ISSN: 1220-3173; E-ISSN: 2457-7618

THE THERAPEUTIC EFFICACY OF EPRECIS (EPRINOMECTIN) AND IVOMEC PLUS (IVERMECTIN AND CLORSULON) AGAINST PULMONARY NEMATODES IN SHEEP FROM VALEA ȘIEULUI, TRANSYLVANIA

EFICACITATEA TERAPEUTICĂ A PRODUSELOR EPRECIS (EPRINOMECTINĂ) ȘI IVOMEC PLUS (IVERMECTINĂ ȘI CLORSULON) ÎN TRATAMENTUL NEMATODOZELOR PULMONARE LA OVINE DIN VALEA ȘIEULUI, TRANSILVANIA

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ABSTRACT | REZUMAT

Grazing sheep are commonly infected by various parasites, mainly due to their permanent contact with both intermediate hosts and the parasite's developmental stages. Prevalence is significant in small ruminants worldwide. The pulmonary strongylatosis complex in sheep is produced by different species of nematodes from the genera *Dictyocaulus*, *Protostrongylus*, *Mullerius*, *Cystocaulus,* and *Neostrongylus,* posing animal welfare and economic concerns. Diagnostic methods in grazing animals usually include faecal examination and, in severe cases, necropsies. Macrocyclic lactones and benzimidazoles are commonly used to treat lungworm infections. It is therefore the aim of our study to identify the species infecting grazing sheep as well as to evaluate the efficiency of eprinomectine and ivermectine in the therapy of parasitic pneumonia in northern Transylvania, Romania. The study was carried out on two flocks of sheep originating from two locations within Bistrița-Năsăud County. A total of 144 sheep were examined in each flock. The study included three lots/flock, each consisting of three age groups, with each group made up of 12 sheep. For each group of 12 treated sheep/age group, 12 control sheep were used. Statistical analysis was done using the SPSS software by employing the student's t-test and Welch's t-test. Four species were identified by way of faecal examination: *Dictyocaulus filaria*, *Protostrongylus rufescens*, *Muellerius capillaris*, *Cystocaulus ocreatus*,*P.rufescens,* and *M. capillaris* were most prevalent. While both molecules showed a good efficiency against these species, Ivomec Plus appeared more reliable, mainly 28 days post-therapy. No statistically significant differences could be observed between the two. Due to the advantages eprinomectin offers farmers, it could gradually replace ivermectins in the future. More in-depth studies would be required to also assess anthelmithic resistance in grazing sheep before categorically recommending one of the two products.

Keywords: *Dictyocaulus filaria, Muellerius capillaris, Protostrongylus rufescens*, *Cystocaulus ocreatus,* macrocyclic lactones

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Ovinele la pășunat sunt frecvent infestate de o varietate mare de paraziți. Motivul principal rezidă în contactul permanent al ovinelor atât cu gazdele intermediare cât și cu stadiile de dezvoltare ale paraziților. Prevalența acestora la nivel global este semnificativă. Complexul strongilatozelor pulmonare la ovine este produs de diferite specii de nematode din genurile: *Dictyocaulus*, *Protostrongylus*, *Mullerius*, *Cystocaulus* și *Neostrongylus*, ridicând probleme economice fermierilor și de bunăstare a animalelor afectate. Metodele de diagnostic la rumegătoarele mici includ, de obicei, examenul coproparazitologic precum și cel morfopatologic, în cazuri severe. Lactonele macrociclice și benzimidazolii sunt grupele cele mai utilizate în tratamentul strongilatozelor pulmoare. Prin urmare, scopul studiului nostru este de a identifica speciile care infestează ovinele la pășunat, precum și de a evalua eficiența eprinomectinei și a ivermectinei în terapia pneumoniei parazitare, la animale din nordul Transilvaniei. Studiul a fost realizat pe două turme de oi provenind din două localități din județul Bistrița-Năsăud. Un număr total de 144 de oi din fiecare turmă au fost examinate.Turmele au fost divizate în 3 loturi, fiecare lot la rândul lui fiind divizat în 3 grupe de vârstă, fiecare din acestea fiind alcătuite din 12 ovine. Pentru fiecare grupa de 12 oi tratate s-au utilizat alte 12 oi ca și control. Analiza statistică a fost realizată folosind software-ul SPSS prin aplicarea testelor student's t-Test și Welch's t-Test. Au fost identificate patru specii de nematode pulmonare ca urmare a examenului coproparazitologic: *Dictyocaulus filaria*, *Protostrongylus rufescens*, *Muellerius capillaris*, *Cystocaulus ocreatus*. *P. rufescens* și *M. capillaris* au fost cele mai prevalente dintre acestea. Deși ambele molecule au demonstrat o bună eficiență împotriva acestor specii de nematode, Ivomec Plus s-a dovedit relative mai potent, în special la 28 zile post-terapie. Totodată nu au putut fi observate diferențe statistice semnificative între eficiența celor două molecule. Cu toate acestea, datorită avantajelor pe care eprinomectina le oferă fermierilor, aceasta ar putea înlocui treptat ivermectinele în viitor. Studii mai amănunțite sunt necesare pentru a evalua și rezistența la antihelmintice la aceste animale înainte de a recomanda categoric unul dintre cele două produse.

Cuvinte cheie: *Dictyocaulus filaria, Muellerius capillaris, Protostrongylus rufescens*, *Cystocaulus ocreatus*, lactone macrociclice

Grazing small ruminants are hosts to a variety of parasites, mainly due to persistent contact with their intermediate hosts as well as their eggs and larvae (2). From a clinical and economic standpoint, the most impactful parasitic diseases in sheep are coccidiosis (*Eimeria* spp), fasciolosis (*Fasciola hepatica*), haemonchosis (*Haemonchus contortus*), echinococcosis (*Echinococcus granulosus*), and parasitic pneumonia (30, 39). The pulmonary strongylatosis complex, the basis of parasitic pneumonia, typically includes the following genera of nematodes: *Dictyocaulus, Protostrongylus, Mullerius, Cystocaulus*, and *Neostrongylus* (24). Conversely, roe deer and red deer appear to harbour the same genera of gastrointestinal parasites, while the most common lungworm species are limited to *Muellerius capilaria*, *Dictyocaulus cervi*, *D. skrjabini,* and *Varestrongylus capreoli* (31, 37). On the other hand, infections with *D. filaria* and *D. viviparus* are less common in cervids (24). Prevalence of lungworms in sheep appears to be influenced by lush vegetation (29), which in turn, in the case of Protostrongylidae, creates a favourable habitat for molluscs (as intermediate hosts) (28). Moreover, as in the case of *D. filaria*, young animals are more susceptible to infection, this event often occurring in the second half of their first grazing season (26), whereas older animals develop lasting immunity to reinfection (42).

Clinical signs can vary, based on the species involved, and are often non-specific (43). The main findings include coughing, stunted growth, weakness, diarrhoea in young animals, and decreased production in adults. (2,17,26,43). Coinfections with various bacteria or viruses are frequent; however, fatalities are uncommon (13, 36). Diagnosis mainly relies on the presence of clinical signs during high-risk periods of the year, the presence of larvae in faeces, and post-mortem examination of animals from the same herd (3). Treatment costs and production losses can have significant economic and social impacts on the animal industry (8). Therapeutic options for parasitic pneumonia in sheep commonly include oral administration of ivermectin, oral or subcutaneous administration of moxidectin and spot-on eprinomectin (13, 33). While Ivermectin and Moxidectin effectively reduced lungworm infections and faecal excretion of larvae (27, 34), withdrawal times are a concern.

The present study aimed to evaluate the efficacy of two modern molecules in the treating the main pulmonary helminthiasis in sheep from central Transylvania.

MATERIALS AND METHODS

Between September 2022 and March 2023, 288 sheep originating from two distinct flocks (144 sheep/flock), located in Bistrița-Năsăud County, Romania, were examined for the presence of pulmonary nematodes (Fig. 1).

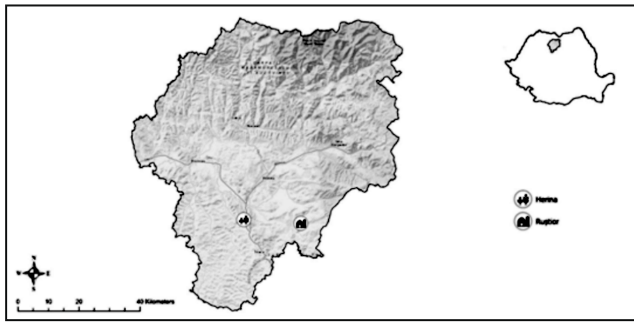

Fig. 1. Geographical locations for the two flocks in the current study (Bistrița-Năsăud County, Romania)

Two experimental lots were allo-cated for each flock of sheep, each consisting of 3 groups. Groups were established based on age and consisted of 12 sheep/group: adult sheep (AS), youth from the previous year (S-PY), and youth from the current year (S-CY). Furthermore, a control lot (Lot 3), for each of the aforementioned experimental ones, was established. Control groups were annotated with (C) while treated

Table 1

Structure of sheep lots, therapeutic product, dose, and markings for each flock

Lot no	Age group	Therapeutic product	Dose	Animal number	Markings
1	AS	EPRECIS* (20 mg/ml)	0.7 ml/animal	12	Red spray on head, single red dot
	S-PY		0.7 ml/animal	12	Red spray on withers, single red dot
	S-CY		0.4 ml/animal	12	Red spray on saddle, single red dot
2	AS	IVOMEC Plus** (10 mg/ml)	1.5 ml/animal	12	Red spray on head, two red dots
	S-PY		1.5 ml/animal	12	Red spray on withers, two red dots
	S-CY		0.8 ml/animal	12	Red spray on saddle, two red dots
3***	AS	None	None	24	Blue spray on head
	S-PY			24	Blue spray on withers
	S-CY			24	Blue spray on saddle

*The product was administered subcutaneously in the axillary region of the left forelimb.
**The product was administered subcutaneously in the thigh region, on the medial face of the right limb.
***Control lot, consisting of 1 group for each therapeutic product/age group.

groups were annotated with (T). Two macrocyclic lactones were chosen for the experiment, namely Eprinomectin (4'-(epiacetylamino)-4'-deoxy-avermectin B1) as Eprecis 20 mg/ml, and a combination of ivermectin and clorsulon, commercially known as Ivomec Plus. Each group was marked accordingly. The structure of each lot, along with their markings and therapeutic agent administered, are detailed in Table 1.

Faecal samples were collected on day 0, 12, and 28 of therapy. Lightly powdered latex gloves and polyethylene bags appropriately labelled for each individual were used to collect these samples from both control and treatment-subjected groups. The method of collection was via rectal swab from each individual. Parasite identification and quantification of parasitic load were conducted using flotation (Willis and McMaster method), modified Ziehl-Neelsen stained faecal smears, centrifugal sedimentation, modified Blagg technique, Baermann method, and faecal cultures. Morphological identification was done using the keys by Levine (21), Divina et al. (11), and Mircean et al. (23). All parasitic forms were examined via the Olym-

Table 2

Prevalence of infections in sheep from the experimental groups treated with EPRECIS and IVOMEC PLUS

Medication	Flock	Age group	Parasites	Day 0 (%)		Day 12 (%)		Day 28 (%)		*Total prevalence D0
				C	T	C	T	C	T	
Eprecis	Herina	AS	*P. rufescens*	75	83.33	83.33	0	75	0	79.17
			D. filaria	66.66	66.66	75	8.33	83.33	0	66.66
		S-CY	*M. capilaris*	66.66	75	75	25	66.66	8.33	70.83
			P. rufescens	0	58.33	66.66	0	66.66	0	29.17
			C. ocreatus	0	0	8.33	0	8.33	0	0.00
		S-PY	*D. filaria*	100	100	100	100	100	41.66	100.00
			M. capilaris	75	83.33	91.66	0	83.33	0	79.17
			P. rufescens	25	16.66	0	0	0	0	20.83
	Ruștior	AS	*D. filaria*	91.66	58.33	83.33	50	66.66	16.66	75.00
			M. capilaris	58.33	50	58.33	0	66.66	0	54.17
			P. rufescens	50	58.33	50	0	50	0	54.17
			C. ocreatus	16.66	16.66	8.33	0	8.33	0	16.66
		S-CY	*M. capilaris*	83.33	75	83.33	0	75	0	79.17
			P. rufescens	75	83.33	83.33	0	75	0	79.17
			D. filaria	66.66	66.66	75	8.33	83.33	0	66.66
			C. ocreatus	25	25	16.66	0	16.66	0	25.00
		S-PY	*D. filaria*	58.33	83.33	91.66	83.33	75	25	70.83
			P. rufescens	66.66	66.66	58.33	0	66.66	0	66.66
			M. capilaris	58.33	66.66	58.33	66.66	58.33	0	62.50
Ivomec Plus	Herina	AS	*P. rufescens*	75	83.33	83.33	0	75	0	79.17
			D. filaria	66.66	66.66	75	8.33	83.33	0	66.66
		S-CY	*D. filaria*	83.33	58.33	83.33	0	41.66	0	70.83
			P. rufescens	0	41.66	33.33	0	41.66	0	20.83
			M. capilaris	0	16.66	25	0	0	0	8.33
			C. ocreatus	0	0	0	0	8.33	0	0.00
		S-PY	*M. capilaris*	66.66	75	75	25	66.66	8.33	70.83
			P. rufescens	0	58.33	66.66	0	66.66	0	29.17
			C. ocreatus	0	0	8.33	0	8.33	0	0.00
	Ruștior	AS	*M. capilaris*	83.33	75	83.33	0	75	0	79.17
			P. rufescens	75	83.33	83.33	0	75	0	79.17
			D. filaria	66.66	66.66	75	8.33	83.33	0	66.66
			C. ocreatus	25	25	16.66	0	16.66	0	25.00
		S-CY	*P. rufescens*	75	83.33	83.33	0	75	0	79.17
			D. filaria	66.6	66.66	75	8.33	83.33	0	66.63
		S-PY	*M. capilaris*	83.33	75	83.33	0	75	0	79.17
			P. rufescens	75	83.33	83.33	0	75	0	79.17
			D. filaria	58.33	58.33	66.66	8.33	83.33	0	58.33
			C. ocreatus	25	25	16.66	0	16.66	0	25.00

*Total prevalence on day 0 was set as the average of Control (C) and Treated (T) groups on day 0.

pus BX61 microscope (Olympus Corp., Tokyo, Japan) with Cell F version 3.1 software. Practices used for sample collection, animal handling, and drug administration were performed with the verbal consent of the owners and upholding animal welfare standards.

Collection and management of data was done in Microsoft Excel, while analysis used the Statistical Package for Social Sciences (SPSS) software version 17. The student's t-Test and Welch's t-Test were employed to determine if there were any statistically relevant differences between the efficacy of Ivomec Plus and Eprecis on days 12 and 28, as well as for each age group and lungworm species, with respect to lungworms. Mapping was done using the ArcGIS Pro software (ESRI, Canada), version 3.3.0.

RESULTS AND DISCUSSIONS

Pulmonary nematodes were detected in both flocks across all age groups via coproparasitological examination. Morphological identification revealed that the most prevalent genera were *Protostrongylus* sp. and *Muellerius* sp. (66-100%), followed by *D. filaria* (58-66%), and *C. ocreatus* (0-25%). Higher infection rates were observed in the AS and S-PY groups, with similar values reported in both flocks (Table 2). Additionally, other parasitic forms detected included genera such as *Eimeria*, *Dicrocoelium*, *Moniezia*, *Nematodirus*, *Trichuris,* and *Strongyloides,* along with strongyle eggs.

There were no statistically significant differences in the overall efficacy of the two therapeutic options. Furthermore, no differences were observed in the medication efficiency when analysed by parasite species, age groups, or treatment day (Table 3).

Parasitic diseases in sheep significantly impact the economic output of farmers globally by affecting animal welfare and productivity. Parasitic lung diseases notably reduce livestock productivity and reproductive performance, especially when respiratory conditions overlap with immunodepression, stress, pregnancy, lactation, or when affecting very young, old, or debilitated animals (7). Bronchopneumonia is the most common respiratory condition in small ruminants and can be of parasitic, viral, bacterial, mycotic, or toxic origin. The most common pulmonary parasitic diseases are strongylidosis and echinococcosis (2).

From an epidemiological standpoint,pulmonary strongylatosis in small ruminants is less pathogenic than in cattle. It still causes significant losses, mainly in Mediterranean countries, as well as in Australia, across Europe, North America, and Africa (3, 18, 20). Lungworms are more prevalent in animals grazing on lush pastures, with contamination intensifying during rainy periods. Their infective forms thrive at moderate temperatures between 10 and 21°C, with cool and humid environments being particularly favourable (28-30). The prevalence of pulmonary lesions induced by parasitic disease in sheep varied between 5-79% (14, 15, 40, 43). Small ruminants are commonly infected by *M. capillaris*, whereas mixed infections with *M. capillaris* and *D. filaria* seldom appear. Interestingly, *D. filaria* is rarely present as a sole pathogen in sheep (10, 25). In Spain, the overall prevalence of

Table 3

Statistical results of ANOVA, student's t-Test and Welch's t-Test between Ivomec and Eprecis efficacy based on various variables

Comparison		Day 12			Day 28		
		ANOVA *p* value	Student's t-Test *p* value	Welch's t-Test *p* value	ANOVA *p* value	Student's t-Test *p* value	Welch's t-Test *p* value
Overall Efficiency		0.689	0.711	0.713	0.484	0.491	0.491
S-PY	**Overall**	0.578	0.59	0.588	0.65	0.657	0.658
	D. filaria	0.442	0.454	0.452	0.639	0.647	0.645
	M. capilaris	0.612	0.621	0.62	0.593	0.602	0.601
	P. rufescens	0.583	0.594	0.591	0.568	0.578	0.575
	C. ocreatus	0.726	0.737	0.736	0.794	0.803	0.802
S-CY	**Overall**	0.355	0.356	0.359	0.401	0.411	0.414
	D. filaria	0.369	0.379	0.377	0.386	0.395	0.396
	M. capilaris	0.485	0.494	0.493	0.457	0.463	0.464
	P. rufescens	0.494	0.505	0.503	0.499	0.507	0.508
	C. ocreatus	0.784	0.792	0.79	0.796	0.805	0.804
AS	**Overall**	0.516	0.524	0.526	0.462	0.471	0.473
	D. filaria	0.489	0.496	0.494	0.452	0.46	0.463
	M. capilaris	0.521	0.528	0.526	0.515	0.522	0.52
	P. rufescens	0.623	0.631	0.629	0.53	0.538	0.537
	C. ocreatus	0.809	0.818	0.816	0.803	0.811	0.809

protostrongyles in flocks is 11.6%, with *M. capillaris* representing 97.9% of the cases (12, 22). Conversely, sheep from Transylvania harbour a significant variety of lung parasites. An estimated 58.1% of sheep are infected, primarily by *Protostrongylus* spp. and *Muellerius* spp., along with mixed infections with *Strongylus* spp. *Echinococcus* spp. (14.9%) (40). Young animals in the latter half of their first grazing season (late autumn), lacking prophylactic measures, appear more susceptible to infections (33). While these results are also reflected in our study, the increased prevalence of *D. filaria* is concerning, contrasting with the findings of Toma et al. (40). The lower number of lung parasites detected in the S-CY group could be attributed to high temperatures (over 30^{0}C) recorded during the summer and autumn (38).

Clinical diagnosis is challenging due to non-specific symptoms and often requires coprological and morphopathological examinations (3, 9). In our study, owners reported no clinical signs in sheep from any of the three lots. Faecal examination was the reliable diagnostic method for identifying these subclinical infections. One limitation, however, resides in the lack of sensibility of the method in identifying early infections or the complete absence of adults in lung tissue (27, 34). Despite the commercial availability of several therapeutic options in current veterinary practice, the prevalence of lungworms remains high. Some of the potential causes for this could reside in anthelmintic resistance and improper and/or inconsistent administration of these substances. Macrocyclic lactones (ML), primarily eprinomectin, moxidectin, and ivermectin, have shown great promise in treating lungworm infections in large and small ruminants (13, 34). However, as in the case of *H. contortus*, there is growing concern about the emergence of anthelmintic resistance if therapy is misused or overused (5, 35). Recent reports indicate ML resistance in *D. viviparus* in cattle (6). Other therapeutic options include benzimidazoles (4), such as albendazole, with a historic efficiency of 86%. Nevertheless, as pointed out by Cabaret et al. (5), inadequate weighing of the animals can lead to systematic suboptimal dosing. Given that in our study ivermectin showed over 95% efficacy on day 28 against most lungworms, including *M. capillaris*, there is not enough evidence to support the emergence of resistance in these flocks. Despite some reports attesting to the high efficiency of eprinomectin in treating *M. capillaris* and *D. filaria* (100%) (13), albeit in goats, our study found strikingly similar results between eprinomectin and ivermectin. The latter was able to reduce the intensity of *D. filaria* to a broader extent than the eprinomectine (Table 2); however, no statistically significant differences could be identified. Lastly, the efficiency of eprinomectine against *C. ocreatus* is comparable to that of Kircali Sevimli et al. (19), with over 91%. One major advantage that Eprinomectine has over its peers is its 0-day milk and meat withdrawal time (1). Prevention and control of parasitic lung diseases in sheep can be most effectively achieved by integrating the proper use of anthelmintic drugs, immunisation, and adequate feed rations (16). Balanced rations enhance the host's resistance to lung parasites by improving overall immunity. Additionally, pasture control in endemic areas is crucial, along with providing clean drinking water and limiting interactions with intermediate hosts (41). Although these measures significantly impact the prevalence of lungworms and the success of therapeutic interventions, the associated costs can be burdensome for small farmers. Moreover, the scientific community's focus on zoonotic and epizootic infectious diseases, combined with the relatively mild clinical manifestations of parasitic pneumonia, means it often fails to capture the attention of veterinary professionals (8).

CONCLUSIONS

Parasitic infections with pulmonary strongyles in sheep were detected in both flocks from Bistrița Năsăud County. Pulmonary strongylatosis affected all age categories: youth from the current year, youth from the previous year, and adult sheep. The main pulmonary strongyles identified were *Dyctiocaulus filaria, Muellerius capillaris, Protostrongylus rufescens,* and *Cystocaulus ocreatus.* While seemingly the efficacy of Ivomec Plus was superior to that of Eprecis in treating pulmonary strongylatosis in the two flocks, no statistically significant differences could be identified.

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