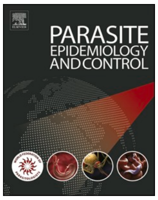
Parasite Epidemiology and Control 27 (2024) e00384

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi



A randomized controlled trial of Tickoff® (*Metarhizium anisopliae* ICIPE 7) for control of tick infestations and transmission of tick-borne infections in extensively grazed zebu cattle in coastal Kenya

Joseph Wang'ang'a Oundo [a,b,*], Shewit Kalayou [a], Gerrit Gort [c], Gebbiena M. Bron [b,d], Constantianus J.M. Koenraadt [e], Quirine ten Bosch [b,1], Daniel Masiga [a,1]

[a] *International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya*
[b] *Quantitative Veterinary Epidemiology, Wageningen University & Research, Wageningen, the Netherlands*
[c] *Biometris, Wageningen University & Research, Wageningen, the Netherlands*
[d] *Norwegian Veterinary Institute, Department of Animal Health, Animal Welfare and Food Safety, Norway*
[e] *Laboratory of Entomology, Wageningen University & Research, Wageningen, the Netherlands*

ARTICLE INFO

Keywords:
Tick
Anaplasma marginale
Theileria parva
Biological control
Entomopathogenic fungus
Cattle
Kenya

ABSTRACT

The entomopathogenic fungus *Metarhizium anisopliae* isolate ICIPE 7 is being developed as an eco-friendly alternative to chemical acaricides in managing natural tick infestation on livestock. Its impact on tick infestation and tick-borne infections in cattle under natural conditions are yet unclear. We conducted a randomized controlled field trial to assess the safety and effects of Tickoff® (a formulation of *M. anisopliae* isolate ICIPE 7) and the chemical acaricide Triatix® on tick infestation and incidence of *Anaplasma marginale* and *Theileria parva* in extensively grazed zebu cattle in coastal Kenya. A total of 217 eligible herds comprising 1459 intent-to-treat zebu cattle were enrolled from 12 villages. The herds were randomly assigned in a 1:1:1 ratio to Tickoff®, Triatix®, or Tickoff® excipients. Tick counts, treatment administrations, and adverse events were registered every two weeks for seven months. The mortality of ticks collected from treated cattle was monitored in vitro. Infections with *A. marginale* and *T. parva* were monitored every two months. No adverse events were reported in either treatment group. Tickoff® did not significantly affect tick infestation ($p = 0.869$) or infection incidence ($p > 0.05$) compared to excipients. Triatix® significantly reduced tick infestation ($p < 0.001$) and incidence of *T. parva* ($p = 0.042$), but not *A. marginale* ($p = 0.509$) compared to the reference Tickoff®. In ticks that were removed from cattle, Tickoff® demonstrated significant pathogenicity in vitro relative to excipients (hazard ratio: 8.50, 95 % CI: 4.67–15.47). Fungus growth and sporulation were also observed on tick cadavers from Tickoff®, but not from excipients. While Tickoff® did not impact tick counts, its delayed, but significant effect on tick mortality may hinder onward pathogen transmission and give rise to indirect (i.e., to untreated animals) epidemiological effects, that were not picked up with this study design. Additionally, adverse environmental conditions

---

Abbreviations: 95 % CI, 95 % confidence interval; ECF, East Coast fever; HR, Hazard ratio; ITT, Intention-to-treat; TBDs, tick-borne diseases.

[*] Corresponding author at: International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya
E-mail address: joundo@icipe.org (J.W. Oundo).

[1] *Equal senior contributions.*

https://doi.org/10.1016/j.parepi.2024.e00384
Received 11 April 2024; Received in revised form 25 August 2024; Accepted 3 October 2024

resulted in low tick abundance and pathogen circulation towards the end of the study period, reducing the power of the study. This work re-emphasizes the challenges of randomized controlled field trials and the complexity of assessing the impact of vector control products on both direct and indirect impacts on pathogen transmission.

1. Introduction

Ticks (Acari: Ixodidae) are responsible for significant economic losses to the livestock industry. This is the result of direct effects through reductions in meat and milk yields, damage to teats, skins and hides, blood loss, and even anemia (Jongejan and Uilenberg, 2004). In addition, ticks have indirect effects through their role as vectors of viral, bacterial, and protozoal agents that cause tick-borne diseases (TBDs) in livestock and humans (Walker et al., 2003). Added to the impact on the livestock industry, ticks and tick-borne diseases significantly affect the socioeconomic conditions of small-scale, resource-poor households by reducing the contribution of livestock to food and nutrition security, household income, and poverty alleviation. In vast areas of Kenya, zebu cattle are kept under a traditional extensive management system which is characterized by a constant high risk of tick infestations and TBD transmission (Gachohi et al., 2012). In this extensive management system, cattle are grazed extensively on fallow or communal grazing fields in natural pastures and share watering points. This uncontrolled sharing of grazing land and water points results in the mixing of herds from different areas. Therefore, sustainable strategies are needed to control tick infestations on cattle and reduce tick-borne pathogen transmission.

Tick species undergo four developmental life stages i.e., eggs, larvae, nymphs, and adults. Except for eggs, these stages vary substantially in the duration and host interaction, depending on whether the ticks are one-host, two-host, or three-host species (Walker et al., 2003). One-host ticks, like those in the *Boophilus* sub-genus of the *Rhipicephalus* genus, complete all stages on a single host, typically within three weeks for feeding and two months for development. Two-host ticks feed as larvae and nymphs on one host, then drop to the ground to molt into adults, which feed on a different host. In contrast, three-host ticks, such as those in the *Amblyomma* genus, feed on a different host at each stage, leading to much longer life cycles, ranging from six months to several years. This manuscript focuses on the control of all on-host ticks and the pathogens they transmit, especially *A. marginale* and *T. parva*.

East Coast fever (ECF) and bovine anaplasmosis are among the most economically important TBDs of cattle in Kenya (Gachohi et al., 2012; Moumouni et al., 2015). East Coast fever is caused by the protozoan *Theileria parva* and transmitted by the three-host tick *Rhipicephalus appendiculatus*. The Cape buffalo (*Syncerus caffer*) is the natural reservoir host for *T. parva* (Gachohi et al., 2012; Nene et al., 2016). The disease is endemic in eastern, central, and southern Africa where it causes considerable economic losses, especially to resource-poor smallholder farmers and pastoralists. Infected cattle can exhibit a mild, moderate, or severe clinical disease, and those that recover following treatment or spontaneous recovery become long-term asymptomatic carriers and can infect ticks (Baylis et al., 1992; Kariuki et al., 1995; Olds et al., 2018). On the other hand, bovine anaplasmosis is caused by the intra-erythrocytic bacteria *Anaplasma marginale,* and occurs mainly in tropical and subtropical areas, causing high morbidity and mortality in susceptible animals (Aubry and Geale, 2011). The pathogen is transmitted biologically by approximately 20 different tick species and mechanically by biting flies or blood-contaminated fomites (Aubry and Geale, 2011). In sub-Saharan Africa, the main tick vectors are *Hyalomma rufipes, Rhipicephalus annulatus, Rhipicephalus decoloratus, Rhipicephalus microplus, Rhipicephalus evertsi,* and *Rhipicephalus simus* (Walker et al., 2003). The severity of *A. marginale* infection in cattle is age dependent. The disease is acute and often fatal in adult cattle over two years of age. Animals between one and two years of age suffer from acute but rarely fatal disease, while animals aged between six to twelve months usually develop mild disease. Calves are less susceptible to clinical disease and the illness is rare under six months of age (Aubry and Geale, 2011). Recovered animals remain persistently infected carriers for life and act as a source of infection to ticks (Aubry and Geale, 2011). The sustained transmission of tick-borne pathogens in a susceptible population is determined by several factors, including the ratio of ticks to cattle and the number of tick bites per day per animal. These factors affect the basic reproduction number (R_0), a metric for transmission efficiency (Hartemink et al., 2008). An R_0 greater than one (>1) allows the pathogen to persist in a population.

Control of the tick population on cattle could potentially reduce the risk of exposure to tick-borne infections and disrupt pathogen transmission cycles (Hoch et al., 2012; Medley et al., 1993). Control of tick populations on cattle has relied heavily on the use of chemical acaricides, but the widespread emergence of resistance in ticks threatens its long-term sustainability (Abbas et al., 2014; Githaka et al., 2022) as well as by contamination of the environment, meat and milk products with toxic residues when withdrawal periods are not respected (De Meneghi et al., 2016). This highlights the need to develop tick control strategies that are safe and efficacious and provide sustainable options for controlling tick infestations on livestock. Biological control of ticks with entomopathogenic fungi is a promising alternative to chemical acaricides. In vitro studies have shown that the pathogenic effects of the fungus *Metarhizium anisopliae* are not limited to the direct effect on tick mortality, but also continue in female ticks by reducing fecundity, egg hatchability and engorgement weight, and increasing engorgement duration, pre-oviposition, oviposition and post-oviposition periods (Camargo et al., 2012; Nana et al., 2015). However, the existing data supporting *M. anisopliae* efficacy under field conditions are limited to small-scale and short-term studies, and with inconsistent reports on control levels (Alonso-Díaz et al., 2022; Barbieri et al., 2023; Correia et al., 1998; Murigu et al., 2016; Samish et al., 2014). Additionally, these studies are often limited by low statistical power and did not include data on epidemiological outcomes, such as the incidence of tick-borne infections in cattle populations. Robust large-scale randomized controlled trials that are the gold standard for providing empirical evidence of efficacy are therefore needed to establish the efficacy of formulations of entomopathogenic fungi under natural field conditions.

The biopesticide Tickoff® is a product based on the entomopathogenic fungus *M. anisopliae* ICIPE 7 and which is being developed as an alternative to chemical acaricides for the control of tick infestation on livestock. The effect of this biopesticide in reducing natural tick infestation on cattle has not yet been established in a large-scale field trial. Knowledge on the effectiveness of this biopesticide in reducing the incidence of tickborne infections in cattle is limited. We therefore conducted a randomized controlled field trial to evaluate the safety and effects of Tickoff® in reducing (1) natural tick infestations and (2) the incidence of *A. marginale* and *T. parva* infections in indigenous zebu cattle (*Bos indicus*) managed under an extensive grazing system in coastal Kenya. We included the synthetic acaricide Triatix® as a positive comparator and the excipients of Tickoff® as a placebo control.

2. Materials and methods

2.1. *Study design*

We implemented the protocol as outlined in supplementary methods (Appendix A). This randomized controlled trial was conducted during the dry and rainy seasons, from December 2021 to July 2022, in twelve villages in Kayafungo ward, Kaloleni sub-county in Kilifi County in coastal Kenya (Fig. 1). The selected villages were easily accessible by vehicle, practiced livestock farming and the region is a known hotspot for tick-borne diseases (Maloo et al., 2001). Herds composed of local zebu cattle and managed under an extensive grazing system were enrolled in the study based on evidence of infestation with live attached ticks, generally in good health, and the owners' willingness to participate in the study. Qualifying herds were stratified by village, herd size, and tick infestation level and randomly allocated in a 1:1:1 ratio to either Tickoff® (Real IPM Ltd., Kenya), Triatix® (12.5 % EC amitraz, CKL Africa Ltd., Kenya), and excipient of Tickoff® (Real IPM Ltd., Kenya). Tickoff® formulation was prepared using *M. anisopliae* ICIPE 7 (4×10^9 conidia/mL) as the active ingredient, mixed with canola oil (95 %), 0.05 % Triton X-100 (1.5 %) and Kerosene (3.5 %). The excipient contains the formulation of Tickoff® without *M. anisopliae* ICIPE 7. The excipient was chosen as a control treatment to ensure blinding of the experiment: the smell of the treatment is similar to that of Tickoff®. However, owing to the color of the products, it was not possible to fully blind the study. Treatment was allocated at the herd level to ensure adequate protection of all cattle in a herd, with measurements of effectiveness conducted at the individual cattle level. The treatments i.e., Tickoff®, Triatix® and the excipient, were diluted as recommended by the manufacturer and were topically applied to the skin using a hand rocker sprayer with a cone-type nozzle and a

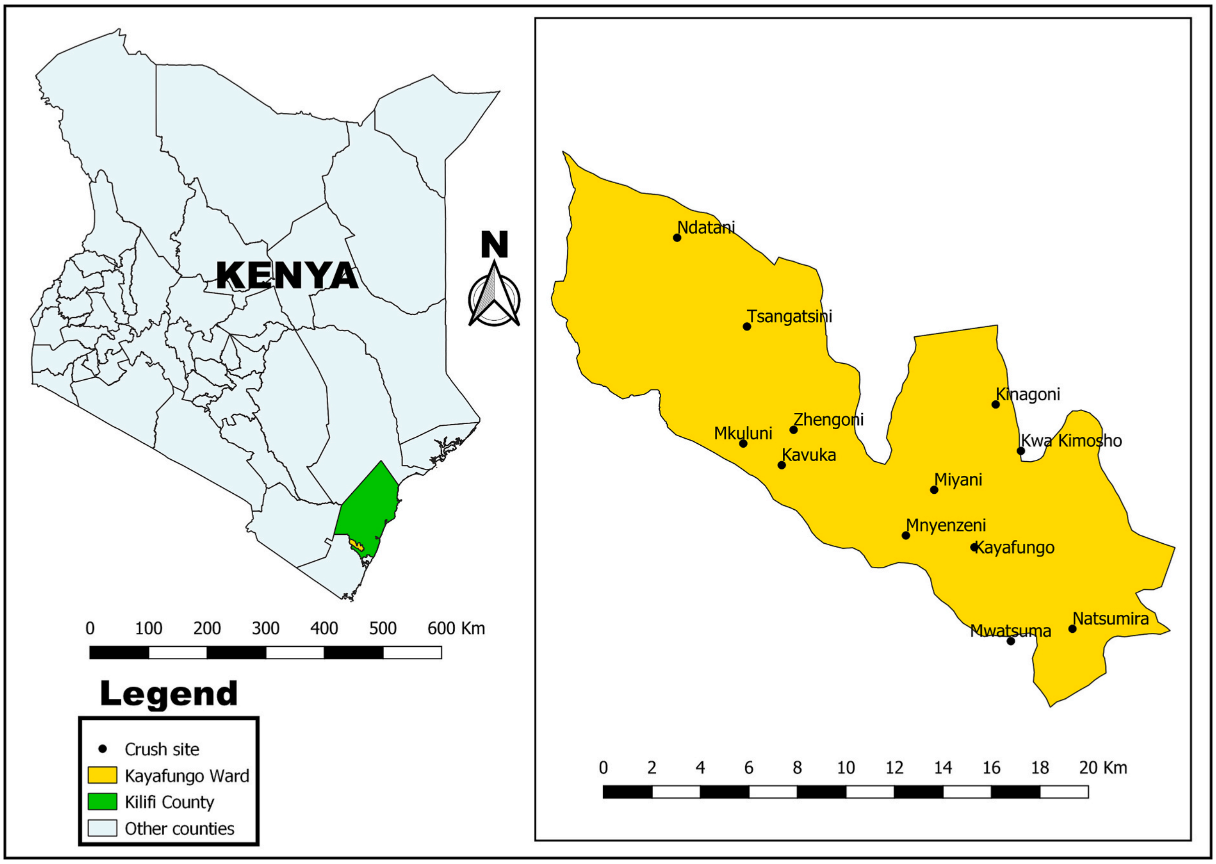


Fig. 1. Map of Kayafungo Ward in Kilifi County in coastal Kenya showing the trial sites. The map was prepared using common-license shape files in QGIS software version 3.10 (QGIS Development Team, 2020).

pressure of 6 kg/cm. Cattle received treatment on day 0, and thereafter every two weeks until the end of the study. Day 0 was defined individually as the day an animal received the first treatment. Whole-body tick counts were also done on day 0, and thereafter at two-week intervals until the end of the study. Blood sampling was done on days 0, 60, 120 and 180 to determine the presence of *A. marginale* and *T. parva* in cattle. The study was conducted in compliance with the study authorizations issued by Kenya's Veterinary Medicines Directorate (Approval reference: MOALF/SDL/VMD/TRIALS/VOL1/14), Directorate of Veterinary Services (no objection ref.: MOALF/SDL/DVS/DS/RES/74), the National Commission for Science, Technology and Innovation (NACOSTI/P/21/6726), and the Pwani University Ethics Review (approval number ERC/EXT/002/2020). The use of trade, brand, or corporation names in this publication is for information and convenience of the reader, and should not be misinterpreted as an endorsement, promotion, or demotion of such products based on their efficacy result.

2.2. *Sample size determination*

This trial aims to assess the performance of Tickoff® in reducing on-host tick counts compared to an existing synthetic chemical acaricide. A weekly application of Triatix® acaricide on tick-infested cattle for four weeks caused a reduction in on-host tick counts by 94.9 % (Murigu et al., 2016). In semi-field experiments, the efficacy of oil-based formulations of *M. anisopliae* (10^8–10^9 conidia/ml) on the reduction of tick counts on cattle ranged between 65 and 92 % depending on the application intervals, study duration, tick species, and life cycle stage (Kaaya et al., 2011; Kaaya and Hassan, 2000; Kaaya and Hedimbi, 2012; Murigu et al., 2016). Similarly, our previous pilot study showed that Tickoff® produced an efficacy of 86.1 % in treated cattle compared to untreated cattle (https://patents.google.com/patent/WO2017216752A1/en). Given the expected variation in the efficacy of oil-based formulations of *M. anisopliae* against ticks, we considered the minimal worthwhile difference in efficacy between the conventional Triatix® acaricide and Tickoff® to be 15 %. An efficacy margin of 15 % below which the fungal formulation would not offer a viable alternative to existing chemical acaricide was considered acceptable given the limited availability of alternatives for tick control and the added advantages of *M. anisopliae* in biological control of ticks, i.e., it being selective and virulent against all tick stages (Hedimbi et al., 2011; Kaaya et al., 2011; Kaaya and Hassan, 2000; Kaaya et al., 1996; Kaaya and Hassan, 2000; Kaaya and Hedimbi, 2012), pathogenic to acaricide-resistant ticks (Murigu et al., 2016), and it being safe for humans, animals and the environment (Zimmermann, 2007). The significance level and power of the study were set at 5 % and 80 %, respectively. Assuming a 94.9 % (~95 %) efficacy in the conventional Triatix® acaricide (Murigu et al., 2016), a minimum sample size of 73 zebu cattle per intervention arm was calculated as follows (Sakpal, 2010):

$$n = \left[\left(Z_{\alpha/2} + Z_{\beta} \right)^2 \times \left\{ \left(\mathrm{p1}\ (1 - \mathrm{p1}) + (\mathrm{p2}\ (1 - \mathrm{p2})) \right) \right\} \right] \Big/ (\mathrm{p1} - \mathrm{p2})^2$$

Where:
n = sample size required in each intervention arm,
p1 = protection efficacy of Triatix® = 0.95,
p2 = protection efficacy of Tickoff® = 0.80,
p1 - p2 = minimal worthwhile difference = 0.15,
$Z_{\alpha/2}$: for 5 % level of significance = 1.96,
Z_{β}: for 80 % power = 0.84.

Herd-level treatments and repeated measurements are likely to enhance the intra-herd clustering of measurements estimated at individual animal levels. Given the variation that may occur among herds, i.e., the clustering effect, inflating the sample size by two- to four folds can account for the potentially large variation among clusters (Thrusfield et al., 2018). We, therefore, inflated the sample size threefold and obtained a total of 219 zebu cattle per intervention arm. A dropout rate of 30 % was included in the calculation to account for potential dropouts during the trial, bringing the total number of cattle per treatment group to 285 and thus 855 zebu cattle in total.

2.3. *Data analysis*

Data analysis was performed using the R software version 4.2.2. The individual cow in each herd was the observational unit, repeatedly measured over time, and the primary endpoint was the live-attached tick count and incidence of tick-borne infections in each intervention arm. The secondary endpoints were the number, type, and severity of adverse events in cattle in each intervention arm. Descriptive statistics were completed for baseline demographic variables of cattle (age, sex, and body weight), herd size, and tick infestation. *P*-values ≤ 0.05 were considered significant for all statistical tests.

2.4. *Tick count analysis*

The analysis of the percent reduction in tick infestation was based on the Intention-to-Treat (ITT) population, comprising all cattle that were randomized to a treatment group and that received at least one dose of either study product. For cattle withdrawn before the final day of the trial, data up to the time of removal were included in statistical summaries and analyses. The percentage reduction in tick infestation was calculated for each post-treatment day as the reduction in live-attached tick counts compared to the pre-treatment counts (recorded on Day 0). Day 0 was defined individually as the day a cow received its first treatment. The percent reduction at each time point was calculated as follows:

$$\%\text{reduction in tick infestation} = \frac{\text{tick count (day 0)} - \text{tick count (post} - \text{treatment)}}{\text{tick count (day 0)}} \times 100$$

A generalized linear mixed model (GLMM) with a negative binomial distribution (log-link function), fitted with the R-package glmmTMB, was used to compare live tick counts post-treatment among the treatment groups. The fixed part of the model contained the linear and quadratic trends of time point per treatment, occurrence of rainfall since previous treatment, cattle age group and number of days since previous treatment. Rain was included in the model as a covariate since rain may wash off the treatments from cattle skin and thus reduce treatment persistence and efficacy. Moreover, rain has been associated with increased tick activity and abundance (Chepkwony et al., 2021). The age group of cattle was included as a covariate in the model because tick counts are expected to differ among cattle of different age groups, while the age distribution was significantly different among the three treatment arms at baseline. As some cattle or herds occasionally skipped the biweekly interval spraying and tick counting session, we added time since previous treatment (i.e., the extra time beyond 14 days) in the model to evaluate if this had a significant association with the outcome measure. The random part of the model included random effects for village, herd within village, and time points within herd. This part was included to respect characteristics of the study design: strata (i.e., villages) and experimental units (i.e., herds which were used to randomize the treatments), and time points per herd. The random part also contained random intercepts and slopes per cattle for the linear and quadratics terms of time, in order to handle the repeated measurements per cattle over time. Finally, there were data collector random effects that may capture the observer bias among the tick assessors. Testing was two-sided at the significance level of $\alpha = 0.05$.

2.5. *Survival analysis of treated ticks*

We carried out in vitro experiments using both lab-reared and field-collected ticks to monitor mortality rates in the Tickoff®, excipients, and untreated treatment groups (Appendix A). Cox Proportional Hazard analysis was used to estimate the hazard ratios (HR) and their 95 % confidence intervals (CI).

2.6. *Epidemiological analysis*

To estimate the epidemiological impact of the treatments, we followed a cohort of recruited cattle for six months at bimonthly intervals, during which their infection status with *A. marginale* and *T. parva* were recorded. Interval-censored survival analysis with left censoring was used to estimate the probability of cattle remaining free of infection. Cox's proportional hazard regression models were fitted to identify the significant predictors of infection occurrence. The model included treatment and age as covariates. Frailty terms for village and herd within the village were included in the model to adjust for clustering within herd and village.

3. Results

3.1. *Cattle demographics*

The ITT population comprised 1459 zebu cattle from 217 herds that were randomized to either Tickoff® ($n = 541$, 37.1 %), Triatix® ($n = 473$, 32.4 %) or excipient ($n = 445$, 30.5 %) groups and received at least one dose of either treatment (Table 1). Most of the cattle were adults ($n = 896$, 61.4 %) followed by juveniles ($n = 410$, 28.1 %) and calves ($n = 153$, 10.5 %). There were 900 (61.7 %)

Table 1
Demographics and baseline characteristics of recruited and treated cattle that were (ITT population).

Demographics	Mazao Tickoff® (n = 541, 37.1 %)	Triatix® (n = 473, 32.4 %)	Excipient (n = 445, 30.5 %)	Homogeneity
Age				
Calf (6 months-1 year)	45 (8.3 %)	50 (10.6 %)	58 (13.0 %)	$\chi^2 = 9.521$, df = 4, $p = \mathbf{0.049}$
Juvenile (1–2 years)	141 (26.1 %)	136 (28.8 %)	133 (29.9 %)	
Adult (above 2 years)	355 (65.6 %)	287 (60.7 %)	254 (57.1 %)	
Sex				
Male	199 (36.8 %)	196 (41.4 %)	164 (36.9 %)	$\chi^2 = 5.080$, df = 2, $p = 0.279$
Female	342 (63.2 %)	277 (58.6 %)	281 (63.1 %)	
Body weight (kg)				
Arithmetic mean ± SD	153.7 ± 54.4	149.3 ± 53.1	151.8 ± 55.6	χ^2 of a Kruskal-Wallis test = 2.764, df = 2, $p = 0.251$
Range	28–375	36–370	26–257	
Herd size				
1–10	60	60	59	χ^2 of a Wald test = 1.431, df = 2, $p = 0.489$
11–20	12	10	10	
21–25	3	2	1	
Day 0 tick count (live attached)				
Median (1st and 3rd quartiles)	10 (4–21)	10 (3–26)	9 (3–18)	χ^2 of a Wald test = 0.814, df = 2, $p = 0.666$

Abbreviations: Df Degree of freedom, *SD* standard deviation, ITT Intention-to-Treat population.

female and 559 (38.3 %) male cattle. All treatment groups from the ITT population showed reasonable homogeneity for sex, body weight, herd size, and median tick counts at baseline. However, there were small yet significant differences in the baseline distribution of age groups among the treatment groups (Table 1).

3.2. Tick infestation, relative reductions in tick counts, and safety of treatments

A total of 91,741 ticks were observed from the 12,222 cattle inspections (Fig. 2, Supplementary Table 1). Compared to baseline, the averages of mean percent reductions in tick counts across all post-day 0 assessments were 72.5 % (range 40.3–93.9 %) in the Tickoff® group, 87.4 % (76.1–94.5 %) in the Triatix® group and 72.7 % (range 28.9–92.7 %) in the excipient group. The animals were healthy throughout the trial period and no physical, behavioral, or physiological change that could be interpreted as an adverse reaction to experimental treatments was observed.

During the entire trial (December 2021 to July 2022), some animals did not have any post-treatment evaluation data (Supplementary Table 1) due to several reasons including loss of contact with the farmer, migration of herds due to prolonged drought, loss of ear-tags, cattle disappearance from home or lost in the forest, withdrawal of consent by the farmer, cattle sold, and death of the animal (because of prolonged drought). In addition, some cattle or herds occasionally skipped treatment sessions and sampling and therefore did not have tick count and epidemiological data generated. Animals received an average of 7.8 treatments out of the 13 treatment rounds.

3.3. Multivariable analysis

We fitted a GLMM to the infestation counts (Fig. 2) and found that, even though substantial reductions in tick infestations were observed, there was no significant difference in tick infestation in cattle treated with Tickoff® compared to the animals in the excipient group (Supplementary Table 2a). When comparing the excipient group to the reference Tickoff® group, the mean tick count (at log scale) at the average time point did not differ ($p = 0.427$), and the change in counts between the two groups over enrolled time was also not significant (interaction term time point linear $p = 0.932$, and interaction term time point quadratic $p = 0.869$). On the other hand, the Triatix® group did have a significantly lower mean tick count (at log scale) at the average time point compared to the reference Tickoff® group ($p < 0.001$). Additionally, the change in tick counts between the Triatix® and the reference Tickoff® groups over the enrolled time was (close to) significantly different, as indicated by the interaction term time point linear ($p = 0.055$) and the interaction term time point quadratic ($p < 0.001$). Younger animals (calf and juvenile) had significantly lower tick infestation when compared to adult cattle (Supplementary Table 2a). Calves have tick counts estimated to be 0.5 times (exp(−0.682)) the values for adults, while juveniles 0.8 times (exp(−0.263)) the values for adults. The effect of rain on tick counts was non-significant ($p = 0.144$). The Wald tests for the main effects and interactions of treatments and post-treatment time points are presented in Supplementary Table 2b. Comparing mean tick counts at biweekly time points, there were no significant differences among the treatment groups at the start of the study (days 0 and 14). However, as the study progressed, the tick count was significantly lower for the Triatix® group from days 28 to 154. By the end of the study, when tick counts were very low, these differences were no longer significant. The Tickoff® and excipient groups showed no significant differences at any point during the study (Supplementary Table 3).

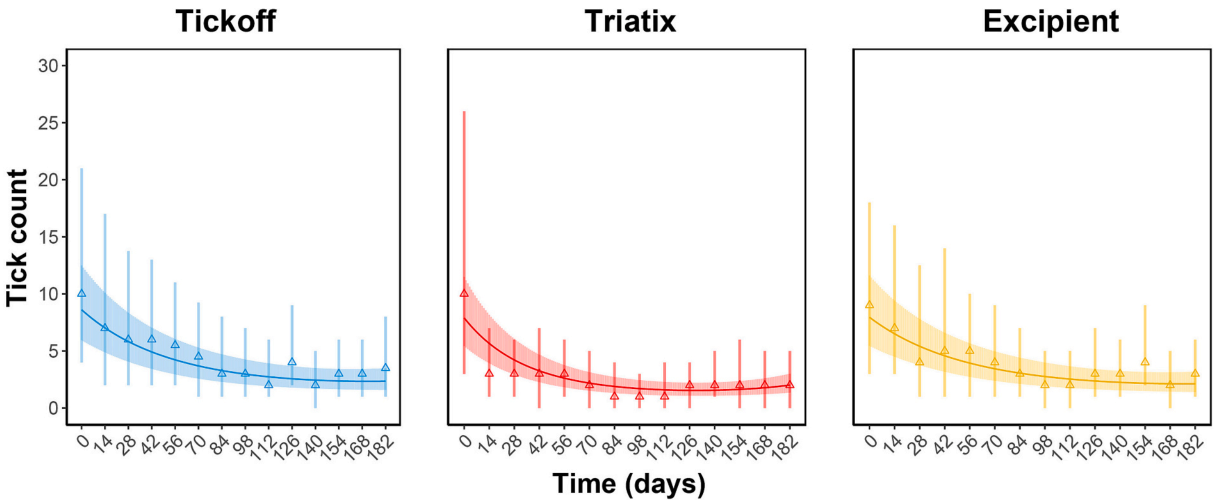


Fig. 2. Observed and predicted tick counts during the trial period based on the fitted model. The continuous lines represent the predicted tick counts (with 95 % CI), while the triangle point-up symbol represents the observed median tick count (with interquartile range) for each treatment group.

3.4. Survival analysis and mycosis

Ticks collected after treatment from Tickoff®-treated cattle in the field and maintained in the laboratory had a median survival time of 13 days (95 % CI: 12–14 days), which was shorter than that of the ticks collected from the excipient group (>21 days) (Fig. 3). The Cox regression model showed that Tickoff® treatment was associated with a significantly higher mortality rate compared to the excipient treatment (HR = 8.50, 95 % CI: 4.67–15.47, $p < 0.001$). Ticks collected from cattle treated with Triatix® and transported to the lab were either dead on arrival, or could not exhibit leg movement or response to external stimuli (e.g. touching with a pen or exhaling air on a tick) and hence were considered dead.

3.5. Monitoring of mycosis development on dead ticks

Fungal growth was observed in over 90 % of ticks that were collected from cattle at three to six hours after Tickoff® application (Fig. 4). No mycosis developed on tick cadavers from excipient and Triatix® treatments.

3.6. Epidemiological impact (survival times to infection)

Blood samples were taken from the cohort of 1488 zebu cattle at 2-month intervals for a duration of six months and tested for the presence of *A. marginale* and *T. parva* infections. Of these, 6.2 % ($n = 92/1488$) and 4.8 % ($n = 71/1488$) had *A. marginale* and *T. parva* infections at baseline, respectively, and were excluded from the analysis as no new infection could be observed. However, cattle positive for *A. marginale* were included in the *T. parva* analysis and vice-versa. During the six months, a total of 69 (4.9 %) new cases of *A. marginale* infection and 51 (3.6 %) new cases of *T. parva* infection were detected in cattle (Fig. 5). We did not detect a significant association between the incidence of *A. marginale* infections in cattle and treatment and the age group. However, Triatix® significantly reduced the incidence of *T. parva* (HR = 0.44, 95 % CI: 0.20–0.97, $p = 0.042$) (Supplementary Table 4).

4. Discussion

We conducted a large-scale randomized controlled trial and coupled it with laboratory experiments to evaluate the safety and effects of Tickoff® biopesticide on tick infestation, tick mortality, and incidence of two tick-borne pathogens in zebu cattle managed under an extensive system. Overall, tick counts in all treatment groups dramatically decreased. The reduction in the Tickoff® group did not, however, differ significantly from that of excipient-treated cattle. The Triatix® group did show a significantly greater reduction in tick counts compared to Tickoff® and excipient. Ticks exposed to Tickoff® biopesticide collected from animals treated with Tickoff® had a significantly shorter survival time compared to ticks exposed to excipient. This increased mortality did not result in a significant effect on the incidence of tick-borne pathogens in cattle in this setting. This was contrary to the effects of the Triatix® acaricide, which was associated with significant reductions in incidence of *T. parva* infection in cattle but not *A. marginale* infection.

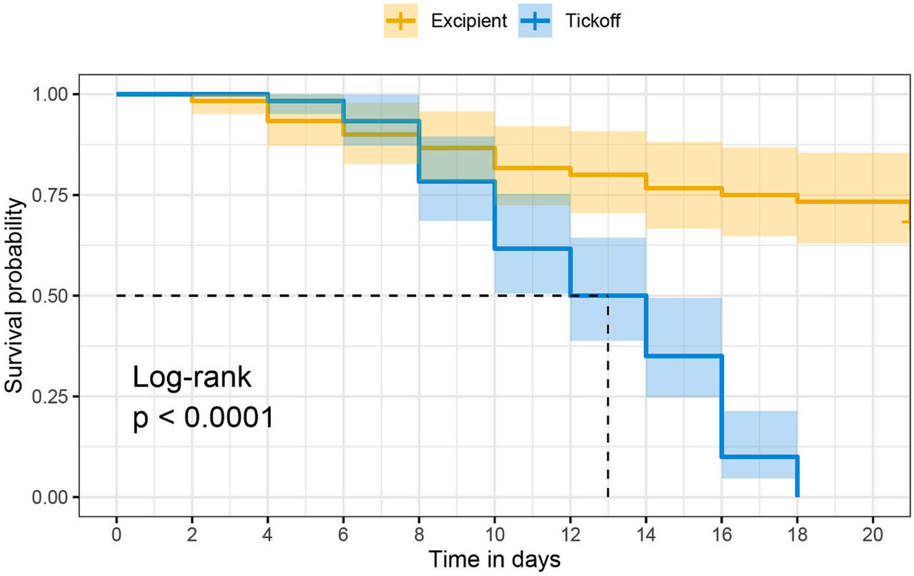


Fig. 3. Kaplan-Meier survival curves for *Rhipicephalus appendiculatus* ticks collected from cattle treated with Tickoff® and excipient.

Fig. 4. Fungal growth on ticks collected from cattle treated with Tickoff® and maintained in the humidity chamber (26 ± 1 °C and 80 ± 5 % RH) in the laboratory.

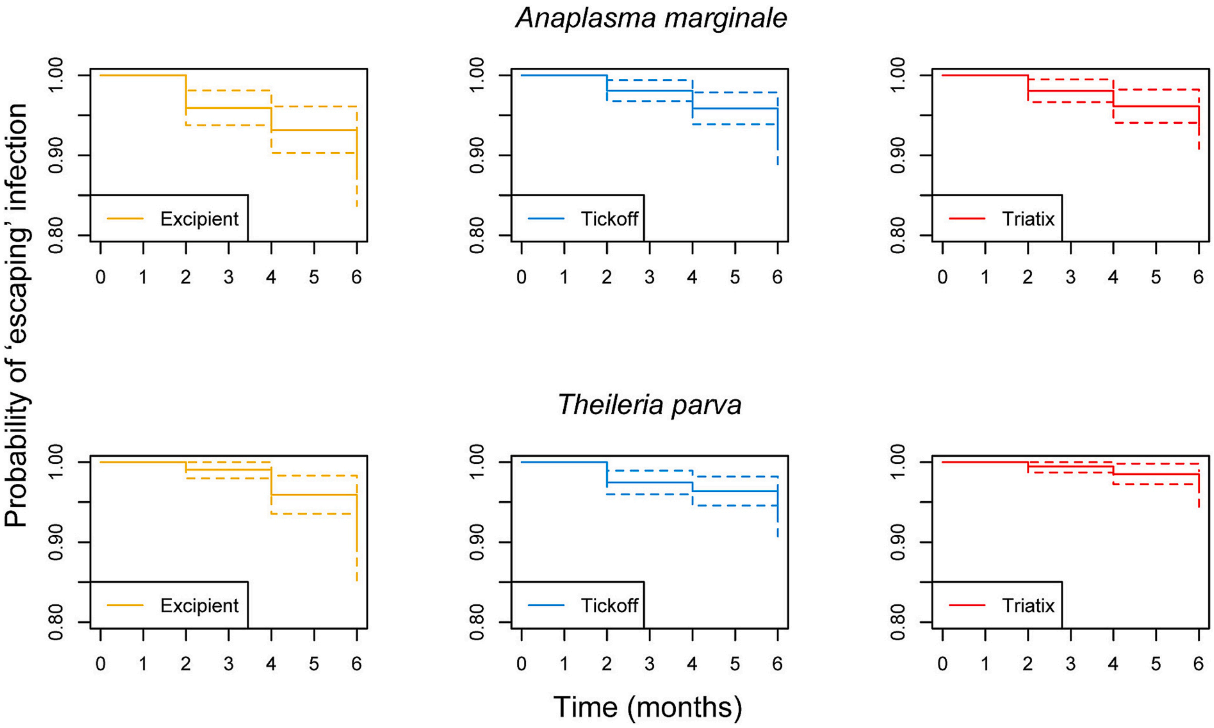


Fig. 5. Estimated probabilities of 'escaping' infection with *Anaplasma marginale* and *Theileria parva* in cattle in different treatment groups.

4.1. Entomological impact

Tickoff® biopesticide showed no significant reduction in tick infestation on cattle when compared to the excipient group. Additionally, the effect of Tickoff® did not change through time as revealed by the non-significant effect of the interaction of time with the treatment. Our findings corroborate earlier studies which also reported a lack of significant effect of fungal formulations (vs. controls) on tick infestation (Correia et al., 1998; Samish et al., 2014). This is, however, in contrast with other studies that reported a significant effect of fungal formulations on the reduction of tick infestation when compared to the respective control groups (Alonso-Díaz et al., 2022; Murigu et al., 2016). Whereas no significant effect of Tickoff® on tick reduction was observed in our field trial, in vitro experiments using both lab-exposed and field-collected ticks showed a clear pathogenic effect of Tickoff® biopesticide on the tick population when compared to the excipient group. In addition, fungus growth and sporulation were observed in all lab-exposed ticks and over 90 % of ticks that were collected from cattle at three to six hours after fungal application. This indicates that Tickoff® could induce elevated mortality in ticks, albeit delayed as compared to Triatix® acaracide. This delayed mortality effect is expected to result in death after the ticks have detached from their treated host animal. This could explain the limited effect on tick infestation of treated cattle. Additionally, the ability of *M. anisopliae* to reduce tick fecundity and egg hatchability as reported in other studies (Camargo et al., 2012; Nana et al., 2015; Rot et al., 2013) may result in a reduction in the next progeny of ticks. Combined, these effects could still contribute to tick control provided a high enough coverage among tick host animals is achieved.

Cattle in the Triatix® group had significantly lower tick infestation than cattle in the Tickoff® group throughout the study period. This significantly higher impact of Triatix® on tick infestation is consistent with earlier studies reporting the efficacy of amitraz-based acaricides when applied as a spray or dip for the control of ticks on cattle (George et al., 1998; Murigu et al., 2016). This effect may be due to their combined lethal and sublethal effects, such as rapid detachment or clearance of attached ticks within 6–30 h after application, and immobilization and killing of detached ticks before they have the chance to lay eggs or molt (Barry Haigh and Gichang, 1980; Davey et al., 1984; Kagaruki, 1996). Despite its effectiveness in this study, this synthetic acaricide should be used with caution for tick management due to its toxicity to humans, its potential to increase contamination of the environment, as well as milk and meat products (De Meneghi et al., 2016), and development of tick resistance (Githaka et al., 2022).

Survival analysis on experiments in the laboratory showed that the excipient treatment, which contains canola oil (95 %), 0.05 % Triton X-100 (1.5 %) and Kerosene (3.5 %) and without the *M. anisopliae* ICIPE 7 (active ingredient of Tickoff®), produced mortality effects on ticks when compared to the untreated control (Appendix C). Such toxic effects of kerosene have been reported before in ticks (George et al., 2004), sand fleas (Enwemiwe et al., 2020) and immature stages of mosquitoes (Djouaka et al., 2007; Ojianwuna and Enwemiwe, 2022). It is believed that kerosene interferes with the physiology of arthropods, by penetrating tissues, causing inflammation and hypoxia, interfering with breathing, suppressing the insect immune system, and causing imbalances in hormones and enzymes (Maiyoh et al., 2015). This could play a role in the observed reductions in tick infestation in cattle in the excipient group. However, in the absence of water as a control treatment, we could not disentangle to what extent these reductions in tick infestation were due to the excipient or were reflective of natural, drought-induced, fluctuations. Additional research is required to determine the extent of tick repellency achieved with low concentrations of kerosene.

In this study, younger animals (calves and juveniles) had significantly lower tick infestation when compared to adult cattle. Similar findings have previously been reported elsewhere, including in Egypt (Asmaa et al., 2014), Pakistan (Rehman et al., 2017), Nigeria (Lorusso et al., 2013), South Africa (Marufu et al., 2011), Colombia (Rocha et al., 2019), among others. The lower tick burdens recorded in calves could be due to a combination of factors, including the frequent grooming of calves, especially head, ears, and neck regions, by their dams and the smaller surface area of younger animals as compared to adults (Mooring et al., 2000).

4.2. *Epidemiological impact*

There was no significant effect of Tickoff® treatment on the incidence of both *A. marginale* and *T. parva* infections in cattle, relative to excipient treatment. Cattle treated with Triatix® did show significantly lower incidence of *T. parva* infection, but not *A. marginale*. This is in line with earlier studies, for instance using Spot-on® (a 10 % deltamethrin pour-on) (Muraguri et al., 2003). Other studies using Vectoid® (an emulsifiable deltamethrin concentrate) failed to show a significant effect on incidence of *T. parva* (Muhanguzi et al., 2014). The attack rates for *A. marginale* and *T. parva* infections were 4.9 % and 3.6 %, respectively, indicating that both pathogens circulated at lower levels. This was likely due to the prolonged drought experienced during the study period and the consequential drop in tick counts. Consequently, we had limited statistical power to detect the epidemiological effects of the treatments on either pathogen.

The observed differences in levels of protection of Triatix® against *T. parva* and *A. marginale* infection could be related to the transmission dynamics of these pathogens. For instance, while *T. parva* is transmitted by *R. appendiculatus* ticks only, *A. marginale* can be transmitted biologically by several tick species in the *Rhipicephalus* and *Hyalomma* genera, but also mechanically by hematophagous arthropods or blood-contaminated fomites if they are not properly sterilized (Aubry and Geale, 2011). The latter transmission routes are not affected by tick control treatments and could therefore result in a smaller potential treatment effect. Indeed, hematophagous arthropods such as the stable flies (*Stomoxys calcitrans*) as well as biological vectors namely, *H. rufipes, R. decoloratus, R. microplus* and *R. evertsi* are present in the region (Oundo et al., 2024). Besides, livestock farmers often used disposable needles on more than one animal, while livestock vaccination drives conducted by county government often used the same set of injection guns on all the animals presented for the vaccination.

4.3. *Safety of Tickoff®*

In this study, no physical or behavioral abnormalities were observed in the Tickoff®-treated cattle at any time during the trial. These results are in agreement with previous studies that reported no adverse reactions in cattle sprayed with fungal formulations of *M. anisopliae* (Alonso-Díaz et al., 2022; Kaaya et al., 2011). This is further exemplified by reports indicating that *M. anisopliae* poses minimal risk to mammals, humans, and non-target organisms (Fischhoff et al., 2017; Zimmermann, 2007).

4.4. Challenges during the study

During the second half of the trial period, a prolonged dry season occurred. This may have contributed to a natural decline in the tick population in the environment and thus resulting in a low infestation abundance. Indeed, such climatic conditions have been associated with decreased survival, development, and questing activity of ticks in the environment (Brown et al., 2014; Jones and Kitron, 2000). The low tick population might have resulted in low circulation of tick-borne pathogens, reducing the power to detect a difference between the treatment groups.

Fungal conidia are sensitive to unfavorable environmental factors such as high temperatures, low relative humidity, and direct ultraviolet (UV) radiation which reduce the germination, viability, and persistence of conidial spores (Fernandes et al., 2012). With the effect of drought, we suspect that these environmental factors might have adversely affected the germination of conidial spores thus affecting the treatment effect of Tickoff®.

4.5. Direct versus indirect effects of Tickoff® biopesticide

Tick control products have varying active ingredients with different modes of action. Depending on the mode(s) of action, these tick control products may produce a range of direct and indirect effects on transmission of tick-borne pathogens. Direct effects are those that protect the treated animal by killing the ticks before feeding thereby preventing pathogen transmission from ticks to tick-infested animals. In contrast, indirect effects protect both treated and untreated animals by killing the ticks after feeding thereby preventing onward transmission to other animals (treated or untreated). *Metarhizium anisopliae* isolate ICIPE 7 (the active ingredient of Tickoff®) kills ticks after feeding and thus may mostly provide community protection and limited direct individual protection. Additionally, *M. anisopliae* ICIPE 7 reduces the reproduction potential of ticks by reducing the tick fecundity and increasing the preoviposition, oviposition, and post-oviposition periods (Maranga et al., 2006; Nana et al., 2015; Nchu et al., 2010). Combined these indirect effects cannot directly protect treated cattle from getting infected by an already attached infectious tick but could result in a reduction in the next generation of ticks which would otherwise become infected and infectious. *M. anisopliae* ICIPE 7 will also kill the newly infected ticks before they molt and become infectious. Such reductions in tick population may reduce the risk of pathogen transmission and thereby reduce the incidence of tick-borne infections in the cattle population, including cattle that were not treated. The impact of these indirect effects will depend on the coverage level in the cattle population, the frequency and duration of treatment application, abundance of alternative hosts for ticks and pathogens that would not be reached by the treatment program, cattle mobility, and alternative pathogen transmission routes other than by an infectious tick bite. All these factors intertwine and make it complex to understand and study indirect effects of this biological control agent. Models can help to improve our understanding of how indirect effects of *M. anisopliae* ICIPE 7 formulation can protect the overall cattle population from tick-borne infections, including those that may not directly receive treatment.

5. Conclusion

Our study demonstrated the significant pathogenic effects of Tickoff® biopesticide on ticks removed from treated cattle and followed up for survival. While delayed mortality may be less effective at conferring direct protection of treated cattle, such effects may disrupt the life cycle of ticks and prevent onward pathogen transmission and reduced tick population sizes. The observed pathogenic effect is promising but was insufficient to result in significant effects of Tickoff® on tick infestation levels or incidence of infection with two tick-borne pathogens. This study was not designed to detect indirect treatment effects and had limited power due to a prolonged drought that occurred during the study period. Before subsequent trials will be rolled out, further efforts on the optimization of the Tickoff® formulation are needed, exploring suitable UV protectants to protect the conidia from damage by UV radiation, and continue with searching for thermo-tolerant strains of the fungi. Thereby, this work presents a next step towards the development of environmentally friendly tick and tick-borne disease control tools.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parepi.2024.e00384.

Funding

We gratefully acknowledge the financial support for this research by the following organizations and agencies: the German Federal Ministry for Economic Cooperation and Development (BMZ) commissioned and administered through the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Fund for International Agricultural Research (FIA), grant number 81235250; the Swedish International Development Cooperation Agency (SIDA); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Federal Democratic Republic of Ethiopia; and the Government of the Republic of Kenya. JWO was supported by The Koepon Foundation Postgraduate Fellowship. The views expressed herein do not necessarily reflect the official opinion of the donors.

Availability of data and materials

All data and code supporting the conclusions of this study will be made available upon publication on Open Science Framework.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Joseph Wang'ang'a Oundo: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Shewit Kalayou:** Writing – review & editing, Methodology, Conceptualization. **Gerrit Gort:** Writing – review & editing, Formal analysis. **Gebbiena M. Bron:** Writing – review & editing, Methodology, Formal analysis. **Constantianus J.M. Koenraadt:** Writing – review & editing, Methodology, Formal analysis. **Quirine ten Bosch:** Writing – original draft, Methodology, Formal analysis. **Daniel Masiga:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare they have no competing interests.

Acknowledgments

Our sincere thanks go to Dr. Caroline Asanyo (participating veterinarian) and Jane Kinyanjui (livestock production officer) for facilitating our fieldwork, and the livestock owners who agreed to enroll their cattle in the study. We are thankful to Jane Njaramba, Kevin Kidambasi and Dickens Ondifu for assistance with laboratory analysis. Further, we thank *icipe*'s Peter Muasa, David Mbuvi, Richard Tumba and Oscar Esibi for technical support. Much appreciation goes to Dr. Charles Omanga for granting us access to laboratory facilities at the Regional Veterinary Investigations Laboratory (RVIL). We are grateful to the staff at RVIL Mariakani for their assistance with fieldwork activities. We also appreciate the logistical support of Caroline Muya of *icipe*.

References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Vet. Parasitol. 203, 6–20. https://doi.org/10.1016/J.VETPAR.2014.03.006.

Alonso-Díaz, M.Á., Fernández-Salas, A., Galindo-Velasco, E., Lezama-Gutiérrez, R., Contreras-Lara, D., 2022. Effect of *Metarhizium anisopliae* (Ma14 strain) against *Rhipicephalus microplus* on cattle infested naturally. Southwest. Entomol 47, 285–290. https://doi.org/10.3958/059.047.0203.

Asmaa, N.M., ElBably, M.A., Shokier, K.A., 2014. Studies on prevalence, risk indicators and control options for tick infestation in ruminants. Beni-Suef Univ. J. Basic Appl. Sci. 3, 68–73. https://doi.org/10.1016/j.bjbas.2014.02.009.

Aubry, P., Geale, D.W., 2011. A review of bovine anaplasmosis. Transbound. Emerg. Dis. 58, 1–30. https://doi.org/10.1111/j.1865-1682.2010.01173.x.

Barbieri, A., Rico, I.B., Silveira, C., Feltrin, C., Dall'agnol, B., Schrsank, A., Lozina, L., Klafke, G.M., Reck, J., 2023. Field efficacy of *Metarhizium anisopliae* oil formulations against *Rhipicephalus microplus* ticks using a cattle spray race. Ticks Tick Borne Dis. 14, 102147. https://doi.org/10.1016/J.TTBDIS.2023.102147.

Barry Haigh, A.J., Gichang, M.M., 1980. The activity of amitraz against infestations of *Rhipicephalus appendiculatus*. Pestic. Sci. 11, 674–678. https://doi.org/10.1002/PS.2780110616.

Baylis, H., Allsopp, B.A., Nene, V., Spooner, P.R., Dolan, T., Morzaria, S.P., 1992. Detection of a carrier state in *Theileria parva*-infected cattle by the polymerase chain reaction. Parasitology 104, 215–232. https://doi.org/10.1017/S0031182000061655.

Brown, L., Medlock, J., Murray, V., 2014. Impact of drought on vector-borne diseases – how does one manage the risk? Public Health 128, 29–37. https://doi.org/10.1016/J.PUHE.2013.09.006.

Camargo, M.G., Golo, P.S., Angelo, I.C., Perinotto, W.M.S., Sá, F.A., Quinelato, S., Bittencourt, V.R.E.P., 2012. Effect of oil-based formulations of acaripathogenic fungi to control *Rhipicephalus microplus* ticks under laboratory conditions. Vet. Parasitol. 188, 140–147. https://doi.org/10.1016/J.VETPAR.2012.03.012.

Chepkwony, R., van Bommel, S., van Langevelde, F., 2021. Interactive effects of biological, human and environmental factors on tick loads in Boran cattle in tropical drylands. Parasit. Vectors 14, 1–8. https://doi.org/10.1186/S13071-021-04683-9/FIGURES/3.

Correia, A.D.C.B., Fiorin, A.C., Monteiro, A.C., Veríssimo, C.J., 1998. Effects of *Metarhizium anisopliae* on the tick *Boophilus microplus* (Acari: Ixodidae) in stabled cattle. J. Invertebr. Pathol. 71, 189–191. https://doi.org/10.1006/JIPA.1997.4719.

Davey, R.B., Ahrens, E.H., George, J.E., 1984. Efficacy of sprays of amitraz against *Boophilus* ticks on cattle. Prev. Vet. Med. 2, 691–698. https://doi.org/10.1016/0167-5877(84)90014-X.

De Meneghi, D., Stachurski, F., Adakal, H., 2016. Experiences in tick control by acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. Front. Public Health 4, 239. https://doi.org/10.3389/FPUBH.2016.00239.

Djouaka, R.F., Bakare, A.A., Bankole, H.S., Doannio, J.M.M.C., Kossou, H., Akogbeto, M.C., 2007. Quantification of the efficiency of treatment of *Anopheles gambiae* breeding sites with petroleum products by local communities in areas of insecticide resistance in the Republic of Benin. Malar. J. 6, 1–6. https://doi.org/10.1186/1475-2875-6-56/FIGURES/4.

Enwemiwe, V.N., Ojianwuna, C.C., Anyaele, O.O., 2020. Assessing the potentials of two local topical ointments as affordable treatment against tungiasis infestation: a self-experimentation in Igbokoda, Nigeria. Parasite Epidemiol. Control 11, e00168. https://doi.org/10.1016/J.PAREPI.2020.E00168.

Fernandes, É.K.K., Bittencourt, V.R.E.P., Roberts, D.W., 2012. Perspectives on the potential of entomopathogenic fungi in biological control of ticks. Exp. Parasitol. 130, 300–305. https://doi.org/10.1016/J.EXPPARA.2011.11.004.

Fischhoff, I.R., Keesing, F., Ostfeld, R.S., 2017. The tick biocontrol agent *Metarhizium brunneum* (= *M. anisopliae*) (strain F52) does not reduce non-target arthropods. PloS One 12, e0187675. https://doi.org/10.1371/JOURNAL.PONE.0187675.

Gachohi, J., Skilton, R., Hansen, F., Ngumi, P., Kitala, P., 2012. Epidemiology of East Coast fever (*Theileria parva* infection) in Kenya: past, present and the future. Parasit. Vectors 5, 194. https://doi.org/10.1186/1756-3305-5-194.

George, J.E., Davey, R.B., Ahrens, E.H., Pound, J.M., Drummond, R.O., 1998. Efficacy of amitraz (Taktic® 12.5% EC) as a dip for the control of *Boophilus microplus* (Canestrini) (Acari: Ixodidae) on cattle. Prev. Vet. Med. 37, 55–67. https://doi.org/10.1016/S0167-5877(98)00098-1.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129, S353–S366. https://doi.org/10.1017/S0031182003004682.

Githaka, N.W., Kanduma, E.G., Wieland, B., Darghouth, M.A., Bishop, R.P., 2022. Acaricide resistance in livestock ticks infesting cattle in Africa: current status and potential mitigation strategies. Current Research in Parasitology & Vector-Borne Diseases 2, 100090. https://doi.org/10.1016/J.CRPVBD.2022.100090.

Hartemink, N.A., Randolph, S.E., Davis, S.A., Heesterbeek, J.A.P., 2008. The basic reproduction number for complex disease systems: defining R0 for tick-borne infections. Am. Nat. 171, 743–754. https://doi.org/10.1086/587530.

Hedimbi, M., Kaaya, G.P., Chinsembu, K.C., 2011. Mortalities induced by entomopathogenic fungus *Metarhizium anisopliae* to different ticks of economic importance using two formulations. Int. Res. J. Microbiol. 2, 141–145.

Hoch, T., Goebel, J., Agoulon, A., Malandrin, L., 2012. Modelling bovine babesiosis: a tool to simulate scenarios for pathogen spread and to test control measures for the disease. Prev. Vet. Med. 106, 136–142. https://doi.org/10.1016/J.PREVETMED.2012.01.018.

Jones, C.J., Kitron, U.D., 2000. Populations of *Ixodes scapularis* (Acari: Ixodidae) are modulated by drought at a Lyme disease focus in Illinois. J. Med. Entomol. 37, 408–415. https://doi.org/10.1093/JMEDENT/37.3.408.

Jongejan, F., Uilenberg, G., 2004. The global importance of ticks. Parasitology 129, S3–S14. https://doi.org/10.1017/s0031182004005967.

Kaaya, G.P., Hassan, S., 2000. Entomogenous fungi as promising biopesticides for tick control. Exp. Appl. Acarol. 24, 913–926. https://doi.org/10.1023/A:1010722914299.

Kaaya, G.P., Hedimbi, M., 2012. The use of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, as bio-pesticides for tick control. Int. J. Agric. Sci. 2, 245–250.

Kaaya, G.P., Mwangi, E.N., Ouna, E.A., 1996. Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*, using the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae*. J. Invertebr. Pathol. 67, 15–20. https://doi.org/10.1006/jipa.1996.0003.

Kaaya, G.P., Samish, M., Hedimbi, M., Gindin, G., Glazer, I., 2011. Control of tick populations by spraying *Metarhizium anisopliae* conidia on cattle under field conditions. Exp. Appl. Acarol. 55, 273–281. https://doi.org/10.1007/s10493-011-9471-3.

Kagaruki, L.K., 1996. The efficacy of amitraz against cattle ticks in Tanzania. Onderstepoort J. Vet. Res. 63, 91–96.

Kariuki, D.P., Young, A.S., Morzaria, S.P., Lesan, A.C., Mining, S.K., Omwoyo, P., Wafula, J.L.M., Molyneux, D.H., 1995. *Theileria parva* carrier state in naturally infected and artificially immunised cattle. Tropl. Anim. Health Prod. 27, 15–25. https://doi.org/10.1007/BF02236328.

Lorusso, V., Picozzi, K., De Bronsvoort, B.M.C., Majekodunmi, A., Dongkum, C., Balak, G., Igweh, A., Welburn, S.C., 2013. Ixodid ticks of traditionally managed cattle in Central Nigeria: where *Rhipicephalus (Boophilus) microplus* does not dare (yet?). Parasites Vectors 6, 171. https://doi.org/10.1186/1756-3305-6-171.

Maiyoh, G.K., Njoroge, R.W., Tuei, V.C., 2015. Effects and mechanisms of kerosene use-related toxicity. Environ. Toxicol. Pharmacol. 40, 57–70. https://doi.org/10.1016/J.ETAP.2015.05.010.

Maloo, S.H., Rowlands, G.J., Thorpe, W., Gettinby, G., Perry, B.D., 2001. A longitudinal study of disease incidence and case-fatality risks on small-holder dairy farms in coastal Kenya. Prev. Vet. Med. 52, 17–29. https://doi.org/10.1016/S0167-5877(01)00235-5.

Maranga, R.O., Hassanali, A., Kaaya, G.P., Mueke, J.M., 2006. Performance of a prototype baited-trap in attracting and infecting the tick *Amblyomma variegatum* (Acari: Ixodidae) in field experiments. Exp. Appl. Acarol. 38, 211–218. https://doi.org/10.1007/s10493-006-0002-6.

Marufu, M.C., Chimonyo, M., Mapiye, C., Dzama, K., 2011. Tick loads in cattle raised on sweet and sour rangelands in the low-input farming areas of South Africa. Tropl. Anim. Health Prod. 43, 307–313. https://doi.org/10.1007/s11250-010-9690-3.

Medley, G.F., Perry, B., Young, A.A.S., 1993. Preliminary analysis of the transmission dynamics of *Theileria parva* in eastern Africa. Parasitology 106, 251–264. https://doi.org/10.1017/S0031182000075077.

Mooring, M.S., Benjamin, J.E., Harte, C.R., Herzog, N.B., 2000. Testing the interspecific body size principle in ungulates: the smaller they come, the harder they groom. Anim. Behav. 60, 35–45. https://doi.org/10.1006/anbe.2000.1461.

Moumouni, P.F.A., Aboge, G.O., Terkawi, M.A., Masatani, T., Cao, S., Kamyingkird, K., Jirapattharasate, C., Zhou, M., Wang, G., Liu, M., Iguchi, A., Vudriko, P., Ybanez, A.P., Inokuma, H., Shirafuji-Umemiya, R., Suzuki, H., Xuan, X., 2015. Molecular detection and characterization of *Babesia bovis, Babesia bigemina, Theileria* species and *Anaplasma marginale* isolated from cattle in Kenya. Parasit. Vectors 8, 496. https://doi.org/10.1186/s13071-015-1106-9.

Muhanguzi, D., Picozzi, K., Hatendorf, J., Thrusfield, M., Welburn, S.C., Kabasa, J.D., Waiswa, C., 2014. Collateral benefits of restricted insecticide application for control of African trypanosomiasis on *Theileria parva* in cattle: a randomized controlled trial. Parasit. Vectors 7, 1–10. https://doi.org/10.1186/1756-3305-7-432/FIGURES/3.

Muraguri, G.R., McLeod, A., McDermott, J.J., 2003. Efficacy of a deltamethrin-based pour-on in the control of tick-borne diseases and trypanosomosis in Kwale District, Kenya. Insect Sci. Appl. 23, 69–74. https://doi.org/10.1017/s1742758400012261.

Murigu, M.M., Nana, P., Waruiru, R.M., Nga'nga', C.J., Ekesi, S., Maniania, N.K., 2016. Laboratory and field evaluation of entomopathogenic fungi for the control of amitraz-resistant and susceptible strains of *Rhipicephalus decoloratus*. Vet. Parasitol. 225, 12–18. https://doi.org/10.1016/j.vetpar.2016.05.026.

Nana, P., Nchu, F., Ekesi, S., Boga, H.I., Kamtchouing, P., Maniania, N.K., 2015. Efficacy of spot-spray application of *Metarhizium anisopliae* formulated in emulsifiable extract of *Calpurnia aurea* in attracting and infecting adult *Rhipicephalus appendiculatus* ticks in semi-field experiments. J. Pest. Sci. 2004 (88), 613–619. https://doi.org/10.1007/S10340-014-0637-8/TABLES/2.

Nchu, F., Maniania, N.K., Hassanali, A., Eloff, J.N., 2010. Performance of a *Metarhizium anisopliae*-treated semiochemical-baited trap in reducing *Amblyomma variegatum* populations in the field. Vet. Parasitol. 169, 367–372. https://doi.org/10.1016/j.vetpar.2010.01.020.

Nene, V., Kiara, H., Lacasta, A., Pelle, R., Svitek, N., Steinaa, L., 2016. The biology of *Theileria parva* and control of East Coast fever – current status and future trends. Ticks Tick Borne Dis. 7, 549–564. https://doi.org/10.1016/J.TTBDIS.2016.02.001.

Ojianwuna, C.C., Enwemiwe, V.N., 2022. Insecticidal effectiveness of naphthalene and its combination with kerosene against the emergence of *Aedes aegypti* in Ika north east, LGA, Delta state, Nigeria. Parasite Epidemiol. Control 18, e00259. https://doi.org/10.1016/J.PAREPI.2022.E00259.

Olds, C.L., Mason, K.L., Scoles, G.A., 2018. *Rhipicephalus appendiculatus* ticks transmit *Theileria parva* from persistently infected cattle in the absence of detectable parasitemia: implications for East Coast fever epidemiology. Parasites & Vectors 11, 1–11. https://doi.org/10.1186/S13071-018-2727-6.

Oundo, J.W., Kalayou, S., Bosch, Ten, Villinger, J., Koenraadt, C.J.M., Masiga, D., 2024. Ticks (Acari: Ixodidae) infesting cattle in coastal Kenya harbor a diverse array of tick-borne pathogens. Ticks Tick Borne Dis. 15, 102266. https://doi.org/10.1016/J.TTBDIS.2023.102266.

QGIS Development Team, 2020. QGIS Geographic Information System. QGIS Association. http://www.qgis.org.

Rehman, A., Nijhof, A.M., Sauter-Louis, C., Schauer, B., Staubach, C., Conraths, F.J., 2017. Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan. Parasites Vectors 10, 1–15. https://doi.org/10.1186/s13071-017-2138-0.

Rocha, J.F., Martínez, R., López-Villalobos, N., Morris, S.T., 2019. Tick burden in *Bos taurus* cattle and its relationship with heat stress in three agroecological zones in the tropics of Colombia. Parasites & Vectors 12, 1–11. https://doi.org/10.1186/S13071-019-3319-9.

Rot, A., Gindin, G., Ment, D., Mishoutchenko, A., Glazer, I., Samish, M., 2013. On-host control of the brown dog tick *Rhipicephalus sanguineus* Latreille (Acari: Ixodidae) by *Metarhizium brunneum* (Hypocreales: Clavicipitaceae). Vet. Parasitol. 193, 229–237. https://doi.org/10.1016/J.VETPAR.2012.11.020.

Sakpal, T.V., 2010. Sample size estimation in clinical trial. Perspect. Clin. Res. 1, 67–69.

Samish, M., Rot, A., Ment, D., Barel, S., Glazer, I., Gindin, G., 2014. Efficacy of the entomopathogenic fungus *Metarhizium brunneum* in controlling the tick *Rhipicephalus annulatus* under field conditions. Vet. Parasitol. 206, 258–266. https://doi.org/10.1016/J.VETPAR.2014.10.019.

Thrusfield, M.V., Christley, R., Brown, H., Diggle, P.J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., Wood, H., 2018. Veterinary Epidemiology, 4th ed. John Wiley & Sons.

Walker, A.R., Bouattour, A., Camicas, J.-L., Estrada-Pena, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M., 2003. Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Bioscience Reports, Edinburgh (UK).

Zimmermann, G., 2007. Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Sci. Tech. 17, 879–920. https://doi.org/10.1080/09583150701593963.