

Full Length Research Paper

An investigation on *Glossina* species and the prevalence of trypanosomosis in cattle in Meatu district, Tanzania

Deusdedit J. Malulu^{1,2*}, Elikira Kimbita², Huruma Tuntufye², Safari Kinungh'i³, Hamisi Nyingilili¹, Togolai Mbilu⁴, Josephat Kaboya⁴, Eugene Lyaruu¹ and Imna I. Malele¹

¹Vector and vector Borne Diseases Institute (VVBD) (Formerly Tsetse and Trypanosomiasis Research Institute), P.O. Box 1026, Tanga, Tanzania

²Department of Microbiology, Parasitology and Immunology, Sokoine University of Agriculture P.O Box 3015, Chuo kikuu Morogoro

³National Institute for Medical Research (NIMR), Mwanza Centre, P.O. Box 1462, Mwanza, Tanzania

⁴National Institute for Medical Research (NIMR), Tabora Centre, P. O. Box 482, Tabora, Tanzania

Received 27 September, 2016; Accepted 16 January, 2017

A cross-sectional study which sought to identify *Glossina* species and the prevalence of trypanosomosis in cattle of Meatu district where sleeping sickness cases have been reported in villages that border protected wildlife parks, was carried out. Four phenols and acetone baited tsetse traps (NGU, NZI, S3 and Biconical) were used in the study and showed that three *Glossina* species occurs in the area namely *Glossina pallidipes*, *Glossina swynnertoni* and *Glossina morsitans*. *G. pallidipes* was the most prevalent species. Four hundred and twenty four (424) blood samples were collected from cattle and subjected to parasitological and hematological analysis. Analysis by microscopic and buffy coat showed an overall prevalence of animal trypanosomosis (AT) of 2.36% (n=10). Identified trypanosome species were *Trypanosoma congolense* (7/10) and *Trypanosoma vivax* (3/10). No mixed infection was identified. The packed cell volume (PCV) for hematological analysis revealed a prevalence of anemia of 8.25%. No statistical evidence implicated animal trypanosomosis as the cause of anemia. Identification of trypanosomes in screened animals implicate AT as a threat to cattle and other domestic and wild animals since the identified trypanosome species affect a wide range of animals. Tsetse control and proper treatment of livestock should be advocated to control the disease.

Key words: *Glossina*, animal trypanosomosis, sleeping sickness, Tanzania.

INTRODUCTION

Trypanosomosis is a disease caused by protozoan parasites of the genus *Trypanosoma* and is transmitted through a bite by tsetse flies of the genus *Glossina*

(Wamwiri and Changasi, 2016). African Trypanosomosis refers to two forms of diseases: Human African Trypanosomosis (HAT) and Animal African

*Corresponding author. E-mail: maluluone@hotmail.com.

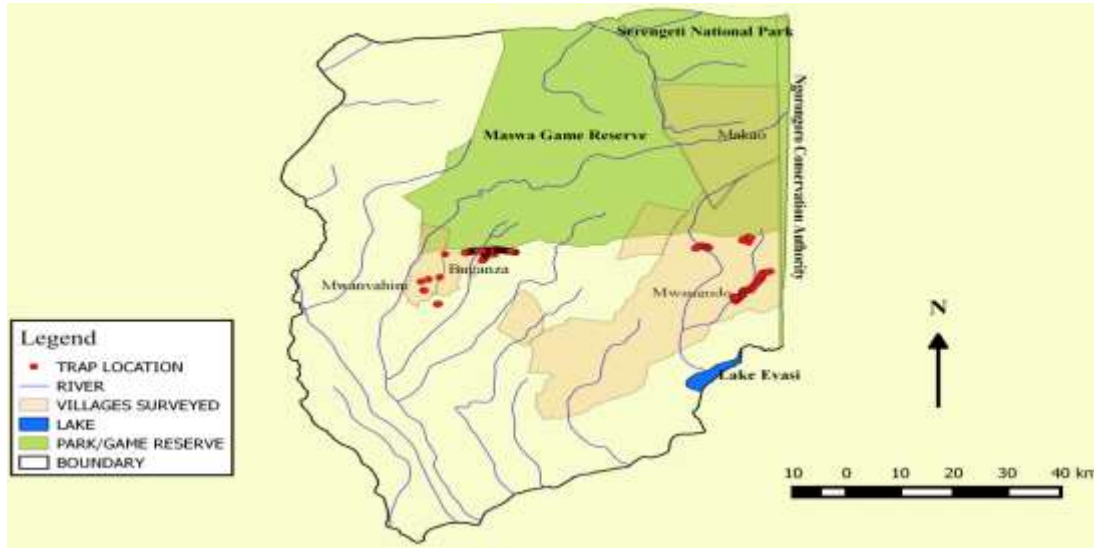


Figure 1. Map of Meatu District showing trap deployment in the villages surveyed.

Trypanosomosis (AAT). Human African Trypanosomosis is caused by *Trypanosoma brucei rhodesiense* (East and Southern Africa) and *T. brucei gambiense* (West and Central Africa) (WHO, 1998). AAT is caused by many trypanosome species including *T. brucei*, *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma simiae* (Kihamia et al., 1991; Leak, 1999). Trypanosomosis have resulted into serious economic impediment in 36 sub-Saharan African countries for years (WHO, 1998). While tsetse flies are well known as biological vectors of the diseases to both animals and human hosts, wildlife animals are also known to be reservoirs of all forms of trypanosomosis (FAO, 1982). Recent studies have confirmed domestic animals like cattle and pigs as being reservoirs of human infective trypanosomes (Haji et al., 2014; Hamill et al., 2013). In Tanzania, 302,465 km² of the land that accounts for thirty-two percent of the total land mass is tsetse infested where seven species of *Glossina* exist (Daffa et al., 2013).

From computations of Shaw et al. (2014), 4.5 Billion TSH (approximately 2 million USD) losses occur annually resulting from cattle mortality and reduced cattle productivity. The losses may be higher than 4.5 billion TSH if costs incurred by livestock owners in treating sick animals are included.

Nevertheless, one hundred cases of sleeping sickness cases are also being reported annually and the majorities are from Kigoma, Rukwa and Tabora regions (Simmaro et al., 2010).

Regular disease surveillance in the trypanosomosis endemic areas for identification of infective trypanosomes in susceptible hosts and key risk factors is one way to justify or estimate tsetse and trypanosomosis burden. The findings can be useful in influencing and guiding strategic vector and disease control for the protection of

livestock and people. Therefore, determining population dynamics of the vector, that is, species, age-sex composition and identifying pathogens in the host, provides essential evidence on the transmissibility of pathogens between vector and hosts (Woo, 1970; Murray et al., 1977).

The aim of the current study was to identify *Glossina* species, establish their density and to determine the prevalence of trypanosomosis in cattle.

The domestic animal host was selected as it is abundant in the study area and it is known to be the reservoir of human infective trypanosomes (Simukoko et al., 2007).

MATERIALS AND METHODS

Study area and design

Meatu district is located at 34°41'8.34"E and 3°28'58.68"S. The district is among the five districts of Simiyu region; with an area of about 8,871 sq. km². In 2012, Meatu district had a population of about 296 616 people (NBS, 2012). The district has a bi-modal rainfall whereby short rains starts from October and ends in December and long heavy rains starts in March and ends in May. The average annual rainfall ranges from 600 to 900 mm. Its topography is characterized by flat, gently undulating plains and lowly sparsely vegetation and in some places, is covered with Miombo woodlands.

A cross-sectional study was conducted in Meatu district because of its proportion of land being occupied by the Maswa game reserve than other districts of Simiyu region and due to the fact that sleeping sickness was reported in previous years (district unpublished report). Entomological as well as parasitological data were collected in Buganza, Mwanvuhina, Mwangudo and Makao villages in September 2015.

Figure 1 shows the surveyed villages selected based on their history of endemicity for trypanosomosis, vicinity to the protected areas and relative cattle population.

Table 1. Mean tsetse catches in Meatu district by villages.

Village	Overall mean	Tsetse species		
		<i>G.pallidipes</i>	<i>G. swynnertoni</i>	<i>G. morsitans</i>
Buganza	22.17±20.35	40.63±25.51	12.50±8.86	13.88±6.80
Mwanyahina	15.46±11.77	23.5±13.14	13.25±10.79	9.63±6.89
Makao	56.06±56.83	43.88±24.29	68.25±73.52	0
Mwangundo	51.02±21.18	59.30±25.65	42.74±12.09	0

Table 2. Tsetse apparent densities in Meatu district by village.

Village	Total catches	Traps	Trapping days	Apparent density (FTD)
Buganza	532	8	3	22.2
Mwanyahina	371	8	3	15.5
Mwangundo	816	8	3	34.0
Makao	897	8	3	37.4

Data collection

Collection of tsetse samples

Entomological data on tsetse species, sex and abundance were collected using Phenol[®] and Acetone[®] baited NZI (Mihok, 2002), NGU (Brightwell et al., 1987), S3 (Ndegwa and Mihok 1999) and Biconical (Challier et al., 1977) traps. Two sites were identified in each village in which the traps were randomly deployed at 100 m apart according to the vegetation type likely to influence visibility. Traps were rotated every day for three consecutive days and tsetse harvested after every 24 h. Tsetse species and sex were determined by taxonomic key as in FAO (1982) tsetse manual. After identification, a total of five non-teneral flies were pooled and preserved in 253 sterile vials containing absolute ethanol for further laboratory analysis.

Parasitological screening for trypanosome infection

Blood samples were collected from cattle after first obtaining oral consent from herd owners. A total of 424 cattle were sampled by collecting blood from the jugular vein using ethylene diamine tetra acetic acid (EDTA) vacutainer tubes, labeled and stored in ice packed cool box. Collected blood samples were later examined for packed cell volume (PCV) (Woo, 1970), buffy coat (Murray et al., 1977), and microscopically for the presence of trypanosomes in the field by the Giemsa-stained thick blood smears method. Cut off point of ≤24% PCV (Marcotty et al., 2008) was used for considering cattle anemic. A total of 100 buffy coat samples (including positives) were extruded on Whatman FTA[®] cards matrix, air dried before being stored for further analysis in the laboratory.

Data analysis

Both parasitological as well as entomological data collected from tsetse and animals from the four villages were entered in Microsoft Excel (2007) and Fly per Trap per Day (FTD), prevalence of nagana and anemia were computed. Furthermore, the records were

transferred to Epi Info7 (CDC, 2014) analytical software for analysis. One-way analysis of variance (ANOVA) was used to analyze the variations of tsetse catches and cattle PCV in the villages.

Tsetse counts were used as the dependent variable while trap type, tsetse species, vegetation type and village were used as the independent variables. The overall comparison of tsetse species and the trap types regardless of the tsetse fly species was done using generalized linear model analysis. Packed cell volume was used as the dependent variable whereas nagana, species and village were independent variables. Separation of means was done at 95% confidence interval (CI) and the significance level of 5% ($P < 0.05$) in all statistical tests.

RESULTS

Collection of tsetse samples

The overall mean tsetse catches for the four villages was 654.08, three species namely *Glossina pallidipes*, *Glossina swynnertoni* and *Glossina morsitans* were identified. *G. pallidipes* was the most abundant tsetse species followed by *G. swynnertoni* and *G. morsitans* in descending order. *G. morsitans* was found at Mwanyahina and Buganza villages only. The highest scores were observed at Makao and Mwangundo villages, while the lowest scores were observed at Mwanyahina and Buganza villages (Table 1). The differences in catches among species and between villages were highly significant ($P < 0.000$). High FTD was observed at Makao (37.4) while low apparent density was observed at Mwanyahina (15.5) (Table 2).

The performance of traps in tsetse catching is shown in Figure 2. NGU trap obtained the highest score for the two species: *G. morsitans* and *G. swynnertoni* while *G. pallidipes* were mostly trapped by NZI trap. S3 and

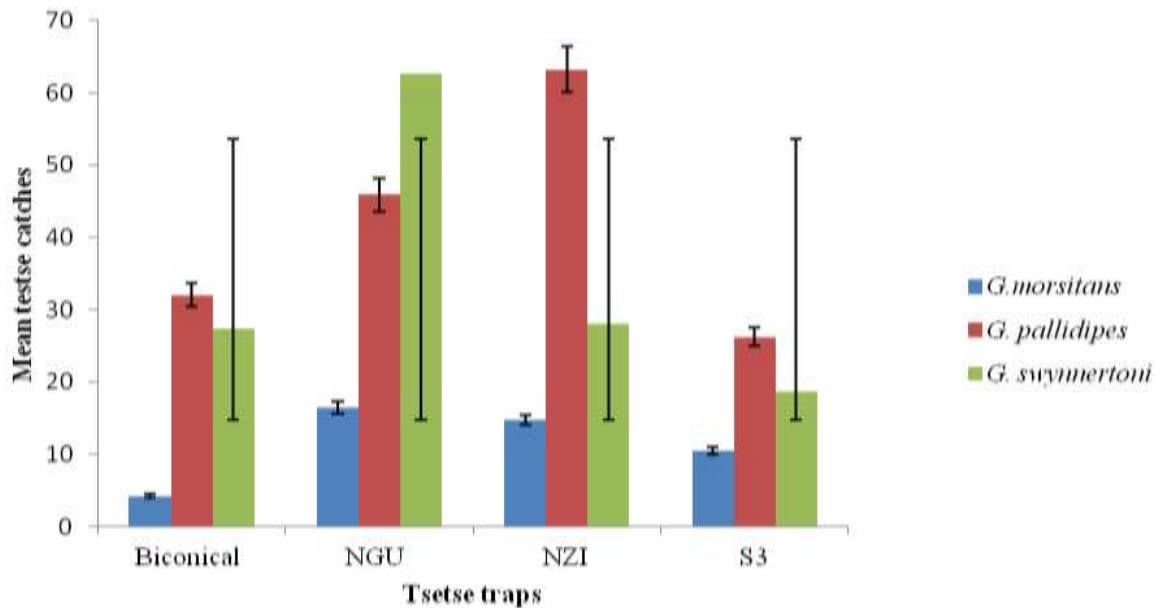


Figure 2. Performance of traps in catching *Glossina* species.

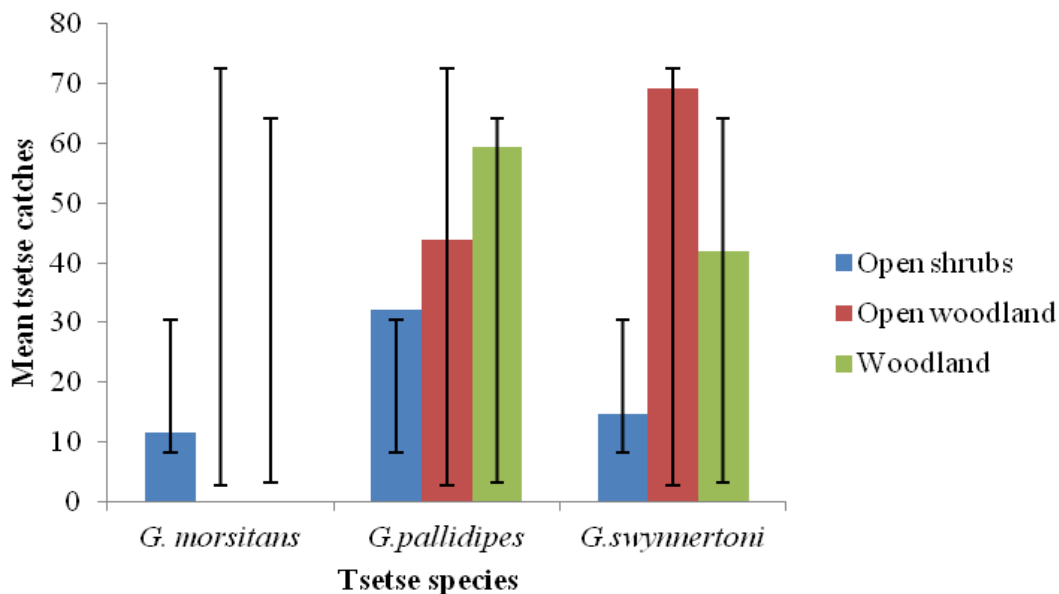


Figure 3. Mean tsetse catches in relation to vegetation where traps were deployed.

Biconical trapped the lowest numbers of all three species (Figure 2). Three vegetation types were found in the villages surveyed. Open and scattered shrubs (Mwanyahina and Buganza) open woodland and woodland (Makao and Mwangudo). *G. pallidipes* and *G. swynnertoni* were trapped in all vegetation types, except for *G. morsitans* which were caught in open shrubs only (Figure 3). The catches of tsetse in the three vegetation types were significantly different ($P < 0.001$).

Parasitological screening for trypanosome infection

Table 3 summarizes PCV scores, the prevalence of trypanosomiasis (nagana) and the prevalence of anemia in cattle in the sampled villages. The overall prevalence of trypanosomiasis in cattle was 2.36% ($n=10$), being highest at Makao village (3.50%) and lowest at Mwangudo village (1.23%). Morphological identification (Uilenberg, 1998) revealed *T. congolense* (7/10) and *T.*

Table 3. Haematological and parasitological attributes of cattle in Meatu district by village.

Village	Anaemia	Nagana	Mean PCV
Buganza	6.56%	2.31%	29.78
Mwanyahina	5.88%	1.43%	31.69
Mwangudo	13.70%	1.23%	29.61
Makao	9.35%	3.50%	29.29
Overall	8.25%	2.36%	29.86

Table 4. Comparison of the effect of nagana on cattle PCV and anaemia.

Class	Level	Mean PCV (\pm SD)
Anaemia status	Normal	30.58 \pm 4.01 ^{ab}
	Anaemic	21.83 \pm 2.84 ^{ab}
Infection status	Nagana negative	30.60 \pm 4.03 ^{aa}
	Nagana positive	30.13 \pm 3.31 ^{aa}

vivax (3/10). *T. congolense* was the most dominant tsetse species. Mean percent PCV of the animals sampled was 29.86%, while the prevalence of anemia was 8.25%. Two anemic cattle were found to be infected with *T. vivax*. Since a PCV value of $\geq 25\%$ was used as a cut of point for cattle to be considered normal, a comparison between normal and anemic cattle in Table 4 revealed a significant difference ($P < 0.001$). There was no statistical evidence for significant differences in PCV values between cattle which were nagana positive and those which were nagana negative ($P = 0.3103$).

DISCUSSION

The findings of this study clearly substantiates the existence of three species of *Glossina*; *Glossina pallidipes*, *Glossina* and *swynnertoni* at Mwanyahina, Buganza, Makao and Mwangudo villages which are adjacent to Maswa Game reserve, whereas *G. morsitans* was trapped at Mwanyahina and Buganza villages only. An investigation carried out by Salekwa et al. (2014) in Simanjiro and areas of Tarangire National park reported that *G. morsitans* were the most abundant species as compared to *G. swynnertoni* and *G. pallidipes*. This difference may be attributed to type of vegetation cover. These results are contrary to the study carried out by Malele et al. (2007) who reported existence of four tsetse species namely *G. swynnertoni*, *G. pallidipes*, *G. m. morsitans* and *G. brevipalpis*. Whereby, *G. swynnertoni* was the most dominant species, a study that included some of the sites surveyed during the present study. It is

clear that the Fly per Trap per Day (FTD) is higher at Mwangudo and Makao than at Mwanyahina and Buganza villages, posing higher tsetse bite risk in the former village than in the later. These findings confirm the existence and transmission of trypanosomosis in the study area and hence the risks of exposure to people and their livestock. Climate change and associated effects such as shortage of feeds for livestock, shortage of food for increased human population create pressure to move into new virgin and fertile lands (McDermott et al., 2001) which in most cases are protected parks and game reserves. The villages surveyed and their crop farms are located within few meters from the Maswa game reserve which increases the chances of contracting the vector-borne diseases. The good performance of NGU traps relative to other traps which in line with findings of the study performed by Malele et al. (2016) validates further its suitability for survey and control purposes in the Serengeti ecosystem.

Parasitological screening for trypanosome infection

This study provides an explanation on the presence of animal trypanosomosis and not human trypanosomosis in cattle by microscopy, where *T. congolense* and *T. vivax* were identified. Similar findings have been reported by Haji et al. (2014). No significant statistical difference was observed in PCV between trypanosome infected and non-infected cattle, and there was no relationship between nagana and PCV. The observation of trypanosome infection in cattle is an evidence of cyclical

transmission of trypanosomes by tsetse but infection of *T. vivax* suggests transmission by other biting flies like *Tabanus* species which were also trapped during the study and have been reported elsewhere in Desquesnes and Dia (2003). *T. vivax* and *T. congolense* are not the only pathogens of importance to cattle but also other livestock species as well as in wildlife (Kaare, 2007; Anderson et al., 2011).

Conclusion

The aim of this study was to identify *Glossina* and trypanosomes species present in cattle in the study area, and the findings also gave the picture of the vector composition and trypanosome species in cattle. Further studies will be done at the VVBD to identify the pathogens in the vectors and cattle using more sensitive molecular tools.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGEMENT

The authors acknowledge the invaluable logistic support provided by VVBD-Tanga and The Director of NIMR-Mwanza Centre during the implementation phase of this study. They also sincerely thank the district authorities of Meatu district, particularly the Livestock and Fisheries Development Officer (DALDO) of Meatu district in Simiyu region for their kind assistance during the fieldwork. Last but not the least, they thank the village authorities and communities for agreeing to participate in this study. Financial support provided by the Tanzania Commission for Science and Technology (COSTECH) Grant No: CST/RA.56/1426/2013 is greatly appreciated.

REFERENCES

- Anderson NE, Mubanga J, Fevre EM, Picozzi K, Eisler MC (2011). Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl. Trop. Dis.* 5(6):e1211.
- Brightwell R, Drans field RD, Kyorku C, Golder TK, Tarimo, SA, Mungai D (1987). A new trap for *Glossina pallidipes*. *J. Trop. Pest Manag.* 33:151-189.
- Centre for Disease Control and Prevention (CDC) (2014). Epi Info. 7 User Guide viewed 10 December 2015. Available at: <https://wwwn.cdc.gov/epiinfo/user-guide>
- Challier A, Eyraud M, Lafaye A, Laveissiere C (1977). Improvement of the yield of the biconic trap for tsetse (Diptera, Glossinidae) by the use of a lower blue cone. *J. Entomol. Med. Parasitol.* 15:283-286.
- Daffa J, Byamungu M, Nsengwa G, Mwambembe E, Mleche W (2013). Tsetse distribution in Tanzania: 2012 status. *Tan. Vet. J.* 28:12-20.
- Desquesnes M, Dia ML (2003). Mechanical transmission of *Trypanosoma congolense* in cattle by the African tabanid *Atylotus agrestis*. *Exp. Parasitol.* 105:226-231.
- Food And Agriculture Organisation (FAO) (1982). Training Manual for Tsetse Control Personnel. pp. 1-105.
- Haji IJ, Malele I, Namangala B (2014). Occurrence of haemoparasites in cattle in Monduli district, northern Tanzania. *Onders. J. Vet. Res.* 81(1):1-4.
- Hamill LC, Kaare MT, Welburn SC, Picozzi K (2013). Domestic pigs as potential reservoirs of human and animal trypanosomiasis in Northern Tanzania. *J. Par. Vect.* 6:322.
- Kaare MT, Picozzi K, Mlengeya T, Fevre EM, Mellau LS, Mtambo MM, Cleaveland S, Welburn SC (2007). Sleeping sickness – a re-emerging disease in the Serengeti. *Trav. Med. Infect. Dis.* 5:117-124.
- Kihamia CM, Komba A, Mella PNP, Mbwambo HA (1991). Trypanosomiasis. In: Health and Disease in Tanzania. Mwaluko G, Kilama WL, Mandara MP, Murru M, Macpherson CNL (eds). Haper Collins Academic, London. pp. 133-143.
- Leak SGA (1999). Tsetse Biology and Ecology. CABI publishing, New York.
- Malele II, Kinung'hi SM, Nyingilili HS, Matemba LE, Sahani JK, Mlengeya TD, Wambura KM, Kibona SN (2007). *Glossina* dynamics in and around the sleeping sickness endemic Serengeti ecosystem of northwestern Tanzania. *J. Vect. Ecol.* 32(2):263-268.
- Malele II, Ouma JO, Nyingilili HS, Kitwika WA, Malulu DJ, Magwisha HB, Kweka EJ (2016). Comparative performance of traps in catching tsetse flies (Diptera: Glossinidae) in Tanzania. *Onders. J. Vet. Res.* 83(1):1057.
- Marcotty T, Simukoko H, Berkvens D, Vercruysse J, Praet N, Van den Bossche P (2008). Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Prev. Vet. Med.* 87:288-300.
- McDermott JJ, Kristianson PM, Kruska RL, Reid RS, Robinson TP (2001). Effects of Climate, Human Population and Socio-Economic Changes on Tsetse-Transmitted Trypanosomiasis to 2050. In: Black SJ, Seed JR, editors. The African trypanosomes. Boston: Kluwer Academic Publishers. P 17.
- Mihok S (2002). The development of a multipurpose trap (the NZI) for tsetse and other biting flies. *Bull. Entomol. Res.* 92(5):385-403.
- Murray M, Murray PK, McIntyre WIM (1977). An improved parasitological technique for the diagnosis of African trypanosomiasis. *Trans. Royal Soc. Trop. Med. Hyg.* 71:325-326.
- National Bureau of Statistics (NBS) (2012). National Population and Housing Census.
- Ndegwa PN, Mihok S (1999). Development of odour-baited traps for *Glossina swynnertoni* (Diptera: Glossinidae). *Bull. Entomol. Res.* 89:255-261.
- Salekwa LP, Nnko HJ, Ngonyoka A, Estes AB, Agaba M, Gwakisa PS (2014). Relative abundance of tsetse fly species and their infection rates in Simanjiro, Northern Tanzania. *Liv. Res. Dev.* 26:213.
- Shaw APM, Cecchi G, Wint GRW, Mattioli RC, Robinson TP (2014). Mapping the economic benefits to livestock keepers from intervening against bovine trypanosomiasis in Eastern Africa. *Prev. Vet. Med.* 113(2): 197-210.
- Simarro PP, Cecchi G, Paone M, Franco JR, Diarra A, Ruiz J A, Fèvre EM., Courtin F, Mattioli, RC, Jannin, JG (2010). The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *Int. J. Health Geogr.* 9(1):1-57.
- Simukoko H, Marcotty T, Phiri I, Geysen D, Vercruysse J, Van den Bossche P (2007). The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Vet. Parasitol.* 147(3-4):231-238.
- Uilenberg G (1998). A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis. Food and Agriculture Organisation Available at: <http://www.fao.org/docrep/006/x0413e/X0413E00.htm#TOC>.
- Wamwiri FN, Changasi RE (2016). Tsetse Flies (*Glossina*) as Vectors of Human African Trypanosomiasis: A Review. *BioMed. Res. Int.* 2016: 8
- Woo PT. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Act. Trop.* 27(4):384-386.
- World Health Organization (WHO) (1998). Control and Surveillance of African Trypanosomiasis. Report of WHO Expert Committee. WHO.