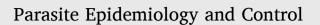
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# Seasonal prevalence of bovine trypanosomosis and trypanosome species distribution in Jimma Horo district, Oromia regional state, Western Ethiopia

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## ARTICLE INFO

Keywords: Bovine Ethiopia Kellem Wollega: Prevalence Oromia Trypanosome

## ABSTRACT

A study was conducted to determine the prevalence of bovine trypanosomosis in rainy (June 2019) and dry (February 2020) seasons in Jimma Horoo district, Kellem Wollega Zone, Oromia Regional State, Ethiopia. A total of 720 blood samples were examined using buffy coat and thin blood smear techniques. The packed cell volume (PCV) of each animal was determined. The overall bovine trypanosomosis prevalence was 4.3% ( $\chi^2 = 1.25$ , P = 0.26). The prevalence was 5% (95% CI = 4.1-8.3) and 3.3% (95% CI = 2.7-6.3) in the dry and rainy reasons, respectively (P > 0.05). Trypanosoma congolense, T. vivax and T. b. brucei were detected in (60%), (33.3%) and (6.7%) of infected cattle, respectively. The highest trypanosome prevalence was observed in Burka Gudina (7.6%), and the lowest in Melka Nega village (2.1%). There were significant variations between trypanosome prevalence in relation to body condition scores ( $\chi^2 = 23.16$ ; P =0.0.00) of examined cattle. No significant difference (P > 0.05) was observed between seasons, age, and sex categories of cattle. The PCV values of trypanosome infected (22.94%) was significantly lower than non-infected cattle (26.47%) ( $\chi^2 = 19.60$ ; (P < 0.05). The prevalence of bovine trypanosomosis in Jimma Horo district was low and it can be controlled by treatment of infected cattle using sanative pairs of trypanocidal drugs with additional application of deltamethrin pouron in the dry season. Further, in view of poor sensitivity of buffy coat technique used the use of molecular techniques should be encouraged.

## 1. Introduction

Animal trypanosomosis is one of the most important vector-borne protozoan parasites of domestic and wild animals that multiply in blood and tissue fluids of their mammalian hosts. In bovine it is caused by extra-cellular protozoan parasites of genus trypanosoma, and characterized by progressive loss of body condition, lymphadenopahty, anemia, decreased milk production and often terminates in death when untreated (Stijlemans et al., 2016). Tsetse and other biting flies have been implicated in the cyclical and mechanical transmission of bovine trypanosomosis and the distribution of the disease corresponds to the distribution of these flies (OIE, 2013). In Africa, tsetse flies and animal trypanosomosis distributed over a wide range of habitats covering over 10 million km<sup>2</sup> in 38 sub-Saharan African countries, exposing >70 million cattle and 160 million small ruminants to the risk of disease (WHO, 2013).

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https://doi.org/10.1016/j.parepi.2022.e00280

Received 26 March 2022; Received in revised form 4 August 2022; Accepted 26 November 2022

Available online 30 November 2022

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Animal trypanosomosis has been endemic for a long time and remains the greatest obstacle to livestock development in Africa (Giordani et al., 2016; Tegegn et al., 2021). The parasite affects a wide range of domestic and wild animals; however, from an economic point of view trypanosomosis is most important in cattle, but it can also cause serious losses in pigs, camels, goats, sheep, and it also affect wildlife and human in Africa (Simwango et al., 2017; Spickler, 2018). In cattle, trypanosomosis is caused by *T. vivax, T. con-golense* and *T. b. brucei*, (Auty et al., 2012). Bovine trypanosomosis affects not only the well-being of the population, but also efficient food production with 3 million cattle deaths per year (Giordani et al., 2016). African animal trypanosomosis has a serious effect on animal health and production, resulting in the loss of 10 to 50% of cattle, 2 to10% of agricultural production, 5 to 30% of meat and10 to 40% of milk production and also limits the full potential of livestock production. The annual losses resulting from direct and indirect effects of the disease can be estimated to 4.5 billion USD (MLDF, 2017).

Even though, Ethiopia has the largest livestock population in Africa, this sub-sector contributes only for about 15–17% of GDP and 35 to 49% of agricultural GDP (CSA, 2017), and 37 to 87% of the household incomes (Sintayew et al., 2010). The development and intensification of livestock productivity is hindered by low genetic potential of local breeds, lack of modern veterinary services and animal diseases such as animal trypanosomosis. In Ethiopia, trypanosomosis is one of the most important endemic live stalk diseases that have been recorded in five of the nine Ethiopian regional states between longitude  $33^0$  and  $38^{\circ}E$  (Tilahun and Schmidt, 2012), and distributed in an estimated area of 220,000 km<sup>2</sup> of fertile lands (Leta et al., 2016). In these areas, 14 million cattle, an equivalent number of small ruminants and >7.5 million equines and 1.2 million of camels are at moderate to high risk of contracting the disease (CSA, 2017). According to Taye et al. (2012), the economic losses due to trypanosomosis in Ethiopia is estimated USD \$200 million.

Western Ethiopia is one of the tsetse-infested areas where livestock development has been severely constrained by bovine trypanosomosis (Degneh et al., 2019). Currently, four species of tsetse flies: *Glossina pallidipes, G.m. submorsitans, G. f. fuscipes,* and *G. tachinoides,* and three species of trypanosomes are known to occur in western Ethiopia (Degneh et al., 2017). Even though a number of studies have been so far undertaken in Western Ethiopia to determine the occurrence of bovine trypanosomosis (Duguma et al., 2015; Degneh et al., 2019b), in Jimma Horo district, systemic studies have not yet been carried out on seasonal prevalence and disease associated risk factors. Therefore, the aim of this study was to investigate the prevalence of bovine trypanosomosis and the host-related risk factors in the area.

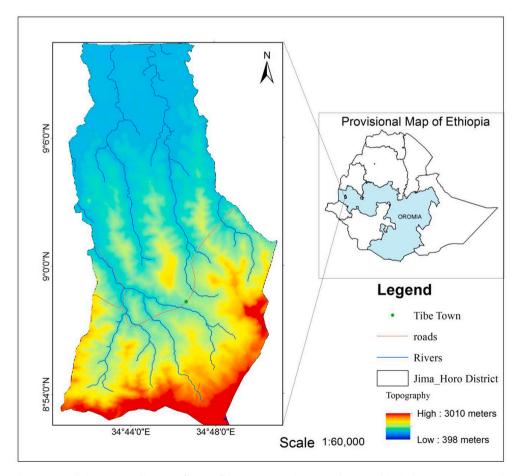


Fig. 1. Map of Jimma Horo district, Kellem Wollega Zone, Oromia Regional State, Ethiopia (KWZAHFRDO, 2019).

## 2. Materials and methods

## 2.1. Study area

This study was carried out in Jimma Horo district, Kellem Wollega Zone, Oromia Regional State, Western Ethiopia (Fig. 1). The Jimma Horo district is found about 640 km to the west of Addis Ababa. The district has a total surface area of 50, 2000 ha with a mean annual rainfall of 900 mm and an altitude of 1400 to 1800 m above sea level, respectively. The district is bounded to the east by Gawo Kebe, to the west by Gidami, to the south by Yamalogi Walal and to the north by Begi districts. The district has two distinct seasons: a dry season extending from November to May and a rainy season which extends from June to October, with an average annual average temperature of 23 °C. Based on altitude the district is subdivided in to three climatic zones: highland 20%, midland 48% and lowland 32% (JHDAHFRDO, 2018). The Livestock and crop production are the main sources of livelihood in the Jimma Horo district.

## 2.2. Study population

The study animals consisted of cattle above one year of age, randomly selected from five villages. The cattle in the district are local breeds that are kept under traditional extensive husbandry systems. The district has an estimated livestock population of 66,300 cattle, 19,400 sheep, 13,647 goats, 7960 equines, and 68,400 poultry (CSA, 2017).

## 2.3. Study Ddesign and sample size determination

A cross-sectional study was conducted in the wet (June 2019) and dry (February 2020) seasons. The sample size was calculated based on Thrusfield and Christley (2018), with an expected prevalence of 14.08% at a 95% confidence interval and a 5% desired absolute precision. Even though 185 samples were required for one season, we increased the sample size to 360 to increase the precision and because of favorable environment for sample collection during the study period. Thus, blood was collected from 720 randomly selected cattle (360 in dry and 360 in rainy) seasons from five villages (144 from each village). At the time of sampling, the age, sex, and body condition score of each animal were recorded. Studied animals were grouped into poor, medium, and good body condition scores (Nicholson and Butterworth, 1986) and as well as young ( $\leq$ 3 years) and adult (>3 years) age categories (Pasquini et al., 2003).

#### 2.4. Blood collection and diagnostic methods

Blood samples were collected by piercing the marginal ear vein into sterile hematocrit capillary tubes. The capillary tubes were then spun in a micro-hematocrit centrifuge at 12,000 rpm for 5 min. After centrifugation, the packed cell volume (PCV) was determined by Samdi et al. (2010). Animals with a PCV of <25% were considered anemic in this study (Douglas and Wardrop, 2010). Thus, the centrifuged capillary tubes were cut with a diamond pencil, and the uppermost layer of red blood cells of each sample were extruded onto a microscope slide and examined to reveal the presence of motile trypanosomes (OIE, 2008). For species identification, from trypanosome positive samples, thin blood smears were made, stained with Giemsa and examined under oil immersion (Buscher et al., 2009). The trypanosomes were identified to the species level according to their movement patterns and morphological characteristics (OIE, 2009).

## 2.5. Data analysis

The data was analyzed with the statistical software STATA14 and SPSS version 20.0. Trypanosome prevalence was calculated as the number of infected individuals divided by the number of individuals sampled x 100. Logistic regression was used to compare the association between the prevalence of infection and body condition scores, sex, and age of animals. A Student's *t*-test was used to assess the difference in mean PCV between trypanosome positive and negative animals. The *P*-values of the results were analyzed (using P = 0.05 as a cut-off value) at 95% confidence intervals.

Table	1
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An overall prevalence of bovine trypanosomosis in different study villages, Jimma Horo district, KellemWollega Zone, Oromia Regional State, Western Ethiopia.

Villages	No. of animals examined	No. of positive animals	Prevalence (%)	Chi-square	p-value
Gombo	144	6	4.2		
Hambashe	144	4	2.8		
Burka Gudina	144	11	7.6	5.21	
Nadi Gudina	144	7	4.8		0.26
Melka Nega	144	3	2.1		
Total	720	31	4.16		

#### 3. Results

Out of 720 (411 females and 309 males) cattle examined, 31 were infected with trypanosomes. The overall prevalence of trypanosome infection was 4.3%. The prevalence of bovine trypanosomosis was higher during the dry season (5%) than in the rainy season (3.3%), but the difference was not varied significantly (P > 0.05). *Trypanosoma congolense*, *T. vivax*, and *T. b. brucei* were detected in the studied cattle. Out of all positive cattle, 60% were infected with *T. congolense*, 33.3% with *T. vivax*, and 6.7% with *T. b. brucei* infections.

As shown in Table 1, the highest prevalence of bovine trypanosomosis was observed in Burka Gudina (7.6%), followed by Nadi Gudina (4.8%) and Gombo (4.2%), and the lowest in Ambashe (2.8%) and Melka Nega (2.1%)

The seasonal variations of trypanosome infections were summarized in Table 2. The prevalence of trypanosome infections varied among body condition scores, different age groups, and sex categories of cattle. However, a statistically significant difference was observed only among the body condition scores of cattle (P 0.05). In contrast, there was no significant difference in the prevalence of bovine trypanosomosis among different age groups and sex categories of cattle (P > 0.05).

The packed cell volumes (PCV) of examined cattle ranged from 11% to 43%. Of the total 720 blood samples, 37.22% had a PCV value of <25%. The overall mean PCV was 25.84% 4.57 on average. Taking into account the mean PCV value cut off of 25%, 73.3% of infected and 38.8% of non-infected cattle was found anemic. The overall mean PCV value of trypanosome infected cattle (22.27%) was significantly lower than that of non-infected cattle (25.99%) (Fig. 2). however, 26.6% of trypanosome infected cattle have a PCV value in the normal range (PCV > 25%).

## 4. Discussion

Bovine trypanosomosis remains one of the most important parasitic diseases limiting livestock health and production in Jimma Horo district, Oromia Regional State, Ethiopia, with a reported prevalence of 4.3%. Comparable results have been reported by Teka et al. (2012) and Hundessa et al. (2021) in South-West and Southern Ethiopia, who reported an overall 4.4% and 5%, respectively. However, this finding is much lower than a range of studies conducted in Ethiopia: 12.41% in Metekel and Awi zones, North-West Ethiopia (Mekuria and Gadisa, 2011) and 14.08% in Gidami district, Western Ethiopia (Degneh et al., 2017). The low prevalence reported in our study could be because of the low sensitivity of the buffy coat method used (Swai and Kaaya, 2012). On the other hand, the prevalence reported here is higher than the 2.86% reported in Dale Wabera district, Kellem Wollega zone, Oromia regional state, Western, Ethiopia (Biyazen et al., 2014). Differences in agro-ecology practice of trypanocidal drug usage, and fly control operations may impact the epidemiology of bovine trypanosomosis (Majekodunmi et al., 2013; Geiger et al., 2015). The highest numbers of trypanosome infections were indicated in Burka Gudina (10/30) and Nadi Gudina villages (3/30) (Table 1). These villages are mainly covered by savannah vegetation, which creates the ideal conditions for tsetse and biting flies' infestation.

The most notable causes of animal trypanosomosis identified in cattle are *T. congolense*, *T. vivax*, and *T. b. brucei*. These trypanosome species are the main causative agents of trypanosomosis in a wide range of domestic animals, including cattle, horses, and monkeys (OIE, 2013). Our findings showed the predominance of *T. congolense* (60%) over *T. vivax* and *T. b. brucei* infections (P < 0.05). The dominance of *T. congolense* infection in cattle was reported in studies conducted in Ethiopia (Duguma et al., 2015; Kassaye, 2015; Degneh et al., 2017), and various parts of Africa (Mwandiringana et al., 2012; Simo et al., 2015), suggesting that this trypanosome species is the major cause of AAT and the most prevalent trypanosome in cattle.

As expected, the overall prevalence of trypanosome infections varied significantly among body condition scores of cattle. In the present study, cattle with poor body condition recorded a higher trypanosomosis prevalence rate (9.5%) than in medium (2.3%) and good body condition (1.6%) (P < 0.05), which was not surprising since trypanosomosis is present as a chronic and wasting disease in animals. This finding was in agreement with the studies of Degneh et al. (2017), who found the highest prevalence of trypanosomosis in poor body condition cattle. This could be attributed to immune suppression due to blood parasites (Takele and Gechere, 2019) or related to the progressive weight loss arising from the debilitating nature of the disease (Radostits et al., 2007).

Table 2

An overall prevalence of bovine Trypanosomosis based on different risk factors, Jimma Horo district, Kellem Wollega Zone, Oromia Regional State,
Western Ethiopia.

Variables	No. animals examined	No. infected animals	Prevalence (%)	X <sup>2</sup> test	P- Value
Season					
Rainy	360	12	3.3		
Dry	360	18	5	1.25	0.26
Body condition					
Poor	221	21	9.5		
Medium	374	8	2.1	23.15	0.00
Good	125	1	0.8		
Sex					
Male	309	11	3.6	0.46	0.50
Female	411	19	4.6		
Age					
$\leq$ 3 years	200	5	2.5	1.80	0.19
>3 years	520	25	4.8		

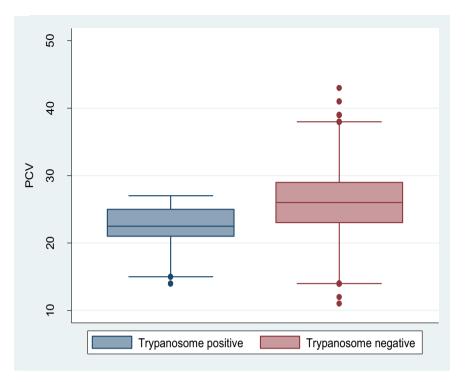


Fig. 2. An overall mean PCV values of trypanosome positive and negative cattle, Jimma Horo District, Kellem Wollega Zone, Oromia Regional State, Western Ethiopia.

In the current study, we assessed potential risk factors such as season, age, and sex of cattle, but significant differences were not observed (P > 0.05). However, the overall prevalence of trypanosomosis was higher in males (39.5%) than in female cattle (36.3%), in the dry season (5%) than in the wet season (3.3%), and in adults (4.8%) than in younger cattle (2.5%), respectively. The relatively higher prevalence in the dry season and in adult cattle could be associated with the chronicity of the parasites (Franco et al., 2014), low nutritional levels during the dry season (Eamin et al., 1998), or grazing behavior which exposed older animals to the vectors (Alemayehu et al., 2012). Likewise, the relative increase in prevalence in female cattle could be due to physiological differences (Torr et al., 2006) or the higher number of sampled females as compared to males.

Despite other anemia-causing confounding factors, trypanosome-infected cattle had a significantly lower mean PCV than non-infected. The significant association between AAT and PCV levels in infected and non-infected cattle is consistent with research findings in Ethiopia (Alemayehu et al., 2012), and other African countries (Mbewe et al., 2015). In addition no significant difference was observed between mean PCV values of *different species of trypanosomes*. Taking into account the mean PCV value cut off at 25%, 73.3% of the infected cattle were found to be anemic. This suggests that the low mean PCV of trypanosome-infected cattle could still be used as an indicator of AAT in endemic areas. In contrast, 38.8% of all non-infected cattle were found to be anemic, which could be because of other factors like blood-sucking gastrointestinal parasites and nutritional deficiencies (Eyasu et al., 2021) or low sensitivity of the buffy coat method used (Marcotty et al., 2008). The fact that 26.6% of trypanosome-infected cattle have PCV values in the normal range (PCV > 25%) could be attributed to recent infection. However, further studies need to address the influence of tsetse and other biting flies' abundance (Biyazen et al., 2014), the frequency of trypanocidal drug usage and drug resistance (Degneh et al., 2019), and molecular identification of trypanosomes to sub-species level (Moti et al., 2015).

# 5. Conclusion

In conclusion, this study confirmed that three trypanosome species are present, and poses a threat to livestock production in the study area. The prevalence of bovine trypanosomosis in Jimma Horo district was low, and it can be controlled by treating infected cattle with a sanative pair of trypanocidal drugs, with an additional application of deltamethrin pour-on during the dry season. Furthermore, due to the low sensitivity of the buffy coat technique, the use of molecular techniques should be encouraged.

## Funding

This research was partially supported by the sub-thematic research project Trypanosomosis, located at Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Ethiopia.

#### Authors' contributions

ED drafted the proposal and the manuscript, collected, analyzed and interpreted the data. TK facilitated and supervised the data collection and edited the manuscript. NK and TW supported the analyses and interpretation of the results and edited the manuscript. All authors reviewed and approved the final manuscript.

## **Ethics** approval

Ethical values were considered, starting from the approval of the proposal. Participants' involvement in the study was on a voluntary basis; the purpose of study was explained to the owners of the animals. Humane handling of animals was followed and the sampling sites were thoroughly disinfected before and after sampling.

## **Declaration of Competing Interest**

The authors declare that they have no competing interests.

## Data availability

All of the data mentioned in this article could be provided to whoever it may concern while adhering to ethical procedures and scientific confidentiality.

## Acknowledgements

The authors would like to acknowledge Aklilu Lemma Institute of Pathobiology, Addis Ababa University, for partially supporting to conduct the study. We also thank management and technical staff of Bedele Regional Veterinary Laboratory for cooperation during sample collection. Jimma Horo district Livestock Development and Health Office is also acknowledged for their invaluable support and collaboration during the study period.

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