Global Veterinaria 20 (1): 19-25, 2018
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2018.19.25

Parasitological Prevalence and its Associated Risk Factors of Bovine Trypanosomosis in Sire District, Western Ethiopia

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Abstract: A cross-sectional study design was conducted from November 2016-March 2017 to estimate the current parasitological prevalence of bovine trypanosomosis in cattle and associated risk factors in Sire district of Eastern Wollega Zone of Oromia Regional State, Western Ethiopia. From 384 randomly selected animals blood sample was collected for different parasitological and haematological techniques. Out of 384 cattle examined 11 (2.86%) were found to be infected with trypanosomes. The prevalence in terms of trypanosome species was 1.82% *Trypanosoma congolense*, 0.78% *T. vivax* and 0.26% *T. brucei.* The proportion of trypanosome species was 63.64% (7/11) *T. Congolense*, 27.27 % (3/11) *T. vivax* and 9% (1/11) *T. brucei.* The prevalence of trypanosomosis in relation to sex, age group and body condition score was not statistically significant ($P > 0.05$). The overall anemia prevalence in the study district was 19.27% (74/384). The anemia prevalence was significantly higher in trypanosome positive cattle (54.54%) than in non-infected cattle (18.23%) ($P < 0.05$). Of 19.27% anemia prevalence, 1.56% (6/384) was trypanosome infected animals. However, large number of animals 17.7% (68/384) had anemia (PCV < 24) without having trypanosome infection. Therefore, to improve livestock production and agricultural development interred control method against the parasite and their vectors should be strengthened.

Key words: Anemia · Buffy Coat · Prevalence

INTRODUCTION

Ethiopia has huge and diverse livestock population that plays an important role in the economy and livelihoods of farmers and pastoralists. Among livestock, cattle are the primary resource for people and government of Ethiopia. Despite the large animal population, productivity in Ethiopia is low and even below the average for most countries in eastern and sub-Saharan African countries, due to poor nutrition, reproduction insufficiency, management constraints and prevailing animal diseases [1].

Animal trypanosomosis is an important livestock disease in Africa which is considered as a threat to the ongoing effort on poverty alleviation in the continent [2]. It is a serious disease in domestic livestock that causes a significant negative impact in food production and economic growth in Africa [3]. The disease is distributed over approximately 10 million km^2 of sub Saharan Africa between latitudes 14°N and 29°S which directly coincide with distributions of tsetse fiies, which is the main vector of the disease [4].

In sub-Saharan Africa, about three million livestock die every year due to tsetse fly transmitted trypanosomosis. The wide occurrence of this disease in people and livestock retards agricultural and economic development in Africa and 30% of the continent cattle population, estimated to be 160 million and comparable numbers of small ruminants are at risk of trypanosomosis [5].

The tsetse flies are widely distributed in the Western Southern and South Western low lands and River Valleys and 15% of the land believed to be suitable for livestock production is affected by one or more of the following species of tsetse flies; *Glossina morsitans sub morsitans, G. paulidipes, G. tachinoides, G. fuscipes fuscipes* and *G. longipennis [6]*. The distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation and presence of suitable hosts [7].

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In Ethiopia, trypanosomosis is one of the most important disease limiting livestock productivity and agricultural development. Estimates made decades ago reported that 180, 000 - 220, 000 km land in the western and south western parts of the country to be suitable for tsetse [8]. Recent estimates indicate that, about 140, 000 km fertile agricultural land which is roughly 12% of the country's land mass is found to be a suitable habitat for tsetse fly [9].

In Ethiopia, the most important tsetse born trypanosomes inficting economic losses in domestic livestock are *T. congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats. Camels are affected by *T. evansi* which is common species in camel rearing areas of the country while equines mainly horses are affected by *T. equiperdum* in some highland parts of the country [10].

Trypanosomosis which is distributed in South-Western administrative region in distribution, animal trypanosomosis is among the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of South West and North West part of the country following the greater River Basins of Abay, Omo, Ghibe and Baro, which has a high potential for agricultural development [11]. Over 6 million heads of cattle and equivalent number of other livestock species are at risk of contracting the diseases. More than 20,000 heads die per annum and annual loss attributed to the diseases is estimated to be over US $236 million, whereas loss due to reduce meat, milk and draft power is not applicable [12].

Even though the disease is endemic in the country especially in Eastern Wollega zone, there is no well documented information about Bovine trypanosomosis and associated risk factors. But, baseline data collection and regular investigation on the prevalence of the parasites is essential to know the burden of the disease at different geographic locations and to enable the measurement of the impact of any control options that will be introduced later for determination of trypanosome infection status. Hence, the study was designed to determine the parasitological prevalence of bovine trypanosomosis, to identify trypanosome species involved during study period and assess potential risk factors in Sire district of Eastern Wollega zone of Oromia Regional State, Western Ethiopia.

MATERIALS AND METHODS

Study Area Description: The study was conducted in Sire District of Eastern Wollega Zone of Oromia Regional State, Western Ethiopia. The area is found at 300 km of west of Addis Ababa, the capital city of Ethiopia. The area lies between 08° 25' 56"N to 08°58'05"N and 34° 33' 41"E to 35° 28' 48"E and has average altitude of 1150 meters above sea level. The area has temperature 33-35°C with more agricultural crops and people in rural of the country. The climatic condition alternates with long summer (May to August) and short rainy seasons from (March to April) and the winter dry seasons (November to February) with mean annual rainfall of 1200 mm [13].

Study Population: The study population constitutes 384 indigenous zebu cattle (*Bos indicus)* of different sex, age groups and body condition scores and managed under smallholder mixed crop-livestock farming system. The age of the animals was grouped as young (<2 years), adults (2–5 years) and old (greater than five years) based on dentition according to the classification used by DeLahunta and Habel [14] and the body condition score was categorized as poor, medium and good based on the appearance of ribs and dorsal spines applied for zebu cattle [15].

Sampling and Sample Size Determination: A cross-sectional study design was conducted from November 2016-March 2017) to estimate the current prevalence of trypanosomosis in cattle in the area. The animals were sampled randomly involving both sexes, all age groups and all types of body conditions. Since there was no previous study conducted in Sire District to establish the prevalence, the sample size was determined by taking 50% expected prevalence of trypanosomosis using the formula given by Thrusfield [16] with 5% precision and 95% confidence interval. Hence, a total of 384 animals were needed to be sampled.

Parasitological and Hematological Data

Packed Cell Volume (PCV) Determination: Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinized capillary tubes. The tubes were then sealed at one end with crystal seal. The capillary tubes were placed in micro-hematocrit centrifuge and were allowed to centrifuge at 12,000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the capillary tubes were placed in a hematocrit reader. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic [17].

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 onto two glass slide and one slide covered with a 22 × 22 mm cover slip for wet smear. The slide was examined under x40 objective and x10 eye piece examined by dark ground-phase contrast microscope for detection of trypanosome for movement of parasite. Trypanosome species were identified according to their morphological descriptions on Giemsa stained blood film as well as movement in wet film preparations was examined by dark ground-phase contrast microscope for detection of trypanosome [4]. The second slide was with small drop of blood from a micro-hematocrit capillary tube was applied to a clean slide and spread by using another clean slide at an angle of 45° for thin blood smear. The smear was air dried and then fixed for 2 min in methyl alcohol. The thin smear was flooded with Giemsa stain (1:10 solution) for 30 min. Excess stain was drained and washed by using distilled water. Then it was allowed to dry by standing up right on the rack and examined under the microscope (x100) oil immersion objective lens for the purpose of species identification [17].

Data Analysis: Animal and laboratory data were stored in Microsoft-Excel and later exported to SPSS Software Version 20 for Analysis. Pearson's Chi-Square (x^2) was carried out to determine the association of the explanatory variables (Sex, Age and Body Condition) and Student two t-test was used to compare mean PCV of infected and non-infected animals. A statistically significant difference between variables was considered at P<0.05 at 95% confidence level. PCV was categorized as anemic if it is less than 24% and normal if it is greater than or equal to 24%.

RESULTS

Out of 384 cattle examined 11 (2.86%) were found to be infected with trypanosomes. The prevalence in terms of trypanosome species was 1.82% *T. congolense*, 0.78% *T. vivax* and 0.26% *T. brucei.* The proportion of trypanosome species was 63.64% (7/11) *T. congolense*, 27.27 % (3/11) *T. vivax* and 9% (1/11) *T. brucei* (Fig. 1). During the study period mixed infection was not detected.

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Fig. 1: Distribution of the species of trypanosomes among the infected animals.

The prevalence of trypanosomosis was higher in males (3.64%) as compared to female animals (1.45%), highest prevalence was observed in the adult animals greater than 5 years old (Table 1). The prevalence of trypanosomosis between body condition scores was 3.37% in poor, 2.81% in medium and 2.61% in good body condition animals (Table 1). However there is no statistical significance between the risk factors (sex, age and body condition score).

The mean PCV value of 27.7% was registered during the study period. The most frequently recorded PCV value was 28% and was recorded in 35 animals in the district. The mean PCV values of cattle were significantly (P = 0.001) influenced by trypanosome infection as 27.86 and 22.36% PCV values in trypanosome positive and trypanosome negative animals were registered, respectively (Table 2).

The overall anemia prevalence in the district was 19.27% (74/384). The anemia prevalence was significantly higher in trypanosome infected animals (54.54%) than in non-infected animals (18.23%) (P < 0.05). Of 19.27% anemia prevalence, 1.56% (6/384) was trypanosome infected animals. However, large number of animals 17.7% (68/384) had anemia (PCV < 24) without having trypanosome infection. Some animals 1.3% (5/384) were infected by Trypanosome but their PCV was found normal (Table 3).

Table 1: Prevalence of bovine trypanosomosis according to sex, age and body condition score in Sire District.

Risk factors		Number examined	Number positive	Prevalence (%)	P^2	*P* value
Sex	Female	137	2	1.45	1.485	0.223
	Male	247	9	3.64		
	Total	384	11	2.86		
Age	<2 year	25	0	0	3.397	0.183
	2-5 year	158	4	2.53		
	Total	384	11	2.86		
Body condition	Good	153	4	2.81	0.112	0.945
	Medium	142	4	2.81		
	Poor	89	3	3.37		
	Total	384	11	2.86		

Table 2: Mean PCV comparison between infected and non-infected animals.

Condition	Number	Mean	SD	*P*-test	*P* value
Infected	11	22.36	7.3929	3.3020	0.001
Non Infected	373	27.86	5.3814		

Table 3: Proportion of anemia from trypanosome infected and non-infected animals

Trypanosome	Anemia	Frequency	Percent in total Animal	Percent share per strata
Non infected	Negative	305	79.4%	81.8%
	Positive	68	17.7%	18.2%
Infected	Negative	5	1.3%	45.5%
	Positive	6	1.5%	54.5%

DISCUSSION

The overall prevalence of bovine trypanosomosis in the study area was 2.86%. This result is in close agreement with the findings of Abebayehu *et al.* [18], Teka *et al.* [19], Fayisa *et al.* [20], Ayana *et al.* [21] and Kumela *et al*. [22] who reported a prevalence of 2.66%, 4.43%, 4.86%, 2.10% and 4.25% from Western Tigray, Northern Ethiopia, Didesa District, Arbaminch area, Amhara region, Northwest Ethiopian and Ilubabor Zone, Southwestern Ethiopia, respectively.

The finding of the current study is lower than a range of studies conducted previously in Ethiopia: Tafese *et al*. [23] 8.5% in East Wollega zone using Buffy Coat Technique; Mekuria and Gadissa [24] reported 12.41% in Metekel and Awi zones of Northwest Ethiopia; Cherenet *et al*. [25], who assessed cattle trypanosomosis in the *Tsetse*-free and the *Tsetse*-infested zones of the Amhara Region of Northwestern Ethiopia using molecular diagnostic method, reported infection rates of 20.9% and 25.7%, respectively. This result was also lower as compared to Abebe and Jobre [26] at Tsetse infested Areas of Ethiopia (17.67%); Mekuria and Gadissa [24] in Dembecha and Jabitehenan (12%); and Shimelis *et al*. [11] in Metekel District (17.20%).

Lower prevalence was found in this study compared to the works of these authors elsewhere in the Country. This disparity emanates from many factors that explain the lower trypanosomosis prevalence in the study area. There were parasite and vector control programs practiced in the area. Also as the study was conducted during late rainy season it is obvious that the population of flies increases; due to this farmers inject their animals with trypanocidal drugs and also use insecticide spray in this season better than any other time to minimize the effect of the disease. In addition, expansion of veterinary services up to peasant association and deforestation for crop cultivation and settlement might also have contributed to the low prevalence. The lower prevalence observed in this study could also be due to inadequacy of parasite detection method used. It was reported that the buffy coat microscopy technique is relatively an insensitive diagnostic method as it fails to detect 66% of infected [27]. The molecular diagnostic techniques which permit precise identification of the parasite to species level and serological diagnostic methods are more sensitive [28].

Out of the 2.86% overall prevalence of trypanosome infection, 1.82% were due to *T. congolense*, 0.78% were due to *T. vivax* and 0.26% were due to *T. brucei.* The finding of this study showed that of the total trypanosome positive animals 63.6% were found to be infected with *T. congolense*, 27.2% were infected with *T. vivax* and the remaining 9% were infected with *T. brucei.* In the current study mixed infection was not detected. The higher proportion of *T. congolense* in this study was in agreement with the previous results of Abebe and Jobre

[26] for tsetse infested areas of Ethiopia (58.5%) and Murray *et al*. [29] at Mereb abaya, south Ethiopia (66.1%). Moreover, the results of Muturi [30] at Arba minch zuria districts (85.2%) and Woldeyes [31] in Ghibe valley, southwest Ethiopia (84%), had also shown higher results of T. *congolense*.

The predominance of T. *congolense* infection in cattle suggests that the major cyclical vectors or *glossina* species are more efficient transmitters of *T. congolense* than *T. vivax* in east Africa [32] and also due to the high number of serodems of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by infected animals [33]. Different studies [33, 34] have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense* and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues [35]. According to Abebe and Jobre [26], *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in *tsetse* infested and *tsetse* free areas of Ethiopia, respectively.

The prevalence of bovine trypanosomosis was studied in different sex, body condition and age groups of cattle and significant variation was not observed ($P > 0.05$). This might be because of an equal chance of exposure to the parasite. This result is in agreement with the previous researches reported by Mihret and Mamo [36], Abebayehu *et al*. [18], Tafese *et al*. [23] and Stephen [37]. In the present study sex was not found to be the risk factor; both males and females can be affected uniformly in high tsetse challenge areas.

The overall anemia prevalence in the study district was 19.27%. When infected and non-infected animals were compared, the anemia prevalence was significantly higher in trypanosome positive cattle (6/11, 54.54%) than in non-infected cattle (68/373, 18.23%) ($P < 0.05$). This finding was in agreement with previous reports Cherenet *et al*. [25], Mihret and Mamo [36] and Stephen [37]. Of total anemia prevalence (19.27%), 1.56% was trypanosome positive animals. However, large number of animals, 17.7%, had anemia without having trypanosomosis infection. This suggests that even though anemia is characteristic of trypanosomosis, other factors are also anticipated to affect the PCV profile of animals. Diseases such as fasciolosis, gastrointestinal parasitism, vector-borne diseases and nutritional deficiencies can also cause reduced PCV [38]; however there were no previous published research reports of these diseases in the studied area.

Some animals were infected by trypanosome but their PCV was normal and anemia was not recorded in them. This might be due to some infected animals being able to keep their PCV within the normal range for a certain period of time. The appearance of parasitological negative animals with PCV values of less than the threshold value set (24%) may be due to inadequacy of the detection method used [29], other anemia causing diseases [39], or delayed recovery of the anemic situation after current treatment with trypanocidal drugs. Furthermore, the occurrence of positive animals with PCV of greater than 24% might be thought of as recent infections of the animals [40].

The mean PCV value of parasitemic animals was found to be significantly lower (22.36% ± 7.39) than that of aparasitemic (27.86% ± 5.38) animals which is similar to the results obtained by Cherenet *et al*. [25] and Bekele and Nasir [38]. Taking the PCV value 24 to 46% as normal for zebu cattle, 54.5% of the parasitemic and 18.2% aparasitemic animals have registered PCV values less than 24%. Low PCV value may not solely be due to trypanosomosis. However, these factors are likely risks for both parasitaemic and aparasitaemic animals. Therefore the difference in mean PCV value between parasitemic and aparasitemic animals indicates that trypanosomosis is involved in reducing the PCV values in the infected animals

CONCLUSION

This study indicated that trypanosomosis is an important disease and a potential threat that affects the health and productivity of cattle in Sire district. The major species of trypanosomes in the study area were *T. congolense* followed by *T. vivax* and *T. brucei*. Nearly 20% of the sampled animals had a PCV value of below 24% and were thus considered as anemic. The anemia prevalence was significantly higher in trypanosome positive cattle than in non-infected cattle. The mean PCV value of parasitemic animals was significantly lower (22.36% ± 7.39) than that of aparasitemic (27.86% ± 5.38) animals. Therefore, proper strategies have to be designed and implemented to minimize its effect on livestock production in the study area.

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