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Prevalence of Babesia bigemina and Anaplasma marginale infections and their associated risk factors among calves in Narok County, Kenya

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Abstract

Babesiosis and anaplasmosis are major constraints to livestock production in many developing countries including Kenya. Therefore, their epidemiological data needs to be constantly updated. The current study was aimed at estimating the seroprevalence of *Babesia bigemina* and *Anaplasma marginale* and determine their associated risk factors among calves aged 3–12 months in Narok County, Kenya. A cross-sectional study was undertaken in Narok County, Kenya, between February and May 2023. A total of 402 calves from 76 farms were randomly selected from 8 villages in Sub-Counties of Naroosura Majimoto and Ololulunga. Data on individual calf and individual farm factors was collected via closed-ended questionnaires administered to someone who was involved the calves' management. Blood was collected from the calves and processed for microscopy (smears) and serology (indirect ELISA using monoclonal antibodies), respectively. Descriptive analysis was performed for both categorical and continuous variables. Mixed effect logistic regression analysis was used to determine the association between seropositivity of the various risk factors with the random effect being the farm. The overall estimation seropositivity of *B. bigemina*, *A. marginale* and mixed infections of *B. bigemina* and *A. marginale* was 60%, 60% and 38.1% respectively. The overall prevalence on microscopy for *B. bigemina*, *A. marginale* and mixed infections of *B. bigemina* and *A. marginale* was 22.9%, 32.6% and 11.4%, respectively. Factors significantly associated with the seropositivity of the infections were increase in age (OR=2.736 for *A. marginale*, 3.030 for *B. bigemina* and 2.073 for *A. marginale*/*B. bigemina*), calves that receive acaricide treatment (OR=0.445 for *A. marginale* and 0.536 for *A. marginale*/*B. bigemina*) and infection history on the farm (OR=3.803 for *A. marginale*/*B. bigemina*). In conclusion, the seroprevalence of *B. bigemina* and *A. marginale* was relatively high. Control and prevention efforts should be enforced to reduce the risk of clinical diseases from the hemoparasites.

Keywords: *Babesia bigemina; Anaplasma marginale;* seroprevalence; calves; risk factors, Narok

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Introduction

Tick-borne infections such as theileriosis, babesiosis and anaplasmosis have significant epidemiological, economic and social impacts especially in the tropics and subtropics, affecting approximately 80% of the world's cattle population (Emongor *et al*., 2000; Bock *et al*.,

2004). The leading bovine tick-borne infections in Kenya, include diseases caused by *Anaplasma marginale* (*A. centrale* having fewer reports), *Babesia bigemina* and *Babesia bovis* (Wesonga *et al*., 2017; Githaka *et al*., 2022; Shepelo, 2020). The main field vector for the transmission of *Babesia bigemina* and *Anaplasma marginale* is *Rhipicephalus (Boophilus) decolaratus*. However, *Anaplasma marginale* can be transmitted mechanically through contaminated fomites like hypodermic needles and biting flies (Potgieter and Stoltsz, 2004). These tick-borne diseases cause significant economic and production losses associated with reduced milk and meat production, morbidity, mortality and loss of draft power (Mureithi and Mukiria, 2015; Kanduma, 2018), eventually leading to hunger and poverty (Okuthe and Buyu, 2006). These diseases also cause indirect losses through costly control measures that include treatment and acaricide use and loss of cash income and reduced access to market (Minjauw and McLeod, 2003; Homewood *et al*., 2006; Kivaria, 2006; Gachohi *et al*., 2012). Exotic and cross bred cattle are more susceptible to these tick-borne diseases (Gachohi *et al*., 2012) in what is generally referred to as "lost potential". Some of these tick-borne diseases also have zoonotic potential (Beattie *et al*., 2002) hence of public health importance. The increasing vaccination against *Theileria parva* infections (Gachohi *et al*., 2012) may also lead to a relaxation in tick control by farmers, which translates to an increase in the occurrence of babesiosis and anaplasmosis cases.

Ticks, their vector, attach and bite and in heavily infested animals result in a negative economic effect on production and livelihood (Rodríguez-Vivas *et al*., 2017). Mastitis can be caused when tick bites on teat(s) become secondarily infected with bacteria (Abbas *et al*., 2014; Vudriko *et al*., 2016). Other direct impacts of tick infestation include: irritation and chronic stress which alter the animals' behavior and lead to immunosuppression, loss of energy (de Castro, 1997; Abbas *et al*., 2014), anemia due to excessive blood loss and tick paralysis. Tick infestation causes indirect losses that emanate from the cost of tick control and treatment for clinical cases, lost potential due to maintaining less productive tick-susceptible breeds, tick-transmitted pathogen impact (Alim *et al*., 2012; de Castro, 1997), trade restrictions on livestock products and acaricide-contaminated animal products (Kariuki *et al*., 1995; Kivaria, 2006).

There is a widespread limited and not updated information on the epidemiological profile of such tick-borne infections (Pegram *et al*., 1989; Mukhebi *et al*., 1992). Such reports would be necessary in highlighting the need for intensified routine surveillance of vector borne diseases to assess risks to animal and human exposure and identify "hotspots" for targeted control measures. In recent years, a further shift in the epidemiology of tick-borne diseases may have occurred due to human activities such as transboundary animal trade, nomadic pastoralism, deforestation and agricultural intensification and recent climatic changes (Githaka *et al*., 2021). To be able to develop sustainable mitigation efforts for tick-borne infections, a greater comprehension of the patterns of the tick-borne disease in a changing climate, is a requisite (Baylis and Githeko, 2006; Van den Bossche and Coetzer, 2008; Thornton *et al*., 2009).

Therefore, the study of the patterns of the epidemiology of *Anaplasma* and *Babesia* infections in calves in Narok County - Kenya, will be crucial in elucidating the disease burden in both livestock production and society. The information may provide biological evidence for control strategies such as no intervention or dipping, innate resistance exploitation or immunization (Norval *et al*., 1992; Perry and Young, 1995; Jonsson *et al*., 2012). The information gathered from this study may be used to predict disease outbreaks hence early detection and management which will contribute to increased livestock productivity from healthy cattle, thereby hence potentiating livestock's contribution to national GDP (Peter *et al*., 2020). Control strategies derived from the results of this study will have a meaningful positive impact on animal health, food production, economic growth, and public health, as visualized in the Sustainable Development Goals (SDG) in Kenya and surrounding countries. This study was carried out to estimate the prevalence of *Anaplasma* and *Babesia* infections and their associated risk factors among calves in Narok County, Kenya.

Materials and methods

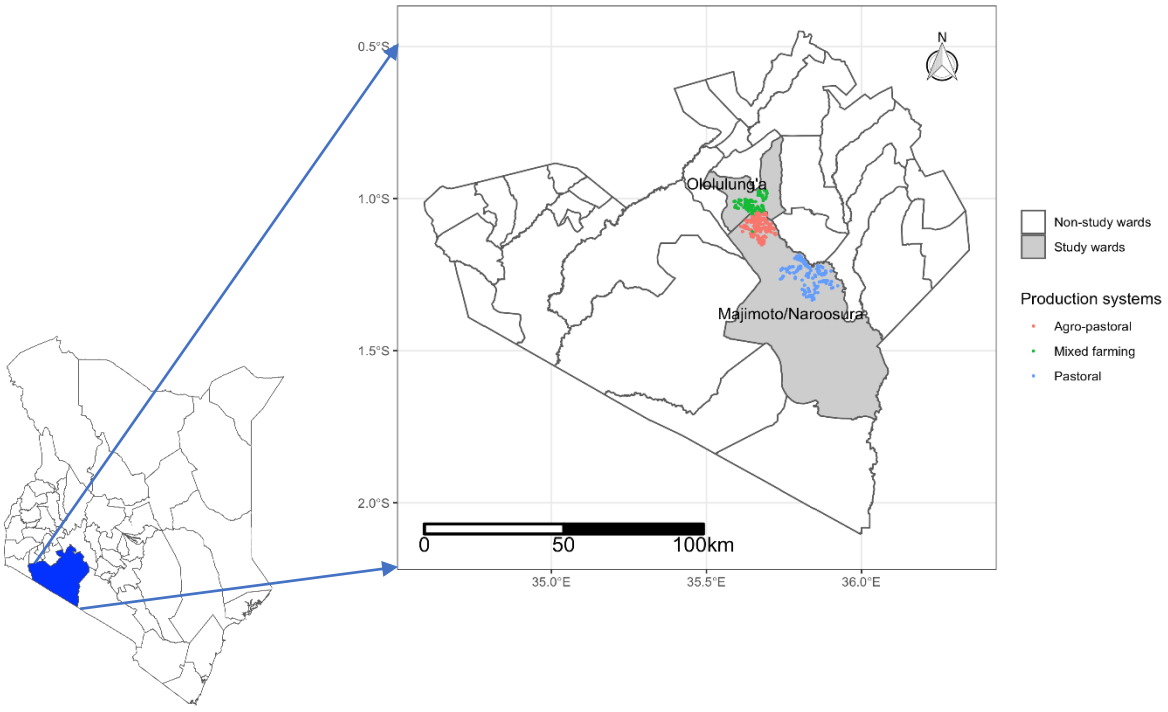
Ethical statement

All the procedures were carried out according to ethical guidelines for use of animals and approved by Biosafety, Animal Use and Ethics Committee (BAUEC) of the Faculty of Veterinary Medicine, University of Nairobi, Kenya (FVM BAUEC/2023/417). The County Veterinary personnel and the local chiefs within the study area, were informed about the aim and protocol of the study. During each farm visit, the study objectives were explained to the farmers to obtain verbal consent for data and sample collection.

Study area

Figure 1

Map of Narok County showing the study sites. The green represents the villages in the mixed production system, the blue represents the villages in the pastoral production system and the red represents villages in agropastoral system.



(Image source – Nge'tich et al., 2023. Accessed via https://www.eajsti.org/index.php/EAJSTI/article/view/765/234)

The study was conducted on farms in Narok County situated in the Rift Valley, south-west Kenya (Figure 1) (1°10′00″ and 2°10′00″ S, 34°14′50″ and 36°10′00″ E) (Ogutu *et al.*, 2011). The region is bordered by the Rift Valley to the East, the international border with Tanzania to the South, and the Siria Escarpment to the West. It includes the world-famous Masai Mara National Reserve (MMNR), a protected area for wildlife (about 1510 km^2) along the border with Tanzania which marks the beginning of the Tanzanian Serengeti National Park. The MMNR is surrounded by community-owned group ranches (4870 km^2), that act as wildlife dispersal

areas in the North and East. Land uses on these ranches include traditional livestock pastoralism, wildlife conservation, tourism and subsistence (maize and wheat) cultivation (Ogutu *et al*., 2011). The Maasai people living in Narok depend on livestock for their livelihoods. Pastoral livestock farming (mainly goats, camels, cattle and sheep rearing) (Gakuya *et al*., 2012) is the dominant production system in this area, which is characterized by intensive wildlife-livestock-human interaction that includes the sharing of pasture and water. Rainfall in the Mara region is bimodal with a short rainfall period in November–December and a longer period in April–June. The long dry season spans July–October and the short dry season January–March. However, these seasons are not fixed and variations occur as the rains become less predictable (Norton-Griffiths *et al*., 1975).

Study design and sample size determination

A cross-sectional study was conducted between February and May 2023. The sample size for the calves in the study was determined according to the method described by Dohoo *et al*., 2014 and Thrusfield, 2018 as follows:

$$n = \frac{1.96^2\, p\,(1 - p)}{L^2}$$

The antibody prevalence of the *Anaplasma* and *Babesia species* infections was not known a priori and so, 50% prevalence and 5% tolerable error were assumed when determining the desired sample size of calves. The z value was 1.96 for the desired confidence level (95%), p was the approximate of the probable prevalence of the infections, L was the desired precision level (5% tolerable error). The total number computed was 384 calves, which was increased to 400 calves in the study to increase the sample size power.

Selection of study areas, farms and animals

To obtain this sample, a sampling frame based on two wards, Naroosura Majimoto and Ololulunga, and three sub-locations, Olenkuluo, Olkiriaine and Ololulunga were purposively selected based on livestock production systems that is, mixed farming, agropastoral and pastoral. Sampling of the villages, households and calves was done through a stratified randomized sampling method. A list of all the households in the chosen villages was used to randomly select households to be recruited for the study, with the assistance of animal health officers, village elders, and research assistants in the respective sub-location to give a total of 76 farms. Since passively-derived colostral antibodies could affect the results of the study, only calves aged 3 months and above were recruited for the study (Burridge and Kimber, 1973; Gitau *et al*., 1997; Swai *et al*., 2005; Gachohi *et al*., 2010).

Data collection

For each of the farm visited, a close ended questionnaire was administered to the animal owner or person normally in charge of livestock using Swahili or Maasai languages with the help of a local translator. The questionnaire had questions on general farm level management factors and calf level factors. In addition, data on farm demographics such as household size, respondents' gender, household main source of income, highest level of education and experience in cattle keeping were collected. Information on knowledge of ticks and tick-borne infections was also collected.

The farm level data collected included grazing system, source and type of fodder, housing practice, herd size, other animals present on the farm, source and introduction process of new animals, frequency of acaricide application, type of acaricide used, method of acaricide application, frequency and source of veterinary services, herd management related information and other disease control activities like vaccination and deworming. The animal-level factors collected included breed, age, sex, source of calf (brought-in or homebred), live weight, body condition score (based on a 1-5 scale as described by Roche *et al*., (2009), source of new animals, tick species present on the calves and any clinical presentation of infections, at the time of sampling as well as disease history (clinical signs manifested and type of treatment).

Sample collection

Blood was collected through the jugular vein using a disposable sterile blood collection needle, after disinfecting the collection site using 70% alcohol-soaked cotton wool, from each selected and well restrained calf, into 4 ml plain vacutainer tubes (Becton Dickinson Vacutainer Systems, UK) and ethylenediaminetetraacetic acid (EDTA) coated vacutainer. The vacutainer

tubes were labelled to indicate a unique individual calf sample number before collection of the blood. Cool boxes containing ice packs were used to store the blood in vacutainers for about 2-5 hours in the field until refrigeration. Sera samples were prepared through centrifugation of blood in plain vacutainers at 4000rpms and stored in the cryovials in a freezer (−20 °C) and labelled correctly in the evening. Blood in EDTA coated vacutainers were used to prepare thin blood smears which were stained with giemsa and later screened under a microscope to detect *Anaplasma* and *Babesia* parasites.

Serological testing of samples

Immunoglobulin G (IgG) antibodies against *Babesia bigemina* and *Anaplasma marginale* present in the samples were detected using an indirect enzyme-linked immunosorbent assay using 200-kDa antigen and 19-kDa antigen (Morzaria *et al*., 1999; Tebele *et al*., 2000), based on a competitive inhibition principle. The procedures for the detection of antibodies by ELISA to *Babesia bigemina* and *Anaplasma* marginale have been described (Morzaria *et al*., 1999; Tebele *et al*., 2000). The tests sensitivity and specificity for *Babesia bigemina* and *Anaplasma marginale* are [97%, 98%] and [90%, 90%] respectively (Morzaria *et al.,* 1999; Tebele *et al*., 2000). The samples were tested in duplicate. The ELISA test plate also had known negative and positive control sera. Using the formula from Wright *et al*., (1993), the optical density (OD) values were expressed as PP (percent positivity), that is, (Optical Density of test serum/Optical Density of strong positive control) × 100. For a reading to be considered positive for antibodies to the *Anaplasma species* and *Babesia species*, it had to be 15 PP or above (Morzaria *et al*., 1999; Tebele *et al*., 2000).

Data handling and statistical analysis

Field and laboratory data were entered, cleaned and coded using Microsoft Excel version 2016 (Redmond, WA, USA). The final data were imported into STATA 18.0 (StataCorp LLC, College station, Texas, USA) for the data analysis. Each individual animal and farm level variables investigated was analyzed independently using univariable mixed effects logistic regression analysis to obtain their association with seropositivity. All variables with significant levels of $P \leq 0.1$ were entered into multivariable mixed effects logistic analysis. Substitution and elimination of these variables was performed to produce the most parsimonious multivariable model in which no variables could be removed without significantly altering model deviance. The level of significance was set as $p \leq 0.05$. The variables were tested for interaction effect using cross-product terms and for multicollinearity using variance inflation factor and variance covariance estimator tests. In order to determine whether each of the created models fitted the data, a non-significant Hosmer and Lemeshow goodness of fit test and Pearson Chi Square ($p \leq 0.05$) were used. Building of the multivariable model also included evaluation for confounding in the form of estimate changes >30% in risk variables (Dohoo *et al*., 2014).

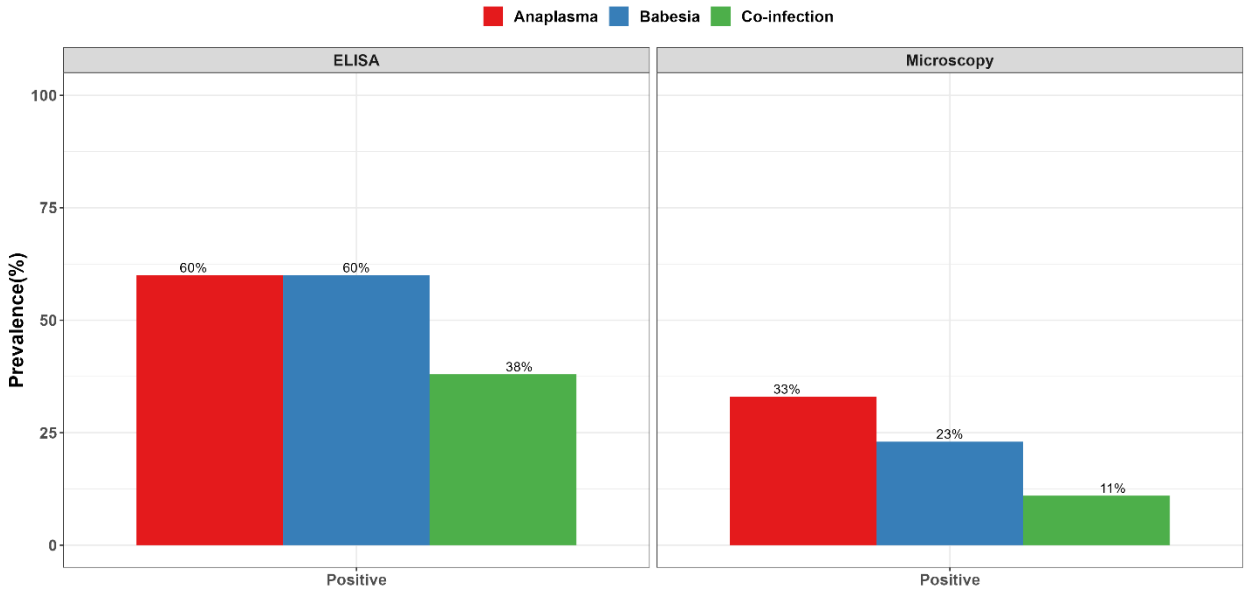
Results

Prevalence of Babesia bigemina and Anaplasma marginale infections

The overall seroprevalence of *Babesia bigemina*, *Anaplasma marginale* and mixed infections were: 60% (241/402; 95% CI 55% - 65 %), 60% (241/402; 95% CI 55% - 65%) and 38.1% (33.3% - 43.0%), respectively (Figure 2). When stratified by the agroecosystems, the seroprevalence of *Babesia bigemina* was 57.1% (108/189), 66.9% (87/130) and 55.4% (46/83) while for *Anaplasma marginale* was 56.1% (106/189), 67.7% (88/130) and 56.6% (47/83) in the agropastoral, mixed and pastoral systems, respectively. The overall prevalence based on microscopy was 22.9% (18.9%, 27.4%), 32.6% (28.1%, 37.4%) and 11.4% (8.6%, 15.1%) for *Babesia bigemina, Anaplasma marginale* and mixed infections of *Babesia bigemina* and *Anaplasma marginale* respectively (Figure 2).

Figure 2

Prevalence of *Babesia bigemina*, *Anaplasma marginale* and mixed *Babesia bigemina* and *Anaplasma marginale* infections based on the ELISA and microscopy among calves between February-May 2023



Univariable analysis of risk factors

The factors significantly associated with *B. bigemina* infections at p ≤ 0.1 were: the age of the calf, the body condition score of the calf, the suckling status of the calf, the presence of mineral supplements in forage, the purchasing of forage, acaricide application, whole body spraying of acaricides, deworming of calves, vaccinating of calves, the weight of the calf, tick infestation and the presence of blue tick (Table 1). Four variables had a positive association, while the other 10 had a negative association.

The factors significantly associated with *A. marginale* infections at p ≤ 0.1 were: the age of the calf, the suckling status of the calf, the weight of the calf, the whole body spraying of acaricides, the presence of brown ear ticks (Table 2). Six variables had a positive association, while the other three had a negative association.

The factors significantly associated with co-infections of *B. bigemina* and *A. marginale* at p ≤ 0.1 were: acaricide application, spraying acaricide on the whole body, feeding of mineral supplements, purchasing of feed, use of wood as shelter material, the calf weight and the presence of brown ear ticks (Table 3). Four variables had a positive association, while the other seven had a negative association.

Risk factors associated with seroprevalence in multivariable analysis

The factors significantly associated with *B. bigemina* seropositivity at p ≤ 0.05 were: calves aged 7 months and above and calves of body condition score between 3 and 4 (Table 4). The calves that were aged 7 months and above were more likely to test seropositive for *B. bigemina* compared to those aged below 7 months (OR = 3.030). The calves with a body condition ranging between 3 and 4 were less likely to test seropositive for *B. bigemina* compared to those with a body condition of 1 and 2 (OR = 0.467). Suckling status (p-value = 0.163) was confounding age and that is why it was included in the model. Suckling status, whether present or absent, will affect age and *B. bigemina* antibodies by 27.8%.

The factors associated with *A. marginale* seropositivity at p ≤ 0.05 were: calves aged 5 months and above and weekly application of acaricide during dry seasons (Table 5). The calves

that were aged 5 months and above were more likely to test positive for *A. marginale* antibodies compared to those aged below 5 months (OR = 2.736). The calves that were raised on farms practicing acaricide application weekly in a month during dry seasons were less likely to test positive for *A. marginale* antibodies compared to those on farms applying acaricides less than 4 times in a month (OR = 0.445).

Table 1

Factors associated with *Babesia bigemina* infections from the univariable mixed effects logistic regression analysis (p-value = 0.1) of calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Tick infestation	Present	1.561	0.995 - 2.450	0.053
	Absent	Reference	----------	-------
Blue tick presence	Present	1.638	1.015 - 2.644	0.043
	Absent	Reference	----------	-------
Purchasing of forage	Yes	0.566	0.358 - 0.896	0.015
	No	Reference	----------	-------
Feeding mineral supplements	Yes	0.541	0.322 - 0.908	0.020
	No	Reference	----------	-------
Dewormed	Yes	0.501	0.278 - 0.901	0.021
	No	Reference	----------	-------
Still suckling	Yes	0.468	0.240 - 0.911	0.025
	No	Reference	----------	-------
Levamisole and oxyclozanide use	Yes	0.404	0.200 - 0.818	0.012
	No	Reference	----------	-------
Vaccinated	Yes	0.404	0.151 - 1.084	0.072
	No	Reference	----------	-------
Whole body acaricide spraying	Yes	0.333	0.096 - 1.150	0.082
	No	Reference	----------	-------
Acaricide application	Yes	0.271	0.065 - 1.124	0.072
	No	Reference	----------	-------
Body condition score	3	0.442	0.232 - 0.842	0.013
	1	Reference	----------	-------
Body condition score	4	0.109	0.021 - 0.573	0.009
	1	Reference	----------	-------
Age	---	1.346	1.204 - 1.504	0.000*
Weight	---	1.027	1.015 - 1.039	0.000*

***These are continuous variables.**

Table 2

Factors associated with *Anaplasma marginale* infections from the univariable mixed effects logistic regression analysis (p-value = 0.1) of among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Age	≥ 5 months	2.780	1.734 - 4.456	0.000
	≤ 5 months	Reference	----------	-------
Weight	≥ 70 kg	2.246	1.393 - 3.621	0.001
	≤ 70 Kg	Reference	----------	-------
Acaricide application	≤ 2 times	1.806	1.013 - 0.220	0.045
	≤ 2 times	Reference	----------	-------
Perceived seasonality	Yes	1.140	0.779 - 1.668	0.500
	No	Reference	----------	-------
Brown tick	Presence	0.602	0.338 - 1.071	0.084
	Absent	Reference	----------	-------
Still suckling status	Yes	0.540	0.272 - 1.073	0.079
	No	Reference	----------	-------
Tick control method	Whole body spray	0.348	0.099 - 1.223	0.100
	Specific parts	Reference	----------	-------

Table 3

Factors associated with *B. bigemina* and *A. marginale* coinfections from the univariable mixed effects logistic regression (p – value = 0.1) among calves in Narok County between February and May 2023

Variable	Levels	Odds ratio	Confidence Interval	p-value
Weight	≥ 70kg	3.534	2.230 - 5.600	0.000
	≤ 70 Kg	Reference	----------	------
Age	≥5 months	3.302	2.079 - 5.243	0.000
	≤ 5 months	Reference	----------	------
Month of samples collection	March	2.907	1.259 - 6.712	0.012
	April & May	Reference	----------	------
Acaricide application frequency	≤2 times	2.216	1.297 - 3.787	0.004
	≤ 2 times	Reference	----------	------
Feeding mineral supplements	Yes	0.603	0.352 - 1.034	0.066
	No	Reference	----------	------
Brown tick	Presence	0.586	0.317 - 1.084	0.088
	Absent	Reference	----------	------
Purchasing of forage	Yes	0.555	0.340 - 0.904	0.018
	No	Reference	----------	------
Wood as shelter material	Yes	0.097	0.009 - 1.094	0.059
	No	Reference	----------	------
Acaricide application	Yes	0.311	0.093 - 1.037	0.057
	No	Reference	----------	------
Whole body spray of acaricides	Yes	0.308	0.102 - 0.925	0.036
	No	Reference	----------	------

Table 4

Factors associated with *B. bigemina* infections from the multivariable mixed effects logistic regression analysis (p – value = 0.05) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Age	≥ 7 months	3.030	1.355 - 6.776	0.007
	≤ 7 months	Reference	----------	-------
Body condition	3 and 4	0.467	0.277 - 0.789	0.004
	1 and 2	Reference	----------	-------

Table 5

Factors associated with *A. marginale* infections from the multivariable mixed effects logistic regression analysis (p – value = 0.05) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence interval	p-value
Age	≥ 5 months	2.736	1.733 - 4.320	0.000
	≤ 5 months	Reference	----------	-------
Acaricide application frequency	4 times a month	0.445	0.275 - 0.722	0.001
	<4 times a month	Reference	----------	-------

Table 6

Factors associated with coinfections of *B. bigemina* and *A. marginale* from the multivariable mixed effects logistic regression analysis (p – value = 0.005) among calves in Narok County between February and May 2023

Variable	Levels	Odds Ratio	Confidence Interval	p-value
Infections history	Present	3.803	1.683 - 8.591	0.001
	Absent	Reference	----------	-------
Age	≥ 5 months	2.073	1.125 - 3.820	0.019
	≤ 5 months	Reference	----------	-------
Acaricide application	≥ 2 times	0.536	0.342 - 0.842	0.007
	≤ 2 times	Reference	----------	-------

The factors associated with *B. bigemina* and *A. marginale* seropositivity at p ≤ 0.05 were calves aged 5 months and above, previous history of the exposure to the hemoparasites on the farm, acaricide application more than twice in a month and the purchasing of feed (Table 6). The calves that were aged 5 months and above were more likely to test seropositive for *B. bigemina* and *A.*

marginale than those below 5 months (OR = 2.073). The calves being raised in farms where acaricide application was being done more than twice in a month were less likely to test seropositive for *B. bigemina* and *A. marginale* than those in farms where acaricide application was less than twice in a month (OR = 0.536). The calves that were raised on farms with a history of the two hemoparasites were more likely to test seropositive for *B. bigemina* and *A. marginale* than those in farms that did not have the history (OR=3.803).

Discussion

The objectives of this study were to determine the seroprevalence of *Babesia* and *Anaplasma* and determine their associated risk factors among calves aged 3 – 12 months in Narok County, Kenya. The findings of this study confirmed the exposure to *Babesia* and *Anaplasma* hemoparasites through the detection of antibodies among calves in Narok County.

The study estimated the seroprevalence for *B. bigemina* at 60% which was higher than similar studies conducted in Kenya: 37.1% in rural Western Kenya (Okuthe and Buyu, 2006), 27.5% in periurban Western Kenya (Okuthe and Buyu, 2006) and 19% in Mbeere District (Gachohi *et al*., 2010). However, this prevalence was closer to the 40.6% prevalence reported in Machakos (Wesonga *et al*., 2017).

From this study, the estimated seroprevalence of *A. marginale* was 60% which was higher than previous reports, 15.8% in Machakos (Adjou *et al*., 2015), 32.1% in peri urban Western Kenya (Okuthe and Buyu, 2006), 19.7% in Western Kenya (Chiuya *et al*., 2021), 10.9% in the Coastal region (Masiga *et al*., 2022). This was close to studies in rural Western Kenya at 50.2% (Okuthe and Buyu, 2006), in Mbeere District at 58% (Gachohi *et al*., 2010), Machakos at 53.4% (Wesonga *et al*., 2017). A high seroprevalence for *A. marginale* has also been reported in other countries including: 57% in Soroti District, Uganda (Kabi *et al*., 2008), 41.1% in Tanga Region, Tanzania (Swai *et al*., 2009) and 50% in Central Equatorial State, South Sudan (Malak *et al*., 2012).

The relatively higher seroprevalence of *A. marginale* and *B. bigemina* reported in this study were associated with continuous infected tick challenge, as the study area is considered to be an endemic area. The study area also practices open and communal grazing, which encourages constant exposure to tick vectors and the subsequent constant challenge of the hemoparasites, hence the presence of antibodies against the hemoparasites.

The seroprevalence of *A. marginale* and *B. bigemina* was similar, possibly because they have the same vector, that is, *R. decoloratus/R. microplus* ticks. These two hemoparasites can be co-acquired and transmitted simultaneously by the same vector tick (Adjou *et al.,* 2015). This also explains the co-presence of antibodies against both A. *marginale* and *B. bigemina*. *Anaplasma* species is reported to have broader transmission sources and dynamics including mechanically through iatrogenic route or hematophagous flies. The finding of a similar seroprevalence seems to suggest that the mechanical transmission route is of less importance in the epidemiology for *Anaplasma* species.

From this study, the seroprevalence estimated suggests a "near endemic stable state" or more accurately endemic instability (Norval *et al*., 1992; Deem *et al*., 1993; Gitau *et al*., 1999; Maloo *et al*., 2001) given that endemic stability is characterized by >70% seroprevalence while endemic instability by < 30% seroprevalence of the hemoparasites.

The probability of endemic status using seroprevalence is only indicated through a single cross-sectional study (Gitau *et al*., 1997; Maloo *et al*., 2001; Rubaire-Akiiki *et al*., 2004; Swai *et al*., 2005; Bazarusanga *et al*., 2007). This prevalence can differ quite considerably with vector tick density, climatic conditions, human-related activities, vector control programs, habitat modification and host population density (Gitau *et al*., 1999; Maloo *et al*., 2001; Rubaire-Akiiki *et al*., 2006; Olwoch *et al*., 2008).

Risk factors associated with Anaplasma marginale and Babesia bigemina seroprevalence

Calves aged 5 months and above had increased odds of testing seropositive for *A. marginale* infections, *B. bigemina* and both *A. marginale* and *B. bigemina* than those aged below 5 months. The relationship between age and *B. bigemina*

seroprevalence has been previously reported (Wray *et al*., 2000; Magona *et al*., 2008; Simuunza *et al*., 2011; Terkawi *et al*., 2011; Hamou *et al*., 2012; Atif *et al*., 2013; M'ghirbi *et al*., 2016; Wesonga *et al*., 2017). All age groups are susceptible to *B. bigemina* and *A. marginale* but the prevalence increases with age (Gachohi *et al*., 2012). The sustained exposure to ticks translates to exposure to *Babesia* species which increases as the calves get older. The grazing of older cattle far in the bush also increases their chance of tick exposure, which consequently increases their chance of exposure to the hemoparasites (Bazarusanga *et al*., 2007).

Calves being raised in farms that were reported to have a history of exposure to both *A. marginale* and *B. bigemina* had a higher odd of testing seropositive for *A. marginale* and *B. bigemina*. This suggests that an animal that survives an acute infection, may develop a persistent infection and serve as a carrier for *A. marginale* and *B. bigemina* in the herd (Kocan *et al*., 2003). To be able to comprehend the epidemiology and control of the two hemoparasites, it is important to identify the chronically infected animals (Figueroa *et al*., 1993; Calder *et al*., 1996; Goff *et al*., 2008).

Calves with body condition above 2 on a scale of 1-5, were less likely to test seropositive for *B. bigemina* compared to those with body condition of 2 and below. Various studies have reported a similar finding (Sitotaw *et al*., 2014; Admassu *et al*., 2015; Hamsho *et al*., 2015; Wodajnew *et al*., 2015; Adugna and Tamrat, 2022). Lowered immunity can be demonstrated in poorly conditioned cattle, which encourages the establishment of *Babesia* and *Anaplasma* infections. Establishing whether the depreciation in body condition is a result of the disease or other potential risk factors can only be possible through a longitudinal study.

From this study, acaricide application frequency was significantly associated with *A. marginale* seropositivity and both *A. marginale* and *B. bigemina* seropositivity. Farms that applied acaricide weekly had lower odds of testing seropositive for *A. marginale* and those that applied acaricide more than twice in a month had lower odds of testing seropositive for *A. marginale* and *B. bigemina*. Acaricide application has been recommended for the prevention and control of infections of *B. bigemina* and *A. marginale* in cattle (Stachurski, 2000; Adjou, 2012; Adehan *et al*., 2016a). Acaricide application effectiveness is shown through this study, as a high proportion (49.3%) of the study calves had no tick infestation.

Conclusion

Findings in this study showed a considerably high seroprevalence of *Babesia bigemina* and *Anaplasma marginale* among calves between 3 months and 12 months in Narok County. Calves aged 5 months to 12 months were reported to have a higher seroprevalence to *Babesia bigemina* and *Anaplasma marginale* compared to those aged 3 months to 5 months. Acaricide application was associated with lower seroprevalence to *Babesia bigemina* and *Anaplasma marginale*.

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