academicJournals

Vol. 6(2), pp. 16-22, February, 2014
DOI: 10.5897/JPVB2014.0139
ISSN 2141-2510 © 2014 Academic Journals
http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Epidemiological significance of major hemoparasites of ruminants in and around Debre-Zeit, Central Ethiopia

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Accepted 15 January, 2014

Prevalence of hemoparasites of ruminants (cattle, sheep and goat) in and around Debre-Zeit was determined using diagnostic records from November, 2010 to April, 2011. A total of 384 blood samples collected from ruminants present in Debre-Zeit town and surrounding peasant associated areas were examined by Giemsa stained, thin blood smears and Diff-quick method for hemoparasite. Packed cell volume (PCV) for each sample was estimated and hematocrit centrifugation (HCT) technique was used to determine the presence of hemoflagellates. An overall prevalence of hemoparasites was 6.3% which consisted of *Anaplasma* species (*Anaplasma ovis* 2.1%, *Anaplasma marginale* 1.6%, *Anaplasma centerale* 0.3%), *Babesia* species (*Babesia bigeemina*, *Babesia bovis* and *Babesia ovis* total of 0.9%) and *Trypanosome* species (only *Trypanosome vivax* 1.6%). The hemoparasite detected alone or in concurrent infection had a significant (P<0.05) effect on PCV of infected animals. The result of this study shows these hemoparasites are not endemic in ruminants in the study area, which may results in serious disease conditions when such animals are subjected to stress full condition.

Key words: Debre-Zeit, hemoparasites, prevalence, ruminants.

INTRODUCTION

Ethiopia is one of the countries with the largest number of livestock in Africa and livestock production plays a major role in the development of Ethiopia's agriculture. Nevertheless, cattle productivity is low (Central Statistics Authority (CSA), 2009). This may be due to improper management, disease, nutritional deficiencies, harsh environment and genetic factors. The presence of diseases caused by hemoparasites is broadly related to the presence and distribution of their vectors. These diseases cause negative effects on the health of the livestock including production and productivity. Arthropod transmitted hemoparasitic disease of cattle is caused by the *Trypanosomae, abesia*, *Theleria* and *Anaplasma* species. The extracellular species belonging to the genus *Trypanosomae* and intracellular species are *Anaplasma*, *Theleria* and *Babesia* in animals (Alekaw, 2000).

Arthropod transmitted hemoparasitic diseases are economically important vector-borne diseases of tropical and subtropical parts of the world including Ethiopia. *Bovine babesiosis* caused by an apicomplexan haemoprotozoan parasite, *Babesia bigemina* (family Babesiidae, order Piroplasmida), is transmitted by brevirostrate tick, *Rhipicephalus microplus*, causing significant morbidity and mortality in cattle and buffaloes. *Trypanosoma evansi*, the causative agent of the "Surra," is mechanically transmitted by tabanid flies (Sumba et al., 1998). Dairy animals, especially bovines, which are bearing production stress along with other diseases, are potential viable host to these infections. Bovines act as reservoir hosts of Surra as the cause of disease remains subclinical (McLeod

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and Kristjanson, 1999).

Animal trypanosomosis and its vectors occur in vast areas of the Sub-Saharan Africa with devastating impact on livestock productivity posing a serious threat to the livestock and communities. It constitutes the greatest single constraint to livestock and crop production by directly contributing to hunger, poverty, protein mal-nutrition and suffering of entire communities in Africa (WHO, 2002). Of the 165 million cattle found in Africa, only 10 million are found within the tsetse fly free belt, and these are mostly low producing breeds which are maintained on high drug management regimes to keep trypanosomosis (Jones and Dávila, 2001). The disease has economic importance due to loss of condition, reduction in milk yield, decrease capacity of work (Reghu et al., 2008).

Prevalence of the disease depends on the rate of exposure, availability of infected animals, the insect reservoir and seasons (Mottelib et al., 2005). In Ethiopia, quite a number of epidemiological studies have been conducted on cattle trypanosomosis and these epidemiological studies were carried out using conventional parasitological techniques such as dark phase buffy coat, thin and thick smear (Dagnachew et al., 2005; Sinshaw et al., 2006). It is the major constraints on livestock production and general agricultural development over an estimated area of 98,000 km^2 in the country (Solomon, 1980).

Babesia species are intra erythrocytic protozoal organisms spread by arthropods like ticks and biting flies transplacentally and by blood transfusion. Babesiosis is a tick born disease of domestic, wild and laboratory animals as well as humans caused by the genus *Babesia*. More than 100 known *Babesia* spp. have been identified which infect many types of mammalian host, out of these, 18 cause disease in domestic animals notably in cattle, sheep, goats, horses, pigs, dogs and cats. Almost any mammals, that serves as a host for a *Babesia* infected tick is a potential reservoir (Homer et al., 2000).

Anaplasmosis is a vector born infectious blood disease in cattle caused by the rickettsial parasites, *Anaplasma marginale* and *A. centrael*. It occurs primarily in worm tropical and subtropical areas. The disease is not contagious but transmitted most commonly by ticks. It can also be transmitted via contaminated surgical instruments, biting flies and mosquitoes. The intracellular parasites destroy the red blood cells. It causes anemia, fever, weight loss, breathlessness, uncoordinated movements, abortion and death (Centers for Disease Control and Prevention (CDC), 2009).

Development and transmission of hemoparasites by tick vectors are phenomena closely synchronized with the tick feeding cycle. In all known life cycles, initial infection of tick tissues occurs in midgut epithelial cells and transmission is effected as ticks feed after parasites have developed and multiplied in salivary glands. Many factors reviewed affect development and transmission of hemoparasites by ticks including age of ticks, artificial temperature, climate and/or season, tick stage or sex, hemoparasite variation, concurrent infection of ticks with other pathogens, host cell susceptibility, transovarial transmission, effect of hemoparasites on tick biology, and the effect of infecting parasitemia level in cattle on infection rates in ticks. Four hemoparasites of cattle, *A. marginale*, *Cowdria ruminantium*, *Theileria parva*, and *Babesia* spp., are all dependent on ticks for biological transmission. *Babesia* is transmitted trans-ovarially, whereas the other three are transmitted transracially. Mechanical transfer of infective blood via formites and mouthparts of biting arthropods is also a major means of transmission for *A. marginale*, but not of the others. Potential control methods for hemoparasites include tick control, vaccines (against ticks and parasites), and drugs (against ticks and parasites). Successful application of control strategies will be dependent upon thorough understanding of parasite developmental cycles, biology of the tick vectors and the immune response of cattle to ticks and to hemoparasites (Kocan, 1995). The most effective control measures are targeted against both ticks and the hemoparasites.

In Ethiopia, various surveys have been carried out on distribution, abundance and prevalence of hemoparasite species on livestock in different regions of the country by various investigations (Adam et al., 1971) including trypanosomosis of ruminants, babesiosis in donkeys, and canine bebasiosis (Tolosa, 2010). Such investi-gations have been conducted in the different regions of the country like Ghibe, Asela, Wolayta, Bahirdar, Gondar, Debre Markos, Arbaminch, Tigray (Mekele), Kefa, West Gojjam, Upper Didesa valley, North Omo, Arjo, Wonago, Gore, Mizan Tefere, Debre-Zeit. The prevalence of trypanosomes in some studies, ranged from 20 to 40% and animal mortality was 16% (Tolosa, 2010). However, the detailed status of the hemoparasitic diseases in ruminants is not thoroughly studied in and around Debre-Zeit and information is so far scanty.

Therefore, the objectives of this study were to study the epidemiological significance of the main hemo-parasitic diseases in and around Debre-Zeit and to indicate the risk factors associated with distribution of hemoparasites in the study area.

MATERIALS AND METHODS

Study area

Debre-Zeit is located in Central Oromia regional state, at a distance of 47 km of the South Eastern part of Addis Ababa. It is the main city of Ada'a-Liban district, situated on two international trade routes, which are connected by Franco-Ethiopia Djibouti railway and Addis-Moyale Nairobi, intercontinental asphalted road transport route. This is the only gate for import/export of livestock and agricultural commodities besides other commercial goods, from or to international market (Central Agricultural Census Commission (CACC), 2003).

Debre-Zeit lies between 9° N latitude and 39° E longitude and an

Table 1. Prevalence of hemoparasitosis in ruminants on the basis of species of animals in and around Debre-Zeit, Ethiopia.

Animal	Laboratory test results	
	No. of animals examined (%)	No. of animals positive and prevalence (%)
Bovine	177	15 (3.9)
Ovine	180	2 (0.5)
Caprine	27	7 (1.8)
Total	384	24 (6.3)

altitude of 1860 meter above sea level (m.a.s.l). It has three agro-ecological locations, 6% Dega greater than 2000 m.a.s.l, 89% Woinadega greater than 1600 m.a.s.l, and 5% Kola greater than 1000 m.a.s.l. It gets an annual rainfall of 871 mm of which 80% is received during long rainy season starting from June to September and the remaining in short rainy season extending from March to May, and the dry season from October to February. The mean annual maximum and minimum temperature are 26 and 14°, respectively with a minimum relative humidity of 63.8% (NMSA, 2005).

Study animals

A total of 384 animals (local and cross breed) were randomly selected for infected and suspected cases, irrespective of their age, breed, sex and agro-ecological set. Mainly concerned animals came to Addis Ababa University, School of Veterinary Medicine open air clinic from and around Debre-Zeit town.

Study design

A cross-sectional study was conducted on prevalence rates of hemoparasite infection of ruminants from November, 2010 to April, 2011 in and around Debre-Zeit.

Sample collection

Blood sample was collected randomly from 177 bovines (local and cross breed), 27 caprine and 180 ovine, from ear-vein, at Addis Ababa University, School of Veterinary Medicine open air clinic.

Parasitological examination

Blood samples collected directly from an ear-vein in a heparinized micro hematocrit capillary tube, thin blood smears were drawn, air dried, fixed (methanol 3 to 5 min) and stained by immersing in jar containing diluted 10% Giemsa solution for 30 min (Kaufman, 1996), and by Diff-quick staining for only 15 s, 5 times dip for each of the 3 types of staining containing jar and then examined under oil immersion ×100 magnification to identify the morphology and type of hemoparasites (Coles, 1986).

Hematological examination

Both parastaemic and aparastaemic blood samples were taken from the ear-vein in the heparinized capillary tubes (75 × 1.2 mm). Each of the two tubes was filled to a height of 66 mm and one end sealed with crystaseal (Hauskyley Ltd. England). After centrifugation, 13000 rpm for 5 min packed cell volume was determined by using micro hematocrit reader, to estimate PCV for checking anemia (Woo, 1971). A separated Buffy coat smear was examined. The capillary tube was put at buffy coat junction with plasma and RBC and buffy coat was taken out on the slide, covered by cover slip and examined under low magnification or by WOO technique (Woo, 1971).

Body condition evaluation and estimation of age

On subjective basis, body condition score of animals were evaluated during sample collection. They were classified as emaciated, moderate and good based on anatomical parts and the flesh and fat cover at different body parts (Nicholson and Butterworth, 1986). The ages of the animals were estimated by history and as described by De-lahunta and Habel (1986).

Data analysis

Data entry was made of Microsoft excel spread and the analysis was performed using chi-square of independence which was employed in the prevalence of hemoparasitic disease in ruminants with respect to sex, age as well as breed to determine the associated risk factors. Analysis of variance (ANOVA) (Thrusfield, 2005) was used to assess the difference in mean PCV% of the animals in hemoparasites infected and non-infected.

RESULTS

Out of 384 blood samples examined (177 bovine, 27 caprine and 180 ovine species) which included 359 samples from the local breeds, 24 samples were found infected on different species of hemoparasites. Among the 25 samples from exotic cross breeds, none was found infected, this may be due to less number of animals included in the present study. The overall prevalence rate of hemoparasites in and around Debre-Zeit is 6.3%. This included 7 different types of hemoparasites. Out of 177 bovine samples, 15 were positive (3.9%), *A. marginale* 6 (1.6%), *A. centrale* 1 (0.3%), *Babesia bigemina* 1 (0.3%), *B. bovis* 1 (0.3%) and *Theleria vivax* 6 (1.6%); out of 27 caprine samples, 2 were positive *Anaplasma ovis* (0.3%) *Babesia ovis* 1 (0.3%) and 180 ovine samples *A. ovis* 7 (1.8%). The mean PCV of parasitized animals (21.4 ± 7.1) was significantly lower ($P<0.05$) than those of non-parasitized animals (27.7 ± 6.9) during dry season (Table 8).

The overall prevalence of hemoparasitosis on the basis of species was 3.9, 0.5 and 1.8% in bovine, caprine and ovine species, respectively (Table 1). There was no statistically significant difference ($P>0.05$) in the prevalence of hemoparasites among the different species of animals examined. Table 2 indicates the species of hemoparasitosis detected and their relative frequencies. There was no statistically significant difference ($P>0.05$) between the prevalence of different species of hemoparasites encountered and species of animals examined.

Prevalence of hemoparasites in ruminants on the basis of age, sex, breeds and body conditions in the study area is indicated in Table 3. The species of hemoparasite and their relative frequencies in ruminants on sex basis in

Table 2. Species of parasites and their relative frequencies of hemoparasitosis in ruminants in and around Debre-Zeit, Ethiopia.

Animal species (No. examined)	Species of parasites						
	***A. marginale* (%)**	***A. central* (%)**	***A. ovis* (%)**	***B. bigemina* (%)**	***B. bovis* (%)**	***B. ovis* (%)**	***T. vivax* (%)**
Bovine (177)	6 (1.6)	1 (0.3)	0 (0)	1 (0.3)	1 (0.3)	0 (0)	6 (1.6)
Ovine (180)	0 (0)	0 (0)	7 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)
Caprine (27)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	1 (0.3)	0 (0)
Total	6 (1.6)	1 (0.3)	8 (2.1)	1 (0.3)	1 (0.3)	1 (0.3)	6 (1.6)

Table 3. Prevalence of hemoparasitosis in ruminants on basis of age, sex, breed and body conditions in and around Debre-Zeit, Ethiopia.

Parameter		No. of animals examined	No. of animals positive and prevalence (%)
Age category	Adult	291	15 (3.9)
	Young	93	9 (2.3)
Sex	Female	241	11 (2.9)
	Male	170	13 (3.4)
Breed	Cross	25	0 (0)
	Local	359	24 (6.25)
Body condition	Emaciated	83	14 (3.6)
	Moderate	167	5 (1.3)
	Good	134	5 (1.3)
Total		384	24 (6.25)

Table 4. Species of hemoparasites and their relative frequencies in ruminants on sex basis in and around Debre-Zeit, Ethiopia.

Sex and No. examined	Species of parasites						
	***A. marginale* (%)**	***A. central* (%)**	***A. ovis* (%)**	***B. bigemina* (%)**	***B. bovis* (%)**	***B. ovis* (%)**	***T. vivax* (%)**
Female (214)	1 (0.3)	0 (0)	5 (1.3)	0 (0)	1 (0.3)	0 (0)	4 (1)
Male (170)	5 (1.3)	1 (0.3)	3 (0.8)	1 (0.3)	0 (0)	0 (0)	2 (0.5)
Total (384)	6 (1.6)	1 (0.3)	8 (2.1)	1 (0.3)	1 (0.3)	1 (0.3)	6 (1.6)

and around Debre-Zeit is shown in Table 4. There was no statistically significant difference (P>0.05) between the species of hemoparasites and their relative frequencies in ruminants on the basis of sex of examined animals. The species of hemoparasite and their relative frequencies in ruminants on breed basis in and around Debre-Zeit is shown in Table 5. There was statistically significant difference (P<0.05) between the species of hemoparasites and their relative frequencies in ruminants on the basis of breed of examined animals.

The species of hemoparasite and their relative frequencies in ruminants on body condition basis in and around Debre-Zeit is shown in Table 6. There was a statistically significant difference (P<0.05) between the species of hemoparasites and their relative frequencies in ruminants on the basis of body condition of examined animals. The mean PCV of animals infected with different species of hemoparasites is shown in Table 7. There was a statistically significant difference (P<0.05) in the mean PCV of animals infected with different species of hemoparasites. A comparative value of mean PCV of parasiteamic and aparasiteamic ruminants in and around around Debre-Zeit is depicted in Table 8. There was a statistically significant difference (P<0.05) in the mean

Table 5. Species of hemoparasites and their relative frequencies in ruminants on the basis of breeds in and around Debre-Zeit, Ethiopia.

Breed and No. examined	Species of parasites						
	***A. marginale* (%)**	***A. central* (%)**	***A. ovis* (%)**	***B. bigemina* (%)**	***B. bovis* (%)**	***B. ovis* (%)**	***T. vivax* (%)**
Cross (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1)
Local (359)	6 (1.6)	1 (1.3)	8 (2.1)	1 (1.3)	1 (0.3)	6 (0)	2 (0.5)
Total (384)	6 (1.6)	1 (0.3)	8 (2.1)	1 (0.3)	1 (0.3)	1 (1.6)	6 (1.6)

Table 6. Species of hemoparasites and their relative frequencies in ruminants based on body condition of the examined animals in and around Debre-Zeit, Ethiopia.

Body condition score and No. examined	Species of parasites						
	***A. marginale* (%)**	***A. central* (%)**	***A. ovis* (%)**	***B. bigemina* (%)**	***B. bovis* (%)**	***B. ovis* (%)**	***T. vivax* (%)**
Emaciated (83)	6 (1.6)	1 (0.3)	0 (0)	1 (0.3)	0 (0)	1 (0.3)	5 (1.3)
Good (167)	0 (0)	0 (0)	5 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Moderate (134)	0 (0)	0 (0)	3 (0.8)	0 (0)	1 (0.3)	0 (0)	1 (0.3)
Total (384)	6 (1.6)	1 (0.3)	8 (2.1)	1 (0.3)	1 (0.3)	1 (1.6)	6 (1.6)

Table 7. Mean PCV of animals affected with different species of hemoparasites in and around Debre-Zeit, Ethiopia.

Hemoparasite species	Mean (%)	No. of animals examined	Standard deviation
A. marginale	18.1	6	5.2
A. central	15	1	-
A. ovis	23.8	8	10.9
B. bigemina	21	1	-
B. bovis	22.5	1	-
B. ovis	18	1	-
T. vivax	23.1	6	2.7
Total	27.4	24	7.1

Table 8. Mean PCV of parasiteamic and aparasiteamic ruminants in and around Debre-Zeit, Ethiopia.

Laboratory test result	Mean PCV (%)	No. of animals examined	Standard deviation
Aparasitemic	27.7	360	6.9
Parasitemic	21.4	24	7.1
Total	27.4	384	7.1

PCV of parasiteamic and aparasiteamic ruminants.

DISSCUSION

In this study, the overall prevalence rate of hemoparasites in ruminants was found to be 6.3%. These results disagree with 25.7% in North-Central Nigeria by Kamani et al. (2010). Trypanosomosis 1.6% consist of only *T. vivax* and tick born hemoparasitosis 4.9% including *A. marginale* (1.6%), *A. centerale* (0.3%), *A. ovis* (2.1%), *B. bigemina* (0.3%), *B. bovis* (0.3%), *B. ovis* (`0.3%). The present results are comparable with different figures reported from different tsetse infected regions of Ethiopia. Trypanosomosis, 51.1% in West Ethiopia (Terefa, 2008),

21% in Southern rift valley areas (Bekele et al., 2010), 17.2% in Metekele (Afework et al., 2000), 17.5% in the Upper Dedessa valley (Afework et al., 2000) and tick born hemoparasitosis 2.1% (Terefa, 2008) and 17% in other African country, Nigeria (Kamani et al., 2010). Furthermore, the study indicates that *A. ovis* 2.1% was the predominant hemoparasite species detected by Giemsa staining and Diff-quick methods as compared to other hemoparasite in the study animals. *A. ovis* infection rate in small ruminants more in sheep (1.8%) as compared to goats (0.3%) in this report.

In the present study, the prevalence rate of babesiosis was lower in the study area (only 0.6%). So results of the present study are not in agreement with previous study by Yismashewa (2005) in Decha Woreda Southern Ethiopia and Seyoum (2007) in Kobo and Girana valley in Amhara region. They reported high prevalence in those areas. The earlier studies were conducted during the late dry and early rainy season but in the present study all sample collected at the dry season. This implies that crucial factors influenced the rate and seasonal variation of vectors, high humidity and temperature. In general, prevalence intensity rate of tick born hemoparasitic disease infestation were generally low during dry season and higher in rainy season (Solomon et al., 2003).

The prevalence rate of hemoparasitism was generally slightly higher in male than female animals in the present study and this can be due to the fact that male animals work exposed in different areas which tends to increase chance of contact with the vectors of these disease as compared to female, disagree with Kamani et al. (2010). The lower prevalence in young animals compared to adults can be attributed to restricted grazing of young animals which tends to reduced their chance of contact with the vectors of these diseases. There was significant difference between the prevalence in local and exotic cross breed of cattle in management practices, where the exotic cross breeds receive more attention in terms of ecto parasite control and feed supplementation and it can be less number of exotic cross breed animals included in this study. The mean PCV of non-infected animals was significantly higher ($p<0.05$) than that of animals with one or more parasites during dry season, which may be related to confounding factors like scarcity of good quality feeds and stress of trekking experienced by most pastoral animals during the dry season of feeds (Obi and Anosa, 1980); this report agree with the previous study (Kamani et al., 2010).

Hemoparasitosis infection in the study animals was statistical significant on basis of body condition of the studied animals and breeds of animals. Also, there was no significant difference between age, sex and species of animals. Moreover, as one of the predominant clinical infestations of hemoparasitosis is anemia and had significant difference lower mean PCV as compared to non-infected animals by hematocrit PCV% determination. This finding indicates that anemia is good indicator for infection with hemoparasites (*Trypanosomae, Babesia, Anaplasma*) than the body condition score of the animals. The anemia could be due to the hemopasites as it has been also indicated by (Laha and Sasmal, 2008) that anemia could be apparent in infected animals as a result of production of haemolysin by trypanosomes resulting into haemolysis of RBCs and extra vascular destruction of RBCs. This destruction may be through the erythro-phagocytosis or may be immune mediated. Depression of erythropoiesis and non-specific factors, which increase red cell fragility, may also be responsible for anemia.

The body condition of infected animals were statically significant ($P<0.05$) as compared to non-infected animals. Loss of body condition not always associated with this disease, can be other chronic disease of other parasitic, bacterial, viral and nutrition deficiency or poor management systems of the farm animals. This study was partly conducted in and around Debre-Zeit town. Thus, it is an area where no tsetse fly was reported and the trypanosome species detected in this area is mainly *T. vivax*, one of the trypanosome species that can be trans-mitted mechanically by biting flies and cyclically by tsetse flies. In the present study, this *Trypanosoma* species is present solely in bovine, but the small ruminants were suspected by this blood parasite. However, in the study area no tsetse flies were reported.

The present report confirms the presence of carrier populations of hemoparasite infected animals which both serve as reservoir of infection for *Trypanosoma* and tick vector and susceptible livestock, and has the potential for clinical relapse under stressful conditions, this report agree with the previous studies conducted in other countries (Sharma et al., 2013; Kamani et al., 2010; Singla et al., 2007).

ACKNOWLEDGEMENTS

The authors appreciate the support by Addis Ababa University. The willingness and cooperation of flock owners were an indispensable input for the accomplishment of this work. All contributions and supports are gratefully acknowledged.

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