

Cross Sectional Study of Bovine Trypanosomosis and Apparent Density of Tsetse Flies in Hurumu District, West Ethiopia

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Abstract: Across-sectional study was conducted from Nov, 2018 to May, 2019 to assess the prevalence of bovine trypanosomosis and apparent density of tsetse flies in three peasant associations of Hurumu district, Ilubabor zone, Western Ethiopia. The overall 6.49% prevalence of bovine trypanosomosis was recorded from 462 blood sample collected from selected animals using buffy coat method. *Trypanosoma congolense* was the dominant species 25(83.33%), while the low infection was *Trypanosoma vivax* 5(16.67%). The highest prevalence 13(9.03%) of the disease was recorded in Gaba peasant association while the lowest 2(1.63%) was recorded in Haro peasant association. The mean packed cell volume (PCV) of parasitemic animals was significantly lower (21.4%) than aparasitemic animals (28.69%) ($P < 0.05$). The Overall apparent density of the flies was 1.342 f/t/d by using Monopyramidal traps. During the study period the *Glossina fuscipes fuscipes*, *G. pallidipes* and *G. morsitans submorsitans* were tsetse flies species encountered in the study area. Generally, the present study came up with low prevalence of bovine trypanosomosis in which the potential impact of this disease on production and productivity of cattle shall not be undermined. Therefore, sustainable community based tsetse and trypanosomosis control program should be implemented.

Key words: Bovine • Trypanosomosis • Buffy Coat • Hurumu and Tsetse Flies

INTRODUCTION

Ethiopia has enormous livestock resource with a total contribution of 15% gross domestic product (GDP) and 33% to agriculture output. Currently estimate of Livestock population shows that there are 41.5 million heads of cattle, 41 millions of sheep and goat, 5.8 millions equine, 1 million camels and over 52 million poultry [1]. Despite the large animal population, productivity in Ethiopia is low and even below the average for most countries in eastern and Sub-Saharan Africa countries, due to poor nutrition, reproduction insufficiency, management constraints and prevailing animal diseases [2].

Trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. Trypanosomosis is a complex disease caused by unicellular parasite (Genus: *Trypanosoma*) found in the blood and other tissue of vertebrate including cattle

(Livestock), wildlife and people [3]. The most important trypanosoma species in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* in cattle, sheep and goat; *Trypanosoma evansi* in camels and *Trypanosoma equiperdium* in horses. Tsetse transmitted animal trypanosomosis still remain as one of the largest cause of livestock production losses in Ethiopia.

Tsetse flies in Ethiopia are confined to South Western and North West region between a longitude 33° and 38°E and latitude of 5° and 12° N [4]. Five species of *Glossina* (*G. msubmorsitance*, *G. pallidipes*, *G. tachinodies*, *G. fuscipes fuscipes* and *G. longipennies*) has been recorded in Ethiopia [5]. All species of *Glossina* transmits trypanosomes in various mammals and also biting flies may act as mechanical vectors, but their significant in Africa is still undefined [6]. Hurumu district is potentially a productive place for agricultural activity and raise livestock.

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Unfortunately the area is infested by medium to high tsetse transmitted trypanosomosis. Therefore, the objective of the study was

- To determine the prevalence of bovine Trypanosomosis
- To identify vector species and their apparent density
- To assess the risk factors associated with the disease

MATERIALS AND METHODS

Study Area: The study area is located in Oromia regional state, Ilubabor zone and lies at 035404 to 035451 E longitudes and 08202 to 08232 latitude and north of equator. Altitude of the area ranges from 1300 to 1820m.a.s.l. The climatology alternates with long summer rain fall (June- Sep), short rainy seasons (March-April) and winter dry seasons (December-February). The district has 28°C maximum temperature and 20°C minimum temperature and 1000mm to 1650mm Rain fall. In the study area the livestock population of bovine is 65000, equine 11200, Caprine 3000 and ovine 7000. The district has a total area of 3000 hector and 10% Dega and 55% Woyena Dega and 35% Kola agro-ecology. The study was conducted in 3 peasant associations (PAs), namely Gaba, Haro and Wangegne. There are river basins which flow throughout the year from the district to Birbir River system, namely Gebba and Sakki and also other seasonal Rivers are also found. The different vegetation type which are found in the district, include *Combratum* Spp, *Pillistigamathonningi*, *Acacia* Spp and *Ficassycomors*. Wild games like buffalos, Bush pig, Kudu, warthog, hippo and crocodiles are the most commonly found in the study area. Agriculture is the main stay of livelihood of people with a mixed farming system and livestock plays an integral role for agriculture [7].

Study Animals: Study animals were zebu cattle kept under extensive traditional husbandry condition. The animals graze the communally owned pasture land throughout the year. They are managed under the same agro-ecology without any additional supplementary feedings. The study was conducted on 462 local breed cattle selected from three peasant associations in the district. Of these animals, 144, 123 and 195 were from Gaba, Haro and Wangegne, respectively. The origin, sex, age and body condition score of the animals were explanatory variables used to associate with prevalence rate.

Study Design: Cross-sectional study was conducted to determine the prevalence of bovine trypanosomosis and apparent density of vectors (tsetse population and other biting flies).

Sample Size Determination: The sampling method applied was simple random sampling. The sample size was calculated at 50% prevalence with the expected precision at 5% and at 95% confidence interval.

The required sample size was 384 animals; however a total of 462 animals were sampled to increase the precision [8].

$$N = a^2_{exp} (1 - p_{exp}) d^2 \\ = (1.96)^2 (0.5) (1-0.5) (0.05)^2$$

where

n = the required sample size

P = the expected prevalence

d = desired absolute precision

a = constant at 95% confidence level

Study Methodology

Entomological Survey: For the entomological study, tsetse flies and other flies were collected from selected sites of the study area. The altitude levels, Peasant Associations, numbers of traps, tsetse species caught, other biting flies, days and vegetation types were recorded during the sampling period. The flies were caught with Monopryamidal baited with acetone, octenol and cow urine. In the selected sites of the study area, about 60 baited traps were deployed at 200-250 meters interval at side of riverine and woody grass land and kept in position for 48 hours. During trapping, acetone and octenol was dispensed from open vials through an approximately, 'O'- sized hole while cow urine from open bottles into which a quarter of tissue paper was used. All odors were placed on the ground about 30cm upwind of the trap. The underneath of each pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The coordinates of each trap position were recorded with a Global Positioning System (GPS). The different fly catches in each trap were counted and identified; the species of tsetse flies and other biting flies were identified based on their morphological characteristics such as size, color and wing venation structure [9].

Determination of Packed Volume: The capillary tubes were placed in micro haematocrit centrifuge with sealed end outer most. The tube was loaded symmetrically to ensuring good balance after screwing the rotators cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12, 000 revolutions per minute for 5 minutes. Tubes were then placed in a haematocrit and readings were expressed as a percentage of red blood cells to the total volume of whole blood. Animals with PCV<24% were considered to be anemic [10].

Buffy Coat Technique: A small blood was collected from an ear vein using heparinized microhaematocrit capillary tube. A haematocrit tube with a whole blood sample and end was sealed with haematocrit clay. The tube was centrifuged at 12000 revolutions per minute for five minutes. After centrifugation trypanosome were usually found in or just above the buffy coat layer. The capillary tube 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 1 mm above to include the plasma. The content of capillary tube was expressed on to side, homogenized on to clean side and covered with cover slip. The slide was under x40 objective x10eye piece for the movement of the parasites [11, 12].

Data Management and Analysis: Data collected from vector fly and trypanosome infection survey was analyzed by using statistical software program (SPSS 20.0). Data collected on PCV values was analyzed by independent Sample t-test to compare the mean PCV

values of parasitaemic and a parasitaemic animal. In all cases differences between parameters were tested for significance at probability levels of 0.05. The risk factors like sex, age and body condition score were compared by using chi-square test. Prevalence (counting positive per total number of cattle examined) for trypanosomosis data and apparent density of tsetse flies were used to analyzed by dividing the number of the tsetse flies caught by number of traps deployed and number of days deployment and it also, expressed as Fly/Trap/Day.

RESULTS

Entomological Survey: A total of 161 tsetse flies, 10 *Tabanus* and 39 *Stomoxys* were caught from the three selected peasant associations during study period. The overall apparent density of tsetse flies was 1.34 f/t/d. Three tsetse species have been identified. 56(34.78%) were *Glossina fuscipes fuscipes*, 42(26.08%) were *Glossina pallidipes* and 63(39.13%) were *Glossina morsitance submorsitance*. From overall the study sites, the highest (2.45 f/t/d) catch was in Haro and the next highest catch (1.31f/t/d) was in Wangegne and the lowest catch (0.655f/t/d) was in Gabapeasant associations. From total tsetse flies trapped females occupied larger proportion and out of 161 tsetse flies caught, 93 (57.76%) flies were female while the rest 68(42.23%) were male as indicated in (Table 1). During this study period 161 tsetse flies were caught by using Monopyramidal traps which indicated in below table (Table 1).

Table 1: Apparent density of flies in different PA's in Hurumu district

Pas	No of trap deployed	<i>G. pallidipes</i>		<i>G. m. submorsitances</i>		<i>G. f. fuscipes</i>		Total	FTD
		M	F	M	F	M	F		
Gaba	15	11	0	9	7	4	8	20	0.655
Haro	10	6	14	6	8	6	9	49	2.45
Wangegne	35	4	7	11	22	19	10	92	1.31
Total	60	21	21	26	37	29	27	161	1.34

PAs: Peasant associations, FTD: Fly per trap per day, F: female, M: male

Table 2: Overall prevalence of bovine trypanosomosis in different PA's of Hurumu district.

Peasant association	Number of animal examined	Infected animals	<i>Trypanosome spp.</i>		Prevalence (%)	X ²	P-Value
			T.c	T.v			
Gaba	144	13	11	2	9.03	6.784	0.034
Haro	123	2	1	1	1.63		
Wangegne	195	15	13	2	7.69		
Total	462	30	25	5	6.49		

Table 3: Prevalence of Trypanosomosis in relation to sex, body condition score and Age of the animals.

Variables	Number of animal examined	Infected animals	Prevalence (%)	χ^2	P-Value
Sex					
Female	251	23	9.16	6.45	0.011
Male	211	7	3.32		
Body Condition					
Good	122	1	0.82	134.76	<0.001
Medium	273	3	1.1		
Poor	67	26	38.81		
Age					
Adult	334	20	5.99	0.50	0.476
Young	128	10	7.81		
Total	462	30	6.49		

Table 4: The mean packed cell volume of examined cattle in Hurumu district

Group	Observations	Mean PCV	SE	SD	95% C
Negative	432	28.69	0.78	3.69	28.34-29.04
Positive	30	21.4	0.69	3.77	19.99-22.81
Total	462	28.22	0.19	4.11	27.84-28.59

SD= Standard Deviation, SE= Standard Error, PCV=Packed cell volume

Parasitological Findings: The overall prevalence of bovine trypanosomosis in the study area was 6.49%. The prevalence of bovine trypanosomosis in each peasant association was determined to be 9.03%, 7.69% and 1.63% in Gaba, Haro and Wangegne respectively. Among those three peasant associations, Gaba peasant association showed the highest prevalence rate (9.03%) and the lowest being in Wangegne (1.63%) as shown in (Table 2). *T. congolense* was dominant species with a proportion of 25 (83.33%), followed by *T. vivax* 5 (16.67%). There was statistically significant difference ($P<0.05$) in prevalence of infection between sexes, Peasant association and higher prevalence rate of 38.81% and 9.32 % in poor body condition score and female, respectively (Table 3).

Hematological Findings: The mean PCV value for the parasitemic cattle was 28.22+4.11SD while the mean PCV value for the aparasitaemic cattle was 28.69+3.69 SD. There was statistically significant difference ($P<0.05$) in mean PCV value between parasitaemic and aparasitaemic cattle (Table 4).

DISCUSSION

The present study revealed that from a total of 462 randomly selected cattle's in the study area, 30(6.49%) of the animal were positive for trypanosomes. Similar findings of 6.77% from Quara district [13] were reported. But this is lower than previous report, 20.4% in Wolyta and Dawero Zone of Southern Ethiopia [14],

7.78% in Lalokile district, kelemwollega , western Ethiopia [15] and 16.9% in Sayo, district, kellemWollega, Western Ethiopia [16]. On other hand, the current study result was higher than 4.86% prevalence in Didesadistrict, Oromia Region [17], 4.43% from Arbaminch [18] and 4.23% from Nonno [19] were reported. The lower prevalence in the current study might due to the use of prophylactic and trypanocidal drugs, application of relatively designed method of tsetse fly control and expansion of cultivation land in the area which in directly affects its vectors.

This study shows that, *T. congolense* was dominant species with a proportion of 25 (83.33%), followed by *T. vivax* 5(16.67%). This result in agreement with the predominance of *T. congolense* infection in cattle as compared to *T. vivax* and may due to the development of better immune response to *T. vivax* by infected animal. Moreover, the most prevalent trypanosome species in tsetse infested area of Ethiopia are *T. congolense* [20].

During the study period, the prevalence of bovine trypanosomosis was assessed between sexes, body condition scores, peasant association (PAs) and age of the animals and there is significant difference ($P<0.05$) between sexes, body condition and PAs in the district. Among 30 trypanosome positive animals, 23(9.16%) of them were female animals and 7(3.32%) of them were male animals. The higher infection rate in female may be attributed to stress factors related to work where animals are used for drought purpose and they have to walk long distance in areas where there is a high risk of tsetse challenge.

In this study, the occurrence of the disease in their different body condition scores (Good, Medium and Poor) animals, shows that statistical significance ($P < 0.05$) variation. The prevalence of trypanosomosis in those animals with poor body condition (38.81%) was higher than those in good (0.82%) and medium (1.1%) body condition. Similar findings were reported in Abay (Blue Nile) base areas of Northwestern, Ethiopia [21] in Bure district, western Ethiopia [22]. On another hand disagreement with the study in Metekel and Awi zone of North West Ethiopia [23]. Obviously, the disease itself result in progressive emaciation of infected animals; never less, non-infected animals under good condition have well developed better immune status that can respond to any foreign protein better than those non infected cattle with poor body condition which can be immune compromised due to other disease or malnutrition, since malnutrition and concurrent infections depress the immune responsiveness in some cases [24].

The present study indicated that the difference between mean PCV values of parasitaemic (21.4%) and aparasitaemic (28.69%) cattle of the study area was significant ($P < 0.05$). This result was in agreement with the previous work done in BiloNophadistrict, south west Ethiopia [25, 26]. Being intracellular blood parasites, trypanosomes result in lowering PCV of cattle because they lyses and destruct the red blood cells. The appearance of trypanosomosis in negative animals with PCV values of less than the threshold values (25%) may be due to the inadequacy of detection method used or delayed recovery of anaemic situation after current treatment with trypanocidal drugs Or due to be anaemic by other complicative cause like malnutrition. Parasitaemic animals with PCV values greater than 25% might be thought of recent infection. Trypanosome infection and mean PCV values obtained in this study in the parasitaemic animals was found to be highly associated. Different authors in southern, northwestern and southwestern Ethiopia [27] also reported similar results. The mean PCV can be affected by many factors including helminth parasites infections, nutritional deficiencies and blood parasites, other than trypanosomosis, however, these factors are likely to affect both trypanosomosis positive and negative animals [28, 29].

The risk of trypanosomosis is also influenced by apparent density of the tsetse flies and type of vector prevailing in the area. In this study, the entomological findings revealed that three species of *Glossina* (*G. pallidipes*, *G. m. submorsitance* and *G. fuscipes*)

out of five reported in Ethiopia. The overall apparent density of *Glossina* species was 1.34 flies/trap/day. These findings lower than the previous report 11.9 f/t/d from Hewa-Gelan district, Oromia region, west Ethiopia [30]. The result also higher than the previous report 1.15f/t/d for tsetse in East Wollega zone [31]. Higher percentage of female 93 (57.76%) tsetse flies was caught than male 68(42.23%) tsetse flies that are in line with various reports from different parts of Ethiopia [32, 33]. This could be adhered to longer lifespan of female tsetse flies than males [34, 35].

CONCLUSION

The present study informed that the presence of the vectors or glossina species which transmits the disease trypanosomosis can reduce handoutly the production and productivity of the livestock in the area. Thus, strategic control of bovine Trypanosomosis including integrated and sustainable vector control should be strengthened to improve livestock production and agriculture development in the area.

ACKNOWLEDGMENTS

The Authors are grateful to the National Tsetse fly and Trypanosomosis Control and Eradication Center, Bedelle (NTTICEC) for providing the required budget and logistics for this study. The cooperation's of Hurumu district, Livestock development and Fisheries and cattle herd owners of the study area are highly acknowledged. At last but not the least, we would like to gratitude the professionals' workers of Hurumu district, Livestock development and Fisheries DrMegersa and MrWase for his help on the field work.

REFERENCES

1. DACA, 2006. standard veterinary Treatment Guidelines for veterinary practice 1 Sted. Drug Administration and Control Authority of Ethiopia.
2. Bekele, J., K. Asmare, G. Abebe, G. Ayelet and E. Gelaye, 2010. Evaluation of Deltamethrin applications in the control of tsetse and trypanosomosis in the southern rift valley areas of Ethiopia. Vet Parasitol., 168: 177-184.
3. Tesfaye, M., 2002. Report of trypanosome infection rate in G.msubmoristans and *G. tachnoides* in Didessa valley from July 29 to September 26. Bedele Ethiopia.

4. Getachew, A., 2005. Trypanosomosis in Ethiopia. *J. Biol. Sci.*, 4: 18-21.
5. NTTICC, 2004. National Tsetse and Trypanosomosis Investigation and Control Center. Bedelle Ethiopia.
6. Urquhart, G.M., J. Armour, J.L. Duncan, A.M. Dunn and F.W. Jennings, 2006. *Veterinary Parasitology*, pp: 212-219.
7. HBOA, 2017. Hurumu district Livestock development and Fisheries Office. Annual Report.
8. Thrufield, M., 2005. *Veterinary Epidemiology*, pp: 233-250.
9. Brightwell, R., R.D. Dransfield, C.A. Korcu, T.K. Golder, S.A. Tarimo and D. Mugnai, 2003. A new trap for *Glossinapallidipes*. *Trop Pest Management.*, 33: 151-159.
10. Morag, G.K., 2002. *Haematology*, pp: 1-25.
11. Codjia, V., W. Mulatu, P.A. Majiwa, S.G. Leak, G.J. Rowlands and E. Authié, 1993. Epidemiology of bovine trypanosomosis in the Ghibe valley, southwest Ethiopia. Occurrence of population of *Trypanosoma congolense* resistant to diminazine, is ometamidium and homidium. *Acta Trop.*, 53: 151-163.
12. Paris, J., M. Murray and F. Mcodimba, 1982. A comparative evaluation of the parasitological technique currently available for the diagnosis of African Trypanosomosis in Cattle. *Acta Trop.*, 39: 307-316.
13. Getaneh, A., 2015. Tewodros. Prevalence of Bovine Trypanosomosis in Quara District, North-Western, Ethiopia. *Global Veterinarian.*, 15: 506- 511.
14. Miruk, A., A. Hagos, H.T. Yacob, F. Asnake and A.K. Basu, 2008. Prevalence of bovine trypanosomosis and trypanocidal drug sensitivity studies on *Trypanosoma congolense* in Wolyta and Dawero zones of southern Ethiopia. *Veterinary Parasitology*, 152: 141-147.
15. Olani, A. and D. Bekele, 2016. Epidemiological Status and Vector Identification of Bovine Trypanosomosis in Lalo-Kile District of Kellem Wollega Zone, Western Ethiopia. *J. Vet. Med. Res.*, 3(2): 1045.
16. Siyum, G., K. Tadele, A. Zelalem and D. Benti, 2014. Epidemiological Survey of Bovine Trypanosomosis in Sayo District of Kellem Wollega Zone, Western Ethiopia. *American-Eurasian Journal of Scientific Research*, 9: 67-75.
17. Gamechu, F., M. Aynalem, H. Birhanu, C. Gemechu and A. Gezahegn, 2015. Epidemiological Status and Vector Identification of Bovine Trypanosomosis in Didesa District of Oromia Regional State, Ethiopia. *International Journal of Nutrition and Food Sciences*. 4: 373- 380.
18. Teka, W., D. Terefe and A. Wondimu, 2012. Prevalence study of bovine trypanosomosis and tsetse density in selected villages of Arbaminch. *Ethio J. Vet. Med. Anim. Hlth.*, 4: 36-41.
19. Dagim, B. and D. Tekalegn, 2019. Prevalence of Bovine Trypanosomosis and Apparent Density of Tsetse Flies in Nonno District, Western Shewa zone, West Ethiopia. *East African Scholars J. Econ. Bus. Manag.*, 2: 7-13.
20. Muturi, K.S., S. Msangi, S. Munstermann, P. Clausen, A. Getachew and T. Getachew, 2000. Trypanosomosis risk assessment in selected sites of the southern rift valley of Ethiopia, pp: 12.
21. Dagnachew, S., K. Arun and G. Abebe, 2006. Assessment of trypanocidal drug resistance in cattle of the Abay (Blue Nile) basin areas, north western Ethiopia. *Ethiop Vet. J.*, 2: 45-63.
22. Mezene, W., B. Ahimedine, Y.S. Moti, D. Efreem and L. Kumela, 2015. Bovine Trypanosomosis and Tsetse Fly Survey in Bure District, Western Ethiopia. *Acta Parasitologica Globalis*, pp: 91-974.
23. Mekuria, S. and F. Gadissa, 2011. Survey on bovine trypanosomosis and its vector in Metekel and Awi zones of Northwest Ethiopia. *Acta*, 117: 146-151.
24. Collins, F.M., 1994. The immune response to mycobacterial infection: development of new vaccines. *Vet. Microbiol.*, 40: 95-110.
25. Tekalegn, D. and L. Kumela, 2018. Trypanosomosis and Apparent Densities of *Glossina* Species in Bilo Nopha District, Southwestern Ethiopia. *European Journal of Applied Sciences*, 10(2): 43-47.
26. Sinshaw, A., G. Abébé, M. Desquesnes and W. Yoni, 2006. Biting flies and Trypanosomavivax infection in three highland districts bordering lake Tana, Ethiopia. *Veterinary Parasitology*, 142(1-2): 35-46.
27. Rowlands, G.J., W. Mulatu, E. Authié, G.D.M. d'Ieteren, S.G.A. Leak, S.M. Nagda and A.S. Peregrine, 1993. Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. *Acta Tropica*, 53(2): 135-150.
28. Tasew, S. and R. Duguma, 2012. Cattle anaemia and trypanosomiasis in western Oromia State, Ethiopia. *Rev. Med. Vet. (Toulouse)*, 163(12): 581-8.
29. Van Den Bossche, P.R.G.J. and G.J. Rowlands, 2001. The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Tropica*, 78(2): 163-170.

30. Fentahun, T., M. Tekeba, T. Mitiku and M. Chanie, 2012. Prevalence of Bovine Trypanosomosis and Distribution of Vectors in Hawa Gelan District, Oromia Region, Ethiopia. *Global Veterinaria*, 9: 297-302.
31. Tafese, W., A. Melaku and T. Fentahun, 2012. Prevalence of bovine trypanosomosis and its vectors in two districts of East Wollega Zone, Ethiopia. *Onderstepoort Journal of Veterinary Research*, 79(1): 1-4.
32. Haile, G., N. Mekonnen, K. Lelisa and Y. Habtamu, 2016. Vector identification, prevalence and anemia of bovine trypanosomosis in Yayo District, Illubabor Zone of Oromia Regional State, Ethiopia. *Ethiopian Veterinary Journal*, 20(1): 39-54.
33. Lelisa, K., D. Damena, M. Kedir and T. Feyera, 2015. Prevalence of bovine trypanosomosis and apparent density of tsetse and other biting flies in Mandura District, Northwest Ethiopia. *Journal of Veterinary Sciences and Technology*, 6: 229.
34. Dyer, N.A., S.P. Lawton, S. Ravel, K.S. Choi, M.J. Lehane, A.S. Robinson and M.J. Donnelly, 2008. Molecular phylogenetics of tsetse flies (Diptera: Glossinidae) based on mitochondrial (COI, 16S, ND2) and nuclear ribosomal DNA sequences, with an Emphasis on the Palpalis.
35. Caljon, G., L. De Vooght and V.D.J. Abbeele, 2014. *The Biology of Tsetse G ÇöTrypanosome Interactions. Trypanosomes and Trypanosomiasis* Springer, pp: 41-59.