

(RESEARCH ARTICLE)



## Comparative analysis of tick burden, diversity, and distribution in intensively managed wildlife estates with and without prescribed burning

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### Abstract

The connection between ticks and zoonotic diseases in Zambia remains unclear due to limited data on tick distribution in private game reserves. This study examines tick control in three ex-situ game reserves across different agro-ecological regions, Lanagani game ranch in the Chibombo district, Chaminuka game ranch in the Lusaka district, and Choma game ranch in the Choma district. The current study evaluated the burden and diversity of ticks across three different management systems concerning the use of fire as a tick control and management tool.

A descriptive cross-sectional study collected 267 ticks from 36 transects across three game ranches in Central, Lusaka, and Southern provinces. These ticks were morphologically identified using the existing taxonomic keys and Olympus DF Plapo 1×4 SN5G 08128 stereomicroscope at the University of Zambia, School of Veterinary Medicine, parasitology laboratory. The Analysis of Variance (ANOVA) was used to compare means between categories and correlation coefficient to determine the strength of the relationship.

The collected species included 11 *Rhipicephalus* species,  $n=6$ , (*Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Rhipicephalus microplus*, *Rhipicephalus decoloratus*, *Rhipicephalus sanguineus*, *Rhipicephalus pulcherus*, *Rhipicephalus zambeziensis*); *Amblyomma* species  $n=2$ , (*Amblyomma variagatum*, *Amblyomma poposum*), and *Hyalomma* species  $n=2$  (*Hyalomma rufipes* and *Hyalomma truncatum*). The most common species were *R. evertsi* (19.4 %), *Amblyomma variagatum* 14%, *Hyalomma truncatum* 11%. Significantly higher ( $p < 0.05$ ) species diversity and burden were observed in the no-burn management system, whilst no significant differences ( $p > 0.05$ ) were observed between late and early burning systems.

These findings have unveiled, for the first time in Zambia, that employing fire as a management tool substantially reduces both tick diversity and burden in enclosed ex-situ conservancy areas. Regular prescribed burning, regardless of the burning regime, was found to remarkably reduce tick populations in game ranches, particularly in the early burning management system. These findings hold promising implications for ecological public health. Considering this, fire as a management tool effectively reduces tick diversity and burden in closed ex-situ conservancy areas. These findings have far-reaching policy implications when it comes to tick control systems that a government may wish to implement.

**Keywords:** Ticks; Ex-situ game reserves; Management systems; Zambia

### 1. Introduction

In recent decades, the intersection of natural resource management and tick-borne disease epidemiology has gained prominence, particularly with the advent of landscape ecology. Understanding the impact of land management practices

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on disease spread and emergence has become crucial(1,2). Spatial considerations are increasingly vital in mitigating tick diseases, as evidenced by the causal link between human-modified landscapes and the prevalence of wildlife diseases (3).

Ticks, as obligatory blood-sucking ectoparasites, rank second only to mosquitoes in transmitting pathogens that cause diseases in humans and animals (4). Their geographical distribution has expanded due to human and animal activities, socio-economic changes, and environmental shifts. The ability of ticks to transmit diseases to both wild and domestic animals pose direct and indirect economic losses. Ticks inject toxins causing paralysis and transmit various pathogens, making them a global threat to human and animal well-being (4).

Globally, ticks pose a substantial threat to human and animal well-being, parasitizing every class of terrestrial vertebrates and transmitting a diverse array of tick-borne diseases (5). In Zambia, tick-borne diseases are a significant public health and economic concern, not only locally but also in Eastern, Southern, and Central Africa (6).

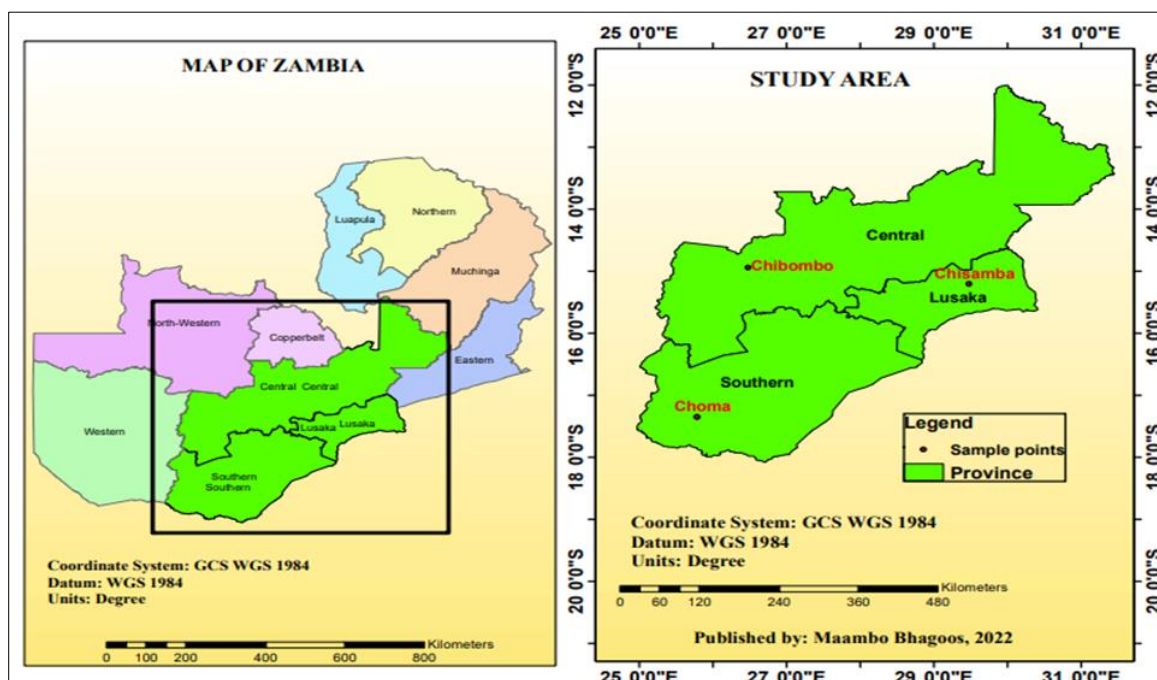
Controlling ticks in free-ranging wildlife is challenging, and current methods involving toxic acaricides and sporozoite infection have limitations and environmental drawbacks. Prescribed burning emerges as a potential solution, offering a nuanced approach to reducing tick populations in-game areas (7).

The study aims to strengthen ecological knowledge of tick distribution in private game ranches and explore the effectiveness of prescribed burning as a management tool.

## 2. Material and methods

### 2.1. Study Area

This study was conducted in three *ex-situ* conservation reserves in Zambia, Lanagani game ranch in Chibombo district (28.014460 E, 15.0431 S), Chaminuka in Chongwe district (28.49870 N, 15.15490S), and a reserve in Choma district (16.6414710 S, 27.0095800N) (figure 1). These areas fall within agro-ecological region IIa, characterized by an annual rainfall below 800 mm in Lanagani and Chaminuka, and a range between 200 mm and 1000 mm in Choma. The mean annual rainfall for Choma is approximately 617 mm (8).



**Figure 1** Geographical set up of three study areas Chisamba, Chibombo and Choma (ArcGIS, version 10.4)

The agro-ecological region IIa experiences three main seasons, a cool-dry period from May to August, a hot and dry period from September to October, and a hot-wet period from November to April. The average midday maximum temperatures range from around 31 °C to 45 °C, particularly high in October, especially in the southern fringes(8).

In Chisamba, the hot season lasts from September 15 to November 22, with an average daily temperature above 30 °C. November is the hottest month, with an average temperature of 30 °C and a low of 19 °C. The cool season spans from May 26 to August 2, with an average daily high temperature below 24 °C. July is the coldest month, with an average low of 9 °C and a high of 23 °C (figure 1).

## 2.2. Vegetation Survey

To assess potential vegetation differences in selected private game reserves, surveys were conducted in mapped transects. A singular point-centered quarter survey, equivalent to 10 points per transect, determined tree compactness. A quarter was considered empty if no trees were within 10 meters of the center point. Marked points were utilized for open woodland vegetation surveys. Ecological assessment index surveys measured open grassland vegetation and tree compactness closure (9).

## 2.3. Study Sample

A total of 267 ticks were collected from 36 transects across three game ranches in Central, Lusaka, and Southern provinces. These ticks were collected across three tick management systems, comprising 52 from late burning, 22 from early burning, and 193 from the no-burning system.

## 2.4. Field Preparation

A white work surface served for sorting ticks, while a waste bucket handled disposal. Falcon tubes (model 352099) stored engorged larvae, flat and engorged nymphs, and flat adult ticks, with snap cap lids used post-collection. The one-meter rule measured sort distances in mapped clusters. Scissors cut drag cloths for tick collection. A white tray stored ticks and falcon tubes, and masking tape captured and disposed of escaped ticks. The Global Positioning System (GPS) mapped clusters/transects, providing coordinates for spatial pattern examination.

## 2.5. Collection of Tick Samples

Tick samples were collected directly from selected *ex-situ* conservancy areas, focusing on peak nymph and adult densities between September to October 2021 and November 2021 to January 2022, respectively (10). Literature sources, on prescribed burning, were referred for comprehensive reviews and identification of existing gaps.(5,7,11–13)





**Figure 2** Picture (a) shows blanket swiping (b) tick collection from the skin (c) collected samples in the field and (d) after transect mapping

The primary dependent variable, tick density, was collected using the drag cloth method in the ecological zones of each private game reserve. This method involved dragging a white corduroy cloth over vegetation, covering a total flagged area of 80 meters per transect (14,15). Tick Samples were obtained from three ex-situ conservancy areas, with 12 transects formed per ranch where wild animals congregate and graze. Collection times were strategically chosen, with engorged female ticks and eggs separated and stored in falcon tubes upon arrival at the field station.

The second collection method utilized carbon dioxide traps (CO<sub>2</sub>-baited pitfall traps), effective for ticks active in the soil or leaf litter. Daily trapping occurred in each transect, deploying five white enamelware pans (18×29×5 cm) buried at 20-meter intervals along mapped ecological zones. These pans functioned as pitfall traps (16). Dry ice (5 to 10 grams) was added to the center of each pan, elevated by two small wooden strips during the warm part of the day; 10 am to 12 pm at major grazing sites and 12 pm to 2 pm in animal resting sites. CO<sub>2</sub> traps successfully captured various life stages of ticks, particularly the actively hunting *Amblyomma variegatum* tick (17).

## 2.6. Tick Identification

Tick samples were morphologically identified in the parasitology laboratory, school of veterinary medicine, university of Zambia, using existing taxonomic keys and an Olympus DF Plapo 1×4 SN5G 08128 stereomicroscope (18). The identification process involved recording tick species, sex, and instar for each specimen. Detailed morphological keys for ticks and their nymphal stages were documented, with adult ticks and nymph specimens photographed using an Olympus DP72 stereomicroscope and Euromex EK-1 fiber optic light source (19). After identification, ticks were preserved in falcon tubes containing 70 percent ethanol, creating a tick bank.

## 2.7. Statistical Analysis

Field data was cleaned, edited, and coded using Microsoft Excel 2021. The prepared file was then imported into STATA 18 software for hypothesis testing, considering a  $p < 0.05$  as statistically significant. Descriptive statistics were calculated for hypothesis categories, and Analysis of Variance (ANOVA) compared means. Quantification of data for prescribed burning and tick diversity distribution enabled the calculation of a correlation coefficient, assessing the strength of the relationship (20).

## 3. Results

### 3.1. Overall burden of ticks across the three management systems

A total of 267 individual ticks were collected across the three tick management systems (Early, late, and no-burning systems). The results indicate that the high tick population and burden was in the area where the management system did not apply any burning control measures, as this accounted for 72.3% of the tick burden across the different

management systems. Early burning had the lowest number of tick burden at only 8.2% of the total tick burden (Table 1).

**Table 1** Mean tick burden by management system

Tick Management System	Total number of Ticks (n=267)	%	MSD
Late Burning	52	19.5%	44.8
Early Burning	22	8.2%	23.3
No Burning	193	72.3%	98.6

**3.2. Correlation comparison of adult ticks by location using Bonferroni correlates**

Significant differences were seen between the early burning management system against the management system where no burning was seen ( $p < 0.05$ ). Significant differences were also seen between the late burning management system against the management system where no burning was practiced ( $p < 0.05$ ). No significant differences were observed between early and late burning (Table 2)

**Table 2** Correlation comparison of adult ticks by location using Bonferroni correlates

	Early Burning	Late Burning
Late Burning	-30 $p = 0.776$	
No Burning	141.167 $p=0.000$	171.167 $p=0.000$

**3.3. Overall tick species identification by proportions**

In total eleven (11) tick species were collected from 36 transects across all three study areas in Chisamba, Chibombo, and Choma. The results show the identified tick species diversity, abundance, and distribution by burning regime (Table 3). Additionally, the overall collected tick species across all the study areas by proportionate in early, late and no burning regime in selected ex-situ conservancies are illustrated in Table 4. The proportion of overall ticks collected by species, were *Hyalomma truncatum* and the *Rhipicephalus appendiculatus*, accounted for most tick species identified at the laboratory each contributing 4 ticks representing 11% at 95% CI (0-21%) (Table 4).

**Table 3** Identified Tick species diversity, abundance and distribution by burning regime

Tick species	Ranch A Chisamba No-burning	Ranch B Choma Late burning	Ranch C Chibombo Early burning
<i>Rhipicephalus evertsi</i>	4	1	2
<i>Rhipicephalus B microplus</i>	1	0	0
<i>Amblyomma variagatum</i>	3	1	1
<i>Rhipicephalus pulcherus</i>	2	1	0
<i>Hyalomma marginatum rufipes</i>	1	0	1
<i>Hyalomma truncatum</i>	2	1	1
<i>Rhipicephalus appendiculatus</i>	1	2	1
<i>Rhipicephalus zambeziensis</i>	2	1	0
<i>Amblyomma pomposum</i>	1	0	0
<i>Rhipicephalus decrolatus</i>	2	0	1
<i>Rhipicephalus sanguineus</i>	3	0	0
Species per transect	61% (22 species)	19.4% (7 species)	19.4% (7 species)

**Table 4** Overall collected tick species across all the study areas by proportionate

Species		(n)	Percentage (%)	95% CI
1	<i>Rhipicephalus evertsi</i>	7	19.4	5.8-33%
2	<i>Rhipicephalus B microplus</i>	1	2.8	0-8.4 %
3	<i>Amblyomma variagatum</i>	5	14	2-25
4	<i>Rhipicephalus pulcherus</i> (Zebra Tick)	3	8	0-17
5	<i>Hyalomma marginatum ruffipe</i>	2	6	0-13
6	<i>Hyalomma truncatum</i>	4	11	0-21
7	<i>Rhipicephalus appendiculatus</i>	4	11	0-21
8	<i>Rhipicephalus zambeziensis</i>	3	8	0-17
9	<i>Amblyomma pomposum</i>	1	2.8	0-8.4
10	<i>Rhipicephalus decrolatus</i>	3	8	0-17
11	<i>Rhipicephalus sanguineus</i>	3	8	0-17

**3.4. Bartlett’s test for equal variances across the 36 transects**

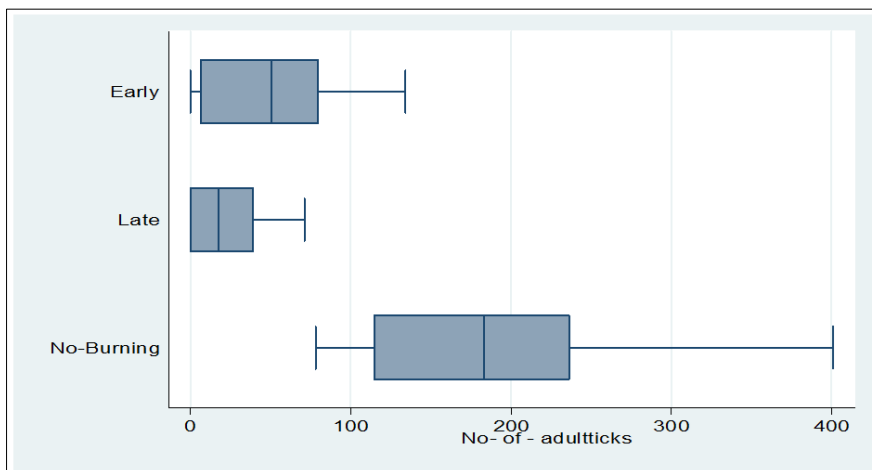
The table below shows the analysis of variance for Bartlett’s test for equal variance with a chi-square = 19.7402 and a *p* = 0.000, indicating significant differences across and within transects in the three management systems (Table 5)

**Table 5** Analysis of variance for Bartlett’s test for equal variances across the 36 transects (12 from each management system)

Sources	Sum of Square	Degrees of Freedom	Mean Square	F	P value
Between groups	200504.222	2	100252.111	24.52	0.0000
Within groups	134923.417	33	4088.58838		
Total	335427.639	35	9583.64683		

Bartlett’s test for equal variances:  $\chi^2=19.7402$  Prob>chi2=0.000

**3.5. Adult tick’s burden**



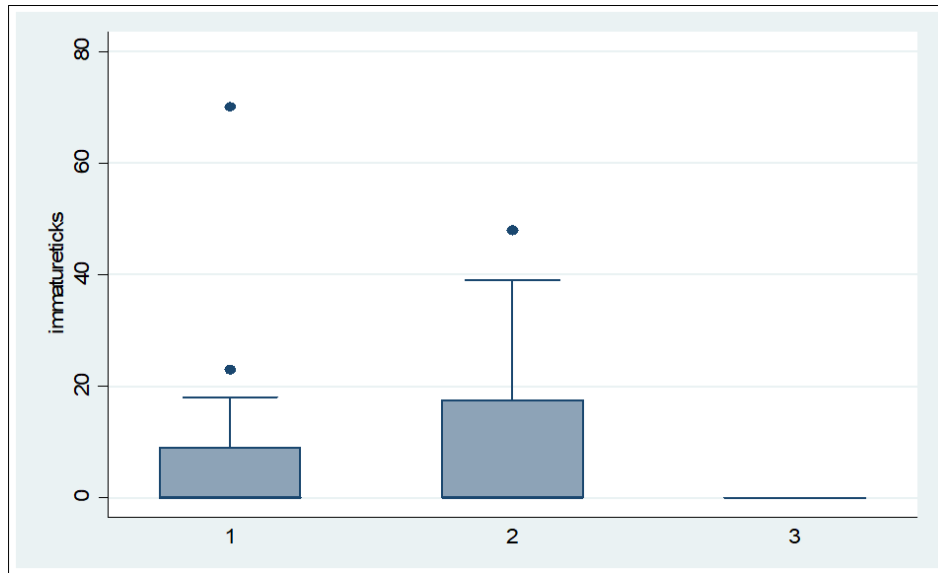
**Figure 3** Tick burden by management system for adult ticks

The results revealed that the heaviest burden was 200 ticks per transect in the no-burning management system according to the box plots at 95% CI (97-400 ticks per transect in the no-burning management system). This was

followed by early burning with a tick burden of (0-120 ticks per transect) at 95% CI. Late burning showed a lower burden at an average of 97 ticks at 95% CI (0-97 ticks per transect) (Figure 3).

### 3.6. Immature tick's burden

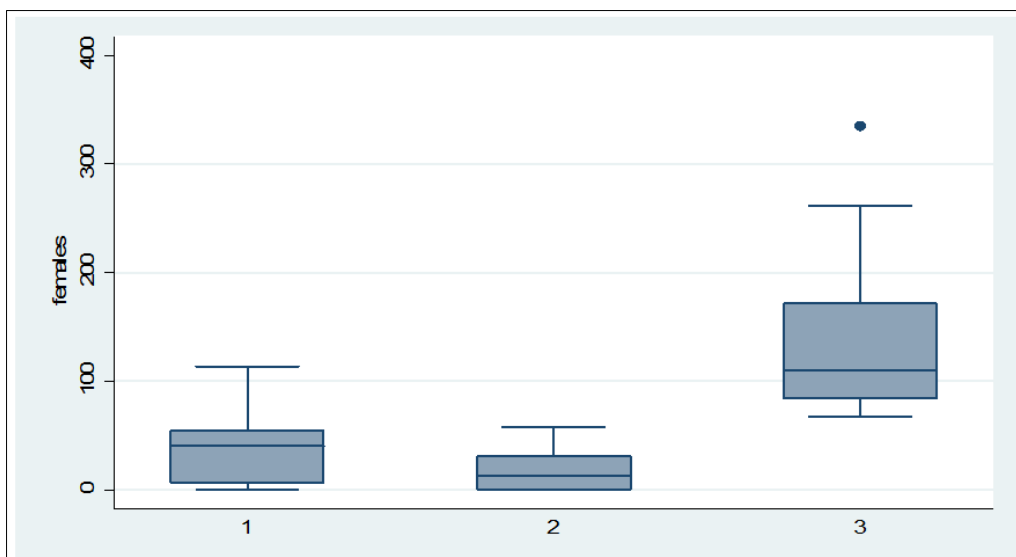
The results indicated that the heaviest burden of immature ticks was in the early burning management system according to the box plots with a 95% CI (0-30 immature ticks per transect). This was followed by late burning with a tick burden of 10 at 95% CI (0 -18) immature ticks per transect). Negligible numbers of immature ticks were accounted for under the no-burning management system in all transects (Figure 4).



**Figure 4** Tick burden by management system for immature ticks (Note: 1 = Late Burning, 2= Early burning, 3= No burning)

### 3.7. Female tick's burden

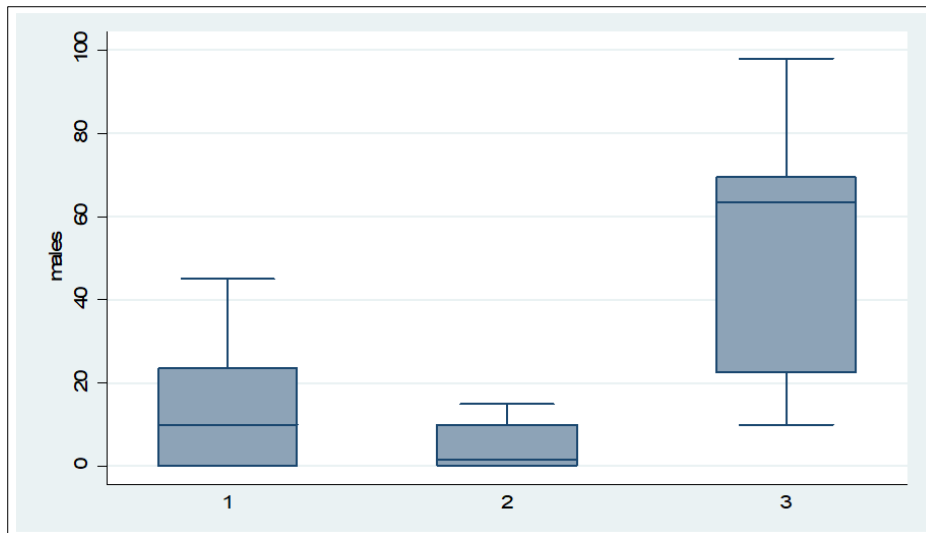
The study showed the highest burden in the no-burning system, with 180 female ticks per transect (95% CI: 95-190). Late burning had a burden of 50 (95% CI: 0-60), while early burning exhibited a lower burden, averaging 25 female ticks per transect (95% CI: 0-30) (Figure 5).



**Figure 5** Tick burden by management system showing burden across the system for female adult ticks (Note: 1 = Late Burning, 2= Early burning, 3= No burning)

### 3.8. Male tick's burden

The study found the highest burden in the no-burning system, with 75 male ticks per transect (95% CI: 20-75). Late burning had a burden of 21 (95% CI: 0-20), while early burning showed a lower burden, averaging 12 ticks per transect (95% CI: 0-15) (Figure 6).



**Figure 4** Tick burden by management system for male adult ticks (Note: 1 = Late Burning, 2 = Early burning, 3 = No burning)

## 4. Discussion

Based on the thrust of this study, the study findings have been able to elucidate the burden and diversity of tick species across three different management systems concerning the use of fire as a tick control and management tool. From the results, *ex-situ* conservancies that do not practice any fire usage on their ranches had the highest burden of ticks and the widest range of species. In total 11 tick species were identified across the three management systems (late, early, and no burning). These tick species were collected directly from mapped transects where wild animals graze.

The species determined under this present study were: *Rhipicephalus microplus*, *Rhipicephalus evertsi*, *Amblyomma variagatum*, *Rhipicephalus pulcherus*, *Hyalomma marginatum ruffipe*, *Hyalomma truncatum*, *Rhipicephalus appendiculatus*, *Rhipicephalus zambeziensis*, *Amblyomma pomposum* and *Rhipicephalus sanguineus*. *Hyalomma truncatum* and *Rhipicephalus appendiculatus*, accounted for the majority of tick species identified at the laboratory.

The finding of these species in various ecological environments in selected *ex-situ conservation* areas has been reported elsewhere although these previous studies did not take into account the different management systems(21–23). The richness of *Rhipicephalus* and *Amblyomma* tick species in continental Africa is reportedly high, and this may in part explain the findings of these species being in greater numbers in our present study than any other tick species(24). Additionally, *R. appendiculatus* tick species is widely distributed in southern, eastern, and central Africa, the distribution ranging from South Sudan to the northern parts of South Africa(25).

Similarly, *R. decoloratus* or African blue tick is widely distributed in most areas of the Africa sub-Sahara, typically within grasslands and wooded areas used as pasture for wildlife and cattle (25). *Rhipicephalus microplus*, an invasive tick species of Asian origin and considered one of the most widespread ectoparasites of wildlife and livestock, was identified from late burning management system in Choma and Chongwe districts.

In our present study, tick abundance in *ex-situ* conservancies or game ranches indicated variations in management systems. Management systems that did not practice either early or late burning were found to have the heaviest burden of ticks. This present finding intimates that prescribed burning at certain times of the year has a significant impact in reducing, as well as keeping the tick burden to a minimum level in the ecosystem. Despite the benefits of reducing the tick burden through prescribed burning, however, this practice has the potential of considerably altering both the abiotic and biotic factors that govern tick survival and reproduction in the environment as well as affecting other micro and macro fauna and flora that are key for the functional balance of the ecosystem (26,27). The findings of significantly



reduced tick burden in *ex-situ* conservation areas that practice both early and late burning are in strong agreement with, Gleim et al (11) who did not observe temporary reductions in tick populations after prescribed fire but rather sustained reductions in tick abundance for two years. However, our study was only cross-sectional and not longitudinal for us to assess the period or length of time that it takes for the tick species to revert to their normal levels after burning.

Intriguingly there were no significant differences observed between early and late burning management systems in this present study. In post-burn habitats, there are significant changes in vegetation cover, temperature, and humidity regimes (28). Whereas the abundance and diversity of vertebrate tick hosts also can be greatly affected (29,30).

Schulze and Jordan (31) in their early studies showed that vegetation cover in grasslands or forest areas is an ecological trait that has serious implications for the conditions of the forest floor, and usage of fire as a management system can seriously alter the ecological determinants. These conditions are major determinants of microhabitat suitability and actual distributions of immature and adult ticks. The results of immature tick distribution indicated a significant deviation from what the adult ticks showed.

What we observed in no-burning areas, was few to no immature ticks, as compared to the higher burden of immature ticks in the burning areas. This observation is difficult to describe in simple terms, however, factors that disturb the microhabitat's stability and suitability for survival of immature ticks may be partly responsible as they are also a function of the ecosystem. The findings of more engorged females in the no-burning system, just before the rainy season, indicated the normal cycle of tick population and survival. This indicates the changes in the tick cycle and survival that fire elicits in the different surviving tick species after the burning of the pastures.

Comparatively, early burning allowed faster regeneration of grass and shrubs compared to late burning. Accordingly, sections that were burnt early were found to hold significantly higher numbers of animals compared to the late burning whilst the no-burning showed an evenly distributed number of animals. This explanation is supported by high herbivore presence in no-burn areas and by the positive correlation between herbivore presence and the number of adult ticks. This is congruent with studies by Allan (32) and Abrams et al. (12) in the very different environment of North American woodland that suggests the time taken for tick re-invasion of burned areas is around two years post burning, hence our findings of low tick burden in either early or late burning management systems.

The findings of the study showed that *R. microplus* was widely distributed in the late burning management system compared to the two other systems. It has been postulated that *R. microplus* was introduced into East and South Africa from Madagascar, where it had originally arrived with cattle from southern Asia (25). The findings showed that this tick species was common in all study areas and was found in comparatively higher numbers. *Rhipicephalus microplus* may be present in variable numbers throughout the year, the largest numbers of adult ticks were collected in November. Theoretically, only larvae of this one-host tick should quest for hosts from the vegetation, but male ticks were also collected from the vegetation, implying that they must have detached shortly before or after molting and were now questing from the vegetation for a second host (10). This tick transmits bovine babesiosis (*Babesia bovis* and *B. bigemina*). *Babesia bovis* infection is acquired by the adults of one generation of ticks and transmitted transovarially by the larvae of the next generation and all infestation is then lost by them (25). *Rhipicephalus Appendiculatus* (brown ear tick) was found to be determined more in woodland and sedge vegetation types.

The current study showed that *R. appendiculatus* survives best in woodland and woodland savanna regions with good vegetation cover and it was mostly determined in the late burning management system as well as the no burning management system. This shows that there is a strong relationship between vegetation cover and the distribution of *R. appendiculatus* in game ranches. This tick species is responsible for the transmission of *Rickettsia conori* to humans. It is hypothesized that the saliva of *R. appendiculatus* contains a toxin and if large numbers of ticks infest an animal with this toxin can interfere with the immune processes of the host resulting in a loss of condition and outbreaks of babesiosis, anaplasmosis, and heartwater in animals that were previously immune to these diseases (33).

These results are similar to the findings of Tripp(13), the study found that higher densities of shrub layers stabilize temperature and increase humidity by limiting airflow between forest strata, as well as reducing predation on ticks. The major vegetation types in sampled areas were mostly Miombo woodland, especially in the no-burning regime (*Combretum* and *brachystegia* species). All in all, the current study areas had termitaria vegetation types as well as dambo vegetation types represented, indicating little or no ecological setup differences.

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## 5. Conclusion

In conclusion, these study findings hold promising implications for ecological public health, revealing that regular prescribed burning significantly reduces tick populations in game ranches, especially in the early burning management system. Particularly, the no-burning system exhibited a high tick burden, emphasizing the effectiveness of prescribed burning. However, the study's cross-sectional nature limited a comprehensive understanding of bioclimatic factors across seasons, reinvasion dynamics, and the lag phase in tick population recovery post-burning. Despite these limitations, the study underscores the diverse tick species' abundance and their role as vectors for significant zoonotic diseases in ex-situ conservancies.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare they have nothing to disclose.

### *Statement of ethical approval*

Clearance was sought from Excellence in Research Ethics and Science Converge (ERES), REF:2022-04-001.

### *Statement of informed consent*

Permission for tick collection and study was granted by the local government and game reserve management, with adherence to wildlife research ethics and ticks are non-humans hence no human consent was applicable.

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