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Research Article

Resistance of Gastrointestinal Nematodes to Anthelmintics in Sheep Production in Zimbabwe

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ABSTRACT

Introduction: Sheep play a crucial role in enhancing the socio-economic welfare of individuals in numerous nations. Within sub-Saharan Africa, productivity levels are hindered by various factors, such as prevalent diseases, inadequate nutrition, climate variations, predators, severe weather, as well as infestations of both external and internal parasites. The purpose of the present study was to assess the extent of anthelmintic resistance (AR) observed on a sheep farm operating for commercial purposes in Zimbabwe.
Materials and methods: Forty female weaner Dorper sheep, averaging 43 kg in body weight, were selected from Ballineety commercial sheep farm in Mashonaland Central, Zimbabwe. These sheep were then divided into four groups of ten each, receiving different treatments including Ivermectin, Albendazole, Levamisole, and no treatment as a control. Each treatment was administered according to the manufacturer's recommendations. Pre-treatment faecal samples (day 0) and post-treatment (day 14) were collected and subjected to the Faecal Egg Count Reduction Test (FECRT). Anthelminthic resistance was suggested (AR) by the presence of faecal eggs after treatment.
Results: All the drugs indicated a decrease in egg counts by less than 90%. The effectiveness of Ivermectin, Albendazole, and Levamisole was measured at 58.5%, 70.1%, and 85.8% respectively. These findings indicate that nematodes have developed resistance to all treatments. The larval cultures of the samples before and after treatment indicated a significant presence of *Haemonchus* spp. in the pooled samples. In contrast, the quantities of *Trichostrongylus* spp. and *Oesophagostomum spp.* were low but present in all treatments. *Cooperia* spp. was recovered in post-treatment larval cultures of groups that received anthelmintic. The present study reports lower-than-expected efficacy for Ivermectin, Albendazole, and Levamisole based on the reduction of egg excreted post-treatment.
Conclusion: A common worms population in sheep are AR in the current study area. Therefore, comprehensive nematode management programs along with complementary eradication strategies are essential for sustainable sheep production.

1. Introduction

Sheep holds significant importance in numerous nations, contributing greatly to the socio-economic welfare of individuals[1]. They serve as a valuable source of income and fulfill various purposes, including the provision of nourishment during cultural ceremonies[2,3]. Sheep are a source of meat, wool, leather, and milk[4]. Due to high disease prevalence, the poor plane of nutrition, climate change, predators, external and internal parasites, and harsh weather conditions in sub-Saharan Africa productivity remains low[5-7]. On a worldwide scale gastrointestinal nematodes (GIN) are one of the major challenges to the economic benefits derived from sheep[8].

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Nematode infections in small ruminants cause low productivity, reduced appetite, low weight gain, anemia, and reduced meat and wool production, leading to condemnation of organs at meat inspection, and subsequently death[9]. Anthelmintics play a vital role in control and treatment of GIN[10]. Anthelmintic resistance can be defined as an increased capability of a particular worm population to withstand and tolerate (< 80% reduction) previously lethal effective doses (> 95% reduction) of a particular compound[10]. However, over the years taking these drugs continuously has resulted in the emergence of anthelmintic resistance (AR) against most classes of anthelmintic drugs[11,12].

Three types of AR have been described to date are side resistance, cross-resistance, and multiple resistances[13]. Side resistance refers to the phenomenon when compounds of similar structure and activity show a decreased efficacy against a resistant strain compared to a susceptible strain of the same species. Cross-resistance describes a phenomenon similar to side resistance but involves compounds of different structures and activities. Multiple resistance is when a resistant strain of nematodes meets with resistance to two or more groups or classes of anthelmintics, either as a result of independent group selection or cross resistance[13,14].

In small scales and communal production systems, AR is an increasing problem. In light of the ever-increasing problem of nematode AR, it is possible that more nematode genera may have developed/acquired resistance over time. Commercial sheep rearing is an established venture in the Southern hemisphere and a reemerging enterprise in Zimbabwe. Thus, the objective of the present study was to determine the efficacy of the most common anthelminthic drugs on the market and show the level of AR in sheep production.

2. Materials and Methods

2.1. Ethical approval

All methods were performed in accordance with the relevant guidelines and regulations of the Department of Veterinary Services, Zimbabwe.

2.2. Study area

The study was conducted from April 2019 to December 2019 at a Ballineety commercial sheep farm in Mashonaland Central, Zimbabwe. The farm is in a Natural Region II covering an area of around 800 hectares with an approximate altitude of 1200 meters above sea level. The area is characterized by a sub-tropical climate with low to moderate rainfall ranging from 650-800 mm distributed from late October to April, with temperatures ranging from 12 °C to 30 °C. May to July are cool and dry months with temperatures ranging from 12 °C to 25 °C. August to October are hot and dry months and temperatures range from 20 °C to 30 °C. These conditions are propitious for the prevalence of sheep nematodes. The predominant vegetation type in the district consists of sparsely scattered trees, mainly *Brachystegia spiciformis*, *Julbernardia globiflora,* and *Combretum molle* species, besides abundant tall tufted grasses, such as *Heteropogon species, Themeda triandra,* and *Hyparrhenia species*. The main agricultural activities in the area are livestock production and cultivation of drought-resistant plant and fodder crops.

2.3. Experimental animals

The flock comprised more than 150 sheep of various age groups. The sheep were allowed to graze a pasture-reinforced (stargrass and pannicum) native rangeland during the daytime. During the dry season, hay and concentrate (commercial) supplements were provided. Sheep were housed in groups (n=10, according to age and sex) of approximately 20 to 30 in a shade tailor-made for sheep housing. Clean tapped water was provided by adding *ad libitum* in water troughs placed randomly within the grazing and overnight shade areas. According to Kaplan[15], sheep with an egg count per gram of faeces (EPG) of 150 per group or more were used. A total of forty female weaner Dorper sheep with an average body weight of 43 kg, aged between six and eight months were selected randomly. All sheep had received anthelminthic therapy two months before the study[8]. According to the WAAVP guidelines, 15 animals per group are recommended for an accurate evaluation of Faecal Egg Count Reduction Test (FECR), on the basis of availability, the number was later reduced to 10 animals per group[16]. In the present study the flock size included 10 animals per group. This choice has been supported by Rinaldi et al.[17], where fecal egg counts from 5, 10, or 20 sheep had a strong correlation with each other and with the mean of the individually examined samples. Animals were vaccinated against Pulpy kidney (PULPYVAX® , Intervet South Africa) disease at weaning at a rate of 1ml per animal.

2.4. Treatments groups

The products to test included, Ivermectin (Oramec®, Beestone Animal Health, Cheshire, UK), Albendazole (Valbazen®, Zoetis, Sandtone, South Africa), and Levamisole (Chanaverm® Agricura , Zimbabwe). The items were administered according to the manufacturer's instructions (Table 1). According to Coles et al.[16], a 14-day post-treatment interval was used in the study. Pre-treatment faecal samples were collected from the sheep's rectum by faecal racking and EPG was determined using the McMaster egg counting technique[9].

2.5. Faecal egg counts

Faecal samples were collected and analyzed 14 days after treatment for *Strongyle* type of eggs using saturated sodium chloride solution of 1.200 specific gravity as a floatation solution[18] at Harare Central laboratory, Zimbabwe. According to the manual of veterinary parasitological laboratory techniques[19], EPGs were

Table 1. Treatment groups, route of drug administration, sheep age, and weight at Ballineety Farm, Zimbabwe

	Treatment			
	Group A (Ivermectin)	Group B (Albendazole)	Group C (Levamisole)	Group D (Control)
Route of administration	Oral (2.5 ml per 10 kg bodyweight)	Oral (0.75ml per 10kg bodyweight)	Oral (0.5ml per 5kgs bodyweight)	-
Age (months)	7.4	7.1	7.6	7.6
Weight (kg)	45.1	42.6	41.3	43.0

obtained using the Modified McMaster method, which has a sensitivity of 50 eggs/gram of faeces. Larval cultures for pre- and post- treatment faecal samples were carried out for the respective groups by pooling the samples for each group and identifying the 3rd larval stages (L3).

2.6. Efficacy evaluation

Efficacy of each drug was assessed by performing the FECRT in accordance with the World Association for the Advancement in Veterinary Parasitology (WAAVP) standards[20].

Efficacy = 100 (1 – (T2/C2))

where T2 is the arithmetic mean EPG for the treated group and C2 is the arithmetic mean of the control group at day 14 of post-treatment[16].

The treatments were classified into three classes according to Geurden[10], as either efficacious, having confirmed anthelmintic resistance, or being inconclusive. Efficacious is the percentage reduction and upper 95% confidence limit. Confirmed anthelmintic resistance is the percentage reduction below 90%, and inconclusive is neither of the above criteria fulfilled.

2.7. Larval identification

Triplicate faecal samples, about 2-3 grams, from each sheep were pooled in each treatment group and incubated at 27°C for 7 days before and after treatment. The L3 will be recovered using the modified Baemann technique[21]. The L3 will be counted and identified according to morphological keys by Van Wyk et al.[22].

2.8. Experimental design

A complete randomized design was used to randomly distribute sheep into four groups with ten animals each according to Kaplan[15] and Close et al.[16]. Group A was treated with macrocyclic lactones (Ivermectin, Oral, 2.5 ml per 10 kg bodyweight), Group B with benzimidazoles (Albendazole, Oral, 0.75ml per 10kg bodyweight), Group C with levamisole (Levamisole, Oral, 0.5ml chanaverm 7.5% per 5kgs bodyweight), and Group D was the control (not treated, Table 1).

2.9. Data analysis

Faecal egg count reduction test was calculated with the arithmetic mean EPGs for treatment groups. ANOVA used to test the mean egg counts at significant level of 0.05 using the Statistics Package for Social (SPSS) Version 24, 2021[23].

3. Results

The mean arithmetic EPGs for all treatment groups was 598.6 EPG (Table 2). The overall prevalence of the *Strongyle* type nematodes in sheep was 100%. The fecal count effectiveness was 58.5%, 70.1%, 85.8% for Ivermectin, Albendazole, and Levamisole respectively, while in the control group the fecal count effectiveness increased by 67.1%. The lowest rate of reduction was observed when ivermectin used ($p < 0.05$). All treatments showed a reduction less than 95% and the upper confidence intervals for all treatments was less than 95%, while the lower confidence interval limit was below 90%, thus all treatments have confirmed anthelminthic resistance.

3.1. Larval identification

Larval identification of the pre-treatment faecal cultures for all the groups indicated a high proportion of *Hemonchus* (*H.) contortus* with very high harvests ranging between 89–100%, while *Oesophagostomum* and *Trichostrongylus* were low in all four groups, with a percentage range from 1-8% (Table 3). Post-treatment was completely effective against *Trichostrongylus* indicating no resistance. Albendazole had a zero effect on *H. contortus* larvae, it significantly reduced the *Cooperia* species infection ($p < 0.05$). *H. contortus* indicated resistance against all treatments, and little reductions were observed for *Oesophagostomum* against all treatments. *Cooperia* species were recovered in post-treatment larval cultures and were resistant to ivermectin and levamisole.

Table 2. The arithmetic faecal egg counts pre and post-treatment for each treatment group at Ballineety Farm in Dorper sheep, Zimbabwe

Treatment group	Mean EPG		Efficacy				P value
	Pre-treatment	Post-treatment	%	U95	L95	Status	
Ivermectin	$757^a \pm 91.5$	$314.0^b \pm 19.5$	58.5	61.6	55.4	CAR	< 0.001
Albendazole	$982^a \pm 19.5$	$293.0^b \pm 19.5$	70.1	72.6	67.8	CAR	< 0.001
Levamisole	$679^a \pm 19.5$	$96.0^b \pm 19.5$	85.8	89.4	58.0	CAR	< 0.001
Control	$429^b \pm 19.5$	$639.0^a \pm 19.5$	-67.1	43.3	54.3	-	< 0.001

ab row means with different superscripts are significant at ($P < 0.05$). EPG: Egg count per gram of faeces, CAR: Confirmed anthelminthic resistance, U95: Upper confidence interval, L95: Lower confidence interval (mean ± standard deviation)

Table 3. The proportion of larva in pre-treatment (day zero) and post-treatment (day 14) faecal samples of Dorper sheep in Zimbabwe

	Pretreatment				Post-treatment			
	Ivermectin	Albendazole	Levamisole	Control	Ivermectin	Albendazole	Levamisole	Control
Hemonchus contortus	96	89	96	92	94	100	88	88
Oesophagostomum spp.	3	3	1	6	2	0	2	6
Trichostrongylus spp.	1	8	3	2	0	0	0	0
Cooperia spp.	-	-	-	-	4	0	8	6

4. Discussion

Based on WAAVP interpretation of FECRT results, when means were greater than 95% faecal reduction, there is lack of resistance[16]. At a reduction between 80% and 95%, resistance is suspected, while a faecal reduction is less than 80% prove AR[8]. It was expected that an effective treatment should result in a zero egg count, thus finding eggs post treatment suggests presence of AR in the current study. The lower efficacy indicated by ivermectin were not expected , however a number of studies have stated AR against this product[24,25].

The findings are contrary to those of Kahiya[26], that no AR was observed from 10 farms. It is therefore possible that, over the years, prolonged use of ivermectin led to the gastrointestinal nematodes developing resistance. In addition, ivermectin is rapidly absorbed irrespective of the administration route (orally or subcutaneously). After absorption it became evenly distributed throughout the body and is particularly concentrated in adipose tissue. The latter may have led to the reduced efficacy as the systemic therapeutic levels meant to kill the worm population may have not reached adequate efficacy levels. It is possible that the recommended manufacturer's dosage rates (to the nearest kilogram), which are not exact, may have led to under dosing among some of the sheep thereby causing reduced efficacy. Nevertheless, the efficacy of ivermectin was very low and highly suggestive of resistance by worms irrespective of the other contributing factors, such as body condition and the manufacturer's recommended dose of a single volume across a range of weight. When Albendazole used, the FECTR was still lower than 80% suggestive of AR to the drug. Intensive production has been muted to increase AR among sheep flocks[27,28]. The copious use of nematicides in the past has led to moderate level of nematode infections in sheep production, however the selection pressure of intensive anthelmintic use resulted in the pasture being laden with resistant parasite population, increasing sheep vulnerability and susceptibility[10].

4.1. Larval identification

Haemonchus spp. were evidently resistant to all the anthelmintics used in the present study. The highest level of resistance was observed with Albendazole treatment. The reason that *H. contortus* is the predominant resistant species might have to do with the high biotic potential for one female, that can lay many eggs per day, about 1200 eggs[27]. On the other hand, Albendazole was completely effective against *Oesophagostomum Trichostrongylus,* and *Cooperia spp.* suggestive of no AR for this drug. According to Saccareau et al.[27], the parasite has developed resistance to virtually all known classes of small molecule anthelminthics and even multidrug resistance, hence very difficult to control. It is known that high parasitic loads are common on farms from peri-parturient infected ewes and does, no wonder kids and lambs are at a high risk. Nonetheless, results from the current study have shown that even weaners are at a high risk from this parasite. Interestingly, Arsenopoulos et al.[29] have reported a drop of the faecal egg counts in cattle, and that with time they acquire immunity against *Haemonchus s*pp. As proposed by Morais-Costa et al.[9] plants containing secondary compounds may provide alternatives for the control of GIN. In sub Saharan Africa, a number of plants are presented with an enormous pool of phytochemicals that warrants further research as to their effectiveness in controlling nematodiasis in ruminants. It is believe that following the current study, more research will be done to evaluate such plants with anthelminthic properties. The presence of *Cooperia spp*. larvae post treatment were also reported by Geurden et al.[10]. However, *Cooperia* species are considered less pathogenic although negative effects have been recorded in cattle[30]. The presence of *Oesophagostomum* in both pre and post treatment samples is highly suggestive of *Oesophagostomum* resistance to Ivermectin and Levamisole. According to the FECRT guidelines, taking post treatment faecal samples within 10-14 days after the treatment prevented differences between anthelmintic bioavailability provided that sampled earlier. While later than 14 days re-infections may be apparent[11,27,28].

5. Conclusion

Nematodes were resistant to all expriented drugs in the current study. Albendazole was completely effective against *Oesophagostomum, Trichostrongylus,* and *Cooperia spp.* Various strategies are needed to minimize the selection pressure for the emergence of anthelmintic resistance in ruminants. The researchers of the present study contend that the persistent utilization and misuse of substances is prevalent within the community of small-scale sheep breeders, consequently resulting in AR. The combination of anthelminthics can enhance the effectiveness of treatment, thereby reducing the likelihood of resistance genes being introduced into the worm population. Nutritional supplementation with tannins, genetic selection of resistant animals, and effective pasture management are essential strategies for eliminating AR in sheep production.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conceptualization, writing the original draft was done by Alice N. Mushonga and Soul Washaya designed the methodology; and did formal analysis supervision and writing, review, and editing. Godfrey B. Nyamushamba was the co-supervisor. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The manuscript contains all datasets generated and/or analyzed in the current study.

Ethical considerations

The ethical concerns of plagiarism, permission to publish, misconduct, data fabrication and falsification, double publishing, submission, and redundancy have all been reviewed by the authors.

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References

1. Mavrot F, Hertzberg H, and Torgerson P. Effect of gastro-intestinal nematode infection on sheep performance: A systematic review and meta-analysis. Parasit Vectors. 2015; 8: 577. DOI: 10.1186/s13071-015-1164-z
2. Jaramillo Hernández DA, Vásquez Trujillo A, and Lesmes Rodríguez LC. *In-vitro* effect of the methanolic extract of Momordica charantia on hatching of eggs of haemonchus sp. Vitae. 2021; 28(1): 345215. DOI: 10.17533/udea.vitae.v28n1a345215
3. Ahbara AM, Rouatbi M, Gharbi M, Rekik M, Haile A, Rischkowsky B, et al. Genome-wide insights on gastrointestinal nematode resistance in autochthonous Tunisian sheep. Sci Rep. 2021; 11(1): 9250. DOI: 10.1038/s41598-021-88501-3
4. Bersissa K, Etana D, and Bekele M. Comparative efficacy of albendazole, tetramisole and ivermectin against gastrointestinal nematodes in naturally infected goats in Ziway, Oromia Regional State (Southern Ethiopia). J Anim Vet Adv. 2010; 9(23): 2905-2911. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20103379412
5. Houndjo DBM, Adjolohoun S, Gbenou B, Saidou A, Ahoton L, Houinato M, et al. Socio-demographic and economic characteristics, crop-livestock production systems and issues for rearing improvement: A review. Int J Biol Chem Sci. 2018; 12(1): 519. DOI: 10.4314/ijbcs.v12i1.41
6. Mthi S, Rust JM, Mpendulo CT, Muchenje V, Goosen WJ, and Mbathsa Z. Basic factors influencing lamb mortality under low input production systems in the Eastern Cape Province , South Africa. S Afr J Anim Sci. 2020; 1(13): 60-68. Available at: https://www.sasas.co.za/AAH&RD/basic-factors-influencing-lamb-mortality-under-low-input-production-systems-in-the-eastern-cape-province-south-africa/
7. Holmøy IH, Waage S, Granquist EG, L'Abée-Lund TM, Ersdal C, Hektoen L, et al. Early neonatal lamb mortality: Postmortem findings. Animal. 2017; 11(2): 295–305. DOI: 10.1017/S175173111600152X
8. Ploeger HW, and Everts RR. Alarming levels of anthelmintic resistance against gastrointestinal nematodes in sheep in the Netherlands. Vet Parasitol. 2018; *262*: 11-15. DOI: 10.1016/j.vetpar.2018.09.007
9. Morais-Costa F, Bastos GA, Soares ACM, Costa EGL, Vasconcelos VO, Oliveira NJF, et al. *In vitro* and *in vivo* action of Piptadenia viridiflora (Kunth) Benth against Haemonchus contortus in sheep. Vet Parasitol. 2016; 223: 43-49. DOI: 10.1016/j.vetpar.2016.04.002
10. Geurden T, Chartier C, Fanke J, di Regalbono AF, Traversa D, von Samson-Himmelstjerna G, et al. Anthelmintic resistance to ivermectin and moxidectin in gastrointestinal nematodes of cattle in Europe. Int J Parasitol Drugs Drug Resist. 2015; 5(3): 163-171. DOI: 10.1016/j.ijpddr.2015.08.001
11. Sanders J, Xie Y, Gazzola D, Li H, Abraham A, Flanagan K, et al. A new paraprobiotic-based treatment for control of Haemonchus contortus in sheep. Int J Parasitol Drugs: Drug Resist. 2020; 14: 230-236. DOI: 10.1016/j.ijpddr.2020.11.004
12. Vadlejch J, Kyriánová IA, Várady M, and Charlier J. Resistance of strongylid nematodes to anthelmintic drugs and driving factors at Czech goat farms. BMC Vet Res. 2021; 17(1): 106. DOI: 10.1186/s12917-021-02819-8
13. Fissiha W, and Kinde MZ. Anthelmintic Resistance and Its Mechanism: A Review. Infect Drug Resist. 2021; 14: 5403-5410. DOI: 10.2147/IDR.S332378
14. Prichard RK, and Geary TG. Perspectives on the utility of moxidectin for the control of parasitic nematodes in the face of developing anthelmintic resistance. Int J Parasitol Drugs: Drug Resist. 2019; 10: 69-83. DOI: 10.1016/j.ijpddr.2019.06.002
15. Kaplan RM. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Vet Clin North Am Food Anim Pract. 2020; 36(1): 17-30. DOI: 10.1016/j.cvfa.2019.12.001
16. Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna G, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol. 2006; 136(3-4): 167-185. DOI: 10.1016/j.vetpar.2005.11.019
17. Rinaldi L, Leveckeet B, Boscoal A, Ianniello D, Pepe P, Charlier J, et al. Comparison of individual and pooled faecal samples in sheep for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC. Vet Parasitol. 2014; 205(1-2): 216-223. DOI: 10.1016/j.vetpar.2014.06.011
18. Comabting anthelminthic resistance in ruminants (COMBAR). Faecal egg count reduction test (FECRT) protocol Gastrointestinal nematodes - sheep and goats. 2021. p. 1-5. Available at: https://www.combar-ca.eu/sites/default/files/FECRT_PROTOCOL_sheep_goats_March%202021.pdf
19. Friedhoff KT. Manual of veterinary parasitological laboratory techniques: Ministry of Agriculture, Fisheries and Food, Agricultural Development and Advisory Service. Technical Bulletin, No. 18. 2nd ed. 1977. p. 16.
20. Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA, et al. World association for the advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol. 1992; 44(1-2): 35-44. DOI: 10.1016/0304-4017(92)90141-u
21. Gelaye W, Williams NA, Kepha S, Junior AM, Fleitas PE, Marti-Soler H, et al. Performance evaluation of Baermann techniques: The quest for developing a microscopy reference standard for the diagnosis of *Strongyloides stercoralis*. PLoS Negl Trop Dis. 2021; 15(2): e0009076. DOI: 10.1371/journal.pntd.0009076
22. Van Wyk JA, Cabaret J, and Michael LM. Morphological identification of nematode larvae of small ruminants and cattle simplified. Vet Parasitol. 2004; 119(4): 277-306. DOI: 10.1016/j.vetpar.2003.11.012
23. Spss Inc. SPSS Trends 24. 2021.

24. Dauparaitė E, Kupčinskas T, von Samson-Himmelstjerna G, and Petkevičius S. Anthelmintic resistance of horse strongyle nematodes to ivermectin and pyrantel in Lithuania. Acta Vet Scand. 2021; 63(1): 1-7. DOI: 10.1186/s13028-021-00569-z
25. Wondimu A, and Bayu Y. 2022; Anthelmintic drug resistance of gastrointestinal nematodes of naturally infected goats in Haramaya, Ethiopia. J Parasitol Res. 2022; 2022: 4025902. DOI: 10.1155/2022/4025902
26. Kahiya C, Mukaratirwa S, and Thamsborg SM. Effects of acacia nilotica and acacia karoo diets on haemonchus contortus infection in goats. Vet Parasitol. 2003; 115(3): 265-274. DOI: 10.1016/s0304-4017(03)00213-9
27. Saccareau M, Sallé G, Robert-Granié C, Duchemin T, Jacquiet P, Blanchard A, et al. Meta-analysis of the parasitic phase traits of Haemonchus contortus infection in sheep. Parasit Vectors. 2017; 10(1): 201. DOI: 10.1186/s13071-017-2131-7
28. Domke AVM, Chartier C, Gjerde B, Höglund J, Leine N, Vatn S, et al. Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. Parasitol Res. 2012; 111(1): 185-193. DOI: 10.1007/s00436-012-2817-x
29. Arsenopoulos KV, Katsarou EI, Mendoza Roldan JA, Fthenakis GC, and Papadopoulos E. Haemonchus contortus parasitism in intensively managed cross-limousin beef calves: Effects on feed conversion and carcass characteristics and potential associations with climatic conditions. Pathogens. 2022; 11(9): 955. DOI: 10.3390/pathogens11090955
30. Aguerre S, Jacquiet P, Brodier H, Bournazel JP, Grisez C, Prévot F, et al. Resistance to gastrointestinal nematodes in dairy sheep: Genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms. Vet Parasitol. 2018; 256: 16-23. DOI: 10.1016/j.vetpar.2018.04.004