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ORIGINAL ARTICLE

Hematological Studies and Management of Lung Worm Infection in a Sheep Flock

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ABSTRACT

Background: Verminous bronchitis in small ruminants is widely prevalent in many regions of India and neighboring countries. The sheep lung worm *Dictyocaulus filaria*, is the principal etiological agent responsible for weight loss, anaemia, hematological changes and causing mortality in sheep.

Aim: Therefore, the present study was conducted to analyze various hematological *factors* in Nellore brown infected sheep with *Dictyocaulus filaria*, the main causative agent of Verminous bronchitis in sheep and its successful control.

Material and Methods: The present study was carried out to record the hematological changes in Nellore brown sheep affected with lung worms. Total 25 adult sheep and 17 lambs were identified and isolated from a sheep flock (n=387), showing symptoms of respiratory illness, dullness, coughing, anaemia, dyspnoea, laboured breathing and depression.

Results: The infection showed an increase in Erythrocyte sedimentation rate (ESR) and total leukocyte count (TLC). However, a decrease in packed cell volume (PCV), Hemoglobin, total erythrocyte count (TEC), blood pH in *Dictyocaulus filaria* infected sheep. All the affected sheep were successfully treated with two doses of levamisole hydrochloride @ 7.5 mg/kg body weight at 21 days interval as well as by minimizing exposer to the contaminated pastures.

Conclusion: The present study reveals that a significant reduction in erythrocyte count, hemoglobin concentration and packed cell volume whereas, a significant

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increase in total leukocyte count and Erythrocyte sedimentation rates in sheep affected with *Dictyocaulus filaria.*

KEYWORDS

• Verminous bronchitis • *Dictyocaulus filaria* • Haematology • Nellore brown sheep

INTRODUCTION

Ovine lung worms are one of the major respiratory parasites of sheep that are widely distributed in temperate, tropical and sub-tropical countries (Tesfaye *et al*., 2015). The lung worms have direct lifecycle they enter the host's body through ingestion of infective larvae, and are located in the respiratory tract of the lungs causing chronic syndrome of coughing, un-thriftiness in kids and lambs (Tesfaye *et al*., 2015). These lung worms suppress respiratory tract immunity in hosts body causing bronchitis or pneumonia resulting in loss of body condition and ultimately death (Gelagay *et al*., 2004). Lung worms cause a severe pulmonary disease commonly called as parasitic bronchitis / verminous pneumonia / husk (Panuska 2006). Incidence of lung worms varies from place to place depending on predisposing factors. The diagnosis may be focused on clinical symptoms, history of grazing as well as recovery of adult worms during post mortem and by detecting L1 larvae in the faeces or in faecal cultures (Hasen *et al*., 2013). Lung worm control and prevention can be accomplished by deworming all animals at the end of the rainy season to avoid heavy burden during grazing and it is important to minimize pollution of pasture (Tewodros, 2015). The current investigation deals with the occurrence of lung worm infestation, haematological alterations and successful treatment in Nellore brown sheep of Anantapur district of Andhra Pradesh.

MATERIAL AND METHODS

The investigation was carried out during May 2023 to October 2023 for a period of six months in a sheep farm (n=387) located at Rapthadu mandal of Anantapur District with latitude of 14^{o} 41'8.0304" north and 77^{o} 35' 43. 4616 east of Andhra Pradesh, India. The average annual rain fall was around 520 mm and annual mean temperature ranging from 24-45^{o}C respectively. The climate is very hot during summer reaching 45^{o}C.

The study animals were Nellore brown sheep of various sexes; age groups were maintained in that farm. All the animals were maintained on semi grazing system of management with 9 hours of grazing in an open pasture, with in sheds with concentrate feed containing 18% crude protein with *ad libitum* of water even in pasture. The animals were dewormed by the animal husbandry assistants yearly twice using Albendazole followed by Praziquantel. All the animals were vaccinated with Enterotoxaemia, PPR, Foot rot and Anthrax vaccines regularly. During lean periods (April to July), all the animals received additional supplementation of sorghum silage. Blood samples were collected from the infected animals as well as uninfected animals for haematological findings (n=387) Nellore brown sheep present in a sheep farm and randomly faecal samples also collected and performed larval culture tests to know the worm burden by calculating larvae per gram (LPG values) in the study area using watch glass and petri dish method (*Figure* 2).

During our investigation period 35 animals (n=387) showed the clinical manifestations suggestive of respiratory illness, coughing with nasal discharge, dyspnoea, weight loss with few mortalities. We recovered adult worms from the respiratory passages viz., trachea, lungs, bronchi and bronchioles during postmortem examination. Lungs of the animals showed marked lung abscesses in the caudal region with adult worms (*Figure* 1). All the worms anterior and posterior ends were cut and examined under microscope for correct identification. After correct confirmation all the affected sheep were isolated and treated with levamisole hydrochloride @ 7.5 mg/kg body weight at 21 days interval to control the lung worm infection.

RESULTS AND DISCUSSION

In the present study, the occurrence of the *Dictyocaulus filaria* infection is recorded from a Nellore brown sheep flock for a period of six months from May to October 2023. In the flock, affected sheep showed the clinical signs of mild

depression, dullness, nasal discharge, moderate coughing to severe dyspnoea, laboured breathing with few deaths. Some animals exhibited reduced weight gains, emaciation and retarded growth which are in accordance with the findings of (Chakraborty *et al*., 2014; Prabhu *et al*., 2023). The severe respiratory illness in small ruminants is caused by lung worms referred as verminous bronchitis or Husk / Hoose. It is worldwide in distribution and more endemic in temperate regions with high rainfall (Taylor *et al*., 2007). Lung worm infestation is known to be potentially increasing and causing extensive troubles in small ruminants in many areas (Ploeger, 2002). In the present study total of 26.74% prevalence (23/387) was recorded (*Table*. 1). The prevalence of this illness varies from place to place depending on climatic conditions prevailed such as rain fall, humidity and temperature in that area, number of infective larvae in the grazing field, management system followed and immune system of the animals are the factors contributing to the severity of this infection (Dar *et al*., 2012).

The lung worms live mainly in the trachea and bronchi and affecting lung tissue due to aspirated eggs, larvae and debris. Hence, they are more pathogenic (Tewodros, 2015). In our study, we recovered adult worms and some larvae during postmortem examination and through larval culture tests in our laboratory from 23 died animals out of 86 infected animals (*Figure* 1). Adult worms were identified as *Dictyocaulus filaria* after finding boot shaped or socks shaped spicules in the posterior end of male lung worms (*Figure* 3) and larvae were identified based on the presence of anterior knob (*Figure* 4) (Soulsby, 1982). The details of sheep infected were shown in *table* 1.

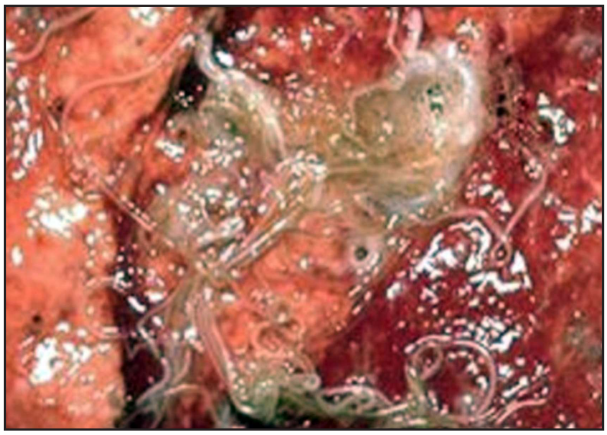

Figure 1: Lungs showing acute infection with Lung worms

Figure 2: Petri-dish and watch glass method

Figure 3: Male worm showing boot/socks shaped spicules

Figure 4: Larva showing bulb at the anterior end

The larval output per gram of faeces and haematological finding were presented in *table* 2. The mean worm recovery and fecal larval output during the study period indicates the progress and status of the disease. The present findings are in confirmation with the reports of (Dhar and Sharma, 1978; Dhar *et al*., 1981). In the temporate regions *D.filaria* infection, the host is known to suffer from

hypoxia and hypoxaemia continuously which generally cause its death (Levine, 1968; Sharma *et al*., 1988). Infection with *Dictyocaulus filaria* in sheep is associated with various hematological changes, notably anemia and alterations in leukocyte counts (Shrama *et al*., 1989). Significantly ($p<0.005$) reduced haemoglobin concentration (Hb), packed cell volume (PCV), Erythrocytes count whereas significantly elevated levels of total leukocytes count, and eosinophil count were noticed. Lungworm infections frequently cause a reduction in red blood cell (RBC) parameters, indicating anaemia in the affected sheep (Mohamed *et al*., 2008; Mohamed and Mottelib, 2020). This reduction in red blood cells contributes to the overall anaemic state in the affected animals (Sharma *et al*., 1989 and Fereig *et al*., 2018). Studies have investigated alterations in erythrocyte membrane constituents during *Dictyocaulus filaria* infection in lambs (Bhat and Sharma, 1989). Furthermore, research has been conducted on the osmotic fragility of sheep erythrocytes due to *Dictyocaulus filaria* infection (Sharma *et al*., 1989). In sheep infected with lung worms a marked decrease in hemoglobin (Hb) concentration and packed cell volume (PCV) values has been observed (Mohamed and Mottelib, 2020; Parmar *et al*., 2019). For instance, one study noted a decline in mean Hb level from 10.83±0.59 to 4.97±0.83 g/dL at five weeks of post-infection in sheep experimentally infected with *Haemonchus contortus* (Parmar *et al*., 2019). Similarly, PCV dropped from 224.08±0.59 to 14.52±1.90 during the same period (Parmar *et al*., 2019). Another study on sheep with various lung affections, including parasitic infestations like Myiasis, also reported a significant decrease in Hb, PCV, and erythrocytes (Dutta *et al*., 2017). This reduction in red blood cell indicates hallmark of anaemia, often characterized as normocytic-normochromic, although some heavier infestations can lead to macrocytic-normochromic anaemia. The anaemia is sometimes attributed to extensive tissue damage caused by larvae, resulting in chronic bleeding from the mucosa (Dobson, 1967).

Lungworm infections typically lead to significant changes in white blood cell (WBC) counts, reflecting the body's immune response to the parasitic challenge (Mohamed and Mottelib, 2020). An increase in the total leukocyte count (leukocytosis) is frequently observed in lungworm-infected sheep (Mohamed and Mottelib, 2020; Fereig, 2007). This general increase in white blood cells suggests an active immune response to the infection (Dobson, 1967). Significant increase in eosinophil counts (Eosinophilia), is a prominent feature in sheep affected by lungworm infections (Mohamed and Mottelib, 2020; Dobson, 1967). This is a typical response to parasitic infestations (Dutta *et al*., 2017). Furthermore, pathological changes in the lungs due to lungworm larvae often involve the influx and activation of eosinophils (Ballweber 2021). In addition to eosinophilia, neutrophilia (an increase in neutrophils) is commonly observed in the lungworms infected sheep (Mohamed and Mottelib, 2020; Parmar *et al*., 2019). Conversely, lymphopenia (a decrease in lymphocytes) is also reported in these animals compared to healthy ones (Dutta *et al*., 2017; Mohamed and Mottelib, 2020).

In the present study, the percent positivity for lung worm infection was reported as 26.74 (Table 1) in Nellore brown sheep flock. In the present study we noticed highest prevalence of *D.filaria* among ewes (n=17) than rams. (Terefe *et al*., 2013). This might be due to production stress in adult ewes resulting in immunosuppression. Whereas, in lamb's ewe lambs shown higher prevalence. The prevalence of lung worms is different based on geographical region and climatic factor. The distribution of *Dictyocaulusfilaria* depends on pasture contamination by carrier animals. The pasture infectivity in turn related to rain fall which stimulates the activity of the larvae (Adem *et al*., 2016). According to Prabhu *et al*., (2023) the infection was present throughout the study period in an organized sheep farm of Tamil Nadu, India. The main mode of transmission is through ingestion of contaminated pasture and due to protective immunity developed in adult sheep after exposure, the infection is more common in young animals during their first grazing season. Successful treatment of parasitic bronchitis in sheep has been achieved with anthelmintics such as fenbendazole (Panuska, 2006). After correct confirmation all the affected sheep were isolated and treated with levamisole hydrochloride @ 7.5 mg/kg body weight at 21 days interval to control the lung worm infection in the sheep flock which is in accordance with the findings of Sevimli *et al*., (2011). Additionally, minimizing exposure to contaminated pastures is crucial for preventing re-infection and controlling the disease (Mohamed and Mottelib, 2020).

A novel combination therapy involving oral triclabendazole or levamisole, followed by subcutaneous ivermectin and florfenicol, has also shown significant efficacy against respiratory diseases in small ruminants, including reductions in clinical signs and mortality (Gholami *et al*., 2023).

Table 1: Details of sheep infected with ***Dictyocaulus filaria*** infection

No. of Nellore brown sheep present	No. of sheep present	No. of sheep examined	Lung worms recovered sheep	% positivity
Rams	33	9	–	–
Ewes	237	123	17	13.82
Ram lambs	14	12	1	8.33
Ewe lambs	103	46	5	12.5
Total	387	86	23	26.74

Table 2: Faecal larval output and Hematological values (Mean± S.D) of *Dictyocaulus filaria* infected sheep

Parameters	Healthy sheep (n=25)	*D. filaria* infected sheep (n=23)
LPG (larvae /gram)	0±0.0 to 38±5.8**	105±6.5 - 887±23.9**
TEC ($x10^3$ /µL)	6.8±0.4*	5. 9±0.4*
TLC($x10^6$/µL)	8.7±0.31*	11.4±0.24*
Hb (g/dl)	11.9±0.4**	7.8±0.4**
PCV (%)	32.5±1.6**	24.7±2.7**
ESR (mm fall / 24h)	16.0±1.8**	7.0±2.9**
Eosinophils (%)	2.8 ± 0.4**	8.8 ± 0.82**
Lymphocytes (%)	65.6 ± 1.82	62.8 ± 2.02
Neutrophils (%)	24.06±0.91	30.12 ± 1.31
Monocytes (%)	2.2 ± 0.3	2.4 ± 0.2

* Significant different (P<0.05), ** Significant different (P<0.01)

CONCLUSION

The study demonstrated that *Dictyocaulus filaria* infection in Nellore brown sheep, causing verminous bronchitis, leads to significant haematological changes including increased erythrocyte sedimentation rate (ESR) and total leukocyte count (TLC), and decreased total erythrocyte count (TEC), haemoglobin (Hb) and packed cell volume (PCV). Levamisole hydrochloride oral solution has shown significant efficacy against lung worms in small ruminants along with pasture management.

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