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Anthelmintic resistance in gastrointestinal nematodes of goats in southern Mozambique

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Small ruminant production is significantly constrained by gastrointestinal parasites once they cause serious production and economic losses for both small-scale and large-scale farmers in the developing world. The control of helminth parasites is most exclusively based on the use of anthelmintics for the economic production of ruminants. However, the resistance of nematodes to the commonly used groups of anthelmintics represents a threat to the production, particularly for small ruminants. The objective of this study was to assess the efficacy of albendazole, the most frequently used anthelmintic in Gaza and Maputo provinces in the southern region of Mozambique, in gastrointestinal nematodes of goats between November and December, 2016. Eleven goat farms in Gaza (n = 5) and Maputo (n = 6) were surveyed. The faecal egg count reduction test was used to assess the efficacy of the drug. The flocks were considered resistant when the reduction in eggs per gram of faeces was less than 95% and the lower limit of the confidence interval was less than 90%. Resistance to albendazole was detected in 60% (3/5) of the farms in Gaza province and 83.3% (5/6) of the farms in Maputo province. The percentage of faecal egg count reduction varied from 51 to 97% in Maputo and from 0 to 100% in Gaza in the farms surveyed. In pre-treatment coprocultures, *Haemonchus* spp., *Oesophagostomum* spp. and *Trichostrongylus* spp. were the predominant nematode species. Post-treatment larval cultures indicated that *Haemonchus* spp. and, to a lesser extent, *Oesophagostomum* spp. and *Trichostrongylus* spp., were resistant to albendazole. This study provided further evidence that anthelmintic resistance of gastrointestinal nematode parasites in goats is currently a problem of great significance in this region of the country and that appropriate measures must be taken to reverse the situation.

Key words: Albendazole, efficacy, gastrointestinal parasites, small ruminants, Mozambique.

INTRODUCTION

Small ruminants make important contributions to human livelihoods in developing countries. In 2012, about 30% of the approximately 1 billion populations of goats in the world were located in Africa (FAO, 2015; reviewed by

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Zvinorova et al., 2016). Gastrointestinal nematode infections of ruminant livestock cause major problems for both small-scale and large-scale farmers in the developing world, with a major impact in sub-Saharan Africa (Sissay et al., 2007; Hussain, et al., 2014; Preston et al., 2014; Pawar et al., 2017; Shija et al., 2014; Singh et al, 2017a; Wondimu and Gutu, 2017), including Mozambique with its tropical and sub-tropical areas where favourable climates for the survival and development of gastrointestinal nematodes prevail (Akkari et al., 2013; Shija et al., 2014). *Haemonchus* spp. are highly pathogenic and economically important gastrointestinal parasites affecting ruminants across the world (Chaudhry et al., 2015; Zvinorova et al., 2016). The control of helminth parasites is often essential for the economic production of animals, especially small ruminants. Current control measures rely heavily upon the use of anthelmintic drugs (Gaba et al., 2012; McArthur and Reinemeyer, 2014). However, the ability of parasites to develop quickly resistance to these drugs is spreading globally (Preston et al., 2014). Anthelmintic resistance is a globally threatening problem to sustainable livestock industry and production (Kenyon et al., 2013; Shalaby, 2013; Chaudhry et al., 2015; Singh et al., 2017b). A strain of *Haemonchus contortus* resistant to all classes of anthelmintics has been reported to have developed in South Africa, a neighbouring country of Mozambique, probably because of frequent use of anthelmintics and inappropriate dosage (Van Wyk et al., 1997). Shalaby (2013) stated also that the factors considered most significant in the development of anthelmintic resistance have been the excessive frequency of treatments and administration of inadequate anthelmintic doses.

Given that the use of anthelmintics is required for an efficient and sustainable control of helminth parasites, the knowledge of the levels of resistance to the drugs commonly used will lead to the implementation of adequate zoo technical management practices to mitigate the spread of anthelmintic resistance. The objective of this study, therefore, was to assess the efficacy of albendazole, the most frequently used anthelmintic, through the faecal egg count reduction test, in goats in Maputo and Gaza provinces, two of the 11 provinces of Mozambique.

MATERIALS AND METHODS

Study locations and animals

The study was carried out between November and December 2016, in seven districts of the southern region of Mozambique, namely, Xai-Xai (farms G1 and G2), Chibuto (farms G3 and G4) and Chókwe (farm G5) in Gaza province, and in Magude (farm G6), Namaacha (farm G7), Boane (farms G8 and G9) and Moamba (farms G10 and G11) in Maputo province. Mozambique is located on the eastern coast of Africa, between Latitudes 10° 20' S and 26° 50' S. The study areas were located between Latitudes 24° 38' S and 26° 02' S, and between Longitudes 33° 39' E and 32° 07' E (Figure 1). The study areas were characterized by a tropical dry climate influenced by the motions of the Indian Ocean, with a hot rainy season from October to March and a cool dry season from April to September. The average maximum temperature is 30°C in January/February, with a maximum of 43°C in January, and the minimum average temperature is 15°C in June/July. The mean maximum precipitation is 152 mm in January, and the minimum average precipitation is 10 mm in June/August (Diniz et al., 2012).

Eleven farms, five in Gaza province and six in Maputo province comprising 1,040 goats, were selected. The number of goats in each of the flocks varied from 26 to 180 animals, and three flocks, two in Gaza (G1 and G3) and one in Maputo (G8), were kept extensively on communal grazing without supplementation throughout the year. The rest of the flocks were kept semi-intensively on private delimited pasture areas with supplementation during the dry season. For the study purpose, only 26 to 30 goats were randomly allocated to two groups of 13 to 15 animals in each flock; thus, a total of 355 goats were surveyed. In order to assess the profile of the zoo technical management systems, a questionnaire was administered to the owners or managers of the farms.

Faecal examination

Faecal collection was done directly from the animals' rectal bulb in order to avoid contamination, and the samples were kept cooled in Coleman thermoelectric cooler until the determination of the eggs per gram of faeces (EPG) was performed at the Central Veterinary Laboratory, Animal Sciences Directorate of the Agricultural Research Institute of Mozambique (IIAM) in Maputo province, and at the Provincial Veterinary Laboratory in Gaza province, respectively.

Reinecke's (1961) modification of the McMaster technique was used for the quantitative determination of nematode eggs in the faecal samples. The number of nematode EPG was calculated using the formula: $EPG = Te_1 + Te_2 \times 100$, where Te_1 is the number of eggs in chamber 1 and Te_2 is the number of eggs in chamber 2 of the McMaster slide (Ueno and Gonçalves, 1998).

One hundred larvae per culture were identified (Gupta and Singla 2012), unless there were fewer than 100 in which case all were identified, using the descriptions of Georgi et al. (1985) and Ueno and Gonçalves (1998).

Determining anthelmintic resistance

The efficacy of oral suspensions of albendazole (5 mg/kg body weight; Albenol-100®, Interchemie, Holland), which is frequently used in Mozambique, was assessed using the faecal egg count reduction test (FECRT). The latter was performed according to the methods recommended by the World Association for the Advancement of Veterinary Parasitology (Coles et al., 1992), described and updated by Coles et al.(2006) and interpreted using RESO® to determine anthelmintic resistance.

Once a flock was selected, 26 to 30 goats were randomly allocated to two groups of 13 to 15 animals each, identified with coded ear tags, and weighed. On each of the 11 farms, an untreated control group was formed to monitor the changes in the nematode egg counts during the test period and a treated group that received oral administration of albendazole (5 mg/kg body weight). Individual faecal egg counts and pooled larval cultures were performed from samples collected on the day of treatment and 14 days after the anthelmintic treatment.

Goats with less than 100 nematode EPG in their pre-treatment samples and those with missing values for either the pre- or post-treatment EPG were excluded from the analyses.

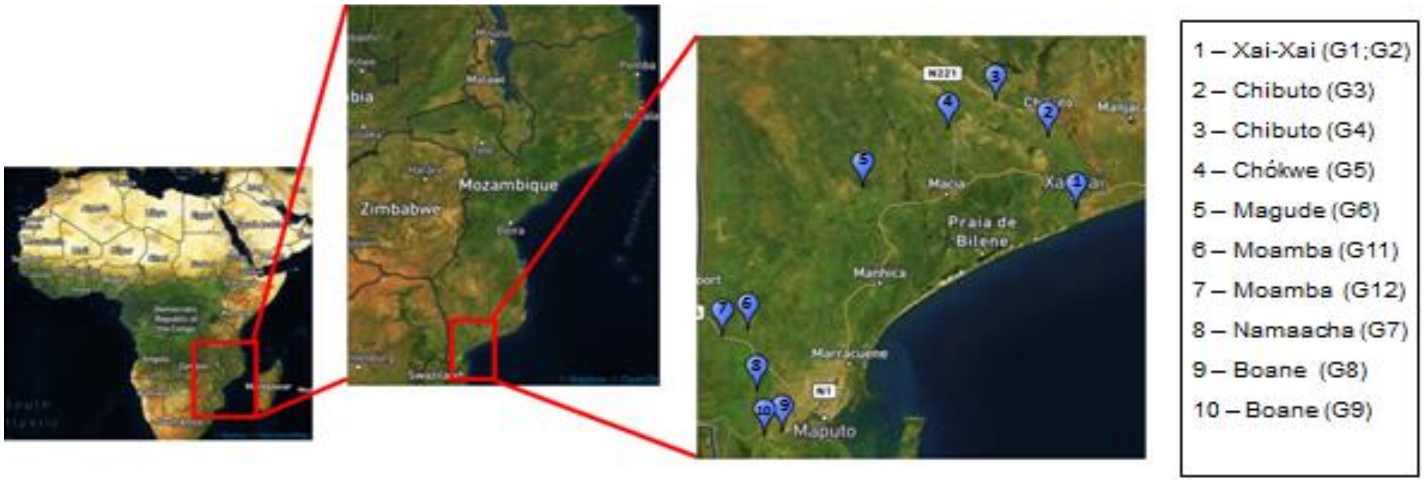

Figure 1. Map of Mozambique indicating the study locations, namely Xai-Xia (G1;G2) and Chokwe (G5) districts in Gaza province and Magude (G6), Namaacha (G7), Boane (G8;G9) and Moamba (G10;G11) districts in Maputo province.

The percentage faecal egg count reduction (%FECR) was calculated, as $100 \times (1 - T2/C2)$, where *T2* was the mean egg count of the treated group at day 14, and *C2* was the mean EPG count of the untreated control group 14 days after treatment. The flocks were considered resistant when the EPG reduction percentage was less than 95% and the CI's lower limit was less than 90%. They were considered suspicious if the flock only complied with some of the aforementioned criteria. Flocks that did not comply with any of the required criteria were considered susceptible (Coles et al., 1992, 2006; Canul-Ku et al., 2012).

Statistical analysis

The Pearson's Chi-square test was performed to check for possible differences between the animals surveyed with reference to an association between EPG and factors such as age, sex, and goat breeds. The comparison of susceptibility and resistance of gastrointestinal nematodes to albendazole between farms relating to the frequency of anthelmintic use, grazing system, location at provincial, district, and locality levels was also performed using the Pearson's Chi-square test with 95% confidence interval (CI). All statistical analyses were performed using SPSS version 21, and $p < 0.05$ was considered significant.

RESULTS

Questionnaire

The analysis of the zoo technical management information as per the questionnaire administered indicated that the main objective in all of the flocks (100%) was to produce goat meat. The animals were kept either in communal or private pastures during day hours and shared the pastures with cattle and/or sheep. The animals were reared under extensive systems in communal pastures during the day and kept in corrals at night in 27.3% of the farms (flocks G1 and G3 in Gaza and flock G7 in Maputo). In the rest of the flocks (72.7%), goats were reared under semi-intensive systems in private pastures and kept in cement or metal slabs at night.

Drenching of animals with anthelmintics was based on visual estimative weight in 90.9% (10/11) of the farms except in flock G7 in Maputo, where animals were weighed to determine the adequate anthelmintic dosage. In 81.8% (9/11) of the farms, all animals were drenched with anthelmintics on each occasion, and no EPG tests were performed to decide which animals should be drenched in all farms. Anthelmintics were chosen without any criteria, no drug combination was used, and no quarantine was undertaken in case of acquiring animals from other countries or from other locations within the country in all the studied farms. The data from the questionnaire indicated also that most of the farms had poor management practices, including inadequate disease control measures, inadequate nutrition during the dry season, and face disease challenges, mainly gastrointestinal parasitism.

Faecal egg counts, percentage faecal egg count reduction, statistical analysis, and larval cultures

The arithmetic means of the faecal egg counts before and after treatment for each of the eleven flocks surveyed in Gaza and Maputo provinces and the %FECR are presented in Tables 1 and 2, respectively. According to Coles et al. (1992), flocks are considered resistant when %FECR is less than 95. Resistance to albendazole was detected in 60% (3/5) of the farms in Gaza and 83.3% (5/6) of the farms in Maputo. The %FECR varied from 0 to 100% in Gaza and from 51 to 97% in Maputo.

Possible differences between the animals surveyed with reference to the EPG and factors such as age, sex and goat breeds were assessed using the Pearson's Chi-square test. No statistical difference was found between

Table 1. The arithmetic means of faecal nematode egg counts before and after treatment and %FECR of five farms (G1 to G5) in Gaza province.

Farm no.	Control			Albendazole			% FECR
	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	
G1	12	425	567	10	560	20	96
G2	13	846	1885	14	707	593	0[R]
G3	12	658	1092	11	786	0	100
G4	11	355	500	12	708	375	34[R]
G5	11	1345	1218	13	1138	577	0[R]

R, Resistant.

Table 2. The arithmetic means of faecal nematode egg counts before and after treatment and %FECR of six farms (G6 to G11) in Maputo province.

Farm no.	Control			Albendazole			% FECR
	No. of goats	Before treatment	After treatment	No. of goats	Before treatment	After treatment	
G6	12	417	500	12	492	183	68 [R]
G7	11	391	245	13	400	108	81[R]
G8	11	245	245	11	209	18	97
G9	12	908	2025	14	886	279	51[R]
G10	10	250	360	11	218	182	68[R]
G11	10	240	290	10	580	160	72 [R]

R, Resistant.

Table 3. The percentages of gastrointestinal nematode species found in pre-treatment and post-treatment larval cultures on five flocks tested in Gaza province.

Farm no.	***Haemonchus* spp. (%)**		***Oesophagostomum* spp. (%)**		***Trichostrongylus* spp. (%)**		***Strongyloides* spp. (%)**	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
G1	37	0	4	0	59	0	0	0
G2	40	84	26	1	31	6	3	9
G3	65	0	24	0	6	0	5	0
G4	95	100	1	0	2	0	2	0
G5	88	100	10	0	0	0	2	0

the animals surveyed in terms of an association between EPG and all analysed factors (p>0.05).

In the pre-treatment larval cultures, *Haemonchus* spp. (37 to 95% in Gaza and 19 to 100% in Maputo) and *Oesophagostomum* spp. (up to 26%) were the predominant nematode species, while *Trichostrongylus* spp. And *Strongyloides* spp. (except flock G9) were present in small numbers, except in two flocks (G1 and G2) in Gaza province where *Trichostrongylus* spp. appeared in large numbers (31 and 59%) as indicated in Tables 3 and 4. Post-treatment faecal cultures indicated that *Haemonchus* spp., and to a lesser extent *Oesophagostomum* spp. and *Trichostrongylus* spp., were resistant to albendazole. However, *Strongyloides* spp. appeared in large numbers in the post-treatment larval cultures of three flocks, one in Gaza (G2) and two in Maputo (G7 and G9).

DISCUSSION

The present study demonstrated that goats from all the flocks surveyed in seven districts of Gaza and Maputo provinces were infected with gastrointestinal nematodes,

Table 4. The percentages of gastrointestinal nematode species found in pre-treatment and post-treatment larval cultures on four of the six flocks tested in Maputo province.

Farm No.	*Haemonchus* spp. (%)		*Oesophagostomum* spp. (%)		*Trichostrongylus* spp. (%)		*Strongyloides* spp. (%)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
G6	58	50	26	50	0	0	16	0
G7	88	100	8	0	4	0	0	9
G8	*	*	*	*	*	*	*	*
G9	19	7.5	2	0	2	0	79	92.5
G10	100	100	0	0	0	0	0	0
G11	*	*	*	*	*	*	*	*

*The larvae collected from coprocultures of the two flocks were reserved for molecular diagnostics since the numbers of larvae were too small.

and eight of the flocks were considered resistant to albendazole.

Knowledge about the species of the gastrointestinal nematodes present and the anthelmintic resistance status of the farms is important for performing control and treatment strategies, and to identify where alternative control measures should be used (Burgess et al., 2012). Laboratory analyses should include knowledge on the reality in the field to make the application of intervention strategies feasible for the farmers (Salgado and Santos, 2016). The faecal egg counts are the most used *in vivo* techniques for detecting infections by nematode parasites, and they help farmers decide when and whether or not to drench animals (Lira, 2005). The chief method for the detection of resistance remains the FECRT, which can be used for all anthelmintic groups. The FECRT estimates anthelmintic efficacy of one or more drugs by comparing the faecal egg counts of animals at the time of treatment and at defined times after treatment, depending on the anthelmintic group used (Coles et al., 1992; Pena-Espinoza et al., 2014). Although the test is considered reliable only if more than 25% of the worms are resistant (Martin et al., 1989; Coles et al., 2006), the FECRT is still the most widely used and most feasible test and is considered the gold standard for detecting anthelmintic resistance *in vivo*.

On all the farms where resistance of the worms to albendazole was detected in both provinces, albendazole had been used over long durations, and the owners had been complaining of the failure of this drug to control nematode infections. Therefore, some of them have dismissed albendazole and used ivermectin or moxidectin to drench animals. According to Van Wyk (2001) and Pena-Espinoza et al. (2014), frequent anthelmintic treatments, use of anthelmintics with similar mode of action for several years, and underdosing are some of the factors that contribute to the development of resistance. Thus, inappropriate use of anthelmintic drugs in small ruminants has led to failures in their effectiveness, leading to a global constraint of anthelmintic resistance (Salgado and Santos, 2016).

On the three farms (G2, G4 and G5) in Gaza province, where resistance to albendazole was detected, most of the goats (mainly Boer breed) were imported from Zimbabwe and South Africa, while the goats (Kalahari red breed) were imported from South Africa in farm G11 in Maputo province. An important feature to note in the referred farms is that animals were neither kept in quarantine nor dewormed after they had been imported before introducing them to their new farms. Shalaby (2013) indicated that introducing newly acquired animals to a farm without prior quarantine and deworming constitutes a faster way to spread resistance of gastrointestinal nematodes.

Indiscriminate use of anthelmintics to treat the entire flocks was observed in the surveyed farms, and this may have probably contributed to the higher levels of anthelmintic resistance in the present study. Das Neves et al. (2014) suggested that the low cost and practicability of anthelmintic administration encourage farmers to treat entire flocks irrespective of the individual's needs, thus resulting in the emergence and rapid spread anthelmintic resistance of gastrointestinal nematodes. Geary et al. (2012) also suggested that, although unsustainable with regard to selection for anthelmintic resistance, routine treatment of the entire flock rather than selective treatment of individuals has become a common practice. Therefore, refugia-based drenching regimes have been widely recommended to slow down the development of anthelmintic resistance (Van Wyk, 2001; Kenyon et al., 2013). Based on the levels of resistance to albendazole detected in the farms studied, "refugia" should be explored to prevent and delay the development of anthelmintic resistance, and even to reverse the situation in flocks where resistance levels are already high.

The occurrence of different levels of resistance to benzimidazoles in gastrointestinal nematode parasites, as evidenced by the FECRT results, is in agreement with other studies undertaken elsewhere, including Denmark (Pena-Espinoza et al., 2014), India (Chaudhry et al. (2015), Mexico (Herrera-Manzanilla et al., 2017), North Ireland (McMahon et al., 2013) and Scotland (Kenyon et al., 2013), where resistance of *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp. to benzimidazoles was registered. Kumsa and Abebe (2009) reported anthelmintic resistance in *Haemonchus* spp. to albendazole and tetramisole in goats in Southern Ethiopia. Chagas et al. (2013) reported the presence of a multiple-resistant strain of *H. contortus* in Southeast Brazil. Borges et al. (2015) reported the occurrence of resistance of *Haemonchus* spp. and *Trichostrongylus* spp. to albendazole in the northeast Bahia State in Brazil.

In the present study, resistance to albendazole was detected in eight (72.7%) of the 11 farms surveyed. The results indicated high levels of resistance to the tested anthelmintic, and demonstrated that there was an alarming increase in the resistance rate compared to the results reported by Atanásio et al. (2002).

Any viable eggs in post-treatment coprocultures indicate that some resistant worms may have been present in the animals at the time of treatment. Evidence of small percentage of survivors may indicate a resistance problem that could develop with further rounds of treatments, and should be monitored (Pena-Espinoza et al., 2014). Since large numbers of infective larvae of *Strongyloides* spp. in the post-treatment larval cultures compared with the pre-treatment larval cultures were observed only in three of the flocks (G2, G7, and G9), it was not possible to determine whether the resistance of this species to albendazole has occurred.

Conclusion

This study provided evidence that anthelmintic resistance of gastrointestinal nematode parasites in goats is currently a problem of great significance and imposes severe constraints on the production of small ruminants in this region of the country. Our findings were in agreement with previous studies that indicated the resistance to benzimidazoles as an emerging problem in southern Mozambique. Different control strategies or appropriate measures including sustainable control strategies using integrated approaches and an alternation strategy of drug groups in order to minimize the pressure for parasite adaptation must be taken to reverse the situation. Refugia-based anthelmintic treatments are recommended to slow down the development of resistance to drugs, and target selective treatments to extend the efficiency of anthelmintics mainly in the farms where the levels of resistance are low or anthelmintic drugs are still effective must be applied. Adoption of strict quarantine procedures should be instituted for all newly acquired animals to prevent the importation of resistant nematodes to the farms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Ethical approval

The clearance by the Ethical Commission on the Use of Animals at the School of Veterinary Medicine-Federal University of Bahia (UFBA), Brazil, has been registered under EMZV-UFBA No. 09/2017.

REFERENCES

Akkari H, Jebali J, Gharbi M, Mhadhbi M, Awadi S, Darghouth MA (2013). Epidemiological study ofsympatric *Haemonchus* species and genetic characterization of *Haemonchus contortus* in domestic ruminants in Tunisia. Vet. Parasitol.193:118-125.

Atanásio A, Boomker J, Sitoe C (2002). A survey on the occurrence of resistance to anthelmintics of gastrointestinal nematodes of goats in Mozambique. Onderstepoort J. Vet. Res. 69:215-220.

Borges SL, Oliveira AA, Mendonça LR, Lambert SM, Viana JM, Nishi SM, Julião FS, Almeida MAO (2015). Anthelmintic resistance in goat flocks from Caatinga and Mata Atlântica biomas.Pesq. Vet. Brasileira. 35:643-648.

Burgess CGS, Bartley Y, Redman E, Skuce PJ, Nath M, Whitelaw F, Tait A, Gilleard JS, Jackson F (2012). A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. Vet. Parasitol. 189:299-307.

Canul-Ku HI, Rodriguéz-Vivas RI, Torres-Acosta JFJ, Aguilar-Caballero AJ, Perez-Cogollo LC, Ojeda-Chi MM (2012). Prevalence of cattle herds with ivermectin resistant nematodes in the hot sub-humid tropics of Mexico. Vet. Parasitol. 183:292-298.

Chagas ACS, Katiki LM, Silva IC, Giglioti R, Esteves SN, Oliveira MC, Junior WB (2013). *Haemonchus contortus:* A multiple-resistant Brazilian isolate and the costs for its characterization and maintenance for research use. Parasitol. Int.62:1-6.

Chaudhry U, Redman EM, Ramen M, Gillead JS (2015). Genetic evidence for the spread of a benzimidazole resistance mutation across southern India from a single origin in the parasite nematode *Haemonchus contortus.* Int. J. Parasitol. 45:721-728.

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, Waller P (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.VP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44:35-44.

Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, Taylor MA, Vercruysse J (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol.136:167-185.

Das Neves JH, Carvalho N, Rinaldi L, Cringoli G, Amarante AFT (2014). Diagnosis of anthelmintic resistance in cattle in Brazil: A comparison of different methodologies. Vet. Parasitol. 206:216-226.

Diniz MA, Bandeira S, Eurico S, Martins ES (2012). Flora and Vegetation of Maputo province: Its appropriation by populations. Proceedings of International Congress on Tropical Knowledge in Mozambique: History, Memory and Science, IICT-JBT:1-2. Available at: https://2012congressomz.files.wordpress.com/2013/08/t10c01.pdf

FAO (2015). The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture [Scherf, B.D. and Pilling, D. (eds)]. FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. Available at: http://www.fao.org/3/a-i4787e.pdf.

Gaba S, Cabaret J, Chylinski C, Sauve C, Cortet J, Silvestre A (2012). Can efficient management of sheep gastrointestinal nematodes of sheep be based on random treatment? Vet. Parasitol.190:178-184.

Geary TG, Hosking BC, Skuce PJ, von Samson-Himmelstjerna G, Maeder S, Holdsworth P, Pomroy W, Vercruysse J, (2012). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guideline: Anthelmintic combination products targeting nematode infections of ruminants and horses. Vet. Parasitol.190:306-316.

Georgi JR, Theodorides VJ, Georgi ME (1985). Parasitology for Veterinarians. 4th Edition, Philadelphia: W.B. Saunders Company. Available at: https://www.abebooks.com/booksearch/isbn/9780721611761/

Gupta SK, Singla LD **(**2012) Diagnostic trends in parasitic diseases of animals. In: *Veterinary Diagnostics: Current Trends.* Gupta RP, Garg SR, Nehra V and Lather D (Eds), Satish Serial Publishing House, Delhi. pp: 81-112.

Herrera-Manzanilla FA, Ojeda-Robertos NF, González-Garduño R, Cámara-Sarmiento R, Torres-Acosta JFJ (2017).Gastrointestinal nematode populations with multiple anthelmintic resistance in sheep farms from the hot humid tropics of Mexico. Vet. Parasitol. 9:29-33.

Hussain T, Periasamy K, Nadeem A, Babar ME, Pichler R, Diallo A (2014). Sympatric species distribution, genetic diversityand population structure of *Haemonchus*isolates from domestic ruminants in Pakistan. Vet. Parasitol.206:188-199.

Kenyon F, McBean D, Greer AW, Burgess GS, Morrison AA, Bartley DJ, Bartley Y, Davin L, Nath M, Jackson F (2013). A comparative study of the effects of four treatment regimens on ivermectin efficacy, body weight and pasture contamination in lambs naturally infected with gastrointestinal nematodes in Scotland. Int. J. Parasitol. Drugs Drug Resist. 3:77-84.

Kumsa B, Abebe G (2009). Multiple anthelmintic resistance on a goat farm in Hawassa (southern Ethiopia). Trop. Anim. Health Prod. 41:655-662.

Lira CMD (2005). 'Willow fodder blocks for growth and sustainable management of internal parasites ingrazinglambs', MSc Thesis, Massey University, New Zealand.

Martin PJ, Anderson N, Jarrett RG (1989). Detecting benzimidazole resistance with faecal egg count reduction tests and in vitro assays. Aust. Vet. J. 1989 66:236-240.

McArthur MJ, Reinemeyer CR (2014). Herding the U.S. cattle industry towards a paradigm shift in parasite control. Vet. Parasitol. 204:34-43.

McMahon C, Barley JP, Edgar HWJ, Ellison SE, Hanna REB, Malone FE, Brennan GP, Fairweather I (2013). Anthelmintic resistance in Northern Ireland II: Variations in nematode control practices between lowland and upland sheep flocks. Vet. Parasitol. 192:173-182.

Pawar PD, Khasnis MW, Hong CD, Khole RP, Mhase PP, Singla LD (2017). Coproprevalence and successful management of parasitic infections in sheep in Pune district of Maharastra.. Proceedings of 25th National Congress of Veterinary Parasitology and International Symposium on Current Concepts in Diagnosis & Control of Parasitic Diseases to Combat Climat Change (15-17 February, 2017) at Veterinary College, Shimoga. P 56.

Pena-Espinoza M, Thamsborg SM, Demeler J, Enemar KH (2014). Field efficacy of four anthelmintics and confirmation of drug-resistant nematodes by controlled efficacy test and pyrosequencing on a sheep and goat farm in Denmark. Vet. Parasitol. 206:208-215.

Preston SJM, Sandeman M, Gonzalez J, Piedrafita D (2014). Current status for gastrointestinal nematode diagnosis in small ruminants: where are we and where are we going? J. Immunol. Res. 2014:1-12.

Reinecke RK (1961). Helminth research in South Africa III. The diagnosis of nematode parasites in ruminants for worm survey purposes. J. South African Vet. Assoc.37:27-31.

Salgado JA, Santos CP (2016). Overview of anthelmintic resistance of gastrointestinal nematodes of small ruminants in Brazil. Rev. Brasileira Parasitol. Vet. 25(1):3-17.

Shalaby HA (2013). Anthelmintic resistance; How to overcome it? Iranian J. Parasitol. 8:18-32.

Shija DSN, Kusiluka LJM, Chenyambuga SW, Shayo D, Lekule FP (2014). Animal health constraints in dairy goats kept under smallholder farming systems in Kongwa and Mvomero Districts, Tanzania. J. Vet. Med. Anim. Health 6(11):268-279.

Singh E, Kaur P, Singla LD**,** Bal MS (2017a). Prevalence of gastrointestinal parasitism in small ruminants in western zone of Punjab, India. Vet. World 10(1):61-66.

Singh R, Bal MS, Singla LD, Kaur P (2017b). Detection of anthelmintic resistance in sheep and goat against fenbendazole by faecal egg count reduction test. J. Parasitic Dis. 41(2):463-466.

Sissay MM, Uggla A, Waller PJ (2007). Prevalence and seasonal incidence of nematode parasites and fluke infections of sheep and goats in eastern Ethiopia. Trop. Anim. Health Prod. 39:521-531.

Ueno H, Gonçalves PC (1998). Handbook for diagnosis of helminthoses of ruminants. 4th Edition, Tokyo, JP: Japan International Cooperation, P 143. Available at: https://r1.ufrrj.br/adivaldofonseca/wp-content/uploads/2014/06/manual_helmintoses-UENO-site-do-CBPV.pdf

Van Wyk JA (2001). Refugia-overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. Onderstpoort J. Vet. Res.68:55-67.

Van Wyk, JA, Malan, FS, Randles, JL (1997). How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics?. Vet. Parasitol. 70:111-122.

Wondimu A, Gutu S (2017). Gastrointestinal nematodes of small ruminants in Guto Gida District, East Wolloega, Ethiopia. J. Vet. Med. Anim. Health 9:83-87.

Zvinorova PI, Halimani TE, Muchadeyi FC, Matika O, Riggio V, Dzama K (2016). Breeding for resistance to gastrointestinal nematodes- the potential in low-input/output small ruminant production systems. Vet. Parasitol. 225:19-28.