Study on Prevalence and Associated Risk Factors of Bovine Trypanosomosis and Identification of the Trypanosomes Species in Dibate Wereda, Benishangul-Gumz Region, Western Ethiopia

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Abstract: A cross-sectional study was conducted from September to November 2009 to determine the prevalence of bovine trypanosomosis, identify the dominant species of trypanosomes and associated risk factors in Dibate woreda of the Benishangul-Gumz Regional State, Western Ethiopia using buffy coat technique. An overall prevalence of bovine trypanosomosis in the study area was 4.9%. The prevalence of the disease in male and female cattle was found to be 6.7% and 3.7% respectively. Prevalence of 2.5%, 9.5% and 4.9% was recorded in Dibate 01, Dibate 02 and Zighi, kebeles respectively. Regarding to age, 4.5%, 3.7% and 5.4% prevalence was registered in animals less than three years, three to six years, and greater than six years of age respectively. Coat color of study animals was also considered as a risk factor and 10%, 3.5% and 5.4% prevalence was recorded in white, red and black colored cattle respectively. The prevailing species of trypanosomes in the study area were *T. congoleanse*, *T. vaivax*, and *T. brucei* with respective prevalence of 41.7%, 33.3% and 25% respectively. The present finding revealed that trypanosomosis was one of the constraints of livestock production and productivity in the study area signaling the need for strategic and integrated approach to mitigate the impact of the disease and further epidemiological study will be conducted to assess the real impact of the disease on livestock production and productivity in the study area.
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1. Introduction

Livestock play crucial role in Ethiopian economy. In addition to its importance as source of food and income for exchange, livestock play significant contribution for crop production as agriculture in the country (IAEA, 1996). However, despite significant role in Ethiopian economy, livestock contribute far below than the expected potential. This is partly because livestock production is constrained by different factors including disease like trypanosomiasis, poor feeding and management practices, and poor genetic potential (Getachew, 1995).

Trypanosomosis is a protozoan disease caused by different species of the genus *Trypanosoma* which affects most species of domestic livestock, many types of wild animals and human. Trypanosomosis occurs in large areas of Africa, Latin America, the Middle East and Asia. In Africa, animal trypanosomosis remains one of the most prevalent and biggest constraints to the development of sustainable livestock production (Mulligan, 1970).

Animals infected by tsetse flies develop fever, anemia and loss weight and progressively become weak and unproductive. Breeding animals frequently abort or may become infertile. Severely affected animals die of anemia, congestive heart failure or intercurrent bacterial infections that frequently take advantage of weakened immune system. Loss associated to trypanosomosis as the result of mortality and morbidity is roughly estimated to be $ 200 million per annum including denying utilization of fertile land for crop live stock production and the cost spend for control (IAEA, 1996).

Bovine trypanosomosis is a serious constraint to agricultural production in large parts of sub-Saharan Africa. Tsetse flies (Glossina sp.), the principal vector of trypanosomes, inhabit on about 10 million km^2 of the continent. Over the years a large amount of tsetse control tools have been developed and proven to be effective but difficult to sustain in areas that are subjected to reinvasion pressure. Although tsetse are vectors responsible for cyclical transmission of trypanosomes, several tabanid and other biting flies can transmit the parasite mechanically and compromise success of a trypanosome eradication programme (Desquesnes and Dia, 2003).

Trypanosomosis is one of the major impediments to livestock development and agricultural production in Ethiopia, contributing negatively to the overall development in general and to food self-reliance efforts of the nation in particular (Langridge, 1976; Mo ARD, 2004). Several studies have been done concerning the prevalence and economic significance of trypanosomosis in Ethiopia and indicated varied infection prevalence from region to region (Habtamu, 1993; NTTICC, 1996; Abebe and Jobre, 1996; Argaw and Abebe, 1988; Afewerk et al., 2000; Abebe, 2005; Lemecha *et al*., 2006; Merid *et al*., 2007).

Ethiopia should give emphasis for trypanosomosis and should design rational prophylactic or control programs against this disease. However, with our present state of knowledge of trypanosomosis, it is difficult to apply rigid rules applicable to all regions. This indicates the need of epidemiological study of trypanosomosis to the level of small areas (Rodostitis, 1994). In this regard, information on current status of trypanosomosis in Dibatea Woreda, Benshangul-Gumz region, is lacking. Therefore, this cross-sectional study was undertaken to estimate the prevalence of Bovine Trypanosomosis, identify species of trypanosomes involved, and associated risk factors of Bovine trypanosomosis in the study district.

2. Materials and Methods

2.1. Study Area

The study was conducted from September to November 2009 in Dibate Woreda, Benishangul Gumuze Region, Western Ethiopia. Dibate is located at 570 km to the west from Addis Ababa. Dibate has altitude range of 1500-1700 meters above sea level and gets 1175mm annual rain fall. The climate is tropical. The main rainy season occurs from May to September. The average annual maximum and minimum temperature reaches 29 ^{o}C and 25 ^{o}C, respectively. In Dibate, there are two important rivers created valleys suitable for insect multiplication. Quarter of the total 368289 square kilometer of the Woreda, is covered by bushes and trees. There are 66265 zebu cattle, 13041 sheep and 33680 goats in the Woreda.

2.2. Study animals

Study animals were local zebu cattle of different age groups involving both sexes managed under extensive farming system. In the study population, the proportion of males to females was very small in any of the herds. Males were used for draught power and for threshing while females were kept for breeding and dairy purpose.

2.3. Study design

The study was a cross-sectional study involving 490 cattle from three sites including Dibate 01, Dibate 02 and Zighi was made. Study animals of both sexes were categorized into different groups based on their dominant coat colour and their age. They were grouped into white, red and black based on the dominant coat colour of studied animals. They were also grouped into three based on their age; animals less than three years of age were included in the first category, the first category contains animals less than three years of age, animals equal to three years but less than six years of age were grouped into the second category, while those animals equal to or greater than six years of age were grouped into the third category. Ages of studied animals were estimated using dentition pattern as described by Steele (1996).

2.3.1. *Sample size determination*

The sample size required for this study was determined 284 cattle based on sample size determination in random sampling for infinite population using 50% expected prevalence of trypanosome and 5% desired absolute precision at 95% confidence level according to Thrusfield (2005) as follows:

$n = 1.96^2 \text{ Pexp } (1- \text{Pexp})/ d^2$

Where

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

However, to maximize accuracy a total of 490 cattle were examined.

2.3.2. Sample collection and laboratory procedure

A total of 490 blood samples were collected from each of 490 randomly selected animals. Heparinized capillary tubes were filled with blood by pricking ear veins of selected animals and maintaining the capillary tubes horizontal to the animal body surface and were sealed with help of soap at the end of which blood was filled.

Capillary tubes were put on hematocrit centrifuge and rotated at 250 revolutions per minute for 3 minutes. Capillary tubes were then attached onto slides by using small adhesive tape. Buffy coats which were observed as grayish narrow space between the plasma and the red blood cells in the capillary tube were examined for presence of trypanosomes under the microscope. When trypanosomes were present, capillary tubes were cut by using diamond pencil towards the red blood cells at the junction between the plasma and the red blood cells (more towards the red blood cells). The Buffy coats containing plasma and some red blood cells were dropped on a clean slide, mixed by using the broken capillary, covered with cover slip, and examined for identification of the species of trypanosomes at x40 magnification.

2.4. Identification of trypanosomes

Trypanosoma vivax and *T. congolense* are monomorphic, unlike *T. brucei* which is pleomorphic, was advantage for identification of these trypanosomes. *Trypanosoma congolense* was identified by its size which is about 8-24µm, medium marginal kinetoplast, and poorly developed undulating membrane. *Trypanosoma vivax* was identified by its large terminal kinetoplast, poorly developed undulating membrane with free flagella and size, about 18-28 µm, which is relatively larger than that of *T. congolense*. *Trypanosoma* brucei was identified by its small subterminal kinetoplast and pronounced undulating membrane.

2.5. Data management and analysis

The data were entered and managed in MS Excel work sheet. The analysis was conducted using Stata version 7 (Stata Corporation, 2000). Prevalence of trypanosomosis was expressed as percentage by dividing total number of samples or animals positive to trypanosome to the total number of samples or total number of animals examined.

3. Results

Out of 490 cattle (194 male and 296 female) examined, 4.9% (24 of 490) were found harbor trypanosomes. Higher prevalence (6.7%) of trypanosomosis was observed in male compared to the prevalence of trypanosomes in female cattle (Table 1).

Table 1: Prevalence of trypanosomes in male and female cattle in Dibate wereda

Sex of animals	No. of animals Examined	Positive (%)
Female	296	11 (3.7)
Male	194	13 (6.7)
Total	490	24 (4.9)

Prevalence of trypanosomes was calculated in cattle in three kebeles, the highest prevalence (9.8%) was observed in Dibate 2 while the lowest (2.4%) was calculated in Zighi (table 2).

Table 2: Prevalence of trypanosomes in cattle in three different kebeles of Dibate wereda

Kebele	No. of animals Examined	Positive (%)
Dibatea 1	163	4 (2.5)
Dibatea 2	163	16 (9.8)
Zighi	164	4 (2.4)
Total	490	24 (4.9)

Highest prevalence of trypanosomes (5.4%) was observed in cattle greater than six years of age while the lowest (3.7%) was observed in cattle between three and six years of age (Table 4).

Table 3: Prevalence of trypanosomes in different age groups of cattle in Dibate Woreda

Age of animals	No. of animals Examined	Positive (%)
< 3 years	110	5 (4.5)
3 – 6 years	81	3 (3.7)
> 6 years	299	16 (5.4)
Total	490	24 (4.9)

Highest prevalence of trypanosomes (10%) was observed in white coloured animals while the lowest (3.5%) was observed in red coat coloured animals (Table 3).

Table 4: Prevalence of trypanosomes in different coat coloured cattle in Dibate wereda

Coat color of cattle	No. of animals Examined	Positive (%)
White	90	9 (10)
Red	344	12 (3.5)
Black	56	3 (5.4)
Total	490	24 (4.9)

Three trypanosome species including *T. congoleanse*, *T. vaivax*, and *T. brucei* were identified with prevalences of 41.7% (10 of 24), 33.3% (8 of 24), and 25% (6 of 24), respectively.

4. Discussion

In this cross-sectional study of trypanosomosis in cattle, over all prevalence of 4.9% (24 of 490) was observed which was lower than the work of Tilahun et al. (1997) who reported trypanosomosis with infection prevalence of 22% at pawe. Higher prevalence (6.7%) of trypanosomes was observed in male compared to the prevalence in female cattle. This result of current study doesn't support the general perception that female animals are more affected by non-sex related diseases as parturition and lactation cause relaxation of the natural immunity of female animals (Craig, 1998).

When prevalence of trypanosomosis was compared among the three study sites, the highest prevalence (9.8%) was observed in Dibatea 2 while the lowest (2.4%) was obtained in Zighi. This might be associated with environmental factors which might be favorable either to the vector or the parasite. It might also be associated with the level of immunity of the host in the respective areas. The environmental factors in Dibate 2 might be more favorable either to the vector or to the parasite than Zighi.

The present result was varied in the selected sites. The reason why this variation happened was that may be due to the presence of well strategic method of these tsetse control and treatment expansion and cultivation which affect flies distribution. Because Dibate 1 and zighi are more near to the district town and Dibate 2 is far and more covered by forests and bushes, expansion of veterinary clinics, awareness of farms towards the control and treatment of the disease were improved relatively. In tsetse infected area of Ethiopia, 20-30% cattle were affected by trypanosomosis and in some high tsetse challenge area the prevalence of the disease reach up to 50% (Getachew, 2005).

Highest prevalence of trypanosomosis was observed in animals of greater than six years of age, followed by animals less than three years of age while the lowest prevalence was observed in animals between three and six years of age. The reason for higher prevalence in relatively young and old animals and lower prevalence in adult animals might be associated with the strength of immune system and less exposure to infection in younger animals. It is likely that very young animals had immature immune system and some others to have weak immune system due to old age. The prevalence varied with the age of animals that agreed with the number of outer (Daya and Abebe, 2008; Jorn et al., 2006) where they have shown an effect f age on the prevalence of trypanosome infections in cattle where calves are the least infected. They also stated that tsetse flies are attracted more by odor of large animals that showed less defensive behavior and least by calves.

Prevalence of trypanosomosis was calculated to study whether coat colour was risk factor for occurrence of trypanosomosis and highest prevalence of trypanosomes (10%) was observed in white coat coloured animals while the lowest (3.5%) was observed in red coat coloured animals.

Three trypanosome species including *T. congolense*, *T. vaivax*, and *T. brucei* were identified with prevalence of 41.7% (10 of 24), 33.3% (8 of 24), and 25% (6 of 24), respectively. Higher prevalence of *T. congolense* compared to the prevalence of *T. vivax* was in agreement with previous works of Rawlands *et al*. (1993), Muturi (1999), Afework (2001), Tewolde (2004) who identified T. congolense with prevalence of 84%, 66.1%, 60.9% and 75% in their study in Ghibe valley, in Merab Abaya, in pawe, and in western Ethiopia, respectively. The reason for the high ratio of *T. congolense* to *T. vivax* could be partly associated with the reason that cattle might more readily develop immunity to T. *vivax* than T. *congolense* (Maclennan, 1970; Dieteren *et al*., 1988; Leak *et a*l., 1993). However, Cherenet *et al*. (2006) reported as T. vivax was responsible for 90.9% of the cattle trypanosome infections in their study in tsetse-free zones of the Amhara Region, northwest Ethiopia while *T. congolense* and *T. vivax* contributed almost equally to the trypanosome infections in tse-tse infested area. This is due to the fact that *T. vivax* can be transmitted by mechanical vectors other than tse-tse.

5. Conclusion

In this cross-sectional study of trypanosomosis in cattle, an overall prevalence of 4.9% was observed. Sex and age of cattle are important factors affecting occurrence of trypanosomosis. Different level of prevalence was recorded in the different study sites. Higher prevalence was observed in male cattle, in animals greater than six years of age, in black coat colored cattle. Three species of Trypanosomes, Trypanosoma *congolense*, *T. vivax* and *T. brucei* were identified. The study indicated that trypanosomosis is one of the constraints of livestock production in the study area. Hence, Farmers should be advised & educated regarding the economic importance and preventive and control measures of trypanosomosis, further epidemiological study should be conducted to assess the real impact of the disease on production and productivity of livestock in general & cattle in particular in the study area.

References

1. Abebe, G. and Jobre, Y. (1996): Trypanosomosis: A Threat to cattle production in Ethiopia Rev. Med. Vet. 147, 896-902.
2. Abebe, G., (2005): Trypanosomosis in Ethiopia. *Ethiop. J. Biol. Sci*., 4(1): 75 – 121.
3. Afewerk, Y; Clausen, P-H; Abebe, G; Tilahun, G. and Mehlitz, D. (2000): Appearance of multiple-drug resistant Trypanosoma congolense populations in village cattle of Metekel district, North-west Ethiopia, *Acta Trop*. 76: 231-238.
4. Argaw, T. and Abebe, G. (1988): A survey of trypanosomiasis in Gamo Gofa Region, Ethiopia. Revue Elev. *Med. Vet.* Pays Trop. 41 (3): 271 – 276.
5. B.Goossens, H. Mbwambo, A.Msangi, D.Geysen, M. Vreysen, 2005. Trypanosomosis prevalence in cattle on Mafia Island (Janzania).
6. Baggish, A. L., hill, D. R. antimicrob. Agebts chemother. 2002, 46, 1163.
7. Carlos Gutierrez, Juana Corbera, Manuel Morales, and Philippe Buscher, 2006. Trypanosomosis in goats current status. Carmichael, J., 1939.
8. Turning sickness of cattle and trypanosome theileri. Parasitology 31, 498-500.
9. Causen, P.,H., adeyemi, I., bauer, B., breloeer, M., salchow, F., staak, C., 1998. Host preferences of tsetse (dipteral:glossini dae) based on blood on bloodmeal identifications. Med. Vet. entomol, 12, 169-180.
10. Claudia Valente, Rui moreira, Rita C. Guedes, Jim Iley, Mohammed Jaffar and Kenneth T. Douglas, 2007. The 1, 4 – naphthoquinone seaffold in the design of cysteine protease in hibitors. http:///www
11. Clause, P.H., adeyemi,l., bauer,B., breloeer,M., salchow, F., staak, C., 1998. Host preferences of tsetse based on blood meal identifications. *Med. Vet. Entomol*. 12, 169-180.
12. Codjia, V., woudyalew, M., majiwa, P.A.O., leak, S.G.A., rowlands, G.J., authie, E., d'iteren, G.D.M., peregrine, A.S., 1993. Occurrence of populations of trypanosima congolense resistant to diminazene, isometamidium and homidium in the ghibe valley, southwest ethiopia. *Acta trop.* 53, 151-163.
13. Daya, T. and Abebe, G., 2008 seasonal dynamics of tsetse and trypanosomosis in selected sites of southern nation nationalities and peoples regiona, l state, Ethiopia. *Ethiopian Veterinary Journal,* 12 (2), P. 72-92.
14. Desquensnes, m., dia, m, l.,2003a. trypanosoma vivax: mechanical transmission in cattle by one of the most common African tabanids, atylos, agrestis, *exp. parasitol*. 105, 226-231.
15. Desquesnes, M., dia M, L., 2003b. mechanical transmission of trypanosome Congolese in cattle by the African tabanid, atylos agrestis. *Exp. Parasitol*. 103, 35-43.
16. Desquesnes, M., dia, M, L., 2004, mechanical transmission of trypanosome vivax in cattle by the African tabanid, atylos fuscipes, *vet, parasitol.* 119, 9-19.
17. Desquesnes, M., Dia, M.L., 2003. Trypanosoma vivax: mechanical transmission in cattle by one of the most common African tabanids, Atylotus agrestis. *Exp. Parasitol*. 103, 35–43.
18. FAO, 1982. In: Pollock, JN. (ed), tsetse control training manual, vol. 1. Tsetsebiology, systematic and distribution, techniques, food and agriculture organization of the united nations, rome, p. 280.
19. Ford, j., 1971. The role of trypanosomiasis in African ecology. A study of tsetse fly problem. Clarendon press, oxford, p. 568.
20. Ford, J., makin, M.J., girmble, R.J., 1976. Trypanosomiasis control programme for Ethiopia. Report to the Ethiopian government of the British technical assistance team appointed to determine a programme for tsetse fly and trypanosomosis control and subsequent land use in south-western Ethiopia. Ministry of overseas development of Great Britain, p. 127.
21. Geets, s., holmes, p.h., 1998 drug management and parasite resistance in bovine trypanosomiasis in Africa. PAAT technical sciences series, no. 1, FAO, rome, p, 31.
22. Getachew, A., 2005. Review article trypanosomosis in Ethiopia. *J. boil. Sci*. 4 (1): 75 -121.
23. Goossens, B., S. osier & S. kora. 11997. Long-term effects of an experimental infection with tryapanosoma conglense on productive performance of trypanotolera NT djallonke ewes and west African does. Res. Vet. Sci. 63: 169-173.
24. Griffin, L. 1978. African trypanosomosis in sheep and goats: a review. Vet.bull. 48: 819-825.
25. Habtamu, B., 1993. Host, trap and odour bait preferebce determination of tsetse flies (g… submorsitans) in the upper didessa vally southwest ehtiopia. Msc thesis. A.A. university school of graduate studies, 65 pp.
26. Harley, J.M.B., 1966 studies on age and trypanosome infection rate in females of glossina pallidipes aust., G. pa; pa; is fuscipes newst. And g.brevipalpis newst. Bull entomol. Res. 57, 23-37.
27. Ilemobade, A.A 1986. tripanodomiasis (nagana, samore, tsetse fly disease). In current veterinary therapy, food animal practice, 2nd ed. J. howaed, ed., 642-645. Wb saunders. Phi; Adelphia.

28. Jordan, A.M., 1986. Trypanosomiasis control and African rural development. Longman, London, p.357.
29. Kramer, J.W. 1996.icidence of trypanosomes in the west African dawarf sheep and goat in nsukka, eastern Nigeria. Bull. Epizoot. Dis.afr.14: 423-428.
30. Kristjanson, P.M., B.M. swallow, G.J. rowlaands, et al. 1999. Measuaring the costs of African animal trypaanosomosis, the potential benefits to control and returns to research. Agr. System 59: 79-98.
31. Kuzoe, F.A.SA 1991 perspective in research on control of African trypanosomiasis. *ann. trop. Med. Parasitol.* 85:33-41.
32. Leak, S.G.A., 1998. Tsetse biology and ecology. In: their role in the epidemiology and control of trypanosomosis, cabi publishing, Kenya, 568pp.
33. Leak, S.G.A., rowlands, G.J., 1997. The dynamics of trypanosome infections in natural populations of tsetse (dipteral: glossindae) studied using wing-fray and ovarian ageing techniques. Bull. Entomol. Res. 87, 273-282.
34. Lemecha, H., W. Mulatu, I. Hussein, E. Rege, T. Tekle, S. Abdicho, W. Ayalew, 2006. Response of four indigenous cattle breeds to natural tsetse and trypanosomosis challenge in the Ghibe valley of Ethiopia. Veterinary Parasitology, 141: 165–176.
35. M Abwambo, H.A., ngovi, C.J., bushiri, P.S., mella, E.S., 2000. Balidation of antibody ELISA for use in combination eith parasitological tests in monitoring trypanosomosis control programmes. In: animal trypanosomosis: diagnosis and epidemiology. Resuls of a FAO/IAEA co-ordinated research programme on the use of immunoassay methods for imptoved diagnosis of trypanosomosiaas and monitoring tsetse and trypanosomosis control programmes, IAE, Vienna, Austria,pp. 165-175.
36. Majiwa, P.A., thatthi, R., moloo,s.k., kyeko,j.h. otieno, l.s., maaloo.S.,1994. Detection of trypanosome infections in the sakuva of tsetse flies and buffy-coat samples from antigenaemic but aparasitaemic cattle parasitology 108, 313-322.
37. Makumyaviri, A.M. 1991`. Epidemiological importance of the experimental host and of the animal reservoir of trypaanosoma brucei gambiense. *Rev. med. vet* 141:873-875.
38. Mehlitz, D., tiethen, U., 1988. Trypanosome infection rates in tsetse midgets using a short-term in vitro vculture technique acta trop. 45, 183-184.
39. Merid Negash, Melaku Girma, Emiru Seyoum, 2007. Spizootiological importance of glossina moristans submorsistant (Diptera: Glossinidae) (Newstead) in the ghibe river valley, south west Ethiopia.
40. Mihok, s., olubayo, r.o., darji, n., zweygarth, e., 1993. The influence of host blood omn infectieon rates in glossina morsitans spp. Infected with trypanosome cngolense, t. brucei and t. stmiae. Parasitology 107, 41-48.
41. Moloo, S.K., 1993. The districutieon of glossina species in Africa and their natural hosts. Insect sci. appl.4, 511-527.
42. Moloo, S.K., kutuza, s.b., desai, J., 1988. Infectieonrates in strile malesa of moritans, pa;pa;is and fusca groups glossina for pathogenic trypanosome species for east and west Africa. Acta trop.45, 145-152.
43. Morlais, I., grebaut, P., bodo, J.M., djoha, S., cuny, G., herder, S., 1998. Detection and identification of trypanosomes by polymerase chain reaction in wild tsetse flies in Cameroon. *Acta trop*. 70, 109-117.
44. Moser. D.R., cook, G.A., ochs, D.E., bailey, C.P., mckane, M.R., donelson, J.E., 1989. Detection of trypanosome congolense aand trypanosome brucei subspecies by DNA amplification using the polymerase chain reaction. Parasitology 99, 57-66.
45. Murray, M., murray, P.K., mclntyre, w.i., 1977. An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans.r.soc. trop. Med. Hyg. 71, 325-326.
46. Murray, M., W.I. Morrison & D.D. whitelaw. 1982. Host susceptibility to African tryapnosomiasis: trypanotolerance. *Aadv. Parasaitol*. 21:1-68.
47. Nishikawa, Y., carr, B. I., wang, M. kar, S., finn, F.. doed, pzheng, Z.B., kerns, l., naganathan, S.J. boil. Chem. 1995, 270, 28304.
48. NTTICC, (1996): National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC), Annual Report, Bedelle, Ethiopia. pp. 29.
49. Ouma, J.O., masake, R.A., masiga, D.K., 2000. Comparativity of dot-ELISA. PCR and dissection method for the detection of trypanosome infections in tsetseflies (dipteral: glossinidae). Actatro. 75, 315-321.
50. Rawlings, P., ceesat, M.L., wacher, T.J., snow, W.F., 1993. The distribution of the tsetse fliesglossina morsitaans submorsitans and g. palplis gambiensis (dipteral: glossinidae) in the gambia and the application of survey results tsetse and trypanosomosis control bull. Ent. Res. 83, 625-632.
51. Rogers, D.J., 1983. Trypanosomiasis ‘risk or challenge: a review with recommendations

livestoke productiveity and trypanotolerance. In: network training manual. ILCA addis ababa, ethiopia. Pp. 101-127.

52. Solano, P., guegan, J.F., Thomas,F., 2001. trying to predict and explain the presence of African trypanosomes in tsetse flies.J. parasiatol. 87,1058-1063.
53. Uileberg. G 1998 afield guaid for the diagnosis, treatment and prevention of African trypanosomiasis, FAO, romme. P. 158.
54. Verber, D.F., Thompson, S.K. curr. Opin. Drug discov dev. 2000, 3, 362.
55. Ward, W.H., hill, MW.M., mazlin, I.D., foster, C.K., 1984. Anaemia associated with a high parasitaemia of trypanosome theileri in a dairy cow. Aust. Vet. J. 61 (10).
56. Wells, E.A., 1972. The importance of mechanical transmission in the epidemiology of naagana: a review. *Trop. Anim. Hlth*. Prod.4, 74-88.
57. Wilson, A.J., dar, FK., paris, J., 1972. A study on the transmission of salivarian trypanosomes isolated from wild tsetse flies. *Trp.anim.hlth*. prod. 4, 14-22.
58. Witola, W.H. & C.E.A. lovelace. 2001. Demonstration of erythrophaagocytosis in trypanosaoma congolense-infected goats. *Vet. parasitol.* 96:115-126.
59. Woom P.T.K. 1996. The haematocrit centrifuge for the detection of trypanosopmes in blood. *Can.j.zool*.47:921-923.
60. Yvonne Gall, Tanja Woitag, Burkhard Baver, Issa Sidibe John McDermott, Dieter Mehlitz, seter Henning clause, 2004. Trypansocidal failure suggested by pcr results in cattle field sample.

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