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## The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya; a cross-sectional study

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### Abstract

The most important tick-borne disease of cattle in eastern, central and southern Africa is East Coast fever (ECF) caused by *Theileria parva* and transmitted by the tick *Rhipicephalus appendiculatus*. Other less-important tick-borne diseases in cattle are benign theileriosis caused by *Theileria mutans*, babesiosis caused by *Babesia bigemina*, anaplasmosis caused by *Anaplasma marginale* and cowdriosis caused by *Cowdria ruminatum*. In Murang'a District, Central Province of Kenya, five agroecological zones (AEZs) are defined according to climate, altitude and agricultural activities. A cross-sectional serological study was conducted on 750 smallholder dairy farms in Murang'a District, selected in a stratified random sampling method. The farms had a total of 362 calves. One hundred and fifty farms were studied from three administrative sublocations in each of the five AEZs. Prevalence of serum antibodies to three tick-borne parasites, that is *T. parva*, *T. mutans* and *B. bigemina*, were determined using the enzyme-linked immunosorbent assay (ELISA) technique. Antibody prevalence values differed across the AEZs. The ranges of means for the prevalences were: *T. parva* (18–72%), *T. mutans* (1.5–28%) and *B. bigemina* (12–49%). The above results serve as indicators of the possible existence of endemic stability in some AEZs for some parasites. © 1997 Elsevier Science B.V.

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## 1. Introduction

Over 90% of the dairy farms in Kenya are owned by smallholder farmers, who are estimated to produce 75–90% of the milk sold in the country (Mbogoh, 1984a, Mbogoh, 1984b; Goldson and Ndeda, 1985; Ministry of Livestock Development, 1985, Ministry of Livestock Development, 1989). These farms, ranging in size from 2 to 5 acres each with an average of two to ten dairy cattle, are located in highland areas where mixed farming is predominant (Mbogoh, 1984a, Mbogoh, 1984b). In these dairy farms, the sale of milk provides a more reliable source of income than that derived from many other farming enterprises. A number of factors limit further development of the dairy industry and these include diseases, poor management, inadequate nutrition and lack of farm inputs (such as capital, farm machinery, etc.) (Bram, 1983; ILRAD, 1984; Goldson and Ndeda, 1985; Norval et al., 1992). Although tick-borne diseases (TBDs) are considered by many to be a priority constraint to dairy development, there are few well-structured studies investigating this.

An important component affecting the efficiency of transmission of tick-borne diseases is the population dynamics of tick vectors. The main field vector for the transmission of ECF is *Rhipicephalus appendiculatus*. For *Theileria mutans*, the main tick vector is *Amblyomma variegatum*, the vector of *Babesia bigemina* is *Boophilus decoloratus*.

An important hypothesis that has been developed during years of observations on ECF and other TBDs in the field is the concept of endemic stability. The term 'endemic stability' has been defined by Norval et al. (1992) as a "climax relationship between host, vector and environment in which all coexist with the virtual absence of clinical disease while endemic instability means an incomplete relationship (between host, vector and environment) in which clinical disease occurs". In the case of endemic stability due to *Theileria parva*, it is defined as "the state in a cattle population (farm, agroecological zone, district, etc.) in which the large majority of that population becomes immune by six months of age and little or no clinical disease occurs" (Norval et al., 1992). Deem et al. (1993) report that serum antibody prevalence may be an indicator of endemic stability status, and the role of antibody prevalence as an indicator of the degree of endemic stability, and thus the levels of losses from tick-borne infection, have been reviewed by Perry (1996). Preliminary results from a recently concluded study in the neighbouring Kiambu District of Kenya show low ECF-specific mortality risks and moderate antibody prevalence to *T. parva*, which may be indicators of the existence of endemic stability in a high proportion of the dairy cattle (O'Callaghan et al., 1994).

The current study was initiated and conducted with the objective of estimating the prevalence of serum antibodies to tick-borne infections in Murang'a District of Kenya by AEZ, cattle type and management system. The purpose was to characterise the potential risks incurred by these factors to help identify potential endemically stable and unstable areas for a subsequent evaluation, by a longitudinal study, of the differences in losses experienced under the two epidemiological states.

## 2. Materials and methods

### 2.1. Study area

The study was conducted in Murang'a District, Central Province, Kenya (Fig. 1). Five major AEZs, defined according to altitude, rainfall levels and agricultural activities, were identified (Jaetzold and Schmidt, 1983). They were: Lower Highlands 1 (LH 1) (designated tea–dairy); Upper Midlands 1 (UM 1) (designated coffee–tea); UM 2 (designated main coffee); UM 3 (designated marginal coffee); UM 4 (designated sunflower–maize). Cattle are raised in all five AEZs. This district has diverse environments due to large variations in elevation (between approximately 1200 and 2500 m above sea level) and annual rainfalls ranging from 800 to 2200 mm. Most smallholder dairy farmers in Murang'a District raise improved Taurine (*Bos taurus*) breeds of dairy cattle in zero-grazing units or on small paddocks. Zero-grazing dairy farming management is a system in which cattle are confined permanently in houses or sheds; feed (especially cut fodder) and water are delivered to the animal. Most farmers are registered members of dairy co-operative societies that market the milk. However, in the lower regions of the district Zebu (*Bos indicus*) breeds of cattle (principally the East African Zebu) and their crosses with Taurine breeds, are predominant and are raised under an open grazing management system.

### 2.2. Selection of sublocations and farms

Smaller study areas (sublocations) and farms within Murang'a District were selected by a stratified random sampling method (Fig. 1). First, all sublocations (which are the smallest administrative subdivisions in Kenya) were classified according to AEZs. Those sublocations which straddled two AEZs were listed once under each; sublocations found exclusively in one AEZ were listed twice. The latter were given a higher chance of selection to minimise selection of those falling on the AEZ boundaries. Three sublocations from each of the five AEZs were selected using random numbers. All sublocations were eligible for selection.

In the second stage, 50 farms per selected sublocation were randomly sampled from a list frame of farms. The lists were obtained from dairy co-operative societies in the western part of the District (Kangema Division) and from diptank registers in other areas as dairy co-operatives were still recruiting more members (with about 50% membership) while dipping was still in place. In Kangema Division, it was easier to obtain names of farmers from dairy co-operative societies as dipping was not widely practised and over 90% of the farmers were members of the dairy societies. However, there was no difference between members and non-members of co-operatives except in the way they marketed their milk.

Relatively few sublocations per AEZ were selected since the average serum antibody prevalences by sublocation was expected to be relatively constant within an AEZ. In some AEZs, farm-to-farm variation for some sublocations was expected. Most of the farms have only one calf and a few have more than one. Since farm-to-farm variation was expected in some cases, there was a need to inflate the number of animals sampled.

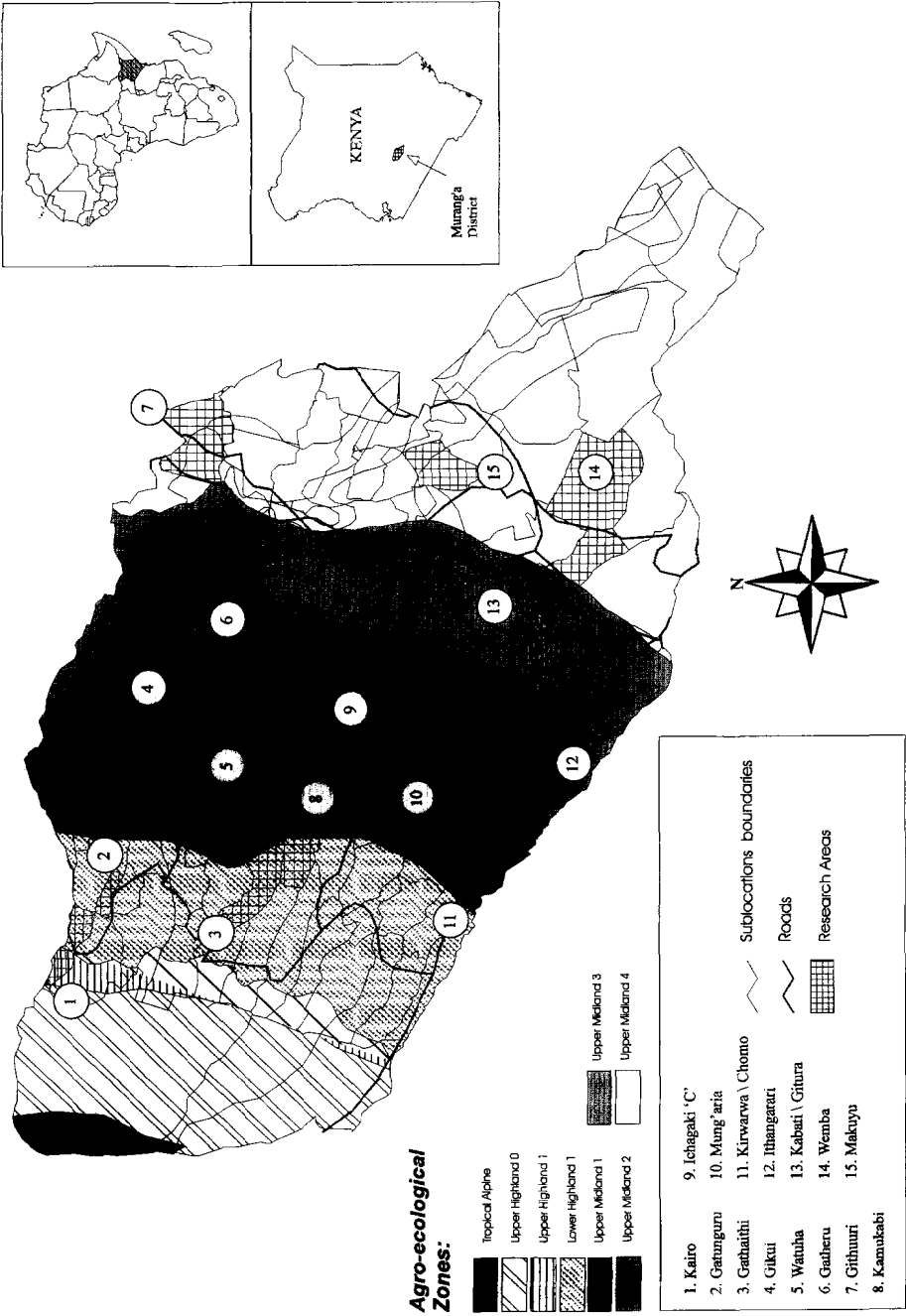


Fig. 1. Map of Murang'a District showing the agroecological zones and the sublocations selected for the tick-borne disease study.

Ordinarily, a doubling of sample size is recommended, but since the number of calves per farm was small, 50% was added. As the antibody prevalence was not known a priori, a 50% prevalence was assumed when calculating the total number of farms required for the study, with a 10% allowable error. The formula ( $n = 4PQ/L^2$ ), which was used to estimate the sample size, was obtained from Martin et al. (1987). The sample size was estimated by AEZ giving a total of 100 farms per AEZ. After 50% addition, a total of 750 farms were selected.

### 2.3. Data and sample collection

For each of the farms visited, a questionnaire was administered to the farmer to gather information on general farm management, on the grazing management system and on specific tick control practices. A separate questionnaire was administered for all calves (without exception) between 6 and 18 months old. For each calf, the breed, age, sex, tick control history (type, initial age and frequency) and disease history were recorded. All questions were presented in a closed format. To maintain consistency, the first author conducted all interviews in the local Kikuyu dialect.

Blood was collected from each calf in two 10-ml plain vacutainer tubes (Becton Dickinson Vacutainer Systems, UK) by jugular venipuncture. Labelling of tubes was carried out and verified before drawing the blood from the calves. After collection, blood samples were stored in ice boxes until they could be refrigerated (usually within 2–6 h). The next day, the sera were separated by centrifugation at  $3000 \times g$  for 20 min and divided into six aliquots of 0.5–1.5 ml and stored in dry ice (solid  $\text{CO}_2$  at approximately  $-160^\circ\text{C}$ ) until they could be transferred to freezers ( $-20^\circ\text{C}$ ) in the laboratory.

### 2.4. Serological tests

ELISAs were used to detect antibodies to *T. parva*, *T. mutans* and *B. bigemina*. For *T. parva* the polymorphic immunodominant molecule (PIM) recombinant antigen was used (Katende et al., 1997). The procedures for the detection of *T. parva* antibodies by this method has been described by Katende et al. (1997). Tests for antibodies to *T. mutans* and *B. bigemina* have been described by Katende et al. (1990, 1997). Each ELISA test plate included predetermined positive and negative control sera. Optical density (OD) readings from the reference highly positive control sera were used to compute the percent positivity (PP) for the test sera (Wright et al., 1993). Percent positivity (PP) for test serum was expressed as the percent of the test serum OD (at optimum dilution of 1:200) divided by the mean OD reading derived from the strong positive control serum on the linear curve (from a curve of OD against the reciprocal of serial dilutions) (Wright et al., 1993; Katende et al., 1997). Any reading of 15 PP or above was considered positive for antibodies to the *T. parva* PIM recombinant antigen. For *T. mutans* and *B. bigemina*, a sample was considered positive if the PP value was 20 or above.

### 2.5. Data storage and analysis

Data files of questionnaires and laboratory results were prepared in Dbase IV Plus (Ashton-Tate Corporation, Torrance, CA, USA). Separate files were prepared for the farm and individual-calf questionnaires. The statistical analyses were conducted in Statistical Analysis Systems (SAS) Institute Inc. software (SAS Institute Inc., Cary, NC, USA). The differences in *T. parva* antibody prevalences were compared across the AEZs using the Mantel–Haenszel chi-square. Confidence limits for the binomial proportions (Snedecor and Cochran, 1989) for each AEZ and grazing system were generated for all antibody prevalences. While generating the confidence limits, most variation in antibody prevalence was expected between calf observations. As the mean antibody prevalences between farms within an AEZ were not expected to be different, clustering between farms was thus ignored while calculating the confidence limits. Furthermore, only one calf per farm was expected, thus minimising the possibility of clustering within farms. Both linear (least squares) and non-linear (logistic) regressions were performed on the antibody prevalence data for *T. parva*, *T. mutans* and *B. bigemina* (using PP values as the outcome variable) against the potential risk factors (dependent variables) listed in Table 1. For the linear regression procedure, the absolute PP values were entered in the model as the dependent variable. For the logistic regression procedure, PP values were converted to dummy variables using a cut-off value as either positive (and coded 1) or negative (and coded 0). Both the regression models were generated according to various calf and management categories e.g. males and females, acaricide use and no use as independent variables (Table 1). The backward elimination procedure in SAS Institute Inc. was applied to generate the final model through step-by-step elimination of non-significant variables from the overall model ( $P < 0.05$ ) based on Wald's tests. The variable AEZ was forced into both models as a dependent variable and also the dummy variable for the intercept.

## 3. Results

Of the original 750 farmers selected, 35 could not be located, 13 were found to be in other subdivisions and three had moved. These were all replaced by the next farms on the lists. During the preliminary visits, three farmers declined to participate. A voluntary participatory rate of 99.6% (747/750 farms) was thus achieved. Of the 747 participating farms, only 305 (41%) had calves (6–18 months old) on the premises at the time of the study. About 86% of the farms had only one calf present on the farm, 12% had two calves present and the other 2% had three to five. Most farms with no calves of the above age category had reported no calving while about 10–15% reported calf deaths. A total of 362 calves were found, of which 148 (41%) were males and 214 (59%) females.

### 3.1. Distribution of risk factors

The distribution of cattle types in the study were: Taurine 73.5% (266/362), Taurine–Zebu 20.4% (74/362) and Zebu 6.1% (22/362) with UM 4 containing

Table 1

Characteristics of 362 selected calves in Murang'a District, in terms of location, breed, age, sex, housing, feeding and tick control

Category	Frequency	Percentage
Agroecological zone		
Lower Highlands 1	67	18.5
Upper Midlands 1	75	20.7
Upper Midlands 2	66	18.2
Upper Midlands 3	64	17.7
Upper Midlands 4	90	24.8
Breed		
Exotic ( <i>Bos taurus</i> )	266	73.5
Zebu and crosses ( <i>Bos indicus</i> )	96	26.5
Sex		
Male	148	40.9
Female	214	59.1
Calf kept before weaning		
Indoors exclusively	230	63.5
Outdoors at least part-time	132	36.5
Calf currently kept		
Indoors exclusively	181	50.0
Outdoors at least part-time	181	50.0
Forage given presently		
Open grazing at least part-time	159	43.9
Cut fodder or grass exclusively	203	56.1
Source of forage feed		
Owner's farm exclusively	162	44.8
Brought outside the farm	262	55.2
Tick treatment method		
None	203	56.1
Any method	159	43.9
Last tick treatment		
≤ 2 weeks	89	24.6
> 2 weeks	273	75.4
No. of times (last 6 months)		
≤ 5	205	31.2
> 5	157	47.1

exclusively Zebu cattle. The mean age for all the calves studied was 10.9 months (standard deviation 3.6). The mean age for females was 11.3 months and for males 10.4 months (standard deviations of 3.7 and 3.4, respectively). Fifty-six percent of the calves in the study (203/362), were reported never to have received any acaricide application. Further details are shown in Table 1.

### 3.2. Serum antibody prevalences of *T. parva*, *T. mutans* and *B. bigemina*

The mean antibody prevalences (with 95% confidence limits for the binomial proportions) for *T. parva*, *T. mutans* and *B. bigemina* by AEZs and grazing system are shown in Figs. 2 and 3. At least one AEZ had prevalences significantly different from the other AEZs for all the parasites ( $P < 0.001$ ).

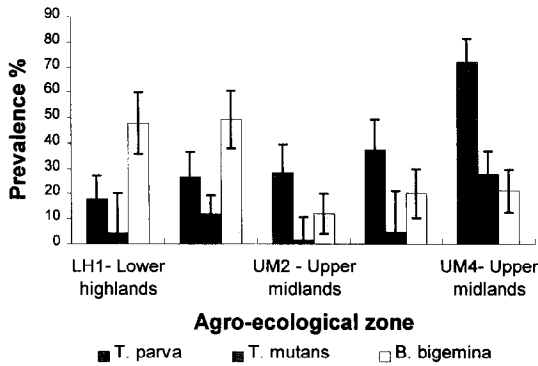


Fig. 2. Mean serum antibody prevalence (with 95% confidence limits) to *Theileria parva*, *Theileria mutans* and *Babesia bigemina* by agroecological zone from the cross-sectional study in Murang'a District, Kenya (March–June, 1994).

3.3. Factors associated with serum antibody prevalence

Three factors were significantly associated with variation in antibody prevalence to *T. parva*: agroecological zone, breed of calf and grazing system ( $P < 0.05$ ) (Table 2). Upper Midlands 3 and 4 had higher prevalences than the other AEZs, indicating that higher-altitude zones had lower PP values than lower-altitude zones. Zebu breeds and their crosses with Taurine breeds were significantly associated with higher prevalences than Taurine breeds. Higher prevalences were significantly associated with calves on open grazing than those on zero-grazing (partially or completely confined).

For *T. mutans*, only the breed of the calf was significantly associated with differences in prevalences ( $P < 0.05$ ). Zebu calves and their crosses with Taurine breeds had significantly higher PP values than Taurine breeds.

For *B. bigemina*, AEZ, grazing system, age of calf, tick control procedure ( $P < 0.05$ ) and sex of calf ( $P < 0.1$ ) were significantly associated with differences in prevalences.

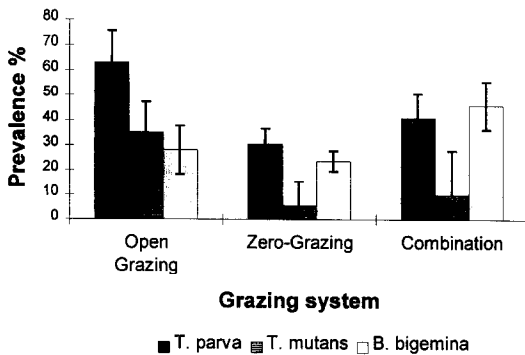


Fig. 3. Mean serum antibody prevalence (with 95% confidence limits) to *Theileria parva*, *Theileria mutans* and *Babesia bigemina* by grazing management from the cross-sectional study in Murang'a District, Kenya (March–June, 1994).

Table 2

Variables associated with *Theileria parva*, *Theileria mutans* and *Babesia bigemina* percent positivity value from the linear regression model for 362 calves in Murang'a district, Kenya (variables considered significant at  $P < 0.1$ )

Variable	<i>b</i>	SE ( <i>b</i> )	<i>P</i> value
<i>Theileria parva</i> <sup>a</sup>			
Upper Midlands 3 (UM 3 = 1, other AEZs = 0)	7.58	2.62	0.004
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	11.54	3.90	0.003
Breed (Exotic = 0, Zebu * crosses = 1)	9.64	3.74	0.010
Grazing system (indoors = 1, outdoors = 0)	- 3.35	2.00	0.095
<i>Theileria mutans</i> <sup>b</sup>			
Breed (Exotic = 0, Zebu * crosses = 1)	- 2.02	0.27	0.0001
<i>Babesia bigemina</i> <sup>c</sup>			
Upper Midlands 4 (UM 4 = 1, other AEZs = 0)	6.51	1.66	0.0001
Grazing system (indoors = 1, outdoors = 0)	- 3.70	1.45	0.011
Age of calf (absolute value)	0.43	0.19	0.023
Sex (males = 0, females = 1)	- 2.25	1.34	0.095
Tick treatment (none = 1, any method = 0)	6.00	2.32	0.010

<sup>a</sup> Tested for antibodies against polymorphic immunodominant molecule (PIM) recombinant antigen.

<sup>b</sup> Tested for antibodies against Protein 32 kDa antigen.

<sup>c</sup> Tested for antibodies against Protein 200 kDa antigen.

Agroecological zone UM 4 was significantly associated with higher prevalences compared with other AEZs. Higher prevalences were significantly associated with calves grazed freely, when compared to those partially or completely confined. Higher preva-

Table 3

Variables associated with *Theileria parva*, *Theileria mutans* and *Babesia bigemina* percent positivity values from the logistic regression model for 362 calves in Murang'a District, Kenya (variables considered significant at  $P < 0.05$ )

Variable	<i>b</i>	SE ( <i>b</i> )	<i>P</i> value
<i>Theileria parva</i> <sup>a</sup>			
Breed (Exotic = 0, Zebu * crosses = 1)	- 2.02	0.27	0.0001
<i>Theileria mutans</i> <sup>b</sup>			
Upper Midlands 4 (UM4 = 1, other AEZs = 0)	- 2.29	0.89	0.010
<i>Babesia bigemina</i> <sup>c</sup>			
Upper Midlands 2 (UM2 = 1, other AEZs = 0)	1.69	0.44	0.0001
Upper Midlands 3 (UM3 = 1, other AEZs = 0)	1.22	0.37	0.0009
Upper Midlands 4 (UM4 = 1, other AEZs = 0)	1.36	0.32	0.0001
Grazing system (indoors = 1, outdoors = 0)	0.61	0.28	0.03
Sex (males = 0, females = 1)	- 0.53	0.26	0.038

<sup>a</sup> Tested for antibodies against polymorphic immunodominant molecule (PIM) recombinant antigen.

<sup>b</sup> Tested for antibodies against Protein 32 kDa antigen.

<sup>c</sup> Tested for antibodies against Protein 200 kDa antigen.

lences were seen in older calves and male calves. Calves that reportedly had never received any acaricide treatment had higher prevalences than those that had received acaricide treatment, regardless of the method used.

The results from the logistic regression model are shown in Table 3. Zebu breeds and their crosses were twice as likely to have the risk of having positive antibody titres to *T. parva* than Taurine breeds (odds ratio: 2.0).

For *T. mutans*, the only significant factor associated with variation in prevalence was AEZ ( $P < 0.01$ ). Calves raised in UM 4 were at twice the risk of being associated with a positive antibody titre than those raised in other AEZs.

Three factors were significantly associated with positive antibody titres for *B. bigemina*. Three AEZs (LH 1, UM 1 and UM 4) had higher odds of seropositivity compared to other AEZs. Also, calves under a free grazing system had higher positive antibody titres than those confined to zero-grazing units. Males calves were more likely to have a positive antibody titre than female calves.

#### 4. Discussion

This study provided a preliminary assessment of serum antibody prevalences to *T. parva*, *T. mutans* and *B. bigemina* in Murang'a District. Calves less than 6 months of age were not sampled to minimise the possibility of detecting passively derived colostral antibodies, which in *T. parva* infection are considered to decline to undetectable levels by 2–4 months of age (Burridge and Kimber, 1973). There was large variability in serum antibody prevalences to the three TBDs across the AEZs suggesting existence of different epidemiological status for these diseases in the District. Serum antibody prevalence values can be applied to assist in determining the presence and degree of endemic stability and instability (Perry, 1996). Endemic stability is more likely to exist where the prevalence of serum antibodies to the infection is high ( $> 70\%$ ), while the antibody prevalence is usually low ( $< 30\%$ ) in the endemic instability state (Norval et al., 1992; Deem et al., 1993; Perry and Young, 1995; Perry, 1996).

Percent positivity values were preferred to optical density readings as the PP values were adjusted for variations associated with inconsistent background activity while performing the ELISA tests (Wright et al., 1993). The test error may arise from varying laboratory conditions such as handling of sera and the preparation of sera dilutions. The performance of the ELISA tests has also been evaluated recently (Katende et al., 1997; J.M. Katende, personal communication, 1996). The sensitivity for ELISA tests for *T. parva* and *T. mutans* has been estimated to be over 99% while that of *B. bigemina* has been estimated to be about 97%, while the specificity has been estimated to be 97% for *T. parva*, 99% for *T. mutans* and 98% for *B. bigemina* (Katende et al., 1997; J.M. Katende, personal communication, 1996).

An endemically stable situation appears to be present in UM 4 for *T. parva*, where serum antibody prevalence is over 70%. This is similar to results from past studies in Kenya conducted in Trans Mara District, (Moll et al., 1986), the Lake Victoria Basin (Morzaria et al., 1988), and Kilifi District (Deem et al., 1993). Prevalences in other AEZs were lower, suggesting that endemically unstable states may exist. For *T. mutans*,

all AEZs showed prevalences suggestive of unstable states. *Babesia bigemina* showed a different pattern, with two AEZs (LH 1 and UM 1) showing intermediate prevalences (approximately 50%) while the other three showed low prevalences, suggesting instability (20% serum antibody prevalence or below). An interesting observation is that while an endemically stable state for *T. parva* appears to occur in the lower altitude zones, the same situation is observed in higher altitude zones for *B. bigemina*. This is possibly explained by the tick dynamics across the AEZs. In lower AEZs, higher number of *R. appendiculatus* ticks are present while higher number of *Boophilus* species are present in the higher AEZs.

Serum antibody prevalences and patterns were also simply correlated with all factors considered, i.e. is grazing management, breed and AEZ. These factors were correlated with each other, often highly. We considered that AEZ and grazing management were more important determinants of serum antibody prevalence than breed in the study area. Three AEZs (UM 1, 2 and 3) were mainly characterised by an intensive grazing system in which virtually all animals are kept under a zero-grazing (strict confinement) management system. In this system, calves are not exposed to open pastures and have minimal contact with adult animals. It is likely that calves raised on pastures are frequently exposed to infected ticks thus developing circulating antibodies.

The association between serum antibody prevalence and the breed of calf may be explained by the distribution of these breeds. There was a high correlation between breed, AEZ and grazing system, with Zebu and their crosses kept under conditions of higher tick exposure (UM 4 and unrestricted grazing). However, after controlling for these two factors (AEZ and grazing management), breed was not associated with differences in prevalence. The UM 4 area is drier than the other four AEZs and Zebu cattle and their crosses are predominant here, especially where the open grazing system is practised. Zebu breeds from endemic-theileriosis areas are known to be less susceptible to the effects of *T. parva* infections; thus, they likely may have a survival advantage in this zone over Taurine breeds and their crosses (Moll et al., 1984, Moll et al., 1986; Perry et al., 1992). The difference in prevalences between sexes for *Babesia bigemina* is possibly explained by the differences in management of female and male calves. Female calves are generally housed and well cared for due to the value attached to them, whereas the males are generally grazed freely due to lower value attached them, thus increasing the risk of their exposure to ticks.

Reported tick control practices were not associated with differences in serum antibody prevalence values. This may be because tick control is generally poor throughout the district, or that reporting was inaccurate. Tick control in highland areas is less-commonly practised than was previously the case (either inadequate or ineffectively applied). Also, as it is still unlawful to move cattle with ticks, some respondents may have been reluctant to report minimal acaricide use. Similar observations were recently made in the coastal area of Kenya (Maloo et al., 1994).

This study shows that different endemic states may exist in Murang'a district for tick-borne diseases and these may result in different production losses. The important factor defining these states is the agroecological zone which is highly correlated with the grazing management and the breed of calf. However, it is difficult to understand clearly the factors associated with acquisition of infection by a cross-sectional study of serum

antibody prevalence and the outcome of these infections. Data on disease incidence and production loss can only be obtained from a longitudinal study which comprises the next portion of the research. The results from this study provided baseline information to guide the design of the longitudinal study currently in progress.

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