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Full Length Research Paper

A study on cattle trypanosomosis and its vectors in Gimbo and Guraferda districts of Southwest Ethiopia

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A cross-sectional study was carried out to determine the prevalence of cattle trypanosomosis and assess the distribution of its vectors in Gimbo and Guraferda districts of Kaffa and Bench Maji zones, respectively, in Southern Nations, Nationalities and Peoples' Regional State (SNNPRS), Ethiopia from November 2011 to March 2012. Simple random sampling technique was used to select 490 local zebu cattle from purposively selected three peasant associations in Gimbo and four peasant associations in Gurafereda districts. Blood samples were examined for trypanosmes by the buffy coat technique (BCT) after determining the packed cell volume (PCV) and traps were employed for collection of tsetse. The overall prevalence of cattle trypansomosis was 14.5%. The prevalence varied between the two districts, that is, Gimbo (9.2%) and Guraferda (19.6%). *Trypanosoma congolense* was the predominant species in the area (62%) followed by *T. vivax* (28.2%). Statistically significant (P<0.05) difference was observed in infection rate for the different trypanosome species, and in prevalence among animals of different body condition scores (p<0.001). However, no significant (P>0.05) difference was noted in prevalence rates among animals of the different age groups, sex and coat colors. The mean PCV per cent value of parasitaemic animals (21.31±5.070) was significantly (p<0.001) lower than that of aparasitaemic animals (27.00±5.097). *Glossina pallidipes* and *Glossina fuscipes* were captured in the study area with an overall apparent density of 3.73 flies/trap/day. In the light of these findings, integrated approaches involving both vector and trypanosome directed measures are suggested for effective management of the problem of cattle trypanosomosis in the study area.

Key words: Bench Maji, bovine, buffy coat, Kaffa, trap, trypanosomosis, tsetse fly.

INTRODUCTION

African animal trypanosomosis, also called 'nagana' is a parasitic disease that causes a serious economic losses in livestock from anemia, loss of condition, emaciation and death in untreated cases (OIE, 2009). It is one of the

major impediments to livestock development and agricultural production in Ethiopia negatively influencing overall development and food self-reliance endeavors of the country. Tsetse transmitted trypanosomosis excludes more than 220,000 km^2 of suitable land for agriculture in the West and South West parts of the country with 14 million head of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting trypanosomosis at any one time (MoARD, 2004).

Ethiopia is home to the largest livestock population in Africa with approximately 47.5 million cattle, 26.1 million sheep, 21.7 million goats, 7.8 million equine, 1 million camels, and 39.6 million chickens (CSA, 2009). Livestock perform multiple functions in the Ethiopian economy by providing food, input for crop production and soil fertility management, raw material for industry, cash income as well as in promoting saving, fuel, social functions, and employment. The contribution of the livestock sub-sector is estimated at 12 to 16% of the total and 30 to 35% of agricultural GDP (AAPBMDA, 1999).

Trypanosomosis is a debilitating and often fatal disease of various domestic animals and humans caused by various species of an extracellular haemo-flagellate parasite infection of genus trypanosome (Juyal et al., 2005; OIE, 2005). Trypanosomes can infect all domesticated animals and more than 30 species in the wild or zoos. In parts of Africa, cattle are the main species affected, due to the feeding preferences of tsetse flies. The host of each trypanosome species may differ, but *Trypansoma congolense*, *Trypansoma vivax* and *Trypansoma brucei* have a wide host range among domesticated animals (OIE, 2009). *T. congolense* infects cattle, pigs, sheep and goats, while *T. brucei* commonly infects cattle, horses, dogs, cats, camels, sheep, goats and pigs (OIE, 2005). *Trypansoma evansi* is commonly noted camels, buffaloes, cattle, horses, etc., and *Trypansoma equiperdum* in horses (Getachew, 2005; Singh and Singla, 2012).

Most trypanosomes must develop for one to a few weeks in tsetse flies, which act as biological vectors. When an infected tsetse fly bites an animal, the parasites are transmitted in the saliva (OIE, 2005). The three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favors the open wood land of the savanna; *Glossina palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favors the high dense forest areas (Aiello and Mays, 1998). Trypanosomosis is also mechanically transmitted by tsetse and other biting flies through the transfer of blood from one animal to another. The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota* and *Stomoxys* flies have also been implicated. In Africa, *T. vivax* has spread beyond the "tsetse fly belts" (Roeder et al., 1984).

Most cases of trypanosomosis are chronic, but acute diseases, which may be fatal within a week, can also occur (OIE, 2009). Susceptibility of cattle to trypanosomosis depends on breed, age, behavior, previous exposure, and pathogen and health status of animal. The indigenous zebus are trypano susceptible and West Africa *Bos taurus* breeds are trypnotolerant, that is, they can survive and be productive without treatment under trypanosomosis risk (Murray and Gray, 1984; Taylor and Authie, 2004).

Earlier study by Abebayehu and Biniam (2010) in Guraferda and Sheko districts indicated the problem of trypanosomosis to be very serious in the area. Another survey carried out by Mizan Teferi (2010) at Regional Veterinary Laboratory in Gimbo and Guraferda districts determined trypanosomosis and its vectors to be the most important livestock development constraints. This necessitates a continued follow-up and evaluation of the status of tsetse infestation and occurrence of trypanosomosis in these and surrounding villages. The objectives of this study were therefore (i) to determine the current parasitological prevalence of cattle trypanosomosis in selected districts of Kaffa and Bench Maji zones, (ii) to identify and determine the predominant trypanosome species infecting cattle in the study areas, and (iii) to assess the prevailing species and density of tsetse flies.

MATERIALS AND METHODS

Study area description

Gimbo and Guraferda districts are geographically located in the southwest part of Ethiopia in Keffa and Bench Maji zones in Southern Nations, Nationalities, and Peoples' Regional State (SNNPRS), which are 430 and 600 km far from the national capital Addis Ababa, respectively. Climatically, the areas are characterized by long rainy season (June to October), short rainy season (February to May), and short dry season (November to January). Gimbo district (latitude: 07˚27' N; longitude: 036°16' E) has a total land area of 87,186.05 km^2 with an altitudinal range of 1270 to 3500 m above sea level (m.a.s.l). The average annual temperature is 25°C and annual rainfall ranges from 750 to 1150 mm. Guraferda district (latitude 6°49'-7°21'N and longitude 34°88'-35°43'E) has a total land coverage of 2,505.80 km^2 and an altitude ranging between 600 and 2500 m.a.s.l. The areas are covered by different vegetation types of savanna grass land, forest and bush lands with many wild lives. The dominant livestock populations are cattle, sheep and goats where all of them are raised under traditional extensive management system. Mixed livestock-crop farming is the dominant production system. The total livestock population of Gimbo district is estimated to be 116,680 cattle, 34,549 sheep, 22,301 goats, and 9,876 equines, while Guraferda district has 101,763 cattle, 41,353 sheep, 15,828 goats and 2,881 equines (MTRVL, 2009).

Study animals

The study animals included all ages and sex groups of local zebu cattle (*Bos indicus*) kept under traditional extensive management system together with other livestock species and fed mainly on natural pasture.

Study design

A cross-sectional survey design was conducted from November 2011 to March 2012 to determine the prevalence of cattle trypanosomosis in Gimbo and Guraferda districts, and to determine the density and species of tsetse flies in the areas.

Sample *size* and *sampling method*

Two district (Gimbo and Guraferda) and seven peasant associations (PA's), namely: Yabekicha wellega, Gojeb, and Chomba from Gimbo district and Kometa, Gobika, Semerta, and Kuki from Guraferda district were purposively selected based on accessibility and expected challenge of trypanosomosis. Simple random sampling technique was followed to select the study animals. During sampling, peasant associations, age, sex, coat colors, and body condition score of the animals were recorded. Body condition for each cattle was estimated based on descriptions given by Nicholson and Butterworth (1986). The age of the animals was determined by dentition (De Launta and Hable, 1986). Coat color was recorded based on visualization of the main coat color such that if a second color was present, the predominant coat color was recorded.

The sample size was calculated according to the formula given by Thrusfield (2005) considering an expected prevalence of 50%, 95% confidence level and 5% desired precision.

$$N = (1.96)^2 \times p_{exp} (1-p_{exp})/d^2$$

where N=number of sample size, p_{exp}=expected prevalence, and d^2=Absolute precision.

Therefore, based on the aforementioned formula 384 were required for the study. But to improve the degree of accuracy and account for some sample loss, a total of 490 samples were taken for the present study.

Sample collection and parasitological examination

Blood collection

Blood samples were collected from the marginal ear vein by pricking it with the tip of a sterile lancet after properly securing the animal and aseptically preparing the area around the ear vein. The samples were collected using two hematocrit capillary tubes to ¾th of the length and sealed with bee wax in one end (OIE, 2008).

Packed cell volume (PCV) determination

The blood in the microhematocrit capillary tubes was centrifuged at 12,000 rpm for 5 min using the Hawksley microhematocrit centrifuge to estimate the PCV (Murray et al., 1983). Animals with PCV less than 24% were considered anemic (OIE, 2008).

Buffy coat technique

After centrifugation and PCV determination, the capillary tubes were cut using a diamond tipped pen 1mm below the buffy coat to include the upper most layers of the red blood cells and 3 mm above to include the plasma. The content of the capillary tube was expressed onto a slide, covered with a cover slip and was examined under 40X objective and 10X eye piece for motile trypanosomes (Paris et al., 1982).

Thin blood smear

Positive samples in the buffy coat technique were further processed by Giemsa-stained thin blood smear prepared from the buffy coat for identification of trypanosome species based on their morphological characteristics (Murray et al., 1983).

Entomological survey

Survey for tsetse and other biting flies was conducted along Gojeb and Akobo rivers which are the main drainage system in the areas, and their tributaries from November 2011 to March 2012. Trap deployment sites were selected to represent all habitats that could be suitable habitat to tsetse fly multiplication, behavior, feeding and other related aspects. During the survey period, 29 NGU and 24 monoconical traps baited with acetone, octanol and cow urine were deployed for 48 h at approximate intervals of 100 to 200 m. The poles of the traps were greased to prevent fly predators like ants. The catches of each trap was collected after 48 h of deployment. The number and the type of traps used, coordinates and altitude of the deployment site were recorded by a global positioning system (GPS). Tsetse and other biting flies caught per trap were counted, species identified, sexed and apparent fly density (F/T/D) were calculated (Mulligan, 1970).

Data analysis

Data were coded and entered in to a Microsoft excel spreadsheets and transferred to SPSS version 15.0 for analysis. Differences in the prevalence of trypanosomosis in animals from different districts, peasant association, age, sex, color, body condition score, and trypanosome species were compared by Pearson's chi-squared test. The mean PCV of infected and non-infected animals were compared with student t-test.

RESULT

Parasitological findings

The overall prevalence of cattle trypanosomosis in the study areas was 14.5%. The prevalence of cattle trypanosomosis in Gimbo and Guraferda districts was 9.2 and 19.6%, respectively. Statistically significant difference (P<0.05) was observed in the prevalence of trypanosomosis in cattle between the two districts. A significant difference (p<0.05) in the prevalence of trypanosomosis was noted between PA's and ranged

Table 1. Prevalence of trypanosome infection in cattle at seven PA's of Gimbo and Guraferda districts.

District	PA	Altitude	No. examined	Positive	Prevalence (%)
Gimbo	Yabekicha wellega	1443	86	11	12.8
	Gojeb	1403	100	9	9.0
	Chomba	1380	54	2	3.7
Guraferda	Kometa	890	54	10	18.5
	Gabika	949	58	14	24.1
	Semerta	1043	59	9	15.3
	Kuki	925	79	16	20.3
Overall	-	-	490	71	14.5

$\chi 2$ = 10.758, P-value = 0.001 for district $\chi 2$ = 14.914, p-value = 0.021 for PA.

Table 2. Prevalence of trypanosome species identified in the study area.

PA	*T. congolense* (%)	*T. vivax* (%)	*T. brucei* (%)	T. c+T.v (%)	T.c+T.b (%)	T.v+T.b (%)
Yabekicha wellega	9 (81.8)	2 (18.2)	-	-	-	-
Gojeb	5 (55.6)	3 (33.3)	1 (11.1)	-	-	-
Chomba	1 (50.0)	1 (50.0)	-	-	-	-
Kometa	8 (80.0)	1 (10.0)	-	1 (10)	-	-
Gabika	8 (57.1)	4 (28.6)	1 (7.1)	1 (7.5)	-	-
Semerta	5 (55.6)	3 (33.3)	-	1 (11.1)	-	-
Kuki	8 (50)	6 (37.5)	-	-	1 (6.25%)	1 (6.25)
Total	44 (62.0)	20 (28.2)	2 (2.8)	3 (4.2%)	1 (1.4%)	1 (1.4)

$\chi 2$=37.469, P-value= 0.402; T.c+T.v: mixed infection of *T. congolense* and *T. vivax*; T.c+T.b: mixed infection of *T. congolense* and *T. brucei*; and T.v+T.b: mixed infection of *T. vivax* and *T. brucei*.

between 3.7% at Chomba and24.1% at Gabika (Table 1). *T. congolense* was the most prevalent species (62.0%) followed by *T. vivax* (28.2%), mixed *T. congolense* and *T. vivax* infection (4.2%), *T. brucei* (2.8%), mixed infection of *T. congolense* and *T. brucei* (1.4%) and mixed infection of *T. vivax* and *T. brucei* (1.4%). However, the observed difference in prevalence among the various species of trypanosomes was not significant (P>0.05) (Table 2). The prevalence of trypanosomosis among the different age groups were: 10.6% in the age group 1 to 2 years, 13.5% in the 2 to 4 years, and 15.8% in those greater than 4 years of age, but the difference was not significant (P>0.05). No statistically significant difference (P>0.05) was observed in the prevalence of trypanosomosis between males and females. The prevalence of trypanosomsis in cattle with good, medium and poor body conditioned animals was 4.3, 9.9 and 26.7%, respectively and the difference was highly significant (P<0.001). There was no significant difference (P>0.05) in prevalence between animals of different coat colors (Table 3).

Hematological findings

The mean PCV values of parasitamic and aparasitamic animals during the study period were 21.3 and 27%, respectively. The difference was highly significant (P<0.001) (Table 4).

Entomological findings

During the study period, 439 flies were caught using 29 NGU and 24 monoconical traps from Yabekicha Wellega, Chomba, Semerta and Kuki peasant associations. Out of these, 396 were *Glossina* species, 45 *Stomoxys* and 2 were *Tabanus* flies. Out of the 396 *Glossina* spp., 357 were *G. pallidipes* and the rest (39) were *G. fuscipes*. The numbers of male and female *Glossina* species were 162 and 234, respectively. The overall apparent density of tsetse flies was 3.73 flies per trap per day (F/T/D). The apparent tsetse fly density in Gimbo and Guraferda

Table 3. Prevalence of cattle Trypaonosomosis by age, sex, and body condition and coat color.

Variable	Total examined	No. of positive	Prevalence (%)	χ2	p-value
Age (Years)					
1-2	66	7	10.6		
2-4	133	18	13.5	1.309	0.520
>4	291	46	15.8		
Sex					
Male	277	42	15.2		
Female	213	29	13.6	0.233	0.630
Body condition					
Poor	180	48	26.7		
Medium	172	17	9.9	35.942	0.000
Good	138	6	4.3		
Coat color					
White	79	8	10.1		
Red	280	37	13.2	5.065	0.167
Black	79	17	21.5		
Brown	52	9	17.3		

Table 4. Mean PCV of parasitaemic and aparasitameic animals.

Status	No. examined	Mean PCV (%)	Standard deviation	t-test	p-value
Parasitaemic	71	21.31	5.070		
Aparasitaemic	419	27.00	5.097	102.758	0.000
Total	490	26.17	5.468		

Table 5. Results of the entomological survey.

District	Altitude	No. of traps	No. of days	*Glossina* spp. caught							*Stomoxys*	*Tabanus*
				G. pallidipes			*G. fuscipes*					
				M	F	T	M	F	T	F/T/D		
Gimbo	1276-1445	26	2	123	171	294	17	18	35	6.32	29	1
Guraferda	858-1030	27	2	22	41	63	-	4	4	1.24	12	1
Total	-	53	4	145	212	357	17	22	39	3.73	41	2

F/T/D: Fly catch per trap per day; M: male, F: female; T: total.

districts was 6.32 and 1.24 F/T/D, respectively (Tables 5).

DISCUSSION

The study found that the overall prevalence of bovine trypanosomosis in Gimbo and Guraferda districts was 14.5%. This finding coincides with the result of Fasil (2004) who reported a prevalence of 15.5% in Guraferda district and Feyissa et al. (2011) who reported a prevalence of 14.2% for bovine trypanosomosis in Humbo district, Southern Ethiopia. By comparison, the

present study revealed higher prevalence of trypanosomosis in cattle than most authors' reports conducted in different parts of the country: Mihreteab and Mubarik (2011) 8.6% prevalence in Hawagelan district, west Wellega zone; Nigatu and Abebe (2009) 10.1% prevalence in Awi zone, north western Ethiopia; and Molalegne et al. (2010) 11.7% prevalence in Jabi Tehenan district, north western Ethiopia. High prevalence of trypanosomosis in the present study can be associated with the presence of favorable environmental conditions for the existence and development of *Glossina* species and other biting flies. Statistically significant difference (P<0.05) was observed in the prevalence of cattle trypanosomosis among the study district where 9.2 and 19.6% prevalence rates were noted in Gimbo and Gurafereda districts, respectively. The low prevalence of trypanosomosis in Gimbo district might be due to awareness of the people towards the control and treatment of the disease and the improved management of animals. High challenge of tsetse fly in the Gimbo district makes the owners self-aware and get initiated local tsetse fly control operation in addition to providing chemotherapeutic drugs to their animals by themselves. The same may not hold true in Guraferda district.

Statistically, significant difference (P<0.05) was observed in the prevalence of trypanosomosis among the study PA's. The high prevalence of the disease especially at Gabika, Kuki, Kometa, and Semerta in Guraferda district and Yabekicha wellega in Gimbo district PA's could be due to the low altitude and the presence of suitable habitat for the vectors which results high fly challenge, respectively. Such variation in tsetse density has also been reported to be the main factor for variations in the prevalence of trypanosomosis (Leak et al., 1993).

T. congolense was the predominant species detected (62%). This result agrees with Shimelis et al. (2011) and Feyissa et al. (2011) who reported 65 and 65.7% prevalence of *T. congolense* compared to 35 and 20% *T. vivax* in Jawi district of Amhara region and Humbo district of southern Ethiopia, respectively. The study of Solomon and Fitta (2011) in Metekel and Awi zone of North West Ethiopia also showed higher result of *T. congolense* (77.6%) compared to 14.9% *T. vivax*. The higher results of *T. congolense* may suggest that *G. pallidipes* caught in the study area, which is a more efficient transmitter of *T. congolense* than *T. vivax,* is the major cyclical vector involved (Langridge, 1976). The present study also found *G. pallidipes* to be present at a relatively higher density. Similar results were reported by Fasil (2004) and Abebayehu and Biniam (2010) in the same study area. Mihreteab and Mubarik (2011) also noted *T. congolense* and *T. vivax* to be the most prevalent trypanosome species that infect cattle in tsetse infested and tsetse free areas of Ethiopia, respectively.

No significant difference (P>0.05) in infection rates was seen among the three age groups of animals though higher infection rate was recorded in animals of 4 years and above. Similar finding were reported by Mihreteab and Mubarik (2011) and Molalegne et al. (2010). This may be attributed to the longer distance older animal have to trek for grazing and drinking bringing them closer to higher tsetse challenge localities. Lower prevalence of trypanosomosis was observed in calves between 1 and 2 years and young animals between 2 and 3 years. This could partly be explained by the protection of maternal antibodies in calves and young animals (Fimmen et al., 1999). There was no significant difference (P>0.05) in prevalence rates among male (15.2%) and female (13.6%) animals. However, higher infection rate was recorded in male animals. According to Shimelis et al. (2011), the possible explanation for the relatively high prevalence in male animals could be associated with the fact that these animals travel long distances for draught as well as harvesting crops into areas with high tsetse challenge. The present study showed statistically significant difference (P<0.001) between animals of poor, medium and good body condition scores. Similar results were reported by Mihreteab and Mubarik (2011) and Feyissa et al. (2011). This may be the effect of the disease itself which results in progressive emaciation of infected animals (Stephen, 1986). There was no significant difference in infection rate among animals of different coat colors although higher infection rate was recorded in black and brown animals. Black and red colors have been found to be more attractive to tsetse species, with a strongest landing response on black surface (Green, 1993).

The present study found a mean PCV of 21.31 and 27.0% in parasitemic and aparasitemic animal, respectively. The mean PCV of parasiemic animals was found to be significantly lower (P<0.001) than that of aparasitemic animals, similar with the observations of Tewelde (2001) and Abebayehu and Biniam (2010). As anemia is the classical symptom of trypanosome pathogenicity, the low PCV in parasitemic animals could be attributed to the parasite infection. Anaemia is one of the most important indicators of trypanosomosis in cattle (Stephen, 1986).

The overall apparent density of tsetse was 3.73 F/T/D. This finding was similar with Abebayehu and Biniam (2010) who recorded a tsetse density of 2.83 F/T/D in the area. But the present finding was lower than that reported by Abebayehu and Gurarra (2010) who recorded 10.5 F/T/D in Guto Gidda district. The apparent density of tsetse was 6.32 and 1.24 F/T/D in Gimbo and Guraferda districts, respectively. The lower value of fly density in the current study might be due to the use of tsetse fly habitats for cultivation by settlers and investors in the two

districts. The season when the study was done might also have contributed for the noted lower density than previous reports of Mizan Teferi Regional Veterinary Laboratory which was done during the rainy season (MTRVL, 2009).

Two tsetse species (*G. pallidipes* and *G. fuscipes*), *Stomoxys* and *Tabanus* flies have been found in the two study districts. The numbers of female tsetse flies were higher than male tsetse flies in the study area. Phelps and Lovemore (1994) associated such higher catches of female tsetse to be attributable to their longer life span (average of 8 weeks) than males living about 4 weeks, so that more catch of females could appear.

CONCLUSION

From the findings, it is concluded that tsetse transmitted cattle trypaonosomosis is a major constraint to cattle production in the study areas. The predominant species of trypanosomes in the study area were *T. congolense* followed by *T. vivax,* mixed infection of the two species and *T. brucei* respectively. *G. pallidipes, Stomoxys* and *G. fuscipes* were the main vectors of the pathogenic trypanosomes in the area.

RECOMMENDATIONS

Based on the aforementioned, there is an urgent need to create awareness about the disease and control methods throughout the community. There is also a great need to supply more trypanocidal drugs and integrated vector and trypaonosomosis management programs. This will assist greatly to reduce the disease prevalence in the areas.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Potency of four different commercial infectious bursal disease (IBD) vaccines and different vaccination schedules in cockerels

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A potency trial was conducted to evaluate the potency of four commonly used infectious bursal disease (IBD) live vaccines to determine the most appropriate vaccination response to establish the most appropriate vaccination programme against IBD in Nigeria. A total of 700 day old cockerels were randomly divided into six groups 1, 2, 3, 4, 5, and 6. Groups 1 to 4 were the vaccination cockerels while Groups 5 and 6 were the positive and negative controls. The chicks were sub divided into five groups A, B, C, D and E representing the different schedules that is, 1 and 3 weeks, 2 and 4 weeks, 3 and 5, 1, 3 and 5, 2, 4 and 6 weeks, respectively. After each vaccination, the chicks were observed for clinical signs of IBD. Two weeks after each vaccination, chicks were challenged with IBD virus, onset of clinical signs, morbidity and mortality rates were observed. Sample of bursa was collected from 5 birds at 3 and 7 days post challenge from dead as well as necropsied birds. The lesions were scored on the scale of 0 to 5 (mild to severe). Vaccine 3 appeared to be the best with the mean of 1.8 for gross lesions, 1.2 for histo-pathological lesions, 0.6 for clinical signs, 0.6 for mortality, 0.2 for antibody titre and 1.8 for bursal body weight ratio. Schedule A (vaccination at 1 and 3 weeks) also was the best for all the vaccines. Therefore, Nigerian poultry farmers are advised base on the aforementioned result to use vaccine 3 and schedule A in the control the outbreaks of IBD.

Key words: Cockerels chicks, live Infectious bursal disease (IBD) vaccines, potency, vaccination schedule, vaccination strategies.

INTRODUCTION

Infectious bursal disease (IBD) is an acute and highly contagious viral disease of chicks that is immuno-suppressive (Iftihar et al., 2001). The virus has lymphoid tissue as primary target with special predilection for bursa of Fabricius (Iftihar et al., 2001). The disease is characterized by trembling, incoordination, inflammation, followed by necrosis and atrophy of the bursa of Fabricius and immunosuppresion (Giambrone, 1983; Abdu, 2007, 2010). The disease was first described by Cosgrove (1962); it causes economic loss in the poultry

industry primarily in the form of mortality and weight loss in affected birds (Abdu, 2010). Chicken 3 to 6 weeks of age are most susceptible to clinical infection (Abdu, 2007). The effective means of controlling infectious bursal disease is vaccination (Abdu, 2007). Infectious bursal disease vaccines had been categorized into mild, intermediate and hot vaccine strain according to the bursa/body weight ratios values following vaccination (Boudaoud and Alloui, 2008). Mild vaccine did not induce bursal lesions and are used in parent chickens to produce primary response prior to vaccination with inactivated vaccine (Babiker and Tawfeeq, 2008); intermediate strains enlarge the bursa twice the normal size. At present there are many imported vaccines and one indigenous vaccine commonly used in Nigeria against IBD. These imported vaccines strains have become popular as they can be used in the presence of maternal antibodies. But experimental results reflected that vaccination of susceptible chickens with such vaccines caused outbreaks of IBD on commercial poultry based on postmortem observations, increased bursal weight to body weight ratio and agar gel precipitation test (Abdu, 1997). IBD has been a persistent problem for the commercial chicken industry worldwide since its discovery in Gomboro District Delaware, USA in 1950, causing huge economic lost mortality and immuno-suppresion (Giambrone, 2008). The present study was designed to evaluate the potency of the four commonly used vaccines (Vaccines 1, 2, 3, and 4) in Nigeria.

MATERIALS AND METHODS

Study area and environmental conditions

The experiment was performed at the livestock farm of the College of Agriculture and Animal Sciences, Ahmadu Bello University, Kaduna located in the Northern Guinea Savanna zone of Nigeria.

Animals and management

The study was approved by the Post graduate Research Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria and conducted according to International guidelines on animal ethics (Farsha, 2006). A total of 700 day-old cockerels were purchased from Zartech Nigeria Limited, Ibadan Nigeria.

Vaccination

Vaccination against IBD was carried out in both control and experimental chicks via oral route administration of National Veterinary Research Institute (NVRI) IBD live vaccine (Batch No 21511063 E).

Ration

Vital feed was obtained from a commercial distributor of the product in Kaduna, Kaduna state. The feed giving to the birds had the following composition in g per 100 g of feed: Crude protein 19, fats 5, crude fibre 5.00, available phosphorus 0.45, calcium 1.00, lysine 1.00, methionine 0.40, and salt 0.3, metabolizable energy 2650 kcal. Feed and water were provided to the chicks *ad libtum.*

Vaccination and blood sampling for antibody titre

The chicks were all bled through the heart at day old to get a base line data of antibody titre. After one week, sub group A and D (Schedule 1 and 3, and 1, 3 and 5, respectively) were bled using insulin syringe to get 3 ml of blood through the heart, the chicks were then vaccinated individually with 0.5 ml of the vaccine. At two weeks, blood was collected from 10 of the vaccinated chicks, while chicks from sub group B and E were bled and vaccinated accordingly. Sera were obtained then stored in the refrigerator until used (2 weeks). At three weeks old, chicks from sub group A, C and D were bled and vaccinated sera were obtained then stored in the refrigerator until used. At four weeks old, chicks from sub group B and E were bled and vaccinated sera were obtained and stored in the refrigerator until used. At five weeks old, chicks were bled and vaccinated. At six weeks old chicks from sub group B and E were bled for antibody. At seven weeks chicks from sub group C and D were also bled and sera were obtained and then stored in the refrigerator until used. Finally, at eight weeks, chicks from sub group E were bled and sera were obtained and stored in the refrigerator until used. The sera at end of 8 weeks were subjected to enzyme linked immune sorbent assay (ELISA).

Challenge virus preparation

The affected bursae of chickens that died from natural IBD were removed, weighed and diluted 1:1(w/v) with PBS (pH 7.6) and ground with a pestle and mortar with the aid of fine sterile sand. The mixture was then frozen, thawed and ground three times and clarified at 2,000 × g for 30 min. Two hundred and fifty I.U. of penicillin and 250 ug of streptomycin were added to the supernatant fluid (2 ml portion) and stored in screw-cap vials at -20°C until used.

Challenge

A 50% suspension w/v of homogenate bursa of fabricius prepared as stated already was used as challenged virus. Five chicks from each sub group were challenged two weeks after each vaccination of the various sub groups (3, 4, 5, 6, 7 and 8 weeks, respectively). Sample of bursa from 5 chickens each at 3 and 7 days post challenged with IBDV, respectively was collected from dead as well as necropsied birds. After challenged, the birds were observed for the development of morbidity, mortality, gross lesions and histopathological studies at post mortem (Babiker and Tawfeeq, 2008) as seen in Table 1.

Blood sample collection

Blood was collected from the chicks before and after vaccination for a period of 8 weeks for antibodies assessment as shown in Table 2. The blood samples collected were kept in a tilted position on a test tube rack for 1 h to get sera.

Enzyme linked immunosorbent assay kit

IDEXX Flock Check Standard ELISA kit, obtained from the IDEX Drive Company (IDEXX LaboratoryWestbrokmaine 04092 U.S.A.) was used to detect antibody titres. The kit consisted of IBD antigen coated plates (5 ml), one bottle of infectious bursal disease positive

Table 1. The clinical signs gross lesion score , histopathological scores , mortality antibody titre, bursal body weight scores for chickens vaccinated with vaccine 1 using different schedules.

Schedule	Clinical signs score	Gross lesions score	Histopat-hologic score	Mortality score	Antibody titre score	Bursal body weight ratio score	Mean score Per schedule
A	0.0	0.0	3.0	0.0	1.0	2.0	1.2
B	0.0	3.6	3.0	0.0	0.0	2.0	1.4
C	1.0	2.5	2.0	1.0	1.0	2.0	1.6
D	1.0	0.0	2.0	1.0	1.0	2.0	1.2
E	1.0	2.7	0.0	1.0	1.0	2.0	1.3
Mean across schedules	1.5	1.8	2.0	1.5	2.0	2.0	1.8

Key: A-Schedule 1 and 3 weeks. B-Schedule 2 and 4 weeks. C-Schedule 3 and 5 weeks. D-Schedule 1, 3 and 5 weeks. E-Schedule 2, 4 and 6 weeks.

control-diluted with sodium azide (1.9 ml), 1 bottle of negative control-diluted chicken sera non-reactive for anti-IBD preserved with sodium azide (1.9 ml), one bottle (goat) anti-chicken. Horseradish peroxidase conjugate preserved with gentimacin (50 ml), 1 bottle sample diluent buffer preserved with sodium azide   (235 ml), 1 bottle TMB substrate (60 ml), one bottle of stop solution (60 ml). Materials used but not provided in the kits included precision pipette and multiple delivery pipetting device with disposable pipette tips, 96-well plate reader, tubes for diluting samples, distilled water and device for the delivery and aspiration of wash solution.

Enzyme linked immunosorbent assay

The enzyme linked immunosorbent assay (ELISA) technique was carried out according to the methods described by IDEXX Laboratories Incorporation, USA. The reagents in the ELISA kit were brought to room temperature (18 to 25°C) prior to the test. The test sample was diluted to five hundred folds (1:500), with sample diluents prior to the assay. One hundred microlitres (μl) of the diluted sample was then dispensed into each well of the plate. This was followed by 100 μl undiluted negative control into wells A1 and A2, 100 μl of undiluted positive control was dispensed into wells A3 and A4. The plate was incubated for 30 min at room temperature. Each well was washed with approximately 350 μl of distilled water, 3 to 5 times. Goat anti-chicken peroxidase (100 ml) was added as conjugate into each well and was incubated for 30 min at room temperature. After incubation the liquid content

was aspirated with a pipette into a waste reservoir and each well was washed 3 to 5 times with about 350 μl of distilled water and then the water was aspirated completely. Tubular basement membrane solution (100 μl) was dispensed with multiple delivery pipette into each well and then incubated for 15 min at room temperature. Finally 100 μl of stop solution was dispensed into each well to stop the reaction. The absorbance values at 650 nm were measured with an ELISA reader and recorded. Infectious bursal disease antibody titre was calculated automatically using a software by Blankfard and Silk (1989)

Clinical evaluation

After challenge, the chickens were monitored for clinical signs and mortalities for one week. At the end of the experiment clinical signs, mortality rates, gross lesions, microscopic lesions antibody titre was calculated as a percentage of the initial number of the birds as describe by Babiker and Tawfeeq (2008). The clinical signs, morality rates, antibody titre, gross lesions, microscopic lesions, bursa body weight ratio were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3), and severe (4) by modified scoring of bursa based on Hair-Bejo (2000) method.

Statistical analysis

Data collected were analyzed using SPSS version 17.0. Analysis of variance was used to compare the means

antibody titre. Descriptive statistic was used for clinical sign, gross lesson, histopathology, mortality and bursal body weight ratio. Turkey's post hoc test was used to compare the means across the groups. P < 0.05 was considered significant.

RESULTS

Vaccine 1

Schedule A (1 and 3 weeks)

Chickens were vaccinated with vaccine 1, at 1 and 3 weeks, the gross lesion score was 0; histopathological score was 3; clinical sign was 0; mortality was 0; antibody titre 1; bursal body weight ratio was 2 and the mean score was 1.2 (Table 1).

Schedule B (1and 2 weeks)

Chickens were vaccinated at 2 and 4 weeks old. The gross lesion score was 3.6; histopathological score was 3.6; clinical sign score was 0; mortality 0; antibody titre was 0 but the bursal body weight ratio was 2 and the mean score was 1.43 (Table 2).

Table 2. The clinical signs Gross lesion score , histopathological scores , , mortality antibody titre, bursal body weight scores for chickens vaccinated with **vaccine 2** using different schedules.

Schedule	Clinicalsigns score	Gross lesions score	Histo-Pathologic score	Mortality score	Antibody titre score	Bursal body weight ratio score	Mean score Per schedule
A	0.0	0.0	2.0	0.0	1.0	2.0	1.0
B	1.0	4.5	3.0	1.0	0.0	2..0	1.9
C	1.0	2.5	3.0	1.0	0.0	2.0	1.6
D	0.0	2.6	4.0	0.0	0.0	2.0	1.4
E	1.0	1.0	4.0	1.0	0.0	2.0	1.5
Mean across schedules	0.6	2.7	3.2	0.6	2.0	2.0	1.5

Key: A-Schedule1 and 3 weeks. B-Schedule 2 and 4 weeks. C-Schedule 3 and 5 weeks. D-Schedule 1,3 and 5 weeks. E-Schedule 2,4and 6 weeks.

Schedule C (3 and 5 weeks)

Chickens were vaccinated at 3 and 5 weeks old. The gross lesion score was 2.5; histopathological score was 2; clinical sign score was 1; mortality 1; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score was 1.58.

Schedule D (1, 3 and 5 weeks)

Chickens were vaccinated at 1, 3 and 5 weeks old. The gross lesion score was 0; histopathological score was 2; clinical sign was 1; mortality 1 antibody titre was 1; but the bursal body weight ratio was 2 and the mean score was 1.17.

Schedule E (2, 4 and 6 weeks)

Chickens were vaccinated at 2, 4 and 6 weeks old. The gross lesion score was 2.7; histopathological score was 0; clinical sign score was 1; mortality 1; antibody titre was 1; but the bursal body weight ratio was 2 and the mean was 1.28 (Table 4).

Vaccine 2

Schedule A (1 and 3 weeks)

Chickens were vaccinated with vaccine 1, at 1 and 3 weeks old. The clinical sign was 0; gross lesion score was 0; histopathological score was 2; mortality was 0; antibody titre 1; bursal body weight ratio was 2 and the mean score for the schedule was 1.0 (Table 2)

Schedule B (1 and 2 weeks)

Chickens were vaccinated at 2 and 4 weeks old. The clinical sign score was 1; gross lesion score was 4.5; histopathological score was 3; mortality 1; antibody titre was 0; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.43 (Table 2).

Schedule C (3 and 5 weeks)

Chickens were vaccinated at 3 and 5 weeks old. The clinical sign score was 1; gross lesion score was 2.5; histopathological score were 3; mortality 1; antibody titre was 0; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.58 (Table 2).

Schedule D (1, 3 and 5 weeks)

Chickens were vaccinated at 1, 3 and 5 weeks old. The clinical sign was 1; gross lesion score was 0; histopathological scores was 3; mortality 1; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.43 (Table 2).

Schedule E (2, 4 and 6 weeks)

Chickens were vaccinated at 2, 4 and 6 weeks old. The clinical sign score was 1; gross lesion score was 1.0; histopathological score was 4; mortality 1; antibody titre was 0; but the bursal body weight ratio t was 2 and the mean scores for this schedule was 1.50 (Table 2).

Table 3. The clinical signs, Gross lesion score , histopathological scores , , mortality antibody titre , bursal body weight scores for chickens vaccinated with **vaccine 3** using different schedules.

Schedule	Clinicalsigns score	Gross lesions score	Histo-Pathologic score	Mortality score	Antibody Titre score	Bursal body weight ratio score	Mean Per schedule
A	0.0	0.0	1.0	0.0	1.0	2.0	0.8
B	0.0	3.3	1.0	0.0	0.0	2.0	1.1
C	1.0	2.0	1.0	1.0	0.0	2.0	1.2
D	1.0	1.0	1.0	1.0	0.0	2.0	1.0
E	1.0	1.0	2.0	1.0	0.0	1.0	1.0
Mean across schedules	0.6	1.8	1.2	0.6	0.2	1..8	1.0

Key: A-Schedule1 and 3 weeks. B-Schedule 2 and 4 weeks. C-Schedule 3 and 5 weeks.

Vaccine 3

Schedule A (1 and 3 weeks)

Chickens were vaccinated with vaccine 1, at 1 and 3 weeks old. The clinical sign was 0; gross lesion score was 0; histopathological score was 1; mortality was 0; antibody titre 1; bursal body weight ratio was 2 and the mean score for the schedule was 0.8 (Table 3).

Schedule B (1and 2 weeks)

Chickens were vaccinated at 2 and 4 weeks old. The clinical sign score was 0; gross lesion score was 3.3; histopathological score was 1; mortality 0; antibody titre was 0; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.1 (Table 3).

Schedule C (3 and 5 weeks)

Chickens were vaccinated at 3 and 5 weeks old. The clinical sign score was 1; gross lesion score was 1; histopathological score was 1; mortality 1; antibody titre was 0; but the bursal body weight ratio was 2 and the mean score for this schedule

was 1.2 (Table 3).

Schedule D (1, 3 and 5 weeks)

Chickens were vaccinated at 1, 3 and 5 weeks old. The clinical sign was 1; gross lesion score was 1; histopathological score was 1; mortality 1; antibody titre was 0; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.0 (Table 3).

Schedule E (2, 4 and 6 weeks)

Chickens were vaccinated at 2, 4 and 6 weeks the clinical sign score was 1; gross lesion score was 1.0; histopathological score was 2; mortality 1; antibody titre was 0; but the bursal body weight ratio was 2 and the mean scores for this schedule was 1.0 (Table 3).

Vaccine 4

Schedule A (1 and 3 weeks)

Chickens were vaccinated with vaccine 1, at 1 and 3 weeks old. The clinical sign was 0; gross

lesion score was 4; histopathological score was 3; mortality was 0; antibody titre1; bursal body weight ratio was 2 and the mean score for the schedule was 1.7 (Table 4).

Schedule B (1 and 2 weeks)

Chickens were vaccinated at 2 and 4 weeks old. The clinical sign score was 0; gross lesion score was 4; histopathological score was 1; mortality 0; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.3 (Table 4).

Schedule C (3 and 5 weeks)

Chickens were vaccinated at 3 and 5 weeks old. The clinical sign score was 1; gross lesion score was 3; histopathological score was 4, mortality 1; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score for this schedule was 2.0 (Table 4).

Schedule D (1, 3 and 5 weeks)

Chickens were vaccinated at 1, 3 and 5 weeks

Table 4. The clinical signs, gross lesion score, histopathological scores , mortality antibody titre , bursal body weight scores for chickens vaccinated with using different schedules.

Schedule	Clinical signs score	Gross lesions score	Histo-Pathologic score	Mortality score	Antibody titre score	Bursal body weight ratio score	Mean Per schedule
A	0.0	4.0	3.0	0.0	1.0	2.0	1.8
B	0.0	4.0	1.0	1.0	1.0	2.0	1.3
C	1.0	3.0	4.0	1.0	1.0	2.0	2.0
D	0.0	4.0	3.0	0.0	1.0	2.0	1.8
E	0.0	3.0	3.0	0.0	1.0	2.0	1.5
Mean across schedules	3.6	2.8	0.2	0.2	1.0	2.0	1.6

Key: A-Schedule 1 and 3 weeks. B-Schedule 2 and 4 weeks. C-Schedule 3 and 5 weeks. D-Schedule 1,3 and 5 weeks. E-Schedule 2,4 and 6 weeks

Table 5. The clinical signs, gross lesion scores, histopathological lesion score, mortality antibody titre and bursal body weight ratio scores for unvaccinated challenged. (positive control) and unvaccinated unchallenged (negative control) chickens.

Schedule	Gross lesion score	Histopath lesion score	Clinical sign	Mortality	Antibody titre	Bursal body	Mean weight ratio
Positive	4.9	5.0	1.0	1.0	0.0	6.5	3.1
Negative	1.3	2.0	1.0	1.0	0-0	2.9	1.7
Mean	3.1	3.5	1.0	1.0	0.0	4.7	2.4

old. The clinical sign was 0; gross lesion score was 4; histopathological score was 3; mortality 0; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.7 (Table 4).

Schedule E (2, 4 and 6 weeks)

Chickens were vaccinated at 2, 4 and 6 weeks old. The clinical sign score was 0; gross lesion score was 3; histopathological score was 3; mortality 0; antibody titre was 1; but the bursal body weight ratio was 2 and the mean score for this schedule was 1.5 (Table 4).

Chickens in positive control group were not vaccinated but challenged showed a clinical sign of 1.0; gross lesion score as 4.9; histopathological score 5.0; mortality 1; antibody titre 0 and a bursal body weight ratio of 6.47; while the negative control group had a clinical sign of 1; gross lesion score of 1.25; histopathological score of 2; motality 1; and antibody titre of 0 and bursal body weight ratio of 2.9 (Table 5).

Table 4 shows that for vaccine 1, schedule A (1 and 3 weeks) and scheduled D (1, 3 and 5 weeks) have the lowest mean score of 1.2 each, then followed by schedule E (2, 4 and 6 weeks) with 1.3, then schedule B (2 and 4 weeks) with 1.4, then followed by schedule C (3 and 5 weeks) with

mean scores of 1.6. For vaccine 2, schedule A (1 and 3 weeks) had the lowest mean score of 1.2, followed by schedule D (1, 3 and 5 weeks), then schedule E (2, 4 and 6 weeks) with 1.5, and schedule C (3 and 5 weeks) with mean scores of 1.6 and then schedule B with 1.9. For vaccine 3, schedule A (1 and 3 weeks) had the lowest mean score of 0.8 , followed by schedule D (1, 3 and 5 weeks) and E (2,4 and 6 weeks) with 1.0 each ,then schedule B (2 and 4 weeks) with 1.1, and schedule C (3 and 5 weeks) with mean score of 1.2. For vaccine 4, schedule B (2 and 4 weeks) had the lowest mean score of 1.3, followed by schedule E (2, 4 and 6 weeks) with 1.5, schedule A (1 and 3 weeks) with 1.6, scheduled D (1, 3 and

Table 6. Comparasion of the mean scores of a local and three imported live vaccines and schedules.

Schedule	1	2	3	4	Mean
A	1.2	1.0	0.8	1.6	1.2
B	1.4	1.9	1.2	1.3	1.4
C	1.6	1.6i	1.0	2.0	1.6
D	1.2	1.4	1.0	1.7	1.3
E	1.3	1.5	1.0	1.5	1.3
Means	1.3	1.5	1.0	1.6	1.4

Key: 1- vaccine 1. 2- vaccine 2. 3- vaccine 3. 4- vaccine 4. A-Schedule 1 and 3 weeks. B-Schedule 2 and 4 weeks. C-Schedule 3 and 5 weeks. D-Schedule 1,3 and 5 weeks. E-Schedule 2, 4 and 6 weeks.

5 weeks) with 1.7, and schedule C (3 and 5 weeks) with mean scores of 2.0.

The mean scores for the vaccines across the schedules was recorded with vaccine 3 having the lowest (1.0), followed by vaccine 1 (1.3) with vaccine 2 (1.5) and then vaccine 4 (1.6). While the mean score across schedule was with schedule A (1 and 3 weeks) with 1.2, followed by scheduled D (1, 3 and 5 weeks) and E (2, 4 and 6 weeks) with 1.3 each, schedule B (2 and 4 weeks) has 1.4, and then schedule C (3 and 5 weeks) with 1.6 (Table 6).

DISCUSSION

The study revealed that vaccinating chickens with vaccine 1 (at 1 and 3 weeks) appeared to be the best schedule. This finding is consistent with what was reported by Segal (2009) who attributed it to the fact that the first vaccination at one week might have neutralized the MDA (Abdu, 1997). It is also important to note that the interval of two weeks before the second vaccination might have given the bursa enough time to recover from the effect of the first vaccination as reported by Abdu (1997). The best schedule for vaccine 2 was also observed at 1 and 3 weeks. The chicks may withstand the infection despite the fact that they were vaccinated at 1 week at an interval of 14 days. This is not surprising because previous researchers have suggested double vaccination against IBD (Segal, 2009). Since the level of MDA usually decline at about 3 week and chicks are highly susceptible, giving the 2nd vaccination at 3 weeks may therefore boost the birds immunity against IBD (Chansiporanchi, 2009).

It was also observed that schedule A was the best schedule for vaccine 3, and was even better than the all the vaccines as the clinical signs, mortality rate, gross lesion scores, histopathological scores, were negligible. This finding agrees with the report of Giambrone (1983). It was also observed that vaccinating chicks at 2 and 4 weeks of age was the best vaccination schedule for

vaccine 4 (B). However, despite the fact that this schedule is the most widely adopted by poultry farmers in Zaria, outbreaks of IBD have been reported in vaccinated flocks as reported by Abdul (1997).

The antibody titre observed with vaccine 1 was lower than with vaccines 2, 3 and 4. This implies that the vaccine did not stimulate antibodies to appreciable level; hence its efficacy in vaccination programme may be doubtful (Abdu, 1997; Babiker and Tawfeeq, 2008). In contrasts to the findings of Abdu (1985), the finding in this study was consistent with the report by Naqi et al. (1983) who also observed marked difference in the titre of the antibody produced against IBD by different vaccines.

The clinical signs observed with vaccine 1 was significantly higher (p < 0.05) when compared to vaccine 2, 3 and 4. The least clinical sign scores was also recorded in vaccine 4. This also agrees with the work of Babiker and Tawfeeq (2008). The finding that vaccine 3 showed the least lesions score on the bursa implies that the vaccine is safe and may protect the birds against IBD, while vaccine 1 exhibited the highest lesions and hence the protective ability of this vaccine is questionable, as reported by Abdu (1997). The finding that the gross lesions were highest with vaccine 1 implies that this vaccine may not protect chicks against IBD. It may suffice to also say that this vaccine contained live viruses incapable of multiplying in the bursal cells of the chicks to illicit an immune response. Chickens vaccinated with such vaccine might remain susceptible to IBD outbreaks which may occur following natural exposure to virulent field viruses. These vaccination failures have continued to cause severe psychological stress and huge financial losses to poultry farmers as reported by Abdu (1997). It might have been possible that vaccine 1 had lost its potency through poor handling, transportation, storage or administration, eventhough vaccine 1 was in a freeze-dried state, a condition that improves the thermo stability of live vaccines (Spradbrow, 1992). In addition, the manufacturer of the vaccine 1 stated that the field dose may still be available to birds even after inoculation at 37°C for seven days. Moreover, IBDV is fairly resistant to heat (Benton et al., 1967).

The mortality rate as well as the bursal body weight ratio was worst with vaccine 1. This implies that the vaccine may not be immunogenic. This could be that the long continuous passage of the stock IBDV in the chick's embryo fibroblast cells may have adversely affected the quality of the batches of vaccine produced. High number of passage might have probably rendered the vaccine virus less able to easily infect and multiply in the bursa and less immunogenic. In contrast vaccine 3 was observed to be immunogenic as the antibody titre was good, the clinical signs, the histo-pathological lesions of the bursa, the gross lesions as well as bursal body weight ratio were low implying that probably the vaccine had maintained its potency. It also implies that the vaccine had been handled, transported, stored and administered appropriately and contained live virus capable of

multiplying within bursal cells of the bird to induce an immune response.

Conclusion

From the finding of this work, it may be concluded that vaccine 3 was the most potent of all the four live vaccines used and schedule A was the best schedule for vaccines 1, 2 and 3 and Schedule B was the best for vaccine 4. While appropriate handling, transportation, storage and administration is hereby re-emphasized, it is also recommended that the strain (type) of IBD vaccines produced should be defined as either mild, moderate or hot IBD vaccines.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Study on prevalence of bovine trypanosomosis and density of its vectors in three selected districts of Wolaita Zone, Southern Ethiopia

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The study was conducted from November, 2013 to March, 2014 in three selected districts of Wolaita zone, Southern Ethiopia with objective of determining the prevalence of bovine trypanosomosis and density of its vectors. Blood samples from 480 randomly selected cattle of both sexes different age, coat color and body condition score groups were collected and examined with conventional hematological and entomological techniques. Out of total 480 cattle examined, 32(6.67%) were found to be positive for *Trypanosoma congolense* and *Trypanosoma vivax* with the individual prevalence of 5% (24/480) and 1.67 (8/480), respectively. This indicated 75% of the infection was caused by *T. congolense* while only 25% was by *T. vivax*. The areal distribution of the trypanosomosis infection was found to be 5.6, 7.3 and 7.1% in Humbo, Duguna fango and Damot woyde districts respectively. The risk factors analysis revealed that the likelihood of the occurrence of the trypanosomosis in male (p=0.046, OR=2.3, 95%CI=1.0, 5.3), age category of 3 to 7 years old (p=0.019, OR=3.2, 95% CI=1.2, 8.3), poor body conditioned animals (p=0.001, OR=12.5, 95%CI=2.8, 50.0), black coat colored animal (p = 0.02, OR=12.5, 95%CI=1.5, 117.7) was higher when compared to female, other age categories, medium and good body condition and with other coat colored animals respectively. One way ANOVA used to compare the PCV values of parasitemic and aparasitemic animals revealed mean PCV values in animal infected by *T. congolense* (21.38%) and *T. vivax* (23.75%). Accordingly, one way ANOVA employed to compare the mean PCV value among the three categories (Negative, Positive for *T. congolense* and positive for *T. Vivax*) revealed significant (p=0.000, F=25.8) difference in PCV values were observed. Likewise, the *Bonferroni* multiple comparisons test indicated the existence of significant difference (p=0.000) in the PCV value between negative group and positive group for *T. congolense* (p=0.000). From 90 traps deployed for three consecutive days at 6 kebeles in three districts, a total of 328 flies were caught. Of these, 37 (11.28%) belong to *Glossina pallidipes*, the remaining were 193 (58.84%) *Tabanus* and 98 (29.87%) *Stomoxy*s. The overall apparent tsetse fly density was 0.14 flies/trap/days (F/T/D).

Key words: Cattle*, T. congolense, T. vivax, Trypanosomosis, Glossina pallidipes,* Southern, Ethiopia.

INTRODUCTION

Ethiopia has high livestock resource potential with estimated number of 40.9 million head of cattle, 22.5 million heads of sheep, 23.4 million heads of goats and more than 7.5 million equines and 2.3 heads of camels (CSA, 2007). However, much of the livestock resources are not fully utilized to maximum potential due to various constraints. Trypanosomosis is one of the major animal diseases affecting sub Saharan African countries in

general, and Western and South western part of Ethiopia in particular (NTTICC, 2004; Enwezor et al., 2006).

Trypanosomosis is a serious haemoprotozoan disease caused by different species of uni-cellular eurykaryotic parasite of the genus trypanosome found in the blood and other tissues of vertebrates including livestock, wild life and people and transmitted cyclically by tsetse flies of *Glossina* species and many other insects mechanically (Tesfaheywet and Abraham, 2012; Kumar et al., 2012). Animal trypanosomosis is an important livestock disease in Africa which is considered as a threat to the ongoing effort on poverty alleviation in the continent (Wint et al., 2010). It is a serious disease in domestic livestock that causes a significant negative impact in food production and economic growth in many parts of the world (Kumar et al., 2012) particularly in sub-Saharan Africa (Taylor et al., 2007; Cecchi et al., 2008).

Animal trypanosomosis is among one of the most important diseases limiting livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of South West and North West part of the country following the greater river basins of Abay, Omo, Ghibe and Baro, which has a high potential for agricultural development (Shimels et al., 2005). Over 6 million heads of cattle and equivalent number of other livestock species are at risk of contracting the diseases. More than 20,000 heads die per annual, and annual loss attributed to the disease is estimated to be over US$236 million, whereas loss due to reduced meat, milk and draft power is not applicable to this figure (OAU, 2002). The most important *Trypanosoma* species affecting cattle in Ethiopia are *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* (Abebe, 2005).

The tsetse flies are widely distributed in the Western, Southern and South western low lands and river valleys and 15% of the land believed to be suitable for livestock production is affected by one or more of the following species of tsetse flies; *Glossina morsitans sub morsitans, Glossina pallidipes, G. tachinoides, Glossina fuscipes fuscipes* and *Glossina longipennis* (Abebe, 2005). Apart from cyclical transmission of trypanosomosis by *Glossina* species, mechanical transmission is a potential threat to livestock productivity in some parts of Ethiopia (Abebe and Jobre, 1996). *T. vivax* infection can be transmitted mechanically by several *Tabanide* and large number of biting flies (Chernet et al., 2006). Among domestic animals, cattle are the most susceptible to *T. congolense*, *T. vivax* and *T. brucei* infections (Radostits et al., 2007).

Currently, the livestock production and productivity of southern region is highly affected by the high incidence of trypanosomosis. The communities in the region in general and in low lands lying along Ghibe and Omo river basins in particular expand a lot of money to purchase trypanocidal drugs.

Therefore, taking this into an account this study was designed with the following specific objectives.

To determine the prevalence of bovine trypanosomes on the basis of age, sex, body condition score and color of the animals and on area basis.

To determine the dominant species of trypanosome in study areas.

To investigate the epidemiological distribution of bovine trypanosomosis and to determine the abundance of tsetse fly in selected districts.

MATERIALS AND METHODS

Study area

The study was conducted from October 2013 to April 2014 in three selected districts of Wolaita zone, Southern Ethiopia which is located about 390 km south of Addis Ababa at an altitude of 700 to 2950 meters above sea level. It has an average annual rain fall ranging from 450 to 1144 mm. The rain over much of the area is typically bimodal with the major rainy season extending from June to September and the short rainy season occurs from February to April. The mean annual maximum and minimum temperature of the area is 34.120°C and 11.4°C, respectively. The predominant farming system is mixed crop-livestock production. The livestock population of Wolaita zone is estimated to be 886, 242 bovine; 117,274 ovine; 99,817 Caprine; 41160 equines and 442,428 poultry. The zone consists of 12 districts of which Humbo, Damot woyde and Duguna Fango were selected for the study based on available information that they are tsetse infested. The information was obtained from the zones office of agriculture (CSA, 2007).

Study design

Study population and study type

The study constituted zebu cattle of various sexes, age groups, body condition scores and different coat color managed under smallholder mixed crop livestock farming system a type of study employed was cross-sectional study type.

Sample size and sampling method

Simple random sampling technique was followed to select the study animals. During sampling age, sex, color and body condition score of animals was recorded. The age was categorized into three groups: less than 3years, 3 to 7years and greater than eight years old and the body condition score was grouped into good, medium, and poor based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986). The desired sample was also calculated according to the formulae given by Thrusfield (2005) as follows:

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$$n=1.96^2 xPexp\ (1-Pexp)/d^2$$

Where n=required sample
P=expected prevalence
d=desired absolute precision

Hence, with 14.2% expected prevalence rate which is done by Feyissa et al. (2011), desired absolute precision of 5 and 95% level of confidence, the sample size was calculated to be 187. But to increase the precision, 480 animals were sampled during the study period.

Study methodology and procedures

Parasitological Study

Buffy coat technique (BCT): Heparinized micro-hematocrit capillary tubes, containing blood samples were centrifuged for 5 min at 12,000 rpm. Buffy coat was drained on to microscope slide by cutting the capillary tube with sharp pointed diamond pencil 1mm below the Buffy coat to include the plasma after which it was covered with a 22x22 mm cover slip on microscope slide and examined under phase contrast or dark field microscope (40x power objective) to see motile parasite. Trypanosoma species were identified according to their morphological descriptions on Giemsa stained blood film as well as their motility in wet blood film preparations provided.

Hematological study

PCV determination: Blood samples were taken by puncturing marginal ear vein with a lancet and added directly into a pair of heparinized capillary tubes to their three-fourth of length. The tubes were then sealed at one end with crystal seal, placed in micro-hematocrit centrifuge and centrifuged at 12,000 revolutions per minute (rpm) for 5 minutes. Then capillary tubes were placed in a hematocrit reader and PCV estimated as a percentage of the total volume of blood to demonstrate the general health status of the animal.

Entomological study

Entomological data collection and recording commenced with collection of base line data on the description and density of tsetse before the start of trial. Tsetse flies were sampled by deploying traps along suspected habitat baited with three week old bovine urine and acetone into two different dispending bottles. Traps were set at approximate intervals of 200 to 250 meters and deployed preferably in shade in a visible manner. The different flies going to be caught in each trap were counted and identified. The species of tsetse fly, characteristic morphology and similar other mechanical vectors were also identified.

Data analyses

Raw data generated from this study were entered into MS-Excel database and the prevalence of bovine trypanosomosis in different age, color, sex and body condition groups and different localities or sites were analyzed using logistic regression analysis test. Mean PCV values between parasitaemic and aparasitemic cattle were compared by using one way ANOVA analysis test. Also the *Bonferroni* multiple comparisons test used to indicate the existence of significant difference in the PCV value between negative group and positive group for *T. congolense* and *T. vivax.* Flies per trap per day (F/T/D) analysis were used to calculate an apparent tsetse and biting flies densities.

RESULT

Prevalence of trypanosome infection

Out of the total 480 cattle examined for trypanosomosis, 32 (6.67%) were found to be positive for *T. congolense* and *T. Vivax* with the relative proportion of 5 (24/480) and 1.67% (8/480), respectively. This indicated that 75% of infection was caused by *T. congolense* while only 25% was by *T. vivax*. The area distribution of the trypanosomosis infection was found to be 5.6, 7.3 and 7.1% in Humbo, Duguna Fango and Damot woyde districts, respectively (Table 1).

Analysis of the risk factors

The analysis of risk factors revealed significant difference in the occurrence of trypanosomosis among different sex, age, body condition score and color. That is, the likelihood of the occurrence of the trypanosomosis in male (p = 0.046, OR = 2.3, 95%CI = 1.0, 5.3), age category of 3 to 7 years old (p = 0.019, OR = 3.2, 95% CI = 1.2, 8.3), animal with poor body condition score (p = 0.001, OR = 12.5, 95%CI = 2.8, 50.0), animal with black color (p = 0.02, OR = 12.5, 95%CI = 1.5, 117.7) was higher when compared to female animals, other age categories, animal with medium and good body condition scores and animals with other coat color. No significant difference was observed in the occurrence of the disease among different districts (Table 2).

Hematological findings

The recorded PCV value of animals was analyzed using one way ANOVA analysis to compare the PCV value of parasitemic and aparasitemic animals. There was a significant difference (p = 0.000, F = 49.2) in mean PCV value between infected and none infected animals in which infected animals have low mean PCV value (21.97) than non-infected ones (26.93) (Table 3).

Additionally, the mean PCV value in the animal infected by *T. congolense* (21.38) and *T. vivax* (23.75) was analyzed (Table 4). Accordingly, the one way ANOVA analysis was employed to compare the mean PCV value among the three categories (Negative, Positive for *T. congolense* and positive for *T. vivax*) and significant (p = 0.000, F = 25.8) difference in PCV values were observed. Likewise, the *Bonferroni* multiple comparisons test indicated the existence of significant difference (p = 0.000) in the PCV value between negative group and positive group for *T. congolense* (p=0.000). No significant difference in PCV was observed between the negative

Table 1. Prevalence of trypanosome infection and species identified in different study areas, sex, age BCS and coat color.

Risk factors	No. examined	No. positive	Prevalence (%)	*T. cong* (%)	*T. vivax* (%)
District					
Humbo	144	8	5.6	6 (4.2)	2 (1.4)
Duguna Fango	96	7	7.3	5 (5.2)	2 (2.1)
Damot Woyde	240	17	7.1	13 (5.4)	4 (1.7)
Sex					
Female	223	10	4.5	10 (4.5)	0
Male	257	22	8.6	14 (5.4)	8 (3.1)
Age					
<3 year	165	6	3.6	3 (1.8)	3 (1.8)
(3, 7) year	232	22	9.5	19 (8.2)	3 (1.3)
>8 year	83	4	4.8	2 (2.4)	2 (2.4)
BCS					
Poor	103	14	13.6	12 (11.7)	2 (1.9)
Medium	245	16	6.5	11 (4.5)	5 (2.0)
Good	132	2	1.5	1 (0.8)	1 (0.8)
Color	49	7	14.3	5 (10.2)	2 (4.1)
Black White/bulla	80	2	2.5	1 (1.3)	1 (1.3)
Gray/mixed	75	1	1.3	1 (1.3)	0
Red	276	22	8.0	17 (6.2)	5 (1.8)

Table 2. Logistic regression analysis of the prevalence of trypanosomosis with assumed risk factors.

Risk factors	No. examined	No. +ve (%)	COR(95% CI)	AOR(95% CI)	P-value
District					
Humbo	144	8(5.6)	1	1	
Duguna Fango	96	7(7.3)	1.3(0.5,3.8)	2.3(0.7,7.4)	0.15
Damot Woyde	240	17(7.1)	1.3(0.6,3.1)	1.5(0.58,3.9)	0.391
Sex	2				
Female	23	10(4.5)	1	1	
Male	257	22(8.6)	1.9(0.9,4.3)	2.3(1.01,5.3)	0.046
Age					
<3 year	165	6(3.6)	1	1	
>8 year	83	4(4.5)	1.3(0.4,4.5)	1.1(0.3,4.3)	0.916
(3,7)year	235	23(9.5)	2.8(1.1,7.1)	3.2(1.2,8.3)	0.019
BCS					
good	132	2(1.5)	1	1	
Medium	245	16(6.5)	23(1.1,4.8)	2.86(12.5,6.3)	0.012
Poor	103	14(13.6)	1.0(2.3,4.6)	12.5(2.8,50)	0.001
Color					
Gray/mixed	75	1(1.3)	1	1	
Red	276	22(7.8)	1.9(0.8,48)	1.8(0.7,4.8)	0.241
White/bulla	80	2(2.5)	1.9(1.3,33.3)	1.7(1.2,33.33)	0.030
Black	49	7(14.3)	12.5(15,100)	12.5(1.5,117.7)	0.02

Table 3. Mean PCV value for parasitemic and aparasitemic animals.

Status	Mean PCV	Std. Dev.	Freq.	F	P-value
Aparasitaemic	26.93	3.85	448		
Parasitaemic	21.97	4.04	32	49.2	0.000
Total	26.60	4.05	480		

Table 4. Mean PCV value for trypanosome species.

Status	Mean PCV	Std. Dev.	Freq.	F	P-value	Bonferroni multiple comparison test Categories	P-value
Negative	26.93	3.85	448			Negative and *T. congolense*	0.000
Positive for *T.congolense*	21.38	4.13	24	25.8	0.000	Negative and *T. vivax*	0.064
Positive for *T. vivax*	23.75	3.37	8			Positive for *T.congolense* and Positive for *T. vivax*	0.397

Table 5. Results of entomological survey.

District	No. PA	Altitude (mtrs)	No. traps	No. days	*Glossina Pallidipes* No. of male	No. of female	U sexed	Total	F/T/D	Tabanus	Stomoxys
Humbo	2	1215-1275	30	3	2	6	2	10	0.11	113	38
Duguna Fango	1	1242-1259	12	3	8	8	2	18	0.5	35	15
Damot Woyde	3	1285-1457	48	3	3	6	0	9	0.063	45	55
Total	6	-	90	-	13	20	4	37	0.14	193	98

F/T/D= apparent fly density per trap per day, No.M =number of males, No.F =number of female, U-sexed=unknown sex.

group and the infected group with *T. vivax*. Similarly, there was no significant difference in PCV between the group infected with *T. congolense* and *T. vivax*.

Entomological survey

From 90 traps deployed for three consecutive days at 6 PAs (kebeles) in three districts, a total of 328 flies were caught. Of these, 37 (11.28%) belong to *Glossina* specie (Tsetse flies), the remaining were 193 (58.84%) *Tabanus* and 98 (29.87%) *Stomoxys*, which belongs to biting flies. Furthermore, all *Glossina* species caught were identified to be *G. pallidipes.* The overall apparent tsetse fly density was 0.14 flies/trap/days (F/T/D). The overall fly density at district level was 0.11, 0.5 and 0.063 F/T/D in Humbo, Duguna Fango and Damot Woyde, respectively. In Damot Woyde district, which is located at altitude range of 1285 to 1457 m.a.s.l., the fly density is relatively lower (0.0625 F/T/D) when compared to Duguna fango (0.5 F/T/D) and Humbo (0.11 F/T/D) districts, which are located at altitude range of 1215 to 1275 m.a.s.l and 1242 to 1259 m.a.s.l, respectively. The sex category indicated 13 (35.14%) male, 20 (54.05%) females and 4

(10.81%) unknown sexed (U-sexed) tsetse flies. The summary of entomological survey is indicated in Table 5.

DISCUSSION

The overall prevalence of bovine trypanosomosis in this study was 6.7% which was in agreement with the previous findings by Habtwolde (1995), Feyisa et al. (2011) and Bitew et al. (2011) who reported 9.3% at Humbo Larena of Wolaita zone, 6.3% at Humbo district , and 11.7% at Jabi Teheran district, West Gojam of Amhara regional state, respectively. However, this finding was relatively lower than the reports of Terzu (2004), Mesfin and Getachew (2001), and Amare (1995) who reported 15.8, 35.5 and 21.0% prevalence of bovine trypanosomosis respectively at Omo river basin of South Western Ethiopia.

The possible explanation for the lower report in the current study could be attributed to the fact that action of Southern Valley Tsetse and Trypanosomosis Eradication (STEP) project, expansion of cultivation in the area which directly affects fly distribution, expansion of veterinary clinic, and awareness towards the control and treatment

of the disease. There was no significance difference in the selected districts, Humbo (5.6%), Duguna Fango (7.3%) and Damot Woyde (7.1%), since they are located relatively at similar agro ecology and tsetse belt of Ethiopia.

T. congolense was the most prevalent trypanosome species in the study area that accounts for the overall percentage of about 75% (24/32). This result was in line with Abebe and Jobre (1996) for tsetse infested areas of Ethiopia (58.5%); Muturi (1999) at Southern rift valley of Ethiopia (66.1%); Afework et al. (2001) at Pawe, North West Ethiopia (60.9%); Terzu (2004) in selected site of southern region (63.4%) and Bitew et al. (2011) in West Gojam (54.3%). An increased proportion of infection with T. congolense in the study area may be due to the major cyclical vectors of Savannah tsetse flies, (Glossina morsitans and Glossina pallidipes) which are effective transmitters of T. congolense and T. vivax (Langride, 1976) since the study area is located at tsetse belt of Ethiopia. Another reason also may be due to high number seroderms of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* infected animals (Leak et al., 1999; MacLennan, 1970).

Higher infection rate was observed in male animals than in females and the significant difference was also observed between two sex groups. Similar results have been reported by different works (Afework, 1998; Muturi, 1999; Tewolde, 2001; Mulugeta et al., 2013). The possible explanation from the present finding would be that the male animals are more exposed to traction power and also cross different vegetation for grazing and watering where tsetse challenge is higher than females.

In the present study, there was a statistically significant difference among age groups. The higher infection rate was observed in adults (3 to 7) years) than young's (<3years) and older (≥8years) animals. This result was in agreement with the previous research result reported by Sinshaw (2004). This could be due to the fact that adult animals travel long distance for grazing and draft as well as harvesting of crops to the tsetse challenged areas. Similar to the case in report by Rowlands et al. (1999) in Ghibe valley, suckling calves do not go out with their dams but graze at homesteads until they are weaned off. Young animals are also protected to some extent by maternal antibodies (Fimmen et al., 1999). This could result in low prevalence of trypanosome of that was observed in calves. *T. congolense* is usually higher in adult animals than the young ones (McDermott et al., 2003).

In the present study, body condition has shown to have significant effect on prevalence of trypanosome infection (p<0.05) with high prevalence recorded (13.59%) at poor body conditioned animals. Animals with poor body condition score were more associated with disease as compared to animals with medium (6.53%) and good (1.52%) body condition. This was in agreement with Habtwolde (1995), Dawud and Molalegne (2011) and Abiy (2002). Obviously, the disease itself results in progressive emaciation of the infected animals; nevertheless, non-infected animals under good condition have well developed immune status that can respond to any foreign protein better than those of non-infected cattle with poor body condition score which can be immune compromised due to other diseases or malnutrition and concurrent infections depress the immune responsiveness in the same cases (Collins, 1994).

Comparison conducted between the different skin color of cattle indicated that higher prevalence was observed in cattle's having black skin color (14.3%) followed by 8% in red, 2.5% in white/bulla and 1.3% in gray/mixed skin color. Tsetse flies by nature are attracted toward a black color, so in animals having black skin color there is high prevalence of trypanosomosis recorded (Teka et al., 2012).

One of the main symptoms of the disease is anemia (Murray, 1997) consequently the present study also indicated significant difference between mean PCV values of infected and non-infected cattle. Out of the observed animals, 32 of them were positive and their mean PCV value was 21.97±4.04, and 448 of them were negatives and their mean PCV was 26.93±3.85. The result of this study was in accordance with Rowlands et al. (1999) who observed an increase in PCV value, the proportions of positivity decreases and hence mean PCV was a good indicator for the health status of animals in an endemic area. The lower mean PCV value in parasitemic animals than the aparasitemic animals was reported by several authors (Leak et al., 1999; Afework, 1998; Muturi, 1999; Tewolde, 2001). Comparison of the mean PCV of infected animals within species of trypanosome out of 32, twenty four were infected with *T. congolense* and their mean PCV was 21.38±4.13 and eight were infected with *T. vivax* and their mean PCV was 23.75±3.37. Mostly, *T. vivax* invades other tissues in addition to blood such as lymph node, eyes and heart (Hoare, 1972; Stephen, 1986; Whitelaw et al., 1988), but *T. congolense* confined in the blood that might results low PCV values. Other than this, it can also be assumed that numerous concurrent diseases like helmenthiasis, tick borne diseases and nutrition imbalance cause anemia in both trypanosome positive and negative animals. There is a significance difference (p<0.05) in none infected and *T. congolense* positive animals.

The overall apparent density of tsetse and biting flies were 0.14 and 1.14 flies/trap/day respectively. There was no a great variability in tsetse apparent density between the study area (selected districts). This may be due to their similarity in agro ecologies and similar tsetse control measures done by Southern Tsetse Eradication Project (STEP). The lower apparent density of tsetse flies might be due to high temperature and low relative density of the dry period, which could limit the spread as described by Pollock (1982).

In the present study, the only *G. Pallidipes* were caught. The result of this study agreed with that of Leak et al. (1999), who found higher apparent density *G. Pallidipes* in Ghibe valley, which was followed by *G. Morsitans*. The present study disagreed with research by a team from NTTICC (2004), specific species of tsetse flies were recovered in Abay valley and its tributaries.

Finally, the study added knowledge to the overall prevalence of trypanosomosis in selected districts (Humbo, Duguna Fango and Damot Woyde) in Wolaita zone, Southern Ethiopia. It also indicated that the dominant trypanosome species in the study area was *T. congolense.* The host risk factors analysis of the study showed higher prevalence in males than females; in adult cattle than in younger and older and in animals with poor body condition score. The black coats colored animals were highly prevalent with disease than other color groups. The mean PCV of aparasitemic animals was higher than parasitemic animals. The overall apparent density of tsetse and biting flies was 0.14 and 1.14 flies/trap/day, respectively.

Conclusion

The study found that bovine trypanosomosis was economically an important disease that affects the health as well as the productivity of cattle in selected districts, and the findings may be used in the design of appropriate control and treatment strategies for existing problem.

Conflict of interests

The authors have not declared any conflict of interests.

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