THE PREVALENCE OF DONKEY TRYPANOSOMOSIS AND ITS ASSOCIATED RISK FACTORS IN AND AROUND ASOSSA

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ABSTRACT: A cross-sectional study was conducted in Asossa Districts of Benishangul Gumuz Regional State, Western Ethiopia from September 2024 to Febuary, 2025 to determine donkey trypanosomosis prevalence, prevailing trypanosomes species, and associated risk factors. Blood samples collected from (n= 384) randomly sampled. Dark phase contrast buffy coat procedures were used for determining prevalence. Whereas, haematocrit method was used for packed cell volume (PCV) values determination. Out of total 100 samples, 20/100 (20%) were found trypanosome positive. *Based on Predominant trypanosome species among recorded were Trypanosome congolense (36.4*%), *Trypanosome vivax* (6.81%), *Trypanosome brucei* (4.54%) and mixed infection (2.27%). There were statistically significant differences concerning existing trypanosome species (P< 0.05). Mean packed cell volume (PCV) value of the parasitic animals was lower (19.04%) than a parasitic animals (25.01%) and the variation was statistically significant (P< 0.05). Sex groups, study sites, and age categories (P> 0.05) were demonstrated non-significant risk factors, however; body conditions, pcv status and trypanosome species were found significant (p<0.05). In conclusion, the current study showed high trypanosomosis prevalence in the area reflecting the need for strategic control measures.

[Asmamaw Aki, Belete Habteleu. **THE PREVALENCE OF DONKEY TRYPANOSOMOSIS AND ITS ASSOCIATED RISK FACTORS IN AND AROUND ASOSSA.** *Researcher* 2025;17(7):26-35]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). http://www.sciencepub.net/researcher. 03. doi:10.7537/marsrsj170725.03

***Key words**: Asossa town; Donkey; Trypanosomosis; prevalence; Risk factors*

INTRODUCTION

Donkeys constitute 70% of the African equine population, and are predominantly found in the arid and semi-arid areas providing a reliable, environmentally friendly and renewable source of draught power to millions of poor communities' worldwide (Fielding, D., Pearson, R. A. (Eds.) (Mesele, F., L *et al*., (2010**).** Primarily, *Trypanosoma evansi* and *T. equiperdum* are trypanosomes which have the equines (horses and donkeys) as their natural host. However, several reports have revealed that other trypanosome species infect donkeys in natural conditions and these species include: *Trypanosoma vivax, T. brucei brucei and T. congolense* (Assefa, E., Abebe, G. (2001); Mesele, F., L *et al*., (2010**).**

Trypanosomosis cause significant loss in animal production and it greatly affect people and animal settlement in considerable parts of world (Ermias Assefa and Getachew Abebe, 2005). Over 10 million square kilometer areas of Africa greatest agricultural potential are infested by tsetse fly, which is the main vector of disease (Dargant *et al*., 2001). Trypanosomiasis that occurs across more than a third of Africa is arguably the most significant disease (ILRAD, 1994). Trypanosomiasis is probable the most serious veterinary and animals production problem in subSahara Africa and prevent, or seriously crucial, the keeping of ruminant and equine (Dhollander *et al*., 2006 and Auty *et al*., 2008).

In Ethiopia, trypanosomiasis is one of the most important disease limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of south west and north west part of the country following the greater river basins of Abay, Omo, Ghibe, and Baro with a high potential for agricultural development. Currently, about 220,000 square kilometer areas of the above mentioned area infested with five species of tsetse flies including *G. pallidipes, G. morsitans submorsitans, G. fuscipes, G. tachinoides and G. longipennis*. Part of the Amhara region is tsetse infested, *G. morsitans sub morsitan*s and *G. tachinoides* are reported (Langridge, 1976). Donkeys are considered as beast of burden in many developing countries including Ethiopia that stands the second largest donkey population in the world (Alemayehu M *et al*., 2001).

Tsetse transmitted animal *Trypanosomiasis* is one of the major constraints to socio-economic development in Africa. Tsetse flies infest approximately 10 million km^2 of the continent affecting 38 countries. It is considered that 7

million km^2 of this area would otherwise be suitable for livestock and or mixed agricultural development. About 30% of the 147 million cattle in countries affected by tsetse are exposed to the disease. The situation with regard to sheep, goats, pigs, horses, donkeys and camels is probably similar but is less well documented. Data available at present indicate that the overall situation is deteriorating. Since the 1950's the areas of savanna tsetse infestation have continued to increase. As a result there is increasing pressure on tsetse-free pasturages (Getachew A., 2005).

In Ethiopia, tsetse transmitted animal *Trypanosomosis* is a serious constraint to livestock production and agricultural development, exorcising farmers and livestock keepers out of areas having very high potential for growth, and forcing them to live on a highly degraded highlands of the country (Abebe, 2005). The problem caused by tsetse and *Trypanosomosis* is not only limited to inflicting diseases but also leading to significant negative impacts such as losses due to mortality and morbidity in domestic animals, cost of livestock treatment and tsetse control, and getting rid of draught animals from their infestation areas (Juyal *et al*., 2005).

Trypanosomiasis is a devastating disease of livestock caused by protozoal parasites of the genus T*rypanosoma* that inhabits blood and other tissues of vertebrates including animals, wildlife and human (Adam *et al.*, 2003; Gupta *et al*., 2009; Bal *et al*., 2014). It is a vector borne disease that is transmitted biologically by tsetse flies and mechanically by other biting flies (FAO, 2002; OIE, 2009). It is a major constraint contributing to direct and indirect economic losses to crop and livestock production (Abebe, 2005) and has a significant negative impact on economic growth in many parts of the world (Taylor *et al*., 2007; Sharma *et al*., 2013), particularly in sub-Saharan Africa (Cecchi *et al*., 2008). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax*, and *Trypanosoma brucei* in cattle, sheep and goats, *Trypanosoma evansi* in camels and *Trypanosoma equiperdium* in horses (Abebe G, 2005).
The Diagnosis of trypanosome infection is based on clinical signs; but the clinical signs of the African Animal trypanosomosis are indicative but are not sufficiently pathognomonic. Therefore, standard methods have been developed and applied practically to diagnose the disease in animals. The methods include: direct microscopic examination of blood, either by the wet film method; but it is insensitive (Getachew, 2005). Stained thin and thick smear techniques permit detailed morphological studies and identification of different *Trypanosoma* species by light microscopy. Sensitivity can be improved through parasitological buffy coat techniques of concentration of the parasites by centrifugation and blood inoculating into susceptible laboratory animals (Getachew, 2005).
For equine trypanosomiasis, little information and few studies were available in Ethiopia, and those scarce literature sources suggest that trypanosomiasis is one of the most important disease limiting animal productivity and agricultural development due to its high prevalence in the most arable and fertile land of South west and North West part of the country. The high disease challenges are in the greater river basins of Abay, Omo, Ghibe and Baro with a high potential for agricultural development (Hailegebrael B and Shimelis D.,2012). About 10.7% of trypanosome prevalence in equines has previously been detected in Humbo, Kindo Koysha and Sodo zuria districts in Southern areas of Ethiopia. Two Trypanosome species were identified from equines: *T. congolense* 52.2% and *T. vivax* 26.1% (Addisu E. *et al*.,2011). Besides this, *Assefa T et al.,(2015) in Asossa district reported 6% of donkey Trypanosome prevalence, and Trypanosoma congolense* (56.52%), *T. brucei* (30.43%) and *T. vivax* (13%).
Benishangul Gumuz is one of the five regions of Ethiopia infested with more than one species of tsetse flies (Keno, 2005). Five species of Glossina (*G. morsitans submorsitans, G. Pallidipes, G. tachnoides, G. f. fuscipes and G. longipennis*) have been registered in the region (Keno, 2005; ARAHDL, 2016/17). The tsetse flies (vectors), *G. fusca*; the bush fly, *G. morsitans*, which inhibit principally savannah area and *G. palpalis*; a riverine species, effectively prevent the rearing of the cattle over the large area of the region (NTTICC, 2004). And nearly 31,000 km2 or 62% of the Benishangul Gumuz region's total land area is believed to be infested with tsetse fly (NTTICC, 2004).

In Asossa district, Trypanosomosis in donkeys has not been extensively studied despite that these animals play an important role in the socio-economic life of the rural farming population (Blench, R., de Jode, A., Gherzi, E. (2004) and are susceptible to this endemic disease affecting livestock (Mesele, F., Leta, S. (2010**)** **a**nd also Assefa T *et al*.,(2015) in Asossa district reported 6% of Donkey Trypanosome prevalence. Besides this, the available reports are from the western , southern and other part of the country and hence scant information were available in the region about the research with regard to donkey trypanosomosis. Therefore, the objective of the present study was focusing on the prevalence of donkey trypanosomosis, to identify the Trypanosome species and associated risk factors of donkey trypanosomosis in the Asossa District.

Materials and Methods

2.1 Study area

The study was conducted in Asossa town, BenishangulGumuz Regional State, west Ethiopia. According to CSA (2007), before a year, Assosa town (the capital city/town of BenishangulGumuz Regional State) had four kebeles but currently, the town has changed its administrative structure to two districts (district-1 and district-2). Each districthas five "ketenas". According to BGRSMSC (2020), the town is located at 10º 00'and 10º 03' north latitude and 34º 35' and 34º 39' east longitude. The total population of the town is 62,632 of which 32, 100 are male and 30,532 are female (projection made for the CSA, 2020). The total area of the town is 2361.34 hectares with an altitudinal difference that ranges between 1461- 1641 meters above sea level (BGRSEIB, 2020). The mean annual temperature of Assosa town ranges from a minimum of 14-33^{0}C. However, there is a slight variation of temperature by month. February to May is the hottest months while November to December is the cold months). The total amount of rain fall recorded at Assosa town during the last nine months of (2020) is 1,119 mm (BGRSMSC, 2020). The rainy season starts in March and extends to November with the highest concentration in June, July, and August. The population size of different livestock species in Assosa town are cattle 569, goat 1545, sheep 739, poultry 17676 donkeys 122, and pig 8 total 20,659 livestock populations are found in the town (ATAOA, 2020).

Figure 1: Administrative map of Assosa town (ATAO, 2021)

2.2 Study Design

A cross- sectional study design were conducted from september 2024 to Febuarary, 2025 to determine the prevalence of trypanosome infections and associated risk factors in Donkeys in and around Asossa town based on parasitological and hematological examination.

2.3 Study Animals

The study animals were indigenous breeds of donkeys kept under extensive husbandry which allows free grazing usually mixed with other livestock in the villages. Donkeys are the main types pack animals in the area used to transport peoples, water house hold materials crops and firewoods from one place to another. The body condition scoring of each of the study animals will be scored as good, medium and poor Nicholson MJ, and Butterworth MH (1986). Concurrently, their age of selected animals was determined by dentition (< 2, 2-5, >5) based on De-Lahunta A, and Habel RE (1986) and Crane, M., (1997).

2.4 Sampling Techniques and Sample Size Determination

Indigenous donkey breeds were selected using simple random sampling methods. Kebeles were be selected purposive as convenient based on Asossa woreda agriculture office data. For sample size determination, previously, Assefa T *et al*.,(2015) in Asossa district reported 6% of Donkey Trypanosome prevalence in the study area. So that, the district expected donkey trypanosome prevalence was taken as 6% expected prevalence and it was entered in to Thrusfield (2007) formula with 5% absolute precision at 95% confidence interval. The formula will be given by

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

Where n = sample size required; P_{exp} =expected prevalence; d = desired absolute precision

So, total sample size was 384 cattle was randomly sampled.

$n = \frac{(1.96^2 * 0.06)*(1-0.06)}{0.0025}$ = 90 donkey sample was collected.

However the sample size was increased to 100 inorder to increase the precision.

2.5 Study methods

2.5.1 Packed cell volume (PCV) determination and Buffy coat technique

90 Blood samples were obtained by puncturing the marginal ear vein with a lancet and collected randomly from cattle of the settlement into capillary tube after piercing the marginal ear vein by using a lancet. One end of the capillary tube was sealed and centrifuged at 12,000rpm for 5 minutes to separate the blood cells and to concentrate trypanosomes using centrifugal forces as buffy coat. Then PCV was measured using haematocrit reader (Hermmle Labortechnik, type Z, Germany).

The heparinized microhaematocrit capillary tubes containing blood samples was 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 and expressed on microscopic slide, mixed and covered with 22x 22mm cover slip.The content of the capillary tube pour onto a glass slide, and covered with cover slip. Then the slide was examined under 40x objective of a microscope using dark ground buffy coat technique to detect the presence of the parasite (Murray *et al*., 1977; Paris, 1982). Buffy coat positive samples was stained by Giemsa's in thin blood smears, fixed with methanol for 5 minute and examined under oil immersion using 100x objectives to identify the species of trypanosomes. The length of the packed red blood cells column is expressed as a percentage of the total volume of blood. Trypanosome species was identified according to their morphological descriptions as well as movement in wet film preparations (OIE, 2008) and Animals with PCV less than 24% were considered to be anemic (OIE, 2008).

2.5 Parasitological Diagnostic techniques

Diagnosis of Trypanosomosis in tsetse, humans or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing chemotherapy and for monitoring vector control operations. Accurate diagnosis of trypanosome infection in livestock is required for a proper appreciation of the epidemiology of the disease in any geographical locality. Besides clinical diagnosis, parasitological, serological and molecular methods with varying degrees of sensitivity and specificity are available for the diagnosis of trypanosomosis (Dagnechew S., 2004).

Thick blood smears

- Place drop of blood on slide
- Spread it to a size of about 2 cm in diameter
- Air dry quickly
- Immerse the smear in distilled water 5-10 min
- Fix with methanol alcohol of 75 % for three minutes
- Stain with Giemsa diluted in distilled water 1:10 for 30 min
- Examine under the microscope

Thin blood smear

- Put drop of blood on slide
- Spread the blood on the slide using a cover slip or another slide at an angle of 45 degree
- Dry with air
- Fix with methanol for three minutes
- Stain with Giemsa

- Wash with phosphate buffer at PH 6.8-7.2
- Allow to dry
- Examine under the microscope 1x100 magnification

Dark ground /phase contrast BCT *(Murray method)*

- Collect blood in heparinisd capillary tube
- Seal one end with crystaseal
- Place in microhaematocrit centrifuge
- Allow to spin 12,000 rpm for 5 min
- Cut the capillary tube with diamond pen 1 mm below the buffy coat to include RBC
- Extrude on slide & cover with cover slip
- Examine in dark ground phase-contrast microscope with x40 objective

1. Data Analysis

The collected parasitological and hematological data was entered into a Microsoft excel spread sheets program and then will be transferred to Intercool STATA version 10.0 for analysis. The Prevalence of Trypanosome infection was calculated as the number of positive animals as examined by buffy coat method divided by the total number of animals examined at the particular time. Data collected on PCV values was analyzed by ANOVA to compare the mean PCV values of infected animals against that of uninfected animals. Pearson's chi-square ($\chi 2$) will be used to evaluate the association of different variables with the prevalence of trypanosome infection. In all of the statistical analysis, a confidence level of 95% is used and P-value of less than 0.05 (at 5% level of significance) will be considered as statistically significant.

2. Ethical considerations

This protocol was presented for initial and continuing review and approval by JUCAVM institutional ethical review board. Any changes to the protocol or consent form were approved by all ethics committees. Since the study involves domestic animals subject, the ethical approval for the study were obtained from Ethical Research Board (ERB) of the JUCAVM for domestic animals.

3. RESULT

5.1 Parasitological result

Out of the total animals examined (n=100), 20/100(20%) were found to be infected with trypanosomes (Table-1). The prevalence in terms of trypanosome species was 36.4% for *T. congolense*, 6.81 % for *T. vivax, 4.54% for T.brucei and 2.27 %* was found to be mixed infection and the infection rate was found to be statistically significant (P<0.000) among trypanosome species (Table 1).

Table 1. Species based prevalence of bovine trypanasomosis in Assosa district

Trypanosomes	No. positive	Positive (%)	X^2 (p-value)
T. congolense	16	36.4	70.32(P=0.000)
T. vivax	3	6.81	
T. brucei	2	4.54	
Mixed	1	2.27	
Total	44	100	

X^{2}-- chisquare

5.2 Hematological result

The mean PCV value for all examined animals was 22.03. However, the mean PCV value for non infected and infected animals was 25.01 and 19.04 respectively. The mean PCV values of equine were significantly ($p < 0.000$) influenced by trypanosome infection as 19.04 % and 25.01 % PCV values in trypanosome positive and negative animals were registered, respectively (Table 2).

Table 2: Mean PCV comparison of parasitaemic and aparasitaemic animals

Status	Frequency	Mean PCV (%)	SE	X^2(p-value)
Infected	48	19.04	07.13	22.12(p=0.000)
Non-infected	52	25.01	01.9	
Total	100	22.03	05.0	

PCV: packed cell volume**;** SE: standard Error

5.3 Trypanosomosis associated Risk factors

The highest prevalence (22.09%) of trypanosomosis was recorded in animals >5 years old whilst the lowest prevalence (0 %) was recorded in animals less than 2 years of old and the association was not found statistically significant among the age groups (Table 3). Slightly, higher prevalence was registered in male animals (20.07 %) than in female animals (15.22 %), which was not found to be statistically significant ($p > 0.05$) (Table 6). The highest prevalence of trypanosomosis (35.71%) was found in animals with poor body condition while the lowest (11.11%) and (25%) body conditions was recorded in animals with good and medium body conditions respectively, and the difference was significant ($p<0.02$). The effect of age, sex, and body condition on prevalence of trypanosomosis is summarized in Table 3.

Table 3. The identified sex, age and body conditions associated risk factors

Risk factors	No. examined	No. positive	(%) positive	χ^2 (p-value)
Sex				1.22(P>0.40)
Male	54	13	24.07	
Female	46	7	15.22	
Total	100	20	20	
Age(years)				1.71(P>0.42)
< 2	1	0	0	
2 – 5	13	1	7.69	
> 5	86	19	22.09	
Total	100	20	20	
Body conditions				5.25(P<0.02)
Good	45	5	11.11	
Medium	40	10	25	
Poor	14	5	35.71	
Total	100	20	**20**	

4. DISCUSSION

20 % of donkey Trypanosomosis prevalence were reported in the study area. This finding was high as compared to the previous finding of Assefa T *et al*.,(2015) in Asossa district who reported 6% of Donkey Trypanosome prevalence in the study area. And also This finding was inconsistent with the study conducted by (Aki A *et al*., 2015) who reported 8.96% bovine trypanosomosis prevalence in Kameshi District, Benishagul Gumuz region, western Ethiopia.

However, the present findings were agreed with the study conducted 22.38 % bovine trypanosomosis prevalence was reported by Bayisa *et al*. (2015) in Asossa district, which was high as compared to the current study. Similarly, 26.30% cattle trypanosomosis prevalence was reported by Aki A *et al*. (2017) in Mandura district which were higher than the present findings.

This research showed that the infection was predominantly caused by *T. congolense (36.4*%), *T.vivax* (6.81%), and *T. brucei* (4.54%) and mixed infection (2.27%). This result is in line with the reported proportions of *T.congolense* (75.5 %) followed by *T.vivax* (14.28%) from Metekel and Awi zones (Mekuria *et al.,* 2011). This result was also in consistent with prior reports of (Mulaw *et al.*,2011) who studied on prevalence of major trypanosomes affecting cattle in Assosa district of Benishangul Gumuz Regional State, western Ethiopia and who found proportional prevalence of *T. congolense to be* 66.7% ; (Abraham *et al*., 2012) conducted their study on prevalence of bovine trypanosomosis in selected sites of Arba Minch district, Southern Ethiopia whose result showed proportional prevalence of *T. congolense* to be 61.4%; (Biyazen *et al*., 2014) reported proportional prevalence of *T. congolense to be* 63.64% during their work on trypanosomosis and anemia in cattle population of Dale Wabera district of Kellem Wollega Zone, western Ethiopia.

The high proportional infection rate of *T. congolense* in cattle might be attributable to the high number of serodems of *T. congolense* relative to other species of trypanosomes. It could also be due to the possible development of better immune response to *T. vivax* by the infected animals as demonstrated by (Leak *et al*., 1993). Further, it might be attributed to the efficient transmission of *T. congolense* by cyclical vectors than *T. vivax* in tsetse-infested areas. Previous reports indicated that *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse infested and tsetse free areas of Ethiopia respectively (Leak *et al*., 1999). Different studies (Leak *et al*., 1993; Rowland *et al*., 1995) have indicated that *T. vivax* is highly susceptible to treatment while the problems of drug resistance are higher in *T. congolense.*

The effect of different risk factors such as sex, age categories, study sites, PCV status, trypanosome species, and body conditions on prevalence of donkey trypanosomosis was studied and, statistically significant associations were observed in, PCV status, body conditions and trypanosomes species ($p<0.05$) while sex groups, age categories and study sites were not found to be statistically significant ($P>0.05$). This result is in agreement with previous reports of (Lelisa *et al*, 2015 and Bayisa *et al*, 2015). The overall mean PCV value for examined animals was 22.03. The mean PCV value of infected animals was significantly lower (19.04) than that of non - infected animals (25.01). This result is in alignment with previous works of (Ali *et al*., 2011; Mulaw, 2011).

7. CONCLUSION AND RECOMMENDATIONS

The high prevalence of donkey trypanosmosis (20 %) were remains a major problem that hinders livestock production and productivity in the district. The most widely distributed and dominant species of trypanosomes in the study sites are *T. congolense (36.4%) followed by T.vivax (6.81%), and* to some extent *T. brucei (4.54 %)* which was mainly transmitted by *G. morsitans sub morsitans* and other biting flies. Parameters of study animals such as sex, study sites, and age were not found to be a risk factor for trypanosomosis whereas body conditions and trypanosome species were potential risk factors in this findings. This study showed that trypanosome infection and other factors such as (nutritional, seasonal, concurrent disease) was found to negatively affect the PCV values of animals.

Based on the current findings, the following recommendations are forwarded:-

- Particular attentions towards the identified trypanosome species are essential to control the impact of the disease on donkey that are potential reservoir of the infections.
- Development of control options that could minimize the tsetse fly and biting flies in the study area should be introduced in a wholistic approach.
- Proper and strict follow up of trypanocidal drug distribution, therapeutic strategies and alternative control measures should be implemented by concerned stake holders.
- Care should be taken on identified risk factors
- The farmer in the area should be trained how to control the vector of the disease and provided with materials
- Further study on the trypanosomosis, tsetse fly investigation and on possible factors should be carried out by laboratory experts to give the best strategic control and prevention measures in the study area.

8.REFERENCE

1. Abebe G., (2005): Current situation of Trypanosomosis. In: review article on: Trypanosomosis in Ethiopia. Ethiop. *J Biol.med. Sci* **4(1)**: 75-121
2. Adam KMG, Paul J, Zaman V (2003): Medical and Veterinary Protozoology. Churchill living stone Edinburgh and London.
3. Abraham ZA, and Zeryehun T (2012): Prevalence of Bovine Trypanosomosis in Selected District of Arba Minch, Snnpr, Southern Ethiopia, *Global Veterinaria* **8**(2): 168-173.
4. Bal MS, Sharma A, Ashuma Bath BK, Kaur P and Singla LD (2014): Detection and management of latent infection of Trypanosoma evansi in a cattle herd. Ind. *J. Anim. Res.* **48**(1): 31-37.
5. Blench, R., de Jode, A., Gherzi, E. (2004). Donkeys in Nigeria: history, distribution and productivity. Donkeys, people and development: a resource book of Animal Traction Network for Eastern and Southern Africa (ATNESA), ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) (pp. 210-219), Wageningen, The Netherlands.
6. Cecchi G, Mattioli RC, Slingenbergh J, de la Rocque S (2008): Land cover and tsetse fly distributions in sub-Saharan Africa. *Med. Vet. Entom.* 22: 364-373.
7. Cherenet T, Sani RA, Panandam JM, Nadzr S, Speybroeck N, Van Den Bossche P (2004): Seasonal prevalence of bovine trypanosomiasis in a tsetse-infested zone and a tsetse-free zone of the Amhara region, north-west Ethiopia. Onderstepoort *J. Vet. Res.* **71**(4): 307-312.
8. Crane, M., (1997): Medical. In: The professional hand book of donkey, 3 ed., London: Whittet BooksLimited, 18 Anley Road, W14 OBY, pp: 19-36.
9. Dagnachew, S., (2004): Epidemiology of bovine trypanosomosis in the Abay basin areas of northwest Ethiopia, MSc thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit.
10. Elnasri, H., (2005). Prevalence and Ranking of Bovine trypanosomiasis in Unity State, Sudan, MSc thesis University of Khartoum, *Faculty of Veterinary Medicine, Unity State, Sudanpp1*-76.
11. Bekele, J., K. Asmare, G. Abebe, G. Ayelet and G. Esayas, (2010): Evaluation of Deltamethrin applications in the control of tsetse and trypanosomosis in the southern rift valley areas of Ethiopia. *Vete Parasitol.,* **168**: 177-184.
12. Ezebuiro, O. G. C., Yohanna, J. A., Abdulya, A., Osue, H. O., Abenga, J. N., Yakasai, M. A., Attahir, A. (2012). Haematological changes following acute Trypanosoma brucei brucei infection in rabbits. Curr Res J Biol Sci. 4(4):414-416.
13. Getechew, A., (2005): Review Article trypanasomasis in Ethiopia. Ethiopia. *J. Biol. Sci.*, **27**(1): 1-8.
14. Getachew A. (2005): Trypanosomosis in Ethiopia, A.A.U, *Faculty of Veterinary Medicine.* Debre zeit, pp 18-20.
15. Gupta MP, Kumar H and Singla LD (2009): Trypanosomiasis concurrent to tuberculosis in black bucks. *Ind. Veter. J.* **86**: 727-728.
16. Keno M. (2005): The Current Situation of Tsetse and Trypanosomosis in Ethiopia, Ministry of Agriculture and Rural Development, Veterinary service Department, in proceeding of 28th meeting of International Scientific Council for *Trypanosomosis Research and Control.*
17. Mulaw S, Addis M, and Fromsa A. (2011): Study on the Prevalence of Major Trypanosomes Affecting Bovine in Tsetse Infested Asossa District of Benishangul Gumuz Regional State, Western Ethiopia. *Global Veterinaria 7 (4): 330- 336.*
18. Mekuria S, and Gadissa F (2011): Survey on bovine trypanosomosis and its vector in Metekel and Awi zones of North west Ethiopia. *Acta Tropica*, **117**: 146-151.
19. Stein, J., (2011): Trypanotolerance and Phenotypic Characteristics of Four Ethiopian Cattle Breeds. Doctoral Thesis, Swedish University of Agricultural Sciences, F*aculty of. Vet. Med. Anim. Sci., UppsalaSweden*. pp1-63.
20. NTTICC, (2004): National tsetse and TrypanomososisInvestigation and Control Center report for the period7 July 2001 – 6 July 2002 *Badelle, Ethiopia*, pp: 3.
21. Loses, G. and B. Ikede, (2002): Review on pathology of diseases in domestic and laboratory animals caused by Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense and T. gambiense. *Vet Pathol,* 9: 1-71.
22. Moore, N. and J. Messina, (2010): A Landscape and Climate Data Logistic Model of Tsetse Distribution in Kenya. PLoS ONE **5**(7): e11809. Doi: 10.1371 Ethiopia, pp: 151-157.
23. Swallow, B.M., (2000):Impact of Trypanosomiasis on African Agriculture. Vol. 2, PAAT Technical and Scientific Series, FAO. Rome,.*Int. J. Anim. Veter. Adv.*, **2**(2): 47-50, 201.

24. Omotainse, S.O., J.O. Kalejaiye, P. Dede and A.J. Dada, (2004): The current status of tsetse and animal trypanosomiasis in Nigeria. *Vom. J. Vet. Sci*., 1: 1-9.
25. Shaw, A.P., G. Cecchi, G.R. Wint, R.C. Mattioli and T.P. Robinson, (2014): Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomosis in Eastern Africa. *Prev. Vet. Med.*, 113: 197-210
26. NTTICC (National Tsetse and Trypanosomosis Investigation and Control Centre), (1996): *Annual Report on Tsetse and Trypanosomosis, Survey, Bedele, Ethiopia*. Pp.11-15.
27. NTTICC (2004): National Tsetse and Trypanosomosis Investigation and Control Center. Report for the period 7th June 2003-6th July 2004. Bedelle, p. 3.
28. OIE (2009): Manual of standards for diagnostic tests and vaccines for terrestrial animals, 6th ed. Paris. pp: 813-2008
29. OIE (Office of Internationale des Epizootics), (2008): "Standardized Techniques for the Diagnosis of Tsetse transmitted Trypanosomosis," in *OIE Terrestrial Manual*, p. 49, Rome, Italy.
30. OIE (2009): Manual of Diagnostic Tests and Vaccines for Terrestrial Animal health code http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/ pp 1-5.
31. FAO (1998): A field guide for the diagnosis, treatment and prevention of African Trypanosomes: In Food and Agriculture organization of the United Nations Rome FAO. http://www. Fao.org/ DOCREP/ 006/ X0413/ X0413EOO. HTM. Pp.170.
32. Jemere BH. (2004): Control of Tsetse and Trypanosomiasis in the southern Rift valley (step area): Evaluation of Deltamethrin Applications, Msc thesis , faculty of vet. Medicine AAU., Debrezeit, Ethiopia.
33. IAEA (2002): Campaign launched to eliminate Tsetse fly; which has turned much of Africa in to a Green Desert Press Reports on PATTEC.
34. Thrusfield M. (2005): Veterinary Epidemiology, 3rd edition, Blackwell Science Ltd, Oxford, UK. Pp.233-250.
35. Kassa B. (2005): Standard Veterinary Laboratory, Diagnostic manual, and volume 3 Parasitology. Minister of Agriculture and Rrural Development, Animal Health Department. Addis Ababa, Ethiopia. Pp 32-37.
36. Singla LD, Aulakh GS, Juyal PD, Singh J (2004): Bovine trypanosomosis in Punjab, India. Proceeding of The 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress, 23-27 August 2004, Pet aling Jaya, Malaysia, pp: 283-285. 4.
37. Jemere BH (2004): Control of Tsetse and Trypanosomiasis in the southern Rift valley(step area): Evaluation of Deltamethrin Applications, Msc thesis , faculty of vet. Medicine AAU, Debrezeit, Ethiopia.
38. Juyal PD, Singla LD, Kaur P (2005): Management of surra due to Trypanosoma evansi in India: an overview. Infectious diseases of domestic animals and zoonosis in India. **75**:109-120.
39. Radostits OM, Gay CC,Blood DC, and Hinchelift KW (2000): Disease caused by protozoa – *Trypanosomes*. Veterinary Medicine: *A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses.* 9th ed. *Harcourt Publisher Ltd. London*. Pp 1531-1541.
40. OAU (Organization of African Union) (2001): Trypanosomosis, Tsetse and Africa. The year book report (2001).
41. Sharma A, Singla LD, Ashuma, Batth BK, Kaur P, Javed M, Juyal PD (2013): Molecular prevalence of Babesia bigemina and Trypanosoma evansi in dairy animals from Punjab, India by duplex PCR: A step forward to detection and management of concurrent latent infections. Biomed. Res. Int. Article ID 893862, 8 pp.
42. Taylor MA, RL coop and RL wall (2007): Veterinary Parasitology 3rd ed. Block Well publishing Ltd, oxford. Uk, Pp 96-102, 212-214.
43. Vincendeau, P. and B. Bouteille, (2006): Immunology and Immunopathology of African Trypanosomiasis. Ann. Braz. Acad. Sci., **78**: 645-665
44. Hailegebrael B and Shimelis D. "Study on the prevalence of donkey trypanosomosis in Awi zone northwest Ethiopia". Ethiopian Veterinary Journal 16.2 (2012): 65-76.
45. Addisu E., et al. "A cross-sectional study of equine trypanosomosis and its vectors in Wolaita zone, Southern Ethiopia". Journal of Veterinary Medicine and Animal Health 3.2 (2011): 21-26.
46. Birhanu H., et al. "Epidemiology of Trypanosoma evansi and Trypanosoma vivax in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia". Parasites and Vectors 8 (2015): 212
47. Thrusfield M. Veterinary Epidemiology, 3rd edition. Singapore, black well science (2005): 233.
48. Rahmeto A and Amanuel W. "Preliminary survey on equine trypanosomiasis and its vectors in Asossa and Homosha districts in Benishangul Gumuz regional state, northwest Ethiopia". Livestock Research for Rural Development 22.1 (2010).

49. Fielding, D., Pearson, R. A. (Eds.) (1991). Donkeys, Mules and Horses in Tropical Agricultural Development. Proceedings of a Colloquium, September, 3–6, ISBN 0-907146066, Edinburgh University, Scotland.
50. Assefa, E., Abebe, G. (2001). Drug resistance of Trypanosoma congolense in naturally infected donkeys in North Omo zone Southern Ethiopia. Vet Para. 99: 261–271. https://doi.org/10.1016/S0304-4017(01)00489-7
51. Mesele, F., LS *et al*, (2010). Prevalence rate of tsetse transmitted donkey trypanosomosis in Dale Wabera District, Western Ethiopia. Global Vet. 5(3):180- 183.
52. Jatau, I. D., Lawal, A. I., Agbede, R. I. S., Abdurrahman, E. M. (2012). Response of albino rats to experimental infection with primary and relapsed isolates of Trypanosoma evansi. Sahel Vet Sci. 11(1):49-57.

6/3/3035