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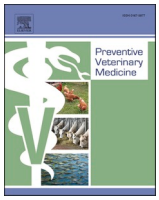

# Epidemiology of tick-borne pathogens of cattle and tick control practices in coastal Kenya

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ABSTRACT

Tick-borne diseases (TBD) are a major constraint to livestock health and productivity in sub-Saharan Africa. Nonetheless, there are relatively few robust epidemiologic studies documenting TBD and its management in different endemic settings in Kenya. Therefore, a cross-sectional study using multi-stage cluster sampling was undertaken to characterize the epidemiology of TBD and management factors among zebu cattle reared under an extensive system in coastal Kenya. Blood samples from 1486 cattle from 160 herds in 14 villages were screened for the presence of tick-borne bacterial and protozoan pathogens using PCR with high-resolution melting analysis and sequencing. Standardized questionnaires were used to collect data on herd structure and herd management practices, and a mixed-effect logistic regression model to identify risk factors for tick-borne pathogens (TBPs). The application of chemical acaricide was the primary method for tick control (96.3%, 154/160), with the amidine group (mainly Triatix®, amitraz) being the most frequently used acaricides. Respondents identified East Coast fever as the most important disease and Butalex® (buparvaquone) was the most commonly administered drug in response to perceived TBD in cattle. The overall animal- and herd-level prevalence for TBPs were 24.2% (95% confidence interval (CI): 22.0–26.4%) and 75.6% (95% CI: 68.2–82.1%), respectively. Cattle were infected with *Anaplasma marginale* (10.9%, 95% CI: 9.4–12.6), *Theileria parva* (9.0%, 95% CI: 7.5–10.5), *Anaplasma platys* (2.6%, 95% CI: 1.9–3.6), *Theileria velifera* (1.1%, 95% CI: 0.7–1.8), *Babesia bigemina* (0.5%, 95% CI: 0.2–1.0), and *Anaplasma* sp. (0.1%, 95% CI: 0.0–0.4). Moreover, 21 cattle (1.4%) were co-infected with two TBPs. None of the assessed potential risk factors for the occurrence of either *A. marginale* or *T. parva* in cattle were statistically significant. The intra-herd correlation coefficients (lCCs) computed in this study were 0.29 (*A. marginale*) and 0.14 (*T. parva*). This study provides updated molecular-based information on the epidemiological status of TBPs of cattle and herd management practices in coastal Kenya. This information can be used in designing cost-effective control strategies for combating these TBD in the region.

## 1. Introduction

Tick-borne diseases (TBD) remain among the most important livestock diseases worldwide due to their impact on livestock health and productivity which result in huge economic losses in the livestock sector (Ocaido et al., 2009). Losses due to TBD are incurred directly through decreased meat and milk production, lost draft power, morbidity and mortality, and indirectly through costly control measures and loss of cash income (Gachohi et al., 2012; Minjauw and McLeod, 2003). These economic losses disproportionately impact small-scale resource-poor households in developing countries, including Kenya, where more people depend on livestock production for financial and nutritional security (Minjauw and McLeod, 2003).

The most important TBD of cattle in Kenya include East Coast fever (ECF), caused by the protozoan *Theileria parva*, bovine anaplasmosis caused by the bacterium *Anaplasma marginale*, and bovine babesiosis

*Abbreviations:* EDTA, ethylene diamine tetraacetic acid; ICC, intra-herd cluster correlation coefficient; PCR, Polymerase chain reaction; TBD, tick-borne diseases; TBP, tick-borne pathogen.

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caused by the protozoa *Babesia bigemina* and *Babesia bovis*. Generally, these TBD pose a greater challenge to the susceptible exotic (i.e., *Bos taurus*) and crossbred cattle (i.e., *B. taurus* × *Bos indicus*), thus representing a major constraint in the improvement of local cattle production (Gachohi et al., 2012). The clinical course of these TBD is usually subclinical in the autochthonous zebu cattle (i.e., *B. indicus*), but high tick infestation combined with other stress factors (e.g., malnutrition, pregnancy, lactation, concurrent infections, etc.) can cause clinically apparent acute disease (Bock et al., 2004; Kocan et al., 2010). Although the impacts of these diseases have not been comprehensively quantified, previous reports from Kenya indicate enormous losses through morbidity, mortality, and productivity losses (Gitau et al., 1999; Kiara et al., 2014; Maloo et al., 2001a; Muraguri et al., 2005; Wesonga et al., 2010).

Potential risk factors associated with TBP infection in cattle include cattle breed, age, agro-ecological zone, livestock production system (Gachohi et al., 2012), inherent resistance of cattle to ticks and TBD (Jonsson et al., 2014; Laisser et al., 2016; Robbertse et al., 2017; Shyma et al., 2013), the frequency of acaricide application (Miyama et al., 2020; Wesonga et al., 2014), tick infestation on cattle (Byaruhanga et al., 2016; Chiuya et al., 2021; Gachohi et al., 2010; Kerario et al., 2017; Wesonga et al., 2014), and distribution of tick vectors and infection rate of ticks (Norval et al., 1992). However, these potential risk factors are highly inconsistent between studies. Some studies did not find any significant association between TBP infection status in cattle and tick control practices (Gitau et al., 1997; Maloo et al., 2001b), age (Byaruhanga et al., 2016; Kerario et al., 2017), sex (Kerario et al., 2017; Okal et al., 2020), frequency of acaricide application (Kerario et al., 2017; Kimaro et al., 2017), and presence of tick infestation among cattle (Simuunza et al., 2011). Therefore, identifying and quantifying risk factors contributing to disease occurrence and characterizing the current epidemiologic states in different endemic settings is essential in designing cost-effective control strategies for combating these TBD.

The recent climatic changes such as the extent and distribution of rainfall, in addition to anthropogenic factors such as agricultural intensification, deforestation, nomadic pastoralism and transboundary animal trade observed in recent years may further lead to a shift in the epidemiology of TBD in Kenya (Githaka et al., 2021). It is, therefore, imperative to regularly update existing epidemiological information on TBD in cattle. Currently, there are few robust epidemiologic studies of TBD in the different endemic settings in Kenya, including the coastal regions, and thus control strategies lack evidence-based guidelines. The available epidemiological studies of TBD in cattle in Kenya have traditionally been based on serological tests (Gachohi et al., 2010; Maloo et al., 2001a), microscopic examination of stained blood smears and smears of lymph node biopsies (Muraguri et al., 2005; Okuthe and Buyu, 2006) or clinical signs (Kanyari and Kagira, 2000). However, all these diagnostic techniques have considerable limitations in terms of sensitivity and specificity (Salih et al., 2015). Microscopy lacks the sensitivity required for detecting low levels of infections in carrier animals, and the pathogens are difficult to identify to species level or distinguish between closely related species. On the other hand, serological methods cannot differentiate between current infections and previous exposures in carrier animals, and reported cross-reactivity of antibodies limits specificity. Therefore, a sensitive and highly specific molecular approach is required to determine the current TBP infection status.

Little contemporary data is available on the management practices of ticks and TBD by livestock farmers following the withdrawal of government-funded veterinary services (Government of Kenya, 2008). To inform more effective and sustainable future management options, it becomes imperative to investigate management practices among cattle owners regarding ticks and TBD. These are no longer strongly informed by government policy but by farmer preferences and affordability. To improve epidemiological knowledge of TBPs in coastal Kenya and present opportunities for strategic disease prevention and control, the objectives of the present study were to 1) estimate the molecular prevalence of species of *Anaplasma, Babesia, Ehrlichia, Rickettsia* and *Theileria* in cattle; 2) assess the potential risk factors for these TBP infections in cattle; and 3) characterize the control practices related to ticks and TBD among cattle owners in coastal Kenya.

## 2. Material and methods

### 2.1. Study setting

The study was conducted in Kayafungo Ward (Kilifi County) and Kinango Ward (Kwale County) in coastal Kenya (Fig. 1) from November to December 2019. The two administrative wards (i.e., Kinango and Kayafungo Ward) were selected purposively based on their potential for livestock production in the region, good accessibility, and the difference in access to veterinary services. Kinango Ward has functional cattle dips sponsored by the local County government or farmer organization groups, unlike Kayafungo. Administratively, Kinango Ward is divided into 4 sub-locations (Kinango, Dumbule, Kibandaongo and Gandini sub-locations). Kayafungo Ward is divided into 6 sub-locations (Tsangatsini, Mnyenzeni, Miyani, Kinagoni, Mbalamweni, Mirimani). Sub-location is the smallest administrative unit in Kenya. The study area is characterized by a semi-arid climate with low and erratic rainfall. The rainfall pattern is bimodal, with most rains between April and June (long rains) and October to November (short rains), but some rain falls nearly every month, especially near the coastline. The average annual rainfall ranges between 500 and 600 mm in the drier hinterland, increasing to 900–1500 mm along the coastal belt. The mean annual temperature in the coastal region ranges between 23 °C and 34 °C. The area faces recurrent droughts and is characterized by extensive rangeland with sparse vegetation. The livestock production system is a predominantly traditional extensive system with the majority of households keeping chickens, cattle, goats, and sheep, which provides a source of income for families through the sale of meat and dairy. The local East African zebu breeds are the predominant cattle in the study area. They are grazed extensively on fallow or communal grazing fields in natural pastures and share watering points. The sharing of grazing land and water points exposes cattle to a high risk of tick infestation and thus increasing the likelihood of TBD outbreaks. Many cattle owners in the region also migrate with their animals, searching for pasture and water during the long dry seasons. This uncontrolled cattle movement results in mixing herds from different areas, thus increasing the risk of disease transmission between herds and new geographical areas (Ekwem et al., 2021).

### 2.2. Study design, sample size, and sampling strategy

The study was a baseline survey of a more extensive operational research project entitled "Improving food and nutritional security through integrated control of tsetse and tick-borne livestock diseases (ICTLD)." A cross-sectional study with multi-stage cluster sampling was used in selecting the study population (Fig. 2). Cluster sampling was chosen due to the unavailability of individual animal sampling frames (Dohoo et al., 2009). All four sub-locations in Kinango Ward and four of the six sub-locations in Kayafungo Ward were purposively selected to increase the geographical spread of the study. Two spatial village clusters (each containing 3 – 6 villages) in each sub-location were then chosen by purposive sampling in collaboration with the respective sub-county's directorate veterinary personnel. The final listing of village clusters was made based on the cooperation of farmers and logistical feasibility (accessibility by vehicle, security, distance). Since cattle from the adjacent villages share common grazing land, route, and watering point, the risk of tick infestation and hence TBPs infection prevalence within those sub-locations was assumed to be similar and therefore few spatial village clusters per sub-location were selected. From selected villages, a group of cattle owned by a household was designated as a herd and was considered the primary sampling unit, and the individual

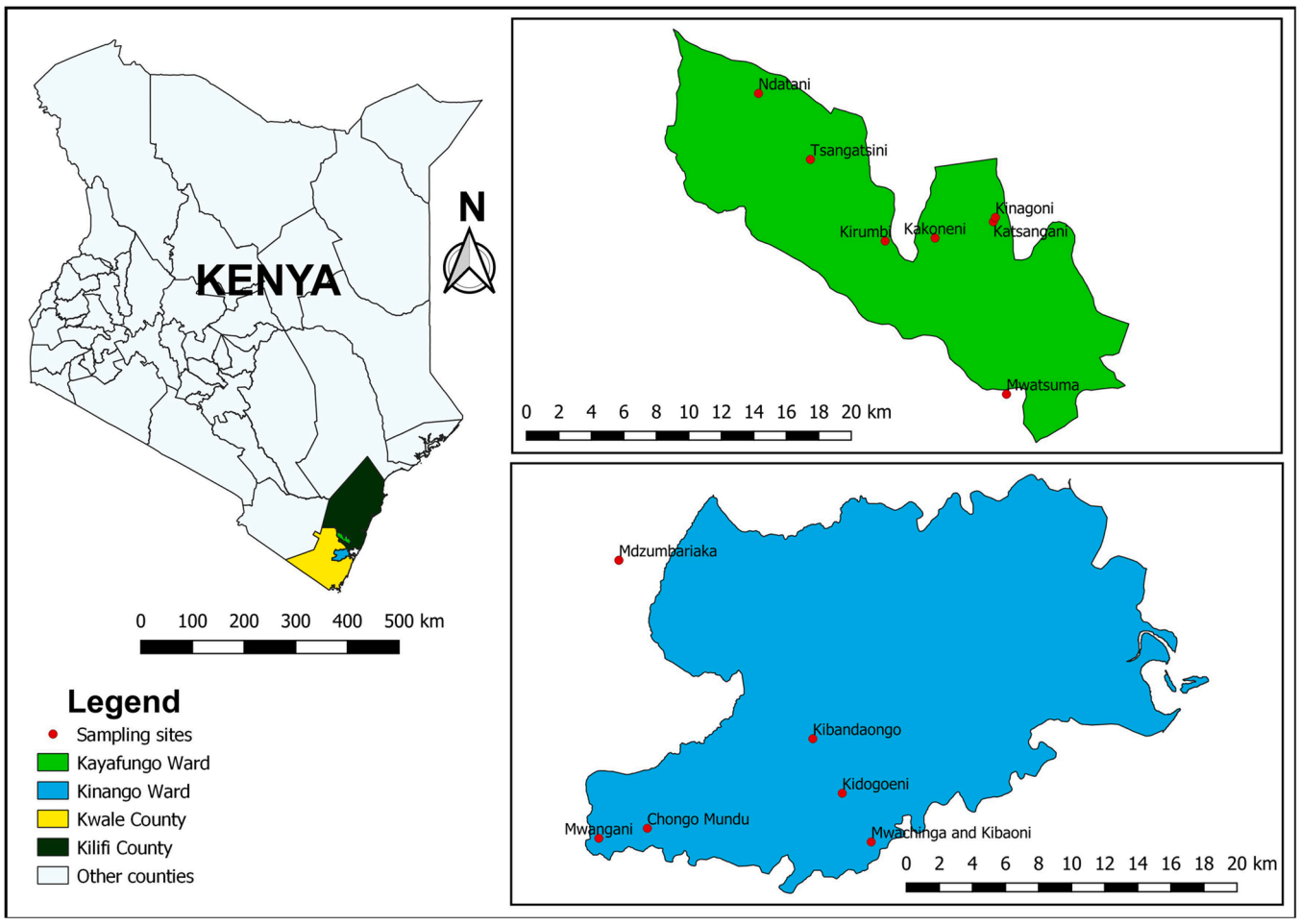


**Fig. 1.** Map of Kayafungo and Kinango wards in coastal Kenya showing the crush sites in each village cluster where the sampling took place. The map was prepared using common-license shape files in QGIS software version 3.10 (QGIS Development Team, 2020).

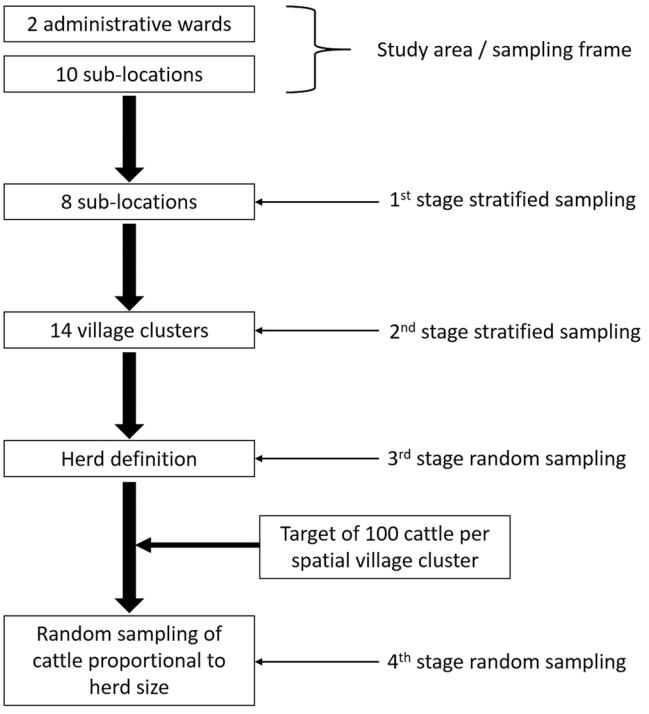


**Fig. 2.** Scheme showing the design and sampling strategy used in this study.

cattle within the herd were considered the secondary sampling unit. Herd selection was randomly made based on their location by village and the willingness of the farmer to participate in the study.

The sample size (n) was determined following a previously described method (Molla et al., 2018):

$$n = gc \quad = \frac{P(100-P)D}{SE^2} \tag{1}$$

The seroprevalence rates for *T. parva, A. marginale* and *B. bigemina* in the region ranged from 14% to 97% (Maloo et al., 2001b). Therefore we used a 50% expected prevalence (P) and a 5% margin of error (SE) and adjusted for design effect (D), which was estimated using formula 2.

$$D = 1 + (g-1)ICC \tag{2}$$

Where g is the average number of individuals sampled per cluster, and *c* is the number of clusters to be sampled. The intra-herd correlation coefficient (ICC) relates to the relatedness of clustered data.

Assuming an ICC of 0.15 and considering the possibility of collecting about 100 blood samples by a team of 4 people per day in a village cluster, *D* equals 16 (formula 2). The ICC estimate was based on the reported intra-herd correlation coefficient for exposure to *A. marginale* (Gachohi et al., 2010). Sampling 100 animals per cluster (village) with an expected disease prevalence of 50% and the desired precision of 5% gave 16 spatial village clusters and thus a total sample size of around 1600 cattle. The clusters and the total sample size were equally distributed among the two study wards. Cattle were sampled randomly, proportional to the herd size. Thus, all cattle were sampled if a herd had

less than ten animals, ten were randomly selected if the herd size was up to 20 animals, and 30% were sampled in herds with more than 20 animals. Each herd was sampled with the informed consent of its owner or authorized agent. There were no sex restrictions, but cattle were not eligible for sampling if they were less than six months of age. Due to logistical challenges, two spatial village clusters were not sampled.

### 2.3. Sampling and data collection

Blood samples were collected from the jugular vein of each cattle using 4-ml vacutainer tubes (BD Vacutainer®) coated with ethylenediaminetetraacetic acid (EDTA). The tubes were gently inverted 4–5 times to mix the blood with the anticoagulant before being transferred to 2-ml sterile cryovials labeled with animal ID, date, and site of collection. These samples were kept in a cool box containing ice packs in the field. At the same time, approximately 125 µl of the collected blood sample was transferred into sodium-heparinized micro-hematocrit capillary tubes to measure packed cell volume (PCV). Briefly, the blood samples in the hematocrit capillary tubes were centrifuged for 5 min at 12,000 rpm using a micro-hematocrit centrifuge, and the PCV was measured using a micro-hematocrit reader. A PCV below the threshold level of 24% was considered anemic. Corresponding records of each sampled animal, including location, ownership, age, sex, breed, live body weight, PCV, and ticks present on cattle, were entered onto a predesigned datasheet. The age of an animal was assessed by the dentition and farmer's information and was categorized as calves (6–12 months of age), juveniles (13–24 months) and adults (over 24 months of age). Sex was categorized as female versus male, while breed was categorized into indigenous and cross classes. The samples in the cool boxes were then transported to the field station for storage in liquid nitrogen before transportation to the Martin Lüscher Emerging Infectious Disease (ml-EID) laboratory at the International Centre of Insect Physiology and Ecology (*icipe*). The samples were stored at − 80 °C awaiting pathogen screening.

A pre-tested questionnaire containing both closed and open-ended questions was administered to the household head or spouse in Kiswahili or the local language. The questionnaire was designed to obtain: (i) sociodemographic information, (ii) herd management-related information, and (iii) tick and TBD-related information. The respondents were asked questions from the questionnaire without having the choices read. The trained data collectors recorded the answers given based on the listed options. This approach was preferred to avoid leading questions that could introduce bias. In cases where the expected responses were deemed not exhaustive or not in the listed choices, an option for "others: please specify" was provided. Open-ended questions were mainly used when a numerical response was expected. In some sections, the participants were allowed to provide more than one answer. For instance, the farmers were allowed to give more than one answer when asked about the type of acaricides they have used in the past 12 months, the symptoms they perceived to be associated with TBD, etc. When the brand name of drugs was the sought response, farmers were asked to verify it by producing a sales receipt or presenting the product or its used packages. The questionnaire took 30–45 min to administer. All this information was collected and managed using Research Electronic Data Capture (REDCap) tools (Harris et al., 2019)hosted at *icipe*.

### 2.4. DNA extraction

The genomic DNA was extracted from EDTA-treated blood samples according to the procedure described by Suguna et al. (2014) with some modifications. Briefly, 300 µl of whole blood was added to a 1.5-ml Eppendorf tube containing 900 µl low salt buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 2 mM EDTA) and 50 µl of 1% Triton X-100. The samples were mixed well by vortexing and incubated for 10 min at 56 °C to lyse the red blood cells. The cells were centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. This step was repeated 2–3 times with a decreasing amount of 1% Triton X-100 until a white pellet of white blood cells was obtained. After the lysis stage, 300 µl of high salt buffer (10 mM Tris-HCl, pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 2 mM EDTA, 400 mM NaCl) and 50 µl of 10% Sodium Dodecyl Sulfate (SDS)were added to the cell pellet, mixed thoroughly and incubated at 56 °C for 10 min. At the end of incubation, 100 µl of 6 M NaCl was added and vortexed to precipitate the proteins before centrifuging at 10,000 rpm for 5 min. The supernatant was then transferred into a new Eppendorf tube containing 500 µl of absolute isopropanol. DNA was precipitated by continuously inverting the Eppendorf tube slowly for 3 min before centrifuging at 10,000 rpm for 10 min to pellet down the DNA. The supernatant was discarded, and 500 µl of ice-cold 70% ethanol was added and mixed slowly to remove any excess salts. Finally, the tubes were centrifuged at 10,000 rpm (4 °C) for 7 min to pellet down the DNA. The supernatant was discarded, and DNA pellets were air-dried. After thorough drying, the DNA pellets were re-suspended in 100 µl of sterile de-ionized distilled water, and the DNA was stored at − 20 °C until further use.

### 2.5. Molecular detection of tick-borne pathogens

The DNA samples were screened by PCR with high-resolution melting (PCR-HRM) analyses for the presence of species of *Anaplasma, Babesia, Ehrlichia, Rickettsia,* and *Theileria*. The PCR-HRM assays were conducted on a Magnetic Induction Cycler (MIC) machine (BioMolecular Systems, Australia) using genera-specific PCR-HRM primers listed in Table 1. The reaction mixture had a final volume of 10 µl, containing 5 µl of PCR grade water, 2 µl of 5xHOT FIREPol EvaGreen HRM mix (no ROX) (Solis BioDyne, Estonia), 0.5 µl of 10 pmol of each primer and 2 µl of the DNA extract. The PCR cycle parameters included an initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 20 s, annealing for 30 s at temperatures listed in Table 1, and extension at 72 °C for 30 s. This was followed by a final extension at 72 °C for 7 min. The PCR cycle was directly followed by HRM analysis with an increasing temperature from 75 °C to 95 °C at 0.1 °C/sec. The positive controls included *Anaplasma bovis, Ehrlichia ruminantium* and *T. parva,* while a master mix without the DNA template was used as a negative control. Representative samples for each unique HRM profile were selected and purified using ExoSap-IT (USB Corporation, Cleveland, OH, USA) according to the manufacturer's protocol and then sent to Macrogen Inc. (The Netherlands) for sequencing in both directions.

Representative samples were further re-amplified for confirmation of positive *Anaplasma* samples using standard PCR primers targeting the major surface protein 4 (*msp4*). The standard PCR reaction contained 4 µl of 5 x HOT FIREPol® Blend Master Mix (Solis Biodyne, Estonia), 1 µl of 10 pmol of each forward and reverse primer, 4 µl of the DNA template, and 10 µl of PCR grade water. The cycling conditions were: initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min. The final extension was at 72 °C for 7 min. This PCR reaction was carried out using SimpliAmp™ Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The PCR products were electrophoresed on a 2% agarose gel stained by ethidium bromide, and expected bands were excised and purified by the QIAquick® Gel Extraction Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol before sequencing.

### 2.6. Sequence and phylogenetic analyses

The obtained sequences were edited using Geneious software version 11.1.5 (Kearse et al., 2012). The sequences were first truncated at the 5'- and 3'-ends to remove low-quality reads and the primer sequences. Identities of the truncated sequences were revealed by querying in the GenBank nr database using the Basic Local Alignment Search Tool (www.blast.ncbi.nlm.nih.gov/). Annotated sequences of the same genus and locus were extracted from the GenBank database and aligned with the MAFFT plugin in Geneious (Katoh and Standley, 2013). The

**Table 1**
PCR primer pairs and annealing temperatures used in this study.

| Genus | Primer | Target gene | Primer sequence (5′–3′) | Annealing temperature (°C) | Amplicon size (bp) | Citations |
|---|---|---|---|---|---|---|
| *Anaplasma* | MSP45<br>MSP43 | *msp4* | GGGAGCTCCTATGAATTACAGAGAATTGTTTAC<br>CCGGATCCTTAGCTGAACAGGAATCTTGC | 60 | 851 | (De La Fuente et al., 2004) |
| *Anaplasma/ Ehrlichia* | 16 S8FE<br>B-GA1B | *16S* rDNA | GGAATTCAGAGTTGGATCMTGGYTCAG<br>CGGGATCCCGAGTTTGCCGGGACTTCTTCT | 60.5 | 448 | (Schouls et al., 1999) |
| *Babesia/Theileria* | RLB-F2<br>RLB-R2 | *18 S* rDNA | GACACAGGGAGGTAGTGACAAG<br>CTAAGAATTTCACCTCTGACAGT | 60.5 | 460–500 | (Georges et al., 2001) |
| *Rickettsia* | Rick-F1<br>Rick-F2 | *16 S* rDNA | GAACGCTATCGGTATGCTTAACACA<br>CATCACTCACTCGGTATTGCTGGA | 55 | 350–400 | (Nijhof et al., 2007) |

phylogenetic analysis was inferred using the maximum likelihood (ml) approach as implemented in PhyML version 3.0 (Guindon et al., 2010) based on the Akaike information criterion (AIC) for automatic model selection. Bootstrap analysis with 1000 replications was used to estimate the confidence of the nodes and branches of the trees.

### 2.7. Statistical analysis

Raw data was entered into Microsoft® Excel 2016 and verified for missing observations and erroneous entries. Incomplete entries were excluded from the analysis (n = 36/1522). Statistical analysis was performed using R software version 4.1.3 (R Core Team, 2022). Descriptive statistics were calculated for all animal-, farm- and area-level variables. Since the two wards (i.e., Kinango and Kayafungo) are autonomous administrative units with different access to veterinary extension services, we calculated the descriptive statistics for demographic characteristics, awareness, perceptions and each element of the control practices at the ward administrative level (cluster of sub-locations). The outcome measure for the prevalence estimation was the presence and absence of the tested TBPs. The individual-level prevalence (proportion of infected cattle out of the total tested cattle) and herd-level prevalence (proportion of herds with at least one positive pathogen divided by the total number of herds tested) for each TBP were calculated. A herd was declared positive if at least one animal tested positive for a pathogen based on PCR-HRM and sequencing results. Only *T. parva* and *A. marginale* pathogens were considered during risk factor analysis due to their economic significance and sufficient data to perform a risk factor analysis. The breed category was also excluded from the analysis due to insufficient data. Analysis of possible risk factors related to *T. parva* and *A. marginale* infection in cattle was performed using a univariable mixed-effect logistic regression model (generalized linear mixed model with a binomial link) using the package '*lme4*' (Bates et al., 2015). Herd and villages with herds nested within villages were included as random effects to account for within-cluster correlation of infection status. The exposure variables considered were age, sex, PCV, frequency of acaricide applications, application of acaricide to other livestock species on the farm, the regular grazing area of the herds, and presence of ticks on cattle when collecting blood samples, and the administrative wards. None of the assessed possible risk variables were statistically significant in the univariate model; therefore, we did not fit a multivariate mixed-effect logistic regression model. Variance estimates associated with the random effects (i.e., herd- and village-level clustering) were used to estimate the intra-herd correlation coefficient (ICC) of *T. parva* and *A. marginale* infections following the latent variable approach (Dohoo et al., 2009). A $p$-value $\leq 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Cattle owner demographics

A total of 160 respondents were interviewed across the 14 villages, and the demographic data are summarized in supplementary table 1. Sixty-seven (41.9%) respondents were from Kinango ward in Kwale county, while 93 (58.1%) were from Kayafungo ward in Kilifi county. The majority of the respondents were male (n = 137, 85.6%). All of these were household heads. A sizable portion of respondents had attained a primary level education (n = 74, 46.3%) or was illiterate (n = 60, 37.5%), practiced crop-livestock mixed farming as their primary occupation (n = 131, 81.9%), and had less than 10 years of farming experience (n = 86, 53.8%). The respondents' ages ranged from 20 to 89 years (median 51.0).

### 3.2. Cattle husbandry and tick control practices

Detailed aspects of cattle husbandry and tick control practices among cattle owners in coastal Kenya are shown in Table 2. All the 160 farms in the survey kept the indigenous zebu cattle, and all farmers relied exclusively on natural breeding services rather than artificial insemination. The cattle were reared for multiple purposes, including draft power, sale, and milk production. All sampled farmers in the survey area practiced extensive grazing where the cattle were left to graze free-range in the open environment. Most farmers grazed their cattle on communal land (55.6%, 89/160) and watered their cattle at a river (54.4%, 87/160). Housing was not provided on 60.0% (96/160) of the farms, with the cattle staying under a tree or next to the houses within the homestead. The frequently reported constraints of cattle production in the study area as perceived by farmers included cattle diseases (90.0%, 144/160), inadequate veterinary services (58.8%, 94/160), inadequate water for livestock (43.1%, 69/160), shortage of feed (40.6%, 65/160) and poor market for livestock products (20.6%, 33/160) (Table 2).

The majority of respondents (56.9%, 91/160) perceived an increase in tick infestation levels on cattle during the rainy season. Almost all farmers (96.3%, 154/160) used chemical acaricides for tick control, with the amidine group (mainly Triatix®, amitraz), being the most frequently used acaricide. Most farmers regularly applied the acaricide following the recommended weekly (30.6%, 49/160) or fortnightly (35.0%, 56/160) application regime, depending on the level of tick infestation. The most commonly used method for acaricide application was spraying (88.1%, 141/160), using either a Knapsack sprayer (38.1%, 61/160) or a hand sprayer (50.0%, 80/160). Dipping was encountered in 8.1% (13/160) of the farms, and they were all in Kinango ward in Kwale County. The majority of farmers (62.5%) also applied acaricide to other animals on the farm, besides cattle. Most farms in Kinango ward (47.8%, 32/67) used bought tap water for acaricide dilution, while most farms in Kayafungo ward (49.5%, 46/93) used water pans and ponds as the main sources of water for acaricide dilution. Farmers widely used a calibrated bottle top to measure the volume of acaricide before dilution (70.6%, 113). A large proportion of farmers (92.5%) bought their acaricides from agro-veterinary shops. The agro-veterinary shop attendants were the farmers' most preferred source of advice and information for tick control (64.4%).

### 3.3. Tick-borne disease control practices

Although all the respondents (n = 160) had heard of TBD, about half

**Table 2**
Cattle husbandry and tick control practices on farms in coastal Kenya.

| Query/item | Response category | Administrative ward | | Total (n = 160 farmers) |
|---|---|---|---|---|
| | | Kinango (n = 67 farmers) | Kayafungo (n = 93 farmers) | |
| Cattle grazing land | Communal land | 48 (71.6%) | 41 (44.1%) | 89 (55.6%) |
| | Own pasture farm | 1 (1.5%) | 9 (9.7%) | 10 (6.3%) |
| | Forest area | 17 (25.4%) | 28 (30.1%) | 45 (28.1%) |
| | Neighbor's plot | 1 (1.5%) | 15 (16.1%) | 16 (10.0%) |
| Cattle watering point | River | 54, 80.6% | 33 (35.5%) | 87 (54.4%) |
| | Others (water pan, water pond, rain water) | 13 (19.4%) | 60 (64.5%) | 73 (45.6%) |
| Housing infrastructure | Shaded | 2 (3.0%) | 4 (4.3%) | 6 (3.8%), |
| | Open but fenced | 20 (29.9%) | 38 (40.9%) | 58 (36.3%) |
| | Open and not fenced | 45 (67.2%) | 51 (54.8%) | 96 (60.0%) |
| Constraints associated with cattle production | Cattle disease | 58 (86.6%) | 86 (92.5%) | 144 (90.0%) |
| | Animal feeds | 19 (28.4%) | 46 (49.5%) | 65 (40.6%) |
| | Water source | 23 (34.3%) | 46 (49.5%) | 69 (43.1%) |
| | Animal health and extension services | 34 (50.7%) | 60 (64.5%) | 94 (58.8%) |
| | Market for live animals and milk | 15 (22.4%) | 18 (19.4%) | 33 (20.6%) |
| Season of high tick infestation | Dry season | 22 (32.8%) | 18 (19.4%) | 40 (25.0%) |
| | Rainy season | 38 (56.7%) | 53 (57.0%) | 91 (56.9%) |
| | All year | 7 (10.4%) | 22 (23.7%) | 29 (18.1%) |
| Tick control practice | No tick control | 0 (0.0%) | 4 (4.3%) | 4 (2.5%) |
| | Hand-picking | 1 (1.1%) | 1 (1.1%) | 2 (1.3%) |
| | Chemical acaricide | 66 (98.5%) | 88 (94.6%) | 154 (96.3%) |
| Frequency of acaricide application | Biweekly | 0 (0.0%) | 6 (6.5%) | 6 (3.4%) |
| | Weekly | 25 (37.3%) | 24 (25.8%) | 49 (30.6%) |
| | Every 2 weeks | 32 (47.8%) | 24 (25.8%) | 56 (35.0%) |
| | Monthly | 5 (7.5%) | 11 (11.8%) | 16 (10.0%) |
| | Depends on presence/level of tick infestation | 4 (6.0%) | 23 (24.7%) | 27 (16.9%) |
| Method of applying acaricide | Spraying | 53 (79.1%) | 88 (94.6%) | 141 (88.1%) |
| | Dipping | 13 (19.4%) | 0 (0.0%) | 13 (8.1%) |
| Equipment used to measure the volume of acaricide before dilution | Calibrated bottle top | 47 (70.1%) | 66 (71.0%) | 113 (70.6%) |
| | Acaricide bottle for dip | 13 (19.4%) | 0 (0.0%) | 13 (8.1%) |
| | Non calibrated bottle top | 0 (0.0%) | 19 (20.4%) | 19 (11.9%) |
| | Syringe | 6 (9.0%) | 3 (3.2%) | 9 (5.6%) |
| Type of water used for diluting the acaricide | Tap water | 32 (47.8%) | 7 (7.5%) | 39 (24.4%) |
| | Borehole/well water | 9 (13.4%) | 23 (24.7%) | 32 (20%) |
| | River water | 22 (32.9%) | 11 (11.8%) | 33 (20.6%) |
| | Water pans and ponds | 4 (6.0%) | 46 (49.5%) | 50 (31.3%) |
| Application of acaricide to other farm animals apart from cattle | Yes | 36 (53.7%) | 64 (68.8%) | 100 (62.5%) |
| | No | 30 (44.8%) | 24 (25.8%) | 54 (33.8%) |
| Brand name of acaricides used in the farm in the past 12 months* | **Trade name (Active ingredient)** | | | |
| | ***Synthetic pyrethroids group*** | | | |
| | Dominex (Alpha-cypermethrin) | 14 (20.9%) | 3 (3.2%) | 17 (10.6%) |
| | Decatix (Deltamethrin) | 0 (0.0%) | 1 (1.1%) | 1 (0.6%) |
| | Sypertix (Alpha-cypermethrin) | 30 (44.8%) | 10 (10.8%) | 40 (25.0%) |
| | Bayticol (Flumethrin) | 1 (1.5%) | 5 (5.4%) | 6 (3.4%) |
| | Ectomin (Cypermethrin) | 3 (4.5%) | 2 (2.2%) | 5 (3.1%) |
| | Delete (Deltamethrin) | 0 (0.0%) | 1 (1.1%) | 1 (0.6%) |
| | ***Co-formulation*** | | | |
| | Duodip (Chlorpyrifos 50% + Cypermethrin 5%) | 5 (7.4%) | 6 (6.5%) | 11 (6.9%) |
| | ***Amidine group*** | | | |
| | Taktic (Amitraz) | 15 (22.4%) | 3 (3.2%) | 18 (11.3%) |
| | Triatix (Amitraz) | 30 (44.8%) | 53 (57.0%) | 83 (51.9%) |
| | Norotraz (Amitraz) | 13 (19.4%) | 34 (36.6%) | 47 (29.4%) |
| | Almatix (Amitraz) | 1 (1.5%) | 6 (6.5%) | 7 (4.4%) |
| | Bimatraz (Amitraz) | 1 (1.5%) | 1 (1.1%) | 2 (1.3%) |
| | Actraz (Amitraz) | 5 (7.5%) | 17 (18.3%) | 22 (13.8%) |
| Where do you buy your acaricide* | Agroveterinary store | 62 (92.5%) | 86 (92.5%) | 148 (92.5%) |
| | Veterinary office | 13 (19.4%) | 0 (0.0%) | 13 (8.1%) |
| | Unofficial source (e.g., market, Dips/crush center, fellow farmer) | 8 (11.9%) | 2 (2.2%) | 10 (6.3%) |
| Source of information/ advice on tick control* | Agroveterinary shop attendant | 36 (53.7%) | 67 (72.0%) | 103 (64.4%) |
| | Fellow farmers | 31 (46.3%) | 58 (62.4%) | 89 (55.6%) |
| | Veterinary officer | 34 (50.7%) | 8 (8.6%) | 42 (26.3%) |
| | Radio/TV | 2 (3.0%) | 1 (1.1%) | 3 (1.9%) |
| | Social media | 1 (1.5%) | 1 (1.1%) | 2 (1.3%) |
| | Farmer group organization | 0 (0.0%) | 4 (4.3%) | 4 (2.5%) |
| | Personal judgement/ decision | 0 (0.0%) | 6 (6.5%) | 6 (3.4%) |

* More than one answer was allowed. The frequency of mention for a given answer response is the percentage of total respondents.

(48.1%, 77/160) could correctly name at least one TBD (Table 3). East Coast fever (locally known as "*ngai*") was the most frequently named TBD and was associated with cattle infections and losses (46.3%), followed by anaplasmosis (3.1%), babesiosis (2.5%), and heartwater (0.6%). A total of 127 (79.4%) respondents perceived TBD as having ever occurred on their farm, while 71 (44.4%) had perceived TBD cases in the past 12 months. A quarter of the farmers seld-diagnosed the TBD when they occurred on their farms (25%, 40/160), and 6.9% (11/160) of the farmers sought the diagnostic services of a veterinary officer. The frequently mentioned drugs used to treat cases of TBD on the farm included the antibiotic adacycline (19.4%, 31/160) and antiprotozoal

**Table 3**
Tick-borne disease management practices on farms in coastal Kenya.

| Query/item | Response | Administrative ward | | Total (n = 160 farmers) |
|---|---|---|---|---|
| | | Kinango ward (n = 67 farmers) | Kayafungo ward (n = 93 farmers) | |
| Ability to name a tick-borne diseases | Yes | 20 (29.9%) | 57 (61.3%) | 77 (48.1%) |
| | No | 47 (70.1%) | 36 (38.7%) | 83 (51.9%) |
| Named tick-borne diseases frequently associated with cattle infection or losses* | East Coast fever (ECF) | 20 (29.9%) | 54 (58.1%) | 74 (46.3%) |
| | Babesiosis/ redwater | 2 (3.0%) | 2 (2.2%) | 4 (2.5%) |
| | Anaplasmosis | 0 (0.0%) | 5 (5.4%) | 5 (3.1%) |
| | Heartwater | 0 (0.0%) | 1 (1.1%) | 1 (0.6%) |
| Occurrence of tick-borne diseases in the farm | Yes | 56 (83.6%) | 71 (76.3%) | 127 (79.4%) |
| | No | 11 (16.4%) | 22 (23.7%) | 33 (20.6%) |
| Occurrence of tick-borne diseases in the farm in the past 12 months | Yes | 26 (38.8%) | 45 (48.4%) | 71 (44.4%) |
| | No | 41 (61.2%) | 48 (51.6%) | 89 (55.6%) |
| Personnel who confirmed the diagnosis when the disease occurred in the farm in the past 12 months | Veterinary personnel (Vet officers, animal health officers, etc.) | 3 (4.5%) | 8 (8.6%) | 11 (6.9%) |
| | Self/ family member judgment | 21 (31.3%) | 19 (20.4%) | 40 (25.0%) |
| | Herdsman/ employee on the farm | 0 (0.0%) | 1 (1.1%) | 1 (0.6%) |
| | Para veterinarians (non-professional but possess the knowledge for drug and vaccine delivery) | 1 (1.5%) | 5 (5.4%) | 6 (3.4%) |
| | Agro veterinary dealer | 1 (1.5%) | 0 (0.0%) | 1 (0.6%) |
| | Fellow farmer | 0 (0.0%) | 12 (12.9%) | 12 (7.5%) |
| Drugs used to treat the animal when they fell ill in the past 12 months* | Butalex | 6 (9.0%) | 27 (29.0%) | 33 (20.6%) |
| | Parvexon | 4 (6.0%) | 0 (0.0%) | 4 (2.5%) |
| | Buperquine | 2 (3.0%) | 0 (0.0%) | 2 (1.3%) |
| | Adacycline LA 20% | 7 (10.4%) | 24 (25.8%) | 31 (19.4%) |
| | Alamycin LA 20% | 0 (0.0%) | 15 (16.1%) | 15 (9.4%) |
| | Bimahistamine | 0 (0.0%) | 2 (2.2%) | 2 (1.3%) |
| | Imochem | 1 (1.5%) | 0 (0.0%) | 1 (0.6%) |
| | Imizol | 3 (4.5%) | 0 (0.0%) | 3 (1.9%) |
| | Diminakel | 0 (0.0%) | 4 (4.3%) | 4 (2.5%) |
| | Veriben (plain) | 4 (6.0%) | 9 (9.7%) | 13 (8.1%) |
| | Veriben + B12 vitamin | 13 (19.4%) | 14 (15.0%) | 27 (16.9%) |
| | Epsom salt | 1 (1.5%) | 0 (0.0%) | 1 (0.6%) |
| | Not sure/ Don't know | 3 (4.5%) | 5 (5.4%) | 8 (5.0%) |
| Source of information/ advice on tick-borne disease control* | Agrovet shop attendant | 39 (58.2%) | 73 (78.5%) | 112 (70.0%) |
| | Fellow farmers | 29 (43.3%) | 62 (66.7%) | 91 (56.9%) |
| | Veterinary personnel (Vet officers, animal health assistants, etc.) | 43 (64.1%) | 14 (15.1%) | 57 (35.6%) |
| | Local/ traditional healers | 1 (1.5%) | 0 (0.0%) | 1 (0.6%) |
| | Paravets (non-professional but trained for drug and vaccine delivery) | 16 (23.9%) | 1 (1.1%) | 17 (10.6%) |
| | Radio/TV/ newspaper/ magazines | 2 (3.0%) | 0 (0.0%) | 2 (1.3%) |
| | Social media | 1 (1.5%) | 0 (0.0%) | 1 (0.6%) |
| | Farmer co-op/ union/group | 0 (0.0%) | 3 (3.2%) | 3 (1.9%) |

* This was a multi-response question. The frequency of mention is expressed as the percentage of group-specific respondents.

Butalex® (20.6%, 33/160). Five percent (8/160) could not remember the name of the drug used. The most common source of awareness regarding TBD was the agro-veterinary shop attendant (70.0%, 112/160), followed by fellow farmers (56.9%, 91/160). Only 2 (1.3%) respondents could not describe the perceived clinical signs and symptoms of TBD on cattle. The most commonly cited symptoms suggestive of TBD were enlarged lymph nodes (53.1%), loss of appetite (49.4%), cough (48.8%), and fever (40.6%) (Supplementary table 2).

### 3.4. Diversity and identity of tick-borne pathogens detected

We detected *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. by PCR-HRM. *Ehrlichia* spp. or *Rickettsia* spp. were not detected. The *Anaplasma* 16 S rDNA sequences detected in this study were identical to reference *Anaplasma* sp., *Anaplasma platys* and *A. marginale* sequences (Supplementary table 3). Sequencing of the amplified *msp4* gene validated the identity of *Anaplasma* sp. and *A. marginale*. The maximum likelihood phylogenetic analysis of the 16 S rDNA and *msp4* sequences from this study showed that all the *Anaplasma* spp. from coastal Kenya clustered together in the same clade with related species found in other parts of the world (Fig. 3). Blast analysis of *Babesia/Theileria* spp. 18 S rRNA sequences identified the presence of *T. velifera*, *T. parva*, and *B. bigemina*.

### 3.5. Prevalence and risk factor analysis of tick-borne pathogens detected

Of the 1486 cattle tested, 359 (24.2%, 95% confidence interval (CI): 22.0–26.4) were positive for at least one TBP (Table 4 and Supplementary table 4). The overall herd levels prevalence was 75.6% (95% CI: 68.1–81.9). The most prevalent pathogen was *A. marginale*, followed by *T. parva, A. platys, T. velifera, B. bigemina,* and *Anaplasma* sp. Dual infections were detected in 1.4% (95% CI: 0.9–2.2) cattle, and the highest frequency of co-infection was recorded for *T. parva* and *A. marginale* (1.0%), followed by *T. parva* and *A. platys* (0.3%), *A. marginale* and *T. velifera* (0.1%), and *A. marginale* and *B. bigemina* (0.1%) (Table 4).

Results from the univariate mixed-effect logistic regression model showed that none of the assessed risk variables were statistically significant for TBP infection in cattle (Table 5). Estimations of ICC values found a substantially higher value for *A. marginale* infection (0.29) compared to *T. parva* infection (0.14).

OR: odds ratio, CI: confidence interval, No.: Number, + ve: Positive.

## 4. Discussion

The current study provides molecular evidence of the diversity of TBP in cattle and information on the management practices relating to ticks and tick-borne diseases among cattle owners in coastal Kenya. The use of chemical acaricide was the primary method for tick control, with the amidine group (mainly Triatix®) being the most frequently used acaricide. East Coast fever was the most important disease and Butalex®

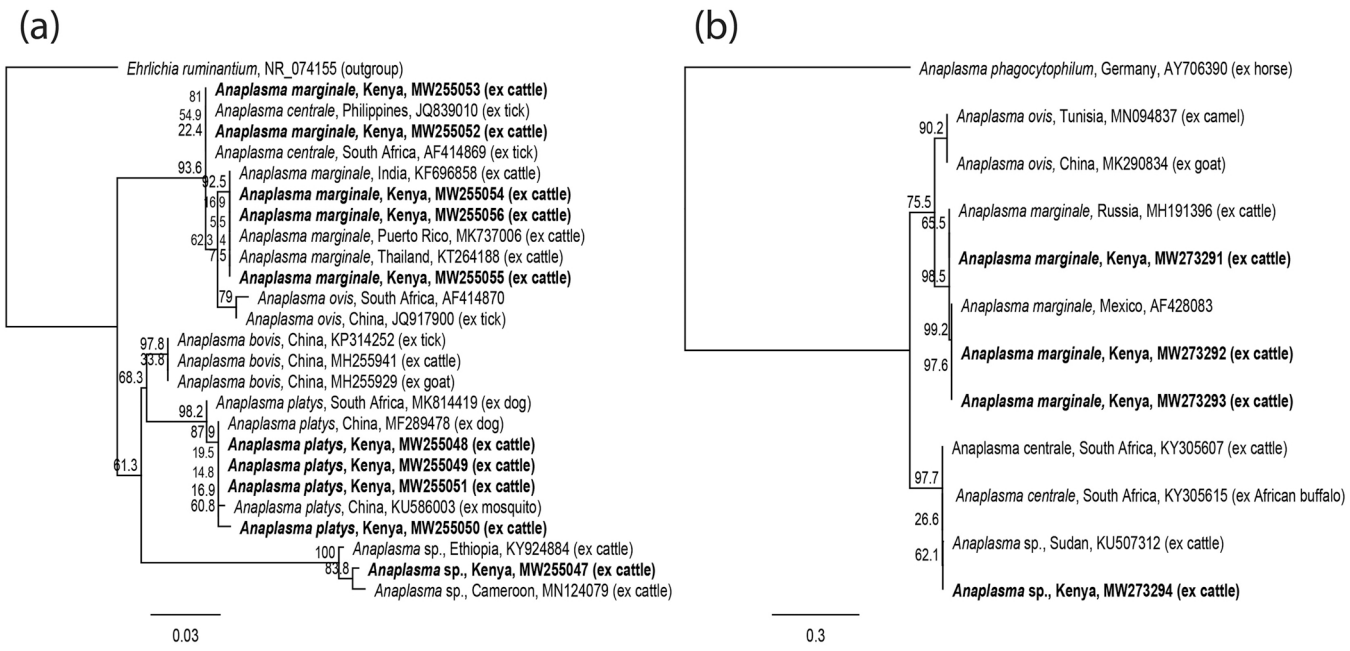


**Fig. 3.** Maximum-likelihood phylogenetic analysis of *Anaplasma* spp. using (a). 16 S rRNA sequences and (b) *msp4* sequences constructed using the Tamura Nei evolutionary model (TN93). The sequences obtained in the present study are highlighted in bold. Numbers on the nodes indicate percentages of 1000 bootstrap replicates. The scale bars represent substitutions per site.

**Table 4**
Individual animal- and herd-level prevalence of tick-borne pathogens in cattle from coastal Kenya.

| Pathogen | Animal-level prevalence | | Herd-level prevalence | |
|---|---|---|---|---|
| | No. of positive cattle[a] | % prevalence (95% CI) | No. of positive herds[b] | % prevalence (95% CI) |
| *Single pathogen infections* | | | | |
| *Anaplasma marginale* | 162 | 10.9 (9.4 – 12.6) | 76 | 47.5 (39.6 – 55.5) |
| *Anaplasma platys* | 39 | 2.6 (1.9 – 3.6) | 27 | 16.9 (11.6 – 23.8) |
| *Anaplasma* spp. | 1 | 0.1 (0.0 – 0.4) | 1 | 0.6 (0.0 – 4.0) |
| *Babesia bigemina* | 7 | 0.5 (0.2 – 1.0) | 6 | 3.8 (1.5 – 8.3) |
| *Theileria parva* | 133 | 9.0 (7.6 – 10.5) | 79 | 49.4 (41.4 – 57.4) |
| *Theileria velifera* | 17 | 1.1 (0.7 – 1.9) | 12 | 7.5 (4.1 – 13.0) |
| Overall | **359** | **24.2 (22.0 – 26.4)** | **121** | **75.6 (68.1 – 81.9)** |
| *Co-infections* | | | | |
| *A. marginale* + *B. bigemina* | 1 | 0.1 (0.0 – 0.4) | 1 | 0.6 (0.0 – 3.4) |
| *A. marginale* + *T. parva* | 15 | 1.0 (0.6 – 1.7) | 12 | 7.5(3.9 – 12.7) |
| *A. marginale* + *T. velifera* | 1 | 0.1 (0.0 – 0.4) | 1 | 0.6 (0.0 – 3.4) |
| *A. platys* + *T. parva* | 4 | 0.3 (0.0 – 0.7) | 4 | 2.5 (0.7 – 6.3) |
| Overall | **21** | **1.4 (0.9 – 2.2)** | **14** | **8.8 (4.9 – 14.2)** |

[a] Total of individual cattle tested positive out of 1486, [b] total number of herds tested positive out of 160 herds.

was the most commonly administered drug in response to perceived TBD in cattle. The present study detected *A. marginale, B. bigemina* and *T. parva,* which are economically important in livestock production in Kenya. Additionally, the study reported *Anaplasma* sp., *A. platys* and *T. velifera,* whose epidemiology and association with clinical disease in cattle in Kenya are still unclear.

### 4.1. Tick and tick-borne disease control practices

As perceived by farmers, the most important constraints to cattle production in the study area included cattle diseases (mainly ECF), inadequate veterinary services, inadequate water for livestock, and shortage of feed, and a poor market for livestock products. Similar constraints have been identified in other cattle production systems in Kenya (Mugambi et al., 2012; Ohaga et al., 2007; Wesonga et al., 2010), Uganda (Byaruhanga et al., 2015), and Tanzania (Swai et al., 2005). There is, therefore, a need to improve access to veterinary extension services in the region to mitigate the impact of these constraints on cattle production.

In the current study, the respondents exhibited a high level of awareness of ticks, as 96.3% of the farmers use chemical acaricides for tick control. Nevertheless, we identified a few malpractices associated with acaricide use, including farmers' failure to adhere to the manufacturer's instructions on the correct acaricide dilution and frequency of application. Indeed, 11.9% of the farms used non-calibrated materials to measure the volume of acaricide for dilution, while another 3.4% of the farms had adopted a shorter acaricide application interval (twice a week) as opposed to the recommended weekly or fortnightly interval. Such malpractices pose a serious threat to public and environmental health and could lead to the emergence and spread of acaricide resistance in the region (De Meneghi et al., 2016; Vudriko et al., 2016).

We also identified several malpractices associated with the diagnosis and treatment of cattle infection on the farms. The farmers treated the sick cattle based on clinical signs without seeking accurate diagnostic services from the local veterinary office that guided rational prescriptions. Instead, most farmers relied on the advice given by local agro-veterinary shop attendants and fellow cattle farmers on the choice of drugs to use. Such malpractices may complicate the control of TBD in the region especially when the wrong information is spread, or an incorrect dosage is prescribed (Irungu et al., 2008).

### 4.2. Tick-borne pathogens identified in cattle

*Anaplasma marginale* was the most prevalent pathogen (10.9%). This bacterium can be transmitted biologically to cattle by infected hard ticks

**Table 5**
Descriptive statistics of cattle (n = 1486) from coastal Kenya and univariable analysis of potential risk factors associated with *T. parva* and *A. marginale* infections using mixed effect logistic regression modeling.

| Risk factor | Category | Total No. (%) | *A. marginale* | | | *T. parva* | | |
|---|---|---|---|---|---|---|---|---|
| | | | No. + ve (%) | *P*-value | OR (95% CI) | No. + ve (%) | *P*-value | OR (95% CI) |
| **Animal variables** | | | | | | | | |
| **Sex** | Female | 898 (60.4) | 89 (9.9) | – | 1.0 | 87 (9.7) | – | 1.0 |
| | Male | 588 (39.6) | 73 (12.4) | 0.33 | 1.2 (0.8–1.7) | 46 (7.8) | 0.114 | 0.7 (0.5–1.1) |
| **Age** | Calf | 115 (7.7) | 11 (9.6) | – | 1.0 | 9 (7.8) | – | 1.0 |
| | Juvenile | 426 (28.7) | 53 (12.4) | 0.547 | 1.3 (0.6–2.6) | 38 (8.9) | 0.474 | 1.3 (0.6–2.9) |
| | Adult | 945 (63.6) | 98 (10.4) | 0.772 | 1.1 (0.6–2.2) | 86 (9.1) | 0.548 | 1.3 (0.6–2.9) |
| **PCV** | ≤ 23 | 240 (16.2) | 30 (12.5) | – | 1.0 | 24 (10.0) | – | 1.0 |
| | ≥ 24 | 1246 (83.8) | 132 (10.6) | 0.636 | 0.9 (0.6–1.4) | 109 (8.7) | 0.705 | 0.9 (0.6–1.5) |
| **Farm variables** | | | | | | | | |
| **Frequency of acaricide application** | None | 31 (2.1) | 3 (9.7) | – | 1.0 | 2 (6.5) | – | 1.0 |
| | Irregular | 242 (16.3) | 21 (8.7) | 0.555 | 0.6 (0.1–3.0) | 14 (5.8) | 0.813 | 0.8 (0.2–4.0) |
| | Regular | 1213 (81.6) | 138 (11.4) | 0.593 | 0.7 (0.1–3.0) | 117 (9.6) | 0.916 | 1.1 (0.2–5.0) |
| **Presence of ticks on cattle when collecting blood samples** | No | 1162 (78.2) | 123 (10.6) | – | 1.0 | 105 (9.0) | – | 1.0 |
| | Yes | 324 (21.8) | 39 (12.0) | 0.758 | 1.0 (0.6–1.5) | 28 (8.6) | 0.758 | 0.9 (0.6–1.5) |
| **Application of the acaricide to other farm animals other than cattle** | No | 521 (35.1) | 56 (10.7) | – | 1.0 | 47 (9.0) | – | 1.0 |
| | Yes | 965 (64.9) | 106 (11.0) | 0.879 | 1.1 (0.6–1.6) | 86 (8.9) | 0.783 | 1.1 (0.7–1.6) |
| **Grazing field** | Own pasture farm | 70 (4.7) | 5 (7.1) | – | 1.0 | 5 (7.1) | – | 1.0 |
| | Shared/common land | 1416 (95.3) | 157 (11.1) | 0.201 | 2.2 (0.6–7.8) | 128 (9.0) | 0.815 | 1.1(0.4 – 3.1) |
| **Area variables** | | | | | | | | |
| **Administrative ward** | Kinango | 781 (52.6) | 66 (8.5) | – | 1.0 | 85 (10.9) | – | 1.0 |
| | Kayafungo | 705 (47.4) | 96 (13.6) | 0.381 | 1.5 (0.6–3.7) | 48 (6.8) | 0.272 | 0.7 (0.3–1.4) |

(*Rhipicephalus* spp.) and mechanically by infected biting flies (*Stomoxys* spp., *Tabanus* spp.) and by blood-contaminated fomites (such as needles, ear tagging, and dehorning) (Aubry and Geale, 2011). This multitude of transmission routes may be responsible for the high prevalence of *A. marginale* in this region. The high molecular prevalence of 10.9% in the present study was not surprising, based on the similarly high sero-prevalences reported in similar settings in Eastern Kenya (58.3%) (Gachohi et al., 2010) and coastal Kenya (81–97%) (Maloo et al., 2001b).

*Babesia bigemina* is the causative agent for bovine babesiosis and is transmitted by *Rhipicephalus* ticks (Bock et al., 2004). Only 0.5% of cattle sampled were positive for the protozoan *B. bigemina,* which is consistent with previous molecular-based study from western Kenya (Njiiri et al., 2015). The low prevalence of *B. bigemina* reported in this study is in agreement with the apparent absence of the *B. bigemina* pathogen in ticks in this region, as found in an earlier study (Oundo et al., 2022).

East Coast fever (ECF), caused by *T. parva*, is the most economically important TBD in Kenya, causing high morbidity and mortality in cattle (Gachohi et al., 2012; Wesonga et al., 2010). The prevalence of *T. parva* (9.0%) recorded here is comparable to the previous molecular finding reported on farms in western Kenya (12.9%) (Njiiri et al., 2015). The low prevalence of *T. parva* in this study area are in agreement with the low infection rates in ticks in this region, as found in an earlier study (Oundo et al., 2022).

This study also confirms the occurrence of an uncharacterized *Anaplasma* sp., *Anaplasma platys* and *Theileria velifera* in cattle. Although their epidemiology and association with clinical disease in cattle in Kenya are still unclear, *T. velifera* is generally non-pathogenic to cattle, while *A. platys* causes canine cyclic thrombocytopenia (Harvey et al., 1978). Therefore, further detailed epidemiological investigations are required to determine their potential pathogenicity on cattle production in coastal Kenya.

*Ehrlichia minasensis*, *Ehrlichia ruminantium* and *Rickettsia africae* were recently detected in *Rhipicephalus* and *Amblyomma* ticks in the same study area (Oundo et al., 2022). However, the present study did not detect any species of *Ehrlichia* or *Rickettsia* in any of the cattle samples analyzed. The absence of *Ehrlichia* in our samples may be attributed to the biology of *Ehrlichia* species, as it mainly resides in endothelial cells and is only periodically found in the bloodstream during the febrile stage of infection (Andrew and Norval, 1989; Steyn et al., 2008). The absence of *R. africae* corroborates previous studies in western Kenya that recorded no evidence of pathogenic rickettsial species in blood samples collected from livestock (Chiuya et al., 2021; Maina et al., 2014; Okal et al., 2020).

Co-infections were detected in 21 blood samples (1.4%) and were mainly due to double infections. The overall co-infection prevalence reported in the present study is lower than in previous studies in Lambwe Valley in Kenya (31.6%) (Okal et al., 2020) and in western Kenya (87.1%) (Njiiri et al., 2015). The most frequent co-occurrences included *A. marginale* and *T. parva,* followed by *A. platys* and *T. parva, A. marginale* and *T. velifera,* and *A. marginale* and *B. bigemina*. These co-infections may have consequences on TBD management in the region as it may complicate the clinical presentation, diagnosis and treatment in cattle with multiple pathogen infections than those with single infections (Diuk-Wasser et al., 2016; Hofmann-Lehmann et al., 2004; Moutailler et al., 2016). Therefore, veterinary practitioners should be aware of co-infections in cattle from coastal Kenya as this may warrant different clinical management strategies.

The epidemiology of TBPs in cattle varies depending on the agro-ecological zone, livestock production system, and individual animal traits such as sex, breed and age (Gachohi et al., 2012). In this study, we

did not find any significant association between TBP infection status and potential risk factors in cattle. This general lack of significant risk factors for TBP positivity in cattle may suggest a relatively uniform distribution of the infections across the study area and that the study population was possibly too uniform in terms of herd management practices, and therefore difficult to detect clear differences in the classical risk factors. Further research is therefore needed to better understand the risk of TBP transmission in other extensive livestock systems of Kenya and to address the potential of control options.

The computed lCCs in this study were 0.29 (*A. marginale*) and 0.14 (*T. parva*), and these were within the previously reported ranges of 0–0.6 for five TBD (i.e., *A. marginale, B. bigemina, E. ruminantium, T. mutans* and *T. parva*) (Deem et al., 1993; Gachohi et al., 2010; Otte and Gumm, 1997). Our computed ICC estimates can inform the design effects needed to adjust for cluster sampling in future TBD surveys in areas with similar agro-climatic and ecological conditions and production systems.

## 5. Conclusions

This study identified several malpractices in the management of ticks and TBD among cattle owners in the coastal region. These included inappropriate acaricide dilution and frequency of acaricide application, and overreliance on unprofessional sources rather than the veterinarians regarding diagnosis and treatment of sick cases on their farms. This study also provides molecular evidence of the existence of highly pathogenic *A. marginale*, *B. bigemina* and *T. parva,* as well as other pathogens, including uncharacterized *Anaplasma* sp., *A. platys*, and *T. velifera* in cattle from coastal Kenya. The general lack of association between the prevalence of *A. marginale* or *T. parva* with the animal-, farm- and area-level variables suggests that the study population was possibly too uniform in terms of herd management practices. There is a need to intensify integrated tick control programs to reduce the risk and burden of disease in the area.

## Ethical statement

This study received ethical clearance from the Institutional Animal Care and Use Committee of *icipe* (IACUC, Reference No. Oundo-icipeACUC-Mar2020), and the Pwani University Ethics Review (approval number ERC/EXT/002/2020). Further approval was sought from Kenya's National Commission for Science, Technology and Innovation (NACOSTI/P/21/6726). The cattle owners were verbally informed of the study objectives and the sampling protocol and provided oral consent for sampling their cattle. This study did not involve endangered or protected species. Any mention of the brand name of an acaricide or drug should not be taken as a promotion or demotion of the product.

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## CRediT authorship contribution statement

**Joseph Wang'ang'a Oundo:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Daniel Masiga:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. **Quirine ten Bosch:** Supervision, Writing – review & editing. **Jandouwe Villinger:** Writing – review & editing. **Constantianus J.M. Koenraadt:** Supervision, Writing – review & editing. **Shewit Kalayou:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

## Competing interests

The authors declare they have no competing interests.

## Data Availability

The data supporting the conclusions of this article are included within the article and its additional file.

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*Consent for publication*

Not applicable.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2022.105777.

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