

Concurrent Naturally Acquired Trypanosome and Gastrointestinal Nematode Infections in Horro Sheep from Anger-Didessa Valley of Western Oromiya, Ethiopia

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Abstract: The study was conducted on 82 sheep flocks to determine the occurrence of concurrent infections of nematode and trypanosome as well as the effect of the parasites on packed cell volume (PCV) from tsetse-affected areas. Faeces were examined using flotation and modified McMaster techniques while blood samples were analyzed using Buffy Coat method and Giemsa-stained smears. The results showed an overall concurrent infection of 2.0% (95% CI: 1.0-3.0%). Among sheep tested parasitological positive for trypanosomes, 40.0% (95% CI: 20.0-60.0%) were concurrently infected with nematode while among trypanosome negative animals, 30.2% (95% CI: 25.0-35.0%) were infected with nematodes and the difference was not statistically significant ($p > 0.05$). The log-transformed mean nematode faecal egg counts (EPG \pm SE) recorded in mixed infection with trypanosome (2.25 ± 0.25) and single infection of nematode (2.30 ± 0.04) were not significantly different ($p > 0.05$). The EPG and PCV values were negatively correlated which was significant ($r = -0.255$, $p < 0.001$). Among concurrently infected animals, 87.5% (95% CI: 60.0 - 100.0%) had anaemia compared to 58.3% (95% CI: 30.0 - 86.0%) and 21.4% (95% CI: 13.0 - 27.0%) recorded in single infections with trypanosome and nematode, respectively. The *Haemonchus contortus* (*H. contortus*) was the most prevalent nematode parasite of sheep followed by *Trichostrongylus* species which accounted for 42.0% and 22.0%, respectively. The trypanosome infection was caused predominantly by *Trypanosoma (T.) congolense* which was responsible for 50.0% of single infection and 25.0% of mixed infection with *T. vivax*. Concurrent infection invariably resulted in severe anaemia in majority of affected animals which presumably has a serious impact on production. To improve and sustain viable sheep production in the areas, control measures directed against nematode and trypanosome parasites should be considered in tsetse-infested lowland plains in the areas.

Key words: Concurrent Infection • Gastrointestinal Nematode • Trypanosome • Horro Sheep • Western Oromiya

INTRODUCTION

As part of a rehabilitation program operated during the year 2003 - 2006, a large number of farming communities transferred from drought stricken areas of Ethiopia and resettled in fertile lowland plains of western Oromiya [1]. Along with the resettlement schemes, expansion of agriculture and restocking took place in the

tsetse-affected areas of upper Didessa and Uke/Anger river valleys. Restocking started with introduction of draught oxen, small ruminants and equines to begin a new life with agriculture. Complaints from the farmers, field reports documented by NTTICC [2] and a recent study from western Oromiya [3] all confirmed the serious challenges confronted from animal trypanosomosis in the newly established resettlement villages. Consequently,

many working animals and small ruminants died from the disease across the villages in the tsetse prefecture. During the long rainy season from May to September a serious challenge with parasitism is experienced in grazing animals in the areas. However, a study on the interaction of gastrointestinal (GI) nematode and trypanosome was not conducted in the areas except some preliminary surveys carried out on trypanosomosis of small ruminants in the upper Didessa valley [4] and Anger valley of East Wollega Zone [5].

A study on the interaction of *T. congolense* and *H. contortus* in Djallonké sheep in the Gambia [6] showed that after exposure to successive experimental infection with both parasites, the animals lost their tolerance to parasites than in single infection with either parasite. Pasture-borne nematode parasites contribute to suboptimal productivity [7]. In tsetse-infested areas, trypanosomosis could play an additive role to the negative impact of GI nematode parasitism in grazing sheep to further deteriorate the productivity and even increase the mortality rate. The present study on trypanosomosis was done in conjunction with the related study carried out on the epidemiology of GI nematodes in sheep flocks kept in tsetse affected resettlement areas. In Anger-Didessa valley, a decrease in the population of wild hosts as a result of the newly expanding farmlands and villagization scheme, tsetse flies forced to feed on new hosts like sheep overrunning their ecology. The grazing management traditionally practiced by some farming communities involved strict tethering with limited rotation in the farmstead which has significant influence on the epidemiology of nematode infections [8]. This practice could subject animals to low plane of nutrition and thus reduce their resistance to parasites [9, 10]. The objectives of this study were to determine the prevalence of concurrent infections with nematode and trypanosome as well as their effects on the packed cell volume (PCV) in naturally infected sheep kept in tsetse-infested places.

MATERIALS AND METHODS

Study Areas: The study was carried out during May 2011 to December 2012 in five resettlement villages namely Kolo Siri and Kerke'a in Bee and Kone in Dabo Hana districts of Ilu Ababora Administrative Zone. Other villages included Abote Didessa in Jimma Arjo and Uke in Guto Gidda districts of East Wollega Administrative Zone.

All places, except for Uke, are located in Didessa valley about 36 km north of Bedele town which is 480 km west of Addis Ababa. Uke is sited in Uke/Anger river valley about 365 km west of Addis Ababa. The areas were selected on the basis of their past history of heavy tsetse challenge and high prevalence of animal trypanosomosis. They are all located below 1500 m above sea level [11] with mean annual temperature range of 15.0-30.0 °C [12]. Two distinct seasons are experienced, the rainy season from May to September with the rainfall peak occurring from July to August and the long dry season from October to February. The places receive a total annual rainfall in exceeding of 1200 mm [13].

Study Animals: A total of 82 flocks comprising 407 indigenous Horro sheep of all age and both sex groups were included in the study. The flock size ranges between 3-7 animals and a cluster sampling was employed in which all animals in a flock were sampled [14] by random selection of households owning the flocks.

Parasitological Study: All faecal samples were taken directly from the rectum of the animals. For coproscopic examination an average of 3 g of faeces was collected into a screw-capped individual bottle from each sheep and transported to the laboratory [15]. When fresh faeces cannot be promptly submitted and processed, they were preserved in 10% formalin to prevent nematode eggs developing and hatching [16]. Samples for pooled faecal cultures were collected separately from a group of 10 to 15 animals based on an average of 3 g of faeces from each animal and thoroughly mixed in the laboratory.

Pooled faecal samples were incubated at room temperature (23-25°C) for 14 to 20 days and the infective larvae (L₃) were recovered using Baermann technique. The larvae were counted and identified based on morphological characteristics according to MAFF [17] and van Wyk *et al.* [18].

Nematode eggs per gram of faeces (EPG) were determined for each sample following the modified McMaster technique as described by MAFF [17] and Coles *et al.* [19] using saturated sodium chloride (specific gravity 1.2) as flotation fluid. The degree of faecal egg count for each sample was categorized as light (50 - 800), moderate (800 - 1200) and heavy (> 1200) based on EPG value recorded [16].

About 5 ml of blood sample was collected from each animal via jugular vein puncture into sterile vacutainer tube containing ethylene diamine tetra acetate (EDTA) as anticoagulant. The samples were properly labeled and transported immediately to Bedele Veterinary Diagnostic Laboratory.

From each vacutainer tube, blood was collected into a capillary tube at one end by capillary attraction until about three-quarters of the length of the tube was filled and sealed with crista seal (Hawksley- Lancing, UK).

The blood in capillary tubes was centrifuged at 12,000 rpm for 5 minutes using haematocrit centrifugation technique (HCT). After centrifugation, the capillary tubes were removed and the packed cell volume (PCV) was determined for each animal on the haematocrit reader [20]. The capillary tube was cut 1mm below the buffy coat junction with a diamond pen to include the top layer of red cells. The content of the capillary tube was expressed onto a clean microscope slide, mixed and covered with a 22 x 22 mm cover slip. The slide was examined for the presence of trypanosomes. Diagnosis of trypanosome species was done after staining thin blood smear with Giemsa and examination with a special oil immersion objective lens [21].

Data Analysis: Data collected on EPG, PCV, larvae counts (L₃), nematode and trypanosome infections were stored in Ms excel spreadsheet. The faecal egg count was logarithm transformed in the form of log₁₀ (EPG+1) to minimize the skewed distribution and used to compare the mean faecal egg outputs of two populations coming from single and mixed infections with both parasites. The independent

samples t-test was employed to compare the mean PCV values of infected and non-infected animals. Pearson correlation test was conducted to analyze the relationship between PCV values and nematode faecal egg count recorded. The chi-square test statistic (χ^2) was used to compare observed prevalence in single and mixed infections of parasites using the statistical procedures of SPSS 16.0 for Windows [22] and IBM SPSS 20.0 for Windows [23]. Percentages were used to summarize results in Table and frequency distribution was employed to present results in Figures. In all analysis, statistical significance was considered at 0.05 or less probability levels.

RESULTS

The results showed that out of 407 faecal and blood samples examined, concurrent infections with both parasites were recorded in 2.0% of animals sampled (Table 1). Among sheep found positive for nematode eggs (125), 8(6.4% [95% confidence interval: 2.0-10.0%]) were concurrently infected with trypanosomes whereas among nematode negative animals (282), 12 (4.3% [95% CI: 2.0-6.0%]) were infected with trypanosomes and the difference between these two populations was not significant. Similarly, among the animals found positive for trypanosomes (20), 8(40.0% [95% CI: 20.0-60.0%]) were concurrently infected with nematode parasites while among parasitological trypanosome negative sheep (387), 117(30.2% [95% CI: 25.0-35.0%]) were infected with nematode parasites and the difference was also not significant (Table 2). The mean worm faecal egg counts

Table 1: Prevalence of concurrent infections with gastrointestinal nematodes and trypanosomes from tsetse-infested lowland valleys in western Oromiya

	Prevalence (%)					
	Nematode		Trypanosome		Mixed	
	Infected	95% CI	Infected	95% CI	Infection	95% CI
No of animals sampled						
407	125 (30.7)	26.0-36.0	20 (4.9)	3.0-7.0	8 (2.0)	1.0-3.0

CI: confidence interval

Table 2: Infection status with nematode and trypanosome in grazing sheep in tsetse affected areas

		Gastrointestinal nematode			χ^2	df	p level
		-	+	Total			
Trypanosome	-	Count	270	117	0.85	1	0.35
		% within trypanosome		30.2%			
	+	Count	12	8	0.85	1	0.35
			% within trypanosome				
		% within nematode	4.3%	6.4%			
Total	Count	282	125	407			

- Parasite absent; + Parasite present

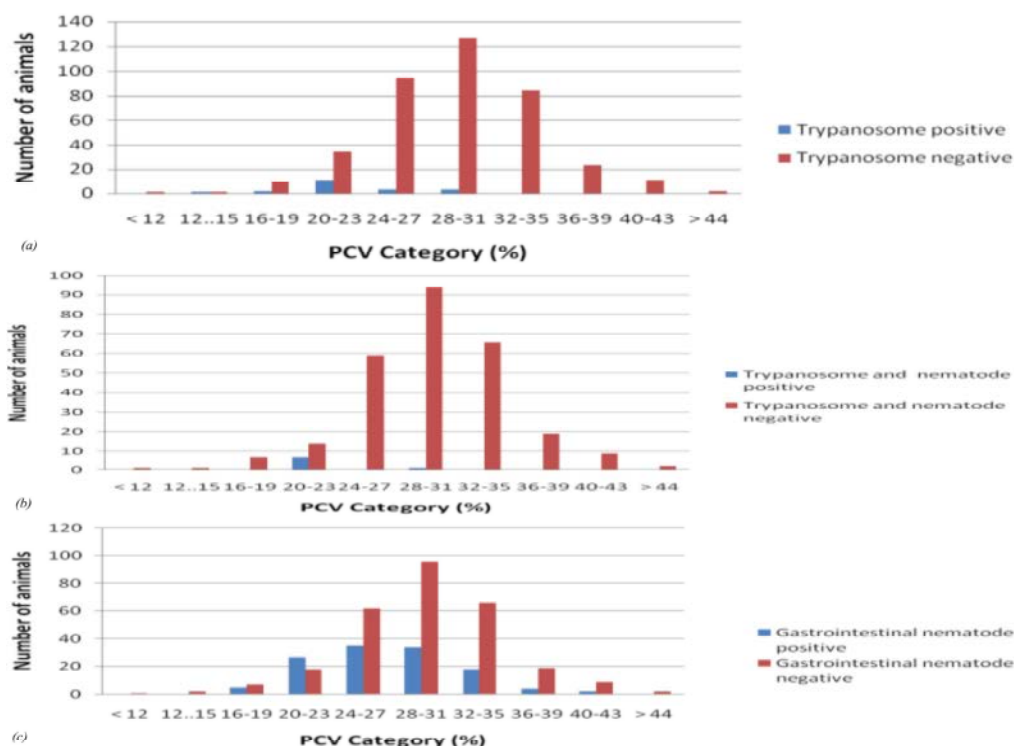


Fig. 1: Packed cell volume distribution: (A) trypanosome positive versus negative animals, (B) trypanosome and nematode positive versus negative animals and (C) gastrointestinal nematode positive versus negative animals

Table 3: Comparison of nematode faecal egg output in parasite infected sheep

Parasite infection	Log ₁₀ -transformed EPG ± SE	t-value	df	p level
Single infection with nematode	2.30 ± 0.04	0.28	123	0.78
Concurrent infection with nematode and trypanosome	2.25 ± 0.25			

SE = standard error of the mean

Table 4: Mean packed cell volume of sheep infected with gastrointestinal nematode and trypanosome parasites from tsetse-infested resettlement areas in western Oromiya

Gastrointestinal nematode	Trypanosome	Mean PCV (%)	95% CI for means	t-value	df	p level
+	+	22.50	19.44-25.56	2.78	123	0.006
+	-	27.43	26.53-28.33			
-	+	22.42	19.63-25.20	5.11	280	0.000
-	-	30.03	29.42-30.64			

+ Parasite present; - Parasite absent

recorded for mixed infections with nematode and trypanosome and single infection with nematode was not significantly different between the two groups in this study (Table 3). A significant negative correlation was observed between mean nematode faecal egg count (log₁₀ [EPG + 1]) and the PCV (Pearson correlation coefficient of - 0.255 and p < 0.001) in parasite infected sheep.

In single infection with trypanosome, 7(58.3% [95% CI: 30.0-86.0%]) of 12 sheep infected were anaemic (PCV < 24.0%) while 7(87.5% [95% CI: 60.0-100.0%]) of 8 animals which were positive for mixed infections with

both parasites had anaemia. On the other hand, only 25(21.4% [95% CI: 13.0-27.0%]) of 117 sheep with single infection of nematode were detected anaemic. A further analysis also showed that the mean PCV for concurrently infected animals was 22.50% which was significantly different from 27.43% recorded for single infection of nematodes. Similarly the mean PCV value of sheep infected with trypanosome alone was significantly different from mean PCV of 30.03% recorded for parasitological negative animals (Table 4). The frequency distributions of animals (infected or non-infected) against PCV values recorded are presented in Figure 1.

Table 5: Percentage composition of GI nematode third stage larvae recovered from pooled faecal cultures of sheep

Total larval count	Composition of species (%)				
	<i>H. contortus</i>	<i>Trichostrongylus spp</i>	<i>Oesophagostomum columbianum</i>	<i>Bunostomum trigonocephalum</i>	Other species
100	42.0	22.0	15.0	13.0	8.0

Table 6: Prevalence of trypanosome species in parasitological positive sheep

Total infection	Percentage infection of species (%)		
	<i>T. congolense</i>	<i>T. congolense</i> + <i>T. vivax</i>	<i>T. vivax</i>
20	50.0	25.0	25.0

The average percentage composition of GI nematode larvae (L₃) recovered from pooled faecal cultures showed that *H. contortus* was the dominant parasite followed by *Trichostrongylus colubriformis* and *T. axei* together constituting the next more prevalent species (Table 5). The trypanosome infection was caused predominantly by *T. congolense* accounting for most of single and concurrent infections observed in this study (Table 6).

DISCUSSION

In this study, results showed that gastrointestinal nematode infection and ovine trypanosomosis are prevalent diseases in tsetse-infested resettlement areas in upper Didessa and Uke-Anger valleys in western Oromiya. The overall concurrent infection with nematode and trypanosome were observed to be 2.0%. It was also noted that, among the animals found parasitological positive for GI nematodes, 6.4% were concurrently infected with trypanosomes whereas among nematode negative sheep, only 4.3% were infected with trypanosomes. On the other hand, among sheep found positive for trypanosomes, 40.0% were concurrently infected with nematode parasites while among trypanosome negative sheep only 30.2% were infected with nematode parasites. This infection status noticeably shows that sheep infected with trypanosomes tend to harbour more gastrointestinal nematode infections. However, these results are not fully in agreement with a finding reported from West Africa [24] in which all trypanosome infected sheep and goats were invariably infected with *H. contortus* and *T. colubriformis* from a concurrent infection study in naturally infected animals.

The GI nematode had high prevalence similar to results recorded in mid altitude area (30.7%) during the same period. Ovine trypanosomosis showed higher prevalence of 4.9% compared to 4.5% and 3.7% infections reported in sheep and goats, respectively in upper Didessa valley [4]. This result was also higher when

compared to prevalence of 2.70% in sheep and 1.70% in goat flocks reported from Anger valley of East Wollega Zone [5]. In previous studies, trypanosome infections of 7.6 % in sheep and 3.5% in goats were recorded in Didessa/Ghibe valley of southwest Ethiopia [25]. In spite of the currently expanding agriculture and villagization scheme in the lowland plains, the ovine trypanosomosis is an endemic problem which does not show a significant decrease in the areas. Although no exact estimates of the prevalence can be made, this finding suggests that trypanosomosis contributes significantly to the overall burden of parasitic diseases in grazing sheep in those areas.

In this study, there was no evidence for synergistic pathogenic effects of the two parasites on the mean nematode faecal egg counts. The differences were not significant between mean counts for single and mixed infections. Similar finding was also reported in West African Dwarf goats naturally infected with gastrointestinal nematodes and trypanosomes [26]. Conversely, a significantly higher mean faecal egg count and a rapid fall in PCV values were reported in concurrent experimental infection of goats with *T. congolense* and *H. contortus* [27]. Similarly, in a mixed successive experimental infection of Yankassa sheep first received *T. congolense* followed by *H. contortus*, a higher nematode faecal egg count and a rapid fall in PCV was observed than in animals infected the other way round [28]. However, in the natural infection process of the present study, the sequence of infection with both parasites was not known.

In concomitant infection with gastrointestinal nematode and trypanosome, the additive pathogenic effects of both parasites on PCV values were more pronounced where majority of concurrently infected animals were anaemic. The *H. contortus* was the predominant parasite detected in the study areas and as a super infection with trypanosomes, it seems to have aggravated the condition of anaemia in animals. As PCV values decreased, conversely the mean faecal egg counts increased significantly which showed a strong negative correlation. Also similar observation was reported in a semi-arid region of eastern Ethiopia where *H. contortus* was the most prevalent worm in the sheep flocks [29]. An experimental study involving a successive infection with

T. congolense and *H. contortus* in Djallonkè sheep in West Africa [6] and a mixed infection in goats with both parasites [27] showed similar interaction resulting in mortality of the hosts.

One of the most typical signs of animal trypanosomosis is the development of anaemia which is best measured by determining the PCV value [30]. In the present finding, 87.5% of concurrently infected sheep with nematode and trypanosome had PCV values below 24.0%. In contrast, 58.3% of animals parasitologically positive for a single infection with trypanosome and 21.4% of sheep infected with nematode alone had PCV values less than 24.0% which is taken as a cut-off-point for anaemia in sheep. The mean PCV value for mixed infection (22.5% [95% CI: 19.44-25.56]) was comparable with the value recorded in single infection with trypanosome (22.42% [95% CI: 19.63-25.20]) regardless of the number of animals affected in each infection status. However, the results were less than mean values documented for single infection with nematode (27.43% [95% CI: 26.53-28.33]) and parasitological negative animals (30.03% [95% CI: 29.42-30.64]). The results show that, due to interaction with both parasites, more animals developed anaemia in mixed infection compared to single infection with any of the parasite. This could result in an increased loss of productivity or mortality of the host animal. In an experimental mixed infection of Djallonkè sheep with *T. congolense* and *H. contortus*, the effects developed to more acute or chronic condition depending on the sequence of either parasite received in advance and in both conditions mortalities were recorded as end results than in single infections [6]. The results were in agreement with the literature that the interaction of various parasites or a parasite with another pathogen, resulting in an exaggerated clinical disease has been reported on several occasions [31].

The findings also indicate that a proportion of sheep detected negative for infection of either parasite were found anaemic with PCV values less than 24.0%. The reason for this was not clear but it might be attributed to the high specificity and low sensitivity of the tests used (Buffy Coat and the modified McMaster methods) or other parasites or conditions causing anaemia could exist in the areas. Similar finding was also reported at a survey of bovine trypanosomosis in South Africa [32].

In this study, *H. contortus* was recorded to be the most prevalent gastrointestinal nematode parasite of Horro sheep representing 42.0% of infective larvae identified from pooled faecal cultures in all settlement sites. It was also the dominant species detected in studies

conducted in mid altitude area during the same period in western Oromiya. Among other commonly identified species, *Trichostrongylus colubriformis* and *T. axei* accounted for 22.0% and *Oesophagostomum columbianum* and *Bunostomum trigonocephalem* comprised 15.0% and 13.0%, respectively. Other less commonly encountered species included *Chabertia ovina* and *Strongyloides papillosus*. These species were also frequently reported in grazing sheep in different agro-ecologies all over the country [29, 33-37].

Much of trypanosome infection (50.0%) was caused by *T. congolense* as a single agent while *T. congolense* and *T. vivax* were responsible for mixed infection (25.0%) and *T. vivax* accounted for the rest infection (25.0%) recorded. In mixed infection with GI nematode, *T. congolense* was again responsible for most of cases (62.5%) followed by joint infection with *T. vivax* and *T. congolense* (25.0%) consistent with a finding reported in Nigeria [38]. The *T. vivax* was also implicated in dual infection with nematodes accounting for 12.5% of infection. In prevalence studies conducted in Didessa/Ghibe valley, similarly both *T. congolense* and *T. vivax* were reported to cause trypanosomosis of small ruminants [25]. In another survey carried out in upper Didessa valley, infection with *T. brucei* was reported in addition to *T. congolense* and *T. vivax* [4]. In a similar mixed infection of West African dwarf sheep and goats with gastrointestinal nematodes and trypanosomes, *T. brucei*, *T. congolense* and *T. vivax* were responsible for 50.0%, 43% and 36.0% of infections reported, respectively [24].

Conclusively results from this study show that ovine trypanosomosis and gastrointestinal nematode infections were endemic which occurred in all resettlement areas located in tsetse belts. The concurrent infections with both parasites invariably resulted in development of severe anaemia in all affected animals. The subclinical or chronic attributes of majority of infections did not call for the attention of farmers to embark on control measures on time which eventually could lead to further production losses. Therefore, to improve and sustain viable sheep production in the study areas, control measures directed against both nematode and trypanosome parasites should be considered.

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