https://doi.org/10.52973/rcfcv-e35590 Revista Científica, FCV-LUZ / Vol. XXXV

Comparison of Free and Liposomal Levamisole Antiparasitic Activity in Sheep

Comparación de la actividad antiparasitaria del levamisol libre y liposomal en ovejas

Mehmet Özüiçli[1], Hasan Susar[2], Murat Çelebi[3], Çağla Çelebi[2], Hüseyin Şen[2], İzzet Karahan[2]*

[1]Department of Parasitology, Faculty of Veterinary Medicine, Balikesir University, Balıkesir, Türkiye.
[2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Balikesir University, Balıkesir, Türkiye.
[3]Department of Laboratory and Veterinary Health, Savastepe Vocational School, Balikesir University, Balıkesir, Türkiye
*Corresponding Author: mehmet.ozuicli@balikesir.edu.tr, phone 0554 334 18 70

ABSTRACT

Worldwide, parasitic organisms residing in the digestive systems of sheep cause substantial economic losses. Various antiparasitic chemicals are employed to combat parasites, mostly levamisole. Nevertheless, parasites have developed resistance to the treatments employed in recent years. Consequently, scientists are currently seeking more effective medicinal compositions. The utilization of liposomes is one of the most extensively studied techniques to enhance pharmaceutical efficacy. This study assessed the antiparasitic efficacy of both free and liposomal levamisole. In this study four groups, each containing 12 animals, were formed: Group 1; free levamisole group (FLOG) received a single oral dose of free levamisole at 7.5 mg/kg; Group 2; liposomal levamisole group (LLOG) received a single oral dose of liposomal levamisole at 7.5 mg/kg; Group 3; (Positive Control: PCG) received a single oral dose of physiological serum at 7.5 mg/kg, and Group 4; served as the negative control (NCG). Fecal specimens were collected from the rectum into sterile containers on days 0, 7, 14, 21, and 28. The McMaster method was employed in the study to quantify eggs per gram of feces (EPG) loading. The efficacy of treatment groups was assessed using the Fecal Egg Count Reduction (FECR) formula. According to the FECR formula, *Strongylids* had a treatment efficacy of 65.36% in the FLOG group and 70.67% in the LLOG group, *Trichuris* had 41.78% and 74.22%, and *Nematodirus* had 52.78% and 71.85%. The efficacy of treatment was higher in the liposomal levamisole group compared to the free levamisole group. This study established the antiparasitic efficacy of liposomal levamisole for the first time. Further research are required to evaluate the antiparasitic effects of liposomal levamisole through the administration of varied and recurrent doses.

Key words: Antiparasitic activity; levamisole; liposome; sheep

RESUMEN

A nivel mundial los organismos parasitarios que residen en el aparato digestivo del ganado ovino causan cuantiosas pérdidas económicas. Para combatirlos se emplean diversos productos químicos antiparasitarios, principalmente levamisol. Sin embargo, en los últimos años los parásitos han desarrollado resistencia a los tratamientos empleados. En consecuencia, los científicos buscan actualmente composiciones medicinales más eficaces. La utilización de liposomas es una de las técnicas más estudiadas para mejorar la eficacia farmacéutica. En este estudio se formaron cuatro grupos, cada uno con 12 animales: Grupo 1; grupo de levamisol libre (FLOG) recibió una dosis oral única de levamisol libre a 7,5 mg/kg; Grupo 2; grupo de levamisol liposomal (LLOG) recibió una dosis oral única de levamisol liposomal a 7,5 mg/kg; Grupo 3; (control positivo: PCG) recibió una dosis oral única de suero fisiológico a 7,5 mg/kg, y Grupo 4; sirvió como control negativo (NCG). Los días 0, 7, 14, 21 y 28 se recogieron muestras fecales del recto en recipientes estériles. En el estudio se empleó el método McMaster para cuantificar la carga de huevos por gramo de heces (EPG). La eficacia de los grupos de tratamiento se evaluó mediante la fórmula de reducción del recuento de huevos en heces (FECR). Según la fórmula FECR, *Strongylids* tuvo una eficacia de tratamiento del 65,36% en el grupo FLOG y del 70,67% en el grupo LLOG, *Trichuris* del 41,78% y del 74,22%, y *Nematodirus* del 52,78% y del 71,85%. La eficacia del tratamiento fue mayor en el grupo de levamisol liposomal en comparación con el grupo de levamisol libre. Este estudio estableció por primera vez la eficacia antiparasitaria del levamisol liposomal. Se requieren más investigaciones para evaluar los efectos antiparasitarios del levamisol liposomal mediante la administración de dosis variadas y recurrentes.

Palabras clave: Actividad antiparasitaria; levamisol; liposoma; oveja

Recibido: 16/12/2024 Aprobado:19/02/2025 Publicado: 15/04/2025

INTRODUCTION

Türkiye's economy and agricultural sector are heavily dependent on livestock farming [1]. Parasitic infections result in substantial losses in meat, milk, wool, and leather production, hence impacting economic input [2]. Türkiye's geographical position and diverse climate create an ideal setting for parasitic infections. Epidemics produced by endoparasites in domestic animals are prevalent in Türkiye, resulting in considerable damage to the local sheep (*Ovis aries*) industry and the national economy [3]. The prevalence of helminths in sheep has been documented to range from 0.2% to 100% globally, according to studies utilizing necropsy and fecal analysis [4]. The prevalence of trematodes, cestodes, gastrointestinal nematodes, and lung nematodes in Türkiye was reported as 3.1-72.6% [5], 7.56-21% [6], 0.39-100% [7], and 7.8-34% [8], respectively.

Levamisole (Tetramisole) is a compound belonging to the Imidazothiazole derivative class of anthelmintic agents, particularly effective against nematodes that invade the gastrointestinal tract and lungs [9]. It has been used in the control of *Haemonchus* spp., *Trichostrongylus axei*, *Ostertagia* spp., *Nematodirus* spp., *Cooperia* spp., *Bunostomum trigonocephalum*, *Strongyloides* spp., *Oesophagostomum* spp., *Chabertia* spp., and *Dictyocaulus* spp. Levamisole is a combination of D- and L- isomers of Tetramisole, exhibiting a nematodicides activity and resembling pyrantel in its mechanism of action [10]. To ascertain that the anthelmintic efficacy of the drugs is attributable to the L-isomer, the toxic effects associated with the D-isomer were mitigated, and the confidence interval was expanded by employing solely the L-isomer. Levamisole functions as an agonist at nicotinic-cholinergic neuromuscular junctions, targeting nematodes in people and animals at low doses, leading to muscle contraction and subsequent paralysis of the parasites. At elevated concentrations, it interferes with intermediate metabolism by inhibiting the enzyme fumarate reductase [11 , 12].

Resistance to antiparasitic drugs is becoming a significant public health issue. With prolonged use at advised dosages, resistance may gradually develop against antiparasitic medications capable of significantly reducing the parasite population within the host. Prolonged medication administration can result in the development of resistance in parasites over time. Novel pharmaceuticals must be produced to address this issue [13 , 14].

The potential ways by which liposomes may prevent parasite resistance might be briefly stated as follows: Diverse phospholipids possess the capacity to act as intracellular messengers in regulating innate and adaptive immune responses via various mechanisms, including the activation of antimicrobial and antiparasitic enzymatic pathways, the regulation of fusion-fission events between endosomes influencing phagosome maturation and/or the antigen presentation pathway, and the modulation of the inflammatory response [15 , 16].

This study aimed to examine the parasiticidal effects of free levamisole versus liposomal levamisole. As parasites acquire resistance to existing medications, novel pharmacological agents are required. This investigation has resulted in the development of a liposomal formulation of levamisole, which has potential for application in veterinary medicine, and its efficacy has been established.

MATERIALS AND METHODS

Study area

The study was conducted in Balıkesir province in Türkiye. Balıkesir is at latitude 39.6484° N and longitude 27.8826° E coordinates.

Preparation of free levamisole solution

The mean weight of the animals (Merino sheep from Türkiye, aged 1 to 2 years) included in the study was 60 kg. Considering that levamisole was to be administered at a dosage of 7.5 mg/kg, 450 mg of the substance was measured. The measured levamisole was dissolved in 10 mL of distilled water and delivered orally to the animals. Levamisole was used orally in this study. Levamisole has been used orally in previous studies. In sheep, Gokbulut et al. [17] 7.5 mg/kg and Fernandez *et al.* [18] 5-10 mg/kg doses were studied and no side effects were reported.

Preparation of liposomal levamisole formulation

Liposomes were synthesized using the specific technique [19]. Soya lecithin (L-α-Lecithin, Sigma – Aldrich, USA) and Cholesterol ((CL), Acros Organics, Belgium) were measured in a 3:1 ratio. 450 mg of levamisole ((LVM), Santa Cruz Biotechnology – sc – 205730, USA) was included into this mixture. Chloroform (Sigma-Aldrich, USA) and methanol (Sigma-Aldrich, USA) were combined in a 1:1 ratio to solubilize the compounds. The mixture was placed in a volatilisation flask and subjected to volatilisation in a rotary evaporator (Isolab Laborgeräte GmbH 605.01.001, Germany) at 37 °C for 60 min at a speed of 8.77 G to produce a lipid film. To eliminate the lipid coating, 10 mL of phosphate-buffered water (Oxoid – BR0014G UK) was introduced, and the flask was spun without applying vacuum. The entryway of the evaporation flask was securely sealed with parafilm, vortexed (Vortex MS 3 basic ika 3617000, Germany) for 2 min, and subsequently placed in an ultrasonic bath (MEDISSON, Türkiye) for 5 min to diminish the particle size. The liposomes were centrifuged (ALLEGRA–X64R, USA) at 3043.76 G for 1 h to remove free levamisole that was not incorporated into the liposomes. The liposomal fraction that precipitated at the bottom was extracted and preserved in a refrigerator (Arçelik 270530EB, Türkiye) at 4°C. The steps of liposome preparation are shown in FİG. 1.

Analysis of liposomal levamisole formulation

Particle size (PS), polydispersity index (PDI), zeta potential (ZP) and encapsulation efficiency (EE) analyses are based on a predefined methodology [20].

Animal management

The research procedure was performed with the permission of Balıkesir University Animal Experiments Local Ethics Committee (Date: 01.08.2024, Decision Number: 2024/8-5). Forty-eight Merino sheep, aged 1 to 2 years, were utilized on a sheep farm situated in Türkiye.

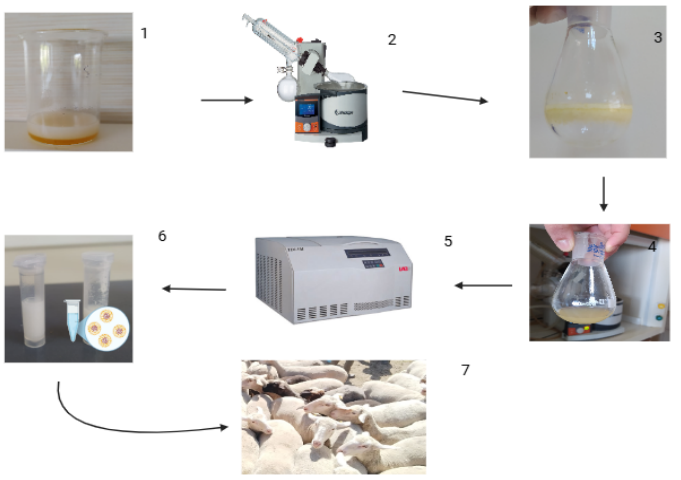

FIGURE 1. Steps of Levamisole Lyposomal Preparation. **1:** Dissolution of substances, **2:** Obtaining lipid film in rotary evaporator, **3:** Obtained lipid film, **4:** Removal of lipid film, **5:** Centrifugation to remove free drug, **6:** Liposomal levamisole, **7:** Oral administration animals

Experimental Design

Identification of positive and negative animals

Fecal samples, approximately five g each, were collected from the rectum of 100 Merino sheep aged 1-2 years, all of which had not undergone antiparasitic treatment prior to sampling, using sterile gloves or carrier bags. Fecal samples were examined using the Fulleborn saturated saline technique [21].

Determination of eggs loads of fecal samples

To evaluate the effectiveness of free and liposomal levamisole against gastrointestinal parasites in both treatment groups, fecal samples were collected weekly for up to four weeks after treatment. Egg counts, expressed as eggs per g of feces (EPG), were measured using the modified McMaster's technique, which has a sensitivity of 50 EPG [22]. With this method, egg loads were assessed for each animal on day 0 (baseline) and at 7, 14, 21, and 28 days post-treatment.

Treatment and control groups

The study involved 48 animals, categorized into four groups according to their egg counts, with 12 animals assigned to each group. The study groups were structured as outlined below: Group 1 (FLOG) was administered a single oral dose of 7.5 mg/kg free levamisole; Group 2 (LLOG) received a single oral dose of 7.5 mg/kg liposomal levamisole; Group 3 (Positive Control: PCG) was given a single oral dose of 7.5 mg/kg physiological serum; and the negative control group (NCG) was included. Following the collection of feces in the morning, samples were transported to the laboratory under cold chain conditions, where the McMaster method was applied. The treatment groups and positive control group were designed to be homogeneous regarding total EPG count. Fecal samples were taken from 100 sheep and 48 animals were included in the study considering the amount of EPG.

Treatments application day

All treatment protocols were administered to the treatment groups on day 0 (baseline) as a single oral dose.

Calculation of treatment efficacy

The efficacy of treatment groups was assessed using the subsequent formula: Fecal Egg Count Reduction (FECR)% = 100 – $(\frac{Final\ Number\ of\ Parasite\ eggs}{Initial\ number\ of\ Parasite\ eggs} \times 100)$ [22]. Day (d) 28 was designated as the conclusive count of parasite eggs, whereas d 0 was recognized as the initial quantity (baseline) of parasite eggs.

Statistical analysis

All data were evaluated by SPSS 25.0 (IBM Corp, Armonk, NY, USA). The Friedman test was applied for intra-group comparisons, while the Kruskal-Wallis test was utilized for inter-group comparisons. P-values below 0.05 signify that the difference is statistically significant [23].

RESULTS AND DISCUSSIONS

In this study, the mean particle size of liposomal levamisole 156.4±2.8 nanometer (nm), with a zeta potential of -47.6, encapsulation efficiency of 92.47%, and a polydispersity index of 0.128 were found. The quality control assessments of the prepared liposomes indicated their suitability for animal use. They exhibited a diminutive particle size, elevated electrokinetic potential, monodispersity, and colloidal stability.

Due to drug resistance, liposome-encapsulated pharmaceuticals studies on levamisole have gained momentum. Numerous studies have examined the antiparasitic effects of levamisole, leading to the development of various formulations for animal use [24, 25, 26, 27]. Nevertheless, no research has compared the antiparasitic efficacy of free and liposomal formulations of levamisole.The advancement of novel drug delivery systems is crucial for establishing therapeutic alternatives that enhance pharmacological responses and reduce side effects [28].

This study evaluated a liquid liposome formulation as a delivery system for levamisole hydrochloride to address parasitic infections. Liposomal levamisole represents a promising alternative for the treatment and prevention of parasites in sheep. The present study yielded superior results compared to previous studies. In this study, the particle size of liposomal levamisole was determined to be 156.4±2.8 nm, with a zeta potential of -47.6 mV, an encapsulation efficiency of 92.47%, and a polydispersity index of 0.128. Researchers found that the PDI of alizarin liposomes was 0.445-0.609, ZP was between -51.8 and -38.6 milivolt (mV), EE was 4-45%, and PS was 451-1031 nanometer (nm) [29]. Another researchers found the PDI value of astaxanthin liposomes to be 0.31±0.04, the ZP value to be -37.23 mV, the EE value to be 89.45%, and the PS value to be 101.21±16.67 nm [30]. Also, the ZP of ciprofloxacin liposomes to be 23.2±2 mV, the EE to be 76.17±1.8% and the PS to be 183±3 nm were found [31]. The substances and amounts used may have contributed to the different results.

In this study, *Strongylids*, *Trichuris*, and *Nematodirus* groups, the efficacies in the FLOG and LLOG treatment groups were determined as (65.36%-70.67%, 41.78%-74.22%, 52.78%-71.85%), respectively. In the study, liposomal levamisole was found to have more treatment efficacy than free levamisole. It was thought that the reason for this may be the increase in the efficacy of levamisole due to the encapsulation process with liposome.

A study was conducted to assess the efficacy of a triclabendazole-levamisole combination in treating endoparasitic infections in sheep. The number of helminth eggs in feces was evaluated before and after treatment, and the efficacy of triclabendazole-levamisole was found to be 96.9% for *Fasciola* spp., 87.1% for *Moniezia* spp., 83.3% for *Trichuris ovis*, and 99.9% for gastrointestinal nematodes [32]. Additionally, the effectiveness of levamisole in treating gastrointestinal nematodosis in sheep was examined. A total of 30 sheep with fecal egg counts exceeding 150 eggs per gram (EPG) were randomly selected. The study reported a significant reduction ($P<0.01$) in the mean fecal egg count (FEC) in the treated groups compared with the pre-treatment FEC. In contrast, the difference between the pre-treatment and post-treatment FEC in the control group was not statistically significant. In this study, levamisole was 99.52% effective against gastrointestinal nematodes in Garole sheep [33].

In a different study, the combined treatment of moxidectin and levamisole was investigated for its efficacy against multi-resistant gastrointestinal nematodes. The researchers observed efficacy rates of 84.3% (*Haemonchus contortus*), 100% (*Teladorsagia circumcincta* and *Trichostrongylus axei*), and 97.4% (*T. colubriformis*) in the first year of drug administration. Following four years of repeated administration of the combined treatment, efficacy remained high (100%) for all species, except for *T. colubriformis*, where efficacy decreased to 58%. In the initial application, efficacy was 99% for MOX, 85% for LEV, and 100% for MOX+LEV. The co-administration of MOX and LEV was found to result in a significantly higher anthelmintic effect (87%) than either MOX (42%) or LEV (69%) alone over the four-year trial period [34].

According to the results of this study, *Strongylids* (FLOG FECR=65.36%, LLOG FECR=70.67%), *Trichuris* (FLOG FECR=41.78%, LLOG FECR=74.22%), and *Nematodirus* (FLOG FECR=52.78%, LLOG=71.85%) were found in TABLES I, II, and III, respectively. In this study, the efficacy of levamisole was found to be lower than the efficacies found in the studies mentioned above. This difference may have been caused by the different sheep breeds studied, the season in which the study was carried out, humidity and rainfall rates depending on the season, the method of administration of the drugs. The development of parasite resistance to levamisole may also have contributed to this issue. A study assessed the efficacy of ivermectin, albendazole, and levamisole, revealing effectiveness rates of 58.5, 70.1, and 85.8%, respectively. These findings demonstrate that nematodes have acquired resistance to all treatments [35]. In another study, anthelmentic resistance was detected in sheep flocks [36]. To elucidate this situation, drug resistance studies must be conducted on a greater number of animals and herds across various regions within the same country, or even in different countries, by diverse researchers, with subsequent comparison of the results.

In this study, administration of free and liposomal levamisole orally at a dose of 7.5 mg/kg to animals did not result in any local or systemic adverse effects. In a study, the horse (*Equus caballus*) displayed symptoms of levamisole toxicity, including depression, recumbency, frequent urination, and the presence of signs indicative of labor. Additionally, the horse exhibited hyperemia of the conjunctivae, a palpebral reflex, constricted pupils, and lacrimation [37]. A flock of sheep also showed signs of acute levamisole-fenbendazole intoxication. The animals displayed symptoms such as mucosal congestion, depression, anorexia, convulsions, ataxia, and drooling. Of the animals, 12 died prior to treatment, and five died after treatment [38]. Friesian calves (*Bos taurus taurus*) exhibited a range of severe nicotinic-type symptoms, including hypersalivation, foaming at the mouth, muscle spasms, a tendency to lie down, and rapid respiration [39]. In this study, no clinical signs was observed in the treatment and control groups and no animal loss was experienced.

The EPG loads and treatment efficiencies of *Strongylids*, *Trichuris*, and *Nematodirus* infection at days 0, 7, 14, 21, and 28 were displayed in TABLE I, TABLE II, and TABLE III, respectively. Also, positive and negative control groups total (*Strongylids*, *Trichuris*, and *Nematodirus*) EPG loads were given in TABLE IV.

In both the FLOG and LLOG treatment groups of *Strongylids*, the total number of EPGs decreased from d 0 to d 28. In the study, the 0-28 d FECR value was 65.36% in the FLOG group and 70.67% in the LLOG group (TABLE I).

TABLE I. The EPG loads and treatment efficiencies of Strongylids infection at days 0, 7, 14, 21, and 28

Days	FLOG EPG Loads	LLOG EPG Loads
D 0	76.950	72.800
D 7	60.650	48.650
D 14	50.450	40.900
D 21	38.450	32.750
D 28	26.650	21.350
Days	Treatment Groups' Efficacies Determined According to the Days	
0–7	FLOG FECR % = 21.18	LLOG FECR % = 33.17
0–14	FLOG FECR % = 34.43	LLOG FECR % = 43.81
0–21	FLOG FECR % = 50.03	LLOG FECR % = 55.01
0–28	FLOG FECR % = 65.36	LLOG FECR % = 70.67

D: Day, EPG: Eggs per gram of feces, FLOG: Free levamizole group, LLOG: Liposomal levamizole group, FECR: Fecal egg count reduction

In both the FLOG and LLOG treatment groups of *Trichuris*, the total number of EPGs decreased from d 0 to d 28. In the study, the 0-28 d FECR value was 41.78% in the FLOG group and 74.22% in the LLOG group (TABLE II).

TABLE II. The EPG loads and treatment efficiencies of Trichuris infection at days 0, 7, 14, 21, and 28

Days	FLOG EPG Loads	LLOG EPG Loads
D 0	68.450	75.450
D 7	62.450	60.250
D 14	55.750	45.550
D 21	44.650	25.350
D 28	39.850	19.450
Days	Treatment Groups' Efficacies Determined According to the Days	
0–7	FLOG FECR % = 8.76	LLOG FECR % = 20.14
0–14	FLOG FECR % = 18.55	LLOG FECR % = 39.62
0–21	FLOG FECR % = 34.76	LLOG FECR % = 66.40
0–28	FLOG FECR % = 41.78	LLOG FECR % = 74.22

D: Day, EPG: Eggs per gram of feces, FLOG: Free levamizole group, LLOG: Liposomal levamizole group, FECR: Fecal egg count reduction

In both the FLOG and LLOG treatment groups, the total number of EPGs decreased from d 0 to d 28. In the study, the 0-28 d FECR value was 52.78% in the *Nematodirus* FLOG group and 71.85% in the LLOG group (TABLE III).

TABLE III. The EPG loads and treatment efficiencies of Nematodirus infection at days 0, 7, 14, 21, and 28

Days	FLOG EPG Loads	LLOG EPG Loads
D 0	65.550	72.850
D 7	60.250	58.350
D 14	53.450	42.750
D 21	40.100	28.350
D 28	30.950	20.500
Days	Treatment Groups' Efficacies Determined According to the Days	
0–7	FLOG FECR % = 8.08	LLOG FECR % = 19.90
0–14	FLOG FECR % = 18.45	LLOG FECR % = 41.31
0–21	FLOG FECR % = 38.82	LLOG FECR % = 61.08
0–28	FLOG FECR % = 52.78	LLOG FECR % = 71.85

D: Day, EPG: Eggs per gram of feces, FLOG: Free levamizole group, LLOG: Liposomal levamizole group, FECR: Fecal egg count reduction

In the negative control group, no parasitic agent was detected on d 0 and 7, but the parasite load increased from d 14 to d 28. In the positive control group, parasite load increased steadily from d 0 to d 28 (TABLE IV). In this study, the parasite load in the positive control group showed a continuous increase during the study period, while the negative group became parasitically positive from the d 14 and the parasitic load gradually increased until the end of the study (TABLE IV). The consumption of food and water contaminated with feces, licking of wool and feathers play an important role in the transmission of parasites between animals on the farm, increasing the risk of contamination [40]. The reason for this situation may be the contamination in the farm.

TABLE IV. Positive and negative control groups total (Strongylids, Trichuris, and Nematodirus) EPG loads

Days	Positive Group EPG Loads	Negative Group EPG Loads
D 0	45.550	0
D 7	56.750	0
D 14	60.150	12.600
D 21	65.600	14.900
D 28	78.900	21.800

D: Day, EPG: Eggs per gram of feces

Strongylids EPG analyses on d 7 indicated a statistically significant difference between groups (P<0.05). No statistically significant difference was observed between the groups on the remaining days (P>0.05). In the LLOG, there was a statistically significant difference between the first *Strongylids* values and those taken on d 7, 14, 21, and 28 (P<0.001). A decrease was observed from the initial mean to the final mean on the 28th d. A statistically significant difference was observed in the *Strongylids* values of the animals in the FLOG on the initial, 7th, 14th, 21st, and 28th d (P<0.001). For FLOG, the initial mean for *Strongylids* was 6525.00±2138.13, and the final mean (28th d) was 1945.83±826.12. A reduction was noted from the initial mean to the final mean (TABLE V).

TABLE V. Intragroup and intergroup comparisons of Strongylids EPG value

		Liposomal Levamizole		Free Levamizole			
		Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	U	p
Strongylids	D 0	7079.17±2164.11	7575 (1250-9500)	6525.00±2138.13	6925 (1000-9300)	1.126	0.260
	D 7	5637.50±1895.46	6100 (800-7600)	4470.83±1646.27	4900 (500-6800)	2.196	**0.028**
	D 14	4745.83±1778.46	4800 (600-6900)	3708.33±1502.85	3800 (300-5800)	1.848	0.065
	D 21	3662.50±1443.34	3750 (350-5500)	2979.17±1135.47	3075 (250-4300)	1.647	0.100
	D 28	1600.00±809.09	1500 (200-3500)	1945.83±826.12	2050 (150-3000)	1.396	0.163
		F=48.000	**P<0.001**	F=48.000	**P<0.001**		

D: Day, F: Firedman Test, U: Mann Whitney U Test, P<0.05, SD: Standart deviation, Min: Minumum, Max: Maximum

The *Trichuris* values on the 21st d indicated a statistically significant difference between the groups (P<0.05). The average *Trichuris* count on d 7 was markedly reduced in the LLOG (2404.17±1120.97) compared to the FLOG (3962.50±1502.14). On the remaining d, no statistically significant difference was observed between the groups (P>0.05). A statistically significant difference was observed in the *Trichuris* values of the animals in the LLOG on the initial d, as well as on the 7th, 14th, 21st, and 28th d (P<0.001). The mean values for the initial d (6870.83±2116.76) and the 28th d (1787.50±775.22) were calculated. A reduction was noted from day 0 to d 28. A statistically significant difference was observed in the *Trichuris* values of the animals in the FLOG on the initial, 7th, 14th, 21st, and 28th d (P<0.001). The mean value for the initial d was 6087.50±2118.33, and the mean value for d 28 was 2275.00±1124.22. A reduction was noted from day 0 to d 28 (TABLE VI).

TABLE VI. Intragroup and intergroup comparisons of Trichuris EPG value

		Liposomal Levamizole		Free Levamizole			
		Mean±SD	Media (Min-Max)	Mean±SD	Media (Min-Max)		
Trichuris	D 0	6870.83±2116.76	7225 (1200-9400)	6087.50±2118.33	6475 (800-8500)	1.271	0.204
	D 7	5479.17±1833.09	5825 (800-7500)	5537.50±1998.76	5800 (700-8000)	0.087	0.931
	D 14	4170.83±1533.63	4400 (700-5900)	4937.50±1854.62	5250 (600-7500)	1.184	0.236
	D 21	2404.17±1120.97	2250 (500-4600)	3962.50±1502.14	4150 (450-5800)	2.600	**0.009**
	D 28	1787.50±775.22	1900 (300-3000)	2275.00±1124.22	2000 (300-4500)	0.961	0.336
		F=47.267	**p<0.001**	F=48.000	**p<0.001**		

D: Day, F: Firedman Test, U: Mann Whitney U Test, P<0.05, SD: Standart deviation, Min: Minumum, Max: Maximum

The d 14 *Nematodirus* value exhibited a statistically significant difference between the groups (P<0.05). The mean *Nematodirus* count on d 14 for the animals in the LLOG (3608.33±1284.49) was significantly lower than that of the animals in the FLOG (4712.50±1750.60). Analysis of *Nematodirus* on d 21 revealed a statistically significant difference between the groups (P<0.05). The average *Nematodirus* count in the LLOG on d 21 (2695.83±989.60) was significantly lower than that in the FLOG (3550.00±1346.21). No statistically significant difference was observed between the groups on the other d (P>0.05). A statistically significant difference in *Nematodirus* values was noted in the animals of the LLOG on the initial, 7th, 14th, 21st, and 28th d (P<0.001). The means of *Nematodirus* on d 0 (6350.00±2132.22) and d 28 (2000.00±921.71) were calculated. A reduction was noted from day 0 to d 28 (TABLE VII). The reason why these differences are evident on the d 14 and d 21 that there is efficacy against adults and not against larvae in the mucosa, as indicated by Muñoz *et al.* [41].

TABLE VII. Intragroup and intergroup comparisons of Nematodirus EPG value

		Liposomal Levamizole		Free Levamizole			
		Mean±SD	Media (Min-Max)	Mean±SD	Media (Min-Max)		
Nematodirus	D 0	6350.00±2132.22	6500 (1000-9000)	5787.50±2144.56	6250 (600-8800)	0.866	0.386
	D 7	5320.83±1806.23	5650 (900-7000)	5312.50±1922.37	5775 (700-7100)	0.260	0.795
	D 14	3608.33±1284.49	3900 (800-4900)	4712.50±1750.60	5175 (400-6500)	2.398	**0.016**
	D 21	2695.83±989.60	2950 (500-4000)	3550.00±1346.21	3725 (300-5000)	2.026	**0.043**
	D 28	2000.00±921.71	1975 (400-3500)	2370.83±902.64	2500 (150-3500)	1.157	0.247
		F=48.000	**p<0.001**	F=46.467	**p<0.001**		

D: Day, F: Firedman Test, U: Mann Whitney U Test, P<0.05, SD: Standart deviation, Min: Minumum, Max: Maximum

CONCLUSION

In this study, the efficacies of FLOG and LLOG treatment groups against adults of *Strongylids*, *Trichuris*, and *Nematodirus* parasites were evaluated, and the results were obtained (65.36%-70.67%, 41.78%-74.22%, 52.78%-71.85%), respectively. The study demonstrated that liposomal levamisole exhibited greater therapeutic efficacy compared to free levamisole. The increase in the efficacy of levamisole is attributed to the encapsulation process with liposomes. This study aimed to examine the parasiticidal effects of free levamisole versus liposomal levamisole. According to the data we obtained, treatment efficacy was lower in FLOG treatment groups compared to LLOG treatment groups. This is an indication that the parasites developed resistance to free levamisole. This study showed that liposomal drugs were more effective on parasites than their free forms.

However, the fact that the study was carried out only on sheep is the limitation of the study; therefore, research should be carried out in different animal species in order to better reveal the effect of liposomal drugs on parasites. This study will shed light on future research in this respect.

Funding

This research was conducted with the support of the Balıkesir University Scientific Research Coordination Office (BAP Project No: 2022 / 084).

Conflict of Interest

The authors have stated that they do not have any competing interests.

BIBLIOGRAPHIC REFERENCES

[1] Aydin KB, Ye B, Brito LF, Ulutaş Z, Morota G. Review of sheep breeding and genetic research in Türkiye. Front. Genet. [Internet]. 2024; 15:1308113. doi: https://doi.org/pcsx

[2] Jacobson C, Larsen JW, Besier RB, Lloyd JB, Kahn LP. Diarrhoea associated with gastrointestinal parasites in grazing sheep. Vet. Parasitol. [Internet]. 2020; 282:109139. doi: https://doi.org/pcsz

[3] Erez MS, Doğan İ, Kozan E, Göksu A. A survey of knowledge, approaches, and practices surrounding parasitic infections and antiparasitic drug usage by veterinarians in Türkiye. Animals. [Internet]. 2023; 13(17):2693. doi: https://doi.org/pcs4

[4] Raue K, Heuer L, Böhm C, Wolken S, Epe C, Strube C. 10-year parasitological examination results (2003 to 2012) of faecal samples from horses, ruminants, pigs, dogs, cats, rabbits and hedgehogs. Parasitol. Res. [Internet]. 2017; 116:3315-3330. doi: https://doi.org/pcs6

[5] Değer MS, Biçek K, Karakuş A. Prevalence of Dicrocoelium dentriticum in sheep and goats slaughtered in Van region (Van municipality slaughterhouse). Van. Vet. J. [Internet]. 2017 [cited 10 Dec 2024]; 28(1):1-24. Available in: https://goo.su/tncfGHg

[6] Aydın A. Hakkâri Belediye Mezbahasında Kesilen Hayvanlarda Anolocephalidae Türlerinin Yayılışı. Van. Vet. J. [Internet]. 2013[cited 10 Dec 2024]; 24(1):5-8. Available in: https://goo.su/UQwsSJU

[7] Sevimli F. Checklist of small ruminant gastrointestinal nematodes and their geographical distribution in Turkey. Turk. J. Vet. Anim. Sci. [Internet]. 2013; 37(4):369-379. doi: https://doi.org/pcs7

[8] Yıldırım A, İça A. Kayseri yöresinde koyunlarda akciğer kıl kurdu enfeksiyonlarının prevalansı. Erciyes Üniv. Vet. Fak. Derg. [Internet]. 2005 [cited 10 Dec 2024]; 2(2):73-78. Available in: https://goo.su/Ag4E2z

[9] Hu Y, Xiao SH, Aroian RV. The new anthelmintic tribendimidine is an L-type (levamisole and pyrantel) nicotinic acetylcholine receptor agonist. PLoS Negl. Trop. Dis. [Internet]. 2009; 3(8):e499. Available in: https://goo.su/vqvpfZZ

[10] Antonopoulos A, Charvet CL, Maitland K, Doyle SR, Neveu C, Laing R. Functional validation of novel levamisole resistance marker S168T in Haemonchus contortus. Int. J. Parasitol. Drugs Drug. Resist. [Internet]. 2024; 24:100524. doi: https://doi.org/pcs8

[11] Gholami MH, Rassouli A, Mirzaei S, Hashemi F. The potential immunomodulatory effect of levamisole in humans and farm animals. JAVAR. [Internet]. 2023; 10(4):620–629. doi: https://doi.org/pcs9

[12] Oliveira CC, Costa DFL, Limeira CH, Nogueira DB, Nascimento BHR, Vaz AFM. Anthelmintic intoxication in goats and sheep: A systematic review. Res. J. Vet. [Internet]. 2022; 152:657–662. doi: https://doi.org/pctb

[13] Selzer PM, Epe C. Antiparasitics in Animal Health: Quo Vadis?. Trends Parasitol. [Internet]. 2021; 37(1):77–89. doi: https://doi.org/ghxn75

[14] Tınar R, Akyol ÇV, Çırak VY, Şenlik B, Bauer C. Investigations on the seasonal patterns of strongyle infections in grazing lambs and the occurarence of antelmintic resistance on sheep and goat farm in Western Anatolia, Turkey. Parasitol. Res. [Internet]. 2005; 96:18-23. doi: https://doi.org/bh5fx2

[15] Aditya NP, Vathsala PG, Vieira V, Murthy RSR, Souto EB. Advances in nanomedicines for malaria treatment. Adv. Colloid Interface Sci. [Internet]. 2013; 201-202:1-17. doi: https://doi.org/gbd74r

[16] Memvanga PB, Nkanga CI. Liposomes for malaria management: the evolution from 1980 to 2020. Malar. J. [Internet]. 2021; 20(1):327. doi: https://doi.org/pctc

[17] Gokbulut C, Yalinkilinc HS, Aksit D, Veneziano V. Comparative pharmacokinetics of levamisole-oxyclozanide combination in sheep and goats following per os administration. Can. Vet. J. [Internet]. 2014; 78(4):316-320. PMID: 25356001 Available in: https://n9.cl/uo77r

[18] Fernández M, García JJ, Sierra M, Diez MJ, Terán MT. Bioavailability of levamisole after intramuscular and oral administration in sheep. N. Z. Vet. J. [Internet].1998; 46(5):173-176. doi: https://doi.org/b9nz8k

[19] Susar H, Çelebi M, Çelebi Ç, Çoban Ö, Şen H, Karahan İ. Preparation and characterisation of liposomal formulations of Levamisole and Albendazole used in Veterinary Medicine. Rev. Cient. FCV LUZ. [Internet]. 2024; 34(2):1-8. doi: https://doi.org/pctd

[20] Çoban Ö, Yıldırım S, Bakır T. Alpha-lipoic acid and cyanocobalamin co-loaded nanoemulsions: development, characterization, and evaluation of stability. J. Pharm. Innov. [Internet]. 2022; 17(2):510-520. doi: https://doi.org/m7xz

[21] Połozowski A, Zawadzki W, Nowak M. Comparison of two fecal flotation techniques for diagnostic of internal parasites infections in swine and dogs. EJPAU. [Internet]. 2006 [cited 10 Dec 2024]; 9(4):39. Available in: https://goo.su/M5SdA

[22] Gökbulut C, Özüiçli M, Akşit D, Aksöz E, Korkut O, Yalcinkaya M, Cirak VY. Comparative plasma and milk dispositions, faecal excretion and efficacy of per os ivermectin and pour-on eprinomectin in horses. JVPT. [Internet]. 2016;39(6):584–591. doi: https://doi.org/f9c9p3

[23] Michael R. Sheldon MS, Michael J, Fillyaw W, Thompson D. The use and interpretation of the Friedman test in the analysis of ordinal-scale data in repeated measures designs. Physiother. Res. Int. [Internet]. 1996; 1(4):221-228. doi: https://doi.org/dchwsz

[24] Pahor-Filho E, Júnior JE, Pilarski F, Urbinati EC. Levamisole reduces parasitic infection in juvenile pacu (*Piaractus mesopotamicus*). Aquac. [Internet]. 2017; 470:123-128. doi: https://doi.org/f9tsx7

[25] Zoghroban HS, Elmansory BM, Issa YA, Eltokhy AK, Abo-Safia HS, Maghraby GM, Salama AM. Novel insights on the therapeutic effect of levamisole on the chronic toxoplasmosis in mice model. Exp. Parasitol. [Internet]. 2023; 248:108515. doi: https://doi.org/pctf

[26] Piña-Vázquez DM, Mayoral-Peña Z, Gómez-Sánchez M, Salazar-Olivo LA, Arellano-Carbajal F. Anthelmintic effect of Psidium guajava and Tagetes erecta on wild-type and Levamisole-resistant Caenorhabditis elegans strains. J. Ethnopharmacol. [Internet]. 2017; 202:92–96. doi: https://doi.org/f96cn3

[27] Feyera T, Ruhnke I, Sharpe B, Elliott T, Shifaw A, Walkden-Brown SW. Comparative therapeutic efficacies of oral and in-water administered levamisole, piperazine and fenbendazole against experimental Ascaridia galli infection in chickens. Vet. Parasitol. [Internet]. 2021; 298:109514. doi: https://doi.org/pctg

[28] Bruxel F, Laux M, Wild LB, Faraga M, Koester LS, Teixeira HF. Nanoemulsions as parenteral drug delivery systems. Quim. Nova. [Internet]. 2011; 35(9):1827-1840. doi: https://doi.org/gqtd9q

[29] Bharathi D, Lee JH, Lee J. Enhancement of antimicrobial and antibiofilm activities of liposomal fatty acids. Colloids Surf. B. Biointerfaces. [Internet]. 2024; 234:113698. doi: https://doi.org/pcth

[30] Zhong C, Liu T, Diao J, Li X, Liu M, Wang Y. Preparation and characterization of astaxanthin-loaded liposomes by phytosterol oleate instead of cholesterol. Food Chem. [Internet]. 2025; 462:41008. doi: https://doi.org/pctj

[31] Bhat UM, Khan NA, Raza SN, Ali M, Mehdi S, Mohiuddin I, Shakeel F, Bhat ZA, Bader GN, Chashoo IA, Din-Wani SU. Ciprofloxacin hydrochloride-loaded ocular silk fibroin liposomes: formulation, characterisation, *in vitro* cytotoxicity, and antimicrobial activity. Heliyon. [Internet]. 2024; 10(22):e38777. doi: https://doi.org/pctk

[32] Nazmi-Yüksek TE, Yüksek N, Altuğ N, Gül A. Koyunlarda endoparazit enfeksiyonlarında Triklabendazol - Levamizol kombinasyonunun tedavi etkinliği. Van Vet. J. [Internet]. 2007 [cited 10 Dec 2024]; 18(1):19-24. Available in: https://goo.su/Gex14

[33] Pandit S, Baidya S, Jas R. Effects of levamisole on haemato-biochemical profiles of naturally occurring gastrointestinal nematodosis in Garole sheep. IJRD. [Internet]. 2017 [cited 10 Dec 2024]; 2(1):1-6. Available in: https://goo.su/NP8TMB

[34] Luque S, Lloberas M, Cardozo P, Virkel G, Farias C, Viviani P, Lanusse C, Alvarez L, Lifschitz A. Combined moxidectin-levamisole treatment against multidrug-resistant gastrointestinal nematodes: A four-year efficacy monitoring in lambs. Vet. Parasitol. [Internet]. 2021; 290:109362. doi: https://doi.org/g7wnns

[35] Mushonga AN, Washaya S, Nyamushamba GB. Resistance of gastrointestinal nematodes to anthelmintics in sheep production in Zimbabwe. FAHN. [Internet]. 2024; 3(1): 22-27. doi: https://doi.org/pctm

[36] Salgado JA, Cruz LV, Olivera da Rocha L, Sotomaior CS, Borges TD, Santos CDP. Implication of the fecal egg count reduction test (FECRT) in sheep for better use of available drugs. Rev. Bras. Parasıtol. V. [Internet]. 2019; (4):700-707. doi: https://doi.org/pctn

[37] İssi M, Gül G, Başbuğ O. Bir atta akut levamizol zehirlenmesi. F.Ü. Sağlık Bilimleri Veteriner Dergisi. [Internet]. 2010 [cited 10 Dec 2024]; 24(1):47-50. Available in: https://goo.su/L7hnDq

[38] Rather MA, Dar MA, Dar TA, Hameed A, Ahmad M, Munawar F, Ahanger SA. Acute levamisole-fenbendazole (LEVOB) poisoning in a sheep flock. Anim. Sci. [Internet]. 2020[cited 10 Dec 2024]; 1(1):31-33. Available in: https://goo.su/cCC1l0

[39] Müller KR, Dwyer C. Suspected levamisole intoxication in calves. NZVJ. [Internet]. 2016; 64(4):257–260. doi: https://doi.org/pctp

[40] Vlassoff A, Leathwick DM, Heath ACG. The epidemiology of nematode infections of sheep. NZJ. [Internet]. 2001; 49(6):213-221. doi: https://doi.org/bnk5tm

[41] Muñoz JA, Angulo-Cubillán F, Ramírez R, Vale O, Chacín E, Simoes D, Atencio A. Eficacia antihelmíntica de doramectina 1%, ivermectina 1% y ricobendazol 15% frente a nematodos gastrointestinales en ovinos de pelo. Rev. Cient. FCV-LUZ. [Internet]. 2008; 18(1):12-6. Disponible en: https://goo.su/DSzZ