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Incidence of Bovine Trypanosomosis and the Distribution of Vector in Chora District of Buno Bedele Zone, Oromia Region, Ethiopia

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Abstract
Bovine Trypanosomosis is one of the most parsimoniously problematic diseases in influencing mammals like cattle and it is a grave haemoprotozoan disease triggered by different species of unicellular eurykaryotic parasite of the genus trypanosome. This study was conducted to assess the incidence of Bovine Trypanosomosis, and density of vector distribution as well as the influence of its associated risk factors on cattle reared in Chora district of Buno Bedele Zone. A Blood sample was collected from a total of 401 cattle of age ranges from 1 to 6 years. The presence of parasites from sampled blood was examined using a method of Buffy Coat Technique and a technique of Giemsa-stained thin blood films was used to identify the existing trypanosome species in the study area. The Packed Cell Volume (PCV) of collected blood samples was determined using haematocrit. A total of 50 traps were deployed to study the entomological survey. In general, 2.99 % of the studied cattle were infected with Trypanosomosis and the T.Vivax (58.33%) was the dominant species of trypanosomosis and followed by T. congulense (41.67%) in the study area. Statistically significant differences ($P<0.05$) were observed due to associated risk factors viz. Body Condition and age of cattle, whereas sampling sites and sex were not significantly affect the prevalence of Bovine Trypanosomosis. The mean PCV value of Parasitaemic and Aparasitaemic animals were 21.37 ± 0.19 and 27.89 ± 0.16, respectively and they were statistically different at ($P< 0.05$). Overall of 0.80 flies per trap per day was recorded from the study area and among the caught flies of Glossina species, 10%, 54% and 36% of them were, G.tachinoides, G.pallidipes and G.fuscipes, respectively. Therefore, the veterinarian's has to continue providing the appropriate medication/treatment for the infected animals per appropriate recommendation and Bedele NTTICC has to take more focus to control the density and distribution of tsetse flies as well as biting flies for the transmission of high incidence of T. Vivax through mechanical ways of transmission of the disease.
Keywords: Cattle, Incidence, Prevalence, T. Vivax, T. Congulense
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Introduction

Among the constraints associated to animal health, Trypanosomosis is one of the important health factor results for low or insignificant livestock production in area of Africa which have the greatest potential for significant increases in domestic livestock productivity [1]. It is a serious haemoprotozoan disease caused by different species of unicellular eurykaryotic parasite of the genus trypanosome found in the blood and other tissues of vertebrates including livestock, wild life and people and transmitted cyclically by tsetse flies of Glossina species and many other insects mechanically [2, 3]. Bovine trypanosomosis is one of the major obstructions to livestock development and agricultural production in Ethiopia as a causative for insignificant development in general and to food self-reliance efforts of the nation in particular [1]. 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.

Vector born trypanosome species are disseminated in most parts of Western and South Western parts of Ethiopia [1, 4]. Tsetse flies are the cause for the transmission of trypanosomosis from one animal to other and widespread in the Western, South and South-western lowland regions and the associated river systems i.e. Abay, Ghibe Omo and Baro [5]. Presently, about 220,000 Km^2 areas of the above listed regions are infested with five species of tsetse flies namely Glossina pallidipes, G. morsitans, G. fuscipes, G. tachinoides and G. longipennis [6]. A major determinant of the distribution and epidemiology of bovine trypanosomosis is the availability of suitable habitat for tsetse. Tsetse is restricted to various geographical areas according to habitat and most of them distributed in forest, riverine and savannah areas. The palpalis and morsitans species are dominantly exist in the major livestock rearing areas and have a great importance in veterinary practices [7]. The most prevalent trypanosome species in tsetse infested areas of Ethiopia were Trypanosoma congolense and Trypanosoma vivax [8]. The reported prevalence varies from locality to locality depending on agro-climatic conditions, seasons, and

as part of activities which were intended to control the impact of the disease [5]. Among all mammals, bovines were dominantly affected by trypanosomosis and it has very importance in regards to economic point views [7].

The economic loss from crop and livestock production was directly or indirectly affected by Trypanosomosis [8]. It is a severe problem for agricultural production in widespread areas of the tsetse infested regions and non-tsetse transmitted trypanosomosis which affects considerable number of animal populations in tsetse free zone of the country [9]. 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 [10, 11]. Even if trypanosomosis is very important disease, not enough studies have been conducted in Chora District of Buno Bedele Zone, Oromia Region. Thus, this study was conducted in Chora District with the objectives of assessing the incidence of bovine trypanosomosis, identifying the influence of associated risk factors and the most dominant causing vectors in the study area.

Materials and Methods

Description of the study area

The study was conducted in Chora District of Buno Bedele Zone of Oromia Regional State, Ethiopia. The center of the district is Chora Town, located at 515 km from Addis Ababa towards South Western direction of Ethiopia. Annual average rainfall distribution is ranging from 1500 to 2300 mm with mean average daily temperature of 10-32 ^{O}C and located at mean altitude of 2300 masl [12]. The study area is covered by different type of vegetation including forests, vast coffee plantation, chat *(Catha edulis)* and different wild animals. Agriculture is the main income source of the livelihood with dominant mixed farming system including rearing of livestock, crop cultivation and honey production [13, 14]

Study Design

A cross sectional study was conducted to determine the prevalence of bovine trypanosomosis in Chora district of Buno Bedele Zone in study period between from 30th of March to 30th 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 study area. The age of the cattle was determined according to the definition characteristics [15] and information from owners of the cattle. The body condition of the study animals were categorized based on the criteria described by [16].

Sample Size Determination and Sampling strategies

Multi stage sampling technique was employed to conduct this study. Purposive sampling technique was employed to select Chora district among the districts under Buno Bedele Zone based on the density of cattle population, road access and the existence of man-made & natural forests in addition to having a plenty of rivers. The study sites or Peasant associations (PA's) involved in the study were chosen using a purposive sampling method based on the density of cattle population and flies distribution. Accordingly, Sololo, Sese and Chirache peasant associations were the selected study sites for this study. Finally, the studied animals were selected using simple random sampling technique. The number of animals required for the study was assessed using the formula given by Thrusfield [17] for simple random sampling.

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

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 and 0.05 desired absolute precision. Based on the formula, the sample size would have been 384 cattle however, 401 cattle were involved as a sample for increasing the precision of the study.

Study Methodology and Procedures

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 ×40 objective and ×10 eye pieces

for the movement of parasite [18].

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 [19, 20].

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 ≤ 24% were considered as anemic [21].

Entomological Survey

A totals of Fourteen seven (47) Monopyramidal, two (2) Ngu and one (1) Biconical traps were positioned in three peasant associations of the study areas. Each of them was placed with an approximate interval of 100 to 200m for 48 hrs. in watering and grazing points in which the fly and the vector are believed to have frequent contacts. A mixture of Acetone, Octenol and Cow urine were used as a bait to attract the flies. Then after 48 hrs of deployment, tsetse flies in the cages were counted and identified based on their habitat and morphology to the genus and species level [22]. The sex of Tsetse flies was identified by observing the posterior end of the ventral aspect of the abdomen using hand lens. Male flies were identified by their enlarged hypopygium in the posterior ventral end of the abdomen. The apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day [23].

Data Analysis

The collected data was analysed 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 of prevalence of trypanosomosis in association with factors such as sampling sites, age, body condition and sex 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.

Result

Parasitological Findings

From total of 401 cattle, 12 of them were detected as positive for infection using a Buffy coat technique with an overall prevalence of 2.99 %. Regarding to the infected animals across the study sites were 3.98 and 3.96 % from Sololo and Sese peasant associations, respectively. However, in contrast to this there were no any infected cattle found in Chirache peasant association. T. Vivax (58.33%) was the most prevalent trypanosome species and the rest 41.67% was recorded for T. Congolense species (Table 1).

Table 1: The overall prevalence of trypanosome in the study area

Variables	Description	Number of Examined	Prevalence in Number (%)
Sampling Site	Sololo	201	8 (3.98)
	Sese	101	4 (3.96)
	Chirache	99	0 (0.00)
	Total	401	12(2.99)
Parasite Species	T. Vivax		7 (58.33)
	T. Congolense		5 (41.67)
	Total		12(100.00)

Prevalence in association to different factors

Effect of different associated factors pertaining to the prevalence of trypanosomosis was shown in Table (2). The prevalence of trypanosomosis in sampling sites of Sololo, Sese and Chirache peasant associations were 3.98%, 3.96% and 0.00%, respectively and it was not significantly different (P>0.05) across different sampling sites. However, even if the association is not statistically different, there was no any occurrence of trypanosomosis in Chirache sampling site during the study periods. Similarly there was no significant difference in trypanosomosis infection among the cattle having different sex. Among 401 sampled cattle, 12.5, 2.08 and 2.33 % of prevalence of bovine trypanosomosis was recorded in cattle having a poor, medium and good body condition, respectively and it was statistically different (p<0.05) across the three body condition of cattle. Prevalence of bovine trypanosomosis was statistically different (P < 0.05) in association to the age categories and the highest record was observed from the older cattle (17.65%) as well as from younger cattle (12.5%).

Hematological results of examined animals

The recorded mean PCV value of aparasitaemic and parasitaemic animals indicated that, out of 401 examined cattle 12 of them were found as anemic (parasitaemic) and their mean PCV value was lower than that of aparasitaemic (non-infected) cattle as well as statistically different at (P<0.05). The observed mean PCV value of animals infected with T. Vivax and T. Congolence as well as non- Infected animals was not statistically different at (P<0.05) (Table:3).

Entomological Survey

A total of 50 traps were deployed for two consecutive days at 3 Peasant Association (PA's) as a sampling site and a total of 241 Glossina Species of flies were caught. Among the caught flies; 10%, 54% and 36% of them were G.tachinoides, G.pallidipes and G.fuscipes, respectively. The overall apparent fly density was 0.80 flies /trap /day. The fly's density per different study sites were 3.03, 1.13 and 2.87 flies/trap/day in Sololo, Sese and Chirache, respectively. The highest density of flies/trap/day was recorded in the Sololo peasant association and lowest in Sese peasant association. Among the caught flies, 23.65% and 76.35% of the flies were male and female, respectively (Table: 4).

Table 2: Prevalence of trypanosome and effect of associated factors

Factor		Number of Examined	Prevalence in Number (%)	X^2	P-Value
Study Sites					
	Sololo	201	8 (3.98)		
	Sese	101	4(3.96)	4.055	0.132
	Chirache	99	0(0.00)		
Sex					
	Male	259	9(3.47)	0.586	0.444
	Female	142	3(2.11)		
Body Condition					
	Poor	32	4(12.50)		
	Medium	240	5(2.08)	10.845	0.004*
	Good	129	3(2.33)		
Age					
	1 year	8	1(12.50)		
	2 years	56	2(3.57)		
	3 years	169	4(2.37)	17.376	0.004*
	4 years	58	0(0.00)		
	5 years	93	2(2.15)		
	6 years	17	3(17.65)		

X^2 = Chi-Square , $P \geq 0.05$ = Non-significant, $P < 0.05$ = significant

Discussions

This study had revealed the overall prevalence of trypanosomosis was 2.99 % (Table 1). The result of this finding was almost near to the result reported by Adane and Gezahagne [24], who reported 3.5% prevalence of trypanosomosis in Dejen District, Amhara Region, Ethiopia. The result of this finding was lower than comparing to other studies conducted elsewhere within Ethiopia; which ranges from 4.43 to 14.97% in a year between 2012 to 2019 (1, 25, 26, 27, 28, 29, 30, 31]. Overall low record of prevalence of trypanosomosis may be due to the impact of parasite and vector control practices have been done by Bedele NTTICC (Bedele National Tsetse and Trypanosomiasis Investigation and Control Center) and the geographical location of the sampling sites; which have equal access for getting an extension services to the farmers regarding to how control the distribution

of vectors, disease control and treatments used for treating the infected animal. The suggestions is similar with [5, 8] who stated that, the prevalence varies from locality to locality depending on activities which were intended to control the impact of the disease.

According to the result presented in Table (1), the proportion of T. Vivax species was in agreement with the findings reported by Tewodros [32] who reported the highest proportion of T. Vivax from Hawa Gelan District, Oromia Region, Ethiopia. According to his report, 45.85 % and 33.33% of T. Vivax and T. Congolence were recorded, respectively. In contrast to this, the highest proportion of T. Congolence was recorded on the report of Megersa *et al.*[31] and Marta *et al.*[28] who reported a high proportion of T. Congolense from Botor Tolay and Chora Districts of Oromiya Region Ethiopia, respectively. The highest proportion of T. Vivax could be due to the ability to adopted itself to the environment and transmitted through mechanical. This possible suggestion is in accordance to the study reported by Leak (22) who stated as the highest proportions of T.Vivax are transmitted mechanically by other biting flies rather than cyclical transmission of trypanosomiasis.

The Prevalence of trypanosomosis in three sampling sites was not statistically different at (P>0.05) (Table 2). The finding of this study is in agreement with the study of Zemedkun [33] and Abayneh [1] those reported from Kindo Koysha District of Wolayita Zone, and three selected districts of Wolayita Zone, Ethiopia. In contrast to this, a significant difference among different study sites was reported by Abebayehu and Biniam [25] from two districts of Bench Maji Zone, South Western Ethiopia. The observed insignificant result could be due to alike controlling measures had been taken to control the flies densities by Bedele NTTICC, the geographical location of sampling sites were being at the same district and having the same flies belt and the same treating strategies. This is in accordance to the suggestion provided by [5, 8] who stated that, the prevalence varies from locality to locality depending on activities which were intended to control the impact of the disease and way of treating the infected animal.

Significant difference was observed in prevalence of trypanosomosis between different age group of cattle during study period (Table 2). The result of this study was in line with the study reported by [28, 31] who found significant difference in prevalence of trypanosomosis among different age group of examined cattle, whereas study reported by [1, 29, and 31] indicated that, insignificant effect of age on prevalence of trypanosomosis on cattle sampled from three selected districts of Wolayita Zone, Kindo Koysha District of Wolayita Zone and Hawa Gelan District of Oromia Region, Ethiopia respectively. The differences in prevalence of trpanosomiasis with respect to age differences could be due to highest degree of resist against to the disease at adult stage and more degree of susceptibility at early younger and older age. There was no significant difference (P>0.05) in prevalence of trypanosomosis between cattle categorized according to their sex (Table 2). The result of this finding is in agreement to the study reported by Eskziaw *et al.* [29] from Gimbo and Guraferda districts of Southern Ethiopia and Megersa *et al.* [31] from Botor Tolay District of Jimma Zone, Ethiopia and Marta *et al.* [28] from Chora District of South Weastern Oromia, Ethiopia. However, significant difference was recorded by Zemedkun *et al.* [33] from three selected Districts of Wolayita Zone, Southern Ethiopia. This could be attributed to similar controlling measures had been taken to control the flies densities by Bedele NTTICC and alike extension services concerning disease control and treating all sex groups of infected animals. The suggestion is similar with [5, 8] who stated that, the prevalence varies from locality to locality depending on activities which were intended to control the impact of the disease. The prevalence of trypanosomosis was statistically different (P < 0.05) across different categories of body condition (Table 2). The result of this finding is consistent to the study reported by Zemedkun *et al.* [33] and Megersa *et al.* [31] who reported as there was significant difference in prevalence of trypanosomosis among cattle categorized according to their body condition from Kindo Koysha District of Wolayita Zone and Botor Tolay District of Jimma Zone, Ethiopia, respectively. In contrast to this, insignificant effect of body condition on the prevalence of trypanosomosis on cattle from Kindo Koysha District of Wolayita Zone, Ethiopia is recorded by [1]. These differences might be due to the disease itself results in progressive emaciation of infected animals [34, 35, and 36].

Table 3: Mean PCV value of Aparasitaemic and Parasitaemic animals and incidence parasite species

Variable	Description	Frequency	Mean PCV ± SE	t	F	P-Value
Anaemic Status	Parasitaemic	164	21.37 ± 0.19	-25.584		0.000*
	Aparasitaemic	237	27.89 ± 0.16			
PSVsNI	T. Vivax	7	23.43 ± 1.67[a]		0.89	0.411
	T. Congolense	5	26.40 ± 1.40[a]			
	Non- Infected	389	25.24 ± 0.21[a]			

[a] The value across the column with the same superscript are not significantly different at (P > 0.05). PSVsNI=Parasite species versus Non-Infected.

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

Sampling Sites	Altitude Range	No. Traps	No. Days	Glossina Species									
				G.morsitans		G.tachinoides		G.pallidipes		G.fuscipes			
				Male	Female	Male	Female	Male	Female	Male	Female	Total	F/T/D
Sololo	1475-1669	20	2	0	0	4	10	19	49	10	29	121	3.03
Sese	1614-1887	15	2	0	0	3	0	3	9	3	16	34	1.13
Chirache	1572-1630	15	2	0	0	1	6	9	41	5	24	86	2.87
Total	-	50	6	0	0	8	16	31	99	18	69	241	0.80

F/T/D = fly /trap/ day

Among the total (401) examined cattle, 164 of them were parasitemic and they found to be anemic (PCV ≤ 24%) compared to aparasitemic (237) cattle and the difference was statistically significant at ($P < 0.05$). The result of this finding is in line to the study reported by Van den Bossche and Rowlands [37] who stated that average PCV of parasitologically negative animals was significantly higher than that of parasitologically positive animals. Thus, trypanosomosis may be involved in adversely lowering the PCV value of infected animals. The low in PCV value of infected animals is may be due to the animals were got sick with disease like as helminthosis, tick-borne diseases, and dietary disparities. It is similar to the suggestion provided by [25]. Whereas, the mean PCV value of cattle infected with T. Congolence, T. Vivax and the non-infected one were not statistically different at ($P > 0.05$). The result of this finding is in agreement with the study report of Zemedkun *et al.* [33] from the three selected districts of Wolayita Zone, Southern Ethiopia.

The apparent density of tsetse flies was about 0.80 flies /trap/day. Different types of traps were designed to catch different species of tsetse flies. The traps availability is indicated based on their efficiency for those species for which the trap has been tested and recommended [40]. Among the total 241 (Glossina Species), 10% (G.tachinoides), 54% (G.pallidipes) and 36 % (G.fuscipes) of them were caught during two consecutives trapping days. Among a total of 50 traps, 47, 2 and 1, of them were Monopyramidal, NUG and Biconical traps, respectively and each of them had different efficiency in catching different species of Glossina Species. The apparent tsetse fly density recorded from Sololo (3.03) and Chirache (2.87) sites were almost close to the report of Abebayehu and Biniam, [25], who reported 2.83 form two districts of Bench Maji Zone, Western Ethiopia. However the lowest density was observed in Sese sampling site (1.13 F/T/D). The overall apparent tsetse fly density of this study was (0.08 F/T/D) and it is almost close to the result found by Zemedkun *et al.* [30] from three selected districts of Wolayita Zone, Southern Ethiopia with the overall apparent density of (0.14 flies/trap/day). In contrast to this, the highest results were recorded by Ayele *et al.* [41] from Daramallo District, Ethiopia with flies/trap/day of 19.14 and 14.97 from different selected study sites of Arbaminch by [42]. This difference might be ascribed to the influence of different biting flies for non-cyclical transmission of trypanosomiasis as well as somewhat differences in their agro-ecological conditions. This possible suggestion is in line with the study report of [31].

Conclusions

The finding of this study showed that, Trypanosomosis is one of the challenging diseases affecting the livestock rearing/farming activity of farmers and it results for low income from rearing cattle in the study area. T. vivax and T. congolense were the type of parasite species recorded in the study area and the former was the predominant species in the study area. This finding indicates, body condition and age of cattle had significant effect on the prevalence of bovine Trypanosomosis, while insignificant differences were also recorded across different sampling sites and sex differences in cattle. The mean PCV value of infected cattle was lower than comparing to that of the non-infected cattle and significant differences was also observed. The Glossina species particularly, G.tachinoides, G.pallidipes and G.fuscipes were the type of Glossina species prevailing in the study area and G.pallidipes was recorded as a dominant species. Therefore, the veterinarian's has to continue providing the appropriate medication/treatment for the infected animals per appropriate recommendation and Bedele NTTICC has to take more focus to control the density and distribution of tsetse flies as well as biting flies for the transmission of high incidence of T. Vivax through mechanical ways of transmission of the disease.

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