

Epidemiology of Bovine Trypanosomosis in Assosa, North-West Ethiopia

Fedasa Mammo, Teka Feyera and Ayalew Niguse

Jigjiga University, College of Veterinary Medicine, P.O.Box 1020, Jigjiga, Ethiopia

Abstract: Cross-sectional study was conducted in Assosa district of Benishangul Gumuz Regional State, North West Ethiopia from November 2011 to March 2012. The objective of this study was to assess the prevalence and incriminated species of bovine trypanosomosis and its possible association with different risk factors. In this parasitological survey, blood samples of 385 cattle were examined using buffy coat technique. The packed cell volume (PCV) value of each animal was also measured using hematocrit reader. In the present study, the overall prevalence of bovine trypanosomosis was found to be 22.6%. The most positive cases were recorded due to *T. congolense* (58.6%), followed by *T. vivax* (17.2%), *T. brucei* (10.3%), *T. congolense* and *T. vivax* (10.3%), *T. congolense* and *T. brucei* (2.3%) and mixed infection of the three species (1.1%) respectively. The PCV value of parasitemic animals is slightly lower than that of aparasitemic animals which is statistically significant ($P < 0.05$). It was found that the prevalence was higher in older age groups when compared to other age groups. This was, however, not statistically significant ($\chi^2 = 0.3.7$). Similarly, no statistically significant association between the two sexes was found ($\chi^2 = 0.817$). The owners' interviews revealed that trypanosomosis was considered a major problem in the study area. From this study, it is possible to conclude that trypanosomosis is an important disease and a potential threat to the health and productivity of cattle in the study area. The species of trypanosomes that affect cattle in the study area include: *T. congolense*, *T. vivax* and *T. brucei*.

Key words: Bovine • Trypanosomes • PCV • Prevalence • Assosa • Ethiopia

INTRODUCTION

Trypanosomosis is a disease of paramount importance both to human and livestock that causes negative impact on food production and economic growth in many parts of the world, particularly in Sub-Saharan Africa [1, 2, 3]. It is caused by unicellular parasites (Trypanosomes) found in blood and other tissues of vertebrates including human, livestock and other wild life [2, 3].

Its epidemiology and impact on cattle production is determined largely by the prevalence and distribution of the disease and its vectors in the affected area [8]. Epidemiologically trypanosomes are distributed in the tropical Africa in the latitude of 14°N and 29°S where they are associated with their vectors Glossina, the tsetse fly [7]. The vectors tsetse fly (Glossina species) inhabits wide-range of habitats in African continent and affecting

37 countries including Ethiopia. According to Feyesa [9], the general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetations and the presence of suitable host animals. Currently, about 220,000km² area is infested with tsetse flies namely Glossina species [10].

In Ethiopia, Trypanosomosis is one of the most important disease limiting livestock production and agricultural development due to its high prevalence in the most arable and fertile land of South-west and North-western part of the country. According to Getachew [6], *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse-infested and tsetse free areas of the country respectively. The prevalence of *T. congolense* is found to be high in tsetse-infested areas of the country and a considerable number of the animals were also harboring *T. vivax*.

Corresponding Author: Ayalew Niguse, Jigjiga University, College of Veterinary Medicine, P.O.Box 1020, Jigjiga, Ethiopia.

Trypanosomosis is transmitted cyclically by tsetse flies, mechanically by other biting flies and also by other means of transmission [2]. The three main species of tsetse flies for transmission of trypanosomosis are *Glossina morsitans*, which favors the open land of the savanna; *Glossina palpalis*, which prefers the shaded habitats immediately adjacent to rivers and lakes; and *Glossina fusca*, which favors the high dense forest areas. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys* and *Chrysops* flies have also been implicated. In Africa, both *T. vivax* and *T. brucei* can spread beyond the “tsetse fly belts”, where transmission is principally by *tabanid* and *hippoboscid* flies. The vector for *T. vivax* in the western Hemisphere remains unknown, but several species of *hematophagous* (especially *tabanid* and *hippoboscid*) flies are believed to serve as mechanical vectors. With the single exception of *T. equiperdum* of equines which is venereal disease, all species have an arthropod vector in which transmission is either cyclical or non-cyclical [7].

The clinical sign manifestation depends on the species and strains of trypanosomes, vectors and resistance of affected breed of infected animal. The major signs are anemia, generalized enlargement of superficial lymph node/Lymphadenopathy, lethargy and progressive loss of body condition. Fever and loss of appetite occur intermittently during parasitemic peaks, the latter becoming marked in the terminal stages of the disease [7]. Photophobia and excessive lacrimation due to *T. vivax* will occur. Trypanoresistant breed can be recovered provide that the nutrition condition is good and the infection severity is low [12].

In Ethiopia, the disease is economically important; and livestock found below 2000 meters contour are exposed to various level of trypanosomes risk [13]. As a result, a total of 14.8 million cattle, 6.12 million sheep and goat, 1 million camels and 1.3 million equine are at risk of constructing trypanosomes in Ethiopia. Accordingly, the overall economic loss due to the disease was estimated to be between US\$1408 and 1540 million annually [14]. The previous study in the North-western parts of the country indicated a prevalence of 24.7% [15] in Mao-Komo special district and 45.1% [16] in Bambasi district of Assosa Administrative Zone was recorded

On the bases of the above existing facts and information, the study was aimed at achieving the objective of estimating the prevalence and incriminated species of bovine trypanosomosis and its possible association with different risk factors.

MATERIALS AND METHODS

Description of Study Area: The present study was conducted on the prevalence of bovine trypanosomosis in Assosa district of Benishangul Gumuz Regional State, which is located in the West and North-western part of Ethiopia, between latitude 9° and 11°North and longitude 34° and 35°East. Assosa is the capital city of Benishangul Gumuz Regional State and it is about 675 km from Addis Ababa. It has common boundaries with Amhara and Oromia in the north, Sudan in the west and Gambela in the South. According to the Assosa district Agricultural and Rural Development Office, the district is divided in to 78 peasant associations (PA) with total populations of 92,144. The district is located in 580- 1500 meter above sea level, wet temperature of 19°C- 34°C. The average rain fall is 900-1200mm annually and the total area of the district is about 2317km². The total livestock of the region is estimated as: 309,629 cattle, 58767 sheep, 200 470 goat 559 horses, 30 952 donkeys, 1394 mules, 15 camels and 981,196 poultry [17].

Study Population: The study animals were cattle in different PA of the Assosa district under extensive management system including both sexes and all ages. Indigenous Zebu breeds are the vast majority of animals included in the study though there are a few exotic breeds which are under intensive management system.

Study Design: Cross-sectional study was the design used for this survey. The study subjects which include all age groups and both sexes were the ones randomly selected for the study. The study animals were classified in different body conditions (good, medium and poor) according to [18], age groups (<2, 2-7 and >7yrs) according to Nicholson and Butterworth [19] and other factor including breed (local and exotic breed) was also used to classify the studied animals.

Sample Size Determination: A total of 385 blood sample were collected from cattle of selected 11 PA of the district. The sample size was determined by using 95% level of confidence interval and expected prevalence of 50% trypanosomosis with desired absolute precision of 5% and simple random sampling method was used [20]. The formula used is shown below:

$$N = \frac{(1.96)^2 \text{Pe} \text{p}(1 - \text{Pexp})}{d^2}$$

Where:

N= required sample size

Pe p= expected prevalence

d² =desired absolute precision

Laboratory and Data Collection Tools

Hematological Examination: The blood sample was obtained and collected into capillary tube; the capillary tube was then placed in microhaematocrit centrifuge with sealed end outer most. The blood sample was allowed to be centrifuged at a high speed (2000 rpm) for five minutes. Finally, the PCV values were read by hematocrit reader, which could be adjusted individually for the length of the blood column in each tube to get a value indication on the presence, absence and degree of anemia [2].

Parasitological Examination: After centrifugation, the capillary tube was cut down using a diamond tipped pen 1mm below the buffy coat to include the upper most layers of the red blood cells and 3mm above to include the plasma. The different species of the parasite were microscopically differentiated by buffy coat technique under 40x objective microscopes and by their morphological features after Giemsa staining of blood smear under 100x objective microscopes. *T. brucei* is pleomorphic, kinetoplast is small, sub-terminal and pointed and move fastly in fresh blood sample. *T. congolense* is monomorphic, no free flagella and move slowly across microscopic field; kinetoplast is medium, marginal and posterior. *T. vivax* is monomorphic, terminal kinetoplast, has free flagella, blunt posterior end [7].

Questionnaire Survey: Five respondents those have large numbers of animals from each selected 11 PA were selected and semi-structured questionnaire form was administered to get additional information about the seasonality of the disease, risk factors and its socio-economic impacts.

Data Analysis: During the study period, address, animal sex, age and body condition were recorded using the animal blood sample collection format and entered into Microsoft Excel spread sheet. Hematological and parasitological data were handled very carefully. The entered data were transferred to SPSS version 15 software and scrutinized as described in the protocol of. Chi-square

test was used to compare the prevalence of trypanosomosis in different variables and to determine the relationship between the categorical variables and the result. The prevalence of bovine trypanosome infection was calculated as the number of parasitological positive animals is examined by buffy coat method to the total population at risk [20].

RESULTS

Overall Parasitological Findings: A total of 385 cattle were selected randomly from the selected 11 PA of the district and cross-sectional study was conducted from November 2011 to March 2012 and examined for trypanosomosis. About 87 (22.6%) animals were infected with various species of trypanosomes out of the total examined animals.

Throughout the study, *T. congolense*, *T. vivax*, *T. brucei* and mixed infections were observed. From the 87 infected animals, 51 (58.6%) were due to *T. congolense*, 15 (17.2%) were due to *T. vivax*, 9 (10.3%) were due to *T. brucei*, 9 (10.3%) were due to mixed infection of *T. congolense* and *T. vivax*, 2 (2.3%) were due to mixed infection of *T. congolense* and *T. brucei* and (1.1%) were recorded for mixed infection of *T. congolense*, *T. vivax* and *T. brucei*. The most infective species of trypanosomes in the study area was *T. congolense* and followed by *T. vivax*, *T. brucei* and mixed infections respectively (Table 1).

Prevalence Within the Sexes: The prevalence of trypanosomosis is varying in both sexes; infection in male is being slightly higher than in the female. The obtained result reveals that 50 (57.5%) male and 37 (42.5%) female animals were infected with trypanosome infection and the prevalence of 22.9% and 22.3% was recorded in male and female respectively. However, this was not statistically significant ($P > 0.05$) (Table 2).

Prevalence among Age Groups: Out of the 385 examined animals, 59 were less than < 2years, 319 were 2-7 years and 7 were the animals with greater than 7 years old age. From the < 2 years (59) sampled animals, 9 (15.3%) were positive, 2-7 years (319) sampled animals, 76 (82.9%) were positive and from the >7 years old (7) sampled animals, 2 (1.8%) were positive for the disease. The current prevalence within the age groups was 15.3%, 23.8% and 28.6% in animals with < 2years, 2-7 years and > 7 years old respectively which has statistically insignificant difference ($P > 0.05$) (Table 2).

Table 1: Proportion of trypanosome species in the study area.

Trypanosomes spp.	No. of positive animals	Prevalence (%)
<i>T. congolense</i>	51	13.2
<i>T. vivax</i>	15	4
<i>T. brucei</i>	9	2.3
<i>T. congolense</i> and <i>T. vivax</i>	9	2.3
<i>T. congolense</i> and <i>T. brucei</i>	2	0.5
<i>T. congolense</i> , <i>T. vivax</i> and <i>T. brucei</i>	1	0.3
Total	87	22.6

Table 2: Prevalence of bovine trypanosomosis between sexes and among age groups

Variables	No. of animals examined	No. of positive animals	Percentage (%)	Prevalence (%)	χ^2 (P-value)
Sex					
Male	218	50	57.5	22.9	0.053 (0.817)
Female	166	37	42.5	22.3	
Total	385	87	100		
Age					
<2 years	59	9	15.3	15.3	2.36 (0.307)
2-7 years	319	76	82.9	23.8	
>7 years	7	2	1.8	28.6	
Total	385	87	100		

Table 3: Prevalence of trypanosomosis in relation with body condition

Body condition	No. of examined animals	No. of positive animals	Percentage (%)	Prevalence in %	χ^2 (P-value)
Poor	48	33	37.9	68.8	75.23 (0.000)
Medium	256	49	56.3	19.1	
Good	81	5	5.8	27.8	
Total	385	87	100		

Table 4: Prevalence of trypanosomosis according to PCV value

PCV value	Positive result	Negative result	Total	Prevalence (%)	χ^2 (P-value)
<25%	75	50	125	60	144.8 (0.000)
=25%	12	248	260	4.6	
Total	87	298	385		

Prevalence Within Body Conditions: Out of the 385 examined animals, 48 (12.5%) were poor, 256 (66.5%) were medium and 81 (21%) were good in body condition. Among the infected animals, 33 (37.9%), 49 (56.3%) and 5 (5.8%) were poor, medium and good in body condition respectively. In relation to the total number of infected animals, the prevalence of the disease is high in the animals with poor body condition which is statistically significant ($P < 0.05$) (Table 3).

Hematological Findings: The other category study considered in this study was recording of PCV value of individuals. From the 385 examined animals, 125 (32.5%) were recorded with PCV value of less than 25% and 260

(67.5%) animals were recorded with PCV value of greater and/or equal to 25%. Out of the infected animals, 75 (86.2%) cattle were with PCV value of less than 25% and cattle with greater and/or 25% PCV value were about 12 (13.8%). The above result indicates that there was statistically significant difference in PCV value between the infected and non-infected animals ($P < 0.05$) (Table 4).

Results of Questionnaire Survey: A questionnaire format was also distributed to get additional information from the owners about the seasonality of the disease, risk factors and its socio-economic impacts. Out of the 55 selected respondents, 40 (72%) owners responded that presence of the disease is detected by coughing, loss of feed intake

and rough hair coat; and the rest 28% (15 / 55) owners depend on loss of body condition to detect the disease. In about 69% (38 / 55) respondents, peak season of the disease is during rainy season, but 17 respondents' response revealed occurrence of the disease is high during the onset of rainy season.

In case where the infection occurred, they would take the diseased animal to the veterinary clinic immediately in of 83.6% (46 / 55) owners, but 9 owners would delay to take their animals to the veterinary clinic for further detection of the disease.

Twenty four respondents reported that there was immediate response following trypanocidal therapy, while 20 and 11 owners responded that there was delayed response to treatment and recurrence of the same clinical sign in a few weeks or some months later respectively. Also 39 owners lost their cattle due to bovine trypanosomosis.

Response of the 30 owners revealed US\$1, while according to 15 and 10 respondents, cost per single treatment per animal is about US\$1.2 and US\$0.75 per single treatment per animal respectively.

DISCUSSION

This study was aimed to investigate the prevalence of bovine trypanosomosis and different risk factors. The overall prevalence of the disease in the present study was 22.6%. According to the previous study, a prevalence of 24.7% [15] in Mao-Komo special district and 45.1% [16] in Bambasi were recorded in neighboring districts which are higher than the present study. This reduction in prevalence may be due to extensive or seasonal clearing of bushes, human expansion and agricultural investment in the area affecting the tsetse ecology; and seasonal factor may also be among the risk factors involved in reduction of the trypanosomosis prevalence.

From the total prevalence in this study, *T. congolense*, *T. vivax*, *T. brucei*, *T. congolense* and *T. vivax*, *T. congolense* and *T. brucei* and mixed infection of *T. congolense*, *T. vivax* and *T. brucei* accounts for about 51 (58.6%), 15 (17.2%), 9 (10.3%), 9 (10.3%), 2 (2.3%) and 1 (1.1%) of the total positive samples respectively. This revealed that the distribution of the parasite species is statistically highly significant difference ($P < 0.05$). The predominant prevalence of *T. congolense* 58.6% in present study was similar with the previous result of Abebe and Jobre [21] in tsetse- infested areas of Ethiopia (58.5%). A similar proportional trend was also reported by

Afework [22] at Pawe, North-west Ethiopia (60.9%), Terzu [23] in selected sites of Southern region (63.4%) and Muturi [24] at Mereb Abaya, South Ethiopia (66.1%). This increased proportion of *T. congolense* indicates that the area is highly infested with tsetse flies. Because, according to Getachew [6], *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in tsetse-infested and tsetse free areas of the country respectively, which the result of the present study agrees with.

The prevalence of the parasite is varied between sexes in this study, in male (22.9%) being slightly higher than in the female (22.3%), but statistically insignificant. This might be due to the fact that both sexes have virtually similar exposure to flies in grazing areas and also the disease is sex independent. The result agrees with the work of Adane [25] in and around Bahir Dar and Molalegne *et al.*[13] in Jabi Tehenan district of west Gojjam.

The prevalence of bovine trypanosomosis was studied in different age groups of cattle. However, there is no statistically significant difference ($P > 0.05$), which may be because of an equal chance of exposure to the parasite. This result also agrees with the work of Alekaw [26] and Molalegne *et al.*[13] who concluded that there is no statistically significant difference in infection between the age groups, yielding a conclusive remark that the disease is age independent. Similar findings were also reported by Cherenet *et al.* [27] and Habtamu, [28] in the Jawi district of the Amhara region, Ethiopia.

On the other hand, considering body conditions, the infection in animals with poor body condition was significantly higher than good body condition animals. This was in agreement with Abiy[29]. On one hand, the disease itself results in progressive emaciation of the infected animals; on the other hand non-infected animals under good body condition are with good 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 diseases or malnutrition, since malnutrition and concurrent infections depress the immune responsiveness in some cases [30]. This might be the indication of association between the parasite and animals body condition.

According to Getachew (2005), the development of anemia is the most reliable indicator of the trypanosome infection. The prevalence of the present study indicates that 75 (86.2%) animals are those recorded with PCV value of less than 25% out of the 87 infected animals and the

PCV value of the rest 12 animals is greater or equal to 25%. There was statistically significant difference between the animals, which agrees with the previous study of Alekaw [26] and Molalegne *et al.*[13] who reported that the mean PCV value of parasitic animals were significantly ($P < 0.05$) lower than that of aparasitic animals. This may indicate the strong association of the infection and PCV value.

The overall results of questionnaire survey indicated the trypanosomosis was considered as a major problem in the study area which causes economic loss due to cost of treatment and death of the animals. This is in line with Taylor [1], trypanosomosis in domestic livestock and costs of treatment or controlling the disease causes a significant negative impact in food production due to indirect losses.

Seasonality of the disease is at the introduction of heavy rain and during rainy season. This might be due to occurrence of tsetse flies and other biting flies in large numbers. This agrees with Feyesa, [9], the general distribution of the tsetse flies is determined principally by climate and influenced by altitude, vegetation and the presence of suitable host animals.

Response to the treatment varies among the treated animals. Some animals give response after the few days of the treatment while response in some animals is delayed and some animals show the same clinical sign after cured from the first infection which might be due to drug resistance. Response of the owners revealed US\$1 per single treatment per animal and most of the owners lost their cattle due to the disease. This agrees with NTTICC, 2002 [14], which shows the overall economic loss of the country due to the bovine trypanosomosis.

CONCLUSIONS

Bovine trypanosomosis was a threat to livestock production and productivity in the study area. It was also evidenced that local farmers were very curious of Trypanosomosis threat. Overall, a high prevalence of the disease was recorded in the study area with high occurrence of *T. congolense*, followed by *T. vivax* and *T. brucei* in decreasing order revealing a remarkable degree of mixed infections. Trypanosomosis was also negatively related to PCV and body condition of study animals. Integrated control of Tsetse and trypanosomosis through vector control, chemotherapy and prophylaxis and use of trypanotolerant animals necessitate to tackle the high burden posed by the disease in the area.

REFERENCES

1. Taylor, K.A., 1998. Immune response of cattle to African trypanosomosis: protective or pathogenic. *Inf. J. parasitol.* 28: 219-240.
2. Uilenberg, G., 1998. A field guide for diagnosis of treatment and prevention of African Animal Trypanosomosis. FAO corporate Document Repository. ISBN: 92 : 510-42381.
3. Tesfaye, M., 2002. Report of Trypanosome infection rate in G.M. Mortals and G. tachiods in Didessa valley from July 29 to September 26/2002, Bedelle.
4. Smith, B.F., 2002. Trypanosomosis 3rded. Large Animal Medicine. USA. Mosby, pp: 918-920.
5. Abebe, G., 2005. Trypanosomosis in Ethiopia, *Ethiop. J. Biol. Sci.*, 4: 75-1.
6. Getachew, A., 2005. Trypanosomosis in Ethiopia, AAU, Faculty of Veterinary Medicine, Debre Zeit, pp: 18-20.
7. Urquhart, G.M., J. Armou, A.M. Dunn and F.W. Jennings, 1996. *Veterinary Parasitology* 2nd ed. Black Well publishing, pp: 212-217.
8. PATTEC, 2001. Pan African Tsetse and Trypanosomosis Eradication Campaign. Plan of action. June, 2001. pp: 28-37.
9. Feyesa, R., 2004. Current epidemiological situation of bovine trypanosomosis in Limmu shaye Tsetse control area of upper Didessa valley. MSc thesis, AAU, Debre Zeit, Ethiopia.
10. MOA, 1995. Federal Democratic Republic of Ethiopia, Ruminant livestock development strategy. Addis Ababa, Ethiopia, pp: 13.
11. FAO, 1998. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. African animal trypanosomes. Africa, pp: 170.
12. Brown, C., D. Hunter and A. Lukins, 1990. Disease caused by protozoa. *Hand Book on Animals Disease in Tropics* (Swell and Brocklesby), 4th ed. Bailer, Tindall, London.
13. Molalegne, B., A. Yeshitla, Z. Tilahun and D. Hailu, 2010. Trypanosome infecton rate in *Glossina pallidipes* and *Glossina fuscipes fuscipes* in Gojem valley, South-west Ethiopia, *Global Veterinaria*, 6(2): 131-135.
14. NTTICC, 2002. National Tsetse-fly and Trypanosomosis Eradications Control Center.
15. Dawud, A., 2011. Clinical prevalence of bovine trypanosomosis in and around Mao-Komo special district of Benishangul Gumuz region, North-west Ethiopia. DVM Thesis, Jimma University,

16. Abebe, B., 2010. Clinical prevalence of bovine trypanosomosis in and around Bambasi district of Benishangul Gumuz region, North West Ethiopia. DVM Thesis, Mekele University
17. CSA, 2003. Central Statistics Agency of Ethiopia.
18. Nicholson, M.J. and M.H. Butterworth, 1986. A guide to condition scoring of zebu cattle. ICCA. Addis Ababa, Ethiopia.
19. MAAFRMD, 1998. Ministry of Agriculture Animal Fishery Resources Development Main Department. Fettering Extension manual. Fourth livestock development project, pp: 26-29.
20. Thrusfield, M., 2005. Veterinary epidemiology. 3rded, Black well science Ltd, pp: 233-250.
21. Abebe and Jobre Y., 1996. Trypanosomosis: A threat to cattle production to Ethiopia. *Revue de Meicine Veterinaire*. 147: 897-902.
22. Afework, Y., 2001. Field investigations on the appearance of drug resistant population of trypanosomes in Metekel district, Northwest Ethiopia. MSc Thesis, AAU with Freie University at Berlin.
23. Terzu, D., 2004. Seasonal Dynamic of Tsetse and Trypanosomosis in selected site of Southern Nation, Nationalities and peoples Regional States, Ethiopia.
24. Muturi, K.S., 1999. Epidemiology of bovine Trypanosomosis in selected site of Southern rift valley of Ethiopia, MSc thesis, AAU, with Freie University.
25. Adane, M., 1995. Survey on prevalence of bovine Trypanosomosis in and around Bahir Dar. DVM Thesis, AAU, FVM, Debre Zeit, Ethiopia.
26. Alekaw, 2003. Prevalence of trypanosomosis of cattle in three Woreda of Amahara region (un published).
27. Cherenet, T., R.A. Sani, N. Speybroeck, J.M. Panandam, S. Nadzr and P. Van den Bossche, 2006. A comparative longitudinal study of bovine trypanosomiasis in tsetse-free and tsetse-infested zones of the Amhara region, northwest Ethiopia, *Vet. Parasitology*, 140: 251-258.
28. Habtamu, G., 2009. 'Current status of tsetse transmitted trypanosomes in Jawi district of Amhara region, north-west Ethiopia', DVM thesis, Faculty of Veterinary Medicine, Gondar thesis, Gondar University.
29. Abiy, M., 2002. Prevalence of bovine trypanosomosis in Goro woreda, South-west Ethiopia. DVM Thesis FVM, AAU, Debrezeit.
30. Collins, F.M., 1994. The immune response to mycobacterium infection, development of new vaccine. *Vet. Microbiol.*, 40: 95-110.