# ORIGINAL RESEARCH

# Environment and farm factors associated with exposure to *Theileria parva* infection in cattle under traditional mixed farming system in Mbeere District, Kenya

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**Abstract** The objective of this study was to investigate the relationship between seroprevalence to *Theileria parva* infection in cattle and potential environmental and farm-level effects in 80 farms under traditional crop–livestock system in Mbeere District, Kenya. A standardized questionnaire was used to collect the effects characteristics as related to *T. parva* infection epidemiology. Serum samples were collected from 440 cattle of all ages for detection of *T. parva* antibodies by the enzyme-linked immunosorbent assay technique. The association between the variables was assessed using a generalized estimation equation logistic regression model. The overall *T. parva* seroprevalence,

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Biosciences Eastern and Central Africa (BecA)—ILRI Hub, P.O. Box 30709, 00100 Nairobi, Kenya accounting for correlation of responses, was 19.3% (95%) confidence interval (CI) 14%, 25%). Two variables, "administrative division" and "presence of the vector tick on the farm", were significantly associated with the T. parva seroresponse. Respectively, cattle from farms in Gachoka, Evurore, and Mwea divisions were (and their 95% CI) 1.3 (0.36, 4.8), 4.4 (1.2, 15.9), and 15.2 (4.9, 47.1) times more likely to be seropositive relative to those from Siakago Division (P=0.000). Cattle from farms in which the vector tick was present were 2.9 (1.2, 6.7) times more likely to be seropositive (P=0.011). Results of this study suggested that both environmental and farm factors may be associated with T. parva infection epidemiology in Mbeere District. Under such circumstances, characterization of environmental suitability for the vector tick and corresponding environment-specific farm management practices in the district is required both for improved understanding of the disease and in planning disease control programs.

**Keywords** *Theileria parva*  $\cdot$  Seroprevalence  $\cdot$  Environment and farm factors  $\cdot$  Mbeere  $\cdot$  Kenya

# Introduction

*Theileria parva* is a tick-borne protozoan parasite that is transmitted by the three host tick, *Rhipicephalus appendiculatus*, to cause a fatal disease in cattle known as East Coast fever (ECF) in eastern, central, and southern Africa (Norval et al. 1992). Whereas about 28 million cattle are at risk in the region, the costs of tick control using acaricides was estimated to range between US \$6 and US \$36 per adult animal in east Africa (Minjauw and McLeod 2003). In Kenya, *T. parva* infection is considered a major constraint to improved livestock production (Gitau et al. 1999, 2010; Maloo et al. 2001; Muraguri et al. 2005; Okuthe and Buyu 2006; Chenyambuga et al. 2010).

Previous studies in Kenya have identified environmental factors, particularly agro-ecological conditions, livestock production systems, and farm management practices as the main factors associated with the epidemiology of ECF with reported disease morbidity, mortality, and case fatality rates varying significantly by these factors (Deem et al. 1993; Gitau et al. 1999; Maloo et al. 2001; Okuthe and Buyu 2006). Moreover, these factors were often reported to be strongly associated with each other. Thus, suitable AEZs for the vector tick (mainly the lower elevation zones) are characterized by high T. parva infection prevalence and incidence, low incidence of clinical ECF, and ECF-resistant indigenous zebu cattle breeds reared under open grazing systems that allow exposure to infected ticks. This phenomenon has been termed endemically stable (Norval et al. 1992). In the upper elevation zones, there exist suitable to marginal zones for the tick vector that are characterized by low to moderate T. parva infection prevalence and incidence, moderate to high clinical ECF and ECF-susceptible exotic cattle breeds kept under stall feeding system that restricts exposure to infected ticks (Gitau et al. 1999). In between these two zones are areas characterized by graded tick suitability areas, mixed grazing systems, and cattle crosses between the exotic and indigenous breeds among other characteristics. Previous studies selected few sites in this middle zone as part of larger studies involving diverse AEZs (Gitau et al. 1999).

The present study, therefore, focused on the whole of Mbeere District, an area predominantly characterized by traditional crop–livestock system, indigenous cattle breeds, and moderate to insignificant differences in agro-climatic features (Jaetzold and Schmidt 1983; Onduru et al. 2002). The aim of the study was to investigate the factors associated with *T. parva* infection seroprevalence in cattle in the district. The information generated will aid in development of disease control practices, particularly in similar areas characterized by homogenous ecosystems and farming systems.

# Materials and methods

# Study area

Mbeere District is located in Eastern Province, Kenya, and lies between latitudes 0°20' and 0°50' S and longitude 370°16' and 370°56' E. The district is covered by three main AEZs: lower midlands 3 (LM3), lower midlands 4 (LM4), and lowlands 5 (L5) (Jaetzold and Schmidt 1983). Mbeere District has no major diverse environments despite large variations in elevation and annual rainfall (Jaetzold and Schmidt 1983).

#### Study design

#### Sample size determination

The sample size was determined according to the method described by Martin et al. (1987) as follows:  $n = [1.96^2 \times$  $p(1-p)/L^2$ , where 1.96 was the z value for the desired confidence level (95%), p was an estimate of the probable prevalence, and L was the tolerable error. As the antibody prevalence to T. parva infection was not known a priori, 50% prevalence and a 5% tolerable error were assumed. A reconnaissance visit in the district in November 2006 indicated that the average number of cattle in farms was around 5. Therefore, we required at least 80 farms to achieve the target sample size (400). Thus, sub-locations (the smallest administrative units in Kenya) and farms were selected by stratified random sampling method by first classifying all sub-locations according to all four divisions (Siakago, Gachoka, Evurore, and Mwea). All sub-locations were eligible for selection. Two sub-locations from each of the four divisions were then selected using random numbers to give a total of eight sub-locations. In the second stage, through collaboration with the assistant chief (the sublocational administrator) and village elders, a list of all farms owning cattle was composed and ten of them per sublocation were randomly sampled using random number tables to give a total of 80 farms.

Sampling of individual cattle used proportional allocation approach. A constant 50% proportion of animals in each farm were selected using systematic random sampling. In farms that had six or less cattle, all the cattle in that farm were sampled. All cattle ages were eligible for selection except calves less than 4 months of age to minimize the possibility of detecting passively derived colostral antibodies (Gitau et al. 1999). A total of 440 animals were sampled in this study. The study was conducted in March 2007.

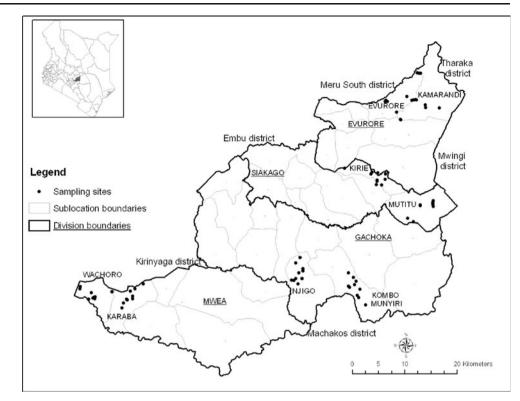
# Administration of questionnaire

For each farm visited, a standardized questionnaire was used to summarize the farm's management practices that were likely to be related to *T. parva* transmission. The grid location of each study farm was geo-referenced and Arcview version 3.3 (ESRI, Buckinghamshire, UK) used to create a detailed map of the study area (Fig. 1).

# Collection of ticks, sera, and serology

During the farm visit, all cattle in a farm were first examined for presence of ticks which were categorized into species according to Kaiser et al. (1988). For this study, our main target was *R. appendiculatus*. After blood collection, serum samples were separated and stored

Fig. 1 Map of Mbeere District showing divisional boundaries, sampled sub-locations, and sampling sites (farms)



at  $-20^{\circ}$ C until they were analyzed at the International Livestock Research Institute, Nairobi. *T. parva* indirect enzyme-linked immunosorbent assay (sensitivity, 99%; specificity, 94–98%) was used to detect antibodies to the parasite (Katende et al. 1998).

# Statistical analysis

# Estimating T. parva seroprevalence

Animal-level *T. parva* seroprevalence was estimated accounting for correlation of responses (Dohoo et al. 2003). In estimating farm-level *T. parva* seroprevalence, a farm was considered positive if at least one animal in the farm tested positive for antibodies against *T. parva* and negative if none was positive.

# Associations between explanatory variables and T. parva seroprevalence

Association between explanatory variables (environmental and farm variables; see "Results" section) were calculated using chi-square statistics. The association between the explanatory variables and the response variable (*T. parva* serostatus) was assessed using generalized estimation equation (GEE) approach of Zeger and Liang (1986) to account for correlation of responses from multiple animals within farms. Univariable analysis was first performed using all explanatory variables. Variables with  $P \le 0.1$  were selected for inclusion in the multivariable model which was built by manual backward variable selection process based on the Wald's chi-square statistic (P<0.05). Throughout the modeling process, "administrative division" was maintained in the model to account for the study design of random farm selection from within each administrative division. The model regression coefficients were exponentiated into odds ratios (OR). The OR is a relative measure of risk that describes how much more likely it is that an animal which is exposed to the factor under study will develop the outcome as compared to an animal which is not exposed. Confounding and interaction were assessed as described by Dohoo et al. (2003). All statistical analyses were performed using STATA Version 10 (StataCorp 2007).

# Results

# General results

Serum samples were collected from 440 animals representing 677 cattle from 80 farms. The mean ( $\pm$  SD) number of animals in the 80 farms was 8.5 ( $\pm$  6.4), ranging from 2 to 30. Table 1 shows the distribution of the major characteristics of the sampled cattle. Although the district is covered by three main AEZs, that is, LM3, LM4, and L5, the selected farms fell into only LM4 and L5 zones after overlaying the selected farms on district's AEZ map as the sampling had been done by division.

Table 1 Distribution of the major characteristics of the 440 selected cattle in Mbeere District, Kenya, March 2007

Variable	Levels	Frequency	Percentage
Breed	Zebu and crosses	405	92.1
	Exotic	35	7.9
Age	Calves	88	20
	Yearlings	76	17.3
	Adult	276	62.7
Sex	Male	161	36.6
	Female	279	63.4
Agro-ecological zone	Lower midlands 4	278	63.2
	Lowlands 5	162	36.8
Division	Siakago	102	23.2
	Gachoka	133	30.2
	Evurore	103	23.4
	Mwea	102	23.2

Farm-level characteristics across divisions and AEZs

*R. appendiculatus* vector was more likely to be found in farms in Mwea and Gachoka divisions (P=0.000) and in farms in LM4 zone (P=0.002) relative to other divisions and L5 zone, respectively. Similarly, majority of farmers in Mwea and Gachoka divisions (P=0.000) and in LM4 zone (P=0.000) were more likely to initiate tick control on calves at an earlier calf age than those in other divisions and L5 zone, respectively. Calf tick control was more frequent in farms from Mwea and Gachoka divisions (P=0.000) and indeed was also more likely to have applied tick control measures in the farm within 2 weeks prior to the farm visit (P=0.000) relative to other divisions. However, there was no significant difference among farms in the two AEZs in terms of application of tick control measures within 2 weeks prior to the farm visit (P=0.377) or calf tick control frequency (P=0.671). Farms in Mwea Division (P=0.000) and in LM4 zone (P=0.000) reported higher use of acaricides compared to other drugs (multivitamins and antihelmintics) relative to farms in other divisions and L5 zone, respectively. Farmers from Mwea Division (P=0.000) and in LM4 zone (P=0.000) were more likely to report previous occurrence of ECF syndrome in cattle (generalized lymphadenopathy, fever, dyspnea, and a characteristic cough) relative to other divisions and L5 zone, respectively. R. appendiculatus was found in 15%, 40%, 10%, and 50% of the sampled farms in Siakago, Gachoka, Evurore, and Mwea divisions, respectively, and the difference across divisions was significant (P=0.012) whereas the vector tick was found in 34% and 20% (P>0.05) of the sampled farms in LM4 and L5 zones. respectively. All selected farms in Siakago Division (P= 0.000) and in L5 zone practiced free grazing in comparison with other divisions and LM4 zone (P=0.000), respectively, which practiced various forms of mixed free and stall systems.

# Estimating T. parva infection exposure prevalence

Divisional-specific animal and farm seroprevalence differed significantly across all divisions (P=0.000). Both AEZspecific animal and farm seroprevalence also differed significantly (P=0.001 and 0.028, respectively) across the two zones (Table 2).

#### Univariate analysis

Univariate analysis returned five significant farm factors and both environmental factors (P < 0.1). The significant factors included presence of vector tick, age at which farmers

Table 2       Animal- and         farm-seroprevalence       classification by	Variable	Level Animal seroprevale (%) [95% confiden		Herd seroprevalence (%) [95% confidence level]
environmental variables	Division	Siakago	3.9 a [0.2, 7.6]	15 a [3.2, 37.9]
		Gachoka	9.8 b [7.2, 12.3]	25 a [8.7, 49.1]
		Evurore	18.5 c [11, 26]	30 a [11.9, 54.3]
Values with different letters are significantly ( $P$ <0.05) different for levels of each variable along the column of comparison		Mwea	48 d [38.4, 57.6]	85 b [62.1, 96.8]
	Agro-ecological zone	Lower midlands 4	24.1 a [19, 29.2]	48 a [33.7,62.6]
		Lowlands 5	11.1 b [6.3, 15.9]	23.3b [9.9,42.3]

initiated calf control on calves, the drug most commonly used on the farm, administrative division, and AEZ.

#### Multivariate analysis

When the seven significant variables in the univariate analysis were offered to the multivariable analysis, two of them were significantly (P < 0.05) associated with T. parva seroprevalence (Table 3). Cattle from farms in which the vector was found during the farm visit were 2.9 times (95% CI 1.2, 6.7) more likely to be seropositive (P=0.01) than those from farms in which the vector was not found. Respectively, cattle from farms in Gachoka, Evurore, and Mwea divisions were 1.3 times (95% CI 0.36, 4.8), 4.4 times (95% CI 1.2, 15.9), and 15.2 times (95% CI 4.9, 47.1) more likely to be seropositive relative to those in Siakago Division (P=0.000). We suspected some confounding effect of "administrative division" because removing it from the final model changed the estimate of the variable "presence of vector tick on the farm" by 27% (Dohoo et al. 2003).

As divisional boundaries are administrative in nature, factors that could have been masked by effects of "administrative division" were investigated for their association with T. parva seroprevalence in the district by repeating univariate analysis but in absence of the variable "administrative division". In addition to the presence of the vector on the farm, two additional variables were significant (P < 0.05) and these included AEZ and calf tick control frequency. However, we chose the model in Table 3 as the most parsimonious for this study because (a) we wanted to retain "administrative division" in the model to account for the study design, (b) the suspicion of the confounding effect of "administrative division", and (c) the model's Wald test for composite linear hypotheses was highly significant (P=0.0000) compared to the one without "administrative division" (P=0.0002).

# Discussion

This study offered baseline population-structured crosssectional findings on *T. parva* seroprevalence in cattle in Mbeere District. Although cross-sectional studies are not powerful at showing cause–effect relationships (Dohoo et al. 2003), this study showed evidence of importance of both environmental and farm factors in ECF epidemiology in the district.

The relatively low seroprevalence of 19.3% indicated that T. parva infection most likely existed in the district under endemic instability status. Endemic instability implies an ecological imbalance between the host, tick, and environment where only a small proportion (mostly <70%) of cattle in a population become infected and immune by 6 months of age (Norval et al. 1992). The low prevalence could have arisen out of low infection challenge, probably due to low levels of vector abundance and distribution and/or low tick infection rates among other reasons (Gilioli et al. 2009; Odongo et al. 2009). This is supported by the observation that the low seroprevalence existed under open grazing system that normally permits exposure to infected ticks. However, reliable spatial and/or seasonal data on tick populations in the district are unavailable. Other factors that have been reported to influence distribution of ticks and T. parva transmission and prevalence include rainfall, altitude, host population size and density, habitat modification, vector control programs, and the social environment (Olwoch et al. 2008; Nshimiyimana and Mutandwa 2010).

Farms in divisions and AEZs where the vector was reportedly present or absent practiced higher or lower levels of tick control, respectively, probably as a result of differences in the distribution of (infected) vector ticks and corresponding clinical infection. Farmers tend to apply tick control measures depending on occurrence, perceived incidence, and severity of the clinical disease experiences and economic impacts of the disease at the farm level (Swai et al. 2009; Mugabi et al. 2010; Phiri et al. 2010). Additionally, presence of the tick vector on the farm was a strong predictor of T. parva seroprevalence in this study. This was expected as the distribution of ECF is largely defined by the distribution of the vector. A distinct pattern between R. appendiculatus distribution and T. parva seroprevalence has previously been reported both in crosssectional studies (Deem et al. 1993) and in longitudinal studies (Rubaire-Akiiki et al. 2006).

Table 3	Variables and estimates
from the	final multivariable
GEE mo	del (P<0.05)

Effect	Variable	Level	Odds ratio	95% confidence interval	Р
Farm	Vector tick found on farm	Yes	2.9	[1.2, 6.7]	0.01
		No	1.0	-	
Environment	Division	Siakago	1.0	-	
		Gachoka	1.3	[0.36, 4.8]	0.00
		Evurore	4.4	[1.2, 15.9]	
		Mwea	15.2	[4.9, 47.1]	

Lack of association between tick control practices and *T. parva* seroprevalence and incidence has been reported previously (Gitau et al. 1999; Rubaire-Akiiki et al. 2006; Swai et al. 2009). However, the finding that frequent application of acaricides to calves was associated with higher exposure to *T. parva* would normally be unusual; however, it is likely that farmers tended to react to presence of perceived disease and/or vector burdens that may have constituted a potential risk factor for withinfarm disease transmission. Indeed, seroconversion to *T. parva* parasite has been associated with clinical signs of ECF (Magona et al. 2008). We could not, however, establish the effectiveness of this management practice due to limitation of determining temporal effects in cross-sectional studies.

The large and important effects of environmental variables on T. parva seroprevalence in this study have been previously reported with the seroprevalence and incidence varying across administrative districts and AEZs in East Africa (Deem et al. 1993; Gitau et al. 1999; Maloo et al. 2001; Rubaire-Akiiki et al. 2006; Bazarusanga et al. 2007; Chenyambuga et al. 2010). This clearly reflects different levels of exposure to T. parva infection. Differences in both T. parva seroprevalence and distribution of the vector on farms suggested differential vector environmental suitability across the district. This was supported by the fact that modeling without the variable "administrative division" revealed the importance of its environmental counterpart, AEZ. In Mbeere District, the environmental difference in T. parva seroprevalence could, in part, be explained by ecological differences between the two AEZs (Jaetzold and Schmidt 1983). In total, these findings suggested that even in areas with no major ecological diversity, differential micro-environmental effects may indirectly explain variability of T. parva seroprevalence at the farm level in endemically unstable areas and subsequent differential farm disease management practices. It is under these circumstances that we suspected "administrative division" to be a potential confounder implying that environmental variables alternately explained the observed relationship between the presence of the vector and T. parva response.

In conclusion, although *T. parva* seroprevalence in cattle in Mbeere District was low, suggesting endemic instability, areas where the tick vector was likely to be found corresponded with higher levels of both farm tick control management and levels of exposure to *T. parva* infection. As cross-sectional studies are not powerful at evaluating such relationships, further information on both the tick vector environmental suitability and environment-specific farm management factors in Mbeere District and other areas with similar ecology and farming systems is required for planning targeted ECF control programs. Acknowledgments We are grateful to Mbeere cattle farmers who participated in this study. Logistical support from Mbeere District Veterinary Office is acknowledged. The authors are also grateful to BecA—ILRI Hub and in particular Alice Njeri for laboratory technical assistance. We thank Pamela Ochungo, ILRI, for generating the map of the study area. This work was funded by the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) and Kenya Agricultural Productivity Project (KAPP).

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