



Epidemiology of Bovine Trypanosomosis in Assosa and Bambasi Districts of Benishangul Gumuz Region, Western Ethiopia

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Abstract: A cross-sectional study was undertaken from November 2018 to March 2019 with the objectives to estimate seasonal prevalence of bovine trypanosomosis and to identify the major putative risk factors associated with bovine trypanosomosis in Assosa and Bambasi districts of Benishangul Gumuz region. One-stage cluster sampling strategy was used to select study animals. Blood samples were collected from ear vein of 1,562 head of cattle, 790 in the late rainy season and 772 in the dry period and examined with buffy coat technique; the overall prevalence of trypanosomosis was 7.7% in the late rainy season and 4.8% in the dry period with significant variation ($P < 0.05$). The risk factors; district in the late rainy season as well as season were significantly associated ($P < 0.05$) with bovine trypanosomosis while *rural kebele*, age, sex, body condition were not significant. Three species of trypanosomes were detected during the study, namely *T. congolense* (64%), *T. vivax* (21.3%), *T. brucei* (1.6%) and mixed infection (13.1%) in the late rainy season and *T. congolense* (75.7%), *T. vivax* (13.5%) and mixed infection (10.8%) in the dry season; *T. congolense* being the predominant species followed by *T. vivax*. The mean Packed cell volume of trypanosome infected cattle (23.05 ± 3.40) was significantly lower ($P < 0.05$) when compared to that of non-infected ones (27.73 ± 4.61) in the late rainy season; similarly, it was significantly lower ($P < 0.05$) in parasitemic cattle (23.54 ± 2.58) than aparasitemic ones (28.15 ± 4.58) in the dry season. In conclusion, the parasitological findings revealed that bovine trypanosomosis is widely distributed and endemic in Assosa and Bambasi districts of Benishangul Gumuz region hence designing participatory and integrated control measures including regular surveillance, community based vector prevention and control should be implemented to mitigate the problem.

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1. Introduction

Trypanosomes are extracellular protozoan parasites that cause debilitating diseases called trypanosomosis in animals and sleeping sickness in humans and have great socio-economic impact adversely affecting food production and economic growth in many parts of Africa, particularly in Sub-Saharan Africa (SSA) (Shaw *et al.*, 2014; Taylor, 2015).

The disease caused by these extracellular hemoflagellates in domestic animals is called "Nagana" or African animal trypanosomosis (AAT). The strictly intravascular parasites, *T. congolense* and *T. vivax* are considered to be the most important cause of AAT (Morrison *et al.*, 2016). Yet, also *T.b. brucei* and *T. evansi*, residing both in intravascular as well as extravascular spaces within their host have been documented to contribute to livestock infections (Morrison *et al.*, 2016). In contrast to game animals, where these parasites cause only mild infection, the disease in domestic animals particularly in cattle is

severe and often fatal (Chitanga *et al.*, 2013; Yaro *et al.*, 2016).

Trypanosomosis affects a wide range of host species (spp) where *T. congolense* is considered to be the most pathogenic trypanosome in cattle followed by *T. vivax* (Osorio *et al.*, 2008). *Trypanosoma brucei* is found in various domestic ungulates but it is particularly virulent in dogs, camels and horses, the latter often succumbing to infection within a few months in the absence of treatment. In areas where more than one trypanosome spp is present, mixed infections in domestic animals are often encountered (Auty *et al.*, 2008; Takeet *et al.*, 2013).

In Ethiopia, the most important trypanosome spp affecting livestock include *T. congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats; *T. evansi* in camels and *T. equiperdium* in horses (Abebe, 2005; Alemayehu *et al.*, 2012). Western and southern river basins of Ethiopia are the most severely affected areas by trypanosomosis in the country. In the area specifically in the western part, a wide diversity of

Glossina and trypanosome spp and strains co-exist (Abebe, 2005).

As Benishangul Gumuz is one of the five regions of Ethiopia infested with more than one spp of *Glossina*, three of *Glossina* transmitted trypanosome spp such as *T. congolense*; *T. vivax* and *T. brucei* are found in the region (NTTICC, 2004; Worku *et al.*, 2017).

Assosa and Bambasi are among the districts of Benishangul Gumuz region with a serious problem of bovine trypanosomosis. Even though, the disease is one of the major constraints of cattle production and productivity, studies made on prevalence of bovine trypanosomosis so far in the two districts did not consider seasonal variation in prevalence.

Hence, control of trypanosomosis is a major goal for program aimed at poverty alleviation. In this regards, comprehensive quantification of the occurrence, distribution, seasonal variation and other factors associated with the disease is the primary requisite for the control program. Also, information pertaining to seasonal prevalence is scarce and not well documented in the study areas. Therefore the objectives of the study were: to estimate seasonal prevalence of bovine trypanosomosis and to identify the major putative risk factors associated with bovine trypanosomosis in the study districts.

2. Materials and methods

2.1 Description of the Study Areas

Benishangul Gumuz region is one of the nine regional states established in 1994 by the new constitution of Ethiopia that created a federal system of governance. The region is located in the western end of the country bounded by Sudan Republic in the west, Amhara region in the north and northeast, Oromia region in the east and southeast and Gambella region in the south and found at a distance of about 687 km away from Addis Ababa. According to the current administrative structure, the region is divided in to 3 administrative zones, 20 districts and 482 *rural kebeles* with a total area of approximately 50,380 km² (BGRBoC, 2017).

The region is found at latitude of 9-11°N and longitude of 34-35°E and altitude ranges generally between 580-2731 m.a.s.l., the highest peak being at Belaya mountain (2731 m.a.s.l) and the lowest (580 m.a.s.l) in the extreme west lowlands near the Ethio-Sudan boundary. The mean annual temperature of the region ranges from 17-29°C. Rainfall is uni-modal and occurs for 6 or 7 months between April and October. The mean annual rainfall amount is estimated to be 1275 mm. Higher rainfall period is between May and September, the highest being in July or August (NMSA, 2015).

The region possess 777,915 cattle, 100,013 sheep, 431,216 goats, 2,560 horses, 77,737 donkeys, 1,783 mules and 1,249,578 poultry of which cattle population of the region accounts for only 1.3% of the country (CSA, 2016/17). Similarly, Assosa Zone, in which the present study was carried out, accounts only for 11.3% cattle population of the region.

2.1.1 Assosa district

Assosa district comprises 72 *rural kebeles*, out of which 48 *rural kebeles* possess cattle. The district is located at 9.60°-10.45° N latitude and 34.20°-34.58°E longitude with an altitude that ranges from 580-1544 m.a.s.l. The district has a rainfall that ranges from 850-1200 mm. Its mean annual temperature ranges between 16.75°C and 37.9°C (NMSA, 2015). The total area of the districts is 2317 Km² and its livestock population is 27,850 cattle, 25,943 goats, 5,689 sheep, 5,420 donkeys and 53,185 poultry (ADOOA, 2017). The soil types of Assosa district are mainly silty loam (70%) and sandy loam (30%). Vegetations such as woodlands and shrublands, bushlands, bamboo woodlands, forest and savannah grassland are mainly found in the district. Maize, sorghum, finger millet, teff, mango and coffee are some of the major crops grown in Assosa district. The livelihood of the society largely depends on mixed crop livestock production. Hoha, Affa, Affa Megele, Affa Belbenare and Bildigilu Gambashire are few of small rivers found in Assosa district (BGRBoA, 2017; ADOOA, 2017).

2.1.2 Bambasi district

Bambasi district comprises 38 *rural kebeles* and it is located at 9.45- 9.75°N latitude and 34.35-34.88° E longitude, with minimum and maximum altitude of 1350 and 1770 m.a.s.l., respectively. The total area of the district is 2100 km² and has average minimum and maximum annual rainfall of 900 mm and 1200 mm, respectively; while the average minimum and maximum temperature is 23°C and 32°C, respectively (NMSA, 2015). The total livestock population of the district is 38,964 cattle, 11,990 goats, 3,452 sheep, 1,995 donkeys and 38,442 poultry (BDOOA, 2017).

Clay loam, sandy loam and red soils are the main soil types of the district. Woodlands and shrub lands, bush land, bamboo woodlands, forest and savannah grasslands are the main vegetation types found in the district. Maize, morghum, finger millet, teff and mango are the major crops grown in the district and similar to Assosa district, the livelihood of the society largely depends on mixed livestock and crop production. Some of the rivers found in the district include Affa, Selga, Jema, Qontsa, Sonka and Shebora (BGRBoA, 2017; BDOOA, 2017). Bamboo woodland, one of the most important resources of the

region extensively occurs below 1600 m.a.s.l, the largest part being found in Assosa zone mainly in the

two study districts.

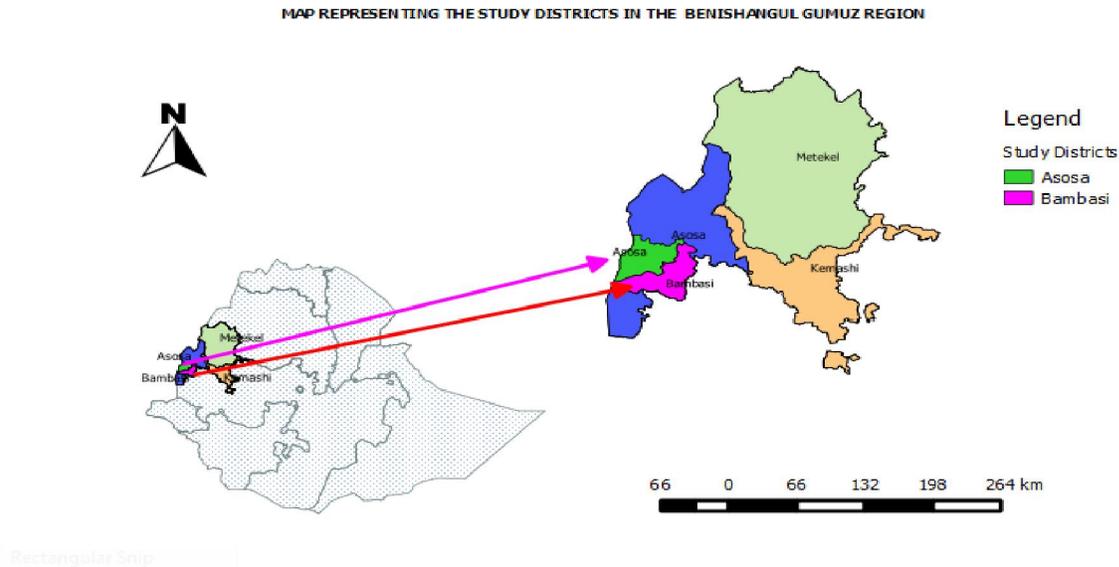


Fig. 1. Map representing the study areas

2.2 Study animals

In this study indigenous zebu cattle kept under traditional smallholder farming system were included. Study animals include 144 herds of 7294 cattle and both sexes and all age groups that were allowed/released for free grazing by their owners. During sample collection, study animals were classified as poor, moderate and good in body condition based on anatomical parts and the flesh and fat cover at different body parts (Nicholson and Butterworth, 1986), and on subjective basis. Concurrently, their age was estimated by history and dental formula as described by De-lahunta and Habel (1989) principles as (≤ 2 years), (2-4 years) and (> 4 years).

2.3 Study design

Cross-sectional study was conducted from November 2018 to March 2019 to estimate seasonal prevalence of bovine trypanosomosis in the late rainy season and during the dry period.

2.4 Sampling methods and sample size determination

Assosa and Bambasi districts were selected purposively for this study because of wide spread occurrence of bovine trypanosomosis and for their huge cattle population among the seven districts of Assosa zone. Nine *rural kebeles* (5 from Assosa and 4 from Bambasi) districts were selected using simple random technique to be incorporated in the study. Since the most recent reports on prevalence of bovine

trypanosomosis in the two districts were 21.5% in Assosa by Ayana and Zerihun (2016) and 9.14 % in Bambasi by Aki and Godesso (2016); these findings were used as an expected prevalence. The sample size for the study was determined according to statistical formula of Thrusfield (2005) at desired absolute precision of 5% and 95% confidence interval. Therefore, the total sample size for the study was calculated as follows:

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

Where: n = required sample size;

P_{exp} = expected prevalence;

d = desired absolute precision; hence

A total of 387cattle (259 from Assosa and 128 from Bambasi) districts were expected to be sampled. However, in the case of cluster sampling, subjects are heterogeneous; as a result, large sample size is required to increase precision. As a rule of thumb doubling the number of animals for simple random sample are needed for cluster sampling (Martin, 1987). Therefore, the optimum sample size for this study was about 774 and hence 790 and 772 cattle were sampled in the late rainy season and during the dry period of the study, respectively.

One-stage cluster sampling strategy was used to select study animals (Thrusfield, 2005) and herds in each *rural kebele* were considered as cluster. Herd (locally called "Tera/Menga") is defined as a group of cattle owned by peoples living together in a village

and their cattle share the same grazing areas and watering points. The sampling frames (list of all herds) were obtained from each *rural kebele* and a total of 144 herds were present in the 9 *rural kebeles* of the study areas of which 15 herds in the late rainy season and 16 herds in the dry period of the study were selected by simple random technique as well. Animals from randomly selected clusters were considered as sampling units and all animals in each selected cluster were sampled. During sampling, parameters like sex, age and body condition score (BCS), *rural kebele*, district as well as owner of the animal were recorded.

2.5 Data collection

2.5.1 Parasitological survey

Blood samples were collected from randomly selected herds by puncture of ear vein using a sterile needles or lancets (Adam *et al.*, 2011) in to dry clean sterile heparinized capillary tubes (75mmx1.2mm). Heparinized microhaematocrit capillary tubes, containing blood samples were centrifuged for 5 minutes at 12,000 rpm. The capillary tubes were 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 a glass slide and covered with a 22x22 mm cover slip and diagnosed immediately under x40 objective and x10 eye piece for movement of parasite. Thin blood smears was prepared and stained with Giemsa staining solution for 30 minutes and examined under light microscope using x 100 oil immersion objective lens for identification of trypanosomes (Murray *et al.*, 2003). Trypanosome spp was identified according to their morphological descriptions as well as movement in microscope field (OIE, 2008).

2.5.2 Packed cell volume determination

During sampling, blood was obtained from each animal by puncturing the marginal ear vein with sterile needle or lancet and collected directly into a pair of heparinized capillary tubes. The tubes were sealed at one end with crystal seal and placed in microhaematocrit centrifuge with sealed end outermost. Then the heparinized capillary tubes were loaded symmetrically to ensure a good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 minutes. After centrifugation, the capillary tubes were placed in a haematocrit reader. The length of the packed red blood cell column was expressed as a percentage of the total volume of blood using PCV reader. Packed cell volume of each animal was compared with the normal range (24-46%) of PCV in cattle (Radostits *et al.*, 2006) and

cattle with PCV < 24% were considered as anemic (OIE, 2008).

2.6 Data management and analysis

Raw data collected were entered into a Microsoft Excel spreadsheet. The data were summarized and presented in tables and analyzed by using STATA version 13.0 for Windows (Stata Corp. College Station, TX). The prevalence of trypanosomosis was calculated for all data as the number of infected individuals divided by the number of individuals examined and multiplied by 100. The association between trypanosome infection and risk factors (age, sex, BCS, *rural kebele*, district and season) was determined by univariate logistic regression. Those risk factors with ($P < 0.25$) by univariate analysis were further analyzed by multivariate logistic regression. Independent t-test was used to assess the differences in mean PCV between trypanosome positive and negative animals. Throughout the analysis, the test result was considered as significant when the calculated P-value was ≤ 0.05 at 95% confidence interval and 5% absolute precision (Thrusfield, 2005).

3. Results and discussion

3.1 Parasitological Survey

Blood samples were collected from ear vein of 1562 heads of cattle, 790 in the late rain season and 772 in the dry period of the study. Out of the total number of samples collected, 61 and 37 cattle were tested positive in the late rainy season and during the dry period of the study, making the overall prevalence of trypanosome infection 7.7% (95% CI= 6.9-8.5) and 4.8% (95% CI= 4.5-5.1), respectively as indicated in Table 4.

Using the same parasitological technique for diagnosis, comparable prevalence to the current finding in the late rainy season was recorded in the neighboring district of Oromia region by Takile *et al.* (2014) who reported an overall prevalence of 7.81% in Guto Gida district. It was also in agreement with studies carried out by Bishaw *et al.* (2012) who reported an overall prevalence of 7.81% in Wemberma district of west Gojjam zone and Aki and Godesso (2016) who reported an overall prevalence of 9.14% in Bambasi district. The result of the dry season in the present study was comparable with previous research work of (Teka *et al.*, 2012; Lelisa *et al.*, 2015) who reported 4.43% and 5.58% prevalence in Arbaminch and Pawi districts, respectively.

Among the 61 positive cattle for trypanosomosis in the late rainy season, 64% (95% CI=50.6-75.8) and 21.3% (95% CI=11.8-33.7) accounted for *T. congolense* and *T. vivax*, respectively as indicated in

Table 1. Similarly, of the 37 cattle infected with trypanosomes during the dry period, *T. congolense* and *T. vivax* accounted for 75.7% (95% CI=58.8-88.2) and 13% (95% CI =4.5-28.8), respectively as shown in Table 2.

The results in the two seasons were in agreement with reported proportion of *T. congolense* (65.33%) and *T. vivax* (26.7%) by Eticha and Aki (2016) in Debate district. It was also in line with (Biyazen *et al.*, 2014) who reported proportional prevalence of *T. congolense* to be 63.64% in Dale Wabera district and (Duguma *et al.*, 2015) who obtained proportional prevalence of 76% *T. congolense* and 18.1% *T. vivax* in southwestern Ethiopia.

The high proportion of *T. congolense* infection in cattle in the present study might be attributable to the possible development of better immune response to *T. vivax* by infected animals as demonstrated by Leak *et al.* (1993). It could also be due to efficient transmission of *T. congolense* by cyclic vectors than *T. vivax* in *Glossina* infested areas. Previous report by Leak (1999) indicated that *T. congolense* and *T. vivax* are the most prevalent trypanosome spp that infect cattle in *Glossina* infested and *Glossina* free areas of Ethiopia, respectively that support the current finding.

Table 1. Species of trypanosome identified in each *rural kebele* in the late rainy season in Assossa and Bambasi districts

<i>Rural kebele</i>	No of cattle		Trypanosome spp					Prevalence (%)
	Examined	Infected	T.c	T.v	T.b	Mixed		
						T.c & T.v	T.c & T.b	
K/ no 2	52	2	1(50%)	1(50%)	0	0	0	3.8
N/K	78	5	2(40%)	1(20%)	0	2(40%)	0	6.4
Sonka	75	3	2(66.7%)	1(33.3)	0	0	0	4.0
Shebora	64	2	1(50%)	1(50%)	0	0	0	3.1
Bambasi total	269	12	6(50%)	4(33.3%)	0	2(16.7%)	0	4.5
Amba 11	127	8	5(62.5%)	2(25%)	0	1(12.5%)	0	6.3
K/27	60	5	3(60%)	1(20%)	0	1(20%)	0	8.3
K/ 28	130	6	5(83.3%)	1(16.7%)	0	0	0	4.6
Abrehamo	80	11	7(63.6%)	1(9.1%)	1(9.1%)	1(9.1%)	1(9.1%)	13.8
Megele 38	124	19	13(68.4%)	4(21%)	0	1(5.3%)	1(5.3%)	15.3
Assosa total	521	49	33(67.3%)	9(18.4%)	1(2%)	4(8.2%)	2(4.1%)	9.4
Total	790	61	39(64%)	13(21.3%)	1(1.6%)	6(9.8%)	2(3.3%)	7.7

T.c =*T. congolense*, T.v=*T. vivax*, T.b= *T. brucei*, K/no 2= Keshimando number 2, N/K= Nebar Keshimando, K/27= Komeshiga 27, K/28= Komeshiga 28

Table 2. Species of trypanosome identified in each *rural kebele* during the dry period in Assossa and Bambasi districts

<i>Rural kebele</i>	Cattle		Trypanosome spp					Prevalence (%)
	Examined	Infected	T.c	T.v	T.b	Mixed		
						T.c & T.v	T.c & T.b	
K/ no 2	56	2	2(100%)	0	0	0	0	3.6
N/K	74	4	2(50%)	1(25%)	0	1(25%)	0	5.4
Sonka	61	1	1(100%)	0	0	0	0	1.6
Shebora	63	1	1(100%)	0	0	0	0	1.6
Bambasi total	254	8	6(75%)	1(12.5%)	0	1(12.5)	0	3.1
Amba 11	130	5	5(100%)	0	0	0	0	3.8
K/27	60	2	1(50%)	1(50%)	0	0	0	3.3
K/ 28	132	3	2(66.7%)	1(33.3%)	0	0	0	2.3
Abrehamo	73	7	5(71.4%)	1(14.3)	0	1(14.3%)	0	9.6
Megele 38	123	12	9(75%)	1(8.3%)	0	1(8.3%)	1(8.4%)	9.8
Assosa total	518	29	22(75.9%)	4(13.8%)	0	2(6.9%)	1(3.4%)	5.6
Total	772	37	28(75.7%)	5(13.5%)	0	3(8.1%)	1(2.7%)	4.8

T.c =*T. congolense*, T.v=*T. vivax*, T.b= *T. brucei*, K/no 2= Keshimando number 2, N/K= Nebar Keshimando, K/27= Komeshiga 27, K/28= Komeshiga 28

The association between trypanosome infection and risk factors such as *rural kebeles*, district, age, sex, BCS and season was analyzed using univariate logistic regression in the late rainy season (Table 3) and during the dry period of the study (Table 4).

Although the association was not statistically significant ($P>0.05$) for *rural kebeles* except for Megele 38 in the univariate logistic regression analysis, the highest infection of trypanosome was registered in Megele 38, which was 15.3% (95% CI=9.5-22.9), followed by Abrehamo 13.7% (95% CI= 7.1-23.3) and the least was recorded in Shebora 3.1% (95% CI= 0.38-10.8) in the late rainy season. Similarly, during the dry period of the study, slightly higher prevalence of trypanosome infection 9.8% (95% CI=5.1-15.4) was registered in Megele 38 and the least was also reported in Shebora 1.4% (95% CI=0.04-8.53), however, the association was not found to be statistically significant ($P>0.05$). This finding revealed that, the odds of cattle in Megele 38 *rural kebele* are 4.5 and 2.9 times higher to be infected with trypanosomes when compared to cattle in Keshimando number 2 in the late rainy season and during the dry period, respectively.

Prevalence of bovine trypanosomosis was significantly higher ($P<0.05$) in Assosa district 9.4% (95% CI=7.0-12.2) when compared to Bambasi district 4.5% (95% CI =2.3-7.7) in the late rainy season and even though, higher prevalence was obtained in Assosa district 5.6% (95% CI= 3.8-7.9) in the dry season when compared to Bambasi district 3.1% (95% CI=1.4-6.1), the association was not significant ($P>0.05$). Univariate logistic regression analysis showed that, the odds of cattle to be infected with trypanosomes are 2.2 and 1.8 times higher in Assosa district than cattle in Bambasi district in the late rainy season and during the dry period of the study, respectively as indicated in Tables 3 and 4.

This variation in prevalence between the two districts might be due to the fact that Assosa district has relatively more unspoiled savannah grass/wood lands when compared to Bambasi district as Bambasi district is one of the potential agricultural investment areas of the region.

Infection with trypanosome was slightly higher in cattle with age group of 2-4 years 9.5% (95% CI= 6.0-14.2) and 7% (95% CI = 3.96-11.25) in the late rainy season and during the dry period of the study, respectively when compared to cattle of either > 4 years 7.9% (95% CI = 5.4-11) or ≤ 2 years 5.1% (95% CI=2.3-9.4) of age in the late rainy season and 4.2% (95% CI =2.4-6.7) and 3.5% (95% CI =1.28-7.4) in the dry period of the study, respectively; however, no significant variation ($P>0.05$) was observed in the two seasons as indicated in Tables 3

and 4. The odds of cattle with age group 2-4 years to be infected with trypanosomes were 2 and 2.1 times higher than cattle with age ≤ 2 years in the late rainy season and during the dry season of the study, respectively.

Similar research results were reported by Bitew *et al.* (2011), Degneh *et al.* (2017), Golassa and Mekonnen (2017), Worku *et al.* (2017) in different parts of the country. As all the cattle sampled during the present survey were those released for free grazing by their owners, they might have equal chance of being getting contact with the vectors while traveling long distances for grazing and watering.

With regard to sex group, univariate logistic regression analysis showed that higher trypanosome infection was recorded in male 9.2% (95% CI=6.1-12.2) in the late rainy season and 5.8% (95% CI =3.3-8.4) during the dry period of the survey, when compared to female which was 6.6% (95% CI =4.3-8.9) in the late rainy season and 4% (95% CI= 2.2-5.8) during the dry period of the survey, with no significant difference ($P>0.05$) as shown in Tables 3 and 4.

This finding was in agreement with earlier research works of (Regasa *et al.*, 2015; Degneh *et al.*, 2017; Batu *et al.*, 2017) who reported higher prevalence of trypanosomosis in males than females in various parts of the country. The fact that trypanosomosis did not depend on gender, could possibly hypothesized that both male and female animals have virtually equal chance of being in contact with fly vectors and ultimately developing the disease under extensive management system.

Relatively higher prevalence of trypanosomosis was registered in animals with poor body condition which was found to be 10% (95% CI=5.6-16.2) in the late rainy season and 6% (95% CI=3.0-11.2) in the dry period of the study when compared to cattle with either medium 8% (95% CI =5.6-11) or good 5.8% (95% CI=3.1-9.7) body condition in the late rainy season and 5% (95% CI=3.1-7.6) and 3.3% (95% CI=1.3-6.7) in the dry period, respectively, however, the association was not statistically significant ($P > 0.05$) as indicated in Tables 3 and 4.

Degu *et al.* (2012) and Kebede (2015) indicated in their research works that, prevalence of trypanosome infection in cattle with poor body condition was higher when compared to cattle with either medium or good body condition with no significant difference ($P > 0.05$) which support the current research work. Haile and Gizaw (2018) and Furgasa *et al.* (2018) reported higher prevalence of trypanosomosis in cattle with poor body condition, with statistically significant association ($P < 0.05$) unlike to the present

research result. The current research result revealed that if animals of poor, medium and/or good body condition were equally exposed to either *Glossina* or other biting fly challenge, they would have equal chance of being infected with trypanosomes.

Univariate logistic regression analysis showed that, higher prevalence of trypanosomosis was seen in the late rainy season 7.7% (95% CI=5.9-9.8) than the dry period of the study 4.8% (95% CI=3.4-6.5) with statistically significant difference ($P < 0.05$) indicating that cattle are 0.6 times less likely to be infected with trypanosomes during the dry season

when compared to the late rainy season (Table 8). The concurrent survey of *Glossina* spp at the same time in the study districts showed that higher apparent density was registered in the late rainy season than the dry season with significant variation ($P < 0.05$).

This finding was in line with prior research work of (Dagnachew, 2004) who reported lower infection rate of cattle with trypanosomes during the dry period. Degneh *et al.* (2017) also reported lower prevalence of trypanosomosis during the dry season in Gidami district of Oromia region.

Table 3. Univariate logistic regression model for rural kebele, district, body condition score, age and sex with buffy coat result in the late rainy season in Assosa and Bambasi districts

Risk factors	Risk Category	No examined	No positive	Prevalence % (95% CI)	OR (95% CI)	P-value
RK	K/no 2	52	2	3.8(0.47-13.2)	-	-
	N/K	78	5	6.4(2.1-14.3)	1.7(0.3-9.2)	0.53
	Sonka	75	3	4.0(0.83- 11.2)	1.0(0.2-6.5)	0.96
	Shebora	64	2	3.1(0.38-10.8)	0.8(0.1-5.9)	0.83
	Amba 11	127	8	6.3(2.1-12)	1.7(0.3-8.2)	0.52
	K/27	60	5	8.3(2.8-18.4)	2.3(0.4-12.2)	0.34
	K/28	130	6	4.6(1.7-9.8)	1.2(0.2-6.2)	0.82
	Abrehamo	80	11	13.7(7.1-23.3)	4.0(0.8-18.8)	0.08
	Megele 38	124	19	15.3(9.5-22.9)	4.5(1.0-20.2)	0.05
Age	≤ 2 years	178	9	5.1(2.3-9.4)	-	-
	2-4 years	220	21	9.5(6.0-14.2)	2(0.9-4.4)	0.10
	> 4 years	392	31	7.9(5.4-11)	1.6(0.7-3.5)	0.22
Sex	Male	338	31	9.2(6.3-12.8)	-	-
	Female	452	30	6.6(4.5-9.3)	0.7(0.4-1.2)	0.19
BCS	Poor	140	14	10(5.6-16.2)	-	-
	Medium	425	34	8(5.6-11)	0.8(0.4-1.5)	0.46
	Good	225	13	5.8(3.1-9.7)	0.5(0.2-1.2)	0.14
District	Bambasi	269	12	4.5(2.3-7.7)	-	-
	Assosa	521	49	9.4(7.0-12.2)	2.2(1.2-4.3)	0.02

BCS= Body condition score, K/no 2= Keshimando number 2, N/K= Nebar Keshimando, K/27= Komeshiga 27, K/28= Komeshiga 28, RK = Rural kebele

Table 4. Univariate logistic regression model for *rural kebele*, district, body condition score, age and sex with buffy coat result during the dry season in Assosa and Bambasi districts

Risk factors	Risk Category	No examined	No positive	Prevalence % (95% CI)	OR (95% CI)	P-value
RK	K/no 2	56	2	3.6(0.44-12.3)	-	-
	N/K	74	4	5.4(1.49-13.27)	1.5(0.3-8.7)	0.62
	Sonka	61	1	1.6(0.04-8.8)	0.4(0.04-5.1)	0.52
	Shebora	63	1	1.6(-1.5-4.9)	0.4 (0.04-4.9)	0.50
	Amba 11	130	5	3.8(0.04-8.5)	1.1(0.2-5.7)	0.93
	K/27	60	2	3.3(0.41-11.53)	0.9(0.1-6.8)	0.94
	K/28	132	3	2.3(0.47-6.50)	0.6(0.1-3.9)	0.62
	Abrahamo	73	7	9.6(3.9-18.8)	2.9(0.6-14.4)	0.20
	Megele 38	123	12	9.8(5.1-16.4)	2.9(0.6-13.5)	0.17
Age	≤ 2 years	173	6	3.5(1.3-7.4)	-	-
	2-4 years	215	15	7.0(4.0-11.25)	2.1(0.8-5.5)	0.14
	> 4 years	384	16	4.2(2.4-6.7)	1.2(0.5-4.3)	0.70
Sex	Male	325	19	5.8(3.6-9.0)	-	-
	Female	447	18	4(2.4-6.3)	0.7(0.3-1.3)	0.24
BCS	Poor	160	10	6(3.0-11.2)	-	-
	Medium	400	20	5(3.1-7.6)	0.8(0.4-1.7)	0.55
	Good	212	7	3.3(1.3-6.7)	0.5(0.2-1.4)	0.18
District	Bambasi	254	8	3.1(1.4-6.1)	-	-
	Assosa	518	29	5.6(3.8-7.9)	1.8(0.8-4.1)	0.14
Season	Late rainy	790	61	7.7(5.9-9.8)	-	-
	Dry	772	37	4.8(3.4-6.5)	0.6(0.4-0.9)	0.02

BCS= Body condition score, K/no 2= Keshimando number 2, N/K= Nebar Keshimando, K/27= Komeshiga 27, K/28= Komeshiga 28, RK = *Rural kebele*

Risk factors such as age, sex and district were incorporated in multivariate logistic regression analysis in the late rainy season ($P < 0.25$) but they didn't show any significant difference ($P > 0.05$) except district as indicated in Table 9. During the dry season, only sex and district were incorporated in the multivariate logistic regression analysis ($P < 0.25$),

however, they did not show significant variation in the multivariate analysis ($P > 0.05$).

Multivariate logistic regression analysis of the current survey in the late rainy season revealed that, the odds of cattle to be infected with trypanosomes in Assosa district are 2 times higher when compared to cattle in Bambasi district with significant variation ($P < 0.05$) as shown in Table 5.

Table 5. Multivariate logistic regression model for age, sex and district with buffy coat results in the two seasons in Assosa and Bambasi districts

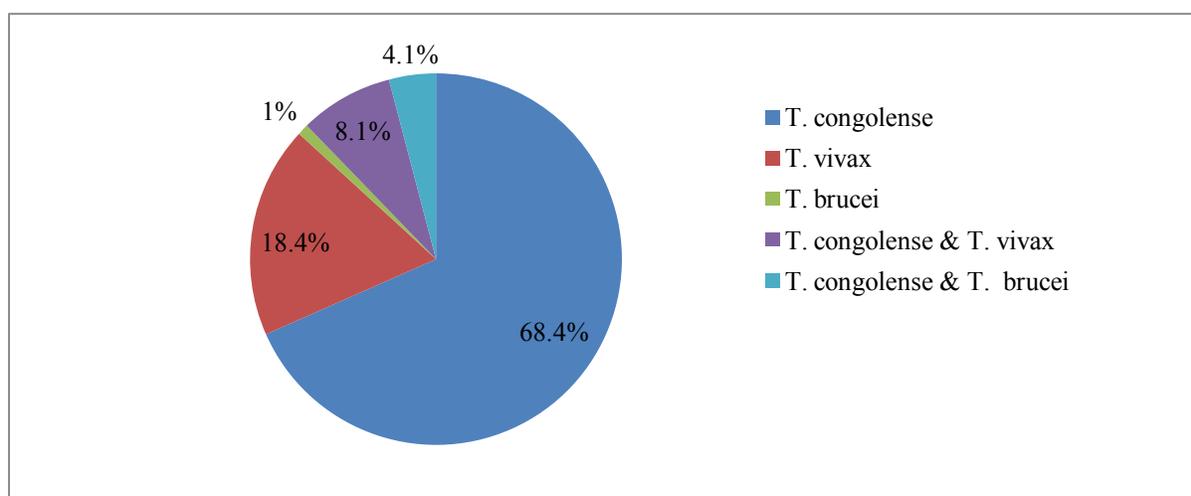
Season	RF	RC	No examined	No positive	Prevalence (%)	OR(95% CI)	P-value
Late rainy		≤ 2 years	178	9	5.1	-	-
	Age	2-4 years	220	21	9.5	1.8(0.8-4.2)	0.15
		> 4 years	392	31	7.9	1.5(0.7- 3.2)	0.31
	Sex	Male	338	31	9.2	-	-
		Female	452	30	6.6	0.7(0.4-1.2)	0.24
	District	Bambasi	269	12	4.5	-	-
Assosa		521	49	9.4	2.0(1.03-3.9)	0.04	
Dry	Sex	Male	325	19	5.8		
		Female	447	18	4.0	0.7(0.4-1.4)	0.30
	District	Bambasi	254	8	3.1		
		Assosa	518	29	5.6	1.8(0.8-3.9)	0.16

RC = Risk category, RF= Risk factor

Herd prevalence was determined in a total of 1,562 cattle sampled from 31 herds, 15 herds in the late rainy season and 16 herds during the dry period of the study. Herd prevalence ranges from 3.1 to 15.6 in the late rainy season and 1.4 to 10.5 in the dry season as well. The overall herd prevalence of the two seasons was 6.3%. All herds sampled during the study were found to be positive for trypanosomosis

which indicated that the disease is widely distributed and endemic among herds of cattle in the study districts.

Of the total 1,562 cattle of 31 herds examined, trypanosome infection due to *T. congolense* was detected in 67 (68.4%, 95% CI=58.2-77.4) of animals while 18(18.4%, 95% CI =11.3-23.5) of the infection was caused by *T. vivax* as shown in the Fig. 2.

**Fig. 2.** Proportion of trypanosome species detected in the two seasons in Assosa and Bambasi districts

3.2 Hematological findings

The mean PCV values (%) of parasitaemic animals were 23.05 ± 3.40 (95% CI = 22.18-23.92) and those of aparasitaemic animals were 27.73 ± 4.61 (95% CI=27.23-27.90) in the late rainy season and it was found to be statistically significant ($P < 0.05$) while during the dry period of the study, it was 23.54 ± 2.58 (95% CI=22.68-24.40) in parasitemic and 28.15 ± 4.58 (95% CI= 27.82-28.49) in aparasitemic animals with significant difference ($P < 0.05$) as shown in Table 6. The overall mean PCV values were also significantly different ($P < 0.05$) between parasitaemic 23.23 ± 3.11 (95% CI=22.61-23.86) and aparasitaemic 27.86 ± 4.60 (95% CI=27.62-28.10) animals indicating that trypanosome infection

resulted in a significant reduction in PCV of study animals even though other factors such as malnutrition and/or other diseases might also affect the PCV values.

Similar research findings were reported by Bitew *et al.* (2011), Haile *et al.* (2016), Eshetu *et al.* (2017) as well as Haile and Gizaw (2018) where the mean PCV values of infected animals were significantly lower than those of non-infected ones. Rowlands *et al.* (2001) in Ghibe area of Ethiopia reported that with a decrease in the PCV values, the proportion of infected animals increased and hence the mean PCV value was a good indicator of the health status of herds in trypanosomosis endemic areas.

Table 6. Mean packed cell volume of parasitemic and aparasitemic animals in the two seasons in Assosa and Bambasi districts

Season	Category	Number examined	Overall PCV	Mean PCV	95% CI	SD	P-value
Late rainy season	Parasitemic	61	1403.9	23.01	22.11-23.92	3.52	0.000
	Aparasitemic	729	20092.7	27.56	27.26-27.86	4.13	
Dry season	Parasitemic	37	871	23.54	22.83-24.25	2.12	0.000
	Aparasitemic	735	20693.2	28.15	27.87-28.44	3.97	
Overall	Parasitemic	98	2276.8	23.21	22.60-23.83	3.06	0.000
	Aparasitemic	1464	40786.9	27.76	27.54-27.98	4.40	

Packed cell volume of parasitemic cattle fall in the range of 12-27%; in aparasitemic animals it ranges from 15-45% in the late rainy season, while during the dry season the PCV values of parasitemic and aparasitemic animals range from 17-27% and 17-42%, respectively. Out of the 729 aparasitemic cattle, 9.2% had PCV<24% and from the 61 parasitemic animals 59% had PCV<24% in the late rainy season while during the dry period of the study, of the 735 and 37 aparasitemic and parasitemic cattle, 8% and 62.2% had PCV<24%, respectively.

This finding was comparable with (Golassa and Mekonnen, 2017) who reported mean PCV value that ranges from 9-37% in parasitemic and 11-45% in aparasitemic animals. Similarly, it was comparable with the finding of (Haile *et al.*, 2016) who reported mean PCV value that ranges from 17-36% and 15-41% in parasitemic and aparasitemic animals, respectively. The aparasitemic cattle with PCV<24% in the present study might be attributed to either the low sensitivity of the buffy coat techniques used in

chronic cases of trypanosomosis or it could be due to other factors such as poor nutrition and other diseases especially parasitic diseases which cause anemia.

In conclusion, the current study revealed the presence of three spp of *Trypanosoma* (*T. congolense*, *T. vivax* and *T. brucei*) which are mainly transmitted cyclically by *Glossina* in the study districts; the predominant spp being *T. congolense* followed by *T. vivax* in both the late rainy season and during the dry season. Significantly higher prevalence of bovine trypanosomosis was registered in the late rainy season when compared to the dry season. In this study, season was found to be an important risk factor for occurrence of bovine trypanosomosis. In general, trypanosomosis is an important disease and a potential threat in hampering productivity, work performance and general health status of cattle in the study districts. Therefore, designing trypanosomosis control strategies focusing on vectors and against the parasites including regular surveillance, community based vector prevention and

control measures should be implemented to mitigate the problem and taking other seasons into consideration, further epidemiological study on seasonal prevalence of bovine trypanosomosis should be conducted in the study districts so as to design the right time to apply preventive and control measures.

Conflict of interest statement

The authors declare that they have no competing interests and have no any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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