The prevalence of serum antibodies to East coast fever and associated risk factors in cattle in the traditional crop-livestock system in Mbeere district, Kenya; a cross-sectional study.

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Abstract

East coast fever (ECF) is the most important tick-borne disease (TBD) of cattle in Kenya. A cross-sectional survey was carried out in the cattle population raised under traditional crop-livestock production system in Mbeere district, Kenya. The objective was to estimate ECF seroprevalence and identify associated risk factors for planning ECF control strategies in the district. A total of 440 cattle of all ages from 80 farms were selected by multistage random sampling. Prevalence of serum antibodies to ECF was determined by enzyme-linked immunosorbent assay (ELISA). Risk factor information was collected at three levels: animal-, farm-(herd) and division-levels. The relationship between ECF seropositivity and the risk factors was assessed by multivariable analysis using logistic regression models. The overall ECF seroprevalence was 19.3% (range: 3.9% to 48% across divisions) in the district [95%CI: 13.7%, 24.9%]. Regression analysis found four major factors associated with seropositivity: presence of the vector tick on the farm (OR=3.8), frequency of calf tick control before 6 months of age (for frequency of >5 times, OR=3.9 relative to frequency of ≤5 times), herd size (OR for herd size category 6-10 cattle = 2.7; OR for over 10 cattle = 0.95 relative to herd size category 1-5 cattle) and division (ORs for Siakago, Gachoka and Mwea divisions = 0.3, 0.21 and 5.1 respectively relative to Evurore division). The low ECF seroprevalence indicates that ECF occurs in the district in an endemic instability state. The significant herd management factors possibly arose out of differential perceptions of ECF occurrence and importance across the district while the wide variation in seroprevalence across divisions was thought to be due to a gradient in vector

tick environmental suitability habitats. These findings suggest that ECF seroprevalence in Mbeere district is mainly influenced by herd and environmental factors.

Key words: ECF, *T. parva,* seroprevalence, Mbeere

1. Introduction

Traditional mixed farming systems, in which both indigenous livestock and crops are integrated on the same farm, are widespread in the rain-fed sub-Saharan Africa (SSA) (Lenné & Thomas, 2005). These systems are perceived to be important in terms of their contribution to the total output of animal products and in enhancing the livelihoods of the poor (Lenné & Thomas, 2005). In Kenya, these systems are common in rangeland semi-arid areas (SAAs) of which Mbeere district is part (Onduru *et. al.,* 2002). These systems have the potential for intensification and for contributing more to overall agricultural productivity and sustainability (McDermott *et al.,* 1999). These systems are, however, faced with varied and different constraints (Lenné & Thomas, 2005). In this regard, Onduru et al. (2002) cited tick-borne diseases (TBDs) and inadequate fodder, in that order, as the main constraints to the livestock sub-sector in Mbeere District. The most important TBD of cattle in Kenya is East coast fever (ECF) caused by *Theileria parva* and transmitted by the brown ear tick *Rhipicephalus appendiculatus.*

An important hypothesis advanced during the course of field research on ECF and other TBDs is the concept of endemic stability. In the case of endemic stability to *T. parva,* it is described as "the state in a cattle population (farm, agroecological zone (AEZ), district, etc.) in which, as a result of low but continuous challenge by ticks with low infection rates, a "climax relationship" between host, vector and environment is achieved, whereby the large majority of that population becomes immune by six months of age and little or no clinical disease occurs" (Norval *et al.,* 1992). Endemic instability describes an incomplete relationship (between the same factors) in which clinical disease occurs. Serum antibody prevalence has been widely used in Kenya as an indicator of endemic stability or instability status (Deem *et al.,* 1993; Gitau *et al.,* 1999; Maloo *et al.,* 2001).

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Majority of epidemiological studies have been conducted in smallholder dairy systems that are mainly practiced in the highlands and coastal areas of Kenya. In these systems, the epidemiology of ECF has been well studied (Deem *et al.* 1993; Gitau *et al.,* 1997, 1999; Maloo *et al.,* 2001). However, there seems to be no well-structured observational studies investigating the epidemiology of TBDs in the traditional crop-livestock systems that are practiced in rangeland areas of Kenya where there are no major contrasting farming systems, cattle breeds and ecological zones. The objectives of the current study were therefore to estimate ECF seroprevalence and quantify the relationships between risk factors and ECF seroprevalence in the cattle population of Mbeere district. The purpose was to provide population-structured quantitative baseline information on seroprevalence and endemic status of ECF in the district for planning cost-effective disease control strategies.

2. Materials and methods

2.1. Study area and sampling strategy

The study was conducted in Mbeere District, Kenya. Detailed description of altitude, latitude, longitude and vegetation and AEZs in the district are provided by Jaetzold and Schimdt (1982). Cattle are raised in all AEZs. Farmers in Mbeere District raise indigenous (*Bos indicus*) zebu breeds of cattle under open grazing management system (Onduru *et al.,* 2002). Farms were selected by a multistage random sampling method. All the four administrative divisions in the district were included in the study. Two sub-locations from each division and 10 farms (herds) per selected sub-location were randomly sampled using simple physical randomization and random number tables respectively from sampling list frames (Figure 1). The optimal cattle sample size (400) was determined as follows: $n = [1.96^2 p\ (1\text{-}p)]/\ L^2$ (Martin *et al.,* 1987). A total of 440 animals were finally sampled.

2.2. Data management

For each of the farms visited, a questionnaire was administered to gather information on animal and general farm level management practices particularly on calves tick control practices and disease history. Blood was collected from each animal and serum samples separated and stored at -20°C. Enzyme-linked immunosorbent

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assay (ELISA) was used to detect antibodies to *T. parva* as described by Katende *et al.* (1998). Data files of questionnaires and laboratory results prepared in Microsoft Excel were exported to Genstat Discovery Edition 2 software (Genstat RELEASE 4.24DE, 2005) for statistical analysis. The outcome of interest was the individual-animal serostatus (positive or negative). The variables were collected at three levels: animal-, herd- and administrative-(divisional) levels.

2.3. Statistical analysis

The distributions of all variables according to the population organization were analyzed descriptively (data not shown). The overall *T. parva* seroprevalence and the corresponding 95% confidence intervals (95% CI) were also calculated. Investigation of risk factors was carried out by fitting the study data into logistic regression models using maximum likelihood estimation process both by univariable and multivariable analysis. The latter model was built accounting for internal correlation at herd level using the Schall method (Schall, 1991). However, as divisional boundaries are only administrative or political in nature, specific attributes within the divisions were investigated for their importance in explaining the *T. parva* seroprevalence variability in the district. The strength of association between the risk factor and the outcome was estimated by odds ratios (OR) (Dohoo *et. al.,* 2003). The OR is a relative measure of risk that describes by how much more likely a subject that is exposed to a factor under study will develop the outcome as compared to a subject that is not exposed. An OR of 1 means that there is no association, whereas an OR>1 means increased risk. An OR<1 means decreased risk.

3. Results

3.1. Sero-prevalence and univariate analysis

The overall ECF seroprevalence was 19.3% (range: 3.9% to 48% across divisions) (Table 1) in the district [95%CI: 13.7%, 24.9%]. Table 1 also classifies cattle and herd prevalence into either more or less than 70% seroprevalence, the indicator of TBDs endemic stability (Norval *et al.,* 1992) at divisional-level. Of the animal-level risk factors, only breed was significantly associated with *T. parva* antibody seroprevalence (*p*=0.067). On the

other hand, all of the herd-level risk factors were significantly associated with *T. parva* antibody prevalence (p≤ 0.1) except grazing type (*p=0.725*). Likewise all area-level variables were also significantly associated with *T. parva* antibody prevalence (p<0.001). An increase of 1% in Normalized Differential Vegetation Index (an area-level variable) values increased the odds of exposure to *T. parva* by 28%.

3.2. Multivariate analysis

Before adjusting for correlation at herd level, the significant predictors in the multivariable analysis included division, frequency of calf tick control by 6 months of age, herd size and presence of *R. appendiculatus* on the farm. On accounting for correlation at herd level, the same four predictors were significant whereas the estimates for the significant variables were more or less similar. Cattle from Siakago and Gachoka divisions were 3.3 and 4.8 times less likely to have *T. parva* antibodies respectively compared to those from Evurore division. On the other hand, cattle from Mwea division were 5.1 times more likely to have *T. parva* antibodies compared to those from Evurore division (Table 2). Cattle from herds whose calves tick control frequency was more than 5 times by 6 months of age were 3.9 times more likely to have *T. parva* antibodies compared to those whose application frequency was less than 5 times (Table 2). Cattle from herd size category '6 to 10 cattle' were 2.7 times more likely to have *T. parva* antibodies compared to cattle from the '1 to 5 cattle' category while those from herd size category 'Over 10 cattle' were 1.1 times less likely to have *T. parva* antibodies compared to cattle from the '1 to 5 cattle' category (Table 2). Cattle from herds in which *R. appendiculatus* had been found were 3.8 times more likely have *T. parva* antibodies compared to those in which no vector had been found (Table 2). On modelling without the variable 'division' three additional effects became significant and these included, age at which tick control in calves was initiated, NDVI and AEZ. However, we chose the model in Table 2 as the most parsimonious for this data.

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4. Discussion

In Mbeere district, the overall *T. parva* seroprevalence was 19.3% though this varied widely across divisions. This apparently low level of exposure suggests that ECF occurs in an endemic instability state in the district. The overall prevalence is comparable to that reported in certain AEZs in the coastal lowland (Deem *et. al.,* 1993; Maloo *et. al.,* 2001) and central Kenya highlands (Gitau *et. al.,* 1999) that are marginal for the ECF vector tick.

Two major factors associated with endemic stability, i.e., indigenous zebu cattle and open grazing system (Norval *et al.,* 1992), predominate in Mbeere District. Additional factors necessary for endemic stability are the low but continuous exposure of calves to ticks and low infection rates of *T. parva* in ticks acquired from low parasitaemias in immune carrier cattle (Norval *et al.,* 1992). However, in this study, *R. appendiculatus* tick was found in only 27% of the farms sampled and only 19.3% of the population was supposedly immune carriers. *Theileria parva* prevalence in a population has been found to be positively correlated with *R. appendiculatus* abundance when serology was used as a diagnostic test (Gitau *et al.,* 1997; Rubaire-Akiiki *et al.,* 2006). These findings therefore suggest that endemic instability in Mbeere district was possibly as a result of an unfavourable or marginal environment for the vector tick in some parts of the district, particularly the two areas with the lowest prevalence, i.e. Siakago and Gachoka divisions. It appeared that ECF would present very low challenge in these divisions compared to the other divisions (Mwea and Evurore).

Almost all herd-level factors investigated in this study were significantly associated with *T. parva* seroprevalence under univariate analyses ($p \leq 0.1$). Three of the four significant factors in the final model were herd-level factors (Table 2). Similar associations between herd management factors and *T. parva* seroprevalence in endemic unstable areas have been reported (Deem *et al.,* 1993; Swai *et. al.,* 2005). In this study, majority of the herd factors investigated were related to specific tick control management practices. Presence of vector tick in the farms was biologically expected to be associated with *T. parva* seroprevalence as the geographic distribution of *T.*

parva is associated with the distribution of the vector tick species (Norval *et. al.* 1992). Interestingly, frequent application of acaricides to calves was related with increased presence of *T. parva* antibodies. Moreover, the highest percentage of farmers in a division who used acaricides most and who started calf tick control measures early enough came from Mwea, Evurore, Gachoka and Siakago divisions in that order and similarly, the seroprevalence levels declined following the same order. These findings suggest that there were differential herd tick control management strategies across the district. These differences could have arisen out of differences in perceptions of importance and occurrence of ECF in their areas possibly due to possible differential levels of tick burdens on their animals, morbidity and/or mortality of their animals as seroconversion has recently been strongly associated with clinical signs of ECF (particularly the more visible lymph node enlargement) (Magona *et al.,* 2008). Moreover, ECF as a constraint to livestock productivity was ranked first only in Mwea division (seroprevalence of 48%) whereas none of the farmers from Siakago division (seroprevalence of 3.8%) ranked ECF either in the first or second position.

For the variable 'herd size', farmers with large herd sizes came from areas with the least seroprevalence (Siakago and Gachoka divisions) and vice versa in Mwea and Evurore divisions. The relationship of herd size and *T. parva* seroprevalence could not, however, be explained by the information available. Whether the relationship between herd sizes and *T. parva* seroprevalence arose from mortality constraints due to ECF needs to be investigated in a longitudinal study.

Variable division was the only area-level predictor significant under multivariable analyses. Moreover, the divisional seroprevalences were all significantly different from each other ($p<0.05$) (Table 1). Similar associations between spatial factors and *T. parva* seroprevalence have been reported (Deem *et al.,* 1993; Swai *et. al.,* 2005; Rubaire-Akiiki *et al.,* 2006). This finding suggested that there existed major differential vector tick suitability habitats among the divisions. However, as divisional boundaries are only administrative in nature, specific attributes within the divisions were, therefore, further investigated by building a model without 'division'.

Subsequently, area-level predictors' counterparts (NDVI and AEZ) became significant. The geographic distribution of ECF is known to be limited by climatic factors and vegetation (Norval *et. al.* 1992), factors that were inferred through the inclusion of AEZ and NDVI in analysis of this study. These findings strongly suggested that environmental differences in tick suitability habitats were important in explaining the variation in *T. parva* seroprevalence across the district.

5. Conclusions

We concluded that ECF occurs in Mbeere district in an endemic instability state possibly due to unfavorable or marginal environment for the vector tick. Under this state, ECF seroprevalence in cattle appeared to be mainly related to both herd-level factors and differences in tick suitability habitats.

6. Recommendations

We recommend creation and sustenance of endemic stability through immunization and subsequent adjustments of acaricide application frequency in two divisions, Mwea and Evurore, within Mbeere district. Further, the geographical distribution and relative suitability of the vector tick need to be clearly defined in Mbeere district and regions with similar geographic characteristics and production systems to aid in designing of control strategies and extrapolation of findings.

7. Acknowledgements

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Table 1: Divisional *T. parva* seroprevalences, classification and ranges derived from cattle ECF data collected in Mbeere District, Kenya, March 2007.

Divisional cattle sample size	Cattle seroprevalence (95% CI)	Herd seroprevance (n= 20 herds/division)		Herd seroprevalence range (%)
		>70%	<70%	
Siakago (n=102)	3.9%[a] [1.4%, 7.6%]	0	20	0 - 33
Gachoka (n=133)	9.8%[b] [4.7%, 14.9%]	0	20	0 - 41
Evurore (n=103)	18.4%[c] [10.9%, 25.9%]	2	18	0 - 83
Mwea (n=102)	48%[d] [38.3%, 57.7%]	6	14	0 - 100

a, b, c, d, Values with different superscript letters are significantly ($p < 0.05$) different for levels of the variable along the column of comparison

Table 2: Fixed-effect estimates from a generalized linear mixed model (GLMM) used to estimate risk for *T. parva* seroprevalence in the cattle population of Mbeere district, March 2007

Risk factor	Level	Adjusted odds ratio
Constant	-	0.032
Division	Siakago	0.3
	Gachoka	0.21
	Evurore	1.0
	Mwea	5.1
Freq of calf tick control[a]	≤ 5	1.0
	>5	3.9
Herd size	1 to 5	1.0
	6 to 10	2.7
	Over 10	0.95
R. appendiculatus seen on farm	Yes	3.8
	No	1.0

Freq of calf tick control[a]: Number of times acaricide applied to calves before 6months of age;

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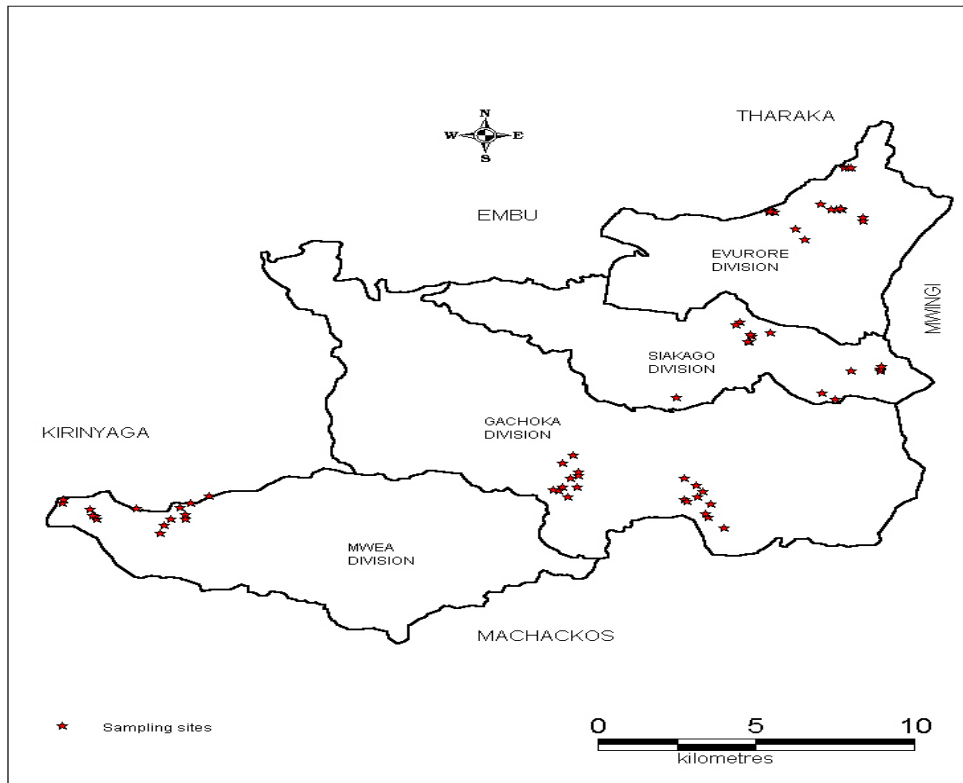J.M. Gachohi,* P.N. Ngumi, P.M. Kitala, R. Skilton

Fig 1: Divisions of Mbeere district showing the sampling sites (location of farms/herds)