

Morphological and Molecular Characterization of Ticks and Detection of *Theileria* spp. from Wild Animals at the Wildlife–Livestock Interface in Matabeleland North Province, Zimbabwe

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Ticks and tick-borne diseases remain a major constraint to livestock productivity in sub-Saharan Africa, particularly in areas where wildlife and livestock interact. This study characterized ticks collected from selected wild animals in Matabeleland North Province, Zimbabwe, using morphological and molecular approaches, and screened ticks for the presence of *Theileria* spp. A total of 16 adult ticks were collected from impala (*Aepyceros melampus*, n = 6), zebra (*Equus quagga*, n = 3), warthog (*Phacochoerus africanus*, n = 3) and waterbuck (*Kobus ellipsiprymnus*, n = 5). Morphological identification was conducted using standard Southern African dichotomous keys, while molecular confirmation employed cytochrome oxidase subunit I (COI) gene and internal transcribed spacer 2 (ITS2) PCR region. Detection of *Theileria* spp. was conducted using genus-specific PCR targeting the 18S rRNA gene. Ticks belonging to three genera (*Rhipicephalus*, *Amblyomma* and *Hyalomma*) were identified, with *Rhipicephalus* spp. accounting for 81.3% of specimens. Genus-specific PCR detected *Theileria* DNA in 5 of 16 ticks (31.3%), exclusively within *Rhipicephalus* species. Although based on a limited sample size, the findings provide baseline evidence of wildlife-livestock interface. The study highlights the importance of incorporating wildlife into tick surveillance and control strategies to support livestock health and agricultural productivity in Zimbabwe.

Keywords: ticks; wildlife–livestock interface; *Theileria*; molecular identification; agriculture; Zimbabwe

1. INTRODUCTION

Tick infestations and tick-borne diseases continue to impose substantial constraints on livestock production in sub-Saharan Africa. Diseases such as theileriosis, babesiosis and anaplasmosis reduce animal productivity, increase mortality and generate significant economic losses for farming households. Recent syntheses have documented a high diversity of protozoan, bacterial and viral tick-borne pathogens circulating within African tick populations, with important implications for rural livelihoods and food security (Djiman et al., 2024).

Wild animals play a central role in the ecology of ticks by providing alternative blood-meal sources and sustaining tick populations independently of livestock. At wildlife–livestock interfaces, ticks may parasitize multiple host species, facilitate pathogen persistence and increase opportunities for spillover into domestic animal populations. Studies from Southern Africa show that livestock grazing near wildlife conservation areas are frequently exposed to diverse tick assemblages and associated pathogens (Raboloko et al., 2020; Makwarela et al., 2024).

Despite this recognized risk to livestock health and productivity, most tick surveillance and control programs in the region focus primarily on domestic animals. Wildlife-associated tick populations remain under-represented in molecular surveillance, particularly in Zimbabwe, where wildlife conservation areas coexist closely with communal livestock systems. This limit understanding of the broader epidemiology of tick-borne diseases at interface ecosystems.

Accurate identification of tick species is fundamental for understanding disease transmission dynamics. Morphological identification remains widely used but can be unreliable when closely related or morphologically similar species coexist or when specimens are damaged. Integrative approaches combining morphology with molecular tools, particularly mitochondrial COI and nuclear ITS2 markers, have been shown to improve taxonomic resolution and reduce misidentification bias (Balinandi et al., 2020; Lieberman et al., 2024).

Information on ticks infesting wildlife and their associated pathogens in Zimbabwe remains limited, especially in Matabeleland North Province. This study therefore aimed to: (i) morphologically identify tick species infesting selected wildlife hosts, (ii) molecularly support morphological identification using COI and ITS2 markers, and (iii) detect the

presence of *Theileria* spp. in wildlife-associated ticks, with direct relevance to livestock health and agricultural disease management.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Nyamandlovu and Mangwe districts, Matabeleland North Province, Zimbabwe. The area represents wildlife-livestock interface, where wildlife conservation zones occur adjacent to communal farming systems. The region lies within a semi-arid ecological zone dominated by savanna woodland and grassland vegetation. Mean annual rainfall ranges between 450 and 650 mm, while average temperatures range from approximately 15°C in winter to over 30°C in summer. Wildlife conservation areas are located within approximately 5 km of communal grazing lands, creating frequent opportunities for tick exchange between wildlife and livestock.

2.2 Tick Collection and Preservation

Ticks were collected opportunistically from wild animals during routine wildlife management and veterinary activities conducted by trained personnel. No animals were captured, restrained, or euthanized specifically for the purposes of this study. Tick samples were obtained from impala (*Aepyceros melampus*), zebra (*Equus quagga*), warthog (*Phacochoerus africanus*) and waterbuck (*Kobus ellipsiprymnus*).

A total of 16 adult ticks were collected, comprising samples from impala (n = 6), waterbuck (n = 5), zebra (n = 3), and warthog (n = 2). Ticks were carefully removed using sterile forceps to avoid damage to diagnostic structures. Each tick was placed individually into sterile 1.5 mL microcentrifuge tubes containing 70% ethanol, transported to the laboratory, and stored at 4°C until analysis.

2.3 Morphological Identification of Ticks

Prior to examination, ticks were rinsed briefly in sterile distilled water to remove residual ethanol. Morphological identification was performed using a stereomicroscope and compound light microscope at x10, x40, and x100 magnifications.

Ticks were identified to genus and species level based on standard morphological characters, including:

- scutal and conscutal ornamentation
- basis capitula
- mouthpart length and structure
- presence and morphology of festoons
- spiracle plate shape and position
- coxal spurs and adanal plates

Identification followed standard Southern African ixodid tick taxonomic keys, including Walker et al. (2003) and Horak et al. (2002). Where morphological characters were insufficient for confident species-level identification, specimens were assigned to genus level only.

2.4 DNA Extraction

Genomic DNA was extracted individually from each tick using the Quick-DNA™ Microprep Kit (Zymo Research, USA) following the manufacturer's instructions. Adult ticks were longitudinally bisected using sterile scapel blades prior to lysis to improve DNA yield.

DNA was eluted in 30 µL of nuclease-free water and stored at -20°C until PCR analysis. DNA concentration and purity were assessed spectrophotometrically, and no pooling of samples was performed.

2.5 Molecular Identification of Tick Species

2.5.1 PCR amplification of the COI gene

Molecular identification of ticks was conducted by amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene using universal arthropod primers:

- LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'
- HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

(Hebert et al., 2003)

PCR reactions were performed in a total volume of 25 µL, containing:

- 12.5 µL of 2x PCR Master Mix
- 0.5 µM of each primer
- 2 µL of template DNA

- Nuclease-free water to volume

Thermocycling conditions were as follows:

- Initial denaturation at 95°C for 5 min
- 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min
- Final extension at 72°C for 7 min

The expected amplicon size was approximately 710 bp. Negative (no template) controls were included in all PCR runs.

2.5.2 PCR amplification of the ITS2 region

The internal transcribed spacer 2 (ITS2) region was amplified using tick-specific primers:

- ITS2-F: 5'-GTGAATTCTGCAGCTGAG-3'
- ITS2-R: 5'-ATGCTTAAATTTAGGGGTAGTC-3'

(Fukunaga *et al.*, 2000; Murrell *et al.*, 2001)

PCR reactions were prepared as described above. Thermocycling conditions included:

- Initial denaturation at 95°C for 5 min
- 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min
- Final extension at 72°C for 7 min

Expected amplicon sizes ranged between 900-1200 bp, depending on tick species.

2.6 Agarose Gel Electrophoresis

PCR products were resolved by electrophoresis on 1.5% agarose gels stained with a nucleic acid intercalating dye. Gels were visualized under ultraviolet illumination using a gel documentation system. A 100 bp or 1 kb DNA ladder was used to estimate amplicon sizes.

2.7 Detection of *Theileria* spp.

Detection of *Theileria* spp. was carried out performed genus-specific PCR targeting the 18S rRNA gene, using primers:

- Theileria-F: 5'-GAAACTGCGAATGGCTCATT-3'
- Theileria-R: 5'-TTGCGACCATACTCCCCCA-3'

(Gubbels *et al.*, 1999)

PCR reactions (25 µL) consisted of the same reagent concentrations described above. Thermocycling conditions were:

- Initial denaturation at 95°C for 5 min
- 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min
- Final extension at 72°C for 7 min

The expected amplicon size was approximately 430 bp. Positive and negative controls were included in each PCR run. Positive amplification was interpreted as evidence of *Theileria* DNA presence. As genus-specific primers were used, differentiation of *Theileria* species and assessment of parasite viability were not possible.

2.8 Study Design and Triangulation Approach

This study employed a cross-sectional, laboratory-based observational design. A convergent triangulation approach was used, integrating morphological identification with molecular diagnostics (COI and ITS2 PCR). Both data streams were generated from the same tick specimens, analyzed independently, and integrated during interpretation to enhance reliability. In cases of uncertainty, molecular evidence was used to support morphological identification.

2.9 Ethical considerations

Tick sampling was conducted during routine wildlife management activities. No animals were intentionally restrained or harmed, or euthanized for research purposes. Sampling was performed by trained personnel, in accordance with accepted veterinary and wildlife ethical standards.

Permission to collect samples was obtained from the Department of National Parks and Wildlife Management, Zimbabwe. All laboratory procedures complied with the biosafety and ethical guidelines of the National University of Science and

Technology, Zimbabwe. The study involved no human participants; therefore, informed consent was not applicable.

3. RESULTS

3.1 Tick Collection and Host Distribution

A total of 16 adult ixoid ticks were collected from four wildlife host species at the wildlife-livestock interface in Matebeleland North Province, Zimbabwe. Ticks were recovered from impala (*Aepyceros melampus*, n = 6), waterbuck (*Kobus ellipsiprymnus*, n = 5), zebra (*Equus quagga*, n = 3), and warthog (*Phacochoerus africanus*, n = 2).

3.2 Morphological Identification of Ticks

Morphological examination identified ticks belonging to three genera: *Rhipicephalus*, *Amblyomma* and *Hyalomma*. The genus *Rhipicephalus* predominated, accounting for 13 of 16 ticks (81.3%). *Amblyomma* and *Hyalomma* accounted for 2 specimens (12.5%) and 1 specimen (6.2%) respectively.



Figure 1a. Diagnostic morphological features of *Rhipicephalus camicasi* (x40) showing basis capitula (B), palp pedicels (P), cervical fields (C), scutum (SC) and spiracle plate (SP).

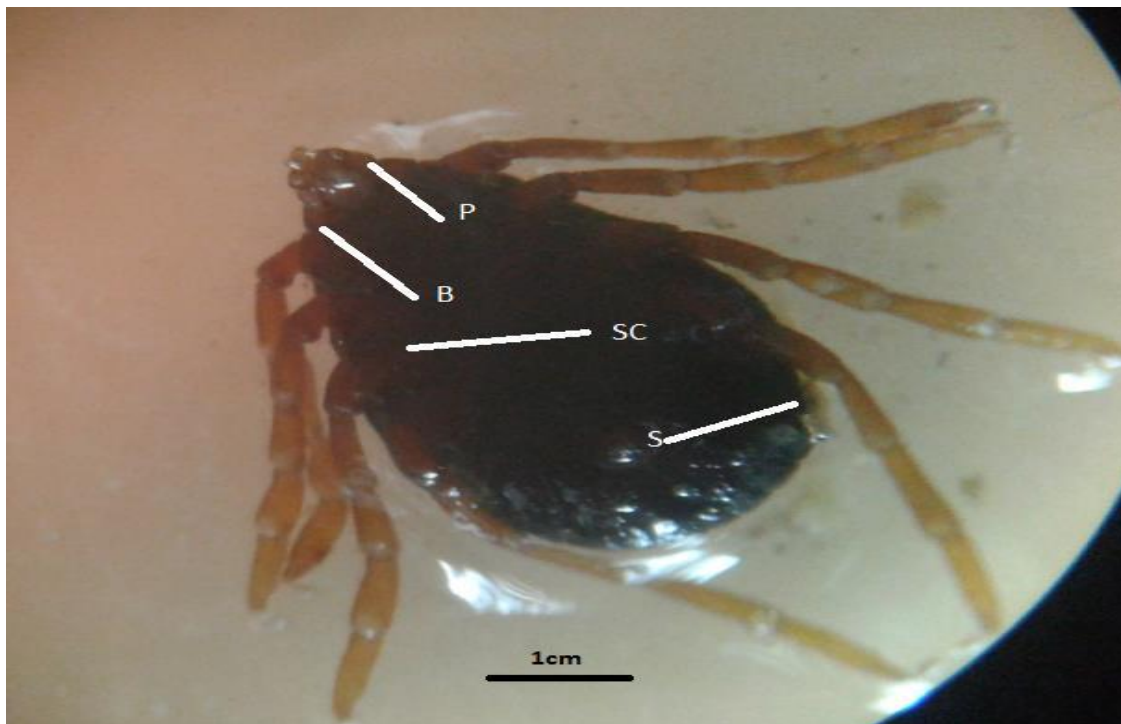


Figure 1b. Diagnostic morphological features of *Rhipicephalus appendiculatus* (x40) showing basis capitula (B), palp pedicel (P), spiracle (S) and consutum (SC).



Figure 1c. Diagnostic morphological features of *Amblyomma variegatum* (x40) showing coxae (C), scutum (SC) and ventral plates (V).



Figure 1d. Diagnostic morphological features of *Rhipicephalus bicomis* (x40) showing coxae (C), mouthparts (M) and spurs (S).

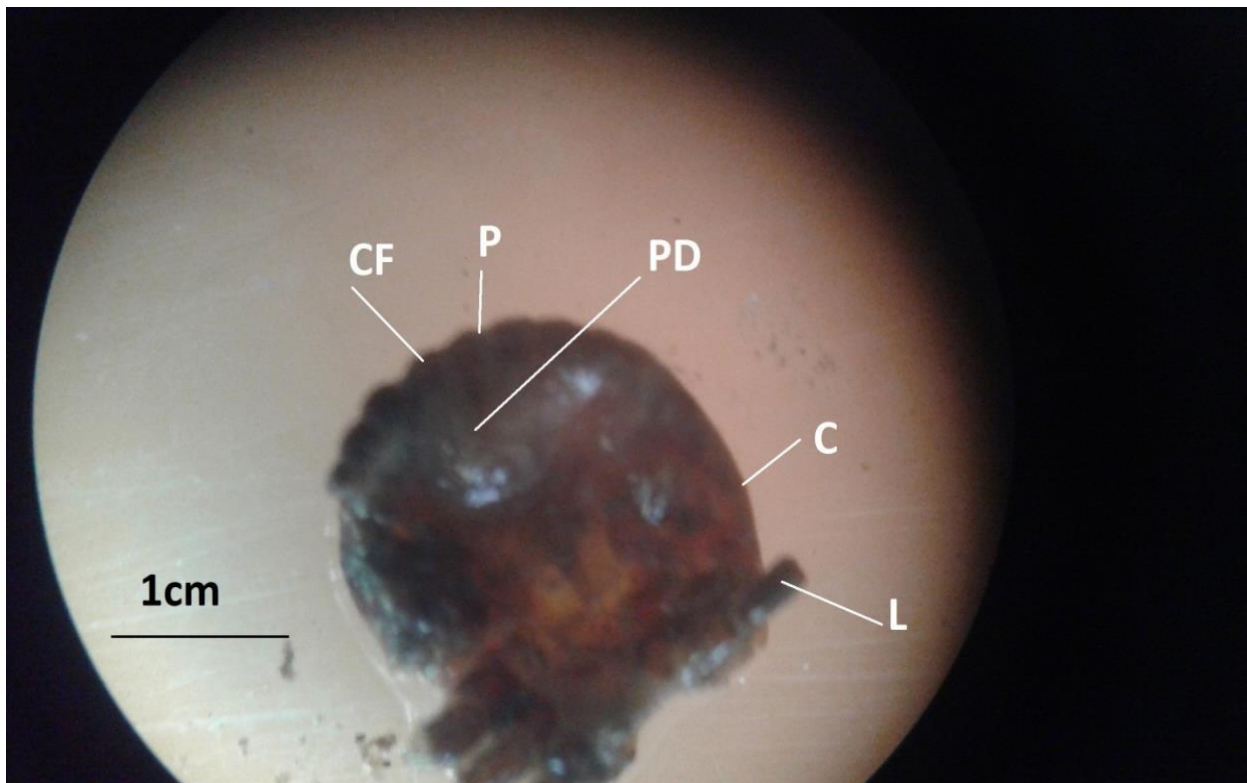


Figure 1e. Diagnostic morphological features of *Hyalomma truncatum* (x40) showing leg (L), cervical field depression (C), punctuation distribution (PD), punctuation size (P) and central festoon (CF).

Six tick species were identified morphologically: *Rhipicephalus bicornis*, *R. camicasi*, *R. lunulatus*, *R. appendiculatus*, *Amblyomma variegatum* and *Hyalomma truncatum*. One specimen was identified only to genus level (*Rhipicephalus* (*Boophilus*) spp.) due to overlapping diagnostic characters.

Table 1: Morphological identification and frequency of tick species collected from wildlife hosts (n = 16)

Tick genus	Tick species	Number of ticks (n)	Percentage (%)
<i>Rhipicephalus</i>	<i>R. lunulatus</i>	3	18.8
	<i>R. bicornis</i>	5	31.3
	<i>R. camicasi</i>	2	12.5
	<i>R. appendiculatus</i>	1	6.2
	<i>Rhipicephalus</i> (<i>Boophilus</i>) spp.	1	6.2
<i>Amblyomma</i>	<i>A. variegatum</i>	2	12.5
<i>Hyalomma</i>	<i>H. truncatum</i>	1	6.2
TOTAL		16	100

3.3 Host-Tick Associations

Ticks of the genus *Rhipicephalus* were recovered from all four wildlife host species. *Amblyomma variegatum* was detected exclusively on impala, while *Hyalomma truncatum* was detected on warthog.

Table 2: Distribution of tick genera across wildlife host species

HOST SPECIES	RHIPICEPHALUS	AMBLYOMMA	HYALOMMA	TOTAL TICKS
IMPALA	4	2	0	6
WATERBUCK	5	0	0	5
ZEBRA	3	0	0	3
WARTHOG	1	0	1	2
TOTAL	13	2	1	16

3.4 Molecular Confirmation of Tick Identity

PCR amplification of the COI and ITS2 gene regions was successful for all 16 tick DNA samples. Amplicons of expected sizes (710 bp for COI and 900 – 1200 bp for ITS2) were observed.

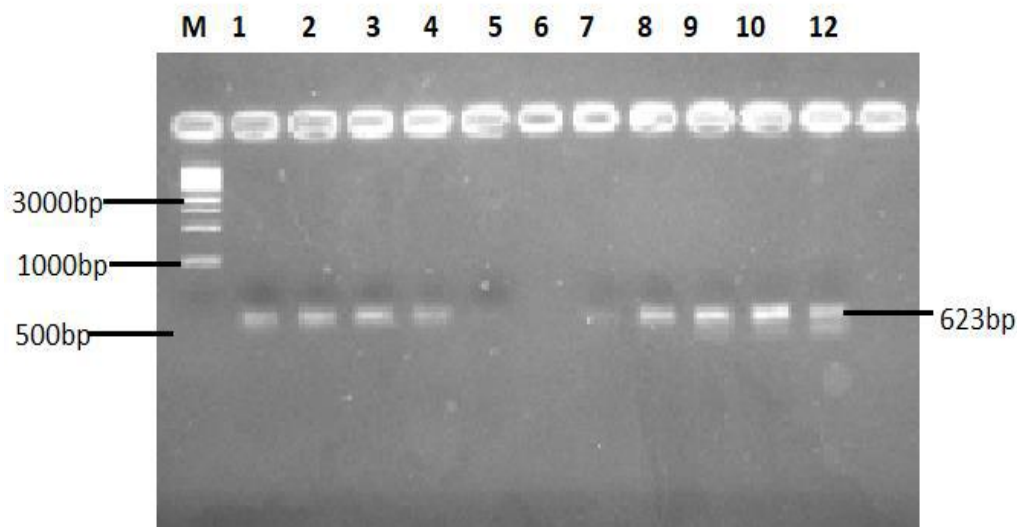


Figure 2. Agarose gel electrophoresis (1.5%) of cytochrome c oxidase subunit I (COI) PCR products from tick DNA samples. Lanes 1-16 represent individual tick samples; M indicates the 1kb ladder.

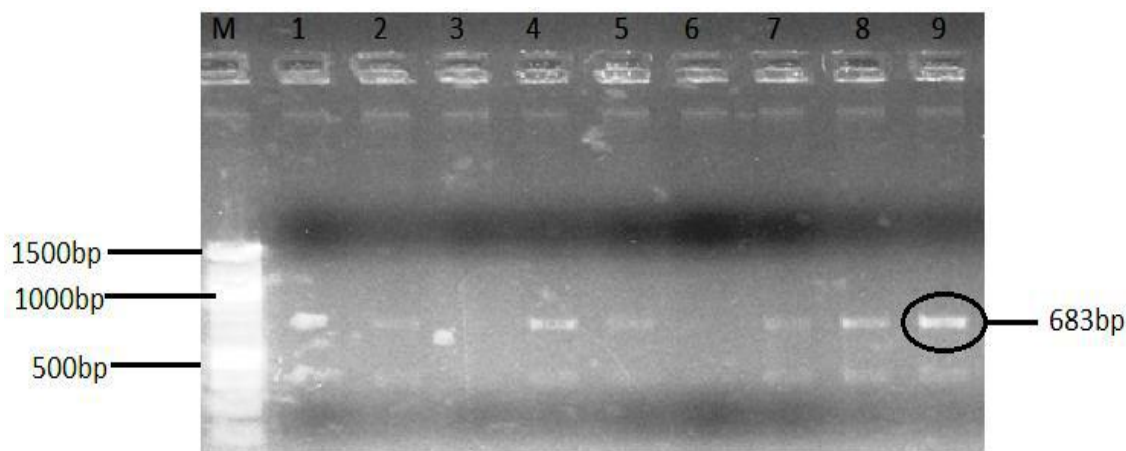


Figure 3. Agarose gel electrophoresis (1.5%) of ITS2 PCR products from tick DNA samples. Lanes 1-16 represent individual tick samples; M indicates the molecular weight ladder.

Molecular results were fully concordant (100%) with morphological identification at the genus level. Species-level assignments based on morphology were supported by corresponding COI and ITS2 band sizes. No discordance between morphological and molecular identification was observed.

3.5 Detection of *Theileria* spp.

Genus-specific PCR targeting the *Theileria* DNA in 5 of 16 tick samples, representing an overall prevalence of 31.3%.

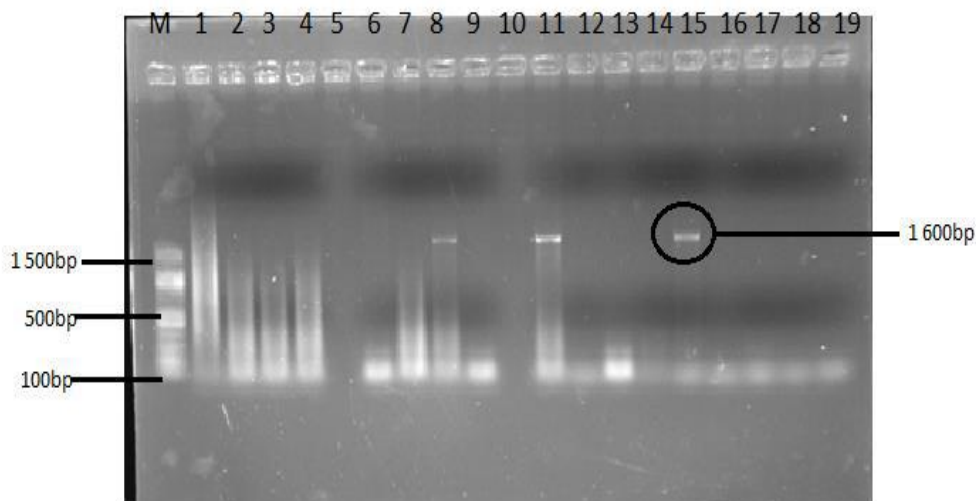


Figure 9. Agarose gel electrophoresis (1.5%) of genus-specific PCR targeting 18S rRNA gene of *Theileria* spp. Lanes indicate individual tick samples; positive samples show bands at 1600 bp and the molecular weight ladder labeled M.

Theileria-positive ticks were detected in multiple *Rhipicephalus* species. No *Theileria* DNA was detected in *Amblyomma variegatum* or *Hyalomma truncatum* specimens.

Table 3: Prevalence of *Theileria* DNA by tick species

Tick species	No. tested	No. positive	Prevalence (%)
<i>R. bicornis</i>	5	2	40.0
<i>R. lunulatus</i>	3	1	33.3
<i>R. camicasi</i>	2	1	50.0
<i>R. appendiculatus</i>	1	1	100.0
<i>Rhipicephalus</i> (<i>Boophilus</i>) spp.	1	0	0
<i>A. variegatum</i>	2	0	0
<i>H. truncatum</i>	1	0	0
TOTAL	16	5	31.3

3.6 Distribution of *Theileria* Positivity by Host Species

Ticks carrying *Theileria* DNA were recovered from impala, waterbuck, and zebra. No *Theileria*-positive ticks were detected from warthog hosts.

Table 4: Distribution of *Theileria*-positive ticks by wildlife host species

HOST SPECIES	NO. OF TICKS	NO. POSITIVE	PREVALENCE
IMPALA	6	2	33.3
WATERBUCK	5	2	40.0
ZEBRA	3	1	33.3
WARTHOG	2	0	0
TOTAL	16	5	31.3

3.7 Summary of Key Quantitative Findings

- 81.3% of ticks belonged to the genus *Rhipicephalus*
- 31.3% of ticks tested positive for *Theileria* DNA
- *Theileria* DNA was detected exclusively in *Rhipicephalus* ticks
- Multiple wildlife host species contributed to *Theileria*-positive tick samples

4. DISCUSSION

This study provides integrated morphological and molecular evidence of tick species infesting wildlife hosts at the wildlife-livestock interface in Matebeleland North Province, Zimbabwe and documents the presence of *Theileria* DNA in a proportion of these ticks. By combining quantitative species distribution data with molecular screening, the study contributes baseline epidemiological information from an under-represented ecological setting.

4.1 Tick species composition and host associations

The predominance of *Rhipicephalus* ticks in this study (81.3% of all specimens) is consistent with findings from wildlife-livestock interface systems across southern Africa, where members of this genus dominate both wildlife and livestock tick assemblages. The recovery of *Rhipicephalus* ticks from all four wildlife host species sampled indicates broad host utilization and reinforces their importance as interface vectors.

The detection of *Rhipicephalus appendiculatus*, although represented by a single specimen (6.2%), is epidemiologically noteworthy. This species is the principal vector of *Theileria parva*, the causative agent of East Coast fever. While the present study did not employ species-species *Theileria* assays, the occurrence of this vector on wildlife hosts suggests potential maintenance of transmission pathways independent of cattle. Given the small sample size, this finding should be interpreted cautiously but remains relevant for livestock disease risk assessment.

Amblyomma variegatum accounted for 12.5% of ticks and was detected exclusively on impala. Although limited in number, the presence of this generalist tick species is important due to its known capacity to parasitize both wildlife and livestock and its role as a vector of multiple pathogens. However, because *A. variegatum* was not recovered from multiple host species in this dataset, conclusions regarding its interface role in the study area should remain conservative.

Hyalomma truncatum was detected in low abundance (6.2%) and only on warthog. This finding aligns with the known ecological association of *Hyalomma* species with wildlife hosts in semi-arid environments. Its limited occurrence suggests a minor contribution to overall tick abundance at the interface during the sampling period.

4.2 Molecular confirmation of morphological identification.

PCR amplification of the COI and ITS2 gene regions was successful for all tick samples and showed complete concordance with morphological identification at the genus level. This agreement supports the reliability of the morphological identifications and highlights the value of integrative taxonomic approaches, particularly when dealing with morphologically similar *Rhipicephalus* species.

It is important to note that molecular confirmation in this study was based on expected amplicon sizes rather than sequence analysis. While this approach strengthens confidence in genus-level assignments and supports morphological species-level molecular confirmation. This limitation is acknowledged and underscores the need for future studies incorporating DNA sequencing and phylogenetic analysis.

4.3 Detection and distribution of *Theileria* spp.

Genus-specific PCR detected *Theileria* DNA in 31.3% (5/16) of tick samples. All positive samples were associated with *Rhipicephalus* ticks, reinforcing the role of this genus in the epidemiology of *Theileria* parasites. The detection of *Theileria* DNA in multiple *Rhipicephalus* species (*R. bicornis*, *R. lunulatus*, *R. camicasi*, and *R. appendiculatus*) suggests broad exposure of wildlife-associated ticks to haemoparasites within the study ecosystem.

Theileria positivity was distributed across three wildlife host species – impala, waterbuck, and zebra – with prevalence ranging from 33.3% to 40.0%. No *Theileria* DNA was detected in ticks collected from warthog hosts. Although sample sizes per host were small, these findings indicate that multiple wildlife species may contribute to the circulation of *Theileria*-infected ticks at the interface.

As genus-specific primers were used, the study cannot distinguish between pathogenic and non-pathogenic *Theileria* species, nor can it determine whether detected DNA represents viable infections or residual blood-meal material. Nonetheless, the molecular signal provides evidence of parasite exposure within the wildlife-associated tick population and should be interpreted as an indicator of potential transmission risk rather than proof of active transmission.

4.4 Implications for wildlife-livestock interface epidemiology

From an agricultural and veterinary perspective, the detection of *Theileria* DNA in nearly one-third of sampled ticks is noteworthy. Even at low sample sizes, such prevalence suggests that wildlife-associated ticks may contribute to pathogen persistence in interface areas. This has implications for communal livestock systems that rely on shared grazing resources adjacent to wildlife habitats.

The findings align with regional and global studies demonstrating that wildlife hosts can sustain diverse tick populations and serve as reservoirs or epidemiological sentinels for tick-borne pathogens. Within a One Health framework, the coexistence of wildlife, livestock, and shared tick vectors highlights the interconnected nature of ecosystem health and agricultural productivity.

5. STUDY LIMITATIONS

The study was limited by a small sample size ($n = 16$ ticks) and opportunistic sampling, which restricts the generalizability of findings. The cross-sectional design captured tick and pathogen presence at a single time point and did not account for seasonal variation. Molecular identification relied on PCR amplicon size rather than sequencing, and genus-level detection of *Theileria* did not allow species-level pathogenic assessment. Despite these limitations, the study provides valuable baseline data from an under-studied wildlife-livestock interface.

6. CONCLUSION

Wild animals at the wildlife-livestock interface in Matebeleland North Province host tick species of agricultural importance, with *Rhipicephalus* ticks comprising 81.3% of collected specimens. Genus-specific PCR detected *Theileria* DNA in 31.3% of ticks, exclusively within *Rhipicephalus* species, indicating exposure of wildlife-associated tick populations haemoparasites.

Although limited sample size, the findings suggest that wildlife may contribute to the maintenance of tick vectors and tick-borne parasites in interface ecosystems. The detection of *Rhipicephalus appendiculatus*, the principal vector of East Coast fever, highlights the potential relevance of wildlife-associated ticks to livestock disease risk. Integrating wildlife surveillance into tick and tick-borne disease control programs is therefore important for safeguarding livestock productivity in Zimbabwe. Future research should employ larger sample sizes, seasonal sampling, species-specific molecular assays, and parallel wildlife-livestock and wildlife to better quantify transmission dynamics.

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Conflict of Interest

The authors declare no conflict of interest.

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