

Epidemiological Study of Nematodes in Adigudom and Mereme'eti Grazing Areas, Southern Tigray, Northern Ethiopia: Larval Nematodes Cattle Pasture Contamination

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Abstract: A study was carried out in Adigudom and Mereme'eti grazing areas of Southern Tigray during the periods of December, 2008 to April, 2009 to identify the major infective nematodes' larvae that contaminate the pasture, to assess the level of pasture contamination and to determine nematodosis prevalence in cattle populations of the area. An area of one hectare by one hectare plot of pasture area was sampled from both sites and 0.5 kg of herbage was collected monthly for identification of larvae. Fecal samples were also collected randomly from cattle grazing in the study sites for measuring level of infestation in cattle population. Larvae of trichostrongylus, haemonchus, strongylus and dictyocaulus were detected as the major contaminants of pasture in the study areas with trichostrongylus larval count being the most prevalent (7400 ± 1437) followed by strongylus (5800 ± 1753), haemonchus (5200 ± 1216) and dictyocaulus (2600 ± 878). The finding also indicated that the mean larval count of Mereme'eti area was higher (6900.0 ± 1327) than that of Adigudom area (3600.0 ± 927.40). Further, fecal samples collected from (n= 234) cattle grazing in the area were examined showing an overall prevalence of 183/234(78.20%). Higher proportion of nematodosis infestation (83.78%) was detected in Mereme'eti than in Adigudom (73.17%), though, the difference in prevalence between the study sites was statistically non-significant. The major nematodes identified from fecal microscopic examination were trichostrongylus (25.21%), haemonchus (11.5%), dictyocaulus (4.7%), strongylus (4.3%), and mixed infection (32.5%). Peak larval count was recorded during the months of small rainy seasons (February and March) then decline to undetectable levels during the dry seasons (December, January and April). It was observed that age and sex categories were not found statistically significant risk factor for parasitic infestation. To wrap up, the study indicated the prevailing of various types of nematode genuses signaling the importance of devising strategic and appropriate control measures to mitigate the parasitic adverse impacts on livestock production and health in the studied areas.

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1. Introduction

Member of the phylum Nematoda, the nematodes, or round worms, are the most numerous and most diverse group of animals on earth. Approximately 10,000 species thrives in very diverse habitat. There are three basic types of nematodes namely: the free living nematodes residing in marine water, freshwater and soil environment, the nematodes that parasitize plants, and the nematodes that parasitize domesticated and wild animals, and humans (Hendrix and Robinson, 2006).

Parasitic nematodes are very prolific in which a single female nematode can produce several thousands of eggs (or larvae) each day. External environmental factors such as temperature, moisture, oxygen and shade and cover are very crucial for larval development. Larval development rate is temperature dependent occurring faster in warm weather whereby 18-20 °c is generally the optimal temperature range for

development of the maximum number of larvae in the shortest feasible time. Larval development is faster at higher temperature making them hyperactive promoting lipid reserve depletion resulting in rise of mortality rate which ultimately ends in few larvae to survive to third stage. Fall in temperature slows larval development causing failure of egg to develop to third stage larvae at 10 °c whereas below 5 °c movement and metabolism of third stage larvae would be minimal favoring survival of many species (Banks *et al.*, 1990; Onyali *et al.*, 1990; Chartier, 1991; Besier and Dunsmore, 1993; Radostitis, *et al.*, 2000; Urquhart *et al.*, 2003).

It has been indicated that adequate moisture in the soil help prevent desiccation while sufficient oxygen aids metabolic process. Further, shade and cover protects against drying and freezing condition. The optimal humidity is 100%, although, some development can occur down to 80% relative

humidity. It should be noted that even in dry weather where the ambient humidity is low, the microclimate in feces or at the soil surface may be sufficiently humid to permit continuing larval development (Urquhart *et al.*, 2003).

The parasites' eggs develop into third-stage (infective larvae) in fecal material from which they must migrate or must be transported to any nearby herbage to be accessible for ingestion by ruminants. Larval migration or transportation could be performed horizontally whereby they usually migrate not more than 5-10cm facilitated by disintegration of fecal material crust as a result of rain or moisture. In addition, larvae could migrate vertically as a case where they move up and down the blades of grass depending on the moisture on the grass reaching grass top when there is rain or dew. They migrate to herbage base and even down to the soil following evaporation, though, heavy rain can also wash them off the herbage and down to the ground. Invertebrates such as dung beetles may also play a role in the transport of larvae onto herbage (Hansen and Perry, 1994).

Amongst the major epidemiological risk factors affecting worm burdens of grazing cattle is the infection rate of pastures which, in turn, is influenced by favorable conditions for egg hatching, larval development and survival (Barger, 1999; Vlassoff *et al.*, 2001).

Therefore, the objectives of the present study were to identify the major infective nematodes' larvae that contaminate the pasture, to determine the level of pasture contamination and to assess nematode parasites' prevalence in the cattle populations of the area.

2. Materials and Methods

Study area: The study was carried out at Adigudom and Mereme'eti grazing sites of Hintalo Wajerat and Enderta districts respectively. Enderta is one of the nearest districts to the capital city of the regional state, Mekelle, and encircling it in all directions. It has an altitude of 1700 meters above sea level with longitudinal range of 39°30'30" to 39°47'30" E and latitudinal range of 30°00' to 13'30" N. Out of the total area of land coverage, 32,649 hectares are farming land and the remaining 4,178 hectares are grazing land. The mean annual rain fall lies between 450-500mm, and the temperature varies from 9°C to 20°C (BONAR, 2007).

Hintalo Wajerat district is located in southern direction of Tigray. It has an altitude of 1500 meters above sea level with longitudinal range of 29°27'26" to 29°35'32" E and latitudinal range of 11°00' to 13'30" N. The mean annual rainfall lies between 400-450 mm and the temperature varies from 12°C to 27°C.

The nature and type of the soil of the areas are characterized by sandy soil (75%), vertisol (15%) and silt soil (10%) having PH ranges from acidic to slightly alkaline. The vegetations cover of the area are bushy and low weeds type, and almost all part of the grazing lands are covered by short grass type.

Study animals: A total of 234 local breed cattle of different age groups and sex categories were sampled randomly from the grazing areas of the study sites to determine the level of infection of nematodes parasite. **Study design and sampling procedures:** An area of pastures approximately one hectare by one hectare of grazing land was randomly selected from the study areas of Adigudom and Mereme'eti. In Adigudom, the number of cattle grazing on a hectare of pasture land is estimated to be 35 whereas it was 47 cattle per hectare in Mereme'eti. Herbage sample was collected from both study areas monthly for five consecutive months (Hansen and Perry, 1994). Infective larvae were recovered from the pasture samples and identified to the genus level (Ministry of Agriculture, Fishers and food, 1986). The number of infective larvae per kilogram of herbage was determined (Hansen and Perry, 1994).

Study methodology

Isolation of ineffective nematodes larvae from herbage: Herbage samples were collected from the study pasture lands using a "W" or "N" collecting route. On monthly basis, five hundred gram (500 gm) of herbage was collected in a plastic bag using scissors. The sample was then transferred into a gauze bag and immersed in a bucket of water for 3-4 hours. After these hours, it was removed, drained and replaced in water several times to agitate. Then, it was left in water at room temperature over night.

In the next morning, the bag was removed and fresh tape water was run over it into the bucket. The content of the bucket was left to sediment for one hour whereas the bag of grass was sun dried and weighed. The supernatant was carefully decanted, leaving one litter of the sediment and then the sediment was suspended and poured into funnel in its stand with the bottom clamp fastened. It was left to stand for an hour with the top clamp closed.

Fifteen milliliters (15ml) of the sediment was poured into a test tube and put in the refrigerator at 4°C to cool for one hour. Next, the supernatant was siphon off and the sediment was stained with iodine for one hour. Following this, a drop of the sediment was placed on a microscope slide and covered with cover slip, and examined under 10× and 40× magnification. Finally, number of larvae per kg of dry herbage was calculated as described by Hansen and Perry (1994).

$$\text{Larval count per kg of dry herbage} = \frac{\text{count} \times 1000}{\text{Herbage weight}}$$

Identification of nematodes from fecal culture:

Twenty gram (20gm) fresh fecal sample was collected from cattle grazing at pasture land and placed in a petri-dish. The feces were broken up with tongue depressor and moisten slightly with tape water. Then it was incubated at room temperature for 7 days. The Petri dish was checked every day for moisture to ensure if droplets of condensed water can be seen on the sides of the Petri dish and if moisture doesn't form, a few drop of water was added and the larval was collected by using Barman technique as described by Hendrix and Robinson, (2006).

Statistical analysis: The data collected was recorded in Microsoft Excel. The independent variable analyzed on herbage sample were animal sampling sites (Adigudom and Mereme'eti), the total larvae count between each months (December, January, February, March and April), and between the genus of infective nematodes larvae (trichostrongylus, haemonchus, strongylus and dictyocaulus) and the data was analyzed by Graph pad software, 2009. The mean third larvae per kilogram of each grazing site were analyzed by unpaired t-test with Welch correction and the mean third larvae per kilogram of each months across the different grazing sites was analyzed by Turkey's multiple comparison test (one way ANOVA). The independent variable analyzed on fecal examination were animal sampling site (Adigudom and Mereme'eti), sex of animals (male and female), age group of animals (less than 3 years, 3-6 years and more than 6 years), genus of parasites (present and absent), and prevalence of parasites divided by the number of examined hosts and was analyzed by SPSS version 15, 2006. Association between the grazing animals and the parasites was analyzed by Person Chi-square tests and a value of $P < 0.05$ was considered to be significant.

3. Results

Microscopic fecal examination: Out of the total animals examined, 183/234 (78.20%) cattle found nematode infected. The higher infestation rate (83.78%) of nematodosis was detected in Mereme'eti than in Adigudom (73.17%) as indicated in table 1. However, the difference in prevalence between the study sites was statistically non-significant. The nematode genus discovered were trichostrongylus (25.21%), haemonchus (11.5%), dictyocaulus (4.7%),

strongylus (4.3%), and mixed infection (32.5%) as depicted in table 2.

Isolation of infective nematodes larvae from herbage: Most of the herbage samples examined from the two study areas were found to be positive for infective stage of nematodes larvae. Four genera of nematodes larvae were identified and these were trichostrongylus, strongylus, haemonchus and dictyocaulus. The infective nematodes larvae count of Mereme'eti area was not significantly different ($p > 0.05$) amongst the genera, however, it was observed statistically significant different in Adigudom area ($p < 0.05$) as shown in table 3. Peak larval count was recorded during the months of small rainy seasons (February and March) with significant decline during the dry seasons (December, January and April) as portrayed in Fig.1.

Nematodes infestation rate in relation to age and sex: The highest (86%) prevalence of nematode infection was recorded in cattle of ages less than three years and the least (71.76%) in those having ages between 3 and 6 years. Higher (79.65%) infection rate was detected in males than females as shown in table 4. Nonetheless, age and sex were found statistically non-significant.

Table 1: Prevalence of nematodes infection between the study sites

Site	Mereme'eti	Adigudom	Overall total
Infected	93	90	183
Non-infected	18	33	51
percent	83.78	73.17	78.20

Table 2: Distribution of nematodes genera in the study areas

Nematode	Infected	Percent
Trichostrongylus	59	25.21
Haemonchus	27	11.5
Dictyocaulus	11	4.7
Strongylus	10	4.3
Mixed	76	32.5
Non-infected	51	21.8
Total	234	100

Table 3: Comparison of the mean and range of nematodes larvae count at the study areas

Infective nematodes (genus level)	Mereme'eti			Adigudom		
	Range	Mean + SE	p-value	Range	Mean + SE	p-value
Trichostrongylus	4000-14000	8000 +1673	0.2043	4000-10000	6800 + 1200	0.019
Strongylus	4000-1200	7600+ 1833		0-8000	4000+ 1673	
Haemonchus	4000-1000	8000+ 1265		0-600	2400+ 1166	
Dictyocaulus	2000-6000	4000+ 1265		0-2000	1200+ 489.9	

Table 4: Prevalence of nematodes in association with age and sex groups

Determinant	No. examined	No. positive	percent
Age			
<3 years	50	43	86
3-6 years	85	61	71.76
>6 years	99	79	79.79
Sex			
Male	113	90	79.65
Female	121	93	76.86

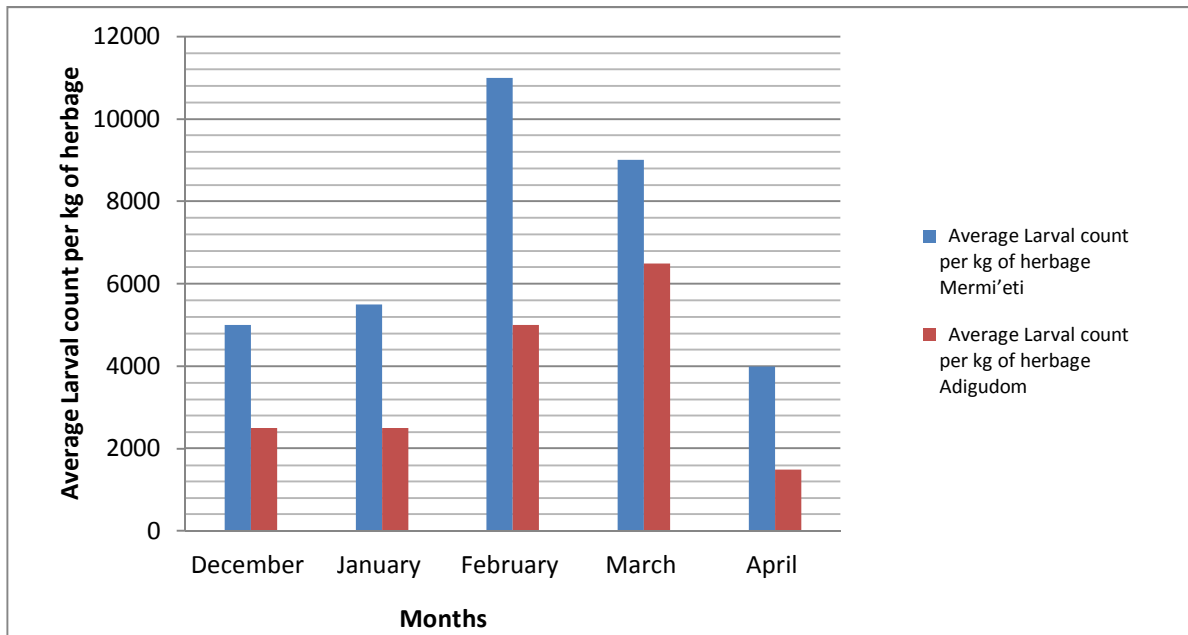


Fig.1. Variations of larval counts during the study seasons

4. Discussions

Out of the total animals examined, 183/234(78.20%) cattle found nematode infected. The higher proportion of infestation rate (83.78%) of nematodosis was detected in Mereme'eti than in Adigudom (73.17%). However, the difference in prevalence between these sites was statistically non-significant. These findings agree with results of earlier studies such as Etsehiwot (2004) who reported an overall prevalence of 82.8 % while studying on bovine gastrointestinal helminthes in dairy cows in and around Holetta. Ahmed *et al.* (2015) also reported 72% prevalence of gastrointestinal parasitism of cattle

in Bangladesh. The nematode genuses discovered microscopically from fecal samples during the study were trichostrongylus (25.21%), haemonchus (11.5%), dictyocaulus (4.7%), strongylus (4.3%), and mixed infection (32.5%). This rise of prevalence and existence of mixed infections could be attributable to the poor control measures, high pasture contamination for overgrazing and, the possible effect of malnutrition and climatic conditions favoring parasitic multiplications (Biffa *et al.*, 2007).

In this study, larvae of trichostrongylus, haemonchus, strongylus and dictyocaulus were detected as the major contaminants of the pasture in

the study areas with trichostrongylus larval count being the most prevalent (7400 ± 1437) followed by strongylus (5800 ± 1753), haemonchus (5200 ± 1216) and dictyocaulus (2600 ± 878). The highest prevalence of trichostrongylus and strongylus could possibly be due to the fact that their embryonated eggs and ensheathed third larvae (L3) are best equipped to survive in adverse conditions such as freezing or desiccation; though, L1 and L2 are particularly vulnerable. Desiccation is generally considered to be the most lethal influence in the larval survival, however, there is increasing evidence that by entering a state of anhydrosis certain larvae can survive severe desiccation (Anderson and Levine, 1968; Banks *et al.* 1990; Urquhart *et al.*, 1998).

In the present study, the total parasitic larvae count was decline during the months of December, January and April which coincides with the dry season. This could be attributed to the fact that there was no rain during this period and the fecal pellets rapidly dried out. The dry condition also contribute to the reduction of herbage coverage resulting in lowered number of animals grazing during this period on the pasture because they concentrate most of the time by feeding straw which, in turn, decrease the pasture contamination by nematodes. Hansen and perry (1994) showed that the survival of larvae in the environment depends upon adequate moisture and shade. Hot and wet weather leads to increased larval movements and higher energy utilization, and thus progressively reduces their longevity (Rose, 1963). Whereas, low temperature and dry conditions prevent active movement by larvae and thus minimizing energy expenditure (Barger, 1999).

In contrast to the above finding, the total infective nematodes larvae counts of both study areas were high during the period of small rainy season (February and March). This finding was in agreement with earlier demonstration of Ng'ang'a *et al.* (2004) who showed that more eggs completed their development and there was mass translocation of larvae into the pastures after contamination during the rainy seasons. On the other hand, heavy rains may wash larvae of herbage and even down in to the soil (Hansen and perry, 1994).

This study showed that the total infective nematodes larvae count was higher in all months in Mereme'eti grazing area than that of Adigudom areas. The finding indicated that the mean larval count of Mereme'eti area was higher (6900.0 ± 1327) than that of Adigudom area (3600.0 ± 927.40). This could possibly be due to the fact that animals graze throughout the year in the Mereme'eti grazing area causing overgrazing which, in turn, could increase the level of pasture contamination by the nematodes parasites. Contrasting to this practice, rotational

grazing was commonly used in Adigudom area which ensures low pastures contamination. These research outcomes were in agreement with the findings of Ng'ang'a *et al.* (2004) who demonstrated the relatively rapid decline in the numbers of infective nematodes larvae count on the pasture during the wet and dry seasons due to the practice of rotational grazing as a means of gastrointestinal parasite control.

The highest (86%) prevalence of nematode infection was recorded in cattle of ages less than three years and the least (71.76%) in those having ages between 3 and 6 years. Nonetheless, age was found statistically non-significant. Higher (79.65%) infection rate was detected in males than females, though, it was not observed statistically significant. Similar findings had been reported by previous research results (Kemal and Terefe, 2013). This might be attributed to the condition that both males and females, and all age groups of animals could have equal opportunity of being infected with the parasites and ultimately developing the disease.

5. Conclusions

The study revealed an overall prevalence of 78.20% nematodosis in cattle population of the study areas. The infestation rate of nematodosis was higher (83.78%) in Mereme'eti than in Adigudom (73.17%). The major nematode genuses identified in the study areas were trichostrongylus (25.21%), haemonchus (11.5%), dictyocaulus (4.7%), strongylus (4.3%), and mixed infections of these parasites (32.5%). The identification of larvae from herbage showed that the total infective nematodes larvae count of the genus trichostrongylus and strongylus was higher than that of the genus haemonchus and dictyocaulus in both study areas during the study periods. Age and sex was not observed to be statistically significant risk factor for nematode infections. In summing up, different genuses of nematode parasites were found highly prevalent in the study areas with subsequent contamination of pasture by their larvae threatening livestock production which seeks devising strategic control measures to combat the adverse effects incurred.

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References

1. Hendrix, M. C and Robinson, E. D., (2006). Diagnostic parasitology for veterinary technicians, 3rd ed., st. Louis, Missouri 63146, Mosby Inc., USA.
2. Banks, D. J; Singh, R.; Barger, I. A.; Pratrapp, B. and Lesambre, L. F. (1990). Development and survival of infective larvae of *haemonchus contortus* and *trichostrongylus colubriformis* on pasture in tropical environment, *international journal of parasitology*, 20: 155-160.
3. Onyali, I. O., Onwulri, C. O. E. and Ajayi, J. A. (1990). Development and survival larvae of *haemonchus* larvae on pasture at Vom plateau state, *Nigeria veterinary research communication* 14: 211-216.
4. Basier, R. B and Dunsmore, J. D (1993). The ecology of *haemonchus contortus* in a winter rain fall climate in Australia, the survival of infective larvae on pasture, *veterinary parasitology*, 45: 293-306.
5. Radostits, O. M., Blood, D. C and Gray, C. G. (2000). *Veterinary Medicine A text book of the Disease of cattle, sheep, pigs, goats, and horse*, 9th ed. ELBS, Tindell, Pp 785-792.
6. Urequehart, G. M., Armour, J., Dunn, A. M., Jennings, F. W., (2003). *Veterinary parasitology*, 2nd ed. Book power, British, Pp 8-9.
7. Hansen, J. and Perry, B. (1994). *The epidemiology, diagnosis and control of gastrointestinal parasites of the ruminants in Africa*, ILRAD, Nairobi, Kenya, 123-141.
8. Barger, I. A (1999). The role of epidemiological knowledge and grazing management for helminthes control in small ruminants, *international journal of parasitology*, 20: 155-160.
9. Vlassoff, A., Leathwick, D. M. and Heath, A. C. G., (2001). The epidemiology of nematodes infection in sheep, *New Zealand veterinary journal*, 49, 213-221.
10. Bureau of agriculture and natural resource of Tigray (2007). Livestock census analysis result, volume I, Mekelle, Tigray.
11. Ministry of Agriculture, Fishery, and Food (1986). *Manual of veterinary parasitology laboratory techniques*, reference book 418, Her Majesty's Stationary office, London, Pp 5-12.
12. Etsehiwot W (2004). Study on bovine gastrointestinal helminthes in dairy cows in and around Holetta. DVM thesis, Debre zeit, Ethiopia.
13. Ahmed1, R., Kumar, P., Barua, M., Md. Abdul, Md., Islam1, K. and Zohorul, Md., (2015) Prevalence of gastrointestinal parasitism of cattle in Banskhali upazilla, Chittagong, Bangladesh, *J. Adv. Vet. Anim. Res.*, 2(4): 484-488. DOI: 10.5455/javar.
14. Biffa D, Jobre Y, Chakka H (2007). Ovine helminthosis: a major health constraint to productivity of sheep in Ethiopia. *Anim. Health Res. Rev.* 7(1/2):107-118.
15. Anderesn, F. L. and Levine, N. D. (1968). Effect of desiccation on survival of the free living stages of *trichostrongylus colubriformis*, *journal of parasitology*, 54,117-128.
16. Rose, J. H. (1963). Observation on the free living stages of the stomach worm *haemonchus contortus*, *parasitology*, 53, 469-481.
17. Ng'ang'a, C. J; Maingi, N.; Kanyari, P. W. N. and Munyua, W. K. (2004). Development, survival and availability of gastrointestinal nematodes of sheep on pastures in a semi-arid area of Kajiado District of Kenya, *Vet. Res. Commun.*, 28:491–501.
18. Kemal, J. and Terefe, Y., (2013). Prevalence Of Gastrointestinal Parasitism Of Cattle In Gedebano Gutazer Wolene District, Ethiopia, *J. Vet. Med. Anim. Health*, Vol. 5(12), pp. 365-370, December, DOI: 10.5897/JVMAH2013.0226.

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