

PREVALENCE AND PATHOLOGICAL FEATURES OF OVINE LUNGWORM IN NILE DELTA

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Abstract: A cross-sectional study was conducted from March 2017 to March 2018, to determine the prevalence of ovine lungworm infection, and to describe the related pathological lesions in naturally infected local breed sheep. A total of 200 whole lungs were collected from fifteen slaughter houses in Nile Delta, Egypt. The overall prevalence for ovine lungworm infection was 4.5%. The prevalence rates in Ossimi, Rahmani, and Barki breeds were 5.55, 4.28, and 2.5%, respectively. The only identified species of lungworms was *Dictyocaulus filaria* (*D. filaria*) (100%). Grossly, the fundamental lesions were multiple patchy consolidated pulmonary tissues with the presence of adult parasites mixed with foamy and sometimes mucous exudate in the lumens of trachea and bronchi. Histopathologically, alveolitis, obstructive bronchiolitis, bronchiolar hyperplasia, emphysema, granulomatous pneumonia, pleural thickening, peribronchiolar lymphoid hyperplasia and trapped different developmental stages and adult parasites besides degenerated and necrotic parasites in the pulmonary tissue were the hallmarks of the histopathological features. In conclusion, ovine lungworms were prevalent in sheep at Nile Delta, Egypt, causing significant damage to the lung and produce characteristic gross and microscopic lesions.

Key words: sheep; lung worm; Nile Delta; *D. filaria*

Introduction

Sheep are multipurpose animals, but in Egypt, they are raised principally for meat production and they participate by about 6.5% of local red meat production (1). Lungworms (dictyocaulosis or husk) had a worldwide distribution with important economic impacts, especially in temperate regions (2-4). Although *Dictyocaulus filaria* (*D. filaria*) is not the most ubiquitous ovine lungworm but it represents an important cause of respiratory disease in sheep particularly in Mediterranean countries (5). Geographically, the prevalence of lungworm

infection in ruminants varies greatly (6). Their prevalence ranges between 46.0 to 66.3% in Ethiopia (6-8), 89 to 95% in France (9,10), 26.6% in India (11) and 4.72% in Egypt (12). The clinical signs and pathological lesions of lungworm infestations are dependent principally on the number of invading larvae, host immunity and stage of infection (5). Mild infections may have no clinical signs and pass unnoticed, but, in general, the most associated clinical signs include coughing, tachypnea, nasal discharge, depression, weight loss, and rarely death (5,13). The main histopathological lesions in foreign breeds were interstitial

pneumonia, granulomatous pneumonia, chronic catarrhal bronchitis, bronchiolitis associated with presence of adult worms, eggs and other developmental stages in the bronchi and large bronchioles (5,11,14). However, the detailed gross and histopathological changes in native breeds present in Nile Delta were not previously described. Therefore, this study was designed to determine the prevalence of ovine lungworm infection, and to describe the related pathological lesions in naturally infected local breed sheep.

Material and methods

Study protocol

A cross-sectional study was conducted in the period from March 2017 to March 2018, on 200 native breed sheep, their ages were over 6 months, slaughtered in fifteen Nile Delta abattoirs. A pre-slaughter inspection for each animal was done where the case history, age, breed, sex and general health condition were recorded. Animals were divided into 2 age groups; young (6 months to one year) and adults (more than one year). The age of animals was determined by dentation (15) and owner's information. The body condition of the slaughtered animals was categorized into 4 groups; obese, fat, average and thin (16). There were no Ossimi (90 animals), Rahmani (70 animals) and Barki (40 animals).

Parasitological and pathological examination

Immediately after slaughter, the whole lungs were collected, and any detectable gross lesions were recorded. Dissections were made along the trachea and bronchial tree and searched for the presence of adult worms and all visual parasites were collected. The recovered nematodes were cleaned with physiologic saline, fixed in 70% ethyl alcohol, then cleared in lactophenol and mounted in emaciated slaughtered animals. Geographically, the study was conducted in Nile Delta region in northern Egypt, between 31°44'N altitude and 30°42'E longitude. The sheep breeds under study were polyol (Sigma-Aldrich Chemie GmbH, Cat. No, 68133-07-3,

Kappelweg 1, 91625 Schnellendorf, Germany). All worms were identified according to morphological and morphometric characteristics of parasites (17,18). For histopathological examination, representative tissue specimens from grossly affected lungs were collected and immediately fixed in 10% neutral buffered formalin. Paraffin sections of 5-micron thickness were prepared, stained with Hematoxylin and Eosin (19) then examined microscopically.

Statistical analysis

All statistical operations were performed using statistical analysis software, SAS statistical system Package V9.1. Univariate logistic regression model was fitted through Maximum Likelihood (ML) procedure to assess the effect of independent variables [age, sex, breed, season and body condition], on the dependent dichotomous variable (incidence of lung worms). Reference category for the comparison of crude odds ratios (CORs) was nominated as R. P-values of less than 0.05 considered to have a statistically significant association with studied traits.

Results

Physical Examination

Pre-slaughter physical examination revealed that all animals were apparently healthy, but 10.5% (21/200) of sheep had mild to minor respiratory signs (tachypnea, nasal discharge, and cough), 17 (80.95%) out of the 21 sheep which had respiratory signs had a thin body condition.

Parasitological examination

Nine out of the 200 (4.5%) native sheep examined in the Nile Delta abattoirs were positive for *D. filaria* infection. The worms (*D. filaria*) were recovered from bronchus and bronchioles of lungs of the slaughtered sheep. They are thin slender, creamy white in color and up to 8-10 cm long. Microscopically they have very small four lips and very small shallow buccal capsule. The posterior end of the adult male *D. filaria* has copulatory bursa having short, stout, dark brown spicules (Figure 1).

Prevalence of lungworm infection

Sheep of all ages were susceptible, but animals less than one year showed lower infection rate (3.33%) than adult (5.45%). Sex had no influence on the prevalence of infection with *D. filaria* in the examined sheep as the prevalence in females was 4.44% and in males was 4.51%. Breed variation was clear where the prevalence of infection in Ossimi sheep was 5.55%, while in Rahmani sheep was 4.28%, and in Bakri sheep was 2.50%. The influence of

seasons on the prevalence of *D. filaria* in sheep was significant ($p < 0.05$) (as the prevalence fluctuated from low in summer (2%) and autumn (2%) to moderate in spring (4%) and high in winter (10%). On the basis of body condition, higher rate of infection with *D. filaria* was observed in animals with thin body condition (23.52%) compared to that having average (6.25%), fat (2.08%), or obese (0.0%) body condition. The prevalence results were summarised in Table (1).

Table 1: Prevalence of ovine lungworms in Nile Delta (March 2017-March 2018)

Risk factors		Examined cases	Positive cases	Prevalence (%)	OR	95 % CI	p-value
Age	Young (\leq year)	90	3	3.33%	1	-	-
	Adult ($>$ year)	110	6	5.45%	1.67	0.41-2.88	0.476
Sex	Male	155	7	4.51%	1	-	-
	Female	45	2	4.44%	0.98	0.15-2.69	0.914
Breed	Ossimi	90	5	5.55%	1	-	-
	Rahmani	70	3	4.28%	0.76	0.18-2.58	0.752
	Barki	40	1	2.50%	0.44	0.05-2.85	0.342
Season	Winter	50	5	10%	1	-	-
	Spring	50	2	4%	0.56	0.21-1.64	0.242
	Summer	50	1	2%	0.18	0.05-0.86	0.049*
	Autumn	50	1	2%	0.18	0.05-0.86	0.049*
Body condition	Obese	39	0	0.0%	1	-	-
	Fat	96	2	2.08%	1.23	0.71-2.73	0.262
	Average	48	3	6.25%	3.46	1.08-4.26	0.034*
	Thin	17	4	23.52%	6.89	3.41-12.60	0.016*

OR= Odd ratios; R= reference value and * Significant differences.

Gross and histopathological examination

Grossly, lungs of affected animals displayed thin cylindrical creamy white worms in a frothy material within the bronchi, pleural adhesions, and patchy consolidated areas, particularly in the caudal lobes. Microscopically, the lesions of verminous pneumonia with variance in the degree of severity were seen in all examined infected lungs. These lesions involved the parenchymal and interstitial tissue. There was alveolitis represented by thickening of the alveolar wall by edema, hyperplasia of type II pneumocytes and mononuclear cell infiltration. The alveolar lumens contained pale eosinophilic material and inflammatory cells mainly eosinophils, with fewer lymphocytes, plasma cells and macrophages (Figure 2A). The

lumens of other alveoli were stuffed with a cluster of embryonated eggs or coiled newly hatched larvae accompanied with interstitial mononuclear cell infiltrations (Figure 2B). Focal emphysematous regions with opening of alveoli to a common space accompanied with thickening of the alveolar walls by fibroplasia were seen adjacent to the areas of alveolitis (Figure 2C). Diffuse perialveolar mononuclear cell infiltrations accompanied with obstructive bronchiolitis due to occlusion of the bronchiolar lumen by parasites, eggs, larvae and desquamated epithelial cells admixed with mucous and cellular exudate comprised of eosinophils, lymphocytes, macrophages and plasma cells were seen (Figure 2D and E). Massive numbers of *D. filaria* filled the bronchial lumens were observed in few cases

(Figure 2F). Marked hyperplasia of the lining epithelium of the bronchioles and bronchi accompanied with peribronchial leukocytic infiltrations were consistent findings (Figure 3A). Goblet cell hyperplasia was seen in some lung specimens (Figure 3B). Peribronchiolar lymphoid hyperplasia with peribronchiolar emphysema was common findings (Figure 3C). Granulomatous pneumonia was frequently detected around dead larvae or eggs with centrally located caseated material surrounded

by macrophages, lymphocytes and giant cells with a delineated layer of fibrous connective tissue at the periphery (Figure 3 D). Occasionally, granulomas coalesced together and replaced large portions of the pulmonary tissue (Figure 3E). Nodular aggregations of mononuclear cells without detected eggs or larvae were also observed. The majority of these aggregations were found subpleural which showed thickening due to edema, mild leukocytic infiltration and fibrosis (Figure 3F).



Figure 1: *Dictyocaulus filaria* detected in lungs of sheep (January, 2018); (A&B) Anterior end, scale bar: 0.4mm, 0.1mm, (C&D) male bursa, lateral and dorsal views, scale bar: 0.3mm. (The posterior end of the adult male *D. filaria* has copulatory bursa having short, stout, dark brown spicules)

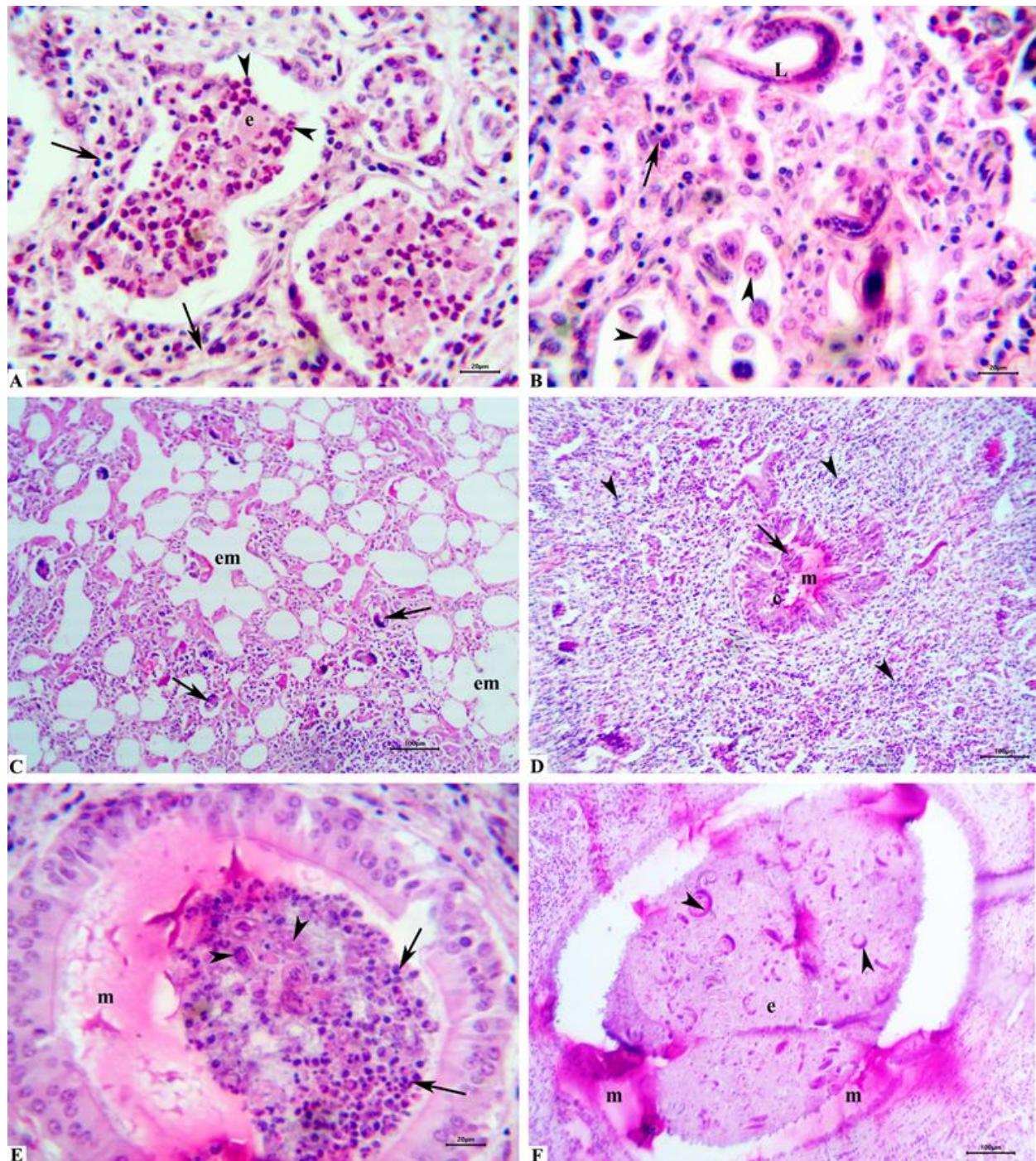


Figure 2: H&E stained sections of lungs of sheep showing; (A) thickening of the alveolar wall by edema, and mononuclear cell infiltration (arrow) and filling of the alveolar lumens with pale eosinophilic material (e) and inflammatory cells predominantly eosinophils (arrowheads), X400, (B) cluster of embryonated eggs (arrowheads) and coiled newly hatched larvae (L) in alveolar lumen accompanied with interstitial mononuclear cell infiltration (arrow), X400, (C) alveolar emphysema (em) adjacent to the areas of alveolitis with presence of eggs and developmental stages in the alveolar lumens (arrows), X100, (D) diffuse mononuclear cell infiltrations (arrowheads) and obstructive bronchiolitis by desquamated epithelial cells (arrow), mucous (m) and cellular exudate (c), X100, (E) occlusion of the bronchiolar lumen by parasites, eggs, larvae (arrowheads), mucous (m) and cellular exudate (arrow), and X400, (F) massive numbers of *D. filaria* developmental stages (arrowheads) admixed in mucous (m) and necrotic eosinophilic material (e) filling the bronchial lumen, X400

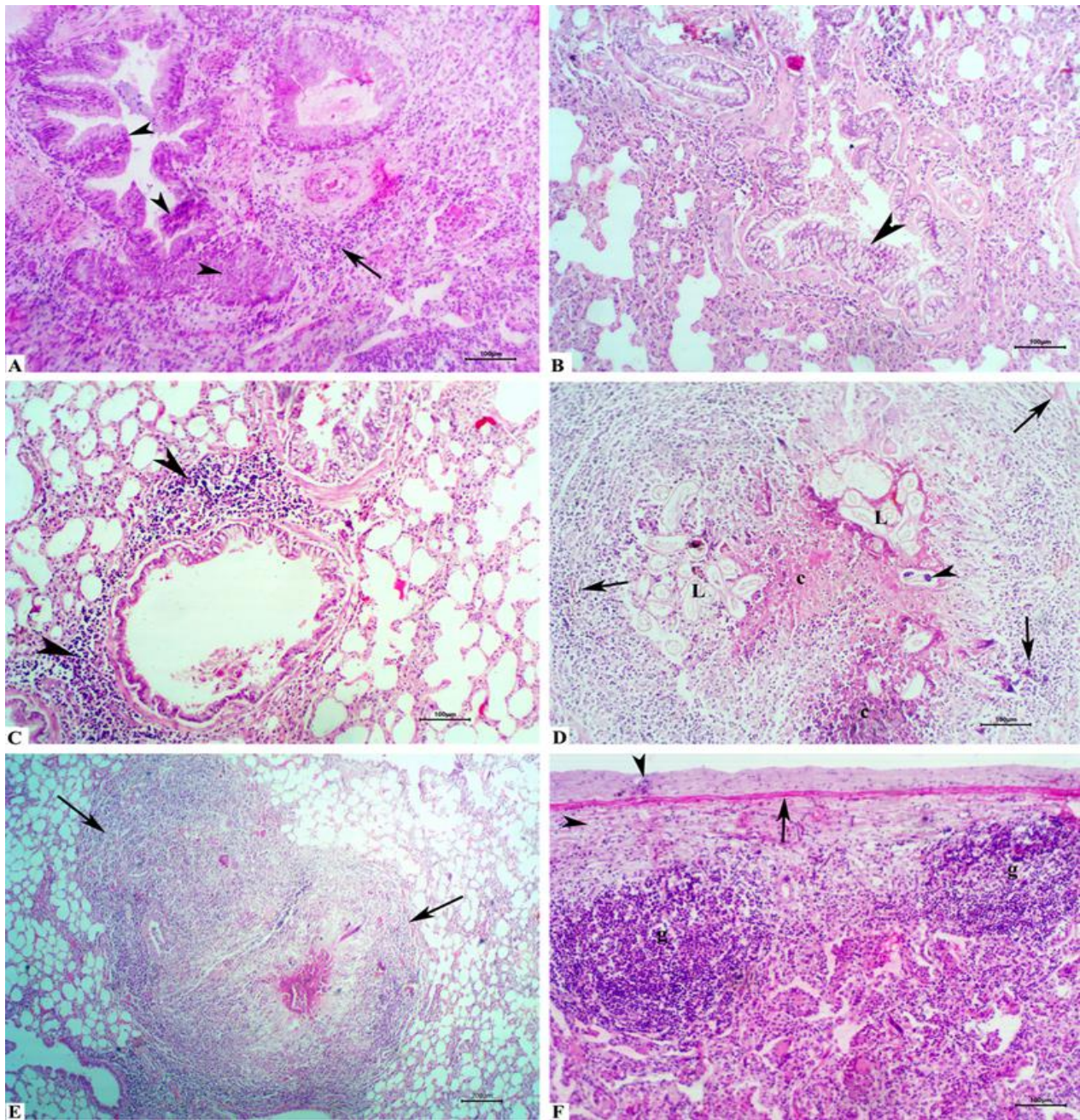


Figure 3: H&E stained sections of lungs of sheep showing; (A) marked hyperplasia of bronchiolar epithelium (arrowheads) and peribronchial leukocytic infiltrations (arrow), (B) goblet cell hyperplasia (arrowheads) (C) peribronchiolar lymphoid hyperplasia (arrows) and peribronchiolar emphysema (em), (D) granulomatous pneumonia consisted of centrally located caseated material (c) with dead larvae (L) and eggs (arrowhead) surrounded by mononuclear cells aggregations and giant cells intermingled with fibrous connective tissue (arrows), (E) Lung showing coalesced granulomas replacing large area of the pulmonary tissue (arrows), (F) Lung showing subpleural granulomas (g) and thickened pleura by edema, mild leukocytic infiltration (arrowheads) and fibrosis (arrow), X100

Discussion

The current study emphasizes that infection with *D. filaria* in sheep caused pulmonary damage and influenced the animal's body

condition. The results revealed that the overall prevalence of *D. filaria* infection in Nile Delta among native breed sheep was 4.5% which is accordant with the prevalence in native breed sheep in upper Egypt "4.72%" (12), in Jordan "3.8%" (20), and Bangladesh "3.3%" (21). Few

reports showed lower infection rates than our findings; 0.19% in Iraq (22), and 2.0% in northeastern Ethiopia (23), while the majority of cross-sectional studies showed higher prevalence rates; 48.8% in Canada (24), 9.3% in Syria (25), 23.5% in Turkey (26), 9% in Iran (27), 26.6-34.09 % in India (28), 46.0-66.3% in Ethiopia (6-8), and 89-95% in France (9,10). The prevalence variations among these reports were multifactorial including geographical location, climate conditions, feeding system, and animal's breed, age, and immunity (29-31). The most important factors for *D. filaria* development were climate conditions and body conditions because a damp and cool environment and low immunity are essential (32), so high infection rates present in countries with mild temperatures (33). The lower prevalence in the current study could be attributed to native sheep are mostly raised in closed farms or farmer's houses and fed on dry ration for long period during the year. Our results indicated that there was insignificant increase ($p < 0.05$) to the infection rates with age. This might be related to increasing the chance of exposure to infection by *D. filaria* with increasing the age. However, many studies mentioned a higher infection rates in young sheep (6,34,35), while others recorded that infection rate of lungworms increased with increasing the age (23,36). The prevalence rate in females was nearly the same in males (18,37), but a higher infection rates in females were also reported (6,23,38), which might be attributed to immune suppression of the female at the time of parturition and during early lactation (39). Our results declared that the highest prevalence of *D. filaria* infection was in winter (10%) related to the cool-environment which are suitable for development of *D. filaria*. The influence of body condition was highly significant ($p < 0.05$) as the highest infection rate (23.52%) was found in animals with thin body condition. Similar findings were reported by many authors (37,40). This could be due to immunosuppression, malnutrition and/or emaciation (41,42). All the identified parasites were *D. filaria* which may be due to absence of intermediate hosts for other lungworms species (29).

Our detected gross and histopathological findings for ovine verminous pneumonia were consistent with previous reports (5,14). Collectively, the histopathological findings could be attributed to the immunological response of the pulmonary tissue to the adult parasite, eggs, and larvae (5). The alveolitis and interstitial leukocytic infiltration were due to an allergic reaction in response to *D. filaria* eggs and larvae in the lung tissue (26). The obstructive bronchiolitis and bronchitis with hyperplasia and desquamation of the lining epithelium were due to the persistent chronic irritation of the respiratory mucosa (13,14), with increased mucous secretion (11,43). The granulomatous pneumonia around the larvae and eggs was due to prolonged existence in the pulmonary tissue with resistance to phagocytosis and failure of acute inflammatory response to remove them (6,44).

Conclusion

In conclusion, the overall prevalence of verminous pneumonia to *D. filaria* infection in the examined native breed sheep in Nile Delta was 4.5%. These pneumonic cases suffered a significant damage to the lung and had a characteristic gross and microscopic lesion.

Conflict of interest

The authors have no conflict to declare.

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