

# Epidemiological, clinical, morpho-pathological, and therapeutic aspects of protostrongylid infections in sheep from an endemic area in Valea Șieului, Bistrița-Năsăud, Transylvania (Romania)

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**Abstract.** The current study addresses gaps in the understanding of pulmonary strongylidosis in sheep, focusing on the epidemiological, clinical, morphopathological, and therapeutic aspects of protostrongylid infections within a flock of 535 Țurcană and mixed-breed sheep in Valea Șieului, Bistrița-Năsăud, Romania. Conducted between autumn 2018 and 2019, the research revealed a higher prevalence of muelleriosis compared to protostrongylosis, with a marked increase in infestations during autumn and winter, followed by regression in the spring-summer period. Clinically, the infections ranged from subclinical to chronic, with the latter characterized by respiratory symptoms such as macro bronchitis, lobar pneumonia, and bronchopneumonia, often accompanied by anemia and weight loss.

Morphopathological examinations of 28 slaughtered sheep predominantly showed pulmonary lesions, including focal bronchopneumonia, nodules at various developmental stages, atelectasis, and bronchiole obstructions, with adult parasites present. Febantel demonstrated the highest therapeutic efficacy against muelleriosis, followed by ivermectin, while tetramisol proved ineffective.

Hematological and biochemical analyses in sheep with protostrongylid infections and concurrent helminthoses revealed anemia and other changes before treatment. Post-treatment with febantel and ivermectin, significant improvements were noted in red blood cell count, hemoglobin, and albumin levels, suggesting a positive therapeutic response. This study underscores the need for tailored therapeutic strategies and further research to optimize diagnostic and prophylactic methods for managing pulmonary nematode infections in sheep.

**Key words:** *Protostrongylus rufescens*, *Muellerius capillaris*, sheep, northern Transylvania, lungworm therapy.

**Rezumat.** Studiul actual abordează lacunele în înțelegerea strongilidozei pulmonare la ovine, concentrându-se pe aspectele epidemiologice, clinice, morfopatologice și terapeutice ale infecțiilor cu protostrongilide într-un efectiv de 535 Țurcană și ovine de rasă mixtă în Valea Șieului, Bistrița-Năsăud, România. Desfășurată între toamna anului 2018 și 2019, cercetarea a relevat o prevalență mai mare a mueleriozei comparativ cu protostrongiloză, cu o creștere marcată a infestațiilor în toamna și iarna, urmată de regresie în perioada primăvară-vară. Din punct de vedere clinic, infecțiile au variat de la subclinice la cronice, acestea din urmă caracterizate prin simptome respiratorii precum macrobronșită, pneumonie lobară și bronhopneumonie, adesea însoțite de anemie și scădere în greutate.

Examenle morfopatologice ale 28 de oi sacrificate au evidențiat predominant leziuni pulmonare, inclusiv bronhopneumonie focală, noduli în diferite stadii de dezvoltare, atelectazie și obstrucții bronhiolare, cu paraziți adulți prezenți. Febantel a demonstrat cea mai mare eficacitate terapeutică împotriva mueleriozei, urmat de ivermectină, în timp ce tetramisolul s-a dovedit ineficient.

Analizele hematologice și biochimice la oile cu infecții cu protostrongilide și helmintoze concomitente au evidențiat anemie și alte modificări înainte de tratament. După tratamentul cu febantel și ivermectină, s-au observat îmbunătățiri semnificative ale numărului de celule roșii din sânge, ale hemoglobinei și ale nivelurilor de albumină, sugerând un răspuns terapeutic pozitiv. Acest studiu subliniază necesitatea unor strategii terapeutice adaptate și cercetări ulterioare pentru a optimiza metodele de diagnostic și profilaxie pentru gestionarea infecțiilor cu nematozi pulmonari la oi.

**Cuvinte cheie:** *Protostrongylus rufescens*, *Muellerius capillaris*, ovine, nordul Transilvaniei, terapie. viermi pulmonari.

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## Introduction

Nematode endoparasites present a significant challenge for sheep farmers worldwide, leading to substantial resource depletion. Infections decrease production (meat, milk, wool) and reproduction, posing a steady threat to animal health and welfare. Even subclinical infections can diminish productivity, as evidenced by performance improvements following treatment (Hamel et al., 2017). Respiratory diseases in small ruminants

account for approximately 5.6% of diseases ailing these animals (Lacasta, 2008), with bronchopneumonia being the most prevalent condition (Panuska, 2006). Parasitic pneumonia, commonly referred to as husk, is a multifactorial disease caused by nematodes from the Strongylida order (Anderson, 2000). Their distribution is ubiquitous, and despite modern therapeutic options, their prevalence remains high (Taylor et al., 2007).

Prophylactic and therapeutic strategies employed globally have led to a decline in the incidence of dictyocaulosis in sheep. However, in recent years, an increase in cases of muelleriosis and protostrongylosis was noted. This trend is attributed to the reduced efficacy of the drugs used and the distinct biology of *Muellerius capillaris* and *Protostrongylus rufescens*, in conjunction with the presence and spread of gastropods, which serve as intermediate hosts (Cabare, 1984; Şuteu, Cozma, 2012; Constantin, 2014). While there is extensive literature on this disease, many aspects remain unclear, mostly concerning pathogenicity, the optimization of diagnostic methods in the context of modern farming, and efficient prophylaxis (Levine, 1980; Constantin, 2014; Deplazes et al., 2019).

Currently, three major groups of anthelmintic (AH) drugs are used in small ruminants: benzimidazoles (e.g., fenbendazole, albendazole, mebendazole), macrocyclic lactones (e.g., ivermectin, eprinomectin, moxidectin), and imidazothiazoles (e.g., levamisole). Additionally, the salicylanilides and substituted phenols group (closantel) are also employed (Bowman, 2014; Antunes et al., 2022). However, the emergence of anthelmintic resistance (AR) has been increasingly reported worldwide across these drug classes (Antunes et al., 2022, Campbell et al., 2024).

The scarcity of specialized studies on the prevalence and epidemiology of protostrongylid infections in the hilly and mountainous regions of Transylvania prompted this investigation. Therefore, we aimed to evaluate the epidemiological aspects of

these infections in sheep, examining clinical and morpho-pathological presentations and assessing the therapeutic efficacy of various treatments. Furthermore, we sought to corroborate the changes in hematological and biochemical blood parameters, pre- and post-treatment, to clinical status in sheep.

## Materials and methods

### *Epidemiology*

The study took place between September 2018 and November 2019. A total of 535 live sheep originating from Valea Şieului, Bistrița Năsăud county along with 28 sheep carcasses were examined for the presence of lungworms. All animals belonged either to the Țurcană breed or mixbred and were part of two flocks, totaling 1100 sheep. Live animals were divided into two age groups, youth of the current year (Y) and adults (A), and examined four times per year (once per season). Morbidity, mortality, and clinical signs were registered for each flock, respective of age group. Coproparasitological examinations were carried out using the Baermann method. Morphological identification of larvae and eggs was done using the keys provided by Divina et al. (2000), Anderson (2000), Panuska (2006), and Mircean et al. (2011). For convenience, the larvae were killed using a formaldehyde solution, as described by Şuteu and Cozma (2012). The carcasses were examined in the early autumn of 2019, within a county slaughterhouse, recording all macroscopic lesions. All carcasses subjected to examination were confirmed during the study as harboring

a lungworm infection, and also exhibiting clinical signs.

The following parameters were calculated: overall prevalence of lungworm infections (P%) per season for each age group, total prevalence per season, 95% confidence interval, and *p* values (Table 1). Statistical analysis was done using Python. The following tests were used: chi-square test, paired t-test, and Spearman's Rho.

### Therapy

In the fall of 2019, 440 sheep belonging to the same two flocks, confirmed to harbor a lungworm infection, were subjected to treatment. Three different molecules were used, namely febantel (1 dose, 6mg/kg), ivermectin 1% (1 dose, 0.5ml/25kg), and tetramisol hydrochloride 20% (powder, 1 dose 0.0375-0.075g/kg). The sheep were divided into three different lots as follows: F (febantel) consisting of 140 adult sheep; I (ivermectin) with 150 adult sheep; and T (tetramisol) consisting of 100 adults and 50 young sheep. The distribution of the lots was dependent on the animal production cycle and withdrawal time considerations for each medication. A parasitological exam preceded therapy, with an additional evaluation done on day 30 post-therapy. Moreover, 10 sheep from lot I, and 9 from lot F were

subjected to a hematological and biochemical examination before treatment, as well as 15 days post-therapy. Seven parameters were evaluated (Table 2).

## Results

### Epidemiology

Prevalence of infections varied with season and age group. Statistically significant differences were observed between seasons and the prevalence in both young animals ( $p < 0.001$ ,  $df = 3$ , chi-square value = 22.45) as well as the entire sample size ( $p < 0.001$ ,  $df = 3$ , chi-square value = 18.62). Furthermore, the results of the Paired t-test revealed significant differences in prevalence between the young and adult groups across all four seasons (Table 1). Using the Spearman's Rho a strong correlation was established between seasonality across both age groups ( $p < 0.05$  and Spearman's Rho coefficient = -0.8 for young animals, and  $p < 0.01$  and Spearman's Rho coefficient = -0.94 for adults), indicating a clear and significant trend of decreasing prevalence of lungworms as the seasons progress. *M. capillaris* was the predominant species in both age groups throughout the year (prevalence 22-50% in A, 3-34% in Y), while *P. rufescens* prevalence consistently varied between 8-15% in A and 2-5% in Y.

**Table 1.** Prevalence of lungworm infections across different statistical categories.

Age group		Y			A			T			Y vs A
Variable		P%	N	95% CI	P%	N	95% CI	P%	N	95% CI	Paired t-test (p value)
Season	Autumn	6%	66	2-11%	50%	64	37-63%	26.5%	130	21-32%	<0.01*
	Winter	34%	62	23-45%	37%	70	26-49%	35.6%	132	29-42%	0.03*
	Spring	6%	52	1-12%	30%	118	23-37%	22.6%	170	18-27%	<0.01*
	Summer	3%	48	0-0,08%	22%	55	12-32%	13.1%	103	9-18%	<0.01*
p value		<0,001*			0,238			<0,001*			

P%- prevalence; N - total number of sheep; Y vs A: comparison between the young and adult groups; \* - statistically significant.

**Table 2.** Morphopathological findings in slaughtered sheep

Organ	Lesions
Mesenteric lymph nodes	Mild hypertrophy
Trachea	Mucus, serous or in rare cases with blood Presence of <i>Dictyocaulus</i> spp.
Bronchi	Mucus, serous or in rare cases with blood
Lungs	Prominent gray-yellow areas with a marbled/ mosaic pattern Increased consistency, sometimes pasty Dense, yellow-dark gray nodules (2-4mm) in the subpleural space Nodules containing larvae Secondary bacterial bronchopneumonia, pulmonary emphysema, and atelectasis

During clinical examination, 34 animals presented visible clinical signs, of which 18 were young animals and 16 were adults. All young animals presented a coinfection with *M. capillaris* and *P. rufescens*, signs including a moderate dry cough that would worsen when transitioning between environments (stall to pasture). Two animals, in particular, also presented increased sensitivity of the thoracic area, and snoring crackles, which evolved into a productive cough with serous nasal discharge. In 4/16 adults a painful cough was reported, and thoracic percussion revealed an area of decreased density, consistent with pulmonary emphysema. In the following 4-6 days the animals developed nasal discharge with soft crackles, while coproparasitological

examination revealed 3 single infections with *M. capillaris* and one coinfection with *M. capillaris* and *P. rufescens*. The remaining 12 adults all presented dyspnea, productive coughs, seromucous nasal discharge, along with densification of thoracic areas. Of these animals, three were infected by *M. capillaris*, while the other presented a coinfection.

### Pathology

Necropsy of the 28 carcasses revealed the presence of *Dictyocaulus filaria*, *Muellerius capillaris*, and *Protostrongylus rufescens* adults within the lungs, along with hydatid cysts. Furthermore, in 72% of animals with these morbidities lesions

consistent with liver echinococcosis, fasciolosis, and intestinal trichostrongylids were also observed. During pre-slaughter clinical examination, infected animals were moderately weak (body score of 1.5/5), with visible mucus around the nostrils,

and clumps of wool missing. Lesions were mild to moderate (Table 2), while three cases were severe. For the latter, findings included: cachectic and anemic carcasses, lobar verminous pneumonia, and catarrhal bronchitis, along with *Dermacentor* spp. infestations.

**Table 3.** Blood parameters evaluated on day 0 and day 15 of therapy with Febantel and Ivermectin

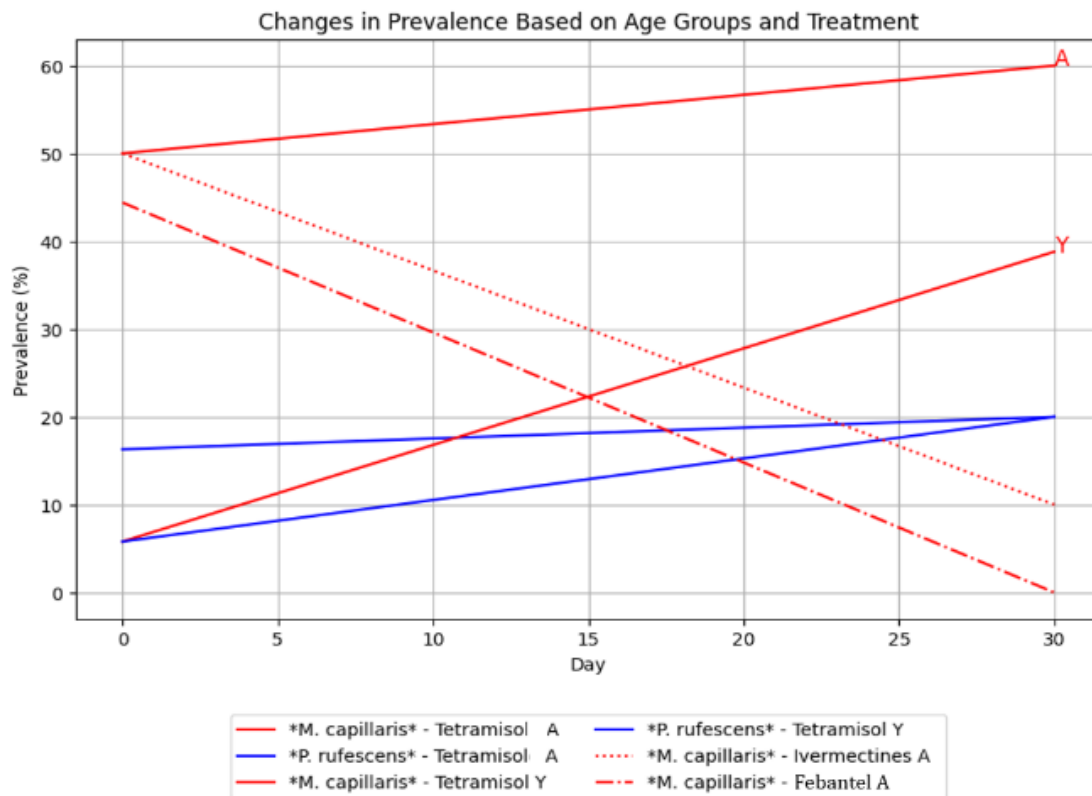
Lot	Parameter	Day 0	Day 15
F	RBC	4.1*10 <sup>12</sup>	5.3*10 <sup>12</sup>
	Hb	7.4g/dl	9.54g/dl
	Hct	27.11%	31.08%
	TP	7.34g/100ml	6.39g/100ml
	Alb	2.81g/100ml	3.47g/100ml
	GB	2.60g/100ml	1.35g/100ml
	Chol	153.3mg%	299.6mg%
I	RBC	4.0 *10 <sup>12</sup> /1	5.1*10 <sup>12</sup> /1
	Hb	7.67g/dl	7.84 g/dl
	Hct	26.42%	25.24%
	TP	7.17g/100ml	6.20g/100ml
	Alb	2.65g/100ml	3.09g/100ml
	GB	1.67g/100ml	1.45g/100ml
	Chol	147.3mg/100ml	296.6mg/100ml

RBC- red blood cells; Hb- hemoglobin, Hct- hematocrit; TP- total proteins; Alb- albumins; GB- gamma globulins; Chol- cholesterol.

### Therapy

Therapeutic efficiency varied between the studied molecules. Tetramisol was the least effective in treating both *M. capillaris* and *P. rufescens* in either age

group, with prevalence increasing 30 days post-therapy. Both ivermectine (80%) and febantel (100%) were effective in the treatment of *M. capillaris* in adult sheep (Fig 1).



**Figure 1.** Prevalence (%) of infections in sheep from the experimental groups treated with tetramisol, febantel, and ivermectin.

Due to the inefficiency of tetramisol in treating these parasitic infections the hematological and biochemical analysis excluded this option. A noticeable increase of RBC in sheep 15 days post-treatment was observed for both febantel (29.3%) and ivermectin (27.5%). On the other hand, Hct varied significantly less for both molecules. Hemoglobin (29%), albumin (23.5%), and cholesterol (95.4%) also increased in sheep following treatment with Febantel. Total proteins and gammaglobuline levels narrowly decreased in both options. While Hct and RBC were mainly within physiological ranges, hemoglobin was 15-20% lower before treatment. Total proteins, albumines, and gammaglobulin levels were within the physiological range as well (Table 2).

## Discussions

Disease prevalence among sheep does not significantly differ between mountainous and hilly areas, but factors such as age and seasonality play a crucial role (Anderson 2000; Panuska 2006; Movsesyan et al., 2023). The distribution of individual species appears linked to altitude, temperature, and humidity, mainly due to intermediate host requirements. However, no single parameter definitively influences prevalence (Pavlovic et al., 2010; Geurden and Vercruysse, 2007). Age dynamics-focused studies imply that protostrongyles more frequently infect adult ruminants due to year-round grazing (Cabaret, 1984). Young animals are more selective of their feed, avoiding the consumption of gastropods (Georgiev and Georgiev, 2002). The overall prevalence of lungworms from

our study varied between 13.1-35.6%, with the highest values observed during the second half of the year. Similar findings were reported in Iceland (Eiríkisdóttir and Skirnisson 2021) and India (Dar et al., 2012). Infections in wild ruminants, particularly in chamois, suggest a different pattern, with peaks in mid to late summer, followed by a steep decline in the autumn (Štefančíková et al., 2010; Yankov, et al., 2022). A plausible explanation for this strong contrast may reside in the limited availability of wildlife sampling during the cold season, as well as their persistent contact with intermediate hosts (Movsesyan et al., 2023). Furthermore, prophylactic deworming before grazing season heavily influences prevalence during spring and early summer. The change in micro and macronutrient balance, due to improved feed, further increases disease resistance during summer months (Nedeva et al., 2022), while during the colder seasons, the immune response diminishes (Dulceanu, 1981). Species dynamics appear to favor the dominance of *M. capillaris* in most small ruminants, attributed to its greater viability, pathogenicity, and ability to infect intermediate hosts, especially in temperate regions (Nicolas et al., 1985). This trend is of particular importance in the context of changing global climates, consequently leading to an increased number of fluke-associated disease reports in countries from western Europe (Taylor et al., 2012) to the gradual decrease of available grazing land throughout the continent (Hennessy et al., 2022). In 2018, the mean yearly temperature in Romania increased by 1.21 °C compared to the 1981-2010 mean, with 9/12 months exceeding the normal historical ranges (ANM, 2018). Therefore, sheep-intermediate host interactions were hastened due to favorable environmental

conditions, likely leading to increased morbidity and clinical signs.

Clinical examination revealed that most infections are subclinical. Adults appear to develop more severe infections, often complicated by secondary bacterial infections. While respiratory symptoms were found in all clinical infections, the immune status of each individual was unknown, limiting their relevance (Vartic, 1979). Dual infections with *M. capillaris* and *P. rufescens* were prevalent, especially in adults, with single infections being rare. Fecal examination for parasitic helminth stages is the most common method for diagnosing patent endoparasitism in domestic animals. Although limited data exist on the correlation between lungworm larval excretion and parasite load, fecal larval counts are often used as indirect markers of infection intensity, particularly in studies of anthelmintic efficacy. One study successfully managed to reveal a significant correlation between fecal larval counts and infection intensity (Rehbein and Hamel, 2022). One potential challenge arises from the phases of the disease, in which the respiratory syndrome usually precedes the fecal larval excretion, therefore complicating early diagnostic methods, particularly in young animals (Cabaret, 1984). Migrating larvae and immature forms of the parasite cause the succession of symptoms, initially digestive and later pulmonary, the severity of digestive disturbances being linked to the number of intermediate hosts ingested over a short period (Benakhla, 1981). One of the key features found across all studies, including ours, is a decrease in productivity along with a poor body score (Cabaret, 1984).



When clinical and paraclinical diagnosis fails to identify the cause, necropsies become the golden standard in providing clarity. Typically, carcasses of animals that succumb to the disease or are slaughtered for diagnostic purposes are emaciated or even cachectic. In early to mid-spring, alopecia is a common finding, the remaining wool losing its natural luster and elasticity (Olteanu, 1977). In *M. capillaris* infections, gray-white, dense subpleural nodules typically appear in the caudal lung lobes. Histologically, these nodules are granulomas composed of macrophages, lymphocytes, and plasma cells surrounding larvae, adults, and embryonated eggs. Differentiating these lesions from those caused by *C. ocreatus*, which shows larger, brown granulomas and severe bronchiolar epithelial hyperplasia, is essential (Uzal et al., 2016). In 80% of the carcasses examined during this study, findings included characteristic lesions caused by *M. capillaris*.

In *P. rufescens* infections, pathological changes are mainly limited to the caudal parts of the lungs and are characterized by oval or polygonal foci or plaques, ranging from 1 to 4 cm in diameter. These lesions are gray, slightly raised on the surface, and well-demarcated from the surrounding tissue (Panayotova-Pencheva and Alexandrov, 2010; Toma et al., 2019). All these pathological features align with those observed in our study. Additionally, some animals were also infected by gastrointestinal parasites, following a similar pattern as that described by Benakhla (1981). A recent study from Algeria identified lung lesions due to parasitic diseases in 33% of their sample size, while 9% were attributed to lungworms (Gherroucha et al., 2022). Other studies in spotted deer, mouflons,

and wild Himalayan bluesheep, revealed similar findings to ours, with poor body condition, anemia, and gray-dark lung nodules extending in the tissue (Panavotov-Pencheva and Alexandrov, 2010; Gopal et al., 2020; Karikalan et al., 2020).

The increasing reports of adenomatosis in some countries, alongside the recent spread of muelleriosis, suggest potential correlations between these conditions, where parasites might play a facilitating (potentiating) role (Adameşteanu, 1971; Şuteu and Cozma, 2012). Ovine pulmonary adenocarcinoma (OPA) is a malignant lung tumor caused by the Jaagsiekte sheep retrovirus (exJSRV) and is reported globally, except in Australia and New Zealand. In Romania, only a few studies focused on the prevalence, morphological characteristics, and diagnostic methods of OPA (Toma et al., 2020; Hodor et al., 2024). Current concerns regarding OPA center on the oncogenic mechanisms driving tumor development. Notably, the study highlighted that a relatively high number of sheep with lung tumors also had verminous pneumonia (n=8), suggesting that the latter might be a contributing factor to lung cancer development in sheep, warranting further research. Additionally, there is a reported correlation between coinfections with OPA and the Maedi-Visna virus, suggesting that the latter may worsen chronic lung diseases in sheep (Hodor et al., 2024).

The efficacy of anthelmintic treatments for pulmonary nematodes in small ruminants varies significantly by drug. Febantel showed the highest efficacy, achieving 100% effectiveness, followed by ivermectin at 80%, whereas tetramisol proved ineffective. This disparity likely arises from the differing

modes of action, with febantel and albendazole targeting alveolar and pulmonary capillaries more effectively, where the parasites reside (Howard, 1993). Ivermectin's effectiveness, although strong, does not surpass that of febantel (Takats, 1986), yet the latter fails to control adult nematode populations consistently (Deplazes et al., 2019). Studies on controlled-release ivermectin capsules showed promising results, with near-total efficacy against most lungworm species, though *M. capillaris* presented challenges, with reduced efficacy over time (Rehbein and Visser, 2002).

The evolution of anthelmintic treatments from early compounds like nicotine sulfate to modern macrocyclic lactones highlights the ongoing need for more effective, long-lasting drugs, especially as resistance rises (Soulsby, 1982). While the current study was focused on treating the disease using an affordable, albeit classical, approach, newer molecules (e.g. eprinomectin) should be encouraged. The 80% efficacy of ivermectin suggests potential resistance, underscoring the need for careful management and the exploration of new anthelmintic strategies (Campbell et al., 2024). Additionally, the decline in grazing land, particularly in Central and Southern Europe, limits non-pharmacological prevention methods,

highlighting the need for integrated parasite management approaches (Deplazes et al., 2019; Hennessy et al., 2022).

Lastly, physiological parameters in ruminants are influenced by various factors including age, sex, feeding, production systems, and season (Farooq et al., 2017). Seasonal variations impact biochemical profiles, which are crucial for maintaining proper body function, and their deficiency can lead to physiological abnormalities (Banerjee et al., 2015). Changes in blood biochemical parameters may predict sheep resistance to climatic conditions and stress factors that affect their welfare (Nedeva et al., 2022). In this study, hematological parameters, although moderately influenced by treatment, have stayed within physiological ranges, except for hemoglobin which improved after treatment. Biochemical parameters also remained normal, while cholesterol levels doubled, however, no clinical relevance could be attributed to it. This increase may be due to deworming medications improving intestinal absorption (Adameșteanu, 1971). In cases of concurrent trichostrongylidosis, temporary hepatic dysfunction caused by these medications may also contribute to the significant rise in cholesterol levels.

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