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Prevalence of *Trypanosoma* spp. on Cattle and Associated Risk Factors in Selected Districts of Borana Zone

Bantayehu Muluneh[1], Dereje Teshome[1], Adem Kumbe[1], Jaldesa Doyo[1], Beshir Hussien[1] and Dereje Kussa[2*]

[1]Yabello Pastoral and Dry land Agriculture Research Center, Oromia Agricultural Research Institute, P. O. Box, 085, Yabello, Ethiopia

[2]International Center of Insect Physiology & Ecology (ICIPE) PO Box 5689, Addis Ababa, Ethiopia

**Corresponding author*

Abstract

A cross-sectional study was carried out to determine the prevalence of bovine trypanosomosis and some associated risk factors, in purposively selected areas of Taltalle and Elawoye districts of borana zone from August 2021 to April 2022. For this purpose, a total of 768 blood samples were collected from cattle using systematic random sampling method considering different age, body condition and coat color; as well as both sex groups of cattle. The packed cell volume (PCV) of each sampled animal was measured using hematocrit reader after centrifugation at 12,000 rpm for five minutes. The overall prevalence of bovine trypanosomosis was found to be 5.08% (39/703). The most prevalent trypanosome species were Trypanosoma congolense (74.35%) followed by Trypanosoma vivax (15.38%) and Trypanosoma brucei (10.25%). The mean PCV recorded as 16.51 ± 6.05 in parasitaemic and 19.56 ± 6.22 aparasitaemic with results showed significant statistical difference (P<0.05) between the two groups of animals. From considered risk factors; district, PCV, age groups and Coat color of animals were statistically significantly (P<0.05) associated with prevalence of bovine trypanosomosis. However, body condition and sex were insignificant (P>0.05). Therefore, the present study indicated that trypanosomiasis is important disease threat animals in the study area, thus it requires outstanding attention

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Bovine, Borana, Prevalence, Risk factors, Trypanosomosis.

Introduction

In Ethiopia, trypanosomosis is one of the major impediments to livestock development, and agricultural production contributes negatively to the overall development of agriculture, in general, and food self-reliance efforts of the nation in particular (NTTICC; 2004). While tsetse-borne trypanosomosis excludes some 180 000 to 200 000 km of agriculturally suitable landing to the west and southwest of the country, 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines, and 1.8 million camels are at risk of contracting trypanosomosis at any 1 time (Dumesa *et al.,* 2015)

Trypanosomosis has recognized as a massive constraint to animal husbandry, livestock production and mixed farming in vast areas of rural sub-Saharan Africa (Oluwafemi, 2014). Ethiopia has known for its large and diverse livestock resource endowments. Livestock are primarily kept on smallholdings where they provide drought power for crop production, manure for soil

fertility and fuels, serve as sources of family diet and cash income (from livestock and livestock products). Despite large livestock population, Ethiopia fails to optimally utilize this resource due to different constrains facing the livestock subsector (Bezabih *et al.,* 2015). Since more than 90% of crop production in Ethiopia are dependent on animal draught power mainly on plugging oxen, many large fields lie fallow due to lack of these animals in trypanosomiasis-infested area (Haile *et al.,* 2016).

Bovine trypanosomosis is one of the economically important diseases that are caused by this flagellated protozoal parasite belonging to the genus *Trypanosoma* which affects all domestic animals (Jember and Mitiku, 2013; Sharma *et al.,* 2012; Singla *et al.,* 2009; Sood *et al.,* 2011). The monitoring of impact assessment is a prime concern for the effectiveness of any control program, which has to meet at least three criteria (Slingenbersh, 1999). First, they should be economically sound and sustainable, secondly the direct and indirect effect on the environment should be minimal and the third and most important is that they should fit into the rural development policy of a country. The impact of tsetse control can be best assessed by comparing changes before and after the implementation of a control intervention or alternatively by cross-sectional comparison of similar agricultural area under different level of trypanosomosis challenge or both (Van den Bossche and Rowlands, 2001).

According to annual report of Ethiopian Veterinary Association in 2008, most budgets allocated to rural development bureau is to buy drugs and spray to treat and control animal trypanosomosis. In Southern region of Ethiopia, the problem of tsetse is common where 75 districts fully or partially infected.

The infected area is about 48,000 km square. In the study area there was no information regarding the prevalence rate of the parasite and vector infestation level. Based on this, the study was conducted with the objectives of determining infection rate of *Trypanosoma* spp. in cattle and determining predisposing factors that contribute to the occurrence of the disease in borana zone.

Materials and Methods

Study area

This study was conducted in Elwaya and Teltelle districts the Borana zone that is predominantly inhabited by the Borana community and extends to the Kenyan border in the South; Somali region in the South East; Southern Nation, Nationalities, and People Region (SNNPR) in the West and North; and Guji zone in the North East. Borana rangeland is characterized by a semiarid to arid climate (Kamara *et al.,* 2005; Haile *et al.,* 2011). Geographically, the area is located between from 4 to 6° N latitude and from 36 to 42° E longitude with altitude ranging from 1000 to 1700 m above sea level. The mean annual rainfall of the area ranges from 250 to 700 mm. The annual mean temperature varies from 19 to over 25 °C. Extensive pastoralism is the main means of livelihoods for the Borana people (Gelagay *et al.,* 2007).

Study animals and study type

Local breeds of cattle with different age groups, body conditions and both sex groups that were kept under pastoral and agro-pastoral management systems were included in this study.

The animals examined were categorized into different age groups as less than 2 years (young), between 2 up to 4 years (medium) and greater than 4 years (adult) according to their teeth dentition (Johnson, 2003).

The body condition score of cattle was determined according to Nicholson and Butterworth's method and classified as poor, medium and good. A cross-sectional study was conducted to determine the current prevalence of bovine trypanosomosis and to estimate the potential risk factors associated with the epidemiology.

Estimation of sample size

The sample size of cattle required for the prevalence was calculated using the formula described by Thrusfield (2005). For this purpose, 95% confidence level, considering 50% expected prevalence and 5% desired precision was used as follow.

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

Where n = required sample size, d = desired absolute precision, P = expected prevalence (50 %). Based on this the desired sample size was found to be 384 cattle. However, additional 384 samples were included to increase the accuracy and hence 768 animals were examined.

Study protocol

Laboratory analysis

The prevalence of *Trypanosoma* infection was determined by buffy coat technique. Blood sample was collected by piercing the marginal ear vein with a sterile lancet and blood drawn by a hematocrit capillary tube. Then one end (the heparinized end) of capillary tubes sealed with crystal sealant and centrifuged at 12,000 rpm for five minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces. Then the Packed Cell Volume (PCV) was determined by PCV reader and recorded.

The PCV values ≥25 and <25 was considered as non-aneamic and aneamic, respectively. The buffy coat dropped on microscopic slide and covered with a cover slip and then examined under 40X magnification power of microscope to identify and detect the presence of the parasites (Getachew, 1993). All parasitological diagnostic tests and procedures were conducted as considered by (Paris *et al.,* 1982 and OIE 2017).

Those positive samples were further processed using geimsa staining for identification into species based on morphological characteristics under oil immersion using 100 x objectives (Parashar *et al.,* 2018)

Data management and statistical analysis

Data obtained from the study entered in to Microsoft Excel spread sheet, screened, coded and after initial validation of data and simple descriptive analyses, data were transferred to statistical packages as Stata Version 13 for statistical analysis. The prevalence of trypanosomosis was calculated as the number of cattle infected with trypanosomes divided by the total number of sampled animals, which was expressed as the percentage.

Logistic regression analyses were conducted using trypanosome infection as outcome variables against each of the responses variables risk factors. Firstly, the association of individual risk factor with an outcome variable was screened by univariable logistic regression and then variables significantly (P<0.05) associated with the responses variable at (P<0.05) in the univariable analysis were recruited for multivariable analysis to see their independent effect. The final multivariable logistic regression models were manually built using a forward stepwise selection approach.

Results and Discussions

Prevalence and trypanosome species identified

From 768 cattle observed by using the buffy coat technique, 5.08% (39/768) of them found infected with various type tryps spp. Consequently, the Giemsa stain blood smears using a 100x magnification lens confirmed the existence of *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei* in the study sites (Figure 2). The most prevalent (74.35%) trps identified was *Trypanosoma congolense* followed by *Trypanosoma vivax* (15.38%) and *Trypanosoma brucei* (10.25%).

Risk factors Associated with trypanosomosis

The prevalence of trypanosomosis found not similar concerning study districts (Table 1). Accordingly, relatively higher prevalence of bovine trypanosomosis recorded in Elawoye district (8.70%) than Taltalle (2.52%) with a significant (P < 0.05) occurrence of the disease among the study districts. Similarly, significant (P > 0.05) difference observed between Anemic and non-Anemic animal and higher prevalence of trypanosome infection observed in anemic animals than their non-anemic counterparts. Moreover, animal age found as one source of significant (P <.05) variation and older animals (age >6 years) found more susceptible than younger age groups. Furthermore, Body color of the study animals were significantly associated (P < 0.05) with trypanosome infection. Accordingly, trypanosome infection was higher in black-coated animals than red, brown, white and mixed-coated animals.

Based on multivariate logistic regression analysis, district, hair coat color, age and anemic animal were significantly (P <.05) associated with trypanosomosis infection (Table 2).

Effect of trypanosomosis on packed cell volume (PCV)

The analysis of PCV results in the cattle studied for trypanosomosis infection revealed that the packed cell volume (PCV) of parasitaemic animals was 16.51 ± 6.05 which was lower than that of non-parasitaemic animals (19.56 ± 6.22) (Table 3). The mean PCV showed significant (P <.05) variation between the infected and non-infected animals with t = 3.06 and P = 0.003.

From the total 768 heads of animals examined, 593 (77.21%) animals' PCV ranged 4 to 24 letting them

anemic, while the rest 175 (22.78 %) animals were possessed PCV range of 25 to 43 indicating they were non-anemic (Figure 3).

The overall prevalence (5.08 %) of the current study found in line with previous study results reported by Zelalem *et al.,* (2017), Hundessa *et al.,* (2021), Alemayehu *et al.,* (2012), Bekele and Nasir., *et al.,* 2011 and Biyazen *et al.,* 2014 in Gimbig district (4.9%), Sodo Zuriya District (5%), the Kaffa zone (6.9%), Didessa district (5.47%), and Dale-Wembera district (2.86%) respectively. On the other hand, higher prevalence reports were specified from Benatsemay District (11.46%), Dugda Dawa district (13.8%), and Benishangul Gumuz regions (13.3%) by Chanie *et al.,* (2012) and Leta *et al.,* (2016) respectively. Similarly, a higher prevalence of 17.2% in Metekel areas, (23.0%) in the Deramallo of Gamo zone and (11.05 %) in Gnangatom district of the South Omo zone was reported by Afewerk *et al.,* (2000), Ayele *et al., (*2012) and Tegegn *et al., (*2021) respectively. Comparing to other tsetse-infested areas, the current study verified a relatively lower prevalence of trypanosomosis. The dissimilarity observed is usually due to a variety of reasons, which includes decreased prevalence of trypanosomosis in this study location, and the study period. The current investigation performed during dry season that confirmed to influence vector population leading to lower prevalence of trypanosomes infection. Moreover, the increase of veterinary services to pastoral areas, the accustomed migratory livestock husbandry practices of the area, frequent use of trypanocidal drugs and the attention given by government fly control program in the area contributed to the observed lower trypanosomes prevalence.

Accordingly, district, PCV, age groups and Coat color of animals were significantly associated with prevalence of bovine trypanosomosis. The significant difference in trypanosomosis prevalence among the study distract might be associated with the difference in location of each study site and their proximity to suitable Glossina habitats in the district. Elawoye distract is located close to segen river which is the origin of the Glossina infestation in between konoso and borana zone. Due to the proximity of this site to close to the Segen River, this is also suspected to be a suitable habitat for tsetse flies so that the animals in the area can easily become infected. In line with the current study, Van den Bossche P. (2001), study also revealed that the prevalence of trypanosomiasis and its impact on livestock productivity vary by location and heavily influenced by the level of interaction between tsetse, domestic, and game animals.

Variations may exist because of differences in vector infection rate, animal susceptibility and practice of trypanocidal drug use and fly control operations that may obviously influence on epidemiological situations of the disease. (Geiger A *et al.,* 2015 Majekodunmi AO *et al.,* 2013, Reifenberg JM *et al.,* 1997)

PCV is one of the most fundamental quantitative measurements to estimate the anemic status of the tested animal. According to PCV, the animals were classified as anemic and non-anemic and animals with PCV less than 24% were considered anemic (OIE 2008, Van den Bossche P and Rowlands GJ 2001, Marcotty T, *et al.,* 2008). Anemia was considered an important clinical sign of trypanosomosis and the reduced performance of infected animals (Radoostitis OM, *et al.,* 2007, Trail JCM, *et al.,* 1993)

Significant difference was observe between mean PCV value of anemic and non-anemic animal (P >.05) and higher prevalence of trypanosome infection was recorded in anemic animals than in non-anemic animals. Similar trends of mean PCV values were reported by other researcher for trypanosome infected and non-infected cattle. In the North Omo Zone, Muturi (1999) found mean PCV values of 16.7% and 28.0%; Alekaw (2004) found 21.60% and 25.40% in districts bordering Lake Tana in northwest Ethiopia, and Feyisa (2004) found 21.65% and 25.54% in southwest Ethiopia for parasitic and non-parasitic cattle, respectively. The significant decrease in mean PCV value of infected than non infected cattle might be due to the erythrocyte destructive nature of trypanosomes in infected animals.

The prevalence of bovine trypanosomosis was statistically significant (P <.05) among age groups, that is, it was significantly higher and more prevalent in older animals (age >6 years) than in young age group. This result consistent with Previous research findings a significant difference in the prevalence of the disease among different age groups, that is, there was lower disease prevalence in young age groups than in older animals that stated Alemayehu *et al.,* 2012, Gona *et al.,* 2016 and Tegegn *et al.,* 2021. However, disagree with the current finding, who stated an insignificant infection rate of trypanosomosis among different age groups by Nigatu, 2004, Ayele *et al.,* 2012 and Tilahun, 2012.

Table.1 Association of trypanosomosis prevalence with potential risk factors

Category	No. Examined	+ve	Prevalence (%)	χ^2	P-Value
Sex					
Male	559	33	5.90	1.31	0.251
Female	170	6	3.52		
Age Groups					
0 – 2 years	109	2	1.83	6.07	0.048
3 – 5 years	255	10	3.92		
>6 years	365	27	7.39		
Body condition score					
Poor	458	22	4.80	0.66	0.717
Medium	204	13	6.37		
Good	67	4	5.97		
Districts					
Elwoye	333	29	8.70	12.22	0.000
Taltalle	396	10	2.52		
PCV					
Anemic	523	35	5.55	6.03	0.014
Non anemic	206	4	1.94		
Coat Color of animals					
Black	43	14	32.55	50.18	0.000
Brown	220	6	2.72		
Mixed	93	6	6.45		
Red	174	6	3.45		
White	199	7	3.52		

Table.2 Multivariate logistic regression analysis of risk factors for trypanosomosis

Factors	Labels	Odds Ratio	Std. Err.	Z	P>|z|	[95% Conf. Interval]
District		1.2076	2.90	2.90	0.004	1.4411- 6.6495
Age group		2.1715	1.23	1.23	0.219	0.5553 - 13.0751
		4.0197	2.17	2.27	0.030	1.1775 - 23.5171
Coat color		0.0470	-4.56	-4.56	0.000	0.0312 - 0.2509
		0.1479	-2.39	-2.39	0.017	0.0918 - 0.7901
		0.0608	-4.07	-4.07	0.000	0.0399 - 0.3244
		0.0549	-4.36	-4.36	0.000	0.0393 - 0.2929
PCV		1.7237	2.04	2.04	0.041	1.0480 - 9.2161
_cons		0.0159	-4.24	-4.24	0.000	0.0025 - 0.1103

Table.3 Analysis of the association of trypanosome infections with mean PcV (%) of cattle

Status	No. examined	Mean pcv ± sd	95% CI	T- value	P- value
Parasitaemic	39	16.51 ± 6.05	14.5488 - 18.4712	3.06	0.003
Non- Parasitaemic	729	19.56 ± 6.22	19.1077 - 20.0123		

Fig.1 Map of borana zone showing the study areas.

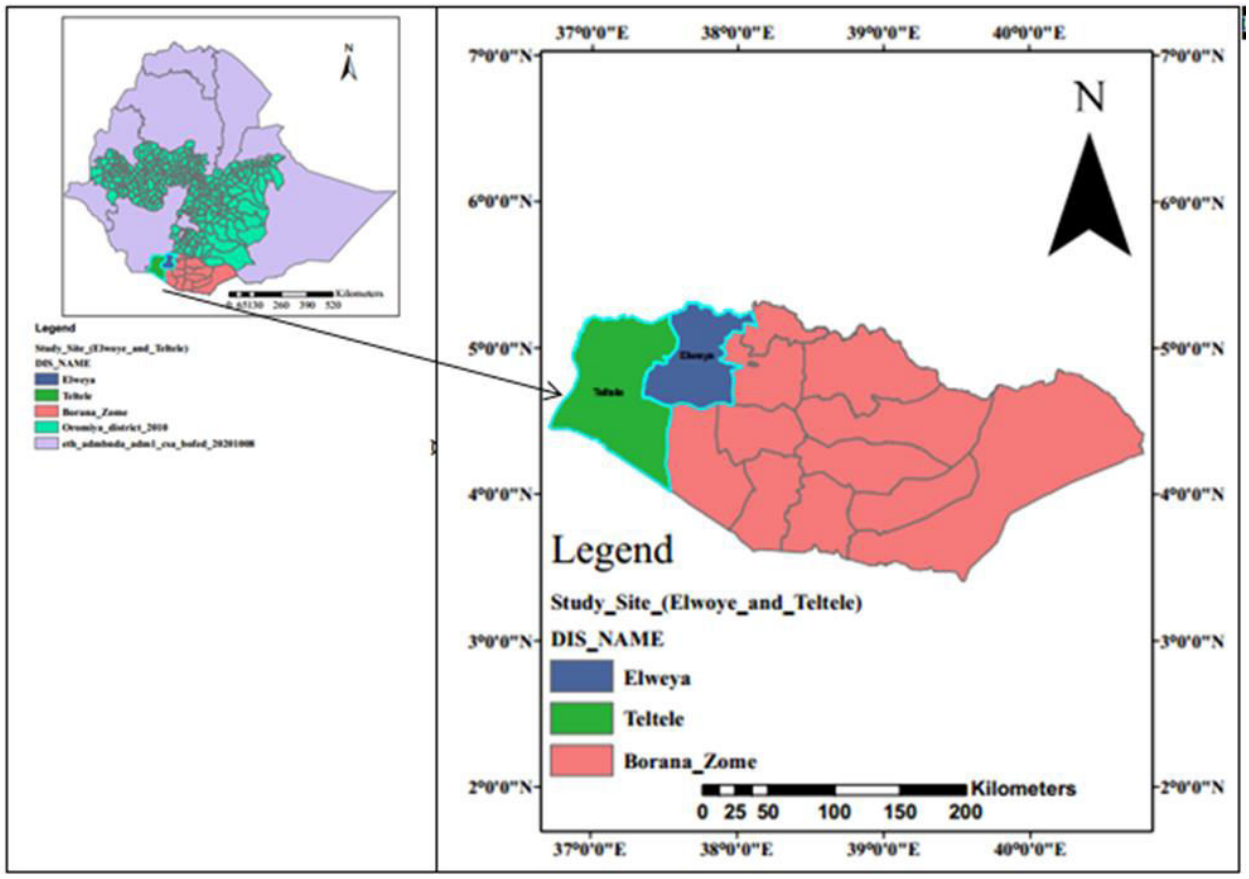


Figure.2 The overall prevalence of trps spp. in the considered districts

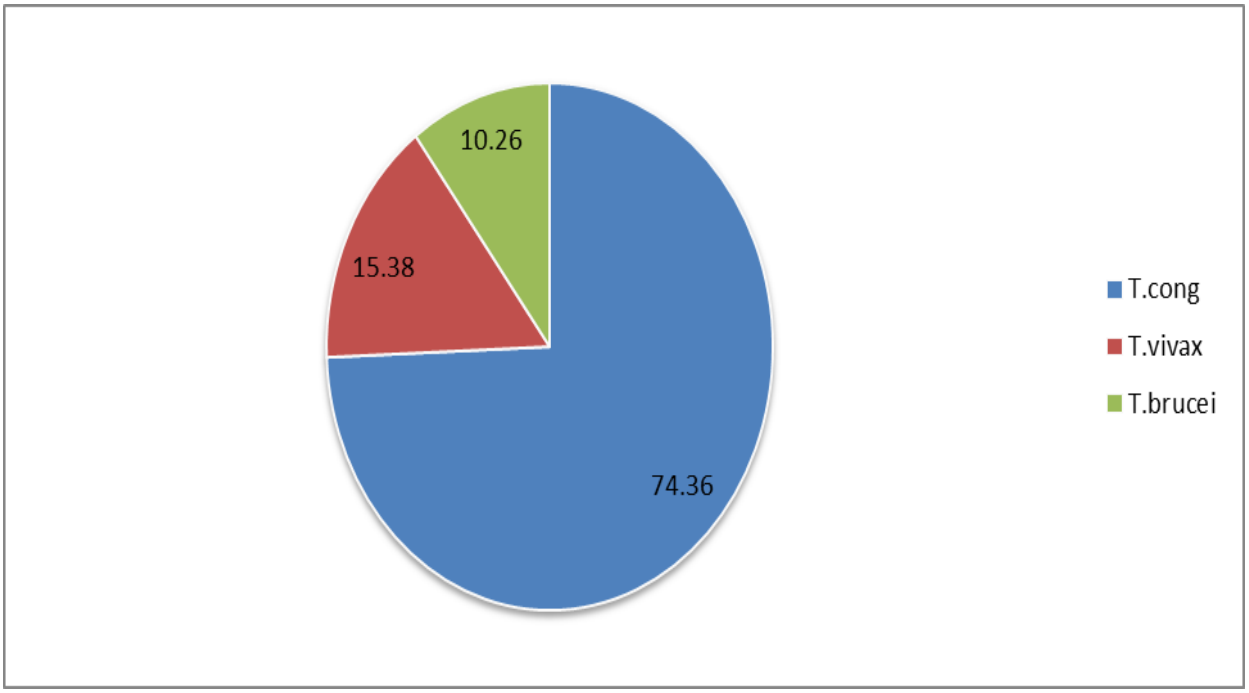
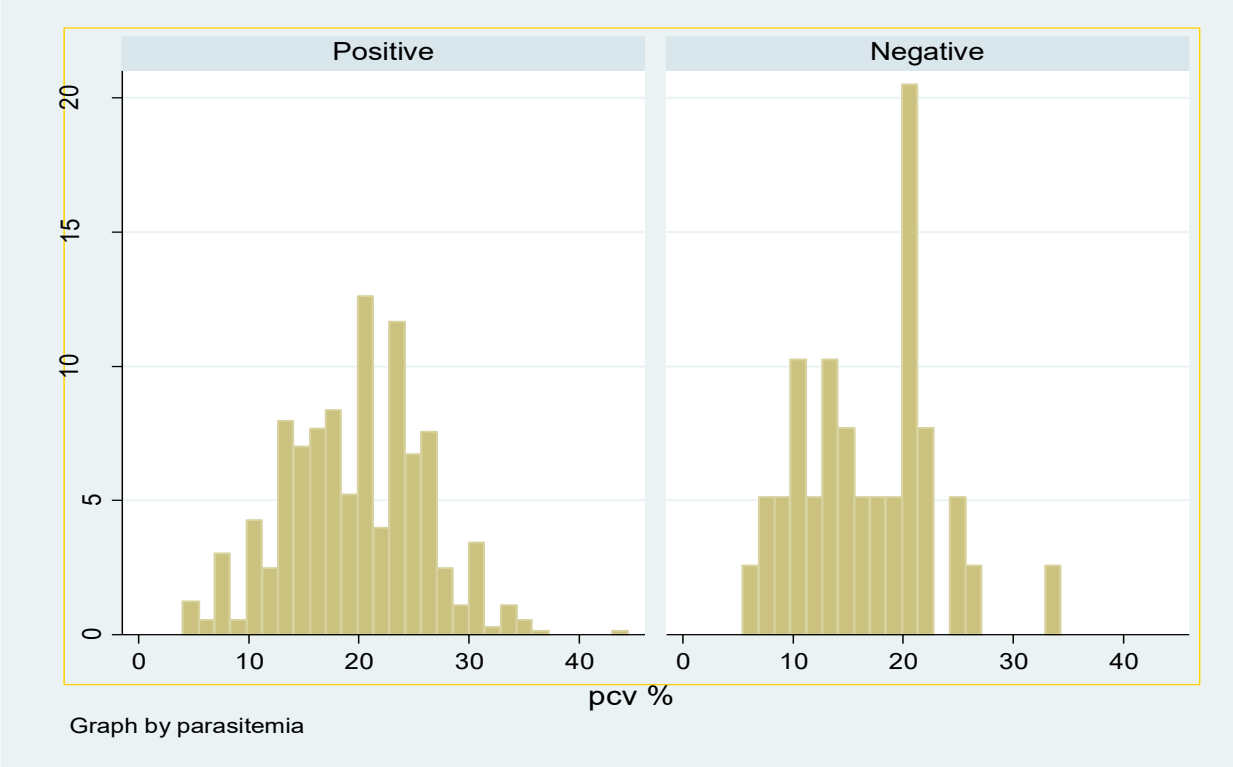


Figure.3 Level of PCV values in associated with Parasitaemic and Non- Parasitaemic animals



This might be due to the effect of maternal immunity in young age groups as well as the preference of tsetse flies to feed on old animals than young animals. Younger calves regularly kept in the homestead and do not move far for grazing and watering, that reducing the risk of tsetse fly exposure.

Body color of the study animals were significantly associated (P <.05) with trypanosome infection. Accordingly, trypanosome infection was higher in black-coated animals than in red, brown, white and mixed-coated animals. This might be associated with a color preference of Glossina vector to feed on black animals than other color types since the tsetse fly was more attracted by black color due to their shade-loving behavior during their flight from one area to another. Similar finding was reported by Ataro *et al.,* 2015, Fuentes A, 2017, significant deference in prevalence of trypanosome among different coat colored animals with the highest prevalence in black hair-coat animals

Conclusion and Recommended

Trypanosomosis is a very important disease that causes economic loss in the livestock industry. Bovine trypanosomosis, which accounts for an overall prevalence of 5.08% is the major livestock constraint in the study area and affects their health, production and productivity. There was (P<0.05) a statistical significant difference in prevalence of bovine trypanosomosis in district, PCV, age groups and Coat color of animals.

Based on the aforementioned the following is recommended:

- Designing and implementation of control strategies of trypanosomosis focusing integrated approach (vector control and chemotherapy) should be continuing in the studied areas.
- The pastoralists in the area should be trained on how to control the vectors of the parasites and the disease properly.
- Expanding an appropriate tsetse control methods (Spot-on and insecticide impregnated targets) to reach tsetse infested area in a sustainable manner.

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