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Prevalence of Bovine Trypanosomosis and Its Associated Risk Factors in Nano and Gudeya Bila Districts of Oromia Regional State, Ethiopia

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Abstract

The prevalence of bovine trypanosomosis, the influence of associated risk factors and relative abundance of tsetse fly was investigated using 891 randomly selected cattle in Nano and Gudeya Bila districts of Oromia regional state, Ethiopia. Blood samples were collected from ear vein and examined by using Buffy coat technique and hematological procedures. The overall prevalence of trypanosomosis was found to be 2.69%, 95%CI. The relative prevalence based on Trypanosome species was 1.79% and 0.90% for T. congolense and T. vivax, respectively. The analysis for the associated risk factors revealed significant difference (P<0.05) in the occurrence of trypanosomosis prevalence between age and sex groups (P>0.05). The mean PCV values of trypanosomosis positive (21.92%) were significantly lower to that of negative animals (27.44%). The apparent density of tsetse fly was 1.08 fly/trap/day. Three species of Glossina including G. Fuscipes (70.0%), G. Palidipes (19.23%) and G. morsistans (10.77%) were captured from the 120 odor baited traps. Therefore, more attention should be given to lessen the pervasiveness of this vector and impact of the disease in the study districts.

Keywords: Glossina, PCV, Risk Factors, T. Congolense, Trypanosomosis, Tsetse Fly **DOI:** 10.7176/JBAH/10-15-05 **Publication date:**August 31st 2020

1. Introduction

Ethiopia has the largest livestock inventories in Africa, including about 59.5 million head of cattle and 60.90 million sheep and goats population according to the survey reported by CSA (2018). Although livestock are the backbone of the rural economy, diseases like African Trypanosomiasis are eminent restraints to livestock production and productivity in many regions of Ethiopia (Kumela et al., 2018). Trypanosomes transmitted by tsetse flies are endemic in a part of sub-Saharan Africa (SSA) called the tsetse fly belt. In Ethiopia, the disease is more prevalent in the southern and western regions where the primary vector exists and it remains as one of the largest causes of livestock production losses. Tsetse fly (*Glossina species*) has a prominent economic impact as the biological vectors of trypanosomes (Tesfaheywet and Abraham, 2012; Kumar et al., 2012). The most prevalent trypanosome species are T. congolense, T. vivax and T. brucei with a wide host range among domesticated animals (Abebe 2005). This prevalence varies depending on agro-climatic conditions, season of year, host preferences or virulence for different species and interventions done to control the impact of the disease (Abebe and Jobre, 1996; Leta et al., 2016).

In Ethiopia, bovine trypanosomosis is widely distributed in western and south-western parts of the country (Abebe, 2005; Leta et al., 2016; Kumela et al., 2018). Bovine trypanosomosis was very importance in regards to economic point views (Meberate et al., 2000). According to Leta et al. (2016) it is estimated that about 10 to 14 million heads of cattle in Ethiopia are exposed to the risk of trypanosomosis. The disease results in loss of livestock and agricultural productivity with severe socio-economic impacts (Mengistu et al., 2019). Trypanosomosis reduces work efficiency of oxen and discourages the introduction of drought animals in to crop farming (Omotainse et al., 2004). It is also a cause for severe and frequently fatal disease of livestock mainly in the poor rural community and it is fairly considered as a foot root cause of poverty in Ethiopia (Abebe, 2005). Among the total regions of Ethiopia, Amhara, Benishangul Gumuz, Gambella, Oromia and SNNPR regions are mostly infected with more than one species of Tsetse flies (Keno, 2005; Tesfaye, 2002). Although livestock trypanosomosis is a well-known constraint to livestock production, its prevalence and the associated risk factors were not well documented in Nano and Gudeya Bila districts. Thus, the major objective of this study was to assess the prevalence of bovine trypanosomosis, the influence of associated risk factors and relative abundance of tsetse fly in Nano and Gudeya Bila districts of Oromia Regional State, Ethiopia.

2. Material and Methods

2.1. Description of the study area

The study was conducted in five peasant associations (PAs) of Gudeya Bila and Nano districts of Oromia Regional State, Ethiopia. Gudeya Bila district is one of the 17 districts located in the East Wollega Zone of Oromia Regional State of Western part of Ethiopia. It is situated at 277 km far away from capital city, Addis Ababa towards West direction and encompasses agro-ecologies of highland, mid-altitude and lowland with proportion of 17.6% and 55.8% and 26.6%, respectively. Rainfall is bi-modal, ranging from 1400 to 2000 mm annually with temperature ranges of 11 to 23°C. The farming system in the district is mainly mixed crop-livestock production. Maize, sorghum, wheat, and teff, barley, and Niger seed are the dominant cereal crops cultivated in the area (CSA, 2018). Nano district is also one of the districts located in the South West Showa Zone of Oromiya Region, Ethiopia. The area is situated at about 230km South West of Addis Ababa within the altitude range of 1500-1600 meters above sea level bordering the Ghibe river system. The district is located at latitude 80° 50'N and longitude 37°45'E. Total area coverage of the district is about 50,000 hectares and the weather condition is characterized by sub-humid climate and a moderately hot temperature with a mean annual temperature of 20°C. The highest average monthly temperature occurs in January with mean maximum temperature of 28°C. The lowest monthly temperature occurs in August with average monthly minimum temperature of 12°c. It receives high and reliable annual rain fall averaging 1100mm/annum with low inter annual variation. Among the livestock species, bovine and caprine are the predominant species in this district and they have been dependent upon communal grazing field as a feed source and watering points are the tributaries of large rivers. Livestock crop (mixed) farming system is the dominant farming system in this area.

2.2. Study Design

A cross sectional study was conducted to determine the prevalence of bovine trypanosomosis in Gudeya Bila and Nano districts of Oromia Regional state, in study period between from 5th of March, 2019 to 25th of June 2019. The study was constituted the local cattle of different age groups, body condition scores and both sex groups of cattle from the selected districts. The age of the cattle was determined according to the definition characteristics (Pasquini et al., 2003) and information from owners of the cattle. The body condition was scored using the method described by Nicholson and Butterworth (1986).

2.3. Sample Size Determination and Sampling strategies

Multi stage random and proportional purposive sampling techniques were employed to select the representative animal from the study areas. Random sampling method was employed to select both Gudeya Bila and Nano districts among the districts of Western part of Oromia Region, Ethiopia. Nano Halo, Halo Dinki, Biftu Jalala, Haro Gudisa and Zangi were the peasant associations (PA's) involved as a study site during the study periods. A proportional purposive sampling method was employed to select the peasant associations (PA's). Among the (PA's) mentioned above, the first three of them were selected from Nano district and the last two of them from Gudeya Bila based on the density of cattle population. Finally, the studied animals were selected using simple random sampling techniques.

The number of animals required for the study was assessed using the formula given by Thrusfield (2007) for simple random sampling.

$$N = \underline{1.96^2 * P_{exp} (1-P_{exp})}{d^2}$$

Where, N = required sample size, P_{exp} = expected prevalence, d = desired absolute precision

The sample size determination was using 95% level of confidence, 50% expected prevalence since there was no previous study conducted in Nono and Gudaya Bila districts and 0.05 desired absolute precision. The sample size would be 384 cattle based on the formula; however, 891 cattle were examined to increase the precision of the study.

2.4. Study Methodology and Procedures

2.4.1. Buffy Coat Technique

Blood was collected from an ear vein using heparinized micro-haematocrit capillary tube and the tube was sealed. A heparinized capillary tube containing blood was centrifuged for 5 min at 12,000 rpm. After the centrifugation, trypanosomes were usually found in or just above the buffy coat layer. The capillary tube was cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed on to slide, homogenized on to a clean glass slide and covered with cover slip. The slide was examined under 40X objective and 10X eye pieces for the movement of parasite (Thrustfield, 2005).

2.4.2. Thin Blood Smear

The trypanosome species were identified using Giemsa-stained thin blood films. A small drop of blood from a

microhaematocrit capillary tube to the slide was applied to a clean slide and spread by using another clean slide at an angle of 45°, air dried and fixed for 2 min in methyl alcohol, then immersed in Giemasa stain (1:10 solution) for 50 min. Drain and wash of excess stain using distilled water, allowed to dry by standing up right on the rock and examined under the microscope with oil immersion objective lens. This technique is the most sensitive of the parasitological tests for the detection of T. vivax and T. congolense (Murray et al., 1977; Murray et al., 1983).

2.4.3. Measuring of Packed Cell Volume (PCV)

Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a capillary tube. The capillary tubes were placed in micro haematocrit centrifuge with sealed end outer most. The tube was loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. Tubes were then placed in haematocrit and the readings were expressed as a percentage of packed red cells to the total volume of whole blood. Animals with PCV \leq 24% were considered as anemic (Van den Bossche et al., 2000).

2.5. Entomological Survey

The apparent densities of tsetse flies were determined based on the mean fly catches in odors baited traps. Mixtures of Acetone, Octenol and cow urine were used as a bait to attract the flies. A total of 120 (110 Monopyramidal, 4 NGU and 6 Biconical traps) were positioned in five peasant association of the selected two districts at an approximate interval of 100 to 200m for 48 hours in watering and grazing points in which the fly and the vector are believed to have frequent contacts. The flies caught per trap were identified, counted and the apparent fly density per trap per day (f/t/d) was recorded.

2.6. Data Analysis

The collected data was analyzed using SPSS (version 20:0). Descriptive statistics was employed to measure the prevalence trypanosomosis and existing parasite species in the study areas. The Chi-square test was employed to test the significant difference in prevalence of trypanosomosis in association to the risk factors such as sampling areas (districts), age, body condition, sex and anemic status of the studied animals. Independent t-test was utilized to compare the mean PCV values of the parasitemic and aparasitemic animals. Single factor ANOVA was employed to test the mean PCV values of animal infected with different parasite species and non-infected animal. Differences between parameters were tested for significance at probability levels of P<0.05 and 95% confidence interval. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day using a descriptive statistics.

3. Results

3.1. Prevalence of Trypanosome and Parasite Species

The overall prevalence of trypanosome and parasite species in the study areas were shown in Table 1. Out of the 891 of cattle examined, only 24 animals were found positive for trypanosomosis and the prevalence in each district was 5.35% (23/430) for Nono and 0.22% (1/461) for Gudeya Bila district. Prevalence, hence, is 2.69% (24/891), 95% CI and the relative prevalence based on Trypanosome species was 1.79% (16/891) and 0.90% (8/891) for T. congolense and T. vivax, respectively.

3.2. Prevalence of Trypanosome and Effect of Associated Risk Factors

The prevalence of trypanosome and effect of associated risk factors in the study areas were presented in Table 2. The analysis for the associated risk factors revealed significant difference in the occurrence of trypanosomosis among different districts, anemic status and body condition of examined animals (P<0.05). However, the prevalence of trypanosome infections did not differ (P>0.05) between sexes and age categories (figure-1).

3.3. PCV Value of Aparasitaemic and Parasitaemic Animals

In this study, the average PCV of the animals infected with trypanosomes was 21.92 ± 0.73 % (Table 3). It was significantly lower (P<0.05) than the average PCV of the animals that were parasitological negative (27.44 ± 0.19%).

3.4. Entomological Survey Results

A total of 520 Glossina flies were caught from 120 odors baited traps deployed for two consecutive days at 5 PAs (kebeles) in two study districts. Almost all, 99.42% (517/520) of the tsetse flies were captured from Nano district (Table 4). The apparent density of tsetse flies captured in the area was 1.08 fly/trap/day. Out of the total caught 520 tsetse flies, 364, 100 and 56 were Glossina Fuscipes, Glossina Palidipes and Glossina morsistans, respectively. Higher proportion, 73.85% (384/520) were females and the rest, 26.15% (1236/520) of them are male tsetse flies.

4. Discussion

4.1. Prevalence of Trypanosome

The overall prevalence of bovine trypanosomosis in the study area was 2.69%, which was lower in contrast to the reports of other authors elsewhere in the country as shown in Table 5. However, a study by Tamiru et al. (2016) indicated that the prevalence of caprine trypanosomosis is only 1% in Nono district. The possible suggestion may be due to the interventions done to control the impact of the disease through Bedele National Tsetse and Trypanosomiasis Investigation and Control Centre (BNTTICC).

4.2. Trypanosome Species

Amongst the trypanosome species, T. congolense (66.67%) and T. vivax (33.33%) were detected in this study. Infection due to T. brucei or mixed infections were not noted. A similar proportional trend was shown in previous reports. For instance, T. congolense (72.73%) and T. vivax (27.27%) were detected in a study by Tafese et al. (2012). Similarly Bitew et al. (2011) found T. congolense (54.3%) followed by T. vivax (45.7%). Bezabih and Bisho (2017) also found T. congolense (60.3%), T. vivax (27.6%) and 12.1% mixed infection at Ouba Debrestahay District of Gamogoffa Zone, Southern Ethiopia. Kumela et al. (2018) also reported that T. Congolense (61.83%), T. vivax (26.83%) and T. brucei (12.75%) were species causing cattle trypanosomosis in Western Ethiopia. However, Mihret et al. (2007) reported T. vivax as the predominant species identified (90.5%) followed by T. congolense (4%) while the remaining (5.5%) were mixed infection. The discrepancy might be due to the differences in on agro-climatic conditions, season of year and interventions done to control the impact of the disease (Abebe and Jobre, 1996; Leta et al., 2016).

4.3. Associated Risk Factors and PCV

The analysis for the associated risk factors revealed that there was a significant difference in trypanosome prevalence between the study sites (P<0.05). Almost all, 99.42% (517/520) of the tsetse flies were captured from Nano district. The altitude of the district ranges from 1375 to 1500 masl. This could favor the presence of suitable habitat for the vectors which results in high fly density. Animals with poor body condition were more victimized for the disease as compared with animals with good body condition scores. Abebayehu & Biniam (2010), Melkamu et al. (2017) and Feyisa et al. (2019) also reported higher infection rate of trypanosomiasis for poor body conditioned animals than good to medium conditioned animals. The higher rate of infection for those poor conditioned and emaciated animals might be attributed to their reduced immunity for the diseases.

In this study, the prevalence of trypanosomiasis was not significantly affected by sex (P>0.05). This shows that both male and female cattle were equally susceptible to trypanosomosis infection. Similarly Daya and Abebe (2008), Abebayehu & Biniam (2010) and Melkamu et al. (2017) observed no significant difference in trypanosome infection between males and females. However, Magona et al. (2008) and Kumela et al. (2018) observed higher prevalence in male than in female animals. The present finding confirms that trypanosomosis is not sex linked.

Trypanosome infection among the different age groups indicated insignificant during the study period, but higher for four year old animals. This finding was in line with Kumela et al (2018) who observed a similar trend as trypanosomosis is not affected by age group of animals. In contrary to the present finding Abebayehu & Biniam (2010) and Melkamu et al. (2017) reported trypanosome infection was more in older cattle compared to young animals.

The present study revealed that anaemic animals were more susceptible to the trypanosomosis infection and the average PCV of the animals infected with trypanosomes was higher than for those of negative animals. This finding was in line with the previous findings of Feyisa et al. (2019). PCV is the most reliable indicator of anaemia in Trypanosomosis (Murray, 1978; Morrison et al., 1981). Several studies reported that Trypanosomosis caused depressed PCV levels in infected animals (Abebe, 2005; Mihret et al., 2007; Marcotty et al., 2008; Bizuayehu et al., 2012).

4.4. Entomological Survey

During the entomological survey, 520 tsetse flies were caught with the apparent density of 1.08 fly⁻¹trap⁻¹day. This finding was relatively higher than previous report of Melkamu et al. (2017) which was 0.83 flies⁻¹ trap⁻¹day in Edja district of Guraghe zone. Abebayehu & Biniam (2010) also reported 2.83 flies⁻¹ trap⁻¹day in Bench Maji zone, South Western Ethiopia. However, Lelisa et al. (2014), Kumela et al. (2018) and Kassaye (2015) reported fly densities of 10.5, 11.98 and 13.01 fly/trap/day from western part of Ethiopia. G. Fuscipes (70.0%), G. Palidipes (19.23%) and G. morsistans (10.77%) species of Glossina were identified from the area. Similarly Abebayehu & Biniam (2010) and Melese et al. (2017) detected G. Fuscipes and G. Palidipes as the dominant tsetse fly species. It had been reported that the *Glossina* species has a prominent economic impact as the biological vectors of trypanosomes (Kumar et al., 2012; Tesfaheywet and Abraham, 2012). In this study, female (73.85%) flies were more in number than male tsetse flies (26.15%) which supports the previous study of Melkamu et al. (2017). This might be attributed to the fact that females live longer lifespan than males as previously stated by lehane (2005)

and Kumela et al. (2018).

5. Conclusions

The result of the present study revealed that trypanosomosis is one of the problematic factors for poor agricultural activity and low production from animal in both Nono and Gudeya Bila districts. The most prevalent parasite species in the study area was T. congolense and T. vivax and the infections significantly affect the PCV values and body condition of the animal. The Glossina species particularly G. Fuscipes, G. Palidipes and G. morsistans had a prominent economic impact as the biological vectors of trypanosomes. Therefore, appropriate control measures and providing treatments based on the veterinarian recommendations should be taken to improve livestock productivity in the study areas, especially at Nono district.

Conflict of Interests

The authors declare that they have no competing interest

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Table 1 The overall	nrevalence of	trynanosome and	narasite sn	ecies in the	study areas
		ii ypanosome anu	parasite sp	cues in the	study al cas

Variables	Description	Number of Examined	Number of Positive	Prevalence (%)
Districts	Nono	430	23	5.35
	Gudaya Bila	461	1	0.22
	Total	891	24	2.69
Parasite Species	T. Vivax		8	33.33
	T. Congolence		16	66.67
	Total		24	100.00

Table 2. The prevalence of trypanosome and effect of associated risk factors in the study areas

Factor	Number of	Number	Prevalence	X ²	P-Value
	Examined	of Positive	(%)		
Study Sites					
Nono	430	23	5.35	22.355	0.000*
Gudaya Bila	461	1	0.22		0.000
Status					
Non - Aneamic	583	4	0.69	25.932	0.000*
Aneamic	308	20	6.49		
Sex					
Male	499	17	3.41	2.201	0.138
Female	392	7	1.79		
Body Condition					
Poor	196	10	5.10		
Medium	512	14	2.73	9.407	0.009*
Good	183	0	0.00		
Age					
1 year	30	0	0.00		
2 years	139	2	1.44		
3 years	266	5	1.88	4.744	0.448
4 years	208	9	4.33		
5 years	213	7	3.29		
6 years	35	1	2.86		

 X^2 = Chi-Square , P ≥ 0.05 = Non-significant, *P < 0.05 = Significant

Table 3: Mean PCV value of Aparasitaemic and Parasitaemic animals in the study areas

Variable	Description	Frequency	Mean PCV ± SE	t	F	P-Value
Result of	Infected	24	21.92 ± 0.73	4.823		0.000*
BCT	Non -Infected	867	27.44 ± 0.19			
PSANI	T. Vivax	8	$20.63\pm0.18^{\mathrm{a}}$		11.952	0.000*
	T. Congolence	16	22.56 ± 1.06^{a}			
	Non- Infected	867	27.44 ± 0.19^{b}			

^{a,b} The value across the column with different superscript are significantly different to each other (P < 0.05). BCT = Buffy Coat technique, PSANI= Parasite species and Non-Infected.

Table 4: Apparent density of flies caught during the study period

		Glossina Species								_		
	No.				Glos mor	sina sitans	Glo Fus	ssina cipes	Glos Pali	ssina dipes		F/T/D
Study	of	Altitude	No.	No.	Μ	F	Μ	F	Μ	F	Total	
Sites	PA's	Range	Traps	Days								
Nono	3	1375 - 1500	60	2	15	39	97	267	22	77	517	4.31
Gudaya-												
Bila	2	1895-1993	60	2	1	1	0	0	1	0	3	0.03
Total	5		120	4	16	40	97	267	23	77	520	1.08

F/T/D = fly /trap/day

Author (Year)	Part of the Country	Area/district	Prevalence
			(%)
Abebayehu & Biniam (2010)	Bench Maji zone, South Western Ethiopia	Guraferda and Sheko	4.4
Melkamu et al. (2017)	Gurage Zone, Southern Ethiopia	Edja	5.25
Feyisa et al. (2011)	Wolayta zone, Southern Ethiopia	Humbo	6.3
Gona et al. (2016)	Wolaita zone, Southern Ethiopia	Humbo, Duguna Fango, Damot Woyde	6.7
Tafese et al. (2012)	East Wollega zone, Western Ethiopia	Diga and Sasiga	8.55
Bitew et al. (2011)	West Gojjam zone	Jabi Tehrain	11.7
Feyisa et al. (2019)	South Western Ethiopia	Botor Tulay	12.24
Kumela et al. (2018)	Western Ethiopia	Dale Wabera	12.28





Figure 1: Prevalence of Trypanosomiasis associated to different age groups of cattle in the study area