STUDY ON MONTHLY DYNAMICS OF TICKS AND SEROPREVALENCE OF ANAPLASMA MARGINALE, BABESIA BIGEMINA AND THEILERIA MUTANS IN FOUR INDIGENOUS BREEDS OF CATTLE IN GHIBE VALLEY, ETHIOPIA

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ABSTRACT: Monthly collection of ticks in the period January through October, 2002 was conducted in the Ghibe Valley of Ethiopia from randomly selected cohort of 60 heifers belonging to four indigenous cattle breeds namely Abigar, Sheko, Horro and Guraghe. The most abundant tick species were Amblyomma variegatum (39.5%), Boophilus decoloratus (39.1%), A. cohaerens (10.5%) and Rhipicephalus evertsi evertsi (6.7%). Species of least abundance include R. praetextatus, Haemaphysalis aciculifer, Hyalomma marginatum rufipes, R. bergeoni, R. lunulatus, R. muhsame and R. pravus, altogether comprising 4.2% of the overall tick species. The monthly abundance of adult ticks, their feeding sites on the hosts and the male to female sex ratio were determined. The seroprevalence of Anaplasma marginale, Babesia bigemina and Theileria mutans was studied using indirect Enzyme Linked Immunosorbant Assay (indirect ELISA) using blood samples collected in April, June, August and October 2002. The period prevalence of antibodies of A. marginale, B. bigemina and T. mutans was found to be 84.2, 87.1 and 54.2 per cent, respectively. There was no significant difference in the prevalence of the diseases among the breeds, except the higher prevalence of A. marginale in Horro (P < 0.05). The overall high seroprevalence values suggest the presence of enzootic stability in the cattle population in the area for A. marginale and B. bigemina infections while the relatively low seroprevalence of T. mutans indicated the state of enzootic instability in the population. The implications of these findings on the health of the cattle and hence the economic impact is discussed.

Key words/phrases: Cattle, enzootic stability, seroprevalence, ticks, tick-borne diseases

INTRODUCTION

Ticks are allong the most important ectoparasites and vectors of animal and human diseases on global scale, particularly in tropical and sub tropical parts of the world. Ticks and the diseases they transmit are considered to be a significant threat to successful livestock production and seriously interfere with the economy of a country. As ectoparasites, ticks are responsible for blood loss, irritations, skin abrasion and loss of udders, which provide portals of entry for secondary bacterial infections and for myiasis inducing larvae. They also secrete toxins which may disseminate to the respiratory organs and cause death of the animal (Sere, 1979; Soulsby, 1982). Tick-borne diseases of livestock whose etiological agents may be protozoa, rickettsia, bacteria or viruses are present throughout the world, but exert their greatest impact in the tropics and subtropics (Fivaz *et al.*, 1992). These diseases generally affect the blood and/or lymphatic system and their impact can be expressed in terms of mortality, loss of production and cost of control (Dreyer *et al.*, 1997). Babesiosis, theileriosis and anaplasmosis are known to be amongst the most important tickborne diseases that hinder livestock production (Fivaz *et al.*, 1992).

In Ethiopia, tick surveys have been carried out in different regions (Hoogstraal, 1956; Morel, 1980; Yilma Jobre *et al.*, 1995; Seyoum Zenebe, 2001). More than 60 species of ticks infesting both domestic and wild animals have been recorded and 33 of these are known to be important parasites of livestock (de Castro, 1994). A few microscopic and serological studies have also

indicated the presence of tick-borne pathogens including anaplasmosis, babesiosis, cowdriosis and benign theileriosis (Pegram et al., 1981; Sileshi Mekonnen et al., 2001). The principal biological vectors of Anaplasma marginale namely Boophilus Hyalomma marginatum rufipes, decoloratus, Rhipicephalus evertsi evertsi are prevalent in Ethiopia. The vectors of Babesia bigemina; B. decoloratus, B. annulatus and R. e. evertsi are common in the country (Pegram, 2001; Solomon Gebre, 1994). The vectors of Theileria mutans, R. e. evertsi and A. variegatum are also commonly found in different regions of Ethiopia (Solomon Gebre, 1994). Cattle living in areas where tick- borne diseases are widespread remain resistant to clinical illness after recovery from their first infection. This situation is termed as enzootic stability of the cattle population (Uilenberg, 1971). Studies on seasonal/ monthly dynamics of ticks and prevalence of tickborne diseases have been performed in few parts of the country (Solomon Gebre, 1994; Seyoum Zenebe, 2001). The aim of this study is to provide baseline data on tick species and their monthly population dynamics in Ghibe valley (Tolle/ Gullele); and further to determine and compare the seroprevalences of A. marginale, B. bigemina and T. mutans in the four breeds of cattle.

MATERIALS AND METHODS

Study area and study animals

The study was conducted at the Ghibe Valley in a joint- project experimental station of International Livestock Research Institute (ILRI) and Ethiopian Institute of Agricultural Research (EIAR) from January to October 2002. The area is 230 km south west of Addis Ababa located between 8°15'N latitude and 37°30'E longitude with altitude ranging between 1300 and 1400 meters above sea level (Fig. 1). The climate consists of a short rainy season in March and April and a main rainy season from late May to October (Markos Tibbo, 1993). The total monthly rainfall and minimum temperature of the study area during the study period, according to the data from the National Meteorology Agency, are presented in Figure 2. The average annual rainfall and minimum temperature were 134mm and 14°C respectively. Vegetation consists mainly of bush savannah with gallery forest along the drainage lines. There is plenty of growth during the wet season but during the dry season vegetation becomes sparse. The major flora include; Acacia spp., broad-leaved deciduous plants

and the dominant grass, *Hyparrhenia spp.* The local farmers are engaged in cultivating maize, sorghum, teff, chickpeas and other cash crops.

A total of 228 young cattle, consisting of four indigenous breeds were brought to the study area in the year 2000 from their original locations in different regions of Ethiopia namely: Abigar, from Gambella area; Guraghe from the Guraghe zone; Horro from Wollega area and Sheko from Sheka zone for trypanotolerance study. Information on the characterization of the breeds was found from **Domestic Animals Genetic Resources Information** System (DAGRIS, 2002). Sixty heifers, 15 from each breed were randomly selected for this study. The cattle were allowed to graze freely with the herd and exposed to natural tick infestation. The animals were regularly vaccinated against anthrax, black leg, pasteurellosis and foot-and-mouthdisease. They were also treated with specific chemotherapeutic agents after proper diagnosis. All of the animals were strategically sprayed with acaricide in the udder and scrotum. This was done after collection of ticks during the months of April and May because of high infestation of the cattle by ticks at this period.

Collection of ticks from cattle

Regular half body tick collections were carried out from the 60 heifers at monthly intervals on alternative body sides. After full restraining of the study animals, visible ticks of all stages were removed by hand and using special forceps, Removal of ticks was done by holding the basis capitulum and screwing so as not to lose the mouthparts of the ticks. The collection was done on eight anatomical sites; head, ear, dewlap, back (side), ventre (abdomen), hoof, tail and ano-vulval areas according to Kaiser (1987) and placed in eight separate universal bottles containing 70% ethyl alcohol. Identification of ticks was subsequently done using light stereoscopic microscope employing standard keys (Hoogstraal, 1956; Matthysse and Colobo, 1987; Morel, 1980). Identified ticks were then counted and recorded by species, sex and instars. The count of ticks from half body zone of each animal was doubled to give the total number of ticks per animal, assuming equal infestation on both sides of an animal. Further confirmatory identification was needed for some specimens and this was done by using the expertise and facilities available at the National Animal Health Research Centre (NAHRC) in Sebata.



Fig. 1. Location of the study area.

Serum collection

Blood samples were collected by jugular venipuncture into plain vacutainer tubes at 2 months interval from April to October, 2002. The samples were then transported to National Animal Health Research Centre and allowed to stand overnight to clot and then centrifuged for 10 minutes at 20,000 rpm. Serum from each sample was separated and kept in sterile, pre-labelled eppendorff tubes at 4°G overnight and then stored at -20°C.

Serological tests

Antibodies against the tick-borne pathogens Anaplasma marginale, Babesia bigemina and Theileria mutans were detected by Indirect Enzyme-Linked Immunosorbent Assay (IELISA) according to the protocol supplied by ILRI - ELISA Diagnostic Unit (Nairobi), also according to Wright *et al.* (1993).

Briefly, the microtiter plates pre-coated with antigens of the above mentioned tick-borne pathogens were reconstituted and filled by blocking buffer (300 µl/well) and incubated for 20 minutes at 37°C. The plates were washed four times with wash buffer (PBS containing 0.5% Tween 20). Test and control sera (150 µl) diluted 100 times for B. bigemina and T. mutans and 40 times for A. marginale were added immediately in duplicate wells and incubated for 40 minutes at 37°C. For each control serum (background, strong positive, weak positive and negative controls) four replicates were used. The plates were washed four times as described above and immediately refilled with wash buffer to soak for 10 min. After soaking, the wash buffer in the wells were flicked and blotted as before. Purified rabbit anti-bovine IgG (150 µl) conjugated to Horse Radish Peroxidase (HRP) enzyme solution was added. After adding

the conjugate, the plates were incubated for 40 minutes at 37°C, and then washed four times. The HRP activities were detected by adding 100 μ l/well of equal volumes of hydrogen-peroxide and tetramethylbenzidine and incubating the plates at 37°C for 15 minutes. The reaction was stopped by adding 100 μ l /well of 1M orthophosphoric acid. The enzyme activities were measured spectrophotometrically at 450nm using Titerteck Multiscan ELISA reader and the readings were transferred to the Computer in a program called PROCOMM. The absorbance values were expressed as percent-positivity (pp) relative to the median absorbance values of the weak positive control (C+) by using the formula:

pp= OD of test sample / median OD of C+ *100, where C+ represents weak positive control serum.

Cut-off values were calculated by computing the mean pp of the negative controls and multiplying by two (Wright *et al.*, 1993).

Data analysis

The abundance of ticks and their monthly variation was determined for each tick species. Abundance of the major tick species over the study period was presented as the total count of ticks of a given species within a breed in a specific month. Period seroprevalences of the tick-borne pathogens were determined and comparisons of the mean seropositivity between breeds was analysed by One way Analysis of Variance followed by Posthoc Scheffe's procedure using SPSS software version 11.0.

RESULT

Abundance and monthly variation of identified tick species

Ticks infesting cattle in the study area belong to the Boophilus, Rhipicephalus, genera Amblyomma, Hyalomma and Haemaphysalis in decreasing order of abundance. The most common tick species were A. variegatum, B. decoloratus, A. cohaerens, R. evertsi evertsi and R. praetextatus. Species of very little abundance include, H. aciculifer, Hyalomma marginatum rufipes, R. bergeoni, R. lunulatus, R. muhsamae and R. pravus, all of which comprised about 0.31 %. of the total adult tick species (Table 1). Overall abundance of adult ticks of all species during the study period was highest in Sheko (12,180) and lowest in Horro (4,736) (Table 1). The general pattern of the activity of all the tick species over the study period showed that ticks were abundant from January to May, when rainfall was lower; and abundance was low from June to October, when rainfall was higher (Fig. 2). Peak count was observed in January, while the lowest count was observed in August and September.



Fig. 2. Pattern of abundance of tick species on the sample herd with respect to rainfall and minimum temperature in Ghibe/ Tolley station over the study period (Jan- Oct 2002).

Tick species	in ing data it gyddin orde yn dde 1,4	Total ticl	Total Count (%)			
	Abigar	Guraghe	Horro	Sheko		
A. variegatum	3,690	3,717	1,814	4,956	14,163 (39.51)	
B. decoloratus	4,974	2,920	1,632	4,432	14,016 (39.10)	
A. cohaerens	1,060	878	531	1,344	3,765 (10.50)	
R. e. evertsi	675	268	576	903	2,395 (6.68)	
R. praetextatus	352	374	159	528	1,388 (3.87)	
R. pravus	18	7	6	2	33 (0.09)	
R. bergeoni	4	13	4	5	26 (0.07)	
H. m. rufipes	13	4	4	4	25 (0.07)	
R. lunulatus	7	2	10	2	21 (0.06)	
H. aciculifer	2	4	0	4	10 (0.03)	
R. muhsame	3	0	0	0	3 (0.01)	
Total	10,798	8,187	4,736	12,180	35,845 (100.00)	

Table 1. Relative abundance of adult tick species identified on the four breeds in Ghibe/Tolley station between January and October 2002.

* Total counts obtained in 15 heifers of each breed over the 10 months.

A. variegatum was the most abundant species over the study period comprising about 39.5% of the total tick count. This tick species was present throughout the study period with peak infestation in May (Fig. 3). In this month, the burden was highest in Sheko breed with total count of 2205 and lowest in Horro with total count of 615 ticks. In Abigar and Guraghe breeds, the total count was 1575 and 1335 respectively. Fully engorged A. *variegatum* female ticks were most abundant in May (n=12) and the highest proportion of them was collected from Sheko breed. The male to female ratio of *A. variegatum* was 3:1 in all the breeds. The body zone with highest frequency of attachment for was the ventral area followed by dewlap and hooves. The frequency of attachment in the ventral site is significantly higher than the other body zones (P<0.05).



*Total counts obtained in 15 heifers of each breed in each month.

Fig. 3. Monthly abundance of A. variegatum in the four breeds in Ghibe/Tolley station between January and October 2002.

The second most important tick species in the study area was *B. decoloratus*. It comprised about 39.1% of the total tick species. The activity of this species was high from January to March and peak count was recorded in February. In January, the average burden was 117 ticks per animal in Abigar, 88 in Guraghe, 39 in Horro and 118 in Sheko (Fig. 4). In March, infestation by *B. decoloratus* became highest only in Abigar breed with an average of 130 ticks per animal. Unlike other species, the proportion of female *B. decoloratus* relative to male was higher in all breeds. The male to female ratio was 1:2 in Abigar, Guraghe and Horro; while it

was 1.3 in Sheko. The attachment site with the highest frequency of this species was the back (side) of the animals (P < 0.05), followed by dewlap, ventre (abdomen) and ear.

R. e. evertsi was present in all months of the study period with highest frequency in January (Fig. 5). The number of fully engorged females was lower than those of *A. variegatum* and *B. decoloratus* throughout the study period. The proportion of males and females of *R. e. evertsi* was equivalent in all breeds. The most preferred anatomical site of this tick species was the anovulva.



Fig. 4. Abundance and monthly variation of Adult *B. decoloratus* in the four breeds in Ghibe/ Tolley station between January and October 2002.



*Total counts obtained in 15 heifers of each breed in each month.

Fig. 5. Abundance and monthly variation of R. e. evertsi in the four breeds in Ghibe/ Tolley station between January and October 2002.

Seroprevalence of tick-borne pathogens

The seroprevalence results showed the presence of the three tick-borne pathogens: A. marginale, B. bigemina and T. mutans in the study area. The period prevalence of A. marginale, B. bigemina and T. mutans were found to be 84.2%, 87.1% and 54.2%, respectively. Variation in point seroprevalences was observed during the study period. In Abigar and Guraghe breeds high seroprevalence rates of A. marginale were obtained with slight decrease in August and October. Upon comparison of the period seroprevalence of A. marginale among the four breeds, highest seropositivity was detected in Horro breed, nevertheless statistically significant difference (P<0.05) was observed only with respect to Guraghe breed. There is also a general trend of decrease in overall seropositivity from June to October (Table 2).

 Table 2. Seroprevalence of Anaplasma marginale,

 based on Indirect ELISA in the four breeds of

 cattle.

	April	June	August	October	Period Pre-
Breed	N (%)	N (%)	N (%)	N (%)	valence (%)
Abigar	15 (100)	11 (7.3.3)	11 (73.3)	9 (60.0)	772
Guraghe	e 15 (100)	15 (100)	8 (53.3)	7 (46.7)	75 ³
Horro	15 (100)	15 (100)	14 (93.3)	12 (80)	931
Sheko	14 (93.3)	15 (100)	13 (86.7)	13 (86.7)	921
Overall	59 (98.3)	56 (93.3)	46 (76.7)	41 (68.3)	84.2

^{1, 2,3} means with different superscripts are different at P< 0.05. N = Number of serologically positive animals.

In all the breeds, the point seroprevalence of *B. bigemina* was higher during the months of April and October than in June and August. However, there was no statistically significant difference in the seropositivity among the four breeds in the period prevalence (Table 3).

 Table 3. Seroprevalence of Babesia bigemina based

 on Indirect ELISA in the four breeds of cattle.

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•	April	June	August	October	Period
Breed	N (%)	N (%)	N (%)	N (%)	Prevalence*
	()			. ,	(%)
Abigar	14 (93.3)	8 (53.3)	12 (80)	14 (93.3)	80
Guraghe	13 (86.7)	12 (80.0)	13 (86.7)	15 (100)	88
Horro	15 (100)	12 (80.0)	14 (93.3)	15 (100)	93
Sheko	15 (100)	11 (73.3)	13 (86.7)	13 (86.7)	87
Overall	57 (95.0)	43 (71.7)	52 (86.7)	57 (95.0)	87.1

* No significant mean difference ($P \ge 0.05$) between the breeds. N = Number of serologically positive animals.

Low seropositivity was found in the cattle with respect to *T. mutans* except in Abigar and Horro

breeds in April. The mean period seroprevalence of *T. mutans* among the four breeds was not significantly different (Table 4).

Table 4. Seroprevalence of *Theileria mutans* based on Indirect ELISA in the four breeds of cattle.

• Breed	April	June	August	October	Overall
	N (%)	N (%)	N (%)	N (%)	prevalence*
					(%)
Abigar	13 (86.7)	5 (33.3)	8 (53.3)	4 (26.7)	50
Guraghe	9 (60)	8 (53.3)	7 (46.7)	8 (53.3)	53
Horro	13 (86.7)	8 (53.3)	8 (53.3)	7 (46.7)	60
Sheko	11 (73.3)	7 (46.7)	8 (53.3)	6 (40)	53
Overall	46 (76.7)	28 (46.7)	31 (51.7)	25 (41.7)	54.2

* No significant mean difference ($P \ge 0.05$) between the breeds. N = Number of serologically positive animals.

DISCUSSION

The tick species identified in the present study were found in previous surveys that were _conducted in western and central parts of Ethiopia (de Castro, 1994; Sileshi Mekonnen et al., 2001). In -all the four cattle breeds, A. variegatum and B. decoloratus were found to be the dominant tick species for the period studied. Previous studies in western Ethiopia also showed that A. variegatum and B. decoloratus were the most widespread tick species in the country (de Castro, 1994). All the breeds showed similar monthly fluctuations in counts of both tick species. Despite the marked decrease in the abundance of adult B. decoloratus in April, there was large number of immature forms per breed in this month leading to peak count of adult tick in May. The reason for the low count of adult tick in April might be due to the seasonal variation of the activity of this tick species.

Resistance to tick infestation expressed by the overall low number of adult tick counts was highest for Horro, followed by Guraghe, Abigar and Sheko. The Abigar and Sheko breeds were infected by two and three times larger number of adult ticks respectively compared to Horro breed indicating better resistance of the Horro breed. Previous comparative study made at Bako research centre on Horro, Boran and their crosses have also shown that Horro cattle were more resistant (Mohammed Ali and de Castro, 1993). Adaptation of a host to various ecological factors such as altitude, climate and vegetation has been shown to affect tick counts on the host (Mohammed Ali and de Castro, 1993). Although, the eco-climatic conditions of the areas from which the four breeds are brought are quite different from Ghibe/Tolley area, all of these animals were brought to the study area 2 years prior the initiation of this study. Thus, the effect of the ecological factors associated with their original location to harbour different tick burden looks insignificant, suggesting the possible genetic and immunological factors for the exhibited difference in tick burden between the breeds

Because of limited baseline data on weather variables (i.e., absence of data on relative humidity and maximum temperature), it was not possible to adequately relate the monthly variation of the ticks with the relevant climatic conditions of the study area. However, based on the available meteorological data, the monthly dynamics of tick species seemed to depend on the ambient temperature and rainfall of the study area. Low rainfall and low minimum ambient temperature in January seemed to be favourable for the activity of ticks. The main rainy season (June-August) was correlated with low tick burden probably due to excessive wetting of the environment unfavourable for development of ticks on the ground.

Regarding the monthly dynamics of *B*. decoloratus, a marked decrease in the abundance of the adult forms in April was observed, followed by increasing burden in May. The decreased burden in April was observed only in adult forms and there was large number of immature forms with mean counts of 203 larval and 354 nymphal forms in this month (data not shown), which led to the second peak of adult tick count in May.

Specificity of ticks to different attachment sites of a host is a common behaviour in most species of ticks (Soulsby, 1982). The most abundant species in the study area, A. variegatum, was predominantly found in abdominal sites. Due to this, the damage of udder and cases of severe myiasis, teat perforation and amputation, and cases of mastitis were observed. Likewise, localized wound infections in the brisket areas of animals infested with A. variegatum were observed especially in the months of April and May. Such wounds were observed to cause inability to move and temporary lameness of the forelimbs of the animals especially in Sheko breed. R. e. evertsi was mainly found on delicate skins of the anal and vulval areas and severe infestation was observed to cause inflammation of these areas. B. decoloratus was mostly attached on the back and dewlap and were observed to cause inflammatory lesions on the attached sites.

In this study the male to female sex ratio of each tick species except that of R. e. evertsi was not equivalent, which is different from the natural condition where equivalent proportions of male

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study dealt with only the on-host population dynamics of ticks, it could not clearly show the abundance of the two-host ticks such as R. e. evertsi, H. m. rufipes, and three-host ticks such as A. variegatum and A. cohaerens which spend some time off their hosts. Although the reason why the proportion of female B. decoloratus ticks was higher than the male is not clear, it is an indication of the possible risk of maintaining a high tick population on the animals.

The vectors for the three pathogens A. marginale, B. bigemina and T. mutans are found to be the abundant species in the study area. The observed higher seroprevalence of A. marginale during the month of April compared to the months of August and October for all the study animals could be due to higher infestation of the cattle population by the major vectors of this disease R. e. evertsi and immature B. decoloratus in April. Although the tick burden was less in June, high seroprevalence has been detected. The reason for this can be explained by the longer half life of immunoglobulin G (IgG), which is 23 days (Benjamini et al., 2000) i.e., some animals that were positive earlier might have remained seropositive when new infection from the vector is minimal.

Previous works revealed that indigenous Zebu and Sanga cattle are more resistant to ticks and various tick-borne diseases (Norval et al., 1988; de Castro and Newson, 1993). In the present study, the similarity in the mean seropositivity of the four breeds to Babesia bigemina and Theileria mutans might be due to the presence of Zebu and Sanga genes in these cattle. The significant difference (P<0.05) in seroprevalence between Guraghe and Horro breeds with respect to A. marginale might be due to the inherent differences in their susceptibility to tick infestation as well as difference in their ability to induce antibody-mediated responses to ticks and tick-borne diseases (Adey Feleke et al., 2007).

Serological studies of tick born diseases provide information that helps classify the immune status of the cattle in an area. Based on the overall seroprevalence of A. marginale (84.2%) and B. bigemina (87.1%), the cattle population can be considered to exist in a state of enzootic stability for these diseases. According to Norval et al (1983), enzootically stable situation is achieved in a certain cattle population when the percentage of positive sera is between 81-100%. In this situation, the inoculation rate of the tick-borne pathogens is adequate to ensure that all are infected while they are protected by innate and/or acquired immunity and thus clinical disease is minimal (de Vos, 1979). A situation approaching enzootic stability to a given disease is achieved when the seropositivity lies between 61–80% in a population. Any seropositivity less than these values is considered as unstable (21–60%), minimal disease situation (1–20%) and disease free situation (0%) (Pegram *et al.*, 1981).

The average seropositivity of *T. mutans* (54.2%) indicates that the population is in the state of enzootic instability. T. mutans is generally known to be benign, although virulent strains have been reported from East and South Africa. It is also reported that virulent strains cause a high piroplasm parasitaemia and haemolytic anaemia in the host (Morzaria, 1989). Retrospective data on the clinical cases from the population of cattle employed in the current study showed that there were cases with clinical signs such as weakness, loss of appetite, rough coat and their diagnosis showed the presence of Theileria spp. in blood smear but no other haemoparasites. Upon treatment of these cases with Oxytetracycline, recovery was observed. This might indicate that the T. *mutans* strains in the cattle population are virulent.

Disruption of enzootic stability is usually associated with drought conditions or intensive tick control by regular application of acaricides (Norval *et al.*, 1983). Since the study area is a dry land with scarce pasture for grazing during long dry season, animals should be supplemented with hay and concentrate. In indigenous cattle, little or no-tick control is required to increase productivity, and mortality due to tick-borne diseases is usually low or insignificant because of enzootic stability. Therefore, it is recommended that the strategic application of acaricide should be practised only when there is peak infestation on the cattle to maintain the enzootic stability established to *A. marginale* and *B. bigemina*.

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