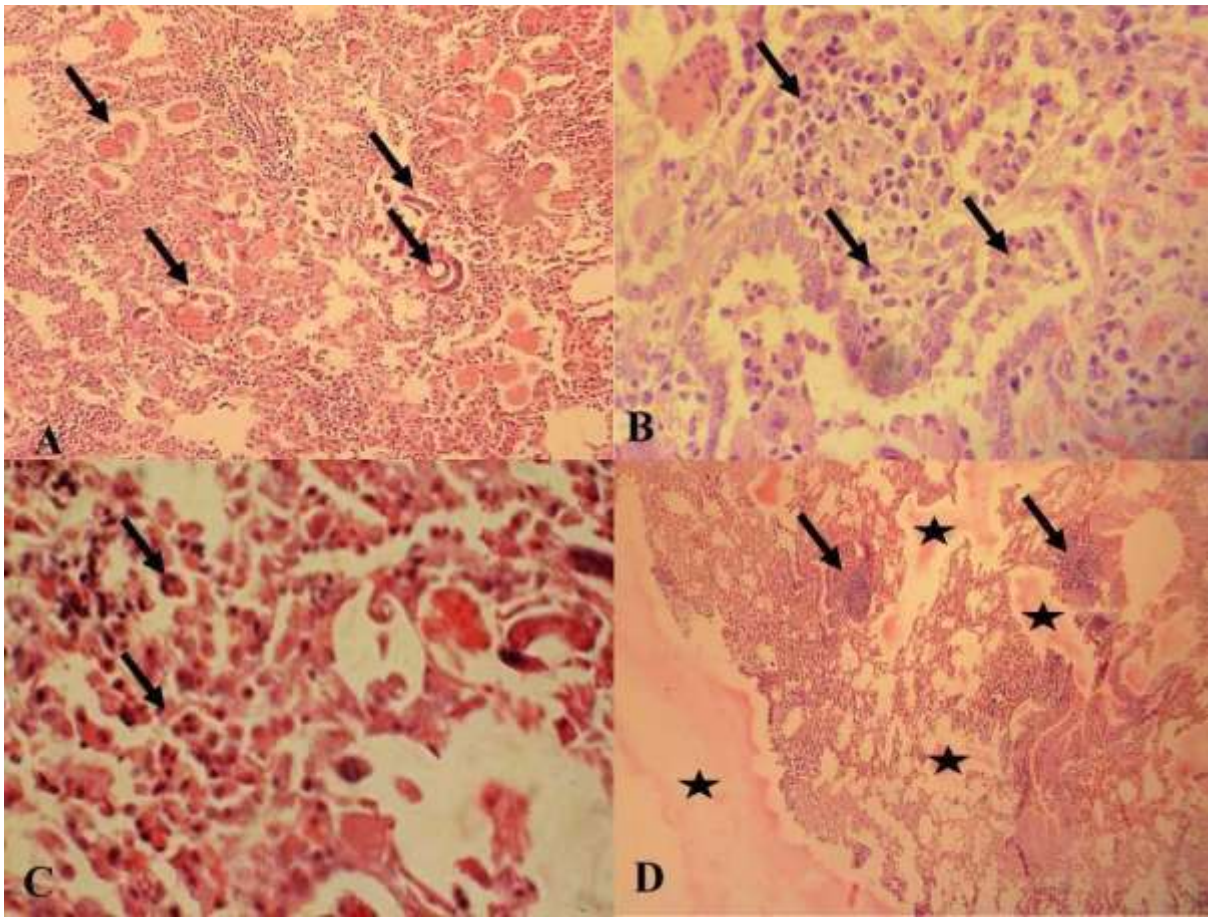

Figure. 5: Histological picture of affected alveoli and interstitium: (**A)**: Great number of parasite forms in different stages (black arrows) (X5). (**B &C)**: Large numbers of granulocytes, including eosinophils, neutrophils, few macrophages and giant cells (black arrows) (X40). (**D**) Sporadic micronodules composed of lymphocytes and plasma cells (black arrows), pale eosinophilic proteinaceous fluid (fibrin, cellular debris and edema) (black stars).

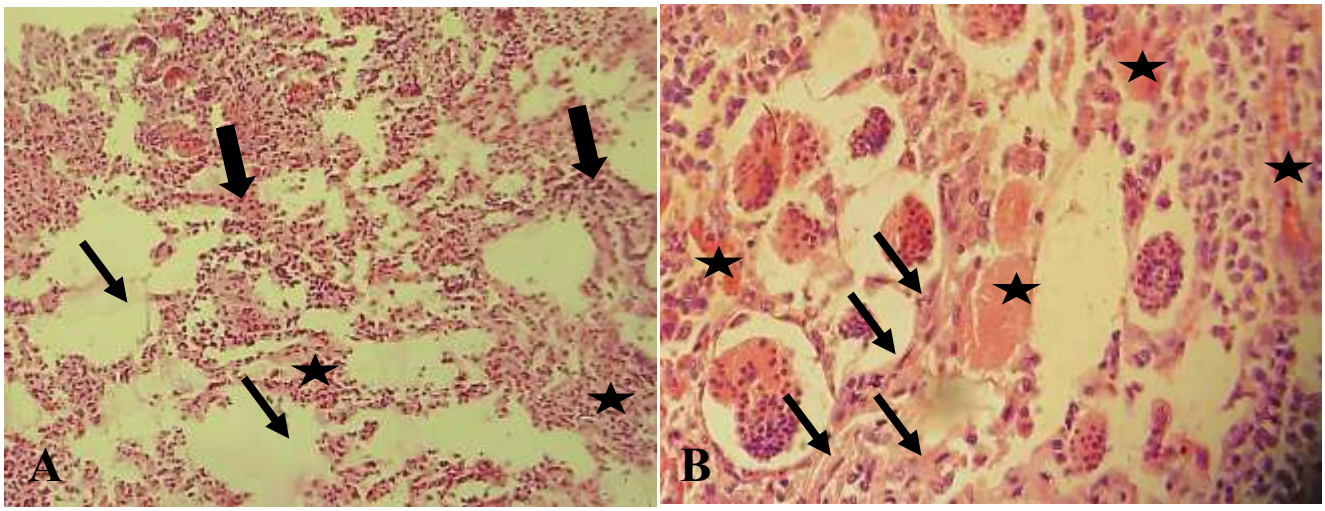

Figure. 6: Histological picture of affected alveoli and interstitium: (**A)**: Thickening of the alveolar septa (thick black arrows), emphysema (thin black arrows) and hyperplasia of alveolar histiocytes (black stars). (**B)**: large numbers of fibroblasts (black arrows) and congestion of the alveolar septa (black stars).

Discussion

Respiratory diseases have been reported as a significant problem for sheep in Libya. The types and frequency of gross and microscopic pneumonia lesions were confirmed by three local studies in recent years. The identification and morphological basis of some lung diseases including pneumonia, ovine pulmonary adenocarcinoma, ovine progressive pneumonia and lung abscesses in sheep slaughtered in Libya were demonstrated through clear evidence (Mohammed and Ibrahim, 2022, 2024; Mohammed et al., 2022; Ali and Mohammed, 2023).

Lungworm infection is a major economic issue for sheep breeder globally. Verminous pneumonia is confirmed by identifying L1 of *M. capillaris* in the faeces of infected animals and examining the cross-section of worm stages in pathological samples (Panayotova-Pencheva and Alexandrov, 2010). As a result, the purpose of this study is to identify the presence of lungworm infection in slaughtered sheep in the eastern north of Libya using parasitological and pathological examinations.

To our knowledge, this study records for the first time the prevalence and determination of pathological features of *M. capillaris* infection in Libyan sheep flocks. 27 out of 342 (7.89%) cases were infected with lungworms, which is comparable with investigations by Ali et al. (2018) in Egypt, where lungworms were discovered in 4.5% (n = 9/200) (Ali et al., 2018), Abdul-Gawad et al. (1993) in Egypt identified lungworms in 4.5% of cases (n = 9/200) (Abo El Hadid and Lotfy, 1993), Maraqa et al. (2005) in Jordan reported lungworms in (3.8%) (Maraqa et al., 2005), Yeasmin et al. (2014) in Bangladesh recorded lungworms in (3.3%) (Yeasmin et al., 2014), and Abdelsalam and Al Sadrani in Saudi Arabia found lungworms in (n = 9; 6.4%) (Abdelsalam and Al Sadrani, 2015). Meanwhile, a few studies have revealed an increase in the prevalence of lungworm infestations. These studies indicated an infection rate of lungworms in sheep of up to 34% in Kirkuk (Yildiz, 2014), 21.83% (110/504) in Afghanistan (Samadi et al., 2019), and 37% (19/51) in Zaire (Samadi et al., 2019).

All of the recorded lungworms in the current investigation were *M. capillaris*, also known as nodular lungworms, which are the most frequent lungworms infecting sheep worldwide (Suarez et al., 2014; Elati et al., 2018). This study's findings are comparable to those reported by Cabaret and Chartier (1989) in Zaire, Alemu et al. (2006), Regassa et al. (2010), Basaznew et al. (2012) in Ethiopia, and Abdelsalam and Al Sadrani (2015) in Saudi Arabia. They identified *M. capillaris* as the only lung worm recovered in sheep (Cabaret. and Chartier., 1989; Alemu et al., 2006; Regassa et al., 2010; Bogale et al., 2012; Abdelsalam and Al Sadrani, 2015). Moreover, research conducted by Orouei in Iran in 2016 and Bekele and Shibbiru in Ethiopia in 2017 reported *M. capillaris* in just 1.5% just 5.6% respectively (Orouei, 2016; Bekele and Shibbiru, 2017) . In contrast, Ali et al. (2018) reported that *Dictyocaulus filaria (D. filaria)* was only species of lungworms (Ali et al., 2018) while and studies by Yildiz (2006) reported of two species *Cystocaulus ocreatus* (50.0%) and *D. filaria* (23.5%) (Yildiz, 2014). This variation of the prevalence of lungworms in small ruminants in different countries seems to be owing to the location, climate conditions, nutrition system, animal's breed, age, presence of intermediate hosts (snails and slugs), and host immunity.

Concerning the pathological findings, Ali et al. (2018) (Ali et al., 2018), Dar et al. (2012) (Dar et al., 2012), and Dar et al. (2013) (Dar et al., 2013) reported multiple patchy consolidated pulmonary, congestion, emphysematous patches, and the presence of adult parasites mixed with foamy and sometimes mucous exudate in the lumens of the trachea and bronchi as gross findings, that were almost the same as in this study.

Also, this study was consistent with previous reports that referred to alveolitis and mixed cellular infiltrations of neutrophils, eosinophils, macrophages, and lymphocytes. Obstructive bronchiolitis and bronchitis with hyperplasia were reported, in addition to desquamation of the lining epithelium, increased mucous secretion and emphysema, are the main histopathological findings for ovine verminous pneumonia (Dar et al., 2012, 2013; Abdelsalam and Al Sadrani, 2015; Samadi, 2019). It seems that the histopathological findings were due to the presence of the stages of parasite and to immunological response of the pulmonary tissue.

Conclusion

To sum up, the results of the present study provided clear evidence of the presence of verminous pneumonia caused by *M. capillaris* within Libyan sheep flocks, and indicated that the morphological characteristics and pathological changes of M. capillaris identified in eastern Libya are consistent with those reported by other African, Asian, and European researchers. However, more studies are needed to investigate the prevalence of other types of lungworms and to screen for verminous pneumonia in sheep during the different seasons of the year, which may contribute

to identifying more details about this disease and thus lead to opening more horizons to learn about the ways of control and prevention.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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