

## Research Article

# Epidemiology of bovine trypanosomiasis and its risk factors in West Gojam and Awi Zone of Amhara regional State, Ethiopia

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## Keywords

• Awi, Buffy coat, Gojam, PCV, Trypanosomiasis

## Abstract

Cross-sectional study was conducted in west Gojam and Awi Zones of Amhara national region of northwest Ethiopia, from September 2020 - March 2021 to determine the prevalence of bovine trypanosomiasis and to evaluate its association risk factors. Blood samples collected from 571 randomly selected cattle were subjected to parasitological and haematological analysis. For the parasitological survey, blood samples were examined using a buffy coat technique. The packed cell volume (PCV) value of each animal was also measured using hematocrit reader. The overall prevalence of trypanosomiasis was 4.7%. The most common trypanosome species identified were *Trypanosomes congolense* 41% (11/27) followed by *T. vivax* 37% (10/27) and 22% (6/27) were mixed infection. The prevalence showed no significant difference in susceptibility between sex and age groups, but has significant difference in body condition and PCV status of animals. The overall anemia prevalence in the area was 38.2% (218/571). The anemia prevalence was significantly higher in trypanosome positive cattle (81.5%) than in non infected animals (36%) ( $P < 0.05$ ). The mean PCV value of the infected animals was lower ( $20.3\% \pm 3.2698\%$ ) compared to non infected animals ( $29.3 \pm 3.7401\%$ ). There was statistically significant difference ( $P < 0.05$ ) in the PCV values of infected and non infected animals. *T. congolense* (18.3%) infection reduced PCV higher than *T. vivax* (21.5%) infection, but it is not statistically significant ( $P > 0.05$ ). In general, this study reveals that trypanosomiasis pretense a risk to cattle production and productivity in the area and also contributed to the occurrence of anemia.

## INTRODUCTION

Trypanosomiasis is main haemoparasite protozoan disease complex in domestic animals and human, which run from a chronic long lasting to an acute. The disease is mainly characterized by anemia, bout of fever, loses of weight, abortion, swelling of the lymph node; lacrimation, hind limb paralysis and unproductiveness [1]. Trypanosomes are flagellated protozoan parasites that live in the blood, plasma, lymph and several tissues of their vertebrate hosts [2]. The parasite is transmitted biologically by the tsetse fly (*Glossina* species) and biting flies and infects animals over an area known as the 'tsetse belt', which extends approximately 10 million km<sup>2</sup> across 37 countries in Africa, from the Sahara Desert in the North to South Africa in the south [3,4].

In Ethiopia, the most important *Trypanosoma* species are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* in cattle, sheep, goats and equines. Camels are affected by *Trypanosoma evansi* which is common species in camel rearing areas of the country while equines mainly horses are affected by *Trypanosoma equiperdum* in some highland parts of the country [5]. In Ethiopia, it is the most important diseases that limit livestock productivity and agricultural development due to its high prevalence in the most arable and fertile and of South west and North West part of the country following the greater river basins of Abay, Omo, Ghibe, and Baro [6]. The

distribution of trypanosomes is dynamic due to climatic change, ecological disturbances, and human interventions. The disease is economically important and livestock found below 2000 meters contour are exposed to various level of trypanosomes risk [7].

The tsetse flies are widely distributed in the Southwestern 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* [5]. Apart from cyclical transmission of trypanosomiasis by *Glossina* species, mechanical transmission is a potential threat to livestock productivity in some parts of Ethiopia [8]. Although trypanosomiasis may be found almost in all parts of the Amhara National Regional State, the problem is much more severe in most areas of West Gojam, part of East Gojam and Awi administrative zones.

In Western part of Amhara Regional State bordering the Abay river, one of the Northwestern tsetse belt areas of Ethiopia, tsetse transmitted trypanosomiasis is becoming a serious threat for livestock production and agricultural activity [6].

As a result tsetse transmitted trypanosomiasis threat a large proportion of the livestock population is forced to reside in tsetse free highland but economically and environmentally fragile and due to climate changes tsetse flies expands to tsetse free area.

Hence regular assessments of trypanosomosis have a paramount importance to plan and implement evidence based interventions. This study was reveal information on the current epidemiological status of trypanosomosis and its associated risk factor so; the objective of this study was to assess the prevalence of bovine trypanosomosis, and to determine its associated risk factor in West Gojam and Awi zones, north – West, Ethiopia.

## MATERIALS AND METHODS

### Study Area

The study was conducted in selected districts of West Gojam and Awi zone of North-West, Ethiopia: Jabi Tehina, Womberma, Jawi and Zigem located in the west Gojam and Awi zone respectively. Jabi Tehina, bordered by Abay River in the South, annual mean temperature is 14-32°C and the elevation varies from 1500-2300 mm above sea level (m a. s. l) with mean annual rain fall of 1250mm [9], Womberma, bordered on the south by river Abbay which separates it from Oromia regional state, on the west by Bure district, on the north and north east by Ankesha district and on the east by Guanga district. The altitude of the area ranges from 800 to 2212 meter above sea level (masl). The area has a rainy season (June to September) and a longer dry season (October to May) with mean annual rain fall of 1115mm. The annual mean temperature for most parts of the district is 14 -26 0c [10]. Jawi district is located approximately 600 km Northwest of Addis Ababa. It lies within the geographical location of 360 - 370E and 100380 to 110 30`N. The district has an altitude range from 648 to 1300 masl. The district has a climate which can be described as tropical with winter dry season. The agro-ecological area of the district has a warm and humid lowland zone around the area of the Belles River. The mean annual temperature varies between 25-400C with mean annual rain fall of 1569 mm. The livestock population of the Jawi district comprises about 70,403 cattle, 6,549 sheep, 24,995 goats, 1,232 equines, 30,997 poultry and 7,520 bee hives [11].

Zigem district: It is found at 405kms and 126kms apart from Addis Ababa and Bahirdar respectively which is located in the direction of North Western part of the country. The mean altitude for the peasant associations selected from the district is 1611 meter above sea level. The average annual rainfall is 2000mm for the district which ranges from the late of May to early September. The land is covered by different vegetation types namely savanna grass lands, forest and bush lands. The annual mean temperature for most parts of the district is 14-26oC according to the report of Zigem district office of Agriculture and rural development of unpublished data 2009.

### Study Animals

The animals used for this study were local zebu cattle (*Bos indicus*); all cattle above 1year of age were included in the study. Cattle in the districts are generally herded under the extensive grazing systems. Herds were composed of between as few as 2 cattle to >50 cattle. Their age was categorized into three age groups (<=2 years, 2-5 years, and greater than five years) based on dentition and the body condition score was grouped into cachectic, lean and good conditioned animals based on the appearance of ribs and dorsal spines applied for zebu cattle [12].

### Sample Size Determination

According to [9, 13, 14 and 15] the prevalence of bovine trypanosomosis in Jabi tehina, Womberma, Zigem and Jawi districts were 15.24%, 7.81%, 7.29% and 11.33% respectively. Therefore 15.24%, 7.81 %, 7.29% and 11.33% expected prevalence were taken for Jabi Tehina, Womberma, Zigem and Jawi district respectively to study the epidemiology of the disease. The sample size was determined using 95% confidence level and 5% desired absolute precision and can be calculated using the formula described by [16].

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

Where: N = required sample size

$P_{exp}$  = expected prevalence

d = desired absolute precision

Accordingly, a total of 567 (198, 111, 104 and 154 cattle from Jabi Tehina, Womberma, Zigem, and Jawi districts respectively) cattle were examined to study the epidemiology of the disease as well as the PVC value of parasitic and a parasitic animals.

## STUDY METHODOLOGY

### Packed Cell Volume (PCV) Determination

Blood samples for parasitological examination were obtained by puncturing the marginal ear vein with a lancet and collected directly into a pair of heparinised capillary tubes and sealed with crystal seal. After centrifugation at 12,000 rpm for 5min in a microhaematocrit centrifuge, the capillary tubes were placed in a hematocrit reader and the length of the red cells column was expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic [17].

**Buffy Coat Technique (BCT):** In each of the kebeles, all animals were screened for trypanosomes using the Buffy - coat technique (BCT) (Murray et al., 1977). Heparinised capillary tubes, containing blood samples, were cut using a diamond tipped pen 1mm below and 3mm above the buffy coat after centrifugation. The content of the capillary tube was expressed on to a glass slide, then covered with cover slip, and examined under  $\times 40$  objectives and  $\times 10$  eye pieces for movement of parasite. Trypanosome species were identified according to their movement in wet film preparations as provided by [17].

**Data management and Analysis:** Data was analyzed using STATA version 13 (StataCorp LP, Texas USA). Prevalence was analyzed by determining total positive cases out of the total number of animals sampled. Infection rate on the basis of sex, age, and body condition was compared using Pearson Chi square ( $\chi^2$ ) test. Mean PCV in parasitemic and aparasitemic animals were compared using student *t*-test. Significance test was set at 5% alpha and 95 confidence interval where  $p < 0.05$  indicates statistically significant.

## RESULTS

### Parasitological findings

Out of 571 cattle examined 4.7% (27/571) were found to be infected with trypanosomes. Prevalence of trypanosomosis

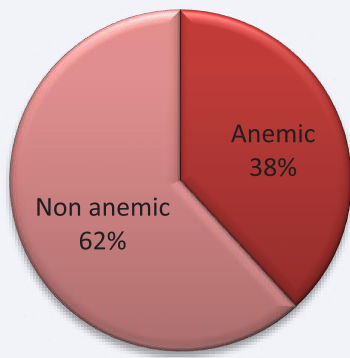


Figure 1 Diagrammatic presentation of PCV status.

**Hematological findings**

**PCV status in studied area:** The mean PCV value of 24.8% was registered during the study period. The most frequently recorded PCV value was 26% and 25% with a record of 50 and 48 cattles respectively from the overall targated animals in the study areas. The mean PCV values of cattle were statistically significant ( $P < 0.001$ ) which was 20.3% and 29.3% PCV positive and negative animals were registered, respectively (Table 3).

**Dynamics of trypanosome infection versus PCV status:** The overall anemia prevalence in the studied area was 38.2% (218/571). The anaemia prevalence was significantly higher in trypanosome positive cattle (81.5%) than in noninfected cattle (36.0%) ( $P < 0.05$ ). Of 38.2% anaemia prevalence, 3.9% (22/571) was trypanosome infected animals.

was almost similar in males (4.6%) and female animals (4.8%). However, the difference was not statistically significant ( $P = 0.874$ ). The highest prevalence was observed in the adult animals greater than 5 years old (5.2%) and the variation in prevalence between the different age groups was also not significant ( $P = 0.567$ ). The prevalence of trypanosomiasis between body condition scores was 8% in cachectic, 5.4% in lean and 1.4% in good body conditioned animals and it was statistically significant ( $P = 0.009$ ) as illustrated in (table 1).

The study revealed that, originally the disease distribution in terms of zone, district, Kebele was very high in Awi zone, Jawi district and in three Kebele of Jawi such as in Babluk, Simedra and workmedra with prevalence of 9.5% (23/242), 12.5% (20/160), 18.5% (10/54), 10.3% (7/68) and 7.9% (3/38) respectively as compared to west Gojam as shown in (table 2).

However, large number of animals 34.3% (196/571) had anaemia (PCV < 24) without having trypanosome infection. Few animals 0.9% (5/571) were infected by trypanosome but their PCV was found normal as indicated in (Table 4).

The prevalence in terms of trypanosome species was 1.9% (11/571) *T. congolense* and 1.8 % (10/571) *T. vivax* and their mixed infection was 1% (6/571). The comparison of anemic status measured by PCV of parasitemic period both infected with *T. vivax* and *T. congolense* infections indicated that *T. congolense* infection reduced PCV significantly higher than *T. vivax* infection ( $P > 0.05$ ) as indicated in (Table 5, Figure 2 and 3).

**DISCUSSION**

The current study revealed that the prevalence of bovine trypanosomiasis in the area was 4.7% (27/571). This finding was to some extent in agreement with the previous studies which

**Table 1:** Prevalence of bovine trypanosomiasis infection according to host related factor.

Host related factor	Number of examined cattle	Number of infected cattle	Prevalence (%)	X <sup>2</sup>	p-value
Sex				0.0250	0.874
Female	330	16	4.8		
Male	241	11	4.6		
Total	571	27	4.7		
Age				1.1365	0.567
<=2years	82	2	2.4		
2-5years	222	11	5		
>5years	267	14	5.2		
Total	571	27	4.7		
BCS				9.5278	0.009
Cachectic	175	14	8		
Lean	184	10	5.4		
Good	212	3	1.4		
Total	571	27	4.7		
PCV				22.5152	<0.001
Anemic	218	22	10.09		
Non Anemic	353	5	1.4		
Total	571	27	4.7		

**Table 2:** Prevalence of bovine trypanosomosis according to environmental related risk factors.

Environmental related risk factors	Number of examined cattle	Number of infected cattle	Prevalence (%)	X <sup>2</sup>	p-value
Zone				21.2627	<0.001
West Gojam zone	329	4	1.2		
Awi zone	242	23	9.5		
Total	571	27	4.7		
District				30.7597	<0.001
Tabi Thena	119	2	1.7		
Wonberema	210	2	0.95		
Zigem	82	3	3.7		
Jawi	160	20	12.5		
Total	571	27	4.7		
Kebele				38.6991	<0.001
Ergib	51	2	3.9		
Adankegn	68	0	0		
Woyntma	87	1	1.1		
Gomer	123	1	0.8		
Kelema	48	2	4.2		
Sonit	34	1	2.9		
Simeda	68	7	10.3		
Babluk	54	10	18.5		
Workmeda	38	3	7.9		
Total	571	27	4.7		

**Table 3:** Mean PCV comparison between infected and noninfected animals.

Conditions	Number	Mean PCV	SD	t-test	P-value
Anemic	218	20.3	3.2698	29.5563	<0.001
Non - anemic	353	29.3	3.7401		
Parasitemic	27	20.1	4.4405	6.8202	<0.001
Non-parastemic	544	26.2	5.5806		

**Table 4:** Proportion of anaemia from trypanosome infected and noninfected cattle population.

Trypanosome	Anemic/Non anemic	Frequancy	Percentage	Percent share per strata
Infected	Positive	22	3.9	81.5
	Negative	5	0.9	18.5
Non - Infected	Positive	196	34.3	36.0
	Negative	348	60.9	64

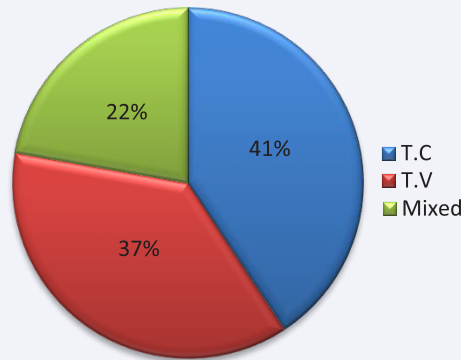
were conducted by [18] who reported 2.18% in Pawe and Jawi district and [19], 2.86 in Dale Wabera district of Kellem Wollega zone. However, [20], who assessed cattle trypanosomiasis in tsetse-free and tsetse infested zones of the Amhara region of Northwestern Ethiopia using molecular diagnostic method, reported infection rates of 20.4% and 25.7%, respectively. This finding is not in agreement with the current study conducted in Eastern and western Gojam zone of the same region. This variation might be attributed to test method applied where BCT failed to detect 66% of infected cattle [21] while molecular

detection is more sensitive. There is also a seasonal and ecological variation which directly influences fly density of both cyclical and mechanical transmitters. In addition to these justification tsetse and trypanosomosis control and prevention office were established in late 2020s' which have a great contribution for the reduction of trypanosomosis in the area due to control and prevention actions taken by the organization.

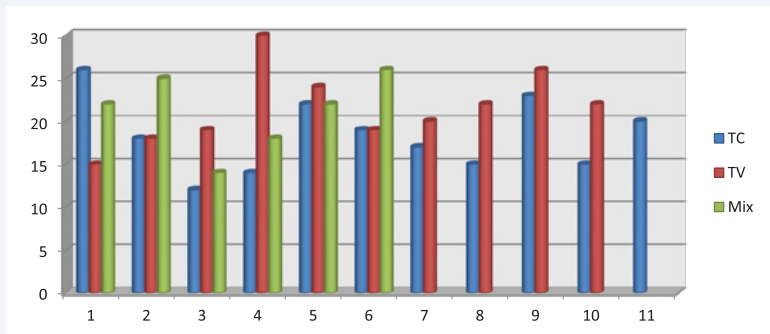
In this study we found 1.7% (2/119), 0.95 (2/210) and 3.7(3/82) prevalence of trypanosomosis in Jabitehna, Womberema and Zigem districts consecutively which were

**Table 5.** Mean PCV values between trypanosome species in parasitaemic animals in study area.

Species	Number	Mean PCV%	SD	t-test	P-value
T. congolense	11	18.3	4.245	0.1300	0.8976
T. vivax	10	21.5	4.491		
Mixed	6	21.2	4.327		



**Figure 2** Proportion of Trypanosome parasites among infected.



**Figure 3** Distribution of PCV among trypanosoma parasites.

lower compared with the previous findings of [15, 9] with (12%) and (15.24%) respectively in Jabitehna, 7.81% in Womberema [13], 7.29% in Ankasha [14]. The difference in prevalence might be occurred due to seasonal variability in the study areas as well as intervention of vectors by deforestation for the purpose of land cultivation and settlement adopted in the time frame. These conditions might led to the reduction of tsetse fly population along with the decline of tsetse borne trypanosomosis in the study area. The prevalence of the disease at Jawi district was likely higher compared to all other study areas (12.5%). This could be due to availability of intact ecological situation relative to other three study areas which favor for the breeding of the vector. The finding at this district was in consistent with the reports of [15] who reported Trypanosoma prevalence at the same area was 11.33%. Similarly, [22] also reported 12.41% prevalence in Metekel and Awi zones of Northwest Ethiopia.

In this study, two species of trypanosomes were retrieved among the overall inspected cattle; namely, *T. congolense* 1.9%, *T. vivax* 1.8% and their mixed infection 1%. The finding of this study showed that of the total trypanosome positive animals 41% (11/27) were *T. Conglense*, 37% (10/27) *T. Vivax* and 22% (6/27) mixed infection for *T. Conglense* and *T. Vivax*. Majority of

infections were also due to *T. congolense*. The higher proportion of *T. congolense* infection in the study areas were in line with the results of [23] at Arba Minch Zuria districts (85.2%) and [24] in Ghibe valley, Southwest Ethiopia (84%). Similarly, Abebe and Jobre, (1996) also reported *T. congolense* infection is found to be higher than other trypanosome species. In the same report it was also indicated that in tsetse free area of highlands, 99% of prevalence was due to *T. vivax*.

The predominance of *T. congolense* infection in cattle suggests that the major cyclical vectors are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa [25] and the development of better immune response to *T. vivax* by infected animals [26]. Different studies [27] 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* also invade the tissues [28]. According to [8], *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of the Ethiopia, respectively.

Animal wise study of trypanosomosis in different sex and age groups of cattle showed no significant variation 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 [18, 29]. According to body condition category cachectic animals were highly prone to the disease 8% (14/175) followed by lean 5.4% (10/184) when compared to good body conditioned animals 1.4% (3/212) which was statistically significant ( $p=0.009$ ). The result is in line with the study of [30] who reported poor body condition animals were significantly higher than that of medium and good body condition animals. Trypanosomiasis is a chronic disease as stated by [31] who deduced that emaciation and weight loss might be caused by the disease itself. However, it would be difficult to conclude either poor body condition predispose to trypanosome infection or trypanosome infection cause loss of body condition based on such cross-sectional study [32], and it should be verified by using a longitudinal study designs.

The overall anaemia prevalence in the studied areas were 38%. When infected and non-infected animals were compared, the anaemia prevalence was significantly higher in trypanosome positive cattle (22/27, 81.5%) than in non infected cattle (196/544, 36.5%) ( $P < 0.001$ ). This finding was in agreement with previous reports [29].

Of 38% anemia prevalence, 3.9% (22/371) was trypanosome positive animals. However, large number of animals, 34.3% (196/571), had anaemia without having trypanosomiasis infection through buffy coat examination. This suggests that even though anaemia is characteristic of trypanosomiasis, 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 [33].

However, Some animals were infected by trypanosome but their PCV was normal and anaemia 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 particularly when stress causing agents are prohibited. The appearance of parasitologically negative animals with PCV values of less than the threshold value set (24%) may be due to inadequacy of the detection method used [34] other anaemia causing diseases [33] or delayed recovery of the anaemic situation after current treatment with trypanocidal drugs.

The mean PCV value of parasitemic animals was found to be significantly lower (20.1%  $\pm$ 4.4405) than that of aparasitemic (26.2%  $\pm$ 5.5806) animals which is similar to the results obtained by [19]. Low PCV value may not solely be due to trypanosomiasis. However, these factors are likely risks for both parasitaemic and nonparasitaemic animals. Therefore, the difference in mean PCV value between parasitemic and aparasitemic animals in this study indicates that trypanosomiasis is involved in adversely lowering the PCV values of infected animals (Girma *et al.*, 2014). This result is in consistent with a survey conducted on cattle in Hawagelan District of West Wellega Zone [29] which revealed that the mean PCV of trypanosome infected animals was significantly lower (20.8 $\pm$ 3.2 %) compared to non-infected animals (24.9 $\pm$ 3.8 %). In Nigeria, domestic ruminants that were naturally infected with trypanosomes had significantly lower ( $p<0.05$ ) PCV and RBC counts compared to uninfected animals [35]. Lower herd average

PCVs for trypanosome positive cattle compared to trypanosome negative cattle have also been reported from Ghana [36], Zambia [21], Cameroon [37] and Gabon [38]. In the present study it was also noted animals which were infected with *T. congolense* have lower mean PCV values (18.3%) than *T. vivax* (21.5%) suggesting that higher impact of *T. congolense* in terms of blood pathology as compared to *T.vivax* which also invades the tissues [28].

In general, Expansion of veterinary services up to peasant association, seasonal variation, deforestation for crop cultivation and settlement might also have contributed to lower the prevalence of the disease. The lower prevalence observed in this study could also be due to sensitivity issue of parasite detection method used (BCT). It was reported that the buffy coat microscopy technique is relatively an insensitive diagnostic method as it fails to detect 66% of infected cattle [21] when compared to molecular diagnostic techniques which permit precise identification of the parasite to species level and serological diagnostic methods are more sensitive [34].

## CONCLUSION

The current study revealed that trypanosomiasis is an important disease and a potential threat that affects the health and productivity of cattle in the area particularly Jawi district. The major species of trypanosomes in the study area were *T. Congolense* followed by *T. vivax*. Nearly 40% of the sampled animals had a PCV value of below 24% either due to trypanosomiasis or PCV reducing infectious and non-infectious agents. The anaemia prevalence was significantly higher in trypanosome positive cattle (81.5%) than in noninfected cattle (36.0%). This indicates that infection with trypanosomiasis negatively affects PCV profile of animals. Therefore, awareness creation, appropriate control and prevention strategies should be designed and implemented to minimize the disease effect on livestock production and productivity in the studied area. It is also recommended to deal with more sensitive test methods for the detection of the parasite to illustrate a more accurate investigation like a molecular or serological detection techniques.

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