

COMPARATIVE EFFICACY OF TWO DIFFERENT BRANDS OF IVERMECTIN AGAINST GASTROINTESTINAL NEMATODES AND ECTOPARASITES OF SHEEP IN GONDAR TOWN, NORTHWEST ETHIOPIA

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ABSTRACT: Gastrointestinal nematodes and ectoparasites are endemic in Ethiopia. Giving appropriate treatment for these infestations will reduce the damage that could occur from them. The current experimental study aimed to see the efficacy of two Ivermectin formulations for the purpose of this parasite in Gondar town. For the study 58 local sheep aged from 6 months to 2 years from small holder farmers were selected and randomly allocated as two treatment and one control groups. Ivermectin bolus and injection formulations were used for this efficacy assessment. For assessment of efficacy against gastrointestinal nematodes, 45 sheep were used and divided into three groups; for ectoparasite evaluation, 13 sheep were involved. Feces from each sheep were taken before and after each drug administration and egg per gram of feces were determined and larval cultures were done on day zero before treatment and on day 14 post-treatment. Ivermectin efficacy was investigated by the Fecal Egg Count Reduction Test (FECRT). Ivermectin injection and bolus were reduced FEC by 95.06% (95%CI: 87.8, 98.5%) and 98.8% (95%CI: 90.3, 100%), respectively. The therapeutic efficacies of both ivermectin formulations against ticks and lice infestations were 100% after 7 days of treatment and remained effective up to 28th days of post treatment. To the contrary, these parasites were increased gradually on 7th, 14th, 21st and 28th days of post treatment in the control group. Coproculture revealed four GIN genera which were *Haemonchus*, *Trichostrongylus*, *Oesophagostomum* and *Trichuris*. The identified genera of ticks and louse were *Bophilus* and *Linognathus*, respectively. The bolus form of ivermectin showed better efficacy against nematodes than the injectable and ectoparasites were cleared totally by the drug. Detailed studies are suggested to verify the efficacy of the formulations and searching optional drugs for those developing resistance.

Keywords: Ectoparasite, Efficacy, Gastrointestinal nematode, Ivermectin

INTRODUCTION

In Ethiopia, small ruminants are important sources of income for the rural communities, however they are affected by many factors (Abebe and Esayas, 2001; Biffa et al., 2006; Sisay et al., 2006). Studies in different parts of the country have shown that ovine gastrointestinal parasites are found to be major problems that are causing considerable losses (Abebe and Esayas, 2001; Biffa et al., 2006; Tembely et al., 1997). There are over 20 different species of gastrointestinal nematodes (GINs) of sheep that can cause clinical or subclinical disease with reduced growth rate, body condition and milk production. Of these, *Haemonchus*, *Strongyloides*, *Trichostrongylus*, *Nematodirus*, and *Trichuris* are the major gastrointestinal nematode species reported in sheep (Razzaq et al., 2013). Besides, GINs, ectoparasites are very common and widely distributed in all agro-ecological zones of Ethiopia (Kumssa and Mekonnen, 2011; Kumssa et al., 2012), causing a wide range of health problems that confront sheep productivity. It is reported that 35% of sheep and 56% of goat skin rejections in Ethiopia are attributed to ectoparasites (Kassa, 2006).

Several anthelmintic preparations imported and distributed in the country; majority of them have been utilized frequently for two or more decades; possible cause of drug resistance (George et al., 2011) and the increase in the prevalence of GIN infections could also be attributed to the development of anthelmintic resistance (AR) (Martínez-Valladares et al., 2015). In small ruminants, GINs can generally be controlled using broad-spectrum

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anthelmintics. Macrocyclic lactones (ivermectin and milbemycins), benzimidazole, and imidazothiazoles levamisole and hydroxyrimidines (pyrantel/morantel) are some of the commonly used anthelmintics in Ethiopia. Among these anthelmintics ivermectin is the one that display a novel mode of action against nematode and arthropod parasites of animals. Its broad spectrum activity against GINs (Egerton et al., 1997) and ectoparasites (Cambell et al., 1983); with a wide safety of margin has made it the drug of choice in cattle, sheep, goat, swine and horses (Ademola et al., 2003). However, in Ethiopia, there are limited reports on the efficacy of these anthelmintics against economically important parasites (Bersissa and Abebe, 2006) and there is no published report on the comparative efficacy of two different brands of ivermectin to control GINs as well as ectoparasites of sheep in North Gondar as well as in Ethiopia. Therefore, the study aimed to evaluate the efficacy of Ivermectin formulations for the treatment of gastrointestinal nematodes and ectoparasites of sheep in Gondar town, Northwestern parts of Amhara regional state, Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in Gondar town; Amhara region, northwest Ethiopia from January 2016 to April 2016. Gondar town is located on 35° 7' N and 13° 8' E and lies at an altitude of 2,200 meter above sea level. It is found 748km north of Addis Ababa. The area receives mean annual rain fall of 1,172 mm mainly in rainy season with average temperature of 19.7 °C. Being a highland area, the city is spread on different mountains, slopes and in valleys and has three small rivers, many streams and a lake. According to Zone Office of Agriculture and Rural Development, the population size of Gondar town in 2008 is about 112,249 out of which 60,883 are males and 51,366 are females [17-18]. The livestock population in North Gondar zone comprises of cattle (2, 771, 7 0 1), sheep (815, 7 1 6), goats (1,251,867), horses (27,248), mules (9, 695) and donkeys (376,841) (CSA, 2013).

Study animals and management

A total of 58 local breed sheep, kept under semi intensive management system sourced from private smallholder farmers with the age between six month and two years were used for the study. The sheep were purposively selected, which have not been treated in the previous 8 to 12 weeks were considered for the study. A young lamb that was not weaned and did not begin feeding on pasture was totally excluded from ivermectin efficacy trial. Sheep allocated for the study were identified by ear tags. Altogether those animals were shepherded together for the most part and grazed on permanent communal pastures. The animals also shared the same watering point during day time and housed in pens during night on their respective farms. The production system was based on traditional practices and no controlled mating (Eda, 2012), breeding occurring year round with two lambing cycles a year for many ewes. Feed supplements were not known except hay, crop residues which were available after harvesting season and a rare supply of common salts (NaCl) for selected animals. The ethical clearance was obtained from the ethical review committee of the college of Veterinary Medicine and Animal Sciences, University of Gondar and permission was obtained from the sheep owners.

Study design and treatment of animals

An experimental study design was conducted from January 2016 to April 2016 to investigate the efficacy of different brands of ivermectin against gastrointestinal nematodes and ectoparasites through fecal egg count reduction test and ectoparasite number with direct physical or visual examination, respectively in naturally infected sheep in Gondar town. Before the experiment fecal samples were collected from all sheep and examined for nematode infestations. The positive sheep were randomly allocated into three experimental groups. The first group was treated with ivermectin of china (1ml/50kg BW) subcutaneously; the second with ivermectin of India in the form of bolus (200mcg/kg BW) orally and the third was left untreated (control). The randomization also holds true for the purpose of the drug efficacy trail for ectoparasites. A control (untreated) group should be used to allow for natural changes in egg/ectoparasite counts during the test.

Sampling procedures and laboratory investigation

Fecal samples were collected from each animal for pre-screening of animals for sufficient egg counts. Feces were collected from each animal directly from the rectum using rubber gloves. The same procedure was followed at the post-treatment sampling. Samples were placed in individually sealed containers and labeled with specific identification mark, then return rapidly to the University of Gondar parasitological laboratory for egg counts. Fecal specimen was also subjected to coprological examination using standard fecal examination techniques (Shah-Fischer and Say, 1989; Hansen and Perry, 1994). Positive samples for parasite eggs was subjected to eggs per gram (EPG) determination using Modified McMaster egg counting method described by Coles et al. (1992).

Many nematode eggs are alike and species differentiations for the genera of *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Bunostomum*, and *Oesophagostomum* cannot be clearly differentiated from the eggs in fecal samples. These parasites, differentiation can be achieved by the use of fecal cultures. Fecal samples from each sheep were collected on each sampling day (before and after treatment) and composite fecal cultures were made for each group. Small amount of water was added to moisten, and the samples left for 14 days at room temperature in sampling bottle by adding small amounts of water as necessary. Third stage larvae (L3) were recovered from the cultures by the Wide-mouthed Jar (Bayou, 2005), differentiated to the generic level, and identified under a compound microscope (at magnification 40x) using morphological keys (MAFF, 1971; Van Wyk et al., 2004).

The ectoparasites (ticks and lice) were detected by physical examination of the animals and the presences of the ectoparasites were visualized and recorded. Tick and lice infestations were examined physically on individual animals. These parasites were counted on different body regions (abdominal and thigh region) and an area of 5×4 i.e. 20 square cm was selected. The selected areas were marked with a permanent color and ticks and lice within this area were counted before treatment (day 0) and post-treatment (7th, 14th, 21st and 28th day) periods. The severity of infestation of ectoparasites (ticks and lice) was observed by counting the number of ectoparasites in a selected area of the individual sheep. Ectoparasites including ticks and lice were collected by hand from their attachment sites, put in universal bottle containing 70% methanol (Soulsby, 1982). Samples were then transported to University of Gondar, Veterinary parasitological laboratory for further identification of the parasites. Tick and lice specimens were placed on Petri-dish and examined under stereomicroscope for morphological species classifications (Walker et al., 2003; Wall and Shearer, 2001).

Fecal egg count reduction test

Fecal egg count reduction test (FECRT) was used for the evaluation of the effects of the drug. The most commonly used field detection methods for anthelmintic resistance or efficacy is the fecal egg count reduction test (FECRT). This method can be adapted for use as a screening agent for Veterinarians and producers to identify less than desired clearance of the parasites after anthelmintic treatment (Gasbarre et al., 2009). The procedure compares the pre-treatment parasite level with the parasite levels after treatment. The efficacy of two brands of ivermectin was determined by comparing the FECRT from a group of animals before and after treatment (Coles et al., 2006).

Arithmetic means of pre and post treatment FECs were used to calculate the percentage efficacy of ivermectin using a formula; $FECR\% = 100 (1 - T_2/C_2)$ where T₂ and C₂ are arithmetic mean egg per gram of feces (EPG) in the treated and untreated groups, respectively at day 14 post treatment. The efficacy of each ivermectin formulation was tested and interpreted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for efficacy evaluations of anthelmintics. Similarly anthelmintic effectiveness was based on the FECR (%) and the lower 95% confidence limits. Reduction in the efficacy and presence of anthelmintic resistance is considered to exist, if the FECRT percentage of an anthelmintic treatment is < 95% and the lower confidence limit for the reductions is <90% (Coles et al., 1992). If only one of the two criteria is met, reduction in efficacy is suspected.

Efficacy of ivermectin against ectoparasite

In this study the efficacy of ivermectin is assessed by the comparison of ectoparasite numbers on groups of treated and untreated sheep. Ectoparasite (tick and louse) counts should be conducted on day 1 or day 0 prior to treatment, on day 7 and at 7-day intervals thereafter until study termination period. Geometric means of pre and post treatment ectoparasite counts were used to calculate the percentage efficacy of ivermectin using a formula as described by Abbott's formula: $100 [(C - T)/C]$ where parasite mean counts of the treated (T) and control (C) occur (Abbott, 1925). In contrast to endoparasites, where an accurate measure of present populations can be made at necropsy, the assessment of louse and tick burdens by examination of selected areas of skin surface is less precise. Therefore, a measurement of response to treatment requires the repeated examination of parasitized areas or previously parasitized areas of the skin surface.

Data management and analysis

While collecting fecal samples from study animals, all data was recorded with pre-designed format and entered into computer using Microsoft excel spread sheet. Mean, standard deviation and reduction percentages were calculated through descriptive statistics. Means of egg count reduction were compared among groups through analysis of variance (One-Way ANOVA) and the difference between treatments was compared using least square method of multiple comparisons. Differences considered significant when P<0.05. Statistical Package for Social Sciences (SPSS) version 20 was used to analyze the data.

RESULTS

Ivermectin efficacy against gastrointestinal nematodes

Mean fecal egg counts and percent reduction. The reduction in mean fecal EPG, after 14 days of post treatment, for ivermectin bolus and injection were 98.8% and 95.06%, respectively. The pre-treatment and post-treatment egg count means, standard deviation and the per cent reduction in the fecal egg counts are presented in (Table 1). There was no statistically significant difference ($P>0.05$) between the egg count of control and treated groups as well as between the two treated groups, ivermectin bolus treated and ivermectin injection treated, before treatment. Statistically post-treatment egg counts and percentage reduction of the drugs were significant ($P=0.04$) between treatment groups as well as there were strict differences ($P=0.00$) in net egg count between treatment and control groups on the post-treatment (Table 2).

Survivor parasites after treatment. Fecal culture was conducted parallel to fecal egg count to differentiate strongly type of eggs both in before and after treatments in each group.

Ivermectin efficacy against ectoparasites

Ivermectin recommended oral dose (200mcg/kg, BW and 0.02ml/kg, BW subcutaneous injection were found 100% effective against tick and lice infestation in sheep. No tick and lice were found within the selected area of sheep on 7, 14, 21, and 28 days after the treatment in both the ivermectin bolus and injection. On the other hand, in the control group, the number of tick and lice increased gradually on 7, 14, 21, and 28 day of treatment (Table 3). The adult ectoparasites (tick and lice) from the control groups were identified at the genus level (Table 4).

Table 1 - Mean fecal egg count and egg count percent reduction by Ivermectin (n=15)

Ivermectin formulations	Mean FEC±STDEV		Reduction (%)	95% Confidence Interval	
	Pre treatment	Post treatment	Mean	Lower	Upper
Bolus	573.3±284.02	6.67±25.82	98.8	90.3	100
Injection	620±298.09	26.67±59.4	95.06	87.8	98.5
Control	420±169.87	540±206.33	NA	NA	NA

FEC: Fecal Egg Count, NA: Not Applicable, n: Number of sheep in each group, STDEV: Standard Deviation

Table 2 - Parasites (GINs) identified in control and treatment groups after ivermectin treatment (N=15)

Group	Ivermectin	Parasite
1	Bolus	NL
2	Injection	<i>Trichuris, Haemonchus,</i>
3	Untreated control	<i>Haemonchus, Trichuris, Oesophagostomum, Trichostrongylus, Strongyloides</i>

N: Number of sheep in each group, NL: No Larvae

Table 3 - Efficacy of Ivermectin (bolus and injection) against ectoparasitic infestation in sheep

Ivermectin	Ectoparasites	Number of ectoparasites (mean± STDEV) (n=5/3)				
		Pre-treatment	Post-treatment period			
		day 0	Day 7	Day 14	Day 21	Day 28
Bolus	Tick	3.4±1.14	Nil	Nil	Nil	Nil
	Lice	5.3±2.1	Nil	Nil	Nil	Nil
Injection	Tick	4.6±2.3	Nil	Nil	Nil	Nil
	Lice	4.8±1.3	Nil	Nil	Nil	Nil
Control	Tick	3.3±1.5	4.3±1.2	6±2.7	6.8±2.9	8.3±4.1
	Lice	4.1±1.16	5.5±2.2	5.8±2.3	7.2±3.5	10±4.9

N: Number of sheep; STDEV: Standard Deviation; Nil: zero (0)

Table 4 - Ectoparasites identified on the control groups of sheep

Group	Ivermectin	Survived ectoparasites
1	Bolus	0
2	Injection	0
3	Untreated control	<i>Bophilus</i> and <i>Linognathus</i>

DISCUSSION

The result showed that the mean fecal egg count reduction test value of ivermectin bolus and injection were 98.8 and 95.06%, respectively. The FECR test indicated that based on World Association for the Advancement of Veterinary Parasitology guidelines of Coles et al. (1992), the ivermectin bolus was effective against GINs of sheep whereas ivermectin injection was suspected reduction in efficacy. This guideline states, Resistance is considered if the percentage reduction in egg counts is less than 95% and the lower 95% confidence level is less than 90%. If only one of the two criteria is met, resistance is suspected. In this study the suspected resistance in the case of ivermectin injection may be due to prior under dosing, lack of anthelmintic class rotation and a high treatment frequency, alone or in combination as it was reported to increase the risk of anthelmintic resistance in Ethiopia, Uganda and in South Africa, and it was evidenced by Macro-cyclic lactones resistance in South Africa (Kumssa and Abebe, 2009; Byaruhanga and Okwee-Acai, 2013; Tsotetsi et al., 2013; Carmichael et al., 1987).

The reason for better efficacy in ivermectin bolus in the present study was probably due to recent introduction and low frequency of treatment. Therefore, majority of nematode parasite populations in sheep remained unexposed to anthelmintic selection, thus remaining susceptible. This is in agreement with work done by Domke et al. (2012) said that limited use of ivermectin seemed to have prevented the development of anthelmintics resistance in GINs of small ruminant in Norway. However, macrocyclic lactone resistance in *Haemonchus* was reported in South Africa (Tsotetsi et al., 2013) and Australia (Jabbar et al., 2013) due to extensive and prolonged use of macrocyclic lactones.

The effectiveness of ivermectin bolus (200µg/kg BW) and injection (0.02ml/kg BW) against ticks and lice were found to be 100% effective at 7th post treatment day and remained effective up to 28th day of post treatment. This result was in agreement with the earlier record made by different authors (Hanif et al., 2005; Aziz et al., 2012; Hassan et al., 2012; Fahima, 2003; Yazwinski et al., 1997; Sangwan et al., 1995; Vizzio and Caro, 1995; Pedroso et al., 1994; Thompson et al., 1994). However, some studies have shown 86.6% (Umur et al., 1993) and 90% (Imrul, 1997) efficacy of ivermectin in sheep and goat, respectively. The intensive use of anthelmintics has led to the development of anthelmintic resistance in small ruminants (Kaplan, 2004; Wolstenholme et al., 2004). Generally, the most likely explanation for the variation in efficacy of ivermectin was probably due to variation in ingredients, prolonged use without rotation, frequent dosing, and incorrect weight calculation which gives a risk of under-dosing that in turn results resistance.

CONCLUSION AND RECOMMENDATIONS

The study revealed that Ivermectin bolus was effective to treat GINs compared to the injectable formulation; whereas both were effective against ectoparasites. Apart from its effectiveness resistant parasites were also recorded, which might contribute for the dynamics of parasites epidemiology and this could urge seeking of optional drugs. Therefore, based on the above concise conclusion newly produced and/or introduced anthelmintics, regular monitoring for anthelmintic resistance, proper use of effective anthelmintics, awareness creation to the farming communities and further studies regarding the drug efficacies are suggested.

DECLARATIONS

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Authors' Contributions

W.A. and A.M. prepared the proposal. W.A. conduct the research and collect the data while W.A., A.M. and A.D. participated in the data analysis and writing of the manuscript. N.M. donated the drugs for the efficacy trial.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

- Abbott WS (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*, 18: 265–267.
- Abebe W, Esayas G (2001). Survey of ovine and caprine gastrointestinal helminthosis in eastern part of Ethiopia during the dry season of the year. *Rev. Med. Vet*, 152: 379-384.
- Ademola O, Benjamin F, Fagbemi O, Olakunle S (2003). Comparative in-vitro studies on the efficacy of ivermectin against gastrointestinal sheep nematode. *Tropical Journal of Pharmaceutical Research*, 2 (2): 235-238.
- Aziz FB, Awal MA, Hasan Q, Rahman MM (2012). Comparative Efficacy of Ivermectin and Diazinon against Ectoparasites in Sheep. *J Anim Vet Adv*, 37: 398-46.
- Bayou K (2005). Standard Veterinary Laboratory Diagnostic manual: Parasitology. Ethiopian agricultural Research Organization: National Animal Health Research Center, Addis Ababa. 3: 1-8.
- Bersissa K, Abebe W (2006). Efficacy of Albendazole and Tetramisole anthelmintics against *Haemonchus contortus* in experimentally infected Lambs. *Intern J Appl Res Vet Med*, No.4, p.2.
- Biffa D, Jobre Y, Chaka H (2006). Ovine helminthosis, a major health constraint to productivity of sheep in Ethiopia. *Anim. Health Res. Rev*.7: 107-118.
- Byaruhanga C, Okwee-Acai J (2013). Efficacy of albendazole, levamisole and ivermectin against gastrointestinal nematodes in naturally infected goats at the National Semi-arid Resources. Research Institute, Serere, Uganda. *Veterinary Parasitology*, 195: 183-186.
- Campbell WC, Fisher MH, Stapley EO, Albers-Schonberg G, Jacob TA (1983). Ivermectin: A potent new antiparasitic agent. *Science* 221(4513): 823–828.
- Carmichael I, Visser R, Schneider D, Soll M (1987). *Haemonchus contortus* resistance to ivermectin. *J. S. Afr. Vet. Assoc*, 58, p. 93.
- Central Statistical Agency (CSA) (2013) Agricultural Sample Survey, 2012/13 (2005 E.C.), Volume II: Report on Livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 570. Federal Democratic Republic of Ethiopia, Addis Ababa.
- Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, et al (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, 44: 35-44.
- Coles GC, Jackson F, Pomroy W E, Prichard KR, Von Samson-Himmelstjerna G et al (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary parasitology*, 136: 167-185.
- Domke AV, Chartier C, Gjerde B, Höglund J, Leine N, et al (2012). Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. *Parasitology Research*, 111: 185-193.
- Edea Z, Haile A, Tibbo M, Sharma AK, Sölkner J, et al (2012). Sheep production systems and breeding practices of smallholders in western and south-western Ethiopia: Implications for designing community-based breeding strategies. *Livest. Res. Rural dev*. 24(7).
- Egerton JR, Ostlind DA, Blair LS, Eary CH, Suhayda D, (1979). Avermectins, new family of potent anthelmintic agents: Efficacy of the B1a component. *Antimicrobial Agents and Chemotherapy*, 15(3): 372–378.
- Fahima BA (2003). Comparative efficacy of Ivermectin and Diazinon against ectoparasite in sheep. MSc thesis Submitted to department of Pharmacology, BAU, Mymensingh, p. 48-80.
- Gasbarre CL, Smith LL, Lichtenfels RJ, Pilit AP (2009). The identification of cattle nematode parasites resistant to multiple classes of anthelmintics in a commercial cattle population in the US. *Veterinary Parasitology*, 166: 281-285.
- George N, Persad K, Sagam R, Offiah VN, Adesiyun AA, et al (2011). Efficacy of commonly used anthelmintics: First report of multiple drug resistance in gastrointestinal nematodes of sheep in Trinidad. *Veterinary Parasitology*, 183, p. 194–197.
- Hanif MA, Mostofa M, Choudhury EM, Awal AM, Rahman MM, et al (2005). Efficacy of ivermectin (pour on formulation) against ectoparasites in sheep. *Bangladesh Journal of Veterinary Medicine*, 3, p. 140-143.
- Hassan MM, Ahasanul MD, Hoque MA, Islam SK, Khan SA, et al (2012). Efficacy of anthelmintics against parasitic infections and their treatment effect on the production and blood indices in Black Bengal goats in Bangladesh. *Turkish Journal of Veterinary Science*, 36(4): 400-408.

- Imrul M (1997). Efficacy of ivermectin (pour on formulation) against gastro-intestinal nematodiasis and ectoparasites in cattle. MS (Vet. science) Thesis, submitted to the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh.
- Jabbar A, Campbell AJD, Charles JA, Gasser RB (2013). First report of anthelmintics resistance in *Haemonchus contortus* in alpacas in Australia. *Parasites & Vectors*, 6: 243.
- Kaplan RM (2004). Drug resistance in nematodes of veterinary importance. *A status report. Trends Parasitol*, 20, P. 477–481.
- Kassa B (2006). 'Cockle, mange and pox: Major threats to the leather industry in Ethiopia. Ethiopian leather industry: Perseverance towards value addition', *Proceedings of the National Workshop*, Addis Ababa, Ethiopia, December 14–15, 2006, p. 71–92.
- Kumsa B, Abebe G (2009). Multiple anthelmintic resistances on a goat farm in Hawassa, southern Ethiopia. *Trop. Anim. Health Prod*, 41(4): 655-662.
- Kumsa B, Mekonnen S (2011). 'Ixodid ticks, fleas and lice infesting dogs and cats in Hawassa, southern Ethiopia', *Onderstepoort Journal of Veterinary Research* 78, Art. #326 4 pages. Available at: <http://dx.doi.org/10.4102/ojvr.v78i1.326> (accessed 19 April 2016).
- Kumsa B, Tamirat H, Tadesse G, Aklilu N, Cassani R (2012). 'Prevalence and species composition of ixodid ticks infesting horses in three agroecologies in central Oromia, Ethiopia', *Tropical Animal Health and Production*, 44, p. 119–124. Available at: <http://dx.doi.org/10.1007/s11250-011-9897-y>, PMID: 21656133 (accessed 22 April 2016).
- Martínez-Valladares M, Geurden T, Bartram DJ, Martínez-Pérez JM, Robles-Pérez D (2015). Resistance of gastrointestinal nematodes to the most commonly used anthelmintics in sheep, cattle and horses in Spain. *Veterinary Parasitology*, 211: 228-233.
- Ministry of Agriculture Fisheries and Food (MAFF) (1971). *Manual of Veterinary Parasitological Laboratory Techniques*. Technical Bulletin, Her Majesty's stationery office, London, 18: 5-19.
- Pedroso PD, Moya BG, Borja GE, Paiva D, Pedroso DE (1994). Ivermectin for the control of *Sarcopromuscapruna* (Shannon and Del Ponte, 1926). (Diptera, Muscidae), carrier of *Dermatobia Homini*, L.Jr. (1781). (Diptera, Cuterebridae). *Revista Brasileira de Parasitologia Veterinaria*, 3: 61-64.
- Razaq AK, Ashraf A, Maqbool MA, Khan Islam M, Khan H (2013). Epidemiology, sero-diagnosis and therapeutic studies on ovine nematodes at district Ioralai, Baluchistan, Pakistan. *J. Anim. Plant. Sci*, 23(6): 1559-1565.
- Sangwan RP, Langholef WK, Cramer LG, Eagleson JS (1995) An ivermectin tablet for sheep, efficacy against gastrointestinal nematodes and a bioavailability comparison with a liquid ivermectin formulation. *Vet Parasitol*, 60: 297-302
- Sissay M, Asefa A, Uggla A, Waller P J (2006). Anthelmintic resistance of nematode parasites of small ruminants in eastern Ethiopia: exploitation of refugia to restore anthelmintic efficacy. *Vet. Parasitol*, 135, p. 337-346.
- Soulby EC (1982). *Helminths, Arthropods, and Protozoa of domestic animals*, 7th ed. Philadelphia: Lea and Febiger. p. 375-502.
- Tembely S, Lahlou-kassi A, Rege J, Sovani S, Diedhiou ML, et al (1997). Epidemiology of nematode infections in sheep in a cool tropical environment. *Vet. Parasitol*, 70: 129-141.
- Thompson DR, Kugg D, Scott PG, Cramer LG, Barrick RA (1994). Rainfall and breed effects on the efficacy of ivermectin jetting fluit for the prevention of fly strike and treatment of infestation of lice in long woolled sheep. *Austr Vet J*, 71: 161-164.
- Tsotetsi AM, Njiro S, Katsande TC, Mayo G, Baloyi F, et al.,(2013). Prevalence of gastrointestinal helminths and anthelmintic resistance on small-scale farms in Gauteng Province, South Africa. *TropicalAnimal Health and Production*, 45: 751-761.
- Umur S, Irmak K (1993). Treatment of natural sarcoptic mange in calves with Ivermectin and phoxin. *Veteriner Fakultesi Dergisi*. Ankara, Universitesi, 40: 301-310
- Van Wyk JA, Cabaret J, Michael LM (2004). Morphological identification of nematode larvae of small ruminants and cattle simplified. *Veterinary Parasitology* 119: 277-306.
- Vizzio EA, Caro RR (1995) Use of Ivermectin and lufenuron in the treatment of dog fleas. *Veterinaria Argentina*, 12: 406-408.
- Walker AR, Bouattour A, Camicas JL, Estrada-Pena IG Horak, AA, et al (2003). Ticks of domestic animals in Africa: A guide to identification of species. Bioscience and Reports. Edinburgh, London, p. 262.
- Wall R, Shearer D (2001). *Veterinary Ectoparasites: Biology, Pathology and Control*. 2nd ed. Black well science.

- Wolstenholme JA, Fairweather I, Prichard R, Samson-Himmelstjerna G, Sangster CN (2004). Drug resistance in veterinary helminths. *Trends in Parasitology*, 20: 10- 12.
- Yazwinski TA, Tucker CA, Featherston HE, Walstrom DJ (1997). Comparative therapeutic efficacy of doramectin and ivermectin against naturally acquired nematode infection in cattle. *Vet Record*, 140: 343- 344.