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Differences in the epidemiology of theileriosis on smallholder dairy farms in contrasting agro-ecological and grazing strata of highland Kenya

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SUMMARY

A prospective cohort study was conducted in five purposively-sampled agro-ecological zone (AEZ)-grazing system strata in Murang'a District, Kenya, between March 1995 and June 1996. The study strata were selected based on a preliminary characterization study to represent the widest range of risks to East Coast fever (ECF) in the District and included zero-grazing and open-grazing farms. In total, 225 calves from 188 smallholder farms were examined from birth to 6 months of age and visited within the first 2 weeks of life and thereafter at bi-weekly intervals for up to 14 visits.

The purpose of the study was to characterize the differences in epidemiology (risks of infection, morbidity and mortality) and potential control of ECF between the selected strata. Evidence of *Theileria parva* infection was assessed by increased antibody levels as measured in an indirect ELISA assay by the percent positivity (PP) of serum samples relative to a strong positive reference serum.

Sero-conversion risks of *T. parva* were highest in the open-grazing strata. Antibody prevalence in adult cattle and ECF morbidity and mortality risks were also highest in open-grazing strata. While different, all five AEZ-grazing strata were considered to be endemically unstable for ECF. East Coast fever challenge was low in all zero-grazing strata and this challenge is likely to remain low due to continuing intensification of smallholder farming in the central highlands. In the open-grazing strata, there was higher challenge and a greater impact of ECF.

INTRODUCTION

Tick-borne diseases are considered a major constraint to dairy farming among the smallholder dairy farms in Kenya, which account for an estimated 75–90 % of all milk produced in the country [1, 2]. The most important tick-borne disease in Kenya is East Coast fever (ECF) caused by *Theileria parva* and transmitted by the brown ear tick *Rhipicephalus appendiculatus*, which has been associated with high mortality in cattle, especially among the exotic breeds of cattle [3].

In Kenya, there is considerable recent evidence

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[4–8] that the prevalence of *T. parva* infections and reported ECF morbidity, mortality and case-fatality can vary significantly by agro-ecological zone and grazing system. These differences have important implications for both the impact and control of ECF. Norval and colleagues [3] and Perry and Young [9] have developed a paradigm for assessing production systems for theileriosis based on their 'endemic stability'. In endemically stable systems, there is an equilibrium between *Theileria* parasites and hosts so that ECF impact is minimal despite high challenge. In unstable situations, ECF impact can be considerable, either through direct loss from ECF or in costs associated with its control. In situations with a sufficient level and continuity of challenge, the goal is to move toward or maintain endemic stability.

In an earlier paper, we described the prevalence, sero-conversion risk and morbidity/mortality risks of ECF for female calves up to 6 months of age in five contrasting agro-ecological zone (AEZ)-grazing system strata in Murang'a District, Kenya [10]. The objective of this study is to use these indicators of theileriosis infection to characterize the epidemiological states of ECF in each of these strata. In addition, available information on theileriosis infection dynamics, such as tick counts and tick infection proportions in AEZ-grazing strata were evaluated. This information was then combined to assess current and potential ECF status in each strata and its implications for future control strategies.

METHODS

Study population and data collection

Calves were purposively recruited in five cohorts drawn from five AEZ-grazing strata as described by [10]. In each selected stratum, farms on which female calves were born between March 1995 and June 1996 and on which the farmers were willing to participate in the study were recruited, giving a total of 225 calves from 188 farms. Briefly, the five AEZ-grazing strata were: a high-elevation AEZ [Upper Midlands (UM) 1] in which both open and zero-grazing farms were present, a medium-elevation AEZ (UM2) in which only zero-grazing farms occurred, and a lower-elevation AEZ (UM4) in which both open and zero-grazing farms were found.

The calves were recruited within the first 2 weeks of life and thereafter visited bi-weekly. Only female calves were monitored since they are more-valued and (as reflected in higher growth rates) are better-managed and better-fed than male calves [5, 11].

Data on routine calf management practices (such as tick-control procedures and access to pastures/grass) were collected during the bi-weekly visits and were recorded in a closed format questionnaire during the visits for each calf. Blood samples (for serum preparation) were taken on every visit. Blood was collected from each calf in two 10-ml plain vacutainer tubes (Becton Dickinson Vacutainer Systems, England) by jugular venepuncture. *Theileria parva* antibodies in sera were estimated by an indirect enzyme-linked-immunosorbent assay (ELISA) test. Other details on the study design and data collection have been described previously [10].

Laboratory determination of *Theileria parva* antibodies

An indirect ELISA technique was used to estimate the level of antibodies to *T. parva* using the polymorphic immunodominant molecule (PIM) recombinant antigen [12]. All the serology was carried out at the International Livestock Research Institute (ILRI) laboratories in Nairobi, Kenya. The performance of the ELISA for detection of *T. parva* antibodies has been described in detail [12].

All the ELISA results were expressed as percent positivity (PP) relative to a reference strong-positive control serum. Optical density (OD) readings from the test serum and the reference strong-positive control serum were used to compute the PP for the test serum as follows: test serum OD divided by the mean OD from the strong positive control serum and expressed as a percent [simply expressed: (OD of test/OD of strong positive) $\times$ 100)] [13]. Any test serum with a PP of 15 or above was considered positive [12].

Theileria parva antibody profile patterns

The PP values of *T. parva* antibody by calf age were graphed as scatter plots for all calves from the date of recruitment to either completion of the study or to the date of withdrawal, for each of the five AEZ-grazing strata. These data were further summarized graphically, by plotting the mean PP values for calves in each strata against the mean age of calves at each bi-weekly visit. The graphs, with 95% confidence limits for each age (visit) interval, were generated.

The prevalence of positive antibody titres for dams in each of the five AEZ-grazing strata were estimated. These were calculated as the number of dams with positive antibody titres in each AEZ-grazing stratum divided by the total number of dams sampled in that stratum.

The proportion of variation in PP values that could be attributed to AEZ-grazing stratum, farm, calf and visit-within-calf differences were estimated in a random-effects models using the Mixed Procedure (PROC MIXED) of the Statistical Analysis Software for Windows (SAS for Windows) version 6.12 (SAS Institute Inc. Cary, NC, USA).

There were two main antibody profile patterns of interest in female calves, (1) the presence and persistence of maternal antibodies and (2) the sero-conversion of calves in response to *T. parva* infections. Any calf that had antibody detected (PP of 15 or above) for both the first two visits was considered to have maternal antibodies and these were considered to persist as long as they remained detectable. Calves were judged to have sero-converted if: (1) they were initially antibody negative and became antibody positive for at least one visit, (2) they had maternal antibody that declined to negative levels and then subsequently became positive for at least one visit, or (3) they had initial maternal antibody that had not yet declined to negative but subsequently increased by at least 15 PP units for at least one visit.

For the time-to-decline of maternal antibody and the time to sero-conversion analyses, the first (recruitment) visit was taken as the start of the first risk interval while the second and subsequent visits were considered the start of subsequent risk intervals. For each ordered visit, the number of calves at-risk, the number with the event of interest, and the number censored were entered as separate columns in a SAS input data set. For the decline-of-maternal-antibody analysis, calves with maternal antibody that subsequently sero-converted before maternal-antibody had declined were considered as withdrawals (censored). The risk during each age interval was calculated as the number of calves with the event of interest divided by the number of calves-at-risk at the beginning of that interval divided by half the number of withdrawals during the interval. The cumulative risks were calculated by the method of Kaplan and Meier [14]. The time-to-event distribution curves were generated using the PROC LIFETEST procedure in SAS. The generalized Wilcoxon test was used to test the homogeneity of the maternal antibody persistence and sero-conversion curves across the five AEZ-grazing strata [15, 16].

Calf morbidity, mortality and ECF-morbidity and mortality risks

Calf morbidity was defined as any calf sickness that had a recognizable clinical manifestation; calf mortality was defined as any death. ECF morbidity and mortality incidents were defined and classified into confirmed and unconfirmed categories. Unconfirmed ECF cases were defined as those in which diagnosis was based on clinical signs of ECF only, while confirmed ECF cases also required serological confirmation. Serological confirmation consisted of sero-conversion when the clinical event occurred.

The cumulative risks of ECF morbidity (suspected and confirmed) for the five AEZ-grazing cohorts and their differences were estimated as described for sero-conversion risks.

Tick counts and tick infection proportions

Tick infestation was estimated by counting the number of *R. appendiculatus* nymphs (total and engorged) and adult ticks (males, females – non-engorged and engorged) on the body of each calf as described by Horak [17] during the bi-weekly visits. Tick infection proportions were estimated from a sample of ticks collected from two open-grazing farms in UM1 and three open-grazing farms in UM4. Adult ticks were collected through pasture flagging using cotton flags as described by Rechav [18] and were later dissected to remove the salivary glands for examination of infection with *T. parva*, as described by Buscher and Otim [19]. Tick infections were later determined through direct microscopy after staining the tick salivary glands with Schiff's (Fuelgen's) reagent to identify infected acini. The intensity of infection was subsequently estimated by counting the number of infected acini. Due to the small number of farms/sites from which ticks were collected, no statistical comparisons were made for the prevalence of tick infections.

Characterization of epidemiological states of theileriosis

The epidemiological state of theileriosis in an area can be considered as either endemically stable or unstable.

Table 1. *Number of calves-at-risk and occurrence of sero-conversion to* Theileria parva *and East Coast fever morbidity by AEZ-grazing strata and visit for a longitudinal study in Murang'a District* (*March 1995–August 1996*)

	Zero-grazing				Open-grazing			
Visit number	Calves at risk*	With-drawn†	Sero-convert	ECF‡/ ECF§	Calves at risk*	With-drawn†	Sero-convert	ECF‡/ ECF§
Upper Midlands 1								
1	34	0	0	0/0	42	0	0	0/0
2	34	0	0	0/0	42	0	0	0/0
3	34	1	0	0/0	42	2	0	1/0
4	33	3	0	0/0	40	4	0	0/0
5	30	0	1	0/0	36	0	1	1/1
6	29	2	0	0/0	35	4	1	1/1
7	27	2	0	0/0	30	2	1	1/0
8	25	0	2	1/1	27	1	0	1/1
9	23	0	1	0/0	26	1	0	1/1
10	22	0	1	0/0	25	1	2	1/1
11	21	0	0	0/0	22	2	2	0/0
12	21	0	0	0/0	18	1	2	0/0
13	21	1	2	0/0	15	1	3	0/0
14	18	2	1	0/0	11	3	3	0/0
Upper Midlands 2								
1	50	0	0	0/0				
2	50	0	0	0/0				
3	50	0	0	0/0				
4	50	1	0	0/0				
5	49	0	0	0/0				
6	49	1	1	0/0				
7	47	1	0	0/0				
8	46	2	1	0/0				
9	43	2	1	0/0				
10	40	3	1	1/1				
11	36	1	2	0/0				
12	33	1	6	0/0				
13	26	2	4	0/0				
14	20	0	1	0/0				
Upper Midlands 4								
1	50	0	0	1/0	49	0	0	1/0
2	50	2	0	0/0	49	1	0	1/0
3	48	2	0	0/0	48	2	0	1/0
4	46	0	0	0/0	46	3	0	1/0
5	46	2	1	0/0	43	1	0	0/0
6	43	3	1	0/0	42	2	4	1/0
7	39	0	1	1/1	36	2	1	1/1
8	38	0	3	2/2	33	2	2	1/1
9	35	3	2	0/0	29	1	4	0/0
10	30	1	1	0/0	24	0	2	2/2
11	28	0	3	1/1	22	2	3	1/1
12	25	1	1	0/0	17	0	1	0/0
13	23	1	0	0/0	16	0	2	1/1
14	22	1	3	0/0	14	1	2	1/0

* Defined as the number of calves at risk for sero-conversion at the start of the period; calves at risk for ECF (suspected or confirmed) can be calculated as [calves at risk$_{t-1}$ withdrawals$_{t-1}$ ECF (suspected or confirmed)$_{t-1}$].

† Withrawal due to farmer withdrawal or study ended before calves had complete follow-up.

‡ All suspected and confirmed ECF cases.

§ All confirmed (by serology) ECF cases.

Endemic stability is assessed based on a combination of indicators, including: (1) antibody prevalence, (2) disease incidence, (3) case-fatality proportion, and (4) age group affected [3, 9]. Tick infection proportions are also considered helpful [20]. According to Norval and colleagues [3], an endemically stable state is characterized by a high antibody prevalence, low disease incidence and case-fatality proportion and a rapid acquisition of infection in young calves. An endemically unstable state is characterized by the opposite: lower antibody prevalence, high disease incidence and case-fatality proportion and primary infections that occur in all age groups. These criteria were used to characterize the endemic stability of the five AEZ-grazing strata investigated.

RESULTS

Follow-up on cohort of calves

The details of numbers of calves initially at-risk (recruited) and those withdrawn, sero-converted and diagnosed with ECF (suspected and confirmed) by bi-weekly visit for each AEZ-grazing stratum are listed in Table 1. The number of withdrawals was not significantly different between strata ($\Pi^2 = 6{\cdot}98$, D.F. $= 4$, $P = 0{\cdot}14$).

Theileria parva antibody profile patterns

Figure 1(*a–e*) shows scatter plots of individual PP measurements of calves by age for each of the five AEZ-grazing strata. High PP values in young calves (< 40 days) are almost exclusively maternal antibodies and high PP values in older calves (> 100 days) were of antibodies produced by the calf in response to *T. parva* infection. The pattern of antibody profiles was different between strata, ranging between the lowest-challenge UM2-zero-grazing stratum and the highest challenge UM4-open-grazing stratum.

Figure 2 shows the mean PP values for calves in the five AEZ-grazing strata by age, fitted with 95% confidence limits. In all the AEZ-grazing strata, the mean antibody levels declined from birth, to reach their lowest point at approximately 94 days of age (visit 7). The antibody levels then rose to the end of the follow-up period. The highest mean PP values were recorded in UM4-open-grazing stratum and the lowest were recorded in UM2 (zero-grazing only).

The proportion of variation in PP values attributed to different measurement classifications was: AEZ-grazing strata 10%, farm within strata 21%, calf within farm 22% and individual visit measurements within calf 47%. While the individual PP measurements over time within calves accounted for the largest source of variability, this variability was generally over the relatively wide range of positive values (< 15 PP) and did not change the assessment of longitudinal trends in positive/negative values (decline of maternal antibody and sero-conversion). It is also important to note that 89% of farms (167/188) had only one calf, so that relatively few farms contributed to the information on calf-within-farm variation.

The prevalence of antibodies presumed to be maternally-derived were as follows: in UM1, 29% (10/34) and 48% (20/42) in zero-grazing and open-grazing farms respectively, 26% (13/50) in UM2 (zero-grazing only) and in UM4, 58% (29/50) and 96% (47/49) in zero-grazing and open grazing farms respectively. For these calves, the vast majority had dams with positive antibody titres: UM1, 9/34 (26%) and 20/42 (48%) in zero-grazing and open-grazing farms respectively, 13/50 (26%) in UM2, and in UM4, 27/50 (54%) and 47/49 (96%) in zero-grazing and open grazing farms respectively. There were also a few cases in which dams had antibodies to *T. parva* that were not transferred at detectable levels to their calves: 0 and 2 in zero-grazing and open-grazing farms of UM1 respectively, 2 in UM2 (zero-grazing only) and 3 and 0 in zero-grazing and open-grazing farms of UM4 respectively.

Figure 3 shows curves of the persistence of maternally-derived *T. parva* colostral antibodies for female calves in each of the five AEZ-grazing strata from age of recruitment to the end of follow-up. There was no significant difference in the persistence of maternal antibody across the five strata (generalized Wilcoxon ($\chi^2 = 7{\cdot}8$, D.F. $= 4$ and $P = 0{\cdot}10$).

Cumulative risks of sero-conversion to *Theileria parva* and East Coast fever

The cumulative risks of sero-conversion to *T. parva* for each of the five AEZ-grazing strata are shown in Figure 4. The cumulative risks of sero-conversion differed between strata ($P < 0{\cdot}05$), with the UM4-open-grazing stratum having a significantly greater cumulative risk of sero-conversion over the follow-up period.

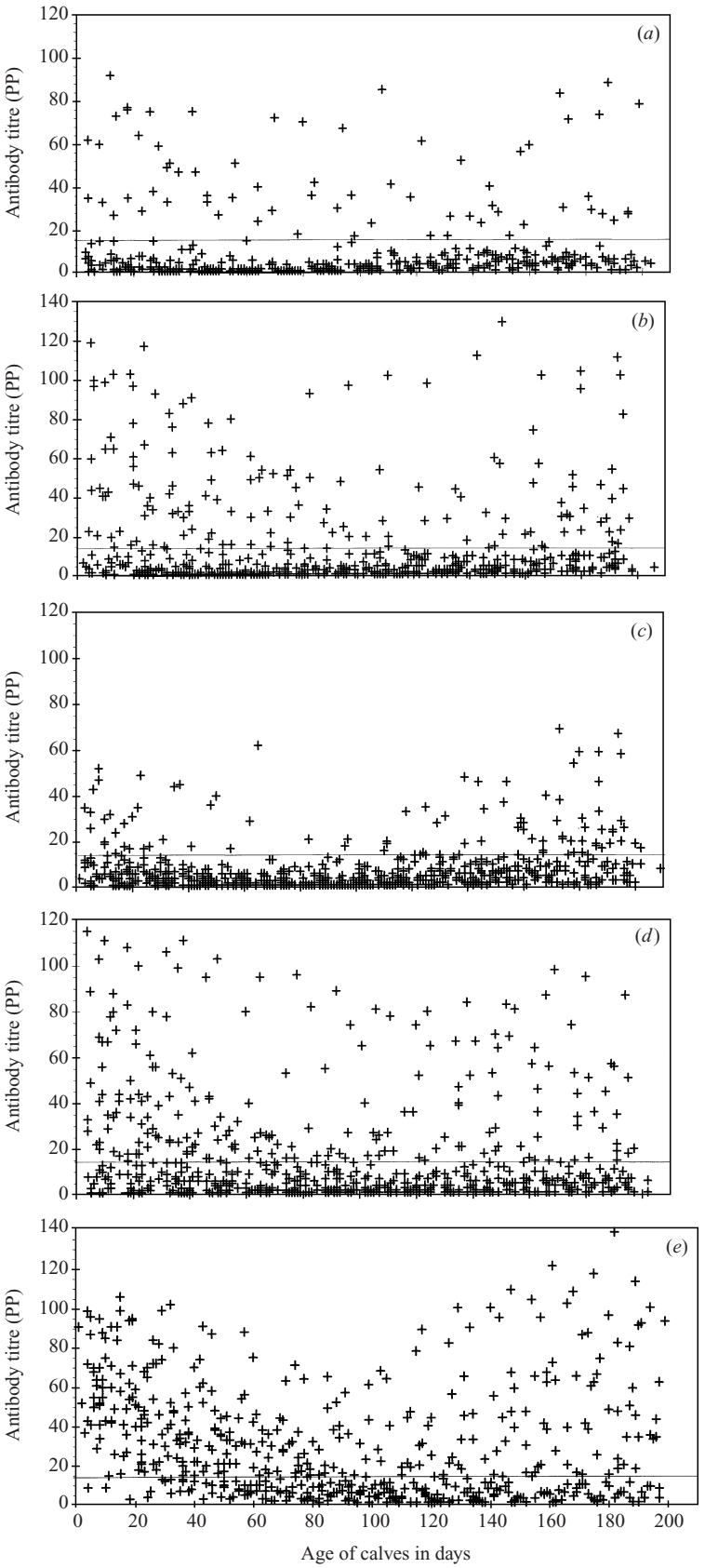

Fig. 1. Distribution of antibody titres [per cent positivity (PP)] by age (in days) in the cohort of female calves in Upper Midlands 1 zero-grazing (*a*), and open-grazing (*b*), Upper Midlands 2 (*c*), and Upper Midlands 4 zero-gazing (*d*), and open-grazing (*e*) from the longitudinal study in Murang'a District, Kenya (March 1995–August 1996). The positive/negative cut-off level of 15 PP is marked.

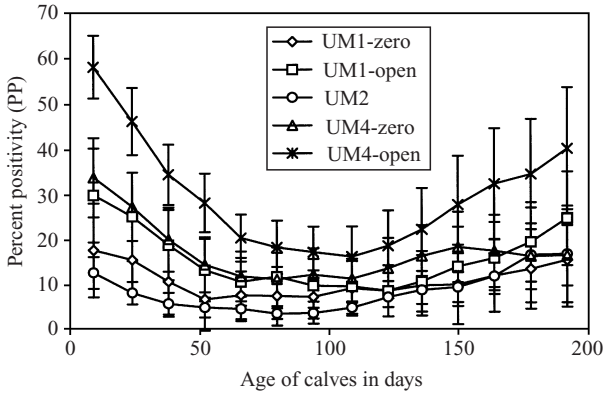

Fig. 2. Mean antibody titres (per cent positivity) for cohort of female calves by visit in the five AEZ-grazing strata fitted with 95% confidence limits, from the longitudinal study in Murang'a District, Kenya (March 1995–August 1996). Key: UM1-zero, Upper Midlands 1 zero grazing system; UM1-open, Upper Midlands 1 open grazing system; UM2, Upper Midlands 2; UM4-zero, Upper Midlands 4 zero-grazing system; UM4-open, Upper Midlands 4 open grazing system. Mean antibody titres significantly different across the AEZ-grazing strata during the first 4 visits only ($P < 0.05$).

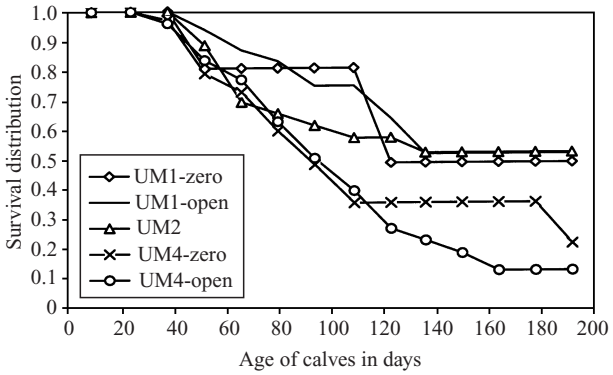

Fig. 3. Persistence of maternal antibodies to *Theileria parva* by age (as visit interval) for the cohort of female calves in the five AEZ-grazing strata from the longitudinal study in Murang'a District (March 1995–August 1996). Abbreviations as in Fig. 2. No significant difference in survival distribution across the AEZ-grazing strata (Generalized Wilcoxon test, $\chi^2 = 7.8$, df = 4, $P = 0.10$).

The cumulative risks for ECF are shown in Figure 5 (*a*, suspected and confirmed cases and *b*, confirmed cases only). Due to the small number of ECF cases, no statistically significant differences between the cumulative risks of ECF for the different strata were found. In both graphs, open-grazing strata had higher cumulative ECF risks and these were greater for UM4 than UM1.

Tick counts and tick infection proportions

The occurrence and distribution of *R. appendiculatus* ticks (both adult and nymphs) are shown in Table 2.

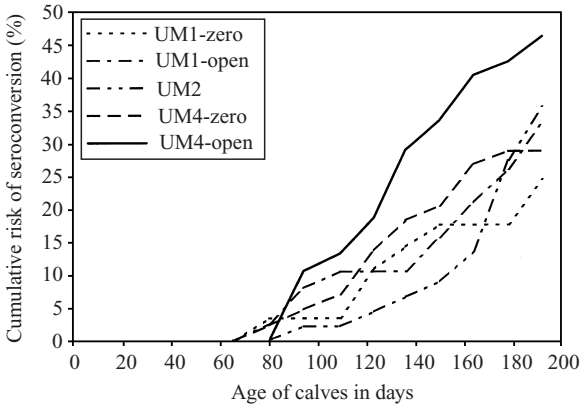

Fig. 4. Cumulative risk for sero-conversion to *Theileria parva* for the cohort of female calves in the five AEZ-grazing strata from the longitudinal study in Murang'a District, Kenya (March 1995–August 1996). Abbreviations as in Fig. 2.

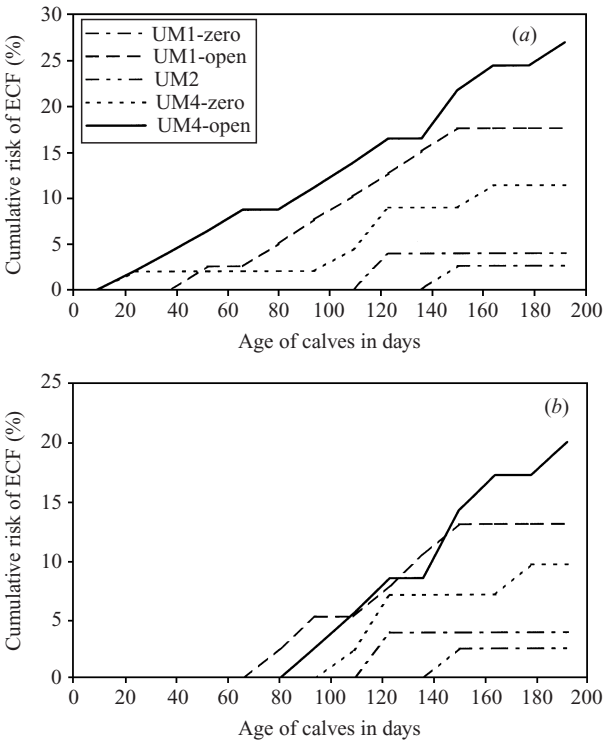

Fig. 5. Cumulative risk for East Coast fever, all cases, (*a*), and (*b*), for confirmed cases only, from the cohort of female calves in five AEZ-grazing strata from the longitudinal study in Murang'a District, Kenya (March 1995–August 1996). Abbreviations as in Fig. 2.

The highest number of tick counts were made in the open-grazing strata. The most prevalent *R. appendiculatus* tick stages were the males and non-engorged females in UM4 open-grazing stratum and these were

Table 2. *Number of calf visits in which* Rhipicephalus appendiculatus *tick stages were observed and range of tick numbers by AEZ-grazing strata on the 225 calves studied in Murang'a district, Kenya (March 1995–August 1996)*

AEZ-grazing stratum	Males	Females		Nymphs	
		Non-engorged	Engorged	Total	Engorged
UM1					
Zero	1/391 (0–3)	2/391 (0–3)	1/391 (0–4)	0/391	0/391
Open	9/515 (0–20)	12/515 (0–15)	12/515 (0–18)	4/515 (0–20)	0/515
UM2					
	0/627	1/627 (0–1)	1/627 (0–1)	0/627	0/627
UM4					
Zero	70/593 (0–120)	74/593 (0–13)	9/593 (0–3)	6/593 (0–4)	6/593 (0–2)
Open	194/524 (0–39)	212/524 (0–39)	64/524 (0–8)	16/524 (0–30)	16/524 (0–5)

Table 3. *Prevalence and intensity of* Theileria parva *infections in* Rhipicephalus appendiculatus *adults collected from pastures in Murang'a District, Kenya (September 1995)*

Agro-ecological zone	Ticks collected	Number dissected*	Number positive (%) for *T. parva* infection	Total acini infected	Total mean intensity
Upper Midlands 1					
Farm/site 1	65	36	9 (25)	85	
Farm/site 2	6	4	0 (0)	0	
Total	71	40	9 (22·5)	85	9·4
Upper Midlands 4					
Farm/site 1	347	148	2 (1·4)	4	
Farm/site 2	125	83	3 (3·6)	4	
Farm/site 3	36	20	0 (0)	0	
Total	508	251	5 (2·0)	8	1·6

* Sexes of infected ticks as follows; 7 males (4 in UM1; 3 in UM4) and 7 females (5 in UM1; 2 in UM4).

observed in 37 and 41% of the bi-weekly calf observations respectively. Table 2 further shows that there was a high variation in the actual number of ticks counts especially in the two open-grazing strata.

Only limited information was available on tick challenge and infection proportions from open-grazing farms in UM1 and UM4. Table 3 lists the numbers of *R. appendiculatus* ticks collected from pastures, the numbers dissected, the proportion infected with *T. parva* and the intensity of those infections (from 2 farms in UM1 and 3 farms in UM4). Ticks were collected in larger numbers in UM 4 than in UM 1. The prevalence of infections in dissected ticks were in the range 0–25% in both UM1 and UM4. The ticks with high infection prevalence in UM1 came from a farm that reportedly had a recent outbreak of ECF prior to tick collection.

Epidemiological states of *Theileria parva* infections

The estimates of indicators used to classify the 5 AEZ-grazing strata for endemic stability to ECF are listed in Table 4. All strata were judged to be endemically unstable; however, the degree of their instability varied. Our assessment of the relative stability of each AEZ-grazing stratum over the range of endemic

Table 4. *Criteria to assess endemic stability to East Coast fever (ECF) in the five AEZ-grazing strata studied in Murang'a District Kenya*

AEZ-grazing stratum	Antibody prevalence*†	Disease incidence*†	Case-fatality*‡	Susceptibility of cattle§	Degree of tick challenge‖	Epidemiologic state*
UM1 zero-grazing	Low (26%)	Very low (3/3)	Low (10/2)	High (100/0/0)	Very low (0.5%)	Unstable
UM1 open-grazing	Medium (46%)	Low (20/14)	Very low (0/0)	High (100/0/0)	Low (2%)	Unstable
UM2	Low (26%)	Very low (2/0)	Very low (0)	High (100/0/0)	Very low (0.2%)	Unstable
UM4 zero-grazing	Medium (53%)	Low (12/9)	Low (2/0)	Medium to high (90/4/6)	Medium (12%)	Unstable
UM4 open grazing	Very high (96%)	Medium (31/20)	Medium (12/2)	Low to medium (67/23/10)	High (40%)	Unstable

* Indicators used by Norval and colleagues, 1992.
† Antibody prevalence based on dam antibody levels for *T. parva*.
‡ (All suspected and confirmed/confirmed only) ECF cases.
§ Breed distribution (%) across AEZ-grazing strata (Exotic/Exotic × Zebu/Zebu).
‖ Proportion of calf observations when ticks were present (based on adult *Rhipicephalus appendiculatus* tick).

stability/instability is indicated in Table 4. The UM4-open-grazing stratum has the highest level of challenge and is the closest to an endemically stable state. All zero-grazing strata and the UM1-open-grazing strata have a very low infection challenge and are unlikely ever to become endemically stable.

DISCUSSION

The purpose of this study was to assess the epidemiological pattern (level of challenge and degree of endemic stability/instability) of ECF in contrasting AEZ-grazing strata in the central Kenyan highlands. The contrasting AEZ-grazing strata were purposively selected based on an initial cross-sectional characterization study [7]. Female calves of up to 6 months of age were the target study group, since we considered the rapid acquisition of infection in calves as the crucial factor in establishing endemic stability to ECF in an area (production system).

This study, as in another recent study in the central highlands of Kenya [8], demonstrated the crucial influence of grazing system on the epidemiology of ECF. Infection challenge was low in all zero-grazing strata, even in the UM4 stratum that was the most ecologically suitable for *R. appendiculatus*. In zero-grazing systems, tick challenge is almost exclusively through the importation of infected *R. appendiculatus* ticks on fodder from neighbouring farms and paddocks. Since there is very little suitable habitat to support tick populations on zero-grazing farms, there is a low probability that ECF cases on these farms will transmit their infections via ticks to other cattle. Another interesting observation on the zero-grazing farms was the relatively low case-fatality proportion, given that cattle on these farms might be expected to be very susceptible (comprising a high proportion of exotic cattle with low-to-medium challenge as calves). The most likely explanation is related to the dose-dependency of clinical ECF. The majority of infected ticks likely became infected by feeding on carrier rather than clinically affected cattle [21]. The subsequent instar would then likely inoculate relatively fewer *T. parva* sporozoites, reducing the case-fatality proportion.

In contrast, open-grazing systems allow for more characteriztic cycles of *T. parva* transmission to occur, both from carrier and clinically affected cattle. In open-grazing systems, the level of infection challenge on a given farm will depend on the classical risk factors such as climatic suitability for ticks, grazing practice and range (and thus mixing with other potentially infected cattle), tick control practices and cattle breed.

Given the high human population pressure and subdivision of farmland in Murang'a and other districts of the Kenyan highlands, the proportion of zero-grazing farms will probably continue to increase. Open-grazing farms will increasingly be restricted to the higher and lower altitude margins (such as the

higher portions of UM1 and even higher AEZs and the lower portions of UM4). Increasingly, zero-grazing farms will be isolated from pastures so that *T. parva* challenge is likely to decrease even further, with continuing low *T. parva* challenge. This level of challenge is a function of the evolving farming system rather than more variable clines such as the degree of tick control. While being endemically unstable in biological terms, the zero-grazing system will have a relatively constant low risk with respect to ECF challenge. However, individual cattle from such zero-grazing farms will be fully susceptible to ECF if moved to higher challenge grazing systems. In Murang'a, higher challenge areas are sometimes as little as 10 km away.

Of the two open-grazing strata sampled in UM1 and UM4, neither is endemically stable. The UM1-open-grazing stratum has a low level of infection challenge and this along with the pattern of low-level ECF morbidity and mortality, is likely to persist due to its marginal ecological suitability for *R. appendiculatus*. The UM4-open-grazing stratum has a much higher challenge and is much closer to endemic stability. The high maternal antibody prevalence (96%) suggests the presence of endemic stability. However, the moderately high incidence of ECF in this zone [10], indicates that this is not the case, and clearly overall antibody prevalence alone is not a reliable indicator of endemic stability. Both UM1 and UM4 appear to be unstable, so morbidity and mortality rates will likely fluctuate from season-to-season and from farm-to-farm. Thus, in these strata, farmers and veterinarians need to pay close attention to varying ECF challenge in making decisions on ECF (and perhaps other tick-borne disease) control.

What are the future prospects of establishing endemic stability in UM4? This is still an unanswerable question. Classic areas of endemic stability, such as the Trans Mara District of Kenya [22], are characterized by very low ECF-fatality proportions and close to 100% seroconversion by 6 months of age. The Trans Mara District is more ecologically suitable than the UM4 strata for *R. appendiculatus* because of its higher elevation and higher and better-distributed rainfall [23]. However, a set of more detailed field studies are needed to estimate the minimum challenge, in terms of both average intensity and continuity of challenge, required to establish endemic stability.

Because there is very little land available in the central highlands of Kenya, most future expansion of smallholder dairy farming will likely be in the lower UM4 highland margins. Farmers are likely to introduce increasing numbers of susceptible exotic cattle into this area and thus, the relatively higher ECF morbidity and mortality risks noted in UM4 are likely to persist or even increase. Thus, the UM4-open-grazing, and also to some extent zero-grazing strata for very valuable cattle, will remain the most important target systems for ECF control in the Kenyan highlands.

The results of this study have important implications for future ECF control in Murang'a and other districts in highland Kenya. In predominately zero-grazing areas, ECF risk is low. Thus, tick control or future vaccination programmes will likely only be used by very risk-averse farmers who wish to be careful in protecting their highly valuable cows from the low risk of ECF mortality. In contrast, for open-grazing systems, particularly in the lower-elevation UM4 zone, the risk of ECF is much greater and probably much more variable. In this system, there will be much greater direct impact of ECF control programmes. In areas where ECF control will be through vaccination, irrespective of the grazing management system, there will be a greater likelihood of the development of endemic stability. Increased vaccination coverage to enhance the development of herd immunity, combined with modification of acaricide control strategies to allow for sufficient challenge, offers the best prospect for establishing endemic stability. It is quite clear from this study that attention will need to be paid to variation in ECF risk, both spatially (since ECF risk changes over relatively short geographic distances) and temporally (seasonally, secularly), to develop optimal combinations of control measures for ECF under different ecological and grazing situations.

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