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A Field Study to Evaluate the Efficacy of Changing the Type of Anthelmintic on Nematodes in Sheep in the Western Area of Libya

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Abstract:
The present experimental work was carried out at the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture, University of Zawia, on Mars in 2023. Twenty-five fecal samples were collected from animals in West Zawia, Assabiriyah, Libya, and sent to the lab for examination. The anthelmintics used in this experiment were Albendazole 2.5%, Ivermectin 0.8%, and Levamisole 2.5%, and all were in drench formulation. The EPG values (Mean ± SD) pre-treatment of the control group was 675± 237.56 on day 0, and the highest EPG value was 1048± 144.77, on day 14, while the EPG values (Mean ± SD) in the post-treatment groups on day 14 were 23± 11.14, 35± 19.52, 185± 61.58, and 385± 161.48 respectively, which was higher than the pre-treatment group 1048± 144.77at day 14. The efficacy of three antihelmintic was significantly higher ($p<0.05$) than Ivomec or Albendazole alone. The EPG values (Mean ± SD) in sheep of a group (2) on days 3, 7, and 14, were 300± 45.91, 73± 33.27, and 23± 11.14 respectively. The highest EPG values (Mean ± SD) were in group 5 (G5) which was treated with Ivomec on all day of the experiment, and the lowest EPG values (Mean ± SD) was in group 2 which was treated with Albendazole on day 0, Levamisole at day 3, and Ivomec at day 7, respectively. The study showed that changing the type of drug at different stages of the treatment days gives significant results in reducing the number of nematode eggs.

Keywords: Efficacy, Antihelmentic, Albendazole, Levamisole, Ivomec, Gastrointestinal Nematodes, Antihelmentic Resistance, Sheep, Assabiriyah, Libya.

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Introduction

Sheep are among the first domesticated animals; livestock has an important role in subsistence and economic growth in Africa [1].
Libya possesses a large population of small ruminants, estimated to be about 6,500,000 heads, however, the immense potential these numbers represent has yet to be realized due to a multitude of factors [2].
The majority of these large populations of animals are distributed in the coastal part [3].
The pastures of the coastal region in the western part of Libya are an important part of the national economy for sheep production, sheep represent an important source of animal protein in third-world countries such as Libya, it's the main source of daily red meat that used for human consumption and in the ceremonial festivities throughout the country [2].

These sheep flocks are exposed to various gastrointestinal parasitic infections parasitism is one of the important issues all over the globe and reducing the productivity of animals [4].
Parasitic helminths are a primary factor in the reduced productivity of small ruminants in many parts of the world, particularly in developing countries where nutrition and sanitation are generally poor [5].
The importance of helminthic infection is enhanced several times in third globe nations like Libya where the economy of people is mainly dependent on their livestock [1].
Gastrointestinal parasitic infection is a serious threat to small ruminant production systems. Most of the economic losses caused by internal parasites are actually due to associated production losses in terms of decreased milk and wool production, cost of prevention, stunted growth, reduced weight gain, poor feed utilization and conversion, low fertility, condemnation of affected organs, cost of treatment and the death of infected animals [6].
Libya is a third-world country and due to the lack of effective helminthic control strategies for small ruminants in these countries, anthelmintics are exclusively used for the management of the adverse effects of nematodes in sheep. However, factors like inadequate dosage, the exclusive use of medications with the same mode of action, poor-quality medications, and inappropriate use have an impact on the effectiveness of anthelmintics. Anthelmintic abuse and smuggling take many different forms in a third-world nation, including illegitimate sales in public marketplaces and arbitrary management [7].
In developing countries, most farmer uses hit and trial method against parasitic infection the development of anthelmintic resistance in field animals is due to the use of drugs having lower efficacy, under-dosing, environmental toxicity, and provision of low protein diet to the animals [8].
Anthelmintic resistance is becoming a significant concern in the world and this phenomenon is not only a problem of livestock in developing countries recently it has been reported in many economically developed countries such as the United States [9], Australia [10], Canada [11], the United Kingdom [12], France, Greece, and Italy [13].
The use of the same anthelmintic medications in helminthic control promotes the development of anthelmintic resistance by placing selection pressure on the parasitic nematodes and accelerating the spread of resistant strains. This happens when an anthelmintic treatment is used at the therapeutic dose but fails to eradicate the exposed population of parasites [14]. As a result, more of the helminth eggs carry resistance genes in the environment, increasing the likelihood that resistant helminths may infect new hosts [15].
Anthelmintic medications are mostly used to manage intestinal parasites, and this will likely continue because there doesn't appear to be a better alternative for helminthic control in small ruminants. The three most significant anthelmintic medication classes utilized globally to eradicate GIT worm infections in sheep are (albendazole) benzimidazoles, (levamisole) nicotinic agonists, and (ivermectin) macrocyclic lactones [16].
Multiple resistances to most of the anthelmintic against gastrointestinal nematodes have also been detected in neighboring countries such as Tunisia [17]. However, the susceptibility of GI nematodes in livestock in the western region of Libya has not been investigated previously.
Diagnosis of anthelmintic resistance in a very early stage requires sensitive tools. For this reason, the present study aimed to find a specific strategy to overcome gastrointestinal nematodes resistance by changing the type of medication during the treatment and the prevention period, and it is very effective against GIT nematodes and it will not give the helminths a chance to form a resistance against the drug.

Material and methods

Study area

The study was conducted in the district, of West Zawia, Assabiriyah, State of Libya. The region is located 59 km west of Tripoli capital of Libya. The area of the study is located at 32.694163°N, 12.625615°E. The district comprises many farms. The sheep populations are estimated to be around 100 to 150 heads per farm. The animals' housings are built near the farmers' homes. The sheep in these farms are usually fed on free pasture for a minimum of four months during the year. The sheep graze on the same pasture and rotational grazing is not followed by the farmers which increases the probability of pasture contamination by Gastrointestinal nematode eggs.

Sources of samples:

One farm was included in the study after the owner visited the veterinary service centers. The samples were obtained from sheep raised on a farm in Assabiriyah city. A total of 25 cases of sheep are diagnosed as heavily infected with an internal parasite. The sheep were examined for the presence of helminths from Mars to April 2023, as the climatic conditions, such as temperature, rainfall, and humidity, are suitable for the survival of nematode larvae at that time.

Ethical approval:

The present experimental work was carried out at the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture Science (Al-Ajeelat), University of Zawia, on the 6th of Mars 2023.

Collection of fecal samples:

The flocks of sheep were visited four times in two weeks for the collection of fecal specimens. Five grams of fecal samples (25 samples) were collected directly from the rectum of sheep using sterile rubber gloves for each sample. The first sampling was just before drug administration while the second fecal collection was 3 days after therapy, the third fecal collection was 7 days after therapy, and the last fecal collection was 14 days after drug

administration. The samples are marked and placed in clean, dry, leak-proof, transparent plastic containers and transported to the laboratory for examination in the Research Laboratory belonging to the Faculty of Veterinary Medicine and Agriculture (Al-Ajeelat), University of Zawia. Samples that did not examine on the same day were refrigerated at 5˚C to be examined on the next day.

Analysis of fecal samples:
The collected fecal specimens were put in cooled containers during transportation to the laboratory, which usually took less than two hours. The samples were then kept refrigerated at 5°C until the egg count was performed. The fecal samples were analyzed by Direct Smear Method and Salt Flotation Technique for the presence of eggs of helminths. The specimens were examined with a Leica DM3000 microscope and imaging system. The (EPG) was conducted in a period that did not exceed five days after sample collection. Eggs per gram (EPG) were counted by using Mac-Master Technique [18]. The examination and identification of helminthic ova were carried out by
Chemotherapy trials:

Table 1. Anthelmintic used against sheep nematodes in the Libyans field.

Trade name	Generic name	Manufacturer	Dose (mg/kg BW)	Route
ALBENDAZOLE AVICO Forte, 125 mg	Albendazole	AVICO/Jordan	2mg/1kg	Per os
LEVAMISOLE AVICO 118 gm.	levamisole	AVICO/Jordan	1ml/15kg	Per os
AVIMEC, 0.8 mg	Ivermectin	AVICO/Jordan	2.5ml/10kg	Per os

Twenty-five animals positive for GIT helminths were chosen from the herd and were randomly divided into five groups G1, G2, G3, G4, and G5 each comprising 5 animals. The animals in each group were marked using suitable identification methods.
The first group (G1) served as the untreated control, while the members of group 2 (G2) were orally given albendazole (ALBENDAZOLE “AVICO” Forte, 5 mg/kg) on day 0 after 3 days samples were collected and animals were orally given levamisole (LEVAMISOLE AVICO 118 gm., 1 ml/15kg), and in day 7 samples were collected then, Ivomec (AVIMEC, 0.8 mg, 2.5ml/10kg) were orally administrated for sheep.
The members of group 3 (G3) were orally given albendazole (ALBENDAZOLE “AVICO” Forte, 5 mg/kg), only for all days of the experiment day 0, day 3, and day 7.
And animals in group 4 (G4) were treated with ivermectin levamisole (LEVAMISOLE AVICO 118 gm., 1 ml/15kg), only for all days same as in group 3.
The animals in group 5 (G5) were given Ivomec (AVIMEC, 0.8 mg, 2.5ml/10kg), only for all days same as in group 3 and group 4.
The drug was orally administered using a calibrated drenching gun and the dosage was calculated based on the individual weight of the sheep.
Egg per gram (EPG) of the sheep in all groups were counted at Day 0 (Pre-treatment) and Days 3, 7, and 14, (post-treatment) using the Mac-Master technique.
[**(**Pretreatment EPG - Post-treatment EPG / Pretreatment EPG] X 100 This formula calculated the efficacy of drugs [19].

Side effects:
No side effects were observed during the trial in the present study.
Statistical analysis:
The collected data were analyzed at different angles by calculating the percentage positivity of helminths. Whereas data on drug efficacy was analyzed by using one-way ANOVA by using SPSS software for Windows (version 27; IBM, USA).

Results and discussion
Infection Rate:
The results of the present study revealed an overall infection rate of GIT helminths in sheep of 75%.
These findings conform with the results of Ahmed, who reported a 60% infection rate of helminths in sheep in Wady El-Hayat, Libya [20].
The infection rate of different gastrointestinal nematodes in sheep is shown in Table 2.
The highest infection rate of nematode species was *Haemonchus* spp (64%), and the lowest spp. was *Strongyloides* spp. (4%).
Haemonchus spp., *Ostertagia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Cooperia* spp., *Chabertai* spp., *Bunostomum* spp., *Strongyloides* spp., and *Nematodirus* spp., were the main nematode species found in sheep.

Table 2. The infection rate of different gastrointestinal nematodes in sheep.

GIT nematodes species	Tested	+ve (%)
Haemonchus spp.	25	16(64)
Ostertagia spp.	25	13(52)
Trichostrongylus spp.	25	11(44)
Oesophagostomum spp.	25	7(28)
Cooperia spp.	25	9(36)
Chabertai spp.	25	6(24)
Bunostomum spp.	25	5(20)
Strongyloides spp	25	1(4)
Nematodirus spp.	25	8(32)

The EPG values (Mean ± SD) in sheep of various groups:

The EPG values (Mean ± SD) pre-treatment of the control group was 675± 237.56 at day 0, and the highest EPG value was 1048± 144.77, on day 14.

The EPG values (Mean ± SD) in the post-treatment groups at day 14 were 23± 11.14, 35± 19.52, 185± 61.58, and 385± 161.48 respectively, which was higher than the pre-treatment group 1048± 144.77at day 14.

The highest EPG values (Mean ± SD) were in group 5 (G5) which was treated with Ivomec on all day of the experiment and the lowest EPG values (Mean ± SD) was in group 2 which was treated with Albendazole on day 0, Levamisole at day 3, and Ivomec at day 7, respectively.

The EPG values (Mean ± SD) in sheep of various groups at days 0, 3, 7, and 14 are shown in Table 3.

Table 3. EPG values (Mean ± SD) in sheep of various groups at days 0, 3, 7, and 14.

Group	EPG at day			
	0	3	7	14
G1	675± 237.56	825± 115.18	898± 138.19	1048± 144.77
G2**	713± 239.47	300± 45.91	73± 33.27	23± 11.14
G3	697± 289.22	521± 163.87	310± 112.47	185± 61.58
G4*	700± 200.53	425± 87.49	85± 24.33	35± 19.52
G5	675± 279.41	538± 183.56	410± 182.21	385± 161.48

** Highly significant, * significant, ± SD $P<0.05$

The Efficacies of Albendazole, Levamisole, and Ivermectin.

The efficacy of various drugs against GIT helminths in sheep on different days shows in Table 4.

The efficacy of Albendazole, Levamisole, and Ivermectin were 45, 88 and 98% on days 3, 7, and 14 respectively in the group (2) which was recorded as the highest efficacy in this study, while the efficacy of Albendazole in the group (3) was 44, 82 and 92% at day 3, 7 and 14, respectively.

The efficacy of Levamisole was 42, 83 and 94% on days 3, 7, and 14 respectively in the group (4), while the efficacy of Ivomec was 41, 80 and 91% on days 3, 7, and 14 respectively in the group (5) which recorded as the lowest efficacy in this study.

Table 4. Efficacy of various drugs against GIT helminths in sheep on different days.

Drug	Group	Efficacy (%) on the day		
		3	7	14
Albendazole, levamisole, Ivomec	G2	45(**A**)	88(**L**)	98(**I**)
Albendazole	G3	44	82	92
Levamisole	G4	42	83	94
Ivomec	G5	41	80	91

A= Albendazole, **L**= Levamisole, **I**= Ivomec.

Results indicate altitude prevalence of gastrointestinal nematodes in sheep in Assabiriyah, Libya. This result might harmonize or diverge from previous studies in the country, and other countries worldwide. The infection rate of *Haemonchus* spp., *Ostertagia* spp., *Trichostrongylus* spp., and *Oesophagostomum* spp., were 64%, 52%, 44%, and 28% respectively which is congruent with the findings of helminths from sheep in Benghazi District and a government sheep breeding farm at Marzouki, Libya [21].

The other study reported *Ostertagia spp., Nematodirus spp, Trichostrongylus spp, Haemonchus spp*., and *Chabertia* species prevalent in sheep [7].
Our results also correlate with the findings from Eastern Nigeria which reported infection with nematodes in sheep GIT mainly by *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp., *Cooperia* spp., and *Bunostomum* spp., [22].
Dyary, reported the common genera of nematodes reported in the study were *Marshallagia* spp., *Nematodirus* spp., *Trichostrongylus* spp., and *Trichuris* spp., which do not agree with our findings [23].

Chemotherapy:
The EPG values and percent efficacy are shown in Tables 2 and 3, respectively.
Albendazole, Levamisole, and Ivomec, significantly reduced the EPG, followed by Levamisole then Albendazole and Levamisole compared with a positive control group.
The efficacy of Albendazole, Levamisole, and Ivomec, in group (2) was 75, 88 and 98% on days 3, 7, and 14, respectively, while the efficacy of Levamisole in group (4), was 42, 83 and 94% at day 3, 7 and 14, respectively.
The lowest efficacy of Ivomec in groups (5), 41, 80, and 91% at day 3, 7, and 14, respectively was observed.
The efficacy of three antihelmintic was significantly higher ($p<0.05$) than Ivomec or Albendazole alone.
The EPG values (Mean ± SD) in sheep of a group (2) on days 3, 7, and 14, were 300± 45.91, 73± 33.27, and 23± 11.14 respectively. The study showed that changing the type of drug from one drug to another at different stages of the treatment days gives significant results in reducing the number of nematode eggs.
Results also showed that the anthelmintic used in this study, albendazole, levamisole, and ivermectin, considerably decreased the EPG of feces in the treated groups on Day 14 of treatment in comparison with EPG on pretreatment time.
Waruiru, reported that albendazole and levamisole significantly reduced the worm burdens in animals infected with GIT helminthes, and these findings are also congruent with the findings of the present study [24].
Dyary, also reported that Levamisole- and Albendazole-treated groups were significantly higher than ivermectin-treated groups, resistance to ivermectin was the highest, and resistance to Levamisole was the lowest of the three therapies used in the study, and these findings are in close alignment with the results of the present study [23].
Njanja [25], reported 100% efficacy of Ivermectin through the reduction in the egg of nematodes in the sheep, Helle [26] obtained similar results except in one sheep, which do not agree with our findings.
The region, climate, sample size, laboratory equipment, management systems, the origin of the animals, the presence of repellent resistance, and the studies conducted locally and, in some countries, could be the causes of the discrepancy in recording different prevalence's less or higher with or close to this study [27].
Additionally, it should be noted that climatic change may have an impact on the parasites' frequency, density, and zone distribution, which directly impacts the stage of their spread in the environment and secondarily impacts the larvae, which live primarily in intermediate families of invertebrates. The distribution range of physiologically varied nematodes is altered by global warming, changing their life cycles [27].
Multiple resistances to most of the anthelmintic against gastrointestinal nematodes have also been detected in neighboring countries such as Tunisia [17]. However, the susceptibility of GI nematodes in livestock in the western region of Libya has not been investigated previously.

Conclusion
The study concluded that the GIT nematode parasite is prevalent in sheep in Assabiriyah, Libya, as in many countries, and it could be observed at various rates.
This study documented Anthelmintic resistance in sheep gastrointestinal nematodes against Albendazole, Ivermectin, and Levamisole.
The results of the present study show that changing the type of anthelminthic during the treatment and the prevention period is a very effective, and very good strategy to overcome gastrointestinal nematodes resistance in sheep, and it will not give the Helminths a chance to form a resistance against the drug.
Technical criteria should be applied for the control of gastrointestinal parasites for example, to maintain and prolong the lifespan of the efficacy of available anthelmintic farmers should be educated by veterinary extension programs about the importance of rational anthelmintic use, such as the correct dose, annual rotation among anthelmintic groups, and avoiding the risk factors that lead to reduced efficacy and anthelmintic resistance.
More studies from Libya are recommended to assess the anthelmintic resistance in other parasite species, and the efficacy status of commonly used front-line anthelmintics in different animal species.

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