academicJournals

Vol. 7(5), pp. 186-192, May 2015
DOI: 10.5897/JVMAH2014. 0372
Article Number: E05C35E52450
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Prevalence of small ruminant trypanosomosis in Assosa and Homosha districts, Benishangul Gumuz Regional State, North West of Ethiopia

Zelalem Ayana[1], Diriba Lemma[2*], Birhanu Abera[2] and Eyob Eticha[2]

[1]Jimma University college of Agriculture and veterinary medicine,Ethiopia
[2]Asella Regional veterinary laboratory, Ethiopia

Received 17 February 2015; Accepted 18 March 2015

A cross sectional study was conducted at Assosa zone of Benishangul Gumuz, North West Ethiopia, to determine the prevalence of trypanosomosis in local breeds of sheep and goats. Blood sample from 384 randomly selected sheep and goats (177 from Assosa and 207 from Homosha districts) of different species, sex, age groups were examined by dark phase contrast buffy coat and thin smear examination for species identification of trypanosome. Among the animals examined during the study period, 10 (2.6%) were infected with trypanosomes. From this survey, *Trypanosoma vivax* was found to be the major cause of trypanosomosis (1.82%), followed by *Trypanosoma congolense* (0.52%) and *Trypanosoma brucei* (0.26%). There was no statical difference (p > 0.05) in infection between sex, species, and among age groups. Mean packed cell volume (PCV) of the parastemic animals was significantly lower than (P < 0.05) that of aparastemic animals. In attempt to identify the vector involved in transmition, tsetse flies group (*Glossina morsitans submorsitans*) and mechanical vectors of trypanosomosis that belonging to Tabanidae (*Tabanus, Stomoxys* and *Haematopota*) were captured in both districts at an altitude range of 1270 to 1507 m above sea level. The results of the prevalence of the disease in small ruminants and its vectors indicate that an effective management and control measures for the disease and transmitting vectors should be designed and implemented.

Key words: Assosa, small ruminant, trypanosomosis, prevalence, vector.

INTRODUCTION

Trypanosomosis is a major constraint on ruminant livestock production in many areas of Africa including Ethiopia. Many animal species can be affected by the different trypanosomes, thus severely impairing the economic efficiency in endemic areas. From an economic point of view, the disease is particularly important in cattle, although other mammals can also be affected (Gupta et al., 2003; Singla et al., 2009; Sharma et al., 2012; Kumar et al., 2012). Although geographical differences have been observed, but the most affected animal species are buffalo, horses, camels, cattle and dog (Singh et al., 2003; Singla et al., 2012; Sharma et al., 2013; Sumbria et al., 2014). Other hosts, including small ruminants, can be affected (Corbera et al., 2006).

Trypanosomosis is a parasite disease caused by species of flagellated protozoa belonging to the genus

*Corresponding author. E-mail: lemmadiriba@yahoo.com.

trypanosome which inhibit the blood plasma, various blood tissues and liquid of vertebrate host (Rodostitis et al., 2000; Juyal et al., 2005). The infection caused by different species of trypanosomes is mainly transmitted cyclically by tsetse flies and mechanically by biting flies belonging to families Tabanidae and Hippoboscidae if they feed on more than one host within a short interval (Radostits et al., 2000; OIE, 2008). It is a serious constraint to agricultural production in extensive areas of the tsetse-infested Ethiopian lowlands (Slingenbergh, 1992). Out of the nine regions of Ethiopia, five (Amhara, beneshangul-Gumus, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (NTTICC, 2004).

The epidemiology of typanosomosis depends on factors such as distribution of vectors, virulence of parasite and response of host. The disease follows the distribution and intensity of various species of tsetse fly (Bursell, 1960). The most important tsetse flies species that are distributed and infested parts of Ethiopia are *Glossina morsitans submorsitans*, *Glossina pallidipes, Glossina fuscipes fuscipes, Glossina tachinoides* and *Glossina longipennis* (Langridge, 1976; Getachew and Hunduma, 2005). Sheep and goats are naturally infected with *Trypanosoma congolense, Trypanosoma vivax, Trypanosoma brucei* and *Trypanosoma evansi* which produce acute, subacute, chronic, or subclinical forms of disease in these animals (Getachew, 2005; Corbera et al., 2006). Even though small ruminants are naturally infected and presenting clinical disease, it is commonly believed that they are highly resistant to infection, but it is only sporadic, and that the disease in these animals is of little economic consequence (Corbera et al., 2006). The role of small ruminant in epidemiology of the disease in nature is still not well known. But the current epidemiological information indicates that the sheep and goats could act as reservoirs for the spread to animal and human trypanosomsis (Dede et al., 2005).

In view of this, the present study was designed to estimate the prevalence rate of typanosomosis in small ruminants, identity species of trypanosomes, determine the packed cell volume (PCV) values on infected and non infected animals and the vector density tsetse and other biting flies of the study area.

MATERIALS AND METHODS

Study area

This study was conducted in two districts (Assosa and Homosha) of Assosa zone of Benishangul Gumuz Regional State. The study was limited to only two peasant associations from each district due to their scatteredness and limited resources. These are Ashura and Bamadon from Homosha district; Ura and Tsetse Adurnon from Assosa district. Assosa is found at 675 km far from capital city, of Addis Ababa, North Western part of Ethiopia at 34° 02' 20E to 36°30'E and 9°30'N to 11°39'N. The maximum and minimum temperature of the area ranges from 35 to 25°C, respectively with the altitude ranging from 600 to 2731 m above sea level and the mean annual rain fall of 1000 mm. The area was gaining the rain for six month duration which starts on April and ends on October. The main occupation of the population is mixed farming practice of crop and livestock. The major livestock reared in the area are bovine, sheep, goats, donkeys and poultry (RSA, 2007). According to the publication bureau of Assosa Zone Agricultural office, the number of animal population in the area were 39133 Cattle, 6977 Sheep, 17675 Goat and Equines 2558 in Assosa district and 3665 cattle, 1350 Sheep, 3752 goat and 317 Equines in Homosha district.

The vegetations type of the area is savanna grass with scattered trees of different species dominated by bamboo trees that covers large area of the region with fat low land. Among the wild game few of them are antelope, bush pig, baboons and warthog (NTTICC, 2004).

Sample size

The sample size was determined using Thrusfield (2005) formula. Totally, 384 samples were collected from four sites of the two districts, namely, Ashura, Bamadon, Ura and Tsetse Adurnon by simple random sampling method.

Study animals

The study was conducted on 384 local breeds of the sheep and goats. From each selected peasant association, a proportional number to the total population of the study animals were sampled. The sample size were 86, 121, 58 and 119 in Ashura, Bamadon, Ura and Tsetse Adurnon, respectively. Examination and evaluation of body condition were accomplished during sample collection. They were classified as very thin (1), thin (2) moderate(3), good(4) and very good (5) by observing the body condition of animals in the field (Cooper and Thomas, 1985). The age of animal were also estimated by examining dentations (Kripali et al., 2010) and information obtained from owner.

Study design

The study was based on entomological and parasitological survey. This cross-sectional study were conducted in two districts.

Parasitological and hematological examination

Blood sample were collected randomly from small ruminants of four sites during the study period. Blood was collected from ear vein using sterile blood lancet and capillary tube. Pair of heparinazed capillary tube were filled with blood from small ruminants to 3/4 of its height and sealed at one end with crystal seal. The capillary tube were loaded on the microhaematocrit centrifuge symmetrically and centrifuged at 12000 rpm for 5 min (Murray et al., 1997). Packed cell volume (PCV) was determined using Haematocrit reader (Woo, 1970). After the PCV has been read, capillary tubes were broken in 1 mm below the Buffy coat which includes red blood cell layer and the content were expressed on microscopic slide and covered with a 22 × 22 mm cover slip. The content was examined under ×40 objective using dark ground Buffy coat technique (Murray et al., 1997). From positive sample, thin smear were made, fixed with methanol for 5 min and stain with geimsa solution for 30 min and examined using oil immersion under × 100 objective to detect the species of trypanosome.

Fly survey

The survey was under taken in two districts of Assosa zone in four

peasant associations. A mono- pyramidal trap was used, to trap different flies, total of 48 h were used and 59 traps were deployed. The collected flies' were counted and identified using hand lenses.

Mono-pyramidal traps baited with acetone, octenol, and cow urine (Brightwell et al., 1987) were used for assessing the fly density. The site selection was to include suitable tsetse habitats like savanna area, river valleys, livestock grazing area and watering points and vicinity to assume wild game reserve area. In all the study sites, a total of 59 mono-pyramidal traps were deployed early in the morning and maintained in position for 48 h. Savanna tsetse (*Glossina morsitans sub morsitans*) can detect odour from about 200 m. So, the traps were spaced at about 200 m interval.

During trapping, acetone and octenol were dispensed from open vials through an approximately O-size hole. While cow urine from open bottle on a piece of tissue paper was included to facilitate odor diffusion. All odors were placed on the ground, above 20 cm up the wind of the trap. The traps poles were greased to exclude insect predators like ants. The different catches in the traps were counted, identified (Langride et al., 1976) and analyzed. The species of tsetse fly was identified based on characteristic morphology (Leak et al., 1993). Other biting flies are separated according to their morphological characteristic, such as size, color, wing venation structure and proboscis at genus level (Wall and Shearer, 1997).

Sexing was done just by observing the posterior end of the ventral aspect of the abdomen by hand lenses. As a result, male fly is easily identified by enlarged hypopygium in posterior ventral part of the abdomen (Challier, 1965). The fly apparent density is the mean catched in traps deployed, expressed as the number of fly catched per trap per day (Leak, 1999).

Data analysis

Statical analysis was employed by Chi-square (χ^2) for data management and analysis using Stata Version 7.0 (2000). The tested hypothesis were prevalence of trypanosomes, PCV value, the relation between PCV value and prevalence of trypanosomes, the relation between age value and prevalence of trypanosome.

RESULTS

During this study, a total of 384 small ruminants of local breeds were examined in both study areas, out of these 207 were from Homosha and 117 from Assosa district. Generally, the overall prevalence rate of the two districts was 2.6% (Table 2.)

The trypapanosome species encountered are *T. vivax,T. congolense* and *T. brucei*. *T. vivax* and *T. congolense* were found in two districts, but *T. brucei* was in Homosha only. The relative proportion of trypanosome species were 1.82, 0.52 and 0.26% for *T. vivax*, *T. congolense* and *T. brucei*, respectively (Tables 1 and 3).

There was no statistically significant difference (P>0.05) between trypanosome infection rates in the village of two Woredas; with prevalence rates of 3.83, 1.64, 3.45 and 2.52% in Ashura, Bamadon, Ura and Tsetse Adurnon, respectively (Table 4).

Rate of infection was 3.82 and 1.78% in ovine and caprine, respectively with an overall infection rate of 2.60%. There is no significant difference (P>0.05) above different species of small ruminant (Table 5).

The body condition of all sampled small ruminant was also evaluated by scoring method indicated by Cooper and Thomas (1985). Out of the infected small ruminant, 3.85% were with thin body condition, 2.68% with moderate body condition, and 2.06% with good body condition (Table 6).

Infection rate was 0 and 2.98% in less than two years and greater than two and/or equal to two, respectively (Table 7). There is no statistically significant difference (P>0.05) in different age groups.

Infection rate between different sexes were 2.25 and 2.9% for male and female, respectively (Table 8). There is no statistically significant difference (p>0.05) in different sex groups.

There was statistically significant (P<0.05) difference in means PCV of parastemic and aparastemic small ruminants. The mean PCV value of parastemic and aparastemic were 20.9 and 27.95%, respectively (Table 9).

DISCUSSION

The results indicated that trypanosomosis to be the important livestock disease in Assosa zone of North West Ethiopia as also reported by Tewolde et al. (2001) in Metekel district, North West of Ethiopia. Even though various conventional diseases induce livestock mortality and results in economic losses in Ethiopia, tsetse transmitted trypanosomosis has a crucial effect which is becoming unchallengable to treat. Vector control action as a strategy option is not widely implemented in Ethiopia (NTTICC, 2004).

The finding of tsetse survey revealed one type of tsetse species at Homosha and Assosa districts Woreda. The main vector were *G. m. submorsitans* which was similar with the previous result in Metekel district (Getachew and Hunduma, 2005). Typical habitat was found in the study area for savanna species, that is, *G. m. submorsitans* prefer savanna grass, riverine and forest ecology. *G. m. submorsitans* was found to be concentrated in low land areas as climatic condition were more favourable. Some flies however, were found as high as 170 m. Earlier works had established the tsetse geographical limit at 1600 m. Later, Slingenbergh (1992) found the inceased limit up to 2000 m.

During the tsetse survey in two Woredas of the Assosa zone 0.0086 and 0.018 flies/trap/day were captured in Homosha and Assosa districts, respectively. Small fly density was obtained because of dry season. There were uncontrolled bush fires few weeks prior to the survey period. Such circumstance might have suppressed the fly density and forced the fly to move the moisture area and river banks of extreme low altitude. The fly density was found to be relatively increased in late rainy season than dry season. This fact is in agreement with result of Leak et al. (1993). According to Leak et al. (1999),

Table 1. Species and number of vectors identified at two districts of Assosa zone.

Village	Altitude (m)	No. of traps	Types and species of flies	Sex		Total	FTD
				Male	Female		
Ashura	1270-1352	14	*G. m. submorsitans*	1	-	1	0.0015
			Stomoxys	-	-	15	0.022
			Tabanus	-	-	6	0.00089
			Haematopota	-	-	8	0.0119
Bamadon	1350-1410	15	*G. m. submorsitans*	4	7	11	0.01553
			Stomoxys	-	-	29	0.04202
			Tabanus	-	-	24	0.0333
			Haematopota	-	-	1	0.0014
Ura	1390-1418	15	*G. m. submorsitans*	1	-	1	0.0014
			Stomoxys	-	-	48	0.0666
			Tabanus	-	-	4	0.00555
			Haematopota	-	-	-	0
Tsetse Adurnon	1460-1507	15	*G. m. submorsitans*	6	19	25	0.034
			Stomoxys	-	-	122	0.1694
			Tabanus	-	-	14	0.0194
			Haematopota	-	-	5	0.0069

Table 2. Prevalence small ruminant trypanosomosis on district basis.

District	No. of animals examined	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Homosha	207	5	2.41		
Assosa	177	5	2.82	0.0592	0.808
Total	384	10	2.6		

Table 3. Relative proportion of different trypanosome species on basis of district.

District	Positive animals	*T. vivax*	*T. congolense*	*T. brucie*	χ^2 cal	P value
Homosha	5	3	1	1		
Assosa	5	4	1	-	1.2087	0.751
Infection rate (%)	-	1.82	0.52	0.26		

Table 4. Prevalence of small ruminant trypanosomosis on the basis of the study peasant associations.

Peasant association	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Ashura	86	3	3.53		
Bamadon	121	2	1.64		
Ura	58	2	3.45	0.901	0.825
TsetseAdurnon	119	3	2.52		
Total	384	10	2.6		

Table 5. Prevalence of small ruminant trypanosomosis based on species.

Animals species	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Ovine	157	6	3.82		
Caprine	227	4	1.76	1.552	0.213
Total	384	10	2.6		

Table 6. Prevalence of small ruminant trypanosomosis on the basis of body condition

Body condition	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Thin	78	3	3.84		
Medium	112	3	2.68	0.059	0.704
Good	194	4	2.06		
Total	384	10	2.6		

Table 7. Prevalence of small ruminant trypanosomosis on the basis of age.

Age category	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
<2 years	48	0	0		
≥2 years	336	10	2.98	0.059	0.809
Total	384	10	2.6		

Table 8. Prevalence of small ruminant trypanosomosis on the basis of sex.

Sex	Examined animals	Positive animals	Prevalence rate (%)	χ^2 cal	P value
Male	178	4	2.25		
Female	206	6	2.9	3.215	0.36
Total	384	10	2.6		

Table 9. Mean PCV value of parastemic and aparastemic small ruminant.

State of the animal	No. of animals	Mean PCV	Minimum	Maximum	SD	% Total N	χ^2 cal	P value
Parastemic	10	20.90	17	25	0.72	97.4	196.126	0.000
aparastemic	174	27.95	18	37	0.14	2.6		
Total	384	-	-	-	-	-	-	-

increased in fly density was due to the growth of vegetation and formation of new habitat in the rainy season.

There was no significant difference ($p > 0.05$) in infection rate among thin, medium and good body condition. This result is similar to the work of Goossens et al. (1998) and Snow et al. (1996). These researchers have indicated that small ruminant are not often selected by tsetse flies. Though these animals do not show signs of trypanosomosis. The same result is revealed by Getachew and Hunduma (2005) at South West of Ethiopia and report that trypanosomosis in sheep and goats is an important disease and small ruminant serve as potential reservoir of infection for other animals and do not show clinical sign and were in good body condition.

The present study revealed that when the age increased, the prevalence rate also increased. This may be due to the immune compromization in very old individuals. The findings from Muturi (1999) and Terzu (2004) support the present finding. Out of the three detected trypanosomes, *T. vivax* stands first and this is due to the fact that its ability is being transmitted by

mechanically as well as cyclically and there is also little number of *Glossina* species when compared with other biting flies. This result is similar to the work of Kalu and Uzoigwe (1996), who reported that the area encountered low density of tsetse flies and *T. vivax* was a predominant species. This suggests that the biting flies would mediate *T. vivax* infection when tsetse fly density is low or absent.

There was no significant difference ($p>0.05$) in the infection rate among species of small ruminant, because in this study, area management, grazing area and nutritional status of the two species were the same. The same result is revealed by Coulibaly et al. (1995) and Defly et al. (1988) indicates that livestock species had a major effect on trypanosome prevalence.

It is known that the development of anemia is the most reliable indicator of the progress of trypanosome infection (ILRAD, 1998). But it can also be assumed that numerous concurrent diseases and nutritional factors interferes with anemic development (O.A.U/S.T.R.C., 1979) and PCV value are reliable indicator of anemia. During PCV determination, a value of 24 to 46 (Radostitis et al., 2000) was considered to be normal.

The mean PCV values of parastemic (20.9%) was found to be statically lower than ($p>0.05$) that of aparastemic (27.95%) small ruminant. Similar result were obtained at South West Ethiopia by (Getachew and Hunduma, 2005). Taylor (1998) indicated that the anemia persists during chronic infection of when parastemia is quite low, probably because of different mechanisms that are involved in its genesis during acute and chronic stage of infection (Singla et al., 1997). Thus suggest that control of parastemia is unrelated in chronic phase when immune system is depressed and anemia is sustained through dyserthropoisis.

Conclusion

Trypanosomosis was prevalent in small ruminant in the study area at low rate of 2.6% with different trypanosome species. Prevalence of mechanical transmitters or biting flies such as *Stomoxys, Tabanus* and *Haematopota* along with cyclically tsetse *G. m. submorsitans* species indicated the vectors for trypanosomes in the study area. Regarding the trypanosomosis in small ruminant, a severe clinical signs does not overt by infection, but they can be considered as an important reservoir for the majority of trypanosomes for other animals and humans. Therefore, in areas where the presence of small ruminants are important and trypanosomosis is prevalent, these animals should be taken into consideration in all programs to control the disease.

ACKNOWLEDGEMENTS

The authors would like to thank Jimma University, College of Agriculture and Veterinary Medicine, Bedelle and Asella Regional Veterinary Laboratories for supporting, and all individual who render help during the study period were highly acknowledged.

Conflicts of interest

Authors have none to declare

REFERENCES

Brightwell R, Dransfield RD, Korku CA, Golder TK, Tarimo SA, Mugna D (1987). A new trap for *Glossina pallidipes*. Trop. Pest Manag. 33:151-159.

Bursell E (1960). The effect of temperature on the consumption of fat during pupal development in *Glossina*. Bull. Entomol. Res. 51:583-598.

Challier A (1965). Method for the determination of physiological age of *Glossina* insect. Physiol. Biol. 6:241-248.

Cooper M, Thomas RJ (1985). Profitable sheep farming, 5th ed., Forming RUN Ltd. pp. 56-85.

Corbera G, Morales JAM, Uscher P (2006). Trypanosomosis in Goats Current Status. Veterinary Faculty, University of LasPalmas, Arucas, Las Palmas, 35416 Canary Islands, Spain. Voice: 34-928451115, New York Academy of Sciences pp. 300-310.

Coulibaly L, Rowlands GJ, Authié E, Hecker PA, d'Ieteren GDM, Krebs H, Leak SGA, Rarieya JM (1995). Effect of tsetse control with insecticide impregnated traps on trypanosome prevalence and productivity of cattle and sheep in northern Cbte d'Ivoire. In: Proc. 22nd Meeting of International scientific Council for Trypanosomiasis Research and Control, Kampala, Uganda, 1993. OAU-STRC Nairobi, pp. 244-250.

Dede PM, Hallid I, Omoogun GA, Uzoigwe NR, Njoku CI, Daniel AD, Dadah AJ (2005). Current tsetse and trypanosomosis situation on Jose Plateau, Nigeria: Epizootiological factors thatmay enhance disease transmission and spread. Rev. Elev. Med. Vet. Pays Trop. 58(1):31-55.

Defly A, Awuome K, Bokavi K, D'Ieteren MGD, Grundler G, Handlos M, Ity P, Leak SGA, Maehl JHH, Mawuena K, Morkramer G, Nagda SM, Paling RW, Rarieya JM, Thorpe W, Trial JCM (1988). Effect of trypanosome infection on livestock health and production in Togo. In: Livestock production in tsetse affected areas of Africa.

Getachew A (2005). Review Article: Trypanosomosis in Ethiopia. Ethiop. J. Biol. Sci. 4(1):75-121.

Getachew A, Hunduma D (2005). Small ruminants trypanosomosis in Southwest Ethiopia. Small Rumin. Res. (In press).

Goossens B, Osaers S, Kora S, Ndo M (1998). Haematological changes and antibody response in trypanotolerant sheep and goats following experimental Trypanosoma congolense infection. Vet. Parasitol. 79(4):283-298.

Gupta MP, Singla LD, Singh KB, Mohan R, Bal MS (2003). Recrudescence of trypanosomosis following administration of dexamthasone in bovines. Indian Vet. J. 80:360-361.

ILRAD (1998). Annual report of International Laboratory for Research on Animal Diseases. p 103.

Juyal PD, Singla LD, Kaur P (2005). Management of surra due to *Trypanosoma evansi* in india: an overview. In: Infectious diseases of domestic animals and zoonosis in India (Tandon V, Dhawan BN (eds.) Proc. Natl. Acad. Sci. India 75(B)-Special Issue:109-120.

Kalu AU, Uzoigwe NR (1996). Tesetse fly and trap on the Jos Plateau: Observation on outbreaks in B/Ladi L.G.A. Trop. Vet. 14:114-126.

Kripali P, Rajput MKS, Jitendra K, Shivani S, Vandna R, Pritee G (2010). Prevalence of helminthes in small ruminants in Tarai region of Uttarakhand. Vet. World 2:265-266.

Kumar H, Gupta MP, Sidhu PK, Mahajan V, Bal MS, Kaur K, Ashuma, Verma S, Singla LD (2012). An outbreak of acute *Tryppanosoma evansi* infection in crossbred cattle in Punjab, India. J. Appl. Anim. Res. 40(03):256-259.

Langridge WP (1976). Tsetse and Trypanosomosis Survey of Ethiopia.

Ministry of Overseas Development, UK and Ministry of Agriculture, Ethiopia pp. 1-40.
Leak SGA (1999). Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomosis. CAB International. Wallingford (UK) p 568.
Leak SGA, Mulatu W, Authie E, d'Ieteren G, Peregrine A, Rowlands GJ, Trail J (1993). Epidemiology of Bovine trypanosomosis in the Ghibe Valley, Southwest Ethiopia, 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle; 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence or recurrent infections. Acta Trop. 53:121-134; 135-150.
Murray M, Murray PK, McIntyre WIM (1997). An improved technique for the diagnosis of African trypanosomiasis. Trans. R Soc. Trop. Med. Hyg. 71:325-326.
Muturi KS (1999). Epidemiology of bovine trypanosomiasis in selected sites of the southern rift valley of Ethiopia. Msc thesis Addis Ababa University with Freie University, Berlin.
National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC) (2004). Annual Report on Tsetse and Trypanosomosis tripanosomiasis and epidemiological surveillance in Survey, Bedelle, Ethiopia.
O.A.U/S.T.R.C (1979). Bulletin of Animal health and production in Africa. 27(3).
OIE (2008). Terrestrial Manual on Trypanosomosis (tsetse-transmitted) pp. 813-820.
Rodostitis OM, Gay CC, Blood DC, Hinchiff KW (2000). A text Book of Disease of Cattle, Sheep, pigs, Goats and Horses. 9thed. Southern company limited, London pp. 1278-1296.
Regional Stastical Abstract (RSA) (2007). Bureau of Finance and Development Division of static's Population, Assosa Zone, Assosa, Ethiopia.
Sharma P, Juyal PD, Singla LD, Chachra D, Pawar H (2012). Diagnosis of *Trypanosoma evansi* in cattle and buffaloes by employing real time PCR using TaqMan assay. Vet. Parasitol. 190:375-382.
Sharma A, Singla LD, Ashuma, Batth BK, Kaur P, Javed M, Juyal PD (2013). Molecular prevalence of *Babesia bigemina* and *Trypanosoma evansi* in dairy animals from Punjab, India by duplex PCR: A step forward to detection and management of concurrent latent infections. Biomed. Res. Int. p 8.
Singh R, Singla, LD, Dhaliwal PS (2003). Dexamethasone flared up trypanosomosis in a dog. Indian Vet. Med. J. 27:93-94.
Singla LD, Juyal PD, Roy KS, Kalra IS (1997). Host responses of cow-calves against *Trypanosoma evansi* infection: haematopathological study. J. Vet. Parasitol. 11:55-63.
Singla LD, Juyal PD, Sharma NS (2009). Immune responses to haemorrhagic septicaemia (HS) vaccination in *Trypanosoma evansi* infected buffalo-calves. Trop. Anim. Health Prod. 42:589-595.
Singla LD, Juyal PD, Sharma NS (2012). Responses to haemorrhagic septicaemia vaccination in *Trypanosoma evansi* infected buffalo-calves. J. Vet. Parasitol. 26(1):39-43.
Sumbria D, Singla LD, Sharma A, Moudgil AD, Bal MS (2014). Equine trypanosomosis in central and western Punjab: Prevalence, haemato-biochemical response and associated risk factors. Acta Trop. 138:44-50.
Slingenbergh JHW (1992). Tsetse control and Agricultural development in Ethiopia. World Anim. Rev. 70/71:30-36.
Snow WF, Wacher TJ, Rowlings P (1996). Observations on the prevalence of trypanosomosis in small ruminants, equines and cattle, in relation to tsetse challenge in The Gambia. Vet. Parasitol. 66:1-11.
Stata Corporation (2000). Intercooled Stata Version 7.0 for Windows 95/98/NT. University Drive East College Station, Texas, USA.
Taylor KA (1998). Immune responses of cattle to African trypanosomes: protective or Pathogenic. Int. J. Parasitol. 28:219-240.
Terzu D (2004). Season dynamic of tsetse and trypanosomiasis in selected sites of Msc Southern Nation Nationalities and Peoples of Regional State (SNNPG) thesis AAU, Debre Zeit, Ethiopia.
Tewolde N, Abebe G, Eisler M, McDermott J, Greiner M, Afework Y, Kyule M, Munstermann S, Zessin KH, Clausen PH (2001). Application of field methods to assess Isometamidium resistance of trypanosomes in cattle in western Ethiopia. Acta Trop. 90:163-170.
Thrusfield M (2005). Veterinary Epidemiology 3nd Ed. Black Well Science Ltd., UK pp. 228-245.
Wall R, Shearer D (1997). Veterinary Entomology. Arthropod Ectoparasites of Veterinary Importance. Chapman and Hall, London. pp. 141-193.
Woo PTK (1970). The haematocrit centrifugation technique for the diagnosis of African trypanosomiasis. Acta Trop. 27(4):384-6.