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# Anthelmintic resistance of gastrointestinal nematodes in sheep in Piramagroon sub-district, Sulaymaniyah/Iraq

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**Abstract.** Infection with gastrointestinal (GI) nematodes is one of the major obstacles that face the pasture-based livestock production globally. The emergence of anthelmintic resistance has been reported from different parts of the world, and the current status on the efficacy of the common anthelmintic drugs in Kurdistan Region – northern Iraq are not well-understood. This study was conducted to evaluate the effectiveness of albendazole, ivermectin, and levamisole to GI nematodes of Sheep under field conditions in Piramagroon sub-district, Sulaymaniyah Governorate, Iraq. Nineteen sheep farms were included in the study and 40 lamb and yearlings from each flock were randomly selected and divided into four groups. The first group was the untreated control. Groups 2–4 were given the recommended doses of ivermectin, albendazole, and levamisole. Fecal Egg Count Reduction Test was conducted to determine the efficacy of the tested drugs. Anthelmintic resistance to the drugs was to be widespread in the studied areas. Results of larval culture showed that *Nematodirus* spp. was the most prevalent parasite in the region, followed by *Trichostrongylus* spp., *Marshallagia* spp., and *Trichuris* spp. The most abundant genus in the treated groups was the larvae of *Nematodirus* spp. The study concluded that anthelmintic resistance to ivermectin, albendazole, and levamisole is widespread in the area and *Nematodirus* was the most common parasite among the resistant genera. Therefore, alternative methods for the management of helminth infections should be implemented to reduce the burden of these parasites on the productivity of sheep in the region.

## INTRODUCTION

Infection with gastrointestinal (GI) nematodes is considered to a great extent to be the most important disease of grazing sheep throughout the globe, leading to loss of weight, diarrhea and even death (Fox *et al.*, 1989; Charlier *et al.*, 2014). The control of these parasites in sheep depends almost entirely on the use of anthelmintic therapy in order to improve the health of the sheep and increase their productivity (Falzon *et al.*, 2013). Dependence on drugs as the main source of helminth control is due to the ease of implementation and low cost, compared to other methods (Mascarini-Serra, 2011).

On a global scale, the three most important anthelmintic drug classes used to eliminate nematode infections in sheep are the macrocyclic lactones (e.g. ivermectin), benzimidazoles (e.g. albendazole), and imidazothiazoles (e.g. levamisole) (McKellar & Jackson, 2004; Lumaret *et al.*, 2012). Extensive use of effective anthelmintic compounds may eventually lead to the emergence of anthelmintic resistance (Wolstenholme *et al.*, 2004; Dyary, 2016). This occurs when an anthelmintic drug fails to kill the exposed population of parasites using the dose that is recommended therapeutically (Coles, 2006; Jabbar *et al.*, 2006). The spread of anthelmintic resistance is a rising problem in livestock production that threatens the success of anthelmintic therapy and reduces animal productivity (James *et al.*, 2009).

Implementation of the same anthelmintic drugs in helminth control accelerates the spread of anthelmintic resistance which poses selective pressure on the parasitic nematodes, favoring the development of resistant individuals. When drug-susceptible nematodes are eliminated from the host body, the resistant helminths that survive the anthelmintic therapy become more prevalent. Consequently, the proportion of the helminth eggs that carry resistance genes becomes higher in the environment, making infection of new hosts with the resistant helminths more likely (Papadopoulos *et al.*, 2012). The continuous rise in the incidence of anthelmintic resistance is not only a problem of livestock in the developing countries. Resistance to different anthelmintics has recently been reported from many of the economically developed countries such as the United States (Kaplan *et al.*, 2004; Torres-Acosta *et al.*, 2012; Kornele *et al.*, 2014), Canada (Falzon *et al.*, 2013), Brazil (Canever *et al.*, 2013), Australia (Playford *et al.*, 2014; Cotter *et al.*, 2015), the United Kingdom (Learmount *et al.*, 2016), France, Greece, and Italy (Geurden *et al.*, 2014).

Understanding the level of distribution of anthelmintic resistance in GI nematodes will provide information about the effectiveness of the available anthelmintic drugs in a region. However, the susceptibility of GI nematodes in livestock in Kurdistan Region of Iraq has not been investigated previously. Hence, this study was conducted to test the effectiveness of ivermectin, levamisole, and albendazole in the elimination of GI nematode infections of sheep under field conditions.

## MATERIALS AND METHODS

### Study area

The study was conducted in Piramagroon sub-district, Sulaymaniyah, Republic of Iraq. The region is located 35 km north-west of Sulaymaniyah. The sub-district comprises of 50 villages. The sheep and goat populations are estimated to be around 70000 heads. The sheep farms are located in the villages and the animals' housings are built near the villagers' homes. The sheep in this sub-district are usually fed on free pasture for a minimum of three months during the year, during which, they usually drink from the nearby water springs. The sheep graze on the same pasture and rotational grazing is not followed by the farmers which increases the probability of pasture contamination by GI nematode eggs. Adequate quarantine measures are also not followed and new animals are continually introduced into the sheep flocks. Since gastrointestinal nematode infection is considered endemic in the area, sheep farmers voluntarily visit the public veterinary service centers in their area during the grazing season, as anthelmintic drugs are provided at prices that are supported by the local government.

The study was conducted between April and June 2017, as the climatic conditions, such as temperature, rainfall, and humidity, are suitable for the survival of nematode larvae at that time. Nineteen farms were included in the study after the owners visited the veterinary service centers. All the selected flocks included more than 40 lambs and yearlings of mixed sexes. The sample size was based on 95% confidence interval (CI) and an error margin of 5%. Forty sheep were chosen from each herd and were randomly divided into four groups, each containing 10 sheep. The animals in each group were marked using suitable identification methods. The first group served as the untreated control, while the other three groups were orally given albendazole (Albendazole "AVICO" Forte, 5 mg/kg), ivermectin (Ivomec, 0.2 mg/kg), and levamisole (AVICO, 10 mg/kg), respectively. The dosage was calculated based on the individual weight of the sheep and the drug was orally administered using a calibrated drenching gun.

### Fecal egg count reduction test

The Fecal Egg Count Reduction Test (FECRT) is the most common method for the detection of resistance to anthelmintic drugs under field conditions and it can be used with all drug groups (Coles *et al.*, 2006; Falzon *et al.*, 2013). In FECRT, nematode eggs are counted in the feces at the time of drug administration and at defined times after therapy. The minimal number that can be detected by this method is 50 eggs per gram (epg) of feces.

The flocks of sheep were visited twice in a period of two weeks for the collection of fecal specimens. Few fecal pellets were taken directly from the rectum of the animals and put in plastic containers. The first sampling was just before drug administration while the second fecal collection was 14 days after therapy. Samples from individual animals were packaged in separate containers.

The collected fecal specimens were put in cooled containers during transportation to the laboratory, which usually took less than four hours. The samples were then kept refrigerated at 4°C until the egg count was performed. The FECRT was conducted in a period that did not exceed five days after sample collection.

Calculation of nematode eggs was conducted by floatation method. The technique used was previously described by Coles *et al.* (1992). The egg count per gram of fecal sample (FEC) was calculated by multiplying the total number of observed nematode eggs by 50.

The Fecal Egg Count Reduction (FECR) was calculated as $100 \times (1 - [\frac{T2}{C2}])$, where T2 and C2 are the average numbers of epg in the treatment and control groups, respectively, 14 days post-treatment. The 95% CI was calculated as $(100 \times \left(1 - \left[\frac{T2}{C2}\right]\right)) \pm 1.96\sqrt{Y2}$, where Y2 is the variance of the FECR. Flocks were considered resistant to a particular drug when the FECR was < 95% and the lower 95% CI was < 90%. If only one of these two criteria were met, the farm was defined as being suspected of resistance development (Falzon *et al.*, 2013).

**Identification of resistant gastro-intestinal nematodes**

Fecal specimens collected 14 days post-treatment were cultured for larval development. The samples taken from different drug group animals (ivermectin, levamisole, and albendazole) in each farm were pooled together by mixing 2 g of feces from each animal in the same treatment group. The fecal material was put in a glass jar and enough water was added to bring the mixture to a suitable consistency. The jar was then incubated at 27°C for seven days. The hatched $L_3$s were harvested and stained with Lugol's iodine as described by Coles *et al.* (1992) and were examined under a light microscope using a 100 × magnification power. The first 100 larvae observed were identified to the genus level following identification keys (van Wyk & Mayhew, 2013).

**Percentage of reduction for specific nematode genera**

The resistant nematode genera were determined in the farms that scored FECRs less than 95%. This was performed to determine the resistant nematodes in the current study. The percentage of reduction in each genus was calculated using the following formula:

$$100 \times \left\{1 - \left(\frac{\text{mean FEC post-treatment}}{\text{mean FEC pre-treatment}}\right) \times \left(\frac{\text{mean FEC pre-control}}{\text{mean FEC post-control}}\right)\right\}$$ (Waghorn *et al.,* 2006).

The genus was considered resistant to a particular anthelmintic agent when the percentage of reduction was < 95%. The genera which scored a mean FEC less than 50 epg in the pre-treatment samples were excluded from the calculations as the inclusion of these genera will not provide reliable results.

**Comparison of the tested drugs' efficacies**

The efficacies of the three anthelmintic agents were compared statistically. The comparison was conducted by calculating the FECR value for each of the tested compounds in 19 farms. The FECR results of the different drugs were then compared statistically using one-way analysis of variance (ANOVA), followed by post hoc (Tukey).

## RESULTS

**Study area**

Nineteen farms from 11 villages of Piramagroon, a sub-district of Dukan, Sulaymaniyah Governorate, were included in the study. The area of the study is located 35.7°N to 35.9°N and 45.1°E to 45.2°E. The flock sizes ranged between 50 and 650 heads, with the average of 231 sheep.

**Fecal egg count in the untreated control groups**

The mean pre-treatment FEC of the control groups was 2640 epg and highest FEC was 3250 epg, while the lowest FEC was 1550 epg. The mean FEC in the post-control groups was 3210 epg, which was 21.6% higher than the pre-treatment FEC. The highest FEC was 4350 epg and the lowest FEC was 2100 epg.

**Fecal egg count reduction test**

Resistance to ivermectin was reported in all of the flocks included in the study (Table 1). Levamisole resistance was reported in 14/19 farms (74%), while the remaining 26% were suspected of resistance development. Albendazole resistance was also reported in 16/19 (84%) farms, and the other 16% were suspected of being resistant.

**Resistant gastrointestinal nematodes**

The post-treatment fecal specimens from all the 19 farms were cultured after they were pooled together since all the pooled samples scored a post-treatment FEC higher than 200 epg. Figure 1 illustrates the percentage of *Nematodirus* spp., *Marshallagia* spp., *Trichostrongylus* spp. and *Trichuris* spp. in the first 100 harvested larvae.

*Nematodirus* was the most prevalent in the untreated control groups in all the 19 farms, making about 53.7 ± 4.6% of the harvested larvae. *Trichostrongylus* was the second most abundant parasite, accounting for 30.8 ± 3.1% of the isolated genera. *Marshallagia* and *Trichuris* made 10.7 ± 1.7 and 4.7 ± 2.7 of the larvae, respectively.

*Nematodirus* was the predominant parasite in the treated animals. The percentages of this genus in the ivermectin-, levamisole-, and albendazole-treated groups were 97.3 ± 2.6%, 95.9 ± 6.4%, and 89.6 ± 10.9%, respectively. *Trichostrongylus* spp. and

Table 1. Fecal Egg Count Reduction (FECR) results after therapy with different anthelmintic compounds in different farms

| Farm | Ivermectin | | Levamisole | | Albendazole | |
|---|---|---|---|---|---|---|
| | Percentage reduction | 95% CI | Percentage reduction | 95% CI | Percentage reduction | 95% CI |
| 1 | 76 | 52-99 | 71 | 54–87 | 78 | 56–99 |
| 2 | 82 | 71–93 | 82 | 68–96 | 69 | 50–88 |
| 3 | 73 | 55–91 | 74 | 56–93 | 72 | 58–86 |
| 4 | 89 | 81–96 | 91 | 84–97 | 90 | 83–96 |
| 5 | 68 | 43–93 | 87 | 72–100 | 85 | 74–97 |
| 6 | 68 | 46–91 | 86 | 69–100 | 77 | 54–100 |
| 7 | 65 | 40–90 | 81 | 73–90 | 77 | 60–94 |
| 8 | 70 | 50–90 | 83 | 71–94 | 79 | 61–97 |
| 9 | 87 | 80–94 | 89 | 82–96 | 83 | 66–100 |
| 10 | 72 | 59–85 | 89 | 77–100 | 83 | 69–96 |
| 11 | 73 | 51–94 | 90 | 80–100 | 91 | 83–99 |
| 12 | 88 | 81–94 | 90 | 80–100 | 90 | 80–99 |
| 13 | 75 | 62–88 | 76 | 54–98 | 78 | 67–88 |
| 14 | 70 | 43–96 | 90 | 84–96 | 76 | 59–92 |
| 15 | 73 | 56–91 | 90 | 84–96 | 91 | 86–97 |
| 16 | 62 | 41–84 | 90 | 82–97 | 85 | 69–100 |
| 17 | 69 | 55–82 | 85 | 68–100 | 76 | 56–97 |
| 18 | 76 | 65–87 | 91 | 87–95 | 90 | 79–100 |
| 19 | 87 | 81–94 | 84 | 74–95 | 90 | 82–97 |

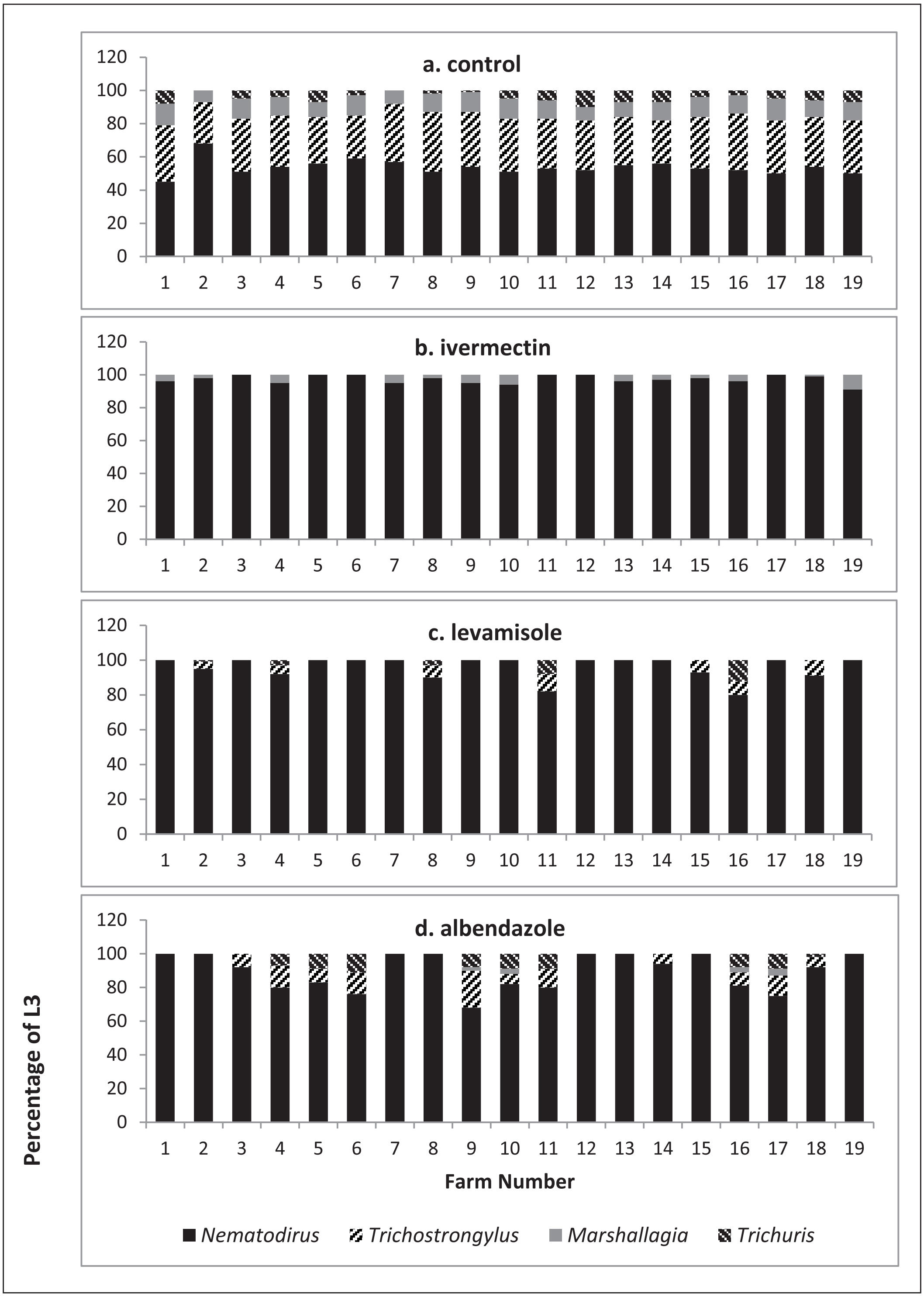


Figure 1. Percentage of *Nematodirus* spp., *Trichostrongylus* spp., *Marshallagia* spp., and *Trichuris* spp. in the first 100 larvae isolated from the pooled post-treatment fecal samples of a. control, b. ivermectin, c. levamisole and d. albendazole.

*Trichuris* spp. were not detectable in the fecal samples of the groups drenched with ivermectin in all the 19 farms. *Marshallagia* was also not detectable in the levamisole-treated groups.

The FECRs for the specific genera are illustrated in Table 2. *Nematodirus* was resistant to all the three anthelmintics in 19/19 farms (100 %). These results indicated that the development of resistance to the three drugs in the studied area was largely due to *Nematodirus* spp.

No resistance to the three treatments was recorded for *Trichostrongylus* spp. The parasite was susceptible in 19/19 farms to ivermectin and levamisole. *Trichostrongylus* was also sensitive to the anthelmintic action of albendazole in 16/19 farms (84%). However, farms number 6, 9, and 17 were suspected of being resistant to this drug.

*Marshallagia* spp. showed no resistance to levamisole and albendazole. Susceptibility of the parasite to ivermectin was reported in 13/19 farms (68%). *Marshallagia* was resistant against ivermectin in farms number 7, 10, and 16, and was suspected of resistance development in farms 13, 14, and 19.

The mean pre-treatment FEC of *Trichuris* was less than 50 epg in farms number 2, 7, and 9. Therefore, the percentage reduction of this parasite was not calculated (Table 2). No resistance of *Trichuris* to ivermectin was recorded on 16/16 farms (100%), while resistance to albendazole was reported on 6/16 farms (38%), and resistance to levamisole was reported on 2/16 farms (13%).

**Efficacies of levamisole, albendazole, and ivermectin**

Levamisole treatment resulted in the highest FECR, compared to the other drugs. The mean FECRs were 85.1 ± 6.1%, 82.0 ± 7.0%, and 74.8 ± 8.0% for the groups treated with levamisole, albendazole, and ivermectin, respectively. The FECRs of levamisole- and albendazole-treated groups were significantly higher than ivermectin-treated groups ($p < 0.05$) while the difference between the FECRs of the two drugs was not statistically significant ($p \geq 0.05$). These results indicated that resistance to ivermectin was the highest and resistance to levamisole was the lowest of the three therapies used in the study.

## DISCUSSION

Clinical practitioners depend on FEC to determine the necessity of anthelmintic therapy in a farm. Some researchers propose that a farm with FECs higher than 500 epg requires anthelmintic therapy (Morgan *et al.*, 2005). However, all the farms included in the current study had pre-treatment FECs higher than 1500 epg and therefore they all underwent the FECRT.

The common genera of nematodes reported in the study were *Marshallagia* spp., *Nematodirus* spp., *Trichostrongylus* spp., and *Trichuris* spp. These genera were also reported as common parasites of small ruminants in the neighboring countries Turkey (Sevimli, 2013) and Iran (Pestechian *et al.*, 2014). The fecal samples were collected in the grazing season (from April to June). Larval counts in the pasture peak during this period of the year in the region. The average daily temperature in April and May in the studied area is between 20°C and 30°C, which is considered optimal for the development of the reported helminths' larvae. The level of humidity during these months also favors the survivability of the larvae on the pasture (O'Connor *et al.*, 2006). This increases the susceptibility of these animals to GI nematode infections and causes higher FECs in the test animals. The high pre-treatment FEC provides better interpretations of the FECRT and more accurate results are gathered about the efficacy of the tested anthelmintic agents (Miller *et al.*, 2006).

Treatment with levamisole resulted in a higher FECR (91.6%), compared to treatment with albendazole (88.5%) and ivermectin (83.5%). Albendazole possesses anti-nematodal, anticestodal and anti-trematodal properties and it is widely used by farmers for the treatment of the widely spread liver fluke infections in the area. The extensive use of albendazole could be the reason for the widespread resistance to this drug.

Table 2. The Fecal Egg Count Reduction, and percentage reductions of *Nematodirus* spp., *Trichostrongylus* spp., *Marshallagia* spp., and *Trichuris* spp. after ivermectin, albendazole, and levamisole therapy in sheep farms from Piramagroon sub-district

| Farm | Ivermectin | | | | | Albendazole | | | | | Levamisole | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Percentage reduction in | | | | | Percentage reduction in | | | | | Percentage reduction in | | | |
| | FECR % | Ne. spp. | St. spp. | Ma. spp. | Tr. spp. | FECR % | Ne. spp. | St. spp. | Ma. spp. | Tr. spp. | FECR % | Ne. spp. | St. spp. | Ma. spp. | Tr. spp. |
| 1 | 76 | 71 | 100 | 96 | 100 | 78 | 74 | 100 | 100 | 100 | 71 | 55 | 100 | 100 | 100 |
| 2 | 82 | 83 | 100 | 97 | n/a | 69 | 70 | 100 | 100 | n/a | 82 | 84 | 98 | 100 | n/a |
| 3 | 73 | 61 | 100 | 100 | 100 | 72 | 63 | 95 | 100 | 100 | 74 | 65 | 100 | 100 | 100 |
| 4 | 89 | 82 | 100 | 95 | 100 | 90 | 87 | 96 | 100 | 84 | 91 | 86 | 99 | 100 | 94 |
| 5 | 68 | 58 | 100 | 100 | 100 | 85 | 85 | 97 | 100 | 87 | 87 | 84 | 100 | 100 | 100 |
| 6 | 68 | 64 | 100 | 100 | 100 | 77 | 81 | 93 | 100 | 47 | 85 | 85 | 100 | 100 | 100 |
| 7 | 65 | 54 | 100 | 83 | n/a | 77 | 68 | 100 | 100 | n/a | 81 | 72 | 100 | 100 | n/a |
| 8 | 70 | 52 | 100 | 95 | 100 | 79 | 67 | 100 | 100 | 100 | 83 | 74 | 97 | 100 | 78 |
| 9 | 87 | 79 | 100 | 95 | n/a | 83 | 83 | 91 | 98 | n/a | 89 | 83 | 100 | 100 | n/a |
| 10 | 72 | 56 | 100 | 88 | 100 | 83 | 77 | 97 | 96 | 74 | 89 | 82 | 100 | 100 | 100 |
| 11 | 73 | 59 | 100 | 100 | 100 | 91 | 90 | 97 | 100 | 90 | 90 | 89 | 98 | 100 | 90 |
| 12 | 88 | 82 | 100 | 100 | 100 | 90 | 85 | 100 | 100 | 100 | 90 | 86 | 100 | 100 | 100 |
| 13 | 75 | 68 | 100 | 92 | 100 | 78 | 70 | 100 | 100 | 100 | 76 | 69 | 100 | 100 | 100 |
| 14 | 70 | 61 | 100 | 94 | 100 | 76 | 68 | 96 | 100 | 100 | 90 | 87 | 100 | 100 | 100 |
| 15 | 73 | 58 | 100 | 96 | 100 | 91 | 86 | 100 | 100 | 100 | 90 | 85 | 98 | 100 | 100 |
| 16 | 62 | 40 | 100 | 88 | 100 | 85 | 80 | 97 | 96 | 65 | 90 | 86 | 98 | 100 | 63 |
| 17 | 69 | 57 | 100 | 100 | 100 | 76 | 76 | 94 | 95 | 71 | 85 | 81 | 100 | 100 | 100 |
| 18 | 76 | 61 | 100 | 98 | 100 | 90 | 87 | 98 | 100 | 97 | 91 | 87 | 98 | 100 | 100 |
| 19 | 88 | 79 | 100 | 91 | 100 | 90 | 83 | 100 | 100 | 100 | 84 | 74 | 100 | 100 | 100 |

FECR % = percentage of Fecal Egg Count Reduction. Ne. = *Nematodirus*, St. = *Trichostrongylus*, Ma. = *Marshallagia*, Tr. = *Trichuris*. n/a = not available because the pre-treatment FEC did not reach 50 epg.

Ivermectin and its chemically-related drugs are used widely for the elimination of external parasitism in sheep and goats in the region. The development of resistance to ivermectin may also be justified by the widespread use of this drug and its analogs by farmers in Piramagroon sub-district, which increases the selective pressure for resistance formation.

Larval cultures of post-treatment fecal samples provide information about the nematode genera that parasitize animals and facilitate the determination of the parasites that did not respond to anthelmintic therapy (Waghorn *et al.*, 2006). Results of the larval cultures revealed that *Nematodirus* was the most prevalent genus in the untreated control groups and the groups treated with anthelmintic drugs. *Trichostrongylus* was the second most abundant in the control and groups treated with albendazole and levamisole, while it was not detected in the ivermectin-treated groups. Farmers in the Piramagroon sub-district and neighboring areas do not follow rigorous quarantine measures; new animals are continually imported to the farm, which could probably bring in resistant nematodes to the flock. The inclusion of these outliers drastically affects the proportions of the genera that were detected after treatment. For example, *Marshallagia* was detected in only four farms (farms 9, 10, 16, and 17) that were treated with albendazole (Figure 1), which increases the probability that outlier animals were included in the tested farms.

All the 19 farms were either resistant or suspected of resistance to the tested drugs, which raises questions about the effectiveness of the anthelmintic agents. Helminths that develop resistance to a drug most probably will become resistant to drugs that belong to the same chemical class (Sangster & Gill, 1999). Hence, farmers should probably consider other methods of controlling GI nematode infection. Techniques such as pasture management, enforcement of strict quarantine measures and the introduction of new anthelmintic drugs that have a different mechanism of action could presumably reduce the selective pressure on the emergence of resistant GI nematodes.

*Nematodirus* spp. was the most prevalent parasite in the untreated control sheep and in the post-treatment samples from three drug groups. This result implied that the majority of resistance occurred was developed by this parasite, although the other genera also showed different levels of resistance development. *Trichuris* resistance to levamisole was detected in 2/16 farms. However, since the percentage of this parasite was initially lower than the other genera in the studied area, the possibility of outliers' inclusion becomes greater. The introduction of an animal that carries resistant parasites to the flock could drastically affect the proportion of these parasites when their percentage is initially low, compared to other genera.

This study investigated the susceptibility of GI nematodes in sheep farms that the owners voluntarily visited the veterinary service centers. Therefore, it may not cover all the parasitic genera that are present in Piramagroon. A larger study area may have been required to provide an accurate prevalence of all the parasitic genera. However, the results concluded that anthelmintic resistance to the commercially available drugs is widespread in the studied farms and producers should be informed about the reduced effectiveness of these treatments.

## CONCLUSION

The FECRT revealed that anthelmintic resistance against ivermectin, albendazole, and levamisole was present on most of the studied farms in Piramagroon sub-district. Resistance to levamisole and albendazole was less common than to ivermectin. Results of the larval culture revealed that *Nematodirus* spp. was the most common parasite both in the untreated control and treated animals. Hence, it is evident that *Nematodirus* spp. is responsible for the widespread resistance to the three drugs. The results indicated that anthelmintic resistance is a serious problem to small ruminant's productivity in this area. Therefore, veterinarians and farmers should practice other methods to tackle GI nematode infections. For example, farmers could probably use anthelmintic agents that act via different modes of action and avoid the widespread use of the currently available drugs. Following stricter quarantine measures to avoid the introduction of carrier animals of resistant parasite species should also be included in the management strategies. A better pasture management by following rotational grazing strategies could also reduce the unnecessary exposure of the small ruminants to the infective nematode larvae and, hence, the probability of resistance development.

**Conflict of interest**

The author declares no conflict of interest.

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