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OVINE LUNGWORMS INFECTION IN TIARET (ALGERIA): PREVALENCE, SPECIES INVOLVED, AND PATHOLOGICAL FINDINGS

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ABSTRACT

Lungworm infections are known to be frequent and responsible for substantial economic losses in ruminants. They are caused by *Dictyocaulus filaria* and various species belonging to the Protostrongylidae family of nematodes. This present study was conducted at the Tiaret municipal slaughterhouse and the parasitology laboratory of the veterinary institute for six months, from November 2016 to April 2017. The study aimed to evaluate the occurrence of lungworm infections and the determination of the circulating species affecting sheep in the region with a pathological study of infected pulmonary tissue sections. The overall incidence of the pathology in sheep was 26 % (240/921). The identified species were *Muellerius capillaris* 43 %, *Neostrongylus linearis* 8 %, *Cystocaulus ocreatus* 4 %, *Dictyocaulus filaria* 3 %, *Protostrongylus rufescens* 1 %, and mixed infestations 42 %. The histological examination of corresponding lesions has revealed pneumonia-type, and bronchopneumonia, inflammatory lesions, with a predominance of mononuclear cells, necrosis of bronchial epithelium, and pulmonary parenchyma as well as hyperplasia of the bronchiolar epithelium. Prevention and control of these parasites is therefore essential for releasing the potential of sheep production.

Key words: ***Cystocaulus ocreatus; Dictyocaulus filaria;*** **histological examination; lungworm;** ***Muellerius cappilaris; Neostrongylus linearis; Protostrongylus rufescens;*** **sheep**

INTRODUCTION

Several factors can influence the productivity of sheep such as the general lack of veterinary care and diseases [27]. Among these diseases, parasitic infestations are the most important with repercussions on fertility, lamb growth, and mortality [19].

Sheep lungworm infection is also called verminous bronchopneumonia, which is a chronic disease characterized clinically by respiratory difficulties breathing like pyrexia, cough, rapid and shallow breathing, and runny nose [5] and pathologically by bronchitis and/or pneumonia [12, 15].

In small ruminants, this disease is caused by a nematode classified under the supper family Trichostronglylodea (Dictyocaulidae family with one species *Dictyocaulus filaria*, located in the bronchial tree), and Metastrongylidea (Protostrongylidae family with four species *Muellerius capillaris*, *Neostrongylus linearis*, *Cystocaulus ocreatus,* and *Protostrongylus rufescens,* which are located in lung parenchyma) [30, 32].

The life cycle of lungworms can have two forms; A direct form (Dictyocaulidae), in which the free-living larvae undergo two moults after hatching and infection is by ingestion of free L3, or an indirect form (Protostrongylidae), in which the first two moults usually occur in an intermediate host (snails or slugs), and infection of the final host is by the ingestion of the intermediate host [28].

Lungworms are widely distributed worldwide but with higher prevalences in tropical and subtropical countries [13].

The search for first-instar larvae in faeces is the simplest and most commonly used technique for laboratory diagnosis of pulmonary infections [31]. Some authors prefer post-mortem examination [14] and others favor coproscopic examination [1, 16]. Thus, the slaughterhouse can be a source of information on the incidence and risk factors of several diseases [26]. The present work has set the following objectives; evaluate the overall incidence of lungworms, by sex, and according to age, identify the different species of lungworms involved, and for the first time evaluate, the histopathological changes of the infected lungs in the study area.

MATERIALS AND METHODS

Study design

The present study was carried out between the municipal slaughterhouse of Tiaret (western Algeria) and the parasitology laboratory of the Institute of Veterinary Sciences of Tiaret, during the period from November 2016 to April 2017. The Tiaret region belongs to the highlands zone (agro-pastoral region characterized by cereal cultivation and dominant sheep farming). This region is characterized by a harsh climate, with a short, cold winter season and a long, hot, dry season that can last 6 months (from May to October).

Samples collection

Nine hundred and twenty-one (921) sheep (447 males and 474 females) slaughtered and eviscerated during our regular visits were examined. After macroscopic examination and inspection of the lungs by the slaughterhouse veterinary inspector, the seizure (partial or total) of the lungs due to lungworm lesions were recovered and placed in bags on which the age and sex were mentioned. The collected samples were transported the same day to the parasitology laboratory of the Institute of Veterinary Sciences of Tiaret for additional investigations. The population was stratified into three age groups: Less than one year, from 1 to 3 years, and more than 3 years.

Macroscopic examination of the lungs at the slaughterhouse

A total of 921 sheep underwent post-mortem inspection of the lungs in search of macroscopic lesions of lungworm infection during regular visits to the slaughterhouse. At the time of evisceration, a first inspection of the lungs was carried out. Those with lungworm lesions were marked with different coloured nodes to be able to distinguish them when they are transferred to the inspection table. The organs are then inspected by the veterinary inspector of the slaughterhouse, where the type of seizure (partial or total) will be pronounced according to the extent of the lesions.

Whole lungs or seized parts are recovered and placed in bags on which the age and sex are mentioned. Then, the samples are sent the same day to the parasitology laboratory of the Institute of Veterinary Sciences of Tiaret for further investigations.

Microscopic examination of the lungs in the laboratory

Identification of lungworms

At the laboratory level, the lungs were explored in more detail. In the case where the seizure is total, we proceeded to a global opening, from the trachea, to the bronchioles in search of the adults. Then, we examined the bronchial mucus in search of the different parasitic structures by scraping the mucous membrane of the sites of predilection of the parasites using a scalpel blade. The nodules were also incised and compressed to allow the exit of these parasitic structures (larvae and portions of adults). Adults are not often identified because the incision risks deforming the posterior end of the male which is useful in species identification. For this, we relied on the identification of the first-stage larvae. The collected mucus was placed on a microscopic slide mixed with a drop of a dye (methylene blue or eosin) to colour the debris and to better see the larvae, then covered with a coverslip and examined under an optical microscope.

All of the nodules were examined to highlight the infestations associated with several species of parasite.

In cases where the lesions are present, but the examination of the products of the scraping does not make it possible to highlight any parasitic structure, we proceed with another method supposed to allow the extraction of the larvae even at very reduced numbers, this is the technique by McKenna [21]. It is based on the positive hydrotropism of living larvae.

For the identification of the larvae, reference was made to the morphologies given by Van Wyk et al. [31]. The portions of adults identified with the copulatory bursa were identified based on their morphologies [8].

Histopathological study

After macroscopic identification of lungworm lesions and microscopic identification of the parasite species involved, samples were taken from the sites where the parasite was isolated, and then placed in vials containing 10 % formalin on which the species of parasite, the age and the sex of the animal are mentioned. It should be noted that the time separating the taking of the samples and the evisceration did not exceed 2 hours. Some of the positive cases were sent to a private histopathology laboratory and the histopathological slides were produced according to the different stages of routine histological techniques.

Statistical evaluation

In the present study, sheep examined were divided according to their sex and age. The statistical analysis was performed by R software (version 3.3.0/2016-05-03). The study of the influence of age groups and sex on infection was analyzed by two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. In addition, $P < 0.05$ was considered statistically significant.

RESULTS

The present study showed that the overall prevalence of lungworm infection in sheep was 26.06 % (Table 1). The infection prevalence of females (37.76 %) was a highly significant difference ($P < 0.001$) from males (13.64 %). Thus, the most affected age group was that of more than 3 years. A highly significant difference ($P < 0.001$) between age groups was revealed.

Table 1. Prevalence of ovine lungworm infection

Sheep examined	Prevalence
Males	13.64 % (61/447)
Females	37.76 % (179/474)
Less than 1 year old	8.55 % (26 /304)
1 to 3 years old	22.07 % (79/358)
More than 3 years old	52.12 % (135/259)
Total	26.06 % (240/921)

Microscopic examination made it possible to identify the five species responsible for lungworm infection in sheep at different stages of development.

M. cappilaris was the most dominant species whether alone (43.33 %), or in association with other species. The second place was occupied by *N. linearis* (7.5 %), followed by *C. ocreatus* (3.75 %), *D. filaria* (2.5 %), and *P. rufescens* (0.83 %) (Table 2). It is important to note that *C. ocreatus* was reported for the first time in the study region.

Table 2. Lungworm species involved

Lungworm species	Prevalence
M. cappilaris	43.33 % (104/240)
N. linearis	7.5 % (18/240)
C. ocreatus	3.75 % (9/240)
D. filaria	2.5 % (6/240)
P. rufescens	0.83 % (2/240)
M. cappilaris+N.linearis	14.58 % (35/240)
M. cappilaris+C. ocreatus	9.17 % (22/240)
M. cappilaris+D.filaria	5 % (12/240)
Mixed infections	13.33 % (32/240)

Histopathological findings

Microscopic examination of sheep lungworm lesions showed variable changes such as pneumonia and bronchopneumonia with a disappearance of the pulmonary architecture following the infiltration of the sites by inflammatory cells (a predominance of mononuclear cells) and the distension of the interalveolar septa (Figure 1). Likewise, this figure highlighted different stages (egg, larva, adult) of the development of *Cystocaulus ocreatus* (arrows). However, Figure 2 shows lung parenchyma necrosis due to *Cystocaulus ocreatus* infection. While hyperplasia of the bronchiolar epithelium was noted in Figure 3. Figure 4 indicated the presence of inflammatory granulomas following infection by *Muellerius capillaris*. However, a disappearance of the interalveolar septa resulting from colonization and distension of the alveoli by the eggs of this species was noted in Figure 5, as well as peribronchial lymphocytic infiltration in Figure 6. Whereas, Figure 7 allowed us to observe hyperplasia and fibrosis at the level of the bronchial glands and around the bronchi. As well as portions of adults of *Dictyocaulus filaria* in the bronchial lumen associated with necrosis of the bronchial epithelium.

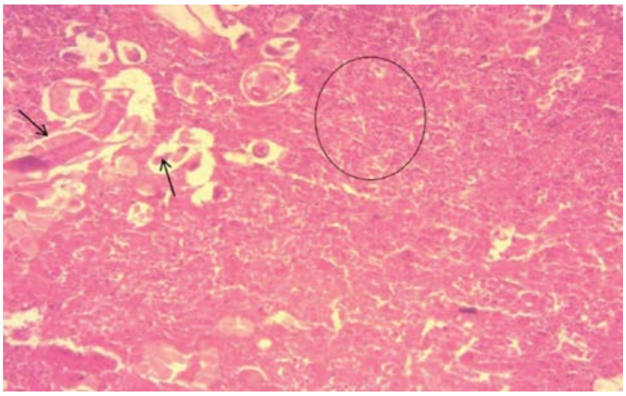

Fig. 1. Lung, ovine; massive infiltration of the lung parenchyma by mononuclear (circle) and different parasitic stages of the *C. ocreatus* species (arrow). H&E. Magn. x 10.

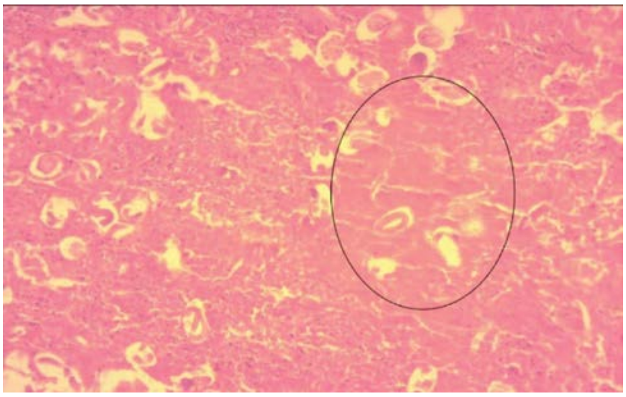

Fig. 2. Lung, ovine; necrosis and disappearance of the pulmonary architecture and the alveoli occupied by parasite fragments (circle). H&E. Magn. x 10.

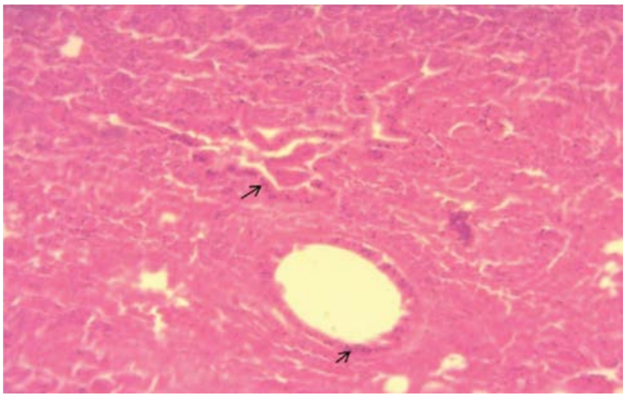

Fig. 3. Lung, ovine; bronchiolar epithelial hyperplasia with condensation of nuclei (arrow). H&E. Magn. x 10.

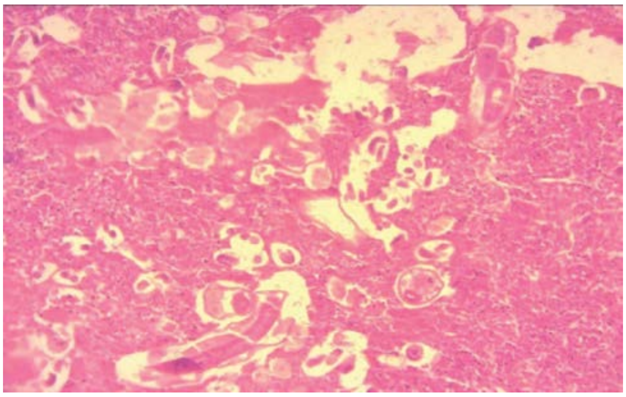

Figure 4. Lung, ovine; *Cystocaulus ocreatus* at different stages of development. H&E. Magn. x 10.

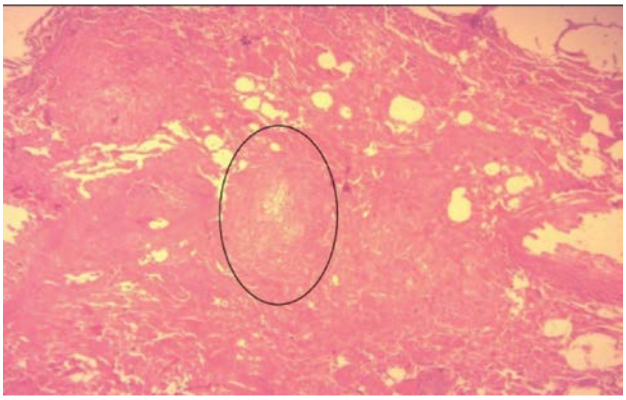

Figure 5. Lung, ovine; inflammatory granulomas (circle) in a lung infected with *Muellerius capillaris*. H&E. Magn. x 10.

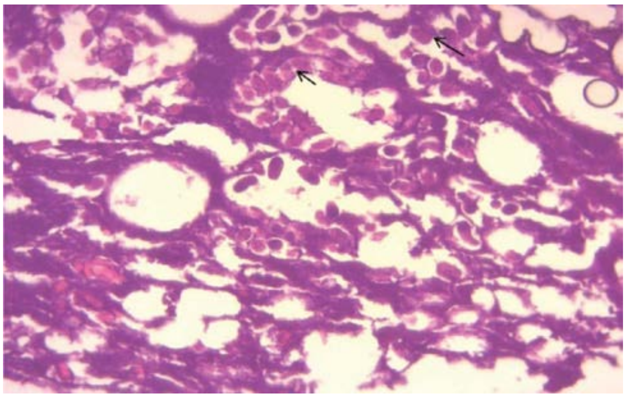

Fig. 6. Lung, Ovine; distension of the alveoli occupied by eggs of *Muellerius Capillaris.* H&E. Magn. x 10.

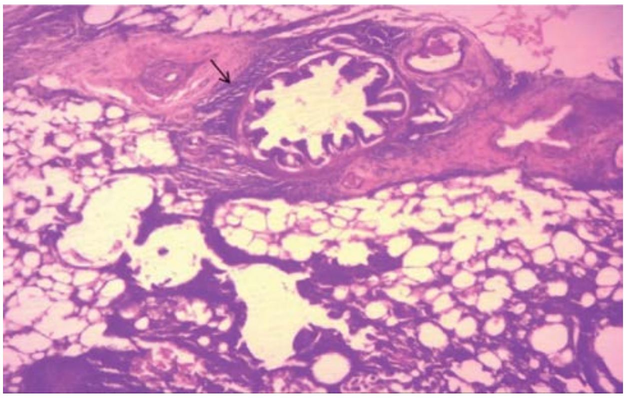

Fig. 7. Lung, ovine; severe bronchitis with mononuclear infiltration all around the bronchiole following infestation by *Muellerius capillaris* (arrow) and alveoli colonized by eggs. H&E. Magn. x 4.

DISCUSSION

The overall infestation rate of 26.06 % reflected certain importance of parasitism by sheep respiratory strongyles in the Tiaret region.

The prevalence of lungworm infection, which is highly dependent on climatic conditions and farming techniques, varies from one region to another [3, 28, 29].

The current prevalence (26.06 %) was close to that reported by Kouidri et al. [18] with a rate of 22 % in a study conducted in the same slaughterhouse for one year. This slight variation between the two studies may be due to the difference in study duration and the size of their sample, which was larger than ours.

The current rate is higher than the rates reported by Nematinejad [22] and Borji [1], which had recorded rates of 16.45 % and 3.80 %, respectively in Iran and that reported by Kassa et al. [16] in 2017 in Ethiopia, which was 7.1 %.

However, our results are lower than the rates of 57.55 % [6], and 39.4 % [10] recorded in Ethiopia, as well as the infestation rates of 62.5 % [23] and 34 % [32] recorded in Turkey. These variations in the prevalence of lungworm infection in different countries are due to differences in nutritional status, level of immunity, and climatic conditions (temperature, humidity), which act by favouring or disfavouring the survival and development of the larval stages and on the biology of the intermediate host mollusks and the seasons during which these studies took place [17]. Intensive or extensive farming techniques are also factors that influence the epidemiology of these pathologies [1]. The level of pasture contamination is a determining factor in the prevalence of lungworm infection.

Dar et al. [9] reported a rate of 26.6 % in India, which is similar to the rate we recorded.

Yimer and Desie [33] found in Ethiopia an infestation rate of 46.6 %. This large discrepancy between our results should be explained by their sample containing more females and the possibility that the latter are in the peripartum period when their resistance to parasites decreases, which consequently allows an increase in the number of larvae excreted [7].

Concerning the influence of sex on the pathology, we found that females are more affected than males, which is consistent with the results of Kouidri et al. [18] who had observed a predisposition of the female sex to pathology. Whereas, Dar et al. [9] and Kassa et al. [16] did not report a difference between the two sexes, although the number of females was greater than that of males. The indifference between the two sexes during these studies may be because females are not in the peripartum phase during the study period [33].

On the other hand, Borji et al. [1] reported that the infestation rate was higher in males than in females and the most predominant parasite species was *Dictyocaulus filaria* where the largest number in the sample were males less than one year old. This partly explains the higher rate in males. The other reason why the males were more affected is that dictyocaulosis is considered to be the disease of the young, especially when they are first brought to the pasture and have no immunity to these parasites, unlike adults. In these circumstances, the pathology takes on an endemic appearance [1].

Regarding the influence of age, we found that the risk of infestation increases with age regardless of gender. Sheep over three years of age infested with parasites belonging to the Protostrongylidae family do not develop protective immunity against subsequent infestations, whereas animals develop acquired immunity against *Dictyocaulus* [10].

We identified during the present study five species of lungworms at varying rates, the most predominant of which was *Muellerius capillaris* alone (43 %) or associated with *Neostrongylus linearis* (15 %), *Cystocaulus ocreatus* (9 %) and *Dictyoculus filaria* (5 %). The predominance of this species has also been reported by other authors, in Morocco, whose environment is similar to the Algerian steppe. They found that the emission of protostrongylus larvae is high all year round, especially those of *Muellerius capillaris*. This is explained by the high fertility of these species compared to other species of protostrongylids [4].

The low rate of infestation by *Dictyocaulus filaria* that we observed (3 %) is comparable to that reported by Boulkaboul and Moulay [2] in the Tiaret region.

However, this low rate could be linked to the high number of old animals which probably benefited from an acquired immunity protecting them from infestations by dictyocaulids [28]. The other possible reason for this low rate of dictyocaulids compared to protostrongylids is the type of evolutionary cycle, dictyocaulids have a direct cycle without an intermediate host which exposes the larvae to external conditions unfavourable to their survival, whereas protostrongylids have an indirect cycle with the intervention of the intermediate host which serves to protect the larvae from external conditions and prolong their lifespan.

The minimal infestation rate by *Protostrongylus rufescens* is probably explained by the fact that this species has a very restricted range of intermediate hosts [1]. The infestation by *Cystocaulus ocreatus* was also low (4 %) contrary to the results of Y i l d i z [32] in turkey.

The high rate of mixed infestations that we recorded (42 %), confirms Mage's theory [19] concerning the simultaneous presence of bronchial and pulmonary infestations and that the animal does not present with a single lungworm infection.

The results of the microscopic examination of the lungs presenting lesions of lungworm infection made it possible to obtain lesion patterns comparable to those obtained by other authors.

The hyperplasia of the bronchial epithelium that we noticed was also reported by M a h m o u d et al. [20]. These lung changes could be related to chronic inflammation that promotes neoplastic processes [8]. As well as the increase in the production of mucus by the cells. The cells increase their number in response to the parasitic infestation [11].

The aggregation of mononuclear populations observed both around the respiratory tract and in the lung, parenchyma that we have been able to see is similar to what was observed during infestation by respiratory strongyles in the study by P a n u s k a [25]. This proliferation of immune cells is probably related to immune responses to egg divisions and migration of larval forms as well as adults [32].

Dictyocaulus is considered to be the major cause of verminous bronchitis. The arrival of this nematode in the lungs activates eosinophils and mast cells, resulting in a reduction in the Caliber of the respiratory tract following oedema and emphysema. They are also responsible for the desquamation of the bronchial epithelium [8], which agrees with our observations.

The formation of inflammatory granulomas in the event of infestation by *Muellerius capillaris* and lymphocyte hyperplasia was also observed by P a n a y o t o v a – P e n c h e v a et al. [24].

CONCLUSIONS

At the end of our study, we conclude:

- The prevalence of lungworms is high, which is an important cause of the seizure of lungs at the slaughterhouse.

- A particular predominance of protostrongylids (*Muellerius capillaris*), which means the abundant presence of intermediate host mollusks in the region, requiring them to be taken into consideration when establishing preventive protocols for lungworm infection.

- Identification for the first time of the species *Cystocaulus ocreatus* in the Tiaret region.

Hence a need to pay particular attention to the latter to limit their role in the dissemination of larvae on pastures and the infestation of young animals when they are put out to pasture.

- Infestations by lungworms cause significant tissue damage that could harm the health and production of sheep.

CONFLICT OF INTEREST

We declare no conflict of interest.

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