

# Prevalence Of Bovine Trypanosomosis In Shebe-Sombo District Of Oromia Regional State, South West Of Ethiopia.

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**Abstract:** The study on prevalence of bovine trypanosomosis was conducted in Jimma Zone Shabe Sombo district from November 2007 to April 2008. In this study area 5 villages were involved. The diagnostic technique used included examination of packed cell volume (PCV), heamatocrit centrifugation technique (buffy coat). From the examined 780 cattle 111 were found infected with different species of trypanosomes with the overall prevalence 14.23%. The predominant species involved in the infections were *Trypanosoma congolense* (79.3%) followed by *Trypanosoma vivax* (9.9%), *Trypanosoma brucei* (1.8%), *Trypanosoma theileri* (0.9%). Mixed infections were also detected which accounted 8.1% out of the total infection rate of 14.23%. Parasitaemic and aparasitaemic animals showed significant difference in PCV values ( $p < 0.001$ ) that is; 32 and 11 are maximum and minimum values of parasitaemic animals whereas 46 to 12 are maximum and minimum values of aparasitaemic animals, respectively. First and above all I would like to thank my savior "Jesus Christ" who saves me from all things in the duration of my study and enabled me to complete this study successfully. I would like to thank Dr Waktole Terfa, Ato Mengistu Namera, Ato Tesfaye Mulatu and Ato Mulugeta Desta for their valuable advice, encouragement and provision of material during my work. I am grateful to staff of Bedelle Regional laboratory and NTTICC, specially help rendered by W/O Fatuma Hassen, Ato Tesfaye, Ato Tadale, Ato Abebe, Ato Getachew, W/O Zelalem, Ato Asfawu, Ato Bultuma, is highly appreciated for the collection of the data used in this investigation. Without their skill and cooperation the work would certainly not have been done. I would like to express my gratitude to Dr Kefelegn Kebede for his assistance in data analysis. Finally I wish to express my thanks to my Mother Maftuku Iddosa and my brothers Teacher Misganu Bula, Gizachew Bula, Tashoma Bula, Lencho Bula Tarafa Bula and my sister Gaddise Bula for their financial support as well as constructive advice during the five years of study period.

**Key Words:** Cattle, Trypanosomosis, Prevalence, Shabe Sombo Woreda,

## 1. INTRODUCTION

Trypanosomosis is one of the major constraints for livestock productivity in sub-Saharan Africa. Only trypanotolerant breeds survive, reproduce and remain productive without treatment in tsetse-infested areas [Murry *et al.*, 1982]. The impact of the tsetse associated disease, trypanosomosis, extends in sub-Saharan Africa over some 10 million km<sup>2</sup> and of this 7 million sq. km are covered by very good grazing area which perhaps fortunately have been protected so far by tsetse fly against grazing [Uilenberg, 1998]. In Ethiopia tsetse transmitted trypanosomosis is widely distributed in western, south-western and southern lowlands and the river valleys cutting into the central highlands of Ethiopia [Getachew, 2005]. Tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33<sup>o</sup> and 38<sup>o</sup> E and latitude 5<sup>o</sup> and 12 N<sup>o</sup> which together amount to 97,855 km<sup>2</sup>. Tsetse infested areas lie in the lowlands of Abay -Didessa, Baro - Akobo, and Ghibe - Omo river system and the area extends from the southern part of the rift valley, around the south-western corner of the country and along the western lowlands and escarpments to the Abay [Nile River]. Further eastward spread is restricted by the cold limit imposed by highlands that rise to the height above which tsetse could not survive, or the semi-desert condition along the southern border east of the rift valley [Langridge, 1976]. Studies conducted on tsetse-transmitted trypanosomosis showed prevalence of 20-30% in cattle [Abebe and Jobre, 1996], 5-7 % in small ruminants [Dinka and Abebe, 2005] and 20-25% in

equines [Assefa and Abebe, 2001]. Among the non-tsetse transmitted trypanosomes, the prevalence of surra ranged from 10-15% in camels [Tekle and Abebe, 2001] and mechanically transmitted *T. vivax* infection in cattle ranged from 6-10% on the highlands of Ethiopia. Seroprevalence of dourine in horses of Arsi-Bale highlands ranged from 20-28% [Ashenafi, 2005]. The distribution tsetse flies and also the prevalence of the associated disease trypanosomosis in a given area show variation through time. Therefore, the main objectives of this study is to determine the current prevalence of bovine trypanosomosis, to compare the degree of anaemia based on packed cell volume [PCV] values of infected and non-infected animals and also to identify the dominating trypanosome species in the studied area; Shabe Sombo district.

## 2. MATERIALS AND METHODS

### 2.1. Study Area and Study population

The study was carried out in Shebe - Sombo district, which is found in Jimma Zone of Oromia region southwest of Ethiopia about 420 km distant from Addis Ababa. Gojeb River which eventually drain into Abay - Didesa river system support the growth of vegetation that could harbor tsetse flies and its reservoir host animals in the studied area. The study population is bovine which is found in the Shabe Sombo district comprising 28,369 [CSA, 1998]. Out of this 780 samples which can

represent the target population were selected with random sampling technique regardless of species, age and sex.

### 2.2. Study Design, Sample Size and Sampling Method

A cross-sectional investigation of bovine trypanosomosis was carried out in selected Peasant associations of Shebe - Sombo district from November 2007 to April 2008. Blood samples were collected randomly using heparinised capillary tube after puncturing ear vein of the sampled animal. The sample size was determined according to the formula given by Thrushfield [1995] ( $n = 1.96^2 \times P \exp. [1-P \exp.] / d^2$ ). Assuming the prevalence expected [P exp.] 21% and the desired absolute precision value [d] 0.03 and precision range being 5% and it become 708 bovines.

### 2.3 Study Methodology of trypanosomosis

Blood samples were collected from an ear vein using a heparinised micro-haematocrit capillary tube. The tube was sealed and centrifuged for five minutes at 12,000 rounds per minute [rpm] and packed red cell volume [PCV] was measured with microhaematocrit reader before the tube was cut about 1mm below the buffy coat. Fresh preparations of the buffy coat were examined microscopically under phase contrast illumination for the presence of live trypanosomes. Giemsa stained thick and

thin blood smears were also prepared and examined microscopically [Murray *et al.*, 1977].

### 3. Data Analysis

Statistical discovery software [JMP5] computer program was used to store the data and to conduct Chi-square statistical analysis.

### 4. Results

In the study area of Shebe - Sombo a total of 780 cattle were examined, out of these 111 animals were found infected with different species of trypanosomes and the overall prevalence rate of trypanosomosis was found to be 14.23% [Table 1]. The trypanosoma species encountered with their respective prevalence rate were *Trypanosoma congolense*[79.3%], *Trypanosoma vivax*[9.9%], *Trypanosoma brucei* (1.8%) and *Trypanosoma theileri*[0.9%] [Table1]. Therefore, *T. congolense* is the predominant species in Shebe - Sombo followed by *T. vivax*, *T. brucei* and *T.theileri* respectively. There was also mixed infection of 8.1% infection rate in the study area [Table 1]. There was significant difference [ $p < 0.001$ ] in PCV value of parasitaemic and aparasitaemic cattle of the study area [Table 2].

**Table 1:** Prevalence of different species of trpanosomosis in cattle of Shabe Sombo Woreda

Study area	Total no. of Animal exam.	Tryps Infection Rate	Distribution of Different tryps Spps (%)				Mixed infection rate <i>T.c</i> + <i>T.v</i> , <i>T.c</i> + <i>T.b</i> , <i>T.b</i> + <i>T.v</i>
			<i>T.c</i>	<i>T.v</i>	<i>T.b</i>	<i>T.th</i>	
Shabe Sombo	780	14.23	79.3	9.9	1.8	0.9	8.1

**Table 2 :** Comparison of mean PCV value of parasitaemic and aparasitaemic cattle in Shabe Sombo.

Site	Parasitaemic Mean pcv	Aparasitaemic Mean pcv	P-Value Significance
shabe Sombo	22.8	25.6	P< 0.001

## 5. DISCUSSION

The percentage prevalence of trypanosomes observed in the study is within the range of other previous reports of studies conducted in neighboring and similar PA'S of present study and varied from 0 to 39.8% [NTTICC, 2004]. The variation in prevalence of trpanosomosis among the PA's in the district, zone and regions of south-western Ethiopia where there are tsetse fly reports is already documented [NTTICC, 2004]. A prevalence study conducted in adjacent site of this study by NTTICC [2004] reported zero prevalence at Cheriko and Medabo to a 35/88 [39.8%] at Walla Kella, 6/97 [6.19%] at Mechi, 15/102 [14.7%] at Gasera Kakaro, 5/71 (7.04%) at Sebeka, 41/117 [35.04%] at Anja Gembo and 20/115[17.39%] at Yanga Dega. Similarly, in a wide survey conducted in Zambia, Sinyangwe *et al.* [2001] reported the prevalence in individual villages varied between 0 and 64% and this prevalence varied widely not only between villages but also between visits. A

prevalence variation that lies between 0 and 43% has also been reported by Mwambo *et al.* [2000] in Tanzania. Except for a single report by Anwar [1993] at Limu Korsa Zuria district that documented a prevalence of 39.8% in one village, other reports are below the 19.2% of the present study. This variation among PA's of prevalence in the present study could be attributed to the biting fly and tsetse population and a type present in each locality, which is dependent on microclimate, animal herd density, distance between herds and other various factors [Foil, 1996]. Results on hematological values reported in the present study where *T. congolense*, *T. vivax*, *T. brucei* and *T. theileri* are the species of *trypanosoma* encountered in cattle of the five PA's .The study also shows *Trypanosoma congolense* is the predominant species in the study areas . Ford [1964] related the predominance of *T. congolense* over *T. vivax* to the prevalence of *glossina* in an area, since the transmission of *T. congolense* is mainly cyclical, requiring the presence of tsetse flies,

whereas the transmission of *T. vivax* is more readily accomplished mechanically by vectors other than tsetse flies. Langridge [1976] describes that in east Africa where tsetse flies of the morsitans group [*Glossina morsitans*, *Glossina pallidipes*] are present later is usually predominant over the farmer, whereas if the palpalis group are the only transmitter, the reverse will be true. *Trypanosoma congolense* is an introvascular parasite of mammals and is unevenly distributed in the blood of its host (Swallow, 2000). And Ssengonga [1980] also indicated that *T. congolense* was confined to the blood vessels particularly the capillaries and was not found in the fluid of the body cavities nor lymph as the *brucei* or *vivax* group trypanosomes which are from his studies in mice and rats. If this is so, the chance of detection of *T. congolense* in peripheral blood of animals infected is higher than to the other group of trypanosomes. It is indicated that *T. vivax* infection represents 9.9% Shabe Sombo out of 14.23% overall prevalence. And the infection rate is very low as compared to the infection of *T. congolense* in the study area. It may be attributed to microscopical examination of blood films for detection of *T. vivax* group infections, particularly in longstanding cases where there is a low parasitaemia has been known for long time. He has further opined that if infections are diagnosed by the blood film technique alone and if gland smear had not been taken at the same time as the blood films, atleast 27% of the infections could have been missed. Another factor which could depress the incidence of *T. vivax* is the use of drugs. In East Africa, *T. vivax* is generally less virulent than *T. congolense* and consequently cattle develop a tolerance to the farmer more easily than to the later [Langridge, 1976]. And there is possibility of self-cure which could partly contribute to the undiagnosis of *T. vivax* during the study. It is known that the development of anaemia is the most reliable indicator of the progress of trypanosome infection [ILCA/ ILRAD; 1988]. But it can also be assumed that numerous concurrent diseases and nutritional factors interfere with the anaemia development [OAU/ STRC; 1979]. And PCV values are a reliable indicator of anaemia, the degree of anemia as measured by the PCV was profound [ $p < 0.001$ ]. Such significant difference in PCV of cattle due to trypanosomosis in ruminants is available in various studies carried out so far and that of trypanosoma infections is given in the literature edited here [Defly *et al.*, 1988; D' Ieteren *et al.*, 1988; Maloo *et al.*, 1988; Mulatu *et al.*, 1988; Ordiner *et al.*, 1988; Getinet, 1994; Mihiret, 1995; Abebe and Jobre, 1996; Kalu, 1996; Enyew and Abebe, 1997; Terefe and Abebe, 1999; Aklilu, 2002]. Taylor [1998] indicated that anemia persists during the chronic stages of infection when parasitemia is generally quite low, probably because different mechanisms are involved in its genesis during the acute and chronic stages of infection. This suggests that control of parasite and control of anemia is unrelated in the chronic phase when immune infections are depressed and anemia is sustained through erythropoiesis. During PCV determination, a value of 24-46 (Blood and Radostits, 1989), was considered to be normal. It is also found from the result [table 2] the difference in the mean PCV of aparasitaemic and parasitaemic cattle was statistically significant. This analysis shows statistically significance differences [ $P < 0.001$ ] between the mean PCVs of parasitaemic and

aparasitaemic cattle in Shabe Sombo. Trypanosomes couldn't be shown in many cattle with low PCV, conversely many cattle having high PCV were found infected. Regarding the case of apparently trypanosoma free cattle with low PCVs could be due to numerous concurrent diseases and nutritional factors aid in the development of anaemia as described above by [OAU/STRC, 1979]. But animals having high PCVs also showed to be infected, this may be attributed to recent infection. And whenever mixed infection of trypanosomosis *T. vivax* and *T. congolense*, *T. congolense* and *T. brucei*, *T. brucei* and *T. vivax* was detected [table 1] parasitaemia caused by either of these three trypanosome species was usually very scanty and there was no an occasion when both of these species were observed to occur in equal proportions in blood, hence, the few numbers of cattle which suffered mixed trypanomes infection might have been attributed in favour of a single infectio; it has been elaborated that mixed trypanosome infections are easily overlooked, since the presence of one species seems to have an inhibitory effect on the other that might be present; with the result that some times one species may suppress the other more or less permanently or else they may have alternate period of predominancy. Trypanosomosis was found in different peasant associations in the district, and it seems that species (host) are important factors for the development of infection and the disease as mostly detected in cattle. In general, the present findings indicate that trypanosomosis due to *T. congolense*, *T. vivax*, *T. brucei* and *T. theileri* are established in the five peasant association affecting cattle productivity.

## 6. CONCLUSIONS AND RECOMMENDATIONS

- Results obtained from this study show that trpanosomosis is a very important disease that brings about great economic losses to livestock owners in Shabe Sombo settlement, by the fact that many animals, particularly cattle, are dying in the area because of this disease. In addition to this in chronically infected animals, milk and meat production is greatly reduced and the animals are too weak to be used for ploughing.
- The disease affected each and every household in the area and thus socio- economic impact of the disease appears to be a single most important constraint to improve livestock productivity in the area.
- In the current trpanosomosis prevalence study I realized the risk of trpanosomosis is higher in PA's like Walla Kella, Mechi, Gasera Kekero, kishae and Hangacha which have high potential for agricultural activities. Therefore attention should be given by all concerned organization to alleviate the problem before the pressures evacuate the settlers.
- Until the responsible body designs a long- term vector control, strengthening the veterinary service to promote appropriate drug treatment in the affected areas is inevitable for the moment.

## 7. CONFLICT OF INTEREST

I have no conflict of interest

## 8. REFERENCES:

- [1] Abebe, G. and Jobre, Y. 1996. Trypanosomosis: A threat to cattle production in Ethiopia *Revue Med.vet.* **147** [12]: 887-902.
- [2] Aklilu, N. 2002. Study on Bovine Trypanosomosis in Selected Sites of Central and Western Tigray, Ethiopia. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia, DVM Thesis.
- [3] Anwar, N., 1993. Prevalence of bovine trypanosomosis in tsetse protected and unprotected areas of the upper Didessa valley. Faculty of Veterinary Medicine, Addis Ababa University, Debre zeit, Ethiopia, DVM thesis.
- [4] Ashenafi, H. 2005. Serological and parasitological survey of dourine [Trypanosoma Equiperdum] in selected sites of Ethiopia. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre zeit, Ethiopia.
- [6] Assefa, E. and Abebe, G. 2001. Drug resistant *T. Congolense* in naturally infected donkey in north Omozone, Southern Ethiopia, *Vet.Parasitol* ; **99**:261-271
- [7] Blood, D.C., and Radostits, O.M., 1989. **Veterinary Medicine**, A Textbook of Diseases of Cattle, Sheep Pigs, Goats, and Horses. 7<sup>th</sup> ed. ELBS. Oxford, pp.1012-1025.
- [8] Central Statistical Authority, 1998. Agricultural sample survey 1997/98. **2**: Statistical bulletin number 193. Addis Ababa, Ethiopia. 267.
- [9] Defly, A., Awuome, K., Bokavi, K., D'Ieteren, M.G.D, Grundler, G., Handlos, M., Itty, P., Leak, S.G.A., Maehl J.H.H., Mawuena, K., Morkramer, G., Nagda, S.M., Paling, R.W., Rarieya, J.M., Thorpe, W., and Trail, J.C.M. 1988. Effect of trypanosome infection on livestock health and production in Togo. In: *Livestock Production in Tsetse Affected Areas of Africa* [ILCA/ILRAD Proceedings, 1987, Kenya], pp. 251-256.
- [10] D'Ieteren, M.G.D, Awuome, K., Bokavi, K., Chema, S., Colardelle, C., Coulibaly, L., Defly, A. Feron, A., Grundler, G., Handlos, M., Hecker, P., Itty, P., Kakiese, O., Leak, S.G.A., Maehl, J.H.H., Maloo, S.H., Mawuena, K., Minengu, M., Morkramer, G., Mukendi, F., Mulatu, W., Mulungo, M., Nagda, S.M., Nankodaba, G., Ngamuna, S., Ordner, G., Paling, R.W., Rarieya, J.M., Shhuetterle, A., Sheria, M., Thorpe, W., Tikubet, G., Trial, J.C.M., and Yangari, G. 1988. Trypanosome infections and other factors influencing PCV in livestock. In: *Livestock Production in Tsetse Affected Areas of Africa* [ILCA/ILRAD Proceedings, 1987 [Kenya], pp.61-167.
- [11] Enyew, M. and Abebe, G. 1997. Bovine Trypanosomosis in South Gonder Administrative Zone Bordering Lake Tana [Ethiopia] in the Apparent Absence of Glossina. *J. of the Ethiopian Vet. Assoc.* **I**. (No.1): pp.19-34.
- [12] 25 Foil, L.D. 1996. Biology and Control of Tabanids and Stable Flies. In: *Proceedings of the first symposium on new world trypanosomes*. Georgetown, Guyana, November 20-22, [1996]. Inter-American Institute for Cooperation on Agriculture [IICA]. Technical Cooperation Agency. In: Barbados Eds. Sandra Vokaty and Marc Desquesnes. pp. 66—71
- [13] Ford, J. 1964. *Bulletin of Epizootic Diseases of Africa*, Pp12-307.
- [14] Getachew, A., 2005. Trypanosomosis in Ethiopia, Faculty of Veterinary Medicine Addis Ababa University. Debre zeit. 18-20.
- [15] Getinet, Y. 1994. Prevalence of Bovine Trypanosomosis in Debre Markos District of Eastern Gojjam Administrative Zone. Faculty of Veterinary Medicine Addis Ababa University, Debre Zeit, Ethiopia, DVM Thesis. ILCA/ILRAD; 1988. *Livestock production in tsetse affected areas of Africa*, Nairobi; Kenya.
- [16] Kalu, U.A. 1996. Current status of tsetse fly and animal trypanosomosis on the Jos plateau, Nigeria. *Prev. Vet. Med.* **27**:107-113.
- [17] Langridge, W.P. 1976. A tsetse and trypanosomosis survey of Ethiopia. Ministry of Overseas Development of British and Ministry of Agriculture of Ethiopia. p 97.
- [18] Maloo, S.H., Chema, S., Connor, R., Durkin, J., Kimotho, P., Maehl, J.H.H., Mukendi, F., Murray, M., Rarieya, J.M. and Trial, J.C.M. 1988. The use of chemoprophylaxis in east African zebu village cattle exposed to trypanosomosis in Muhaka, Kenya. In: *Livestock Production in Tsetse Affected Areas of Africa* [ILCA/ILRAD Proceedings, 1987, Kenya]. pp.283-288.
- [19] Mihiret, A. 1995. Survey on the prevalence of bovine trypanosomosis in and around Bahir Dar, Ethiopia. Faculty of Veterinary Medicine Addis Ababa University, Debre Zeit, Ethiopia, DVM Thesis.
- [20] Mulatu, W., D'Ieteren, M.G.D, Duffera, W., Girma, T., Itty, P., Leak, S.G.A., Maehl, J.H.H., Nagda, S.M., Paling, R.W., Rarieya, J.M., Thorpe, W. and Trial, J.C.M. 1988. Health and performance of zebu cattle exposed to trypanosomosis risk in SW Ethiopia. In: *Livestock Production in Tsetse Affected Areas of Africa* [ILCA/ILRAD Proceedings, 1987, Kenya]. pp. 257-261.
- [21] Murray, M., Morrison, W.I. and Whitelow, D.D. 1982. Host susceptibility to African trypanosomosis: trypanotolerance. *Advan.Parasitol*, **21**:1-68

- [22] Mwambo, H., Ndung'u, J.M., Murilla, G.A., Munga, L., Sanyangwe, L., Machina, N., Holmes, P.H., and Eisler, C.M., 2000. Trypanocidal drug resistance in Tanzania. In: International Scientific Council for Trypanosomosis Research and Control [ISCTRC], 25th meeting, Mombasa, Kenya, 1999. Pub. OAU/STRC. pp. 168—174].
- [23] NTTICC, 2004. Annual report for the period 7<sup>th</sup> June 2003 to 6<sup>th</sup> July 2004. Bedelle. 1
- [24] OAU/STRC.1979 Bull. Anim. Hlth. Prod. Afr. **27**: [No.3]: 43-46.
- [25] Ordiner, G., Colardelle, C., D'Ieteren, M.G.D., Dumont, P., Itty, P., Jeannin, P., Leak, S.G.A.,
- [26] Maehl, J.H.H., Nagda, S.M., Paling, R.W., Rarieya, J.M., Thorpe, W., Trail, J.C.M., and Yangari, G. 1988. Health and productivity of trypanotolerant and susceptible cattle exposed to trypanosomosis in Gabon and the impact of strategic chemoprophylaxis. In: Livestock Production in Tsetse Affected Areas of Africa [ILCA/ILRAD Proceedings, 1987, Kenya]. pp. 310-317.
- [27] Radostits, O.M., Gay.C.C., Blood, D.C, and Hinchcliff, K.W. 2000. Veterinary Medicine, A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9<sup>th</sup> ed. Harcourt Pub, Ltd. London.
- [28] Sinyangwe, L., Machila, N., Mubanga, J., Delespaux, V., Brandt, J., Geerts, S., Holmes, P.H., and Eisler, M.C. 2001. Trypanocidal drug resistance in eastern province of Zambia. In: International Scientific Council for Trypanosomosis Research and Control (ISCTRC), 25<sup>th</sup> meeting, Mombasa, Kenya, 1999. Pub. OAU/STRC. pp. 165-167.
- [29] Ssengago, 1980. A comparative study on the distribution of *Trypanosoma brucei* and *Trypanosoma congolense* in tissues of mice and rats Bull. Anim. Hlth. Prod. Afr. **28**: 312-326
- [30] Swallow, B. 2000. Impact of trypanosomosis on African Agriculture, PAAT Technical and Scientific series. No.2, FAO, Rome. Taylor, K.A., 1998. Immune responses of cattle to African trypanosomes: protective or pathogenic? International J. Parasitol. **28**: 219-240.
- [31] Tekle, T. and Abebe, G. 2001. Trypanosomosis and helminthoses; major health problems of camels (*Camelus dromedaries*) in the southern Rangelands of Ethiopia. J.Com.prac.Res. **8**[1]: 39-42
- [32] Terefe, G. and Abebe, G., 1999. Prevalence of bovine trypanosomosis in two Woredas of western Gojjam Zone, Amhara Region. J. Ethiopian Vet. Assoc. **III** [I]:1-8.
- [33] Thrushfield, A.1995. Veterinary Epidemiology. 2<sup>nd</sup> Ed. Blackwell Pub, Edinburgh. Uilenberg, G. 1998. Veterinary Parasitology, 1<sup>st</sup> ed. Pharmaceutical Press, London.