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Morbidity, mortality, and risk factors associated with ***Theileria parva*** seropositivity in a longitudinal calf study, Narok, Kenya

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A R T I C L E I N F O

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A B S T R A C T

East Coast fever (ECF), caused by *Theileria parva* causes devastating loss to livestock keepers in sub-Saharan Africa. A longitudinal study was carried out in Narok County to estimate the incidence and risk factors associated with *Theileria parva* infection among calves. Calves were recruited within 5 weeks of birth and visited every six weeks to collect farm and calf level factors. A total of 1849 serum samples were collected and tested using an indirect enzyme-linked immunosorbent assay (ELISA). A multilevel multivariable logistic regression was used to determine associations between the seropositivity and different predictor variables. A total of 318 calves were recruited where 62 seroconverted during the follow up period. The overall risk of exposure was 26 %, with ECF-specific morbidity risk of 14.5 %. The cumulative incidence rate was 37 cases per 100 calf-years with higher incidence rate in agro-pastoral system. The crude mortality risk was 5.1 %, but four calves manifested classical signs of ECF resulting to clinical case-fatality risk of 44.4 %. In the final multivariable model, four variables were found to be significantly associated with *T. parva* seropositivity; calf age (OR 6.9; $P < 0.01$), calf sex (OR 1.4; $P = 0.02$), acaricide application (OR 0.5; $P < 0.01$) and spraying specific body parts (OR 2.5; $P = 0.002$). The results present the *T. parva* exposure patterns based on serological response in calves from birth to one year. These results will support evidence-based and effective practices for the management and control of ECF to mitigate the impact on productivity in the livestock sector.

Abbreviations

AEZs	Agro-ecological Zones
CI	Confidence Interval
DF	Degrees of Freedom
ECF	East Coast fever
ELISA	Enzyme-Linked Immuno-Assay
ICC	Intra- Class Correlation
OD	Optic Density
OR	Odds Ratio
PP	Percent Positivity
SSA	Sub- Saharan Africa
TBDs	Tick-Borne Diseases
USA	United States of America

1. Introduction

Cattle production is constrained by Tick-Borne Diseases (TBDs), which substantially decrease the livestock industry's income in sub-Saharan Africa (SSA) (Chiuya et al., 2021). Among these TBDs, East Coast fever (ECF), caused by *Theileria parva* is the most common in cattle in >15 countries in SSA (Oligo et al., 2023). It is transmitted principally by *Rhipicephalus appendiculatus* ticks (Pereira et al., 2022); however, it has been recently detected in areas without the principal vector (Silatsa et al., 2020). The African water buffalo plays a significant role as a reservoir of *Theileria parva* and a source of infection for *R. appendiculatus* ticks, especially at the wildlife-livestock interface that would then transmit to cattle (Allan et al., 2021).

East Coast fever is associated with huge economic losses and high mortality (Omunyin et al., 2014; Gachohi et al., 2012). In Kenya, ECF

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poses a considerable threat to the cattle population, mostly exotic breeds, in intensive and extensive production systems (Ikaal et al., 2020). However, though it may remain clinically inapparent in indigenous cattle herds (due to endemic stability) (Kivaria et al., 2004), the poor innate and adaptive immune response among exotic breeds to ECF results in severe clinical illness (Allan et al., 2021; Oligo et al., 2023).

It is believed that the most significant immune response against infections with *T. parva* and *T. annulata* is cell-mediated immunity. The primary defense against *T. parva* is carried out by bovine major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes, which destroy infected cells (Kamani et al., 2023). Leukocyte infection triggers cytokine production, which starts an immune response and aids in presenting parasite antigen to CD4+ T cells. These cells generate interferon-γ (IFN-γ), which stimulates non-infected macrophages to release tumor necrosis factor α (TNF-α) and nitric oxide (NO), which eliminate cells that are infected with schizont and piroplasm. It has been demonstrated that CD8+ T lymphocytes can identify parasite antigens on the MHC and eliminate infected leukocytes (Kamani et al., 2023). However, many of the clinical symptoms and pathological lesions that define theileriosis are caused by macrophages overproducing cytokines, specifically TNF-α, and the resulting outcome depends on how well the immune system balances its pathogenic and protective functions (Kamani et al., 2023). Antibodies against the polymorphic immunodominant molecule (PIM) develop following a bite from an infected tick and are transferred vertically to calves through colostrum. It is believed that maternal antibodies in calves decline steadily within the first three months and any rising titre is presumably from a primary challenge/exposure (Kiara et al., 2014). However, calves with maternal antibodies are susceptible to *T. parva* infection suggesting minimal humoral protection of calves born from infected dams. Cattle, especially the indigenous breeds that recover from the mild infection, develop a strong immunity to re-infection and therefore become carriers that harbour the parasite (Silatsa et al., 2020).

However, some studies have suggested that antibodies may protect by working in tandem with the cellular immune system by neutralizing the extracellular stages of the pathogen before it can access cells (Jenkins & Bogema, 2016). For *Theileria parva*, there are two instances in which the parasite is extracellular; after inoculation of sporozoites from the tick, and after merogony where the merozoites are released from the lymphocytes into circulation before they invade red blood cells. However, some studies have shown that the sporozoites are rapidly internalized within three minutes of cell attachment (Jenkins & Bogema, 2016). It is believed that at extracellular stage, antibodies may play a role in neutralizing the parasites (Casadevall, 2003). Unfortunately for *Theileria parva* and *Theileria annulata* infections, the damage occurs during the multiplication of the parasite in the lymph nodes and therefore humoral immunity is of less importance. In contrast, this immune mechanism may protect from *Theileria orientalis* and *Babesia bigemina,* which lack the ability to transform leukocytes and, therefore are pathogenic during the intraerythrocytic phase (Jenkins & Bogema, 2016). Nonetheless, animals with acute *T. parva* infection may not survive until seroconversion, but those that do typically develop a persistent immune response and are immune to re-infection in the future (Kiara et al., 2014).

The main risk factors associated with *T. parva* infection and ECF are tick vector dynamics, production system, climatic conditions, agroecological zones (AEZs), tick control measures, management practices and the animal characteristics (breed, sex, age) (Allan et al., 2021; Cassandra et al., 2018). In various production systems, structured longitudinal epidemiological studies have been carried out to estimate the disease events and related risk factors for *T. parva* infection in different regions in Kenya (Gitau et al., 1999; Kiara et al., 2014; Maloo et al., 2001). The main strategies for controlling ECF include the use of chemical acaricides, crossbreeding, and grazing management; however, none of these strategies has shown to be sustainable or effective. To reduce ECF occurrence, vaccination has been developed as a superior substitute (Kamani et al., 2023). However, despite these efforts, East Coast fever remains a significant challenge to Kenyan livestock farmers, impacting their livelihoods and the country's agricultural economy in general.

The aim of this study is estimate the incidence and risk factors associated with *Theileria parva* infection among calves upto one year. The findings will provide critical insights into the epidemiology of the disease with recommendation for improving disease surveillance, early detection and targetted control measures. In the short term, the results will inform on the intervention measures such as vaccination and sustainable tick control measures to reduce disease burden. However, in the long term, the study contributes to the global understanding of *T. parva* transmission dynamics and highlight the gaps for development of more effective, region-specific control programs. Overally, the study adds to the broader body of knowledge on TBDs globally.

2. Materials and methods

2.1. Study area and design

A detailed description of the study area and the participating households has been provided previously (Ngetich et al., 2023). Narok County is in the Southwestern part of Kenya and lies between 1500–2000 m above sea level between latitudes 0° 50′ and 1° 50′ South and longitudes 35° 28′ and 36° 25′ East. It has biannual rainy seasons ranging between 500 and 1800 mm per annum and temperatures ranging from 12 °C to 28 °C. Livestock keeping, crop cultivation, beekeeping, and commerce are the primary economic pursuits of the people living in the study area.

A longitudinal cohort study was undertaken in Naroosura Maji Moto and Ololulung'a wards of Narok South Sub-County, Narok County between December 2022 and December 2023. The two wards were selected from the six wards based on high malnutrition rates, high prevalence of ECF and different production systems (Ngetich et al., 2023). The study site was stratified into three distinct agro-ecological zones; mixed farming, agropastoral and pastoral systems based on parameters previously described by Ngetich et al. (2023).

2.2. Sample size determination and selection/recruitment of calves for the study

The sample size was determined by estimating the number of animal years needed to detect a 2.0 morbidity/mortality rate ratio, with 80 % power to identify relationships between the independent and dependent variables, a 0.05 critical probability, and a 2 design effect (assuming incidence in pastoral system is 15 % and 5 % in mixed farming systems). This will give a minimum of 308 (280 + 28=308, assuming 10 % (28) lost to follow-up) calves (https://clincalc.com/stats/samplesize.aspx)

From the 717 households that participated in an initial cross-sectional study (Ngetich et al., 2023), 109 were recruited into the longitudinal study; 36 from mixed farming, 44 from agro-pastoral and 29 from the pastoral production system. Households were included if they had a calf <5 weeks of age and were willing to participate in the longitudinal phase. From these households, a total of 318 calves were recruited into the study, ear-tagged during the first visit when less than one month old and thereafter visited routinely every 6 weeks for 1 year and a maximum of 10 visits. However, when a calf was reported ill, additional visits were made. The calves were examined, and blood collected for 6 to 10 times depending on the date of recruitment except for those withdrawn from the study. The recruitment was continued when additional calves were introduced either by birth or from other farms through purchases, gifts among others as long as they were <1 month old. The calves that were recruited late were visited at least 6 times as this was adequate period for seroconversion (Rubaire-Akiiki et al., 2006). The recruitment of calves was carried out between December 2022 and April 2023 and follow-up went through to December 2023.

2.3. Animal and farm level data collection

During the six-week interval visits, data on calf and farm management practices including pasture management, tick control, feeding and watering as well as morbidities and mortalities were collected. Information on tick burden was determined by identifying and enumerating the number of all visible ticks on the body and recorded. However, the ticks were not collected for further analysis. The calves were clinically examined and parameters such as rectal temperature, respiration and body weight were recorded.

2.4. Sample collection, processing and Indirect ELISA

During the visits, approximately 4 ml of blood was drawn aseptically (using 70 % alcohol) using jugular venipuncture into a plain vacutainer tube. The blood was labelled and kept in a cool box with icepacks and transported to a mini laboratory in Narok Town for processing. The plain-tube samples were centrifuged at 1000 g for 15 min and serum was transfered using sterile pipettes into a sterile 1.8 ml cryovial and stored at −40 °C awaiting analysis. Indirect ELISA using Polymorphic Immunodorminant Molecule (PIM), was utilized to estimate the level of antibodies to *T. parva* as described previously (Silatsa et al., 2020). The samples were tested in duplicate, and the mean percentage positivity (PP) for each sample was calculated by dividing the optical density (OD) obtained with the sample by the OD obtained with a strong positive control serum (Silatsa et al., 2020) . For this study, the cut-off for the *T. parva*-positive ELISA calves was 20 and above PP.

2.5. Seroconversion for natural infection

The PP results from two consecutive routine 6-week visits were applied as basis for a seroconversion rule. According to the seroconversion rule for natural infection, a calf was deemed to have sero-converted if its antibody titre increased to 20 PP units or higher and remained elevated for a minimum of two consecutive visits (except from those who were lost to follow-up or whose study concluded before a thorough follow-up). Thus, it was determined that the latter had contracted *T. parva* as a primary infection. Any increase of 20 PP units or more from the level of falling maternally derived antibody profile was regarded as sero-conversion for calves who had initially had positive antibody titres (presumed to be due to maternally derived colostral antibodies). This rule has been used elsewhere (Cheryl & Bogema, 2016; Gitau et al., 1999; Kiara et al., 2014). Only observations up to and including the time of the first seroconversion were included in this analysis because it relied solely on the time to the first occurrence of seroconversion. In order to calculate the period at risk, the first visit (recruitment) was regarded as the first risk interval, and the subsequent visits were regarded as the beginning of the subsequent risk intervals. Any calf that sero-converted was excluded in calculating the time at risk in the subsequent risk intervals.

2.6. Morbidity, mortality, rates and risks of T. parva and East coast fever

Morbidity was defined as any sickness that had recognizable clinical signs (for ECF: pyrexia, coughing, nasal discharge, difficulty in breathing, swollen lymph nodes, petechiation on the mucous membrane and harsh lung sounds were key) and mortality as any observed death, irrespective of the cause. Risk was defined as the likelihood or probability of an individual in a defined population developing an infection or disease in a defined period of time (*T. parva* infection or ECF) i.e., the proportion of calves that seroconverted or manifested with classical signs of ECF. Rate was defined as the frequency with which *T. parva* infection or ECF occurred in the calf population over the study period. This was calculated as the number of calves that sero-converted divided by the time at risk resulting in number of cases per unit calf years. Crude or true incidence rates were computed. Most carcasses were unavailable for post-mortem examination as they were immediately offered to dogs or left for wild carnivores to devour. The mortality computed was based on presenting clinical signs before death and laboratory results of seroconversion. The case-fatality rate was defined as the proportion of calves from a specific disease (classical clinical signs) that died. The risks of morbidity and mortality were computed for both suspected and confirmed ECF. The total time at risk was calculated as the accumulated time at risk in weeks for all calves (minus those that had sero-converted) and then divided by 52 to get the total calf-time years at risk. The incidence rate for each interval was calculated by dividing the number of calves that seroconverted by the calf time at risk for each interval and overall incidence rate was generated as the total number of calves that seroconverted divided by the total time at risk for the follow-up period. The number of calves sero-converting divided by the total number of calves at risk at the start of the interval, less half of the withdrawals, was used to calculate the risks of *T. parva* exposure (sero-conversion rate). The number of ECF cases (or deaths) divided by the total number of calves that sero-converted was used to determine the ECF-specific morbidity and case-mortality risks for all calves that sero-converted.

2.7. Data management and statistical analysis

Data cleaning, coding and analysis was conducted in Stata 18 (Stata Corp LLC, College Station, Texas, USA). The data was subjected to normality and homogeneity tests to check for normal distribution before proceeding with statistical analysis. Descriptive statistics were used to determine frequencies, percentages and means of the respective independent variables such agroecosystem, calf's characteristics such as sex, age, bodyweight, tick infestation status, and acaricide application based on seroconversion results.

The odds ratio for risk factors was estimated together with their confidence intervals while the risk factors were found to be significantly associated with seropositivity if the p-value was < 0.05 from the multivariable analysis using Fisher's exact test. The association was first measured using the univariable multilevel mixed-effect logistic regression models for each predictor variable. Subsequently, for each univariable association with $p < 0.20$ on multilevel mixed-effect logistic regression analysis was considered for the multivariable analysis with repeated measures. This methodological approach allowed for the investigation of longitudinal (repeated calf measures) and clustered data for calves nested within farms. The clustering random effect at the farm level was accounted for and the intra-class correlation (ICC) was reported in the final models. The repeated measures trend was displayed as an interaction plot to show antibody response status across the follow-up period. Pairwise correlation was utilized to find correlations between predictor variables, and when two variables had a high correlation (correlation coefficient >0.5), statistical significance and biological plausibility were used to decide the candidate to keep in the final model between two correlated variables. By employing forward stepwise elimination, the final models were constructed, retaining variables with $p < 0.05$. Confounding effects were assessed by checking final model coefficient changes greater than 30 % with and without possible explanatory confounding variables. A variable was deemed a confounder if it had a plausible relationship with both the outcome and the independent variable it confounds. The final multilevel mixed effect model was assessed for goodness of fit using the likelihood ratio test with and without random calf and farm effects.

3. Results

3.1. Distribution of the recruited calves

A total of 318 calves were recruited into the study from 109 farms in the three agroecological zones (Table 1). More calves (39.9 %; 127/318) were recruited from the agro-pastoral system compared to 29.0 % (92/318) and 31.1 % (99/318) from mixed farming and pastoral systems,

Table 1
Distribution of the calves by agroecological zones of 318 calves followed in the longitudinal study in Narok County, Kenya (December 2022-December 2023).

Agroecological zone	Village	Number of farms	Number of calves	% of calves (n=318)
Mixed farming	Masaantare	23	63	19.8
	Olgilai	13	29	9.1
Agro-pastoral	Oloenae	30	85	26.7
	Oloongila	15	42	13.2
Pastoral	Olndonyo-Orasha	8	37	11.7
	Oloorngánayio	9	16	5.0
	Nkitintini	5	27	8.5
	Nkasuriaak	6	19	6.0
Total		109	318	100

respectively. Detailed descriptions of the recruited calves such as the mean age, body weight, sex and geographical location have been described elsewhere (Ngetich et al., 2024).

3.2. Calf retention and withdrawal

Of the 318 calves recruited into the study, 154 (48.4 %) were observed to the end of study period whereas 164 (51.6 %) were withdrawn for different reasons. For all the recruited calves, each had at least one ELISA test giving a total of 1849 results. Most of the withdrawn calves (81.0 %; 133/164) were either due to migration to a far location in search of better pasture or to reduce the stocking density. On the other hand, 7.9 % (13/164) of the calves were withdrawn as farmers did not permit withdrawal of blood, 7.3 % (12/164) died, 2.4 % (4/164) were given away (dowry), 0.6 % (1/164) sold and 0.6 % (1/164) lost the ear tag (Table 2). Excluding the calves that died, more males (55.3 %; 84/152) were lost to follow-up than females (44.7 %; 68/152).

3.3. Calves and dams characteristics

For the calves that were retained in the study, the average weight gain and body condition scores improved throughout the follow-up period. There was a significant difference in the body condition score of the follow-up calves across the different production systems (X^2=22.9, df=8, P = 0.003). Pasture, mainly grown within the farm and mineral supplements were the primary feed/nutrients provided to the calves with a few being supplemented with silage, dairy meal, banana stems and hay as described elsewhere (Ngetich et al., 2024). Similarly, milk production from the dams of the recruited calves increased steadily up to the seventh visit and declined towards the end of the study.

3.4. Seroconversion of calves to Theileria parva

Table 3 below summarizes the number of calves with antibody titer PP of 20 and above and the number that seroconverted. A total of 67 calves seroconverted during the study period after the initial decline of maternal antibodies but five seroconverted multiple times. A higher percentage of calves that sero-converted (59.7 %; 37/62) were in the agropastoral system compared to 21.0 % (13/62) and 19.4 % (12/62) in pastoral and mixed farming, respectively. The seroconversion was not significantly different across the three production systems (X^2=4.9, df=2, P = 0.08). Seropositivity decreased in visit 2 but increased steadily thereafter to peak at visit 6 (50.5 %) and later declined (Fig. 1). Similarly, the first calf seroconverted in visit 3 and a higher proportion of calves seroconverted at visit 6 (13.4 %) though these later declined. Most of the seroconverted calves (67.7 %; 42/62) had visible ticks on them though slightly more than half (53.2 %; 33/62) were reported to have been sprayed. There was a significant difference in tick infestation across the three production systems (X^2=99.6, df=2, P < 0.001).

Fig. 1. Time series marginal plot showing the mean percent positivity of *T. parva* antibody levels at each visit for the calves in the longitudinal study (December 2022 – December 2023)

Table 2
Number of calves withdrawn from the longitudinal study in Narok County, Kenya (December 2022-December 2023).

	Mixed farming (n=92)	Agro-pastoral (n=127)	Pastoral (n=99)	Total calves (n=318)
Reason for lost to follow-up				
Migrated	39 (42.3 %)	36 (28.3 %)	58 (58.5 %)	133 (41.8 %)
Declined	8 (8.7 %)	0 (0.0 %)	5 (5.1 %)	13 (4.1 %)
Died	2 (2.2 %)	5 (3.9 %)	5 (5.1 %)	12 (3.8 %)
Given away	1 (1.1 %)	1 (0.8 %)	2 (2.0 %)	4 (1.3 %)
Sold	0 (0.0 %)	1 (0.8 %)	0 (0.0 %)	1 (0.3 %)
Lost ear tag	0 (0.0 %)	0 (0.0 %)	1 (1.0 %)	1 (0.3 %)
Total lost	50 (54.3 %)	43 (33.8 %)	71 (71.7 %)	164 (51.6 %)

3.5. Incidence rates and risks of Theileria parva infection across the agroecosystems

During the study period, a total of 167.9 animal years at risk was estimated as the total time at risk and this yielded a cumulative incidence rate of *Theileria parva* infection of 37 cases per 100 calf-years (62/167.9 × 100) (Table 3). A higher incidence rate was reported in agropastoral system of 51 calves per 100 calf-years (40/78.9 × 100) compared to 38 calves per 100 calf-years (14/37.2 × 100) and 25 calves per 100 calf-years (13/51.7 × 100) from pastoral and mixed farming, respectively. The overall risk of exposure to *Theileria parva* infection during the follow-up period was 26 % (62/ (318–82) = 62/236×100), however, there was a higher risk of exposure in agropastoral system (38 %; 40/105.5 × 100) compared to 22 % (14/63.5 × 100) and 19 % (13/67×100) from pastoral and mixed farming respectively. Only 14.5 % (9/62) of seroconverted calves manifested classical signs of ECF.

3.6. Calf mortality

The crude mortality risk was 5.1 % (12/318–82) for the 12-month study period. Four of the calves manifested with classical signs of ECF before death resulting in clinical calf fatality risk of 44.4 % (4/9). Based on clinical manifestation and pathologiocal lesions, the mortality of the four calves were likely due to *T. parva* but confirmation of etiological agent was not possible in this study. Of the 12 fatal cases, only one met the sero-conversion rule while the one other had single seropositive result which occurred in the second visit, but this was attributed to the presence of colostral antibody. From the serological results and based on seroconversion rule, only one death could be attributed to infection resulting in 1.6 % (1/62) ECF-specific case fatality risk.

3.7. Factors associated with Theileria parva seropositivity

From the total 1849 serum samples collected, 398 tested positive for *T. parva* on ELISA. Table 4 summarizes the variables that were significantly associated with seropositivity to *T. parva* infection with P < 0.2 for the univariable multilevel mixed-effect model analysis. The results of the final multilevel mixed-effect logistic regression showed that there was significant association between four variables and *T. parva* seropositivity at P < 0.05 (Table 5). The risk of *T. parva* infection increased with age where calves older than 5 months were 6.9 times more likely to test positive for *T. parva* infection compared to those <5 months. Acaricide application protected the calves from infection as calves that were sprayed had a 49 % (OR 0.51) reduction in the probability of testing positive. Female calves had a higher risk of infection (OR=1.4), however, spraying specific body parts increased the risk of infection as the calves that were only sprayed at specific body parts were 2.5 times

Table 3
Number of calves that seroconverted and *Theileria parva* incidence rates in the longitudinal study in Narok County, Kenya (December 2022-December 2023).

Visit number	Number sampled	Number seropositive (%)	Number seroconverted (%)	Time at risk (years)[a]	Incidence rate[b]	Cumulative time at risk (years)[c]	Cumulative Incidence rate[d]
1	318	41 (12.9)	0 (0.0)	0	0	0	0
2	291	11 (3.8)	0 (0.0)	33.6	0	33.6	0
3	261	11 (4.2)	2 (0.4)	29.9	7	63.5	3
4	235	32 (13.6)	4 (1.7)	26.7	15	90.2	7
5	201	69 (34.3)	8 (4.0)	22.2	36	112.4	12
6	186	94 (50.5)	25 (13.4)	18.5	135	130.9	30
7	151	74 (49.0)	10 (6.6)	14.9	67	145.8	34
8	122	46 (37.7)	9 (7.4)	12.9	70	158.7	37
9	64	19 (29.7)	4 (6.3)	6.9	58	165.6	37
10	20	1 (5.0)	0 (0.0)	2.3	0	167.9	37
Total	1849	398 (21.5)	62 (3.4)			167.9	37

[a] Time at risk (years) was calculated as the number sampled minus the number seroconverted* 6/52.
[b] Incidence rate (cases/100 calf years) and calculated as the number of calves that seroconverted/time at risk*100 (years).
[c] Cumulative time at risk was calculated as the summation of time at risk from the preceding intervals.
[d] cumulative incidence rate (cases/100 calf years at risk) and calculated as the number of calves that seroconverted/cumulative time at risk*100k (years).

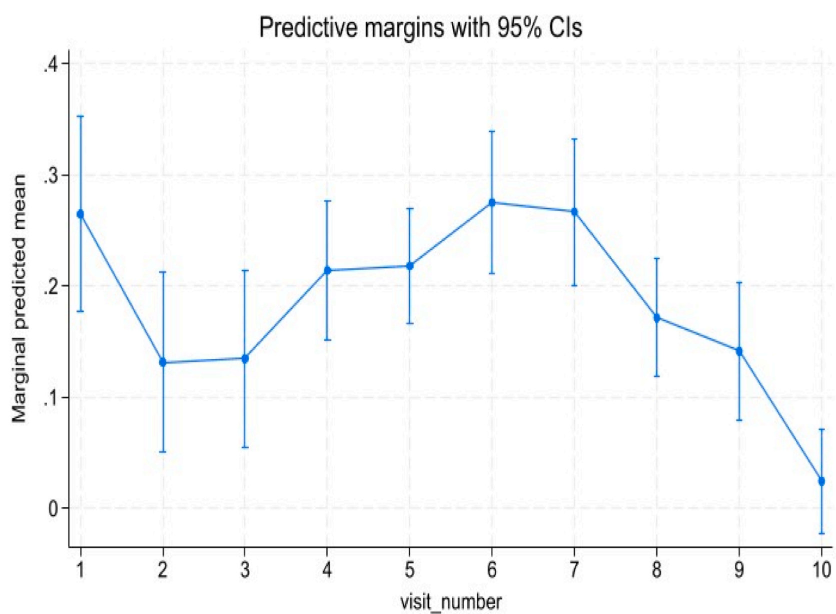

Fig. 1. Time series marginal plot showing the mean percent positivity of *T. parva* antibody levels at each visit for the calves in the longitudinal study (December 2022 – December 2023).

more likely to test positive compared to those sprayed the whole body.

4. Discussion

To the best of our knowledge, this is the first longitudinal seropositivity study carried out in Narok to assess the spatio-temporal incidence of ECF. The results from this study describe the seroconversion of *Theileria parva* and the associated risk factors among young calves followed up to one year of age. The longitudinal serological studies have an innate strength of real-time monitoring of parasitic diseases in livestock. During recruitment, 12.9 % of the calves were seropositive and this was associated with colostral antibodies and agrees with what was reported earlier by Gitau et al. (1999) and Toye et al. (2013). The proportion of the seropositive calves decreased on the second visit and later steadily increased to peak at visit six. Similar findings were reported from longitudinal studies in calves in central (Gitau et al., 1999) and western Kenya (Kiara et al., 2014) where the proportion of seropositive calves decreased steadily from birth to week 16 followed by a general increase until the end of the study. However, the antibody titre declined towards the end of the follow-up period which is in contrast with study by Kiara et al. (2014) that reported steady increase through the end of the one year follow-up period. This can be attributed to the waning of the maternally derived antibodies from circulation and the subsequent increase could be attributed to generation of endogenous antibodies following *Theileria parva* exposure. The use of serum antibody titres to evaluate the exposure to infections may be confounded by the presence of colostral antibodies and therefore the approach in this study was to use two consecutive sera as maternal antibodies are expected to decline over time.

The seroconversion began at visit 3 and increased rapidly reaching the peak at visit 6 (week 36) and diminished towards the end of the study. The above observation agrees with what was reported by Gitau et al. (1999) and Kiara et al. (2014) that seroconversion began from about week 6 and later declined, however, most (77 %) of the calves seroconverted to *T. parva*. Similar findings were reported in the Kenyan Coast where antibody prevalence increased with age to over 90 % in cattle over 18 months under herded grazing system (Maloo et al., 2001). On the other hand, reports from a longitudinal study in Eastern Uganda indicated that average PP values decreased with increasing age up to the fourth month and later increased to the end of the study period

Table 4
Risk factors associated with *Theileria parva* infection in the univariable multilevel mixed-effect logistic regression in calves from the longitudinal study in Narok County, Kenya (December 2022-December 2023).

Variable	Levels	Odds Ratio	95 % CI	P-value
Calf sex	Male	Reference		
	Female	1.3	1.0–1.6	0.033
Calf age	< 5 months	Reference	———	
	> 5 months	9.5	7.2–12.5	<0.01
Calf suckling	No	Reference	———	
	Yes	0.6	0.4–0.9	0.017
Calf weight (kg)	< 45	Reference	———	
	45–140	2.4	1.7–3.3	<0.01
	>140	2.9	1.7–5.2	<0.01
Mineral supplement	No	Reference	———	
	Yes	1.5	1.1–1.9	0.002
Hay	No	Reference	———	
	Yes	0.3	0.2–0.6	0.001
Silage	No	Reference	———	
	Yes	0.3	0.7–1.2	0.09
Feed purchased	No	Reference	———	
	Yes	2.1	1.7–2.7	<0.01
Acaricide application	No	Reference	———	
	Yes	0.4	0.3–0.5	<0.01
Spraying whole body	No	Reference	———	
	Yes	0.4	0.3–0.5	<0.01
Spraying specific parts	No	Reference	———	
	Yes	3.2	2.0–4.9	<0.01
Handpicking ticks	No	Reference	———	
	Yes	2.5	1.1–5.7	0.03
Organophosphates application	No	Reference	———	
	Yes	0.7	0.4–1.2	0.18
Pyrethroids application	No	Reference	———	
	Yes	0.7	0.5–0.9	0.004
Amitraz application	No	Reference	———	
	Yes	0.8	0.6–1.0	0.026
Deworming	No	Reference	———	
	Yes	0.4	0.2–0.8	0.004
Vaccination	No	Reference	———	
	Yes	0.4	0.2–0.9	0.035
Ecozone	Agropastoral	Reference	———	
	Mixed farming	0.8	0.6–1.1	0.13
	Pastoral	0.7	0.5–0.9	0.02
Presence of brown ear tick	No	Reference	———-	
	Yes	0.8	0.5–1.0	0.075
Calf temperature	———	0.9	0.8–1.0	0.069*
Body condition score	———	2.1	1.8–2.4	<0.001*

*Continuous variables.

Table 5
Risk factors associated with *Theileria parva* infection from the final multivariable multilevel mixed-effect logistic regression in calves from the longitudinal study in Narok County, Kenya (December 2022-December 2023).

Variable	Levels	Odds Ratio	95 % CI	P-value
Calf sex	Male	Reference		
	Female	1.4	1.1–1.8	0.016
Calf age	< 5 months	Reference		
	>5 months	6.9	3.4–14.2	<0.01
Acaricide application	No	Reference		
	Yes	0.5	0.4–0.7	<0.01
Spraying specific body parts	No	Reference		
	Yes	2.5	1.5–4.2	0.001

(Rubaire-Akiiki et al., 2006). Similarly, other hemoparasites such as *Babesia bovis* and *Babesia bigemina* have been shown to induce seroconversion in cattle introduced to a property infested with infected ticks within 35 days (Bock et al., 1999). Similar pattern was reported in longitudinal study on dynamic seropositivity of *Toxoplasma gondii* in commercial beef production in Italy where seroprevalence increased from 30.6 % on arrival to 44.6 % at 14th day but declined to 39.3 % by five months (Dini et al., 2024). Based on the serological findings at recruitment of the calves (visit one), a higher proportion of the seropositive calves were from pastoral system (15.2 %) followed by mixed farming (15.1 %) and agropastoral systems (9.5 %) (Ngetich et al., 2024).

Although antibodies against *T. parva* do not confer protection, it has been reported that they may protect by working with the cellular immune response by neutralizing extracellular parasites (Casadevall, 2003; Cheryl & Bogema, 2016). Rubaire-Akiiki et al. (2006) reported that the risk of seroconversion in cattle under communal grazing systems was 10 times compared to those in zero grazing system (mixed farming system) in Uganda. Elsewhere, in Tanzania, a higher prevalence of *T. parva* infection was observed in cattle from agropastoral (27.7 - 77.5 %) compared to those in mixed/zero-grazing (2.7 %) production systems (Laisser et al., 2017). Similarly, a higher incidence risk of *T. parva* was reported in cattle under herded grazing (39 %) compared to those under stall feeding (20 %) in a longitudinal study in the coastal lowland coconut-cassava agro-ecological zone of Kenya (Maloo et al., 2001). This has been attributed to the increased movement of animals in the agro-pastoral system that are likely to interact with infected ticks unlike in mixed farming where pasture farms (paddocks) are demarcated and therefore cattle are isolated from the neighbouring farms or from pastures that would increase the risk of infection. The reporting of higher incidence rate of *T. parva* infection in the agro-pastoral systems agrees with the increased level of awareness among the farmers about ECF in this system compared to the other systems where ECF occurrence is lower as reported in (Ngetich et al., 2023). The ability of the farmers to identify the disease is significant and can go a long way with increased disease reporting and seeking of veterinary services at the right time.

This study only assessed the presence of *T. parva* infection in calves unlike other similar studies that have looked at several tick-borne diseases (TBDs). Comparatively, these studies have shown that ECF contributed approximately 80 % of the TBDs and 62 % of all disease occurrences and was detected in >90 % of the herds from the grazing system and 56 % of the herds in stall feeding system (Maloo et al., 2001). Traditionally, *T. parva* is endemic in Sub-Saharan Africa but it has recently been detected elsewhere; Saudi Arabia (Alanazi et al., 2021) and Iraq (Albakri et al., 2024). The higher seroprevalence >60 % of *Babesia bigemina* and *Anaplasma marginale* from the same study sites in Narok South (Kibet et al., 2024) is an indication of increased abundance and spread of tick vectors that can be attributed to dynamic changes in the climatic conditions that are favourable to their survival. This poses a significant economic threat to cattle keepers in the area as they are required to spend a lot of resources on tick control, management of clinical cases, and the indirect losses resulting from reduced productivity.

There was no antibody response (seroconversion) to *T. parva* in 3 of 4 calves that died having manifested with classical signs of ECF suggesting that serology assays may sometimes miss an infection. This also indicates that an anti-*T. parva* antibody response may be slow and is not a general feature of calves that die of early ECF as the lack of an antibody response in these calves requires caution on the use of serology alone as a diagnostic tool to confirm acute death due to ECF (Thumbi et al., 2013). On the other hand, the presence of maternal antibodies may have inhibited the production of antibodies following exposure and death may have occurred before humoral response (Kiara et al., 2014). However, Ndungu et al. (2005) established that antibodies against *T. parva* can be detected 13–28 days post-infection and death can occur within similar time and therefore antibodies might not be detected in acute infections. From this study, the calves that manifested classical signs of ECF and recovered seemed to have acquired protective immunity as they did not seroconvert thereafter. However, cattle that survived the initial acute phase might succumb to secondary infection from antigenically distinct pathogen or recrudescence of the primary infection because of immunocompromised scenario in animals with co-infections. Antigenic heterogeneity is a common feature of the buffalo-derived *T. parva* infection and strain-specific immunity does not confer immunity and this results in a continuous risk of exposure (Pelle et al., 2011). However, for cattle-derived *T. parva* infection which is more homogenic or in animals that survived the initial infection may develop innate resistance that allows them to mount broad immunity that can protect them against several strains. It was not possible to determine the antigenic strains in this study because serology was used. On the other hand, there was no significant association between the presence of ticks on the body of the calves and seropositivity. Different tick species were observed on the body of the calves including Brown-ear ticks, blue ticks and red-spotted ticks bur there was no significant association between seropositivity and any of the ticks. This can be attributed to the time required for antibody response and the frequent tick control measures that eliminate the ticks before sufficient immune response. Molecular tests would be appropriate to detect active infection. Contrary to the findings of this study, it has been reported in ECF-endemic areas that cattle infested with *R. appendiculatus* have higher odds of testing seropositive compared to those not infested (Pereira et al., 2022; Wesonga et al., 2015). However, with increased transboundary animal movements, *T. parva* has been detected in areas with no history of principal tick species (Silatsa et al., 2020). This has been attributed to the lack of proper screening and treatment of animals before entry to ensure clean animals are introduced into the population (Nyabongo et al., 2021).

The 44.4 % case fatality associated with ECF in this study is lower than 56 % reported in cattle of <18 months of age but higher than 9 % reported in cattle older than 18 months in coastal lowland coconut-cassava agro-ecological zone of Kenya (Maloo et al., 2001). This finding is also slightly higher than 38.6 % ECF case fatality reported in another longitudinal study in western Kenya (Kiara et al., 2014). In Murang'a, ECF was reported as the leading cause of female calves' mortality (Gitau et al., 1999). Swai et al. (2009), reported that 56 % of all the deaths in their longitudinal study of youngstock smallholder dairy cattle in Tanga Tanzania were due to ECF. This indicates that despite the efforts put to prevent/control ECF, it remains an economically significant disease of cattle.

The older calves (>5 months) had a higher risk of infection compared to younger ones which agrees with what was reported among the Maasai pastoral communities where young calves had lower average percent positivity compared to adults (Kimaro et al., 2017). Similarly, Manyenyeka et al. (2022), reported that adult cattle in their study had a higher risk of infection compared to younger ones in Zimbabwe. This can be attributed to the waning maternal antibodies in young calves and the production of endogenous antibodies in adult calves following exposure to infected ticks during grazing or interacting with other infected herds (Gul et al., 2015; Muhanguzi et al., 2014; Kivaria et al., 2004). The findings from this study showed that acaricide application reduced the risk of infection to *T. parva* infection which is expected as this would reduce the tick attachment and reduce the possible parasite transmission. Similar findings were reported in Tanzania where appropriate tick control measures reduced the tick burden resulting in reduced TBDS including ECF (Allan et al., 2021; Ullah et al., 2022). Spraying specific body parts does not fully control/eliminate the ticks on animals and this would increase the risk of infection compared to cattle that are sprayed the whole body. Complete immersion of cattle in the dip wash has been reported to be an effective way of controlling ticks in livestock, however, incomplete immersion or inappropriate acaricide dilution do not effectively control the ticks (Miyama et al., 2020). Female calves had higher odds of testing seropositive from this study and, it has been reported elsewhere that female cattle were found to have a higher risk of infection than males in Burundi and this was attributed to males being provided with better health care due to their purpose for reproduction and meat production (Nyabongo et al., 2021). Similarly, in a longitudinal study in Zimbabwe to determine the spatio-temporal clustering and risk factors associated with *T. parva* infection, female cattle had slightly higher odds (1.2) of testing positive compared to males (Manyenyeka et al., 2022). In the contrary, male calves were reported to have 1.4 relative risk to succumb to ECF compared to females in Tanga, Tanzania (Swai et al., 2009). Kabi et al. (2014) reported that there was no significant difference between the risk of infection between male and female calves in different agro-ecologoical zones in Uganda. Elsewhere, in intensive production systems, it has been reported that female calves remain in the herd for long as replacement heifers and therefore would have higher risk of exposure to infected ticks compared to male calves that are disposed of earlier (Gitau et al., 1999). However, in pastoral setting, this might not be the case though male calves intended for future breeding tend to receive better care than their female counterparts. For this study, more males (55.3 %) were moved (migrated) elsewhere compared to 44.7 % of females, therefore more females completed the follow-up period. At recruitment, more males had maternal antibodies, and this may have protected them from subsequent infection. Unlike the study on the health and welfare of beef cattle during adaptation period in commercial fattening unit in Italy (Masebo et al., 2024), the calves in this study did not have major welfare challenges as they were freely interacting with the main herds. However, during the dry period, they were faced with feed insufficiency and sometimes had to walk for long distance in search of pasture and water.

The limitation of this study was the lack of direct detection to *T. parva* DNA that calls for caution when interpreting the results. The use of serology and clinical manifestation may be confounded by co-morbidities. Similar sentiments were reported in a study in Italy to identify equine piroplasms was to combine different assays to avoid false positive results (Facile et al., 2025). The loss of calves before completion of the follow up period may affect the final outcome. To ensure higher visibility and dissemination of the findings of this study, social media platforms including twitter and Linked In were used to share the preliminary findings. A survey by Lamanna et al. (2025) showed that social media plays a critical role in dissemination of veterinary education where 75.6 % of the general public and 74.5 % of the students reported improved knowledge on dairy cow nutrition and management. They also concluded that 84.3 % of the general public and 77.8 % of the students recommended the use of Instagram to others underscoring the its effectiveness as a digital educational tool.

5. Conclusion and recommendation

The incidence rate of *T. parva* infection was higher in agro-pastoral system compared to other systems. After waning off of the maternal antibodies, seroconversion increases with age to around nine months and later declined. A significant ECF clinical case fatality risk was reported during the follow-up period. Calves in agropastoral production systems had a higher risk of infection than those from other production systems. Acaricide application and spraying the whole animal body significantly reduces *T. parva* infection in calves upto one year. Animal factors including age and sex were significant predictors of *T. parva* infection. Education of farmers on early detection and reporting of ECF and proper tick control measures such as methods of application and dilution ratios of acaricides are recommended to reduce the transmission and impact of ECF. Similarly, farmers should be sensitized to adopt preventive technologies like Infection and Treatment method and tick vaccine to prevent tick-borne diseases.

Ethical approval

The Faculty of Veterinary Medicine's Biosafety, Animal Use and Ethics Committee (REF: FVM BAUEC/2021/316) of the University of Nairobi approved the study protocols and procedures. A signed informed consent was obtained from all the households before the start of the study.

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CRediT authorship contribution statement

Wyckliff Ngetich: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **George Karuoya Gitau:** Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Tequiero Abuom Okumu:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Gabriel Oluga Aboge:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Daniel Muasya:** Writing – review & editing, Visualization, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wyckliff Ngetich reports financial support, article publishing charges, and equipment, drugs, or supplies were provided by USAID. Prof. George Gitau reports a relationship with USAID that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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