Full Length Research Paper

The prevalence of bovine trypanosomosis and associated risk factors in Mareka Woreda of Dawuro Zone, Southern Ethiopia

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A cross-sectional study was carried out to determine the prevalence of bovine trypanosomosis, to identify predominant trypanosome species and some associated risk factors, in purposively selected areas of Mareka district of Dawuro zone, southern Ethiopia from November 2015 to April 2016. For this purpose, a total of 384 blood samples were collected from cattle using systematic random sampling method considering different age, body condition and coat color; as well as both sex groups of cattle. The packed cell volume (PCV) of each sampled animal was measured using hematocrit reader after centrifugation at 12,000 rpm for five minutes. Buffy coat technique was used to determine prevalence of trypanosomal parasites and species was further confirmed by Giemsa stained thin smear. The overall prevalence of bovine trypanosomosis was found to be 8.3% (32/384). The predominant trypanosome species were *Trypanosoma congolense* (62.5%) followed by *T. vivax* (37.5%) with significant statistical variation ($P<0.05$). The mean PCV was recorded as 21.03±3.297 in parasitaemic and 27.98±3.519 in aparasitaemic animals with results revealing significant statistical difference ($P<0.05$) between the two groups. From assessed risk factors; the age, body condition and coat color of animals showed statistically significant variation ($P<0.05$), but animal location and sex were insignificant ($P>0.05$). In conclusion bovine trypanosomosis is one of the major livestock diseases posing continuous threats to the production and productivity of livestock sub-sector in the study area, thus it requires due attention to strengthen an integrated trypanosomosis and vector control.

Key words: Bovine, Ethiopia, Mareka, prevalence, risk factors, trypanosomosis.

INTRODUCTION

Livestock are of enormous importance in Africa, economically, for nutritional and agricultural purposes and socially (FAO, 2002). The size and diversity of Ethiopia's major agro-ecological zones render it suitable for the support of large numbers and class of livestock. Among the livestock population of Ethiopia, there are about 53.4 million of cattle; 25.5 million sheep and 22.78 million goats (CSA, 2011). Cattle are important components

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to nearly all farming systems and provide draught power, milk, meat, manure, hides, skins and other products. They are "Living banks" or "Living accounts" for rural and urban poor farmer or owners, because of they serve as a financial reserve for period of economic distress such as crop failure as well as other cash income. Despite the presence of huge numbers of cattle and their multipurpose; the country is not as such advantageous due to a multitude of problems. This comprises of: Diseases, age old traditional management system, inferior genetic make-up coupled with under nutrition and complicated by malnutrition as well as absence of well-developed market infrastructure. Among the diseases tsetse transmitted animal trypanosomosis has been still remain as one of the largest causes of livestock production losses in the country. As result 14 million heads of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting tsetse- borne trypanosomosis at any one time (MoARD, 2004).

Trypanosomosis is a complex protozoan disease caused by unicellular parasites (trypanosomes) found in the blood and other tissues of vertebrates including cattle and man (Tesfaye, 2002). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei*, in cattle, sheep and goats, *Trypanosoma evansi* in camels and *Trypanosoma equiperdium* in horses (Getachew, 2005).

Researches on the socio-economic impacts of trypanosomosis have revealed that, over 3 million heads of various livestock species in Africa are lost per year by deaths due to the disease. Furthermore, over 35 million doses of trypanocidal drugs are bought annually to treat animals against trypanosomosis and more than 70 million heads are at risk of contracting the disease, so that total direct and potential losses attributable to the disease worth over 4.5 billion dollars per year (Bett et al., 2004). Even if tsetse transmitted animal trypanosomosis has been studied widely in Ethiopia, still remain as one of the largest causes of livestock production losses in the country (NTTICC, 2004). Furthermore, the disease causes direct loss through mortality estimated to amount 1.5 to 2 billion birr per year and indirect losses due to decreasing productivity, and restriction from international livestock trade in Ethiopia (Bizuayehu et al., 2012).

Therefore, trypanosomosis was considered to be an important disease of cattle in different part of the country in general (Shimelis et al., 2005; Bitew et al., 2011) and Dawuro zone in particular, which is within the tsetse belt, bounded by big rivers and tributaries such as: Omo, Ghibe and Gojab and Mareka woreda is under this zone. The previous study conducted in this particular study area (Mareka woreda) spotlighting on the impacts of tsetse challenge on herd composition and mortality, lactation and reproductiveperformance of cattle indicated that, the mortality rate in cattle for one year in the tsetse challenged areas was about 26.73 times higher than in the tsetse free area (Tigicho et al., 2012). However, studies have not yet been fully conducted on the determination of the prevalence and identification of trypanosome species affecting bovine in the study area. Therefore, the present study was accomplished with the general objectives to determine the prevalence of bovine trypanosomosis, identify the species of trypanosomes and assess the risk factors associated with the disease in Mareka district of Dawuro zone.

MATERIALS AND METHODS

Description of the study area

The study was carried out in Mareka district, in Dawuro Zone of SNNPR,Southern Ethiopia from November 2015 to April 2016. The area is located atabout 544 and 277 km south west of Addis Ababa; the capital city of Ethiopia and Hawassa; the administrative town of SNNPR respectively.Tarcha was the former town of the district, but the current town of district is Waka; while Tarcha became the administrative town of Dawuro Zone. The woredais bordered on the South by Loma Woreda, on the West by Gena Bosa Woreda, on theNorth by Tocha Woreda and the Gojeb River which defines its boundary with the Oromiya Region, and on the East by EsseraWoreda. The total land coverage of the woreda is 44050 ha of which 2000 ha (4.5%) is covered by forest, 11500 ha (26.1%) is grazing land, 28140 ha (63.9%) is cultivating land and the remaining 2410 ha (5.5%) comprises bushes, savanna, rivers, springs, stagnant waters and hills. According to the agro-ecological classification criteria the woreda is partioned into three agro-ecological zones; namely high land (Dega), midland (Woinadega), and lowland (kola) with their total land holds of 53, 30 and 17%, respectively. The study area's elevation ranges from 1000 to 2400 m above sea level. The mean annual rainfall ranges from 650 to 1100 mm and the rainfall distribution is bimodal with highest fall at wet season (April to September) and lowest fall at last half of dry season (February and march). The mean daily temperature ranges from 18 to23°C with the highest temperature share at dry season (November to march) and lowest temperature share at wet season. The predominant farming system in the areawas crop-livestock production system. The woreda has a total human population of 126022, of whom 65321 are men and 60701 women. The livestock population consists of:122,084 cattle, 47,438 sheep, 18,854 goats, 4,860 horses, 2,759 mules, 1,699 donkey, and 63,042 poultry and 2,750 traditional and 863 modern bee hives (MWOoA, 2015).

Study population

Local breeds of cattlewith different age groups, body conditions and coat colors as well as both sex groups that were kept under traditional extensive husbandry system with communal herding were considered as study population. The animals examined were categorized into different age groups as less than 2 years (young), between 2 up to 4 years (medium) and greater than 4 years (adult) according to their teeth dentition (Johnson, 2003). The body condition was estimated as per the recommendations of Macintosh (2007) for evaluating the body condition of the zebu cattle. The body condition of animals was recorded by classifying animals in the three groups as good, medium and poor based on the appearance of the ribs and dorsal spines. The examined animals were categorized into five groups according to their coat colour asred, white, mixed, black and gray coat colors to observe whether coat colour of animal have any influence on the disease prevalence (Wondewosen et al., 2012).

Study design

A cross-sectional study was conducted to determine the current prevalence of bovine trypanosomosis and to estimate the potential risk factors associated with the epidemiology.

Sampling method and sample size determination

The study area was selected by convenience sampling method based on previous information on lack of detailed study on bovine trypanosomosis. A total of 384 cattle were selected among cattle brought for deltamethrin pour-onusing systematic random sampling method. During sampling the age, sex, body conditions and coat colors of study animals were considered. The sample size was calculated using Thrusfield (2005) formula and 384 cattle were sampled.

$$N= \frac{1.96^2 \, [Pexp- (1-Pexp)]}{d^2}$$

Where, N is the required sample size, Pexp was the expected prevalence and d is the desired absolute precision. An expected prevalence of 50% was used, because of no previous studies were conducted in the area. In the overall study a 5% absolute precision at 95% confidence level was considered.

Parasitological survey and packed cell volume (PCV) determination

Blood sample collection

The format that includes locations, code number of animal, sex, age, body condition score, coat color, PCV, and parasitological result was prepared. After restraining the animals, 70% alcohol was used to clean ear of cattle then dried with cotton gauze, vein allocated by the tip of thumb, the vein was punctured by sterile lancet and blood sample was collected from marginal ear vein of cattle with heparnized capillary tubes up to ¾ of their length. One end sealed by sealer and placed on capillary holder on sealer by matching sample number with sealer number.

Packed cell volume (PCV) determination

For the measurement of PCV using a micro-hematocrit reader, the capillary tubes filled with blood were placed in micro- hematocrit centrifuge with sealed end outer most. The tubes were loaded symmetrically to ensure good balance. After screwing the rotary cover and closing the centrifuge lid, the specimens were allowed to centrifuge at 12,000 rpm for 5 min. After centrifugation, the capillary tubes were placed in a hematocrit reader. The length of the packed red blood cell column was expressed as a percentage of the total volume of blood; taking the PCV values 24 to 46% as normal for zebu cattle (Blood and Radostits, 2007; Samdi et al., 2011).

Buffy coat technique

After packed cell volume (PCV) determination thecapillary tubes were cut using a diamond tipped pen 1 mm below the buffy coat to include the upper most layer of the red blood cells and 3 mm above to include the plasma. The content of capillary tube was expelled on to slide and covered with cover slip. The slide was examined under ×40 objective for movement of parasite. Trypanosomes species were identified according to their movement in wet film preparations according to Paris et al. (1982).

Thin blood smear

For thin blood smear examination, a small drop of blood from a microhaematocrit capillary tube of buffy coat positive samples was applied to a clean slide and spread by using another clean slide at an angle of 45°. The smear was dried by moving it in the air and fixed for two minutes in absolute methyl alcohol; then it was flooded with Giemsa stain (1:10 solution) for 30 min. Excess stain was drained and washed by using distilled water. Then allowed to dry by standing up right on the rack and examined under the microscope (×100) oil immersion objective lens to confirm the trypanosome species based on their morphology (Murray et al., 1977).

Data analysis

The data collected were recorded properly in a format prepared for this purpose and were collected properly, handled carefully and analyzed systematically. For the analysis of data, statistical software program: SPSS-20 for windows version was used. Prevalence of bovine trypanosomosis was expressed as the number of parasitaemic animals through buffy coat microscopic study to the total animals examined (%). Hematological findings were expressed as percentage of the RBC to the total blood content (%). In all cases, a 95% CI were employed to extrapolate sample results to the target population in the study area. In order to compare trypanosomosis prevalence and the pooled data of mean PCV between aparasitaemic and parasitaemic animals of different factors, a combination of frequency distribution and student's t-test values and correlation were done to compare the relationship of PCV value with trypanosome infection rate.

RESULTS

Parasitological findings

From 384 randomly selected cattle, 32 (8.3%) were found to be positive for trypanosomosis using buffy coat technique. *Trypanosoma congolense* and *T.vivax* are the pre-dominant trypanosome species as indicated in Table 1.

The prevalence with respect todifferent risk factors like location, age, body condition, sex and coat color of cattle was determined. Although, different prevalence was found between both sex groups and among the three selected kebeles, no statistical significance (P>0.05) was observed in overall prevalence of trypanosomosis between sexes and among the kebeles. In contrast, statistically significant (P<0.05) prevalence of trypanosomosis was observed with respect to age, body condition and coat color of examined animals as shown in Table 2.

Hematological result

The mean PCV of individual animals was measured and recorded before buffy coat examination to assess degree

Table 1. Prevalence of trypanosome species among positive cases.

Trypanosome species	No. of positives	Prevalence (%)	Significance
T.congolense	20	62.5	X^2=384.000
T.vivax	12	37.5	P-value=0.000
Total	32	100	

Table 2. Prevalence of trypanosomosis in relation with different associated risk factors.

Risk factors	Category	Cattle examined	No. of negatives	No. of Positives	Prevalence (%)	Significance
Study kebeles	Tarcha Zuria	132	128	14	10.6	X^2=2.191
	Shina Gaburi	126	115	11	8.7	P=0.320
	Shaba Yoyo	126	119	7	5.6	
Sex	Male	169	157	12	7.1	X^2=0.600
	Female	215	195	20	9.3	P=0.464
Age	Young	48	47	1	2.1	
	Medium	127	120	7	5.5	X^2=6.493
	Adult	209	185	24	11.5	P=0.025
Body condition	Poor	73	56	17	23.3	
	Medium	163	154	9	5.5	X^2=26.606
	Good	148	142	6	4.1	P=0.000
Coat color	Red	134	122	12	9	
	White	78	76	2	2.6	
	Mixed	64	58	6	9.4	X^2=21.519
	Black	39	29	10	25.6	P=0.001
	Gray	69	67	2	2.9	

of anemia. As a result, the mean PCV of parasitaemic animals were 21.03±3.297 and aparasitaemic animals were 27.98±3.519 with significant statistical variation (P<0.05) as represented in Table 3. In addition, the mean PCV of infected animals were measured and calculated in order to deduce severity of anemia in relation to trypanosome species. Accordingly, the mean PCV of *T.vivax* infected animals were lower (19.83±2.15) than that of *T.congolense* infected animals (21.75±1.65) as shown in Table 4.

DISCUSSION

The overall prevalence of bovine trypanosomosis obtained in present study (8.3%) was in accordance with previous results recorded as 7.8% at Wemberma district of West Gojjam zone, Ethiopia (Yehunie et al., 2012); 9.3% at Humbo Larena of Wolaita zone, South Ethiopia (Habtewold, 1993); 9.63% at Awi zone, North West Ethiopia (Kebede and Animut, 2009). But, lower than that

of the results reported as 21.33% at Konta special woreda, Southern Ethiopia (Ataro et al., 2015); 14.2% at selected villages of Humbo district, Southern Ethiopia (Feyissa et al., 2011); 19.01% at Ghibe Valley (Shimelis, 2004); 27.5% at selected districts of Arba Minch, Southern Ethiopia (Abraham and Tesfaheywet,2012); 28.1% at tsetse infested Asosa district of Benishangul Gumuz Regional State (Shimelis et al., 2011). The low prevalence observed in present study might be due to anintegrated tsetse and trypanosomosis control program under taken by STEP or due to frequent use of trypanocidal drugs by owners. It might be also due to chronic stage of disease as parasitaemia reach its peak at early acute phase and become low or absent as disease progresses. The chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood (Loses and Ikede, 2002).

This study result is higher than the results documented as 4.2% at South Achefer district, Northern Ethiopia (Denbarga et al., 2012); 4.43% at selected villages of

Table 3. The mean PCV of parasitaemic and aparasitaemic animals.

Conditions	No. of animals	PCV≤24	PCV≥25	PCV range	Mean±SD	Significance
Parasitaemic	32	27(84.4%)	5(15.6%)	14-27	21.03±3.297	T-test =10.35
Aparasitaemic	352	46(13.1%)	306(86.9%)	17-36	27.98±3.519	P-value=0.00
Total	384	73(19%)	311(81%)	14-36	27.40±3.991	

Table 4. The mean PCV of trypanosome infected animals in relation to trypanosome species.

Trypanosome spp	No.posetives	Mean PCV	Significance
T. congolense	20	21.75±1.65	T-test=1.634
T. vivax	12	19.83±2.15	P-value=0.113

Arbaminch, Ethiopia (Wondewosen et al.,2012); 5.43% at Mandura district, Northwest Ethiopia (Kumela et al., 2015); 4.86% at Didessa district (Gamechu et al., 2015); 6.9% at Chena district, Southwest Ethiopia (Bizuayehu et al., 2012). This might be due to the prolonged implementation of an integrated tsetse and trypanosomosis control program by STEP, wich was established earlier in those areas than current study site

The predominant trypanosome species in current study were *T. congolense* (62.5%) followed by *T.vivax* (37.5%) and this result is in consistent with previous reports at Daramallo district of South Western Ethiopia, which is 93% *T.congolense* and 5.3% *T. vivax* (Ayele et al., 2012); Lalo Kile District of Kelem Wollega zone with 75% *T. congolense* and 25% *T. vivax* (Efrem et al., 2013); upper Didessa Valley ofwestern Ethiopia with 81.42% *T. congolense*and 12.85% *T. vivax* (Mulugeta, 2014). In contrast to present study, *T.vivax* was highly predominant species than *T.congolense* as reported at Kindo Koisha district with 71 and 28.4%, respectively (Kidanemariam et al., 2002). The predominance of *T. congolense* in present study might be due to its high number of serodems as compared to *T. vivax* and the development of better immune response to *T. vivax* by infected animals as well as due to the presence of major cyclical vectors Glossina species (*G. pallidepes*) as current entomological result witnessed. Since the transmission of *T. congolense* is cyclical, it requires the presence of tsetse flies, where as *T. vivax is* most readily transmitted mechanically by biting flies (Abebe, 2005). The lower prevalence of *T. vivax* might be due to low distribution of mechanical vectors such as Tabanus and Stomoxys.

No statistically significant prevalence variation (P>0.05) was observed among study kebeles; this might be due to the study was conducted in the same agro-ecological zone (kola) with similar climatic conditions. But, the highest prevalence recorded at Tarcha zuria might be due to the existence of relatively highest apparent density of tsetse flies as present entomological survey revealed. The occurrence of trypanosomosis frequently corresponds

with vector density which in turn dependent on those climatic factors such as; temperature, humidity and vegetation coverage of the area (Abebayehu et al., 2011).

The higher prevalence observed in female animals than male animals in current study was in agreement with previous reports of Konta special woreda; which is 20.7% in female and 17.3% in male animals with no significant statistical variation (Migbaru and Desta, 2015). Similar findings were also reported by Daya and Abebe (2008) plus Tadesse and Tsegaye (2010) and they suggested that the prevalence difference between male and female animals is due to "physiological difference between sex groups". The possible explanation for higher prevalence of trypanosomes in female animals in present study area might be also that female animals were more likely exposed to tsetse flies as they were always released to common grazing site of tsetse infestation, in contrast to male animals as they were kept around house after ploughing and have little chance to be exposed to tsetse flies.

The infection rate was assessed by categorizing animals into different age groups as young (1-2 years), medium (2-4 years) and adult (>4 years) since the age was assumed as one of potential risk factor. As a result the highest infection rate with statistical significance (P<0.05) was observed in adults (11.5%) than medium (5.5%) and young (2.1%) aged animals. This result is in line with the previous reports at Konta Special Woreda with significant variation (P<0.05) of 24.7, 16.7 and 4.8% in adult, medium and young aged animals respectively (Migbaru and Desta, 2015). However, the variation in infection rate could be due to the fact that adult animals travel long distance for grazing and draft as well as harvesting of crops to tsetse challenged areas. In addition to this; Suckling calves do not go out with their dams but graze at home until they were weaned off (Rowlands et al., 1995). Young animals are also naturally protected to some extent by maternal antibodies (Fimmen et al., 1999) this perhaps results in low

prevalence of trypanosome. Moreover, tsetse flies were less successful in feeding from young cattle aged up to 2 years. The lower feeding rate in young animals attributed to higher rate of defense movement; this in turn reduces the risk of contracting trypanosomosis (Torr and Mangwiro, 2000).

Significantly highest infection rate (P< 0.05) was recorded in poor body conditioned animals (23.3%) than medium (5.5%) and good (4.1%) body conditioned animals and was consistent with previous reports at Goro district (Bitew et al., 2011); Konta special woreda (Ataro et al., 2015); selected villages of Humbo district (Feyissa et al., 2015). It is difficult to conclude either poor body condition predisposes to trypanosome infection or trypanosome infection cause loss of body condition. The disease itself results in progressive emaciation of the infected animals; nevertheless, non-infected animals under good body condition have well developed immune status that can respond to any foreign antigen better than those non-infected cattle with poor body condition which can be immune compromised due to either haematopagus parasites, concurrent diseases or malnutrition (Collins, 1994).

Statistically significant variation (P<0.05) was observed between coat color of animals with respective prevalence of 9, 2.6,9.4, 25.6 and 2.9% in red, white, mixed, black and gray colored animals. Similar finding was reported at Konta special woreda as there was significant difference in prevalence among different coat colored animals with the highest prevalence in black hair-coat animals (33.39%) whereas the least prevalence rate was recorded in white hair-coat animals (8.06%) (Ataro et al., 2015). In contrast to this, there was report of slightly higher prevalence in cattle's having mixed skin color (7.25%) followed by 4.88% in red, 3.57% in black, 1.56% in white and 0% in gray skin color (Wondewosen et al., 2012). The possible reason for highest prevalence in black colored animals in current study might be due to the nature of tsetse flies to be attracted toward black color. Tsetse flies have a preference for dark surfaces (Green, 1993).

The mean PCV value of presently studied animals was significantly (P<0.05) varying between parasitaemic (21.03±3.297) and aparasitaemic (27.98±3.519) animals. This result was in agreement with the previous report from Eastern Wollega with lower mean PCV of 20.2±3.0 in infected animals as compared to non-infected animals of 26.5±5.1 (Yibrah, 2012). Likewise, Thrusfield (2005) stated that average mean PCV of parasitologically negative animals was significantlyhigher than those of parasitologically positive animals. Therefore, trypanosomosis may adversely lower PCV value of infected animals, even though other diseases such as helminthosis, thick borne disease and nutritional imbalances contribute to the low PCV values.

Anemia is one of the most important indicators of trypanosomosis in cattle (Stephen, 1986). The level of anemia or PCV usually gives a reliable indication of the disease states and reduced performance of infected animals (Trail et al., 1993). Even though significant difference is found in the study, PCV alone could not be used as diagnostic criteria for trypanosomosis, because there are other factors causing anemia such as worm infestation and nutritional deficiency (Radostits et al., 1994). The occurrence of parasitologically positive animals with PCV greater than 25% might be due to recent infection.

The mean PCV of trypanosome positive animals was also measured and calculated purposively in order to deduce severity of anemia in relation to trypanosome species. Accordingly, the mean PCV of *T. vivax* infected animals was lower (19.83±2.15) than that of *T.congolense* infected animals (21.75±1.65). Therefore *T.vivax* can cause more severe PCV reduction than that of *T.congolense*, this might be due to *T.vivax* can migrate and invade tissues and lymph nodes in addition to blood stream. *T. vivax* usually multiplies rapidly in the blood of cattle and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to beaggregated in small blood vessels and capillaries of theheart, brain and skeletal muscle. Very acute infection with *T. vivax* in cattle causes parasitaemia and dis-seminated intravascular coagulation with hemorrhages, this perhaps result in PCV reduction (Murray and Dexter, 1988).

Conclusion

Bovine trypanosomosis,which accounts for an overall prevalence of 8.3% is the major livestock constraint in the study area and affects their health, production and productivity. The major species of trypanosomes encountered were *T.congolense* followed by *T.vivax*. Infection with trypanosomosis negatively affected PCV and body condition and it is an indication that trypanosome infection of cattle causes loss of body weight and production. Adult, poor body conditioned and black colored animals were most risky group for being affected by trypanosomosis.

RECOMMENDATIONS

Based on the aforementioned the following is recommended:

1. An integrated tsetse and trypanosomosis control action should be strengthened in the area in ordered to minimize direct and potential loss of livestock due to the disease.
2. The government should supply effective trypanocidal drugs and trained man poweras much as feasible to reduce effect of trypanosomosis.

3. Further research should be conducted on drug resistance and epidemiology of disease at different season of the year.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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