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## PREVALENCE OF BOVINE TRYPANOSOMOSIS IN ANFILLO DISTRICT

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### ABSTRACT

A cross-sectional study was carried out from October 2018 to June 2019 to determine the prevalence of bovine trypanosomosis in Anfillo district. The method employed during the study was buffy coat technique for parasitological study. Blood samples were collected from ear vein of 384 heads of cattle to assess trypanosome species. The overall prevalence of the disease was 15.4 %. Out of 59 infected animals 14.1 % cattle were found to be infected by *T. congolense*, (6.8 %) *T. vivax*, (1.6%) mixed parasites (*T. congolense* and *T. vivax*) and (1.3%) *T. brucie*. The study revealed that trypanosomes were widely distributed and prevalent in all body condition scores of animals and in all age and sex groups of cattle in the study area. The results of the study suggested that trypanosomosis in the area was decreasing. However, due to its impact on the livestock, an appropriate tsetse control method should be expanded to reach tsetse infested area in a sustainable manner to alleviate the problem of trypanosomosis in the area.

**Keywords:** Anfillo District, Bovine, Prevalence, Trypanosomosis

## INTRODUCTION

Livestock production constitutes one of the principal means of achieving improved living standards in many regions of the developing world. In sub-Saharan Africa countries livestock plays a crucial role both for the national economy and the livelihood of rural communities. Ethiopia takes the lead in livestock population in Africa, with an estimated 53.99 million cattle population [2]. Livestock fulfill several functions in the Ethiopian economy by providing food, traction power, cash income, fuel and organic fertilizer. Livestock is also an important provider of export commodities such as live animals, meat, hides and skins and over the past few years, livestock and its products has been Ethiopia's second most important source of export, after coffee [17]. However, poor health and productivity of animal due to disease has considerably become the major stumbling block to the potential of livestock industry [11].

Animal diseases in general and infectious ones in particular are the major constraints to crop and livestock production in the humid and sub humid parts of the African continent. Parasitic diseases especially animal trypanosomosis is the most important factor contributing to the sub potential performance of livestock; 10 million heads of cattle and equivalent numbers of small ruminants together with significant equine and camel population are at risk of contracting the disease any time. The disease is a serious often fatal disease of mainly domestic animals that occur in large areas of Africa. It is caused by species of flagellate protozoa belonging to the genus *Trypanosoma* of the family *Trypanosomatidae* that multiply and inhabit in the blood stream, lymphatic vessels and tissue including the cardiac muscle, and central nervous system (CNS) of host, and are transmitted by vectors which are generally haematophagous arthropods (Fischer and Say, 1989). Most cases of animal trypanosomosis (Nagana) are transmitted cyclically by tsetse flies of genus *Glossina* [10].

Trypanosomosis is the most serious veterinary and animal production problem in sub-Saharan Africa and prevents the keeping of ruminants and equines over 10 millions of square kilometers of potentially productive land. Hence, this study is the road map and contribution to the Pan African Tsetse and Trypanosomosis Eradication Campaign agenda [19]. Trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. Currently about 3 million livestock die every year due to tsetse fly transmitted the disease which covers one

third of the continent estimated to be 10 million km<sup>2</sup>. A recent study estimated the direct annual cost of the disease to be about 1.34 billion US\$. African livestock producers are administering an estimated 35 million curative and prophylactic treatments annually which costs the producers and the government at least 35 million US\$ Holmes *et al.* [7].

In Ethiopia, a substantial amount of the national resource is spent annually for control of trypanosomosis through purchase of trypanocidal drugs. An annual loss attributed to the disease exceeds US \$236 million, while losses from reduced milk and meat production and from animal draught power and manure are unquantifiable [4].

According to Getachew *et al.* [6], trypanosomosis is prevalent in two main regions of Ethiopia that is, the North West and the South West regions. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in domestic livestock are the tsetse transmitted species: *T. congolense*, *T. vivax* and *T. brucei*. For the closely related *T. brucei* subspecies, *T. b. rhodensiense*, which causes human sleeping sickness, cattle can be a reservoir host. The other trypanosome species of economic importance are *Trypanosoma evansi* of camels and *Trypanosoma equiperdum* of horses [5].

According to NTTICC [15], tsetse transmitted animal trypanosomosis still remains as one of the largest causes of livestock production losses in Ethiopia. About 10 to 15% of the land believed to be suitable for livestock production is affected by one or two species of the tsetse flies. While tsetse-borne trypanosomosis is excluding agriculturally suitable land of the country; 14 million head of cattle are at the risk of contracting trypanosomosis at any one time [8, 13].

A number of studies have been so far undertaken in different parts of Ethiopia to determine the magnitude of this economically important disease [12, 14]. Nevertheless, very few and limited studies were carried out to assess the prevalence of this disease in Anfillo District. Thus, the objective of this study was to determine the prevalence of bovine trypanosomosis and to identify the prevailing species of trypanosomes and to assess host related risk factors in Anfillo District, Kellem Wollega zone, Oromia Regional State of Ethiopia.

## **MATERIALS AND METHODS**

### ***Study area***

The study was carried out in Oromia National Regional State, Kellem Wellega zone, in Anfillo District which is located at 694km away from Addis Ababa towards the west part of Ethiopia. The major town in Anfillo is Mugi. It's the capital of Anfillo district Kelem Welega Zone of the Oromia region. This town has a latitude and longitude of 8°32'N 34°48'E with an elevation between 1701 and 1827 meters above sea level. Anfillo has a tropical climate and the city remains mostly hot and humid throughout the year. The winter season lasts from December to February and the average temperature during the winter months is around 25 degrees Celsius. Normally the summer months of Anfillo are very hot and the average temperature is around 25 degrees Celsius. However, the temperature may rise up to 38 degrees Celsius on a hot summer day. The area receives an average annual rainfall of 700 to 1100 mm [2].

The total human population of this area is 77156. Out of this total population of 77156 inhabitants, around 39486 were recorded as males and the remaining 37670 were females. Anfillo has a stable and reliable economy and the town is widely known for the production of tej. Anfillo is also a major producer of coffee and more than 500,000 kilos of coffee beans and many other products like wax and animal skins are exported annually. Moreover, many new mining factories and industries are being introduced in the town making it an important commercial center in Ethiopia. The district covers an area of 79,849 hectares and it is bordered by Seyo at east, Yemogi Welele at northeast, Gidami Jimma at north and Gambel Region at southwest [1].

### ***Study animals***

The study animals were cattle of both sexes and different age groups (young and adult) in and around Anfillo district which kept under extensive management system were randomly selected.

### ***Study design***

Cross-sectional study was conducted in Anfillo district, Kellem Wollega zone, Western Ethiopia in dry season from October 2018 to June 2019 to determine the prevalence of bovine trypanosomosis, to identify the prevailing species of trypanosomes and to assess host related risk factors.

### ***Sample size determination and sampling method***

The sample size was calculated according to the formula given by Thrusfield [23] with 50% expected prevalence (considering that no previous study has been done in the area), 95% confidence level and 5% precision. Simple random sampling technique was followed to select individual animals. During sampling, species, age, sex and body condition of the animals will be recorded. Body condition for each cattle will be estimated based on Nicholson and Butterworth (1986) ranging from score 1 (emaciated) to 5 (obese). Though, the required sample size was computed to be 384.

$$N = \frac{1.96^2 \text{pex} (1-\text{pex})}{D^2}, \text{ where, } N = \text{required sample size}$$

D<sup>2</sup>

pex= expected prevalence, D= precision

### ***Study methods and Procedures***

#### ***Direct methods***

a) Usual field methods

i) Blood sampling Trypanosoma species is a parasite of the blood and tissues often inhabiting the deep blood vessels in cases of low parasitaemia. For this reason, it is recommended that blood for diagnosis be obtained from both the peripheral and deep blood vessels. However, it should be realized that less than 50% of infected animals may be identified by examination of peripheral blood. Peripheral blood is obtained by puncturing a small vein in the ear or tail. Deeper samples are taken from a larger vein by syringe. Cleanse an area of the ear margin or tip of the tail with alcohol and, when dry, puncture a vein with a suitable instrument. Ensure that instruments are sterilised or disposable instruments are used between individual animals, so that infection cannot be transmitted by residual blood.

ii) Wet blood films Place a small drop of blood on to a clean glass slide and cover with a cover-slip to spread the blood as a monolayer of cells. Examine by light microscopy ( $\times 200$ ) to detect any motile trypanosomes.

iii) Stained thick smears Place a large drop of blood on the centre of a microscope slide and spread with a toothpick or the corner of another slide so that an area of approximately 1.0–1.25 cm in diameter is covered. Air-dry for 1 hour or longer, while protecting the slide from insects. Stain the unfixed smear with Giemsa's Stain (one drop of commercial Giemsa + 1 ml of phosphate buffered saline [PBS, 2.4 g Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O, 0.54 g NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O, 0.34 g NaCl], pH 7.2), for 25 minutes. After washing, examine the smears by light microscopy at high magnification ( $\times 500$ –1000) oil immersion. The advantage of the thick smear technique is that it concentrates

the drop of blood into a small area, and thus less time is required to detect the parasites. The disadvantage is that the trypanosomes may be damaged in the process, and the method is therefore not suited for species identification in case of mixed infections.

iv) Stained thin smears Place a drop of blood 20 mm from one end of a clean microscope slide and draw out a thin film in the usual way. Air-dry briefly and fix in methyl alcohol for 2 minutes and allow to dry. Stain the smears in Giemsa (one drop Giemsa + 1 ml PBS, pH 7.2) for 35 minutes. Pour off, stain and wash the slide in tap water and dry. Unfixed smears can be stained by covering them with May–Grünwald stain for 2 minutes, then adding an equal volume of PBS, pH 7.2, and leaving the slides for a further 3 minutes. Pour off and add diluted Giemsa for 25 minutes. Pour off, wash the slides with tap water, and dry. Examine at high magnification ( $\times 400$ – $1000$ ) oil immersion. This technique permits detailed morphological studies and identification of the trypanosome species. Rapid staining techniques also exist (Field's stain, Diff Quick®).

### ***Data Analysis***

Collected raw data and results of parasitological and hematological examination was entered in to a Microsoft excel spread sheets program and then was transferred to SPSS version 21 for analysis. The prevalence of trypanosome infection was calculated as the number of positive animals as examined by Giemsa stain of thin blood film and buffy coat method divided by the total number of animals examined at the particular time. Pearson's chi-square ( $\chi^2$ ) was used to evaluate the association of different variables with the prevalence of trypanosome infection. P-value less than 0.05 at 95% level of confidence interval) were considered significant in all analysis.

## **RESULTS**

Parasitological Findings; Out of the total 384 cattle examined, 59 (15.4%) cattle were found positive. The prevalence was (18.3%) in Shabel kebele, (10.6%) in Yati kebele, (17.4%) in Dale koli kebele and (14.0%) in Henache kebele which has no statistically significant difference observed between the implemented kebeles ( $P > 0.05$ ) (Table1).

There was not statistically significant difference observed between the two sex categories of animals ( $P > 0.05$ ). However, there was higher prevalence recorded in male than female animals (Table2).

There was a statistically significant variation in the prevalence of trypanosomosis ( $P < 0.05$ ) among those animals with different age groups (Table 3). There was a statistically significant variation in the prevalence of trypanosomosis ( $P < 0.05$ ) among those animals with different body condition (Table 4).

**Prevalence of species trypanosomes according to age, sex and body condition**

The proportion of trypanosome infection with species level indicate (14.1%) cattle were found to be infected by *T. congolense*, (6.8%) cattle were found to be infected by *T. vivax*, (1.6%) cattle were found to be infected by mixed (*T. vivax* & *T. congolense*) and (1.3%) cattle were found to be infected by *T. Brucei*. Accordingly, *T. vivax* was the most prevalent followed by *T. congolense* and *T. brucei*. *T. vivax* & *T. congolense* were significantly higher in adult than young, *T. brucei* higher in male than female and poor body condition animals were significantly infested by three identified species of trypanosomes than good body condition animals ( $P < 0.05$ ) (Table 5).

Table 1: Prevalence of bovine trypanosomosis in different kebeles of the study area.

Kebeles	No. of examined	No. of positive	Prevalence (%)	X <sup>2</sup>	p-value
Shabel	120	22	18.3	2.71	0.436
Yati	85	9	10.6		
Dale koli	86	15	17.4		
Henache	93	13	14.0		
<b>Total</b>	<b>384</b>	<b>59</b>	<b>15.4</b>		

Table 2: Prevalence of bovine trypanosomosis based on sex group

Sex	No. of examined	No. of positive	Prevalence (%)	X <sup>2</sup>	p-value
Male	106	17	16.0	0.05	0.821
Female	278	12	9.6		
<b>Total</b>	<b>384</b>	<b>59</b>	<b>15.4</b>		

Table 3: Prevalence of bovine Trypanosomosis based on age groups

Age	No. of examined	No. of positive	Prevalence (%)	X <sup>2</sup>	p-value
Young	118	2	1.7	24.48	0.000*
Adult	266	57	21.4		
<b>Total</b>	<b>384</b>	<b>59</b>	<b>15.4</b>		

Table 4: Prevalence of bovine Trypanosomosis based on body condition.

Body condition	No. of examined	No. of positive	Prevalence (%)	X <sup>2</sup>	p-value
Poor	79	21	26.6	11.34	0.003*
Medium	105	17	16.2		
Good	200	21	10.5		
<b>Total</b>	<b>384</b>	<b>59</b>	<b>15.4</b>		

Table5: Prevalence of bovine Trypanosomosis based on host related risk factors

Variables	Risk factors	Species of trypanosomes identified			
		<i>T.congelense</i>	<i>T.vivax</i>	Mixed( <i>T.vivax</i> & <i>T.congolense</i> )	<i>T. brucei</i>
Sex	M (n=106)	17 (16.0%)	4 (3.8%)	2 (1.9%)	4 (3.8%)
	F (n=278)	37 (13.3%)	22 (7.9%)	4 (1.4%)	1 (0.4%)
X <sup>2</sup> /p-value		0.47 (0.492)	2.1 (0.149)	0.1 (0.752)	6.96 (0.008)*
Age	Y (n=118)	10 (8.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	A (n=266)	44 (16.5%)	26 (9.8%)	6 (2.3%)	5 (1.9%)
X <sup>2</sup> /p-value		4.4 (0.036)*	12.4 (0.000)*	2.7 (0.100)	2.2 (0.134)
Body condition	P (n=79)	18 (22.8%)	11 (13.9%)	1 (1.3%)	1 (1.3%)
	M (n=105)	9 (8.6%)	9 (8.6%)	2 (1.9%)	4 (3.8%)
	G (n=200)	27 (13.5%)	6 (3.0%)	3 (1.5%)	0 (0.0%)
X <sup>2</sup> /p-value		7.6 (0.022)*	11.4 (0.003)*	0.1 (0.937)	7.8 (0.020)*
<b>Overall</b>	<b>N=384</b>	<b>54 (14.1%)</b>	<b>26 (6.8%)</b>	<b>6 (1.6%)</b>	<b>5 (1.3%)</b>



## DISCUSSION

The overall prevalence of trypanosomosis investigated in this study area was 15.4% which can be considered as less prevalence due to less vector density which resulted from fly control. The result of the current study was comparable with the reports of disease from different parts of Ethiopia which includes 17.2 % in Metekel and 17.5 % in the Upper Didessa of tsetse infested regions (18, 22).

The result was higher than the report of who observed 5.43% prevalence of the disease in Mandura District, Northwest Ethiopia, with the overall prevalence of 5.3 % in Haro Tatessa settlement area of Upper Dedessa Valley, Illubabor Zone, who reported 6.25 % prevalence of trypanosomosis in Bako Tibe district of West Shoa and Gobu Seyo districts of East Wollega Zone, 6.86% of the disease was also recorded in Lalo Kile District, Kelem Wollega Zone, Western Ethiopia [3, 9, 20, 21].

The result of current finding was also lower than 25 % prevalence recorded in Gawo Dale district and 29 % prevalence done along the escarpment of the Upper Didessa Valley [15, 16].

## CONCLUSIONS

Trypanosomosis is a very important disease that causes economic loss in the livestock industry. *T.congelense*, *T.vivax* and *T.brucie* were found to be the most predominant trypanosomes species in the districts frequently in cattle. The study revealed that trypanosomes were widely distributed and prevalent in all body condition scores of animals and in all age and sex groups of cattle in the study area. The current situation may get not worse as the prevention and control of trypanosomosis is practicing in the area and that is limiting the vector and also chemotherapy. So, the following recommendations were forwarded:

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

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