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Cross section study on the prevalence of bovine trypanosomosis in Dembecha District, Northern Ethiopia

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Abstract
Between December 2018 and June 2019, a cross-sectional study was carried out in the Dembecha region of the Amhara Region in Northwest Ethiopia. In order to ascertain the prevalence of bovine trypanosomosis and associated risk factors, 386 native cattle maintained in a mixed crop-livestock production system took part in the study. Using hematological (packed cell volume measurement) and parasitological (buffy coat technique) procedures, blood samples from 386 randomly chosen cattle (Bosindicus) were examined. Overall, 49/386, or 12.69%, of the trial patients had trypanosomosis. *T. vivax* 29/49 (59.18%) and *T. congolense* 20/49 (40.81%) were the main sources of the infection. Were the sole two species of Trypanosomas discovered in the study area. Although there was no significant link (p>0.05) between the prevalence of trypanosomosis and sex, coat color, or peasant affiliation, there was a significant correlation ($p<0.05$) between the prevalence of trypanosomosis and the animals' body condition, age, and PCV.

In this investigation, the mean PCV value of the infected animals was 22.1±4.611%, significantly lower ($p<0.05$) than the value of the non-infected animals, which was 26.36±6.736%. According to the study's findings, trypanosomosis is a disease that affects cattle economically, and its 12.69% prevalence means that more focus should be placed on developing an integrated disease control plan that takes into account both the parasites and the vector. In conclusion, the present data show a comparatively high trypanosomosis incidence in the studied areas, underscoring the need for a coordinated and integrated strategy to manage the vector and lessen the disease's influence in the area under investigation.

Keywords: Dembecha district, risk factor, packed cell volume, and trypanosomosis

1. Introduction
About 240,000 km^2 are infested by tsetse flies, which are the vector of several Trypanosoma species. Of that region, 25,000 km^2 are located in Ethiopia's southern rift valley. The southern portion of this region contains the majority of this area. About 10-14 million cattle in Ethiopia, 4-6 million in the southern region, and 2-3 million in the southern rift valley are at risk from trypanosomiasis. A complex immunosuppressive disease called trypanosomiasis is caused by trypanosomes, which are heterospecific, unicellular, eukaryotic haemoparasites that infect the blood and other organs of vertebrates, including humans and cattle [1-2].

The disease induced by flagellated protozoa is disseminated by a number of different arthropod vectors. The disease, which covers an area of around 10 million square kilometers across 36 African countries, is linked to the habitat of the tsetse fly (*Glossina* spp.) and other flies that are parasite vectors [3-5] and [7].

Numerous trypanosome species cause trypanosomosis, a disease with a significant economic burden that significantly reduces cattle productivity [8-9]. Many parts of Africa, especially Ethiopia, are home to pathogenic species of salivarian trypanosomes [10]. Livestock disease causes significant losses in terms of death, abortion, lower fertility, decreased milk and meat production, and the animals' ability to work [11].

Bovine trypanosomiasis is one of the most significant protozoan diseases affecting cattle in Ethiopia, affecting the health of these animals. Generally, bovines are susceptible to trypanosome infections such as *T. vivax*, *T. congolense*, T. brucei, and T. evansi [13]. Depending on the species of Trypanosoma, these organisms are spread mechanically by tsetse or other biting insects or cyclically by tsetse flies from the genus *Glossina* [12].

As a result, the goals of this research were to assess the prevalence and risk factors of trypanosomosis in the designated area and to recognize and describe the current species of Trypanosoma present in the region. Consequently, the research was initiated with the following aims: -

1.1 General objectives
To identify the prevalence of bovine Trypanosomiasis and its related risk factors

1.2 Specific Objective
- To assess the prevalence of trypanosomiasis within the study region
- To evaluate risk factors linked to trypanosomosis infection
- To identify the species of trypanosoma related to the illness

2. Materials and Methods
2.1 Study area
The Dembecha district of the North Gojjam Zone in the Amhara Regional State of northwest Ethiopia is where this study was carried out. It lies roughly 220 kilometers southeast of Bahir Dar, the Amhara region's capital, and 370 kilometers northwest of Addis Ababa, the capital of Ethiopia. Latitude 10º30´ North and longitude 37º29´ East are the coordinates of the West Gojjam zone [14]. With an average annual rainfall of 1200-1600 mm, the climate is characterized by a long summer wet season from June to September and a dry winter season from December to March. The research area's average temperature is between 10 and 20 degrees Celsius, and its elevation spans from 1400 to 2300 meters above sea level. Ninety percent of all agricultural activities are currently carried out through crop farming (mixed farming), animal husbandry, and irrigation, both modern and traditional. Sorghum, wheat, teff, maize, and other legumes are the primary seasonal crops that are harvested [15].

2.2. Study population
Local livestock were the subject of the study. In the Dembecha district, these animals were raised in a number of settlements. The cattle examined in this study were housed under a comprehensive management system and represented a variety of randomly chosen Kebeles (the smallest administrative unit), genders, physical states, age groups, and coat colors.

2.3. Study Design
A cross-sectional survey was carried out on 386 randomly chosen animals to investigate the occurrence of bovine trypanosomiasis.

2.4 Sample size and Sampling method
The animals' age, sex, coat color, and body condition score were all documented. According to [15], the animals were divided into three age groups: 0-3 years, 3-6 years, and over six years. Based on the visibility of the ribs and dorsal spines, the body condition score was divided into three categories for Zebu cattle: bad, medium, and good [15]. A 95% confidence level was used to determine the sample size; a desired absolute precision of 0.05 and an expected prevalence of 8.6% were previously reported by [17]. The formula given by [21] was used to calculate the sample size. To increase the study's precision, 386 samples were gathered overall, even though the estimated sample size was 120.

$$N = \frac{g2p\,(1\text{-}p)}{d^2} = \frac{1.96^2(0.086)\,(1\text{-}0.086)}{(0.05)\,(0.05)}$$

N is the sample size (number of samples).
d is the required level of exactness.at 5%, N = 120, p = anticipated prevalence

2.5 Study Methodology
2.5.1 Parasitological Study
2.5.1.1 Thin Blood Smear
2.5.2 Hematological study
2.5.2.1. Packed Cell Volume (PCV) Determination
A micro-hematocrit capillary tube was used to draw a tiny amount of blood from the ear vein. The tube was then put on a sterile slide and spread out at a 45° angle on another sterile slide. The thin smear was immersed in the Giemsa staining solution (1:10 dilution) for 30 minutes after the smear had dried and been fixed in methyl alcohol for two minutes. After removing the excess discoloration, distilled water was used to rinse. The oil immersion objective lens (x100) was then used to examine it under a microscope after it had been left to dry upright on the rack [19].

2.5.3.2 Buffy Coat Technique
Blood samples in heparinized microhematocrit capillary tubes were centrifuged for five minutes at 12,000 rpm. Trypanosomes were usually found in or immediately above the buffy coat layer after centrifugation. A diamond-tipped pen was then used to cut the capillary tube 1 mm below the buffy coat in order to collect the topmost layers of red blood cells, and 3 mm above in order to collect the plasma. The capillary tube's contents were covered with a cover slip. A x40 objective lens and x10 eyepiece were used to view the slide in order to detect any parasite movement [20]. The physical traits of trypanosome species shown on Giemsa-stained blood films and their migration in wet film preparations, as described by [21], were used to identify them.

2.5.4. Data Management and Analysis
Before being imported into the Statistical Package for the Social Sciences (SPSS) version 20.0, the data collected for this study was recorded and saved in Microsoft Excel for Windows 2010. A logistic regression model was used to analyze the prevalence of trypanosomosis across a number of variables, including peasant association, body condition, coat color, gender, and age. Both parasite-aemic and aparasitaemic animals' average PCVs were compared using a student's t-test. The logistic regression model was used to examine the relationships between the explanatory variables (risk factors) and the result (trypanosomosis) for each analytic unit. Odds ratios (OR) were used to assess how strongly the outcome and explanatory variables were related. The number of vectors and comparable agro-ecological conditions across all peasant groups may be the cause of this. On the other hand, because the area is home to a variety of wild animals, the minor variation between the three PAs may be related to unchecked animal movements among peasant associations, as well as favorable conditions

for the vectors and the existence of their preferred hosts, which are not solely domestic livestock [19].

3. Results

In the study locations, the overall prevalence of bovine trypanosomosis was 12.7%. The rise in trypanosomosis prevalence relative to the prevalence found in the earlier study 8.6% by [17] may be associated with the following factors:

1. The absence of a federal tsetse control program in 2018
2. Variations in the number and ecology of villages included in this study

The prevalence rates observed in each peasant association were found to be 9.6% in Enewend, 15.5% in Gedeb, 8.8% in Kendamo, and 18.3% in Melkechaba, with 8.8% in Meqare of Dembecha District. The most common species in the study area was *T. congolense*. Out of a total of 49 cases of trypanosome infections identified, 31 (64.8%) were attributed to *T. congolense*, while the remaining 18 (35.2%) were due to *T. vivax* as shown in Table 1.

Melkechaba had the highest prevalence of bovine trypanosomosis (18.3%), whilst Kendamo and Meqar peasant associations had the lowest (8.8%). The prevalence of trypanosomosis did not, however, differ statistically significantly (p>0.05) between the various peasant associations. The similarities in agro-ecology and the prevalence of vectors in all peasant organizations may be the cause of this. A favorable environment for the vectors and the presence of their preferred hosts, which may not be limited to domestic livestock [21] because the area is home to a variety of wild animals, could be the cause of the slight differences among the three PAs, on the other hand, as well as uncontrolled animal movements between the associations.

The occurrence of trypanosome in various age groups was noted to be 6.2% in young animals (<3 yrs), 32.6% in adults (3-6 yrs), and 61.2% in older animals (>6 yrs). However, the differences in trypanosome infection rates across age groups were not statistically major (p>0.05). Similarly, the rate of trypanosomosis was higher in male cattle (63.3%) compared to females (36.7%), but again, there was no statistically major difference (p>0.05).

According to this study, there was a significant (p< 0.05) correlation between trypanosome infection and BCS. Animals in poor condition had a higher infection rate (44.9%) than those in medium (36.7%) or good (18.4%) health. Animals with low BCS had a 3.1-fold increased risk of trypanosome infection compared to those with high BCS (Table 3). On the basis of this kind of cross-sectional study, it is difficult to ascertain whether animals with poor body condition are more vulnerable to trypanosome infection or whether trypanosome infection causes a decline in body condition [22], and this should be confirmed by longitudinal study designs. This outcome is consistent with research from [23] and [24]. It has been noted that loss of body condition and ongoing emaciation are characteristic indicators of Trypanosomosis [8].

A comparison of various skin colors in cattle revealed that those with black skin had a higher prevalence rate (65.3%), followed by red (20.4%) and white (14.3%) skin. Also, the prevalence of trypanosome infection was found to be considerably linked (p< 0.05) as shown in Table (2). Infected cattle had an average PCV value of 19.2± 3.82 SD, whereas non-infected cattle had an average PCV value of 26.3± 3.1 SD. The mean PCV value of infected and non-infected cattle differed statistically significantly (P<0.05) (Table 4).

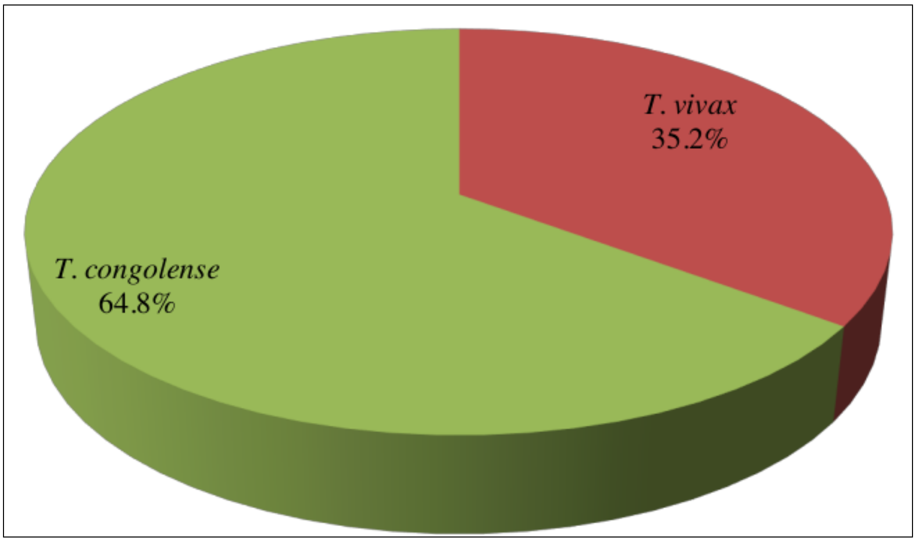

Fig 1: Distribution of the species of trypanosomes among the infected animals

Table 1: Overall trypanosome species predominance

Species	No. positive	Prevalence (%)
T. vivax	18	4.7
T. congolense	31	8
Total	49	12.7

Table 2: The overall prevalence in the study Kebele's

Study kebeles	No Examined	Number positive	Identified species		Prevalence (%) (95% CI)	*P*
			T. vivax	*T. congolense*		
Enewend	73	7	3(43%)	4(57%)	9.6	0.908
Gedeb	104	16	6(37%)	10(62.5%)	15.5	
Kendamo	68	6	2(33.4%)	4(66.5%)	8.8	
Melkechaba	82	15	5(33.3%)	10(66.7%)	18.3	
Meqare	57	5	2(40%)	3(60%)	8.8	
Total	384	49	18(35.2%)	31(64.8%)	12.8	

Table 3: Univariable logistic regression analysis of bovine Trypanosomosis associated risk factors in the study areas

Character	No Examined	Positive	Prevalence	Or (95%CI)	P value
Sex					
Female	254	18	36.7		0.634
Male	130	31	63.3	0.93(0.47-3.48)	
Age					
<3yrs	36	3	6.2		
3-6yrs	147	16	32.6	2.13(0.95-6.81)	0.066
>6yrs	201	30	61.2	2.44(0.96-8.64)	0.069
Coat color					
White	91	7	14.3		
Red	115	10	20.4	1.1(1.29-8.24)	0.032
Black	178	32	65.3	3.41(2.73-9.72)	0.001
Body condition					
Good	65	9	18.4		
Medium	219	18	36.7	1.31(2.34-3.96)	0.022
Poor	100	32	44.9	3.1(5.98-12.64)	0.013

Table 4: Mean PCV comparison of parasitemic and aparasitemic cattle

Health condition	Sample size	Mean PCV±SD	t-test	p-value
aparasitemic	235	26.3± 3.1	1.97	0.00
Parasitemic	49	19.2±3.82		
Overall	384	22.3±0.94		

4. Discussion

It was found that the prevalence of bovine Trypanosomosis in the study area was 12.8%. This discovery is consistent with the findings of [23] and [25], where prevalence rates of 11.7% and 14.68% were reported in JabiTehenan and Dembecha districts, Northwestern Ethiopia, respectively. Nevertheless, the prevalence rate in the present study was higher compared to previous research by [17] and [26], where they found rates of 8.6% and 7.81% in Dembecha and Wemberma districts of Northwestern Ethiopia, respectively. The discrepancies in results could be explained by differences in how the animals are managed, when the study was conducted, the development of drug resistance, a rise in tsetse fly numbers leading to more challenges, and a lack of knowledge among animal owners about the disease in the specific area of the study.

This study mainly discovered two types of trypanosomes: *T. congolense* and *T. vivax*. Out of the 49 trypanosomes found, 64.8% were *T. congolense* and the remaining 35.2% were *T. vivax*. Earlier research by [26], [27], [29], and [30] discovered higher prevalence rates of *T. congolense* (84%, 66%, 60.9%, and 75%) compared to *T. vivax* in Ghibe valley, Merab, Pawe, and western Ethiopia. The higher prevalence of *T. congolense* compared to *T. vivax* may be attributed to the larger number of serodems of *T. congolense* and the stronger immune response of infected animals against *T. vivax*.

However, Cherenet and colleagues (2006) found that *T. vivax* caused 90.9% of cattle trypanosome infections in tsetse-free zones of the Amahara region in Northwest Ethiopia. This is due to the fact that *T. vivax* can be transmitted by other means besides tsetse flies, such as mechanical vectors. On the other hand, *T. congolense* and *T. vivax* equally contributed to the prevalence of trypanosome infections in areas with tsetse flies.

The study found no significant connection between risk factors like the source of animals/PA's, gender, and age and the occurrence of trypanosomosis through univariable logistic regression analysis. The prevalence of trypanosome infection was similar among all peasant associations in the study due to comparable agro-ecological conditions and vector abundance. On the other hand, the slight differences noted in the three peasant groups may be due to unrestricted animal movements between them, and a habitat favorable to disease-carrying vectors and their preferred hosts, such as domestic livestock and numerous wild animals in the area.

No significant relationship was found between sex and trypanosome infection in cattle from the Dembecha district. This outcome aligns with findings from prior studies by [34] and [33] which showed no apparent difference in susceptibility between genders. This phenomenon may be attributed to the fact that both males and females can be affected in a similar and consistent manner in areas with high tsetse challenge.

In animals with poor body condition, the infection rate was significantly higher ($p < 0.05$) compared to animals with good body condition, as stated by [35]. The decreased prevalence of trypanosomosis in medium- and well-bodied animals could be due to their stronger immune systems, which are more effective at fighting off infections due to better nutrition. Moreover, there is a minimal likelihood of infection recurring in animals in optimal physical condition. On the other hand, 68% of animals without fever showed signs of poor body condition, indicating that other factors like diseases, diet problems, and farming methods could have contributed to the cattle's unhealthy state [36].

The present study did not observe a significant age or sex disparity ($p > 0.05$), even though adult and animals over three years old had a higher infection rate. This result aligns with the previous research conducted by Sinshaw [14], possibly due to the fact that mature animals journey long distances to feed, plow, and gather crops in regions with high tsetse fly populations. As per [37], calves in the Ghibe Valley remain at homesteads for grazing until they are weaned instead of accompanying their mothers. Maternal antibodies also offer innate defense to young animals, potentially resulting in a reduced occurrence.

The ratio of the three animals with different hair coat colors in the study area showed a significant variation. Animals that had black fur were the most common (33.39%), while those with white fur were the least common (8.06%). This may be due to the fact that flies prefer certain colors. Tsetse flies prefer animals that are dark in color. This discovery aligns with the findings of Haile (1996) in North Omo and Getnet (2008) in Soddo Zuria Woreda, who both observed a high prevalence of animals with black (7.04%), red (5.09%), and white (3.80%) hair coats.

Cattle infected with trypanosomes showed a significantly reduced average PCV value of 23.29±4.25, compared to uninfected cattle with a value of 25.59±4.23. As stated in reference [27], if an animal tests positive with a PCV level above 24%, it may indicate a recent infection. There could be other reasons besides trypanosomosis for a low PCV value. However, both cattle with parasites and cattle without parasites are likely to be susceptible to these causes. Hence, trypanosomosis is responsible for reducing the PCV value in the cattle with infection, as shown by the variance in average PCV values between the animals with parasites and those without. The outcome, in line with previous findings, indicated that anemia is often a sign of disease severity; the decreased PCV in animals with parasites may have contributed to reducing the average.

5. Conclusion and Recommendation

In this study on trypanosomosis in cattle, a total prevalence of 12.7% was observed, confirming the continued presence of the disease in the area. The predominant species identified were *T. congolense* and *T. vivax*. Based on host risk factors, bovine trypanosomosis was more commonly found in adult cattle than in young cattle. In contrast to animals without parasites, parasitized animals had a significantly lower average PCV value. This indicates that trypanosomosis infection can negatively impact an animal's PCV profile; however, even non-anemic/normal animals (11.7%) can still show positive results for trypanosomosis infection, rendering PCV as an inadequate diagnostic tool on its own.

Based on the earlier results, the below recommendations are provided:

- The expansion of government and private veterinary services is necessary to adequately serve the community.
- A well-planned tsetse and trypanosomosis control program that is based in the community should be developed and put into action.
- Farmers need to be informed about the dangers of drug resistance.
- Further studies are required on the epidemiological characteristics and emergence of drug resistance in pathogenic trypanosomes.

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