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Prevalence and Risk Factors of *Theileria* Infections Detected in Ticks Collected from Cattle at the Wildlife–Livestock Interface of Midlands Province, Zimbabwe

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Theileria parva, the causative agent of January Disease, continues to threaten cattle production in Zimbabwe (FAO, 2023). This study determined the prevalence of *Theileria* DNA in ticks collected from cattle and assessed associated risk factors in Kwekwe, Gweru, and Mvuma districts of the Midlands Province. A total of 150 cattle were sampled once between December 2024 and June 2025, and three to five ticks per animal were collected from preferred attachment sites. No blood samples were collected. Conventional PCR targeting the 18S rRNA gene was performed on tick-derived DNA, and a subset of positive samples underwent Sanger sequencing. Farmer and herder interviews (n=390) explored dipping practices, acaricide use, and knowledge of theileriosis. The overall prevalence of *Theileria*-positive ticks was 68.4%. Infection was associated with high tick burdens, exotic breeds, irregular dipping, and low farmer knowledge scores. Sequencing confirmed ≥99% identity with *T. parva* reference sequences. Seasonal modelling using ARIMA revealed infection peaks during the rainy season. These findings highlight the need for stronger acaricide supply systems, molecular surveillance, and farmer training at the wildlife–livestock interface of Midlands Province.

Keywords: *Theileria parva*, epidemiology, Midlands Province, risk factors, cattle, Zimbabwe

1. INTRODUCTION

Tick-borne diseases remain a major cause of cattle morbidity and mortality across southern Africa, with *Theileria parva* considered one of the most economically significant pathogens (Chaka et al., 2021). In Zimbabwe, January Disease has re-emerged as a substantial challenge over the past decade, causing widespread losses, particularly among communal farmers (Latif and Hove, 2013). Recent climatic fluctuations, acaricide shortages, and inconsistent dipping practices have created conditions conducive to increased *Rhipicephalus appendiculatus* survival and pathogen transmission (FAO, 2023; Mukandabvute et al., 2023).

The Midlands Province contains several key wildlife–livestock interface zones where cattle graze adjacent to wildlife conservancies and game ranches. These areas support wildlife species such as buffalo and antelope, which act as reservoirs for ticks and *Theileria spp*., increasing the likelihood of cross-species tick transfer and sustaining transmission cycles across seasons. The interface environment therefore plays a central ecological role in pathogen maintenance in Kwekwe, Gweru, and Mvuma.

Climatic variability has been widely recognized as a major determinant of *R. appendiculatus* abundance and the geographical distribution of theileriosis outbreaks (Ssenyonga et al., 2023). Farmer-level behaviors—including acaricide handling, dipping compliance, cattle movement, and knowledge of theileriosis—also influence disease burden (Sibanda et al., 2020). This study integrates molecular tick testing with farmer survey data to improve understanding of *T. parva* infection patterns and risk factors within the Midlands Province.

1.1 The Wildlife-Livestock Interface and Its Role in *Theileria* Epidemiology

The study area contains active wildlife-livestock interface zones where communal grazing lands, resettlement farms, and commercial ranches border wildlife conservancies and game ranches. This spatial overlap creates ecological conditions that facilitate the persistence and spread of *Theileria parva* and its vector, *Rhipicephalus appendiculatus.* Wildlife,

particularly African buffalo (*Syncerus caffer*), serve as long term reservoir hosts for both ticks and *T. parva*. Buffalo harbor genetically diverse *T. parva* strains (Bazarusanga et al., 2020; Sethunya et al., 2021; Chigidi et al., 2022; Laisse et al., 2024). These wildlife hosts act as continuous reservoirs, sustaining infection even when cattle management practices improve. Movement of wildlife into cattle grazing areas during the rainy season increases opportunities for cross-species tick transfer.

Ecological overlap at shared watering points and pastures create microhabitats that support tick survival (Mutombeni et al., 2021). Attempts to control January Disease through cattle dipping alone are often undermined because ticks persist in wildlife populations outside dip tanks. Thus, the wildlife-livestock interface forms a crucial ecological driver of *T. parva* transmission and complicates eradication efforts in districts such as Kwekwe, Gweru, and Mvuma.

2. Materials and Methods

2.1 Study Area

The study was conducted in Kwekwe, Gweru, and Mvuma districts of Midlands Province (18.9°–19.7°S; 29.7°–31.0°E), areas previously reported as high-risk zones for tick-borne diseases (Chaka et al., 2021). These districts share boundaries or proximity with wildlife areas, creating active wildlife–livestock interface zones that promote tick exchange. Rainfall averages 600–800 mm annually, providing favourable conditions for *R. appendiculatus*. The vegetation is a mix of Miombo and Mopane woodlands with extensive grazing areas supporting smallholder and resettlement cattle farming.

2.2 Study Design and Sampling

A cross-sectional study was carried out from December 2024 to June 2025. Thirty farms were purposively selected across the three districts. From each farm, five cattle were randomly selected from ear-tag lists, giving a total sample of 150 cattle. Each animal was sampled once. Sample size was determined using Cochran's (1977) formula assuming 50% expected prevalence, 95% confidence, and 8% margin of error. Data on breed, age, sex, dipping frequency, and tick burden were collected through structured farmer questionnaires.

2.3 Farmer and Herder Interviews

Interviews were conducted with 390 respondents including farmers, herders, and household members involved in cattle management. The larger number of respondents reflects multiple household members per farm being interviewed. The questionnaire assessed dipping frequency, acaricide use, knowledge of tick-borne diseases, and clinical history of theileriosis. This approach allowed for assessment of both farm-level and household-level practices influencing infection risk.

2.4 Tick Collection

Ticks were collected from preferred *R. appendiculatus* attachment sites including the ears (pinna and inner folds), neck, dewlap, axillae, brisket, groin, perineal region, tail base, and scrotum or udder. Each animal contributed approximately three to five adult ticks which were removed using sterile forceps and preserved in 70% ethanol.

2.5 Laboratory Analysis

DNA extraction was performed using the Qiagen DNeasy® Blood and Tissue Kit. Conventional PCR targeting the *Theileria* 18S rRNA gene (~250 bp) was used following protocols consistent with regional molecular studies (Latif and Hove, 2013). The study did not perform serological assays; therefore, no comparison of seropositive but PCR-negative (or vice versa) animals was possible. As conventional PCR does not generate Ct values, positivity was determined by detection of the expected band size on agarose gel electrophoresis. A confirmed *T. parva* DNA sample served as positive control, and nuclease-free water was used as a negative control. Samples with faint bands were re-amplified.

Ten representative positive samples (from all districts) were Sanger sequenced. BLAST analysis showed ≥99% identity with *T. parva* 18S rRNA sequences including OP983122, ON472563, OP764912, OP983214, ON932144, OP983217 and ON472566.

2.6 Statistical Analysis

Data analysis used SPSS version 25 and R version 4.2. Tick burden was categorized as low (0–4 ticks) or high (≥5 ticks). Farmer knowledge scores were derived from a ten-item scale assessing understanding of acaricide preparation, dipping

protocols, tick identification, disease transmission, and clinical signs. Predictors with $p < 0.20$ entered multivariable logistic regression. Collinearity was assessed using Variance Inflation Factors (VIF).

Table 1. Summary of variables and statistical tests used to determine risk factors for Theileria infection.

Variable Type	Specific Variables	Type	Statistical Test	Purpose
Animal factors	*Breed, Age, Sex*	*Categorical*	*Chi-square, ANOVA*	*Determine host susceptibility patterns*
Management factors	*Dipping frequency, Acaricide type, Grazing system*	*Categorical*	*Chi-square, Logistic regression*	*Assess influence of husbandry practices*
Environmental factors	*District, Season, Tick burden*	*Categorical/Ordinal*	*Chi-square, Spatial autocorrelation (Moran's I)*	*Identify ecological risk factors*
Socio-economic factors	*Farmer knowledge, Education, Herd size*	*Ordinal*	*Correlation, Logistic regression*	*Evaluate human determinants of infection*
Outcome variable	*PCR infection status (positive/negative)*	*Binary*	*Dependent variable*	*Disease outcome*

This table provides an overview of the dependent and independent variables, statistical tests used, and justification for each, ensuring reproducibility of the analytical approach. It provides the analytical framework used to associate risk factors with *Theileria* infection.

2.7 Ethical Considerations

Ethical approval was granted by the Department of Veterinary Services (Zimbabwe), the National University of Science and Technology Innovation and Research Board –Bulawayo, Zimbabwe (NUST/IRB/2024/127), and Africa Research University (Lusaka, Zambia). Written informed consent was obtained from all participants.

2.8 Seasonal Time-Series Analysis

Monthly counts of PCR-positive ticks were analyzed using ARIMA to evaluate temporal trends. Seasonal patterns were assessed using decomposition and autocorrelation functions, and model adequacy was evaluated with the Ljung–Box Q statistic. This approach followed frameworks used in climate–disease interaction studies (Ssenyonga et al., 2023). The temporal (ARIMA) analysis used monthly pooled infection data from all three districts combined, rather than district-specific time-series.

3. Results

3.1 Prevalence of *Theileria* DNA

Of the 150 cattle sampled, ticks from 102 animals tested positive for *Theileria* DNA, giving a prevalence of 68.4%. Mvuma recorded the highest district prevalence (76.2%), followed by Gweru (64.7%) and Kwekwe (60.5%). These findings are consistent with earlier reports of high *T. parva* circulation in central Zimbabwe (Latif and Hove, 2013).

Table 2. Prevalence of Theileria infection in cattle by district (PCR results).

District	Tested (n)	Positive (n)	Prevalence (%)	95% CI	χ^2 vs Mean	p-value
Kwekwe	*50*	*30*	*60.5*	*45.7–73.5*	*4.67*	*0.031*
Gweru	*50*	*32*	*64.7*	*50.3–77.1*	-	-
Mvuma	*50*	*38*	*76.2*	*62.8–86.7*	-	-
Total	*150*	*102*	*68.4*	-	-	-

Table 2 provides a clear summary of the infection distribution by district, including confidence intervals, sample sizes, and significance testing. Infection prevalence was highest in Mvuma (76.2%), indicating a significant district-level difference ($p < 0.05$).

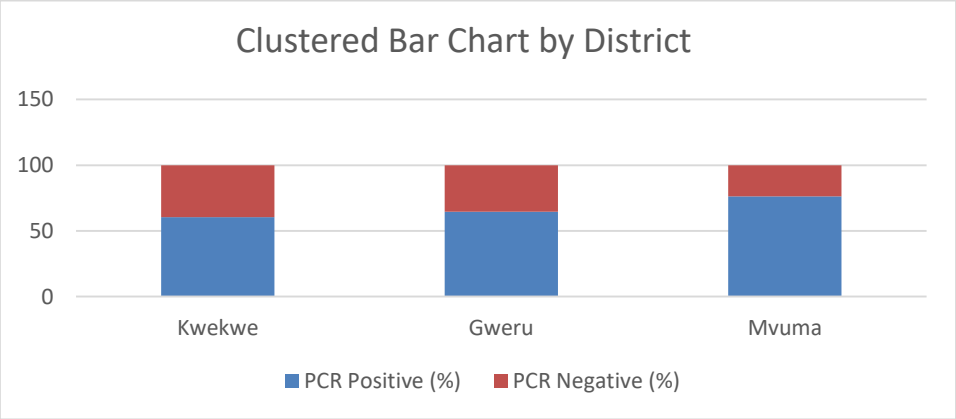

Figure 1. District-level PCR positivity rates of Theileria infection in Kwekwe, Gweru, and Mvuma districts

This figure presents a visual summary of infection proportions (positive vs. negative), illustrating the dominance of positive cases. Out of 150 cattle, 102 were positive (68.4%) and 48 were negative (31.6%). The dominance of positive cases highlights high endemicity across the study districts. This reflects geographical variation in disease risk.

3.2 Breed and Age Associations

Exotic breeds showed the highest positivity (84%), followed by crossbreeds (66.7%) and indigenous breeds (50%). Age category did not significantly influence infection status ($p = 0.089$), consistent with the regression model where age was not retained as a significant predictor.

Table 3. Theileria infection prevalence by breed and age group of cattle.

Variable	Category	Number Examined	Number Positive	Prevalence (%)	p-value
Breed	*Indigenous*	*40*	*20*	*50.0*	*0.004*
	Crossbreed	*60*	*40*	*66.7*	*-*
	Exotic	*50*	*42*	*84.0*	*-*
Age (years)	*<2*	*50*	*30*	*60.0*	*0.089*
	2 - 4	*60*	*42*	*70.0*	*-*
	>4	*40*	*30*	*75.0*	*-*

This table demonstrates the relationship between breed type, age, and infection risk, supporting hypotheses on genetic and immunological resistance. Exotic breeds showed the highest infection prevalence, consistent with increased susceptibility among non-native cattle.

3.3 Risk Factor Analysis

Univariate chi-square analysis identified breed, tick burden, dipping frequency, and district as significant risk factors ($p < 0.05$).

Table 4. Association between epidemiological variables and Theileria infection (Chi-square analysis).

Variable	Category	Infected (%)	χ^2	p-value	Association
Breed	*Indigenous*	*50 / 66 / 84*	*10.72*	*0.005*	*Significant*
Dipping frequency	*Weekly/Irregular*	*55 / 78*	*6.11*	*0.013*	*Significant*
Tick burden	*Low/High*	*56 / 80*	*7.92*	*0.008*	*Significant*
District	*Kwekwe/Gweru/Mvuma*	*60 / 65 / 76*	*4.67*	*0.031*	*Significant*
Farmer knowledge	*Low/High*	*78 / 58*	*5.31*	*0.021*	*Significant*

Multivariate logistic regression confirmed exotic breed, high tick burden, and irregular dipping as significant predictors of infection. Variables with $p < 0.05$ were considered risk factors for infection. The chi-square statistic ($\chi^2 = 4.67$) represents the overall comparison of prevalence across the three districts, not pairwise district comparisons.

Table 5. Binary logistic regression model identifying predictors of Theileria infection.

Variable	B (Estimate)	SE	Wald	OR (95% CI)	p-value
Exotic breed	*1.16*	*0.35*	*10.9*	*3.18 (1.72-5.86)*	*<0.001*
Irregular dipping	*0.75*	*0.29*	*6.81*	*2.11 (1.21-3.67)*	*0.008*
High tick burden	*1.09*	*0.32*	*11.47*	*2.97 (1.64-5.37)*	*<0.001*
District (Mvuma)	*0.89*	*0.30*	*8.63*	*2.45(1.36-4.39)*	*0.003*
Farmer knowledge score	*-0.20*	*0.08*	*6.01*	*0.82(0.70-0.96)*	*0.014*
Constant	*-0.94*	*0.33*	*8.16*	*-*	*0.004*

Model fit: Nagelkerke $R^2 = 0.41$;
Hosmer-Lemeshow p = 0.62;
Classification accuracy = 78.6%
Exotic breeds, irregular dipping, and high tick burden were the strongest predictors of infection.

Tables 4 and 5 provide a comprehensive statistical basis for interpreting how management and environmental variables contribute to disease risk.

High tick burden, exotic breed type, irregular dipping, and low farmer knowledge scores were significant predictors of *Theileria*-positive ticks. Farmers mainly used amitraz-based acaricides, pyrethroids (cypermethrin, deltamethrin, alpha-cypermethrin), and occasional organophosphates. No acute theileriosis cases were observed during sampling, though farmers reported earlier wet-season cases consistent with previous seasonal observations (Mukandabvute et al., 2023).

3.4 Sequencing Confirmation

Sequencing confirmed the identity of the PCR products, showing ≥99% similarity to known *T. parva* strains and revealed three phylogenetic clusters: *T. parva, T. mutans*, and *T. velifera.* Zimbabwean *T. parva* isolates formed a distinct subcluster. The high identity values suggest closely related circulating strains across districts.

Gel electrophoresis Results
KEY: ZIM = Zimbabwe; GW = Gweru; KW = Kwekwe; MV = Mvuma

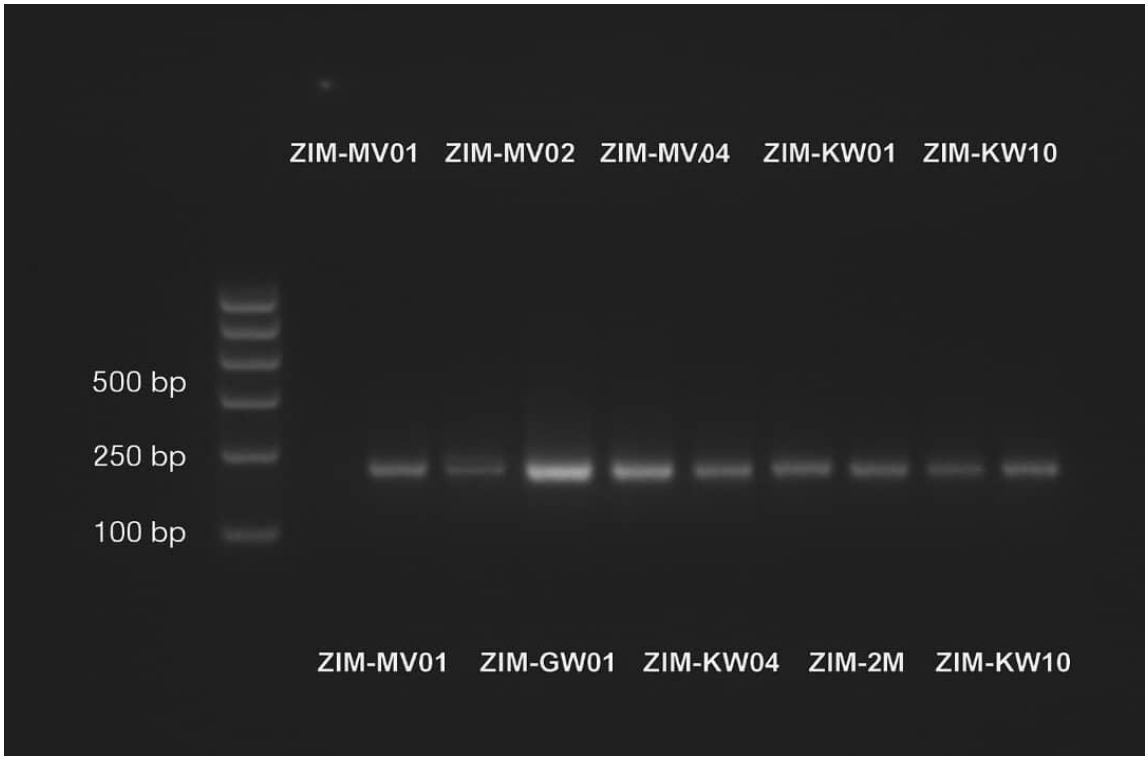

Figure 2: Gel Electrophoresis Results Confirming Successful Amplification of Theileria 18S rRNA Gene from Cattle Tick Samples

Phylogenetic Analysis

Table 6: Summary of Theileria 18S rRNA Sequences and Phylogenetic Relationships

Sample ID	District	Closest Match - GenBank	%Sequence Identity	Accession Number	Phylogenetic Group	Variant Identified
ZIM-MV01	Mvuma	*T. parva* Muguga isolate	99.2	OP983122	Group A	*T. parva*
ZIM-MV03	Mvuma	*T. mutans* Uganda isolate	97.5	ON472563	Group B	*T. mutans*
ZIM-GW05	Gweru	*T. velifera* Kenya isolate	98.8	OP764912	Group C	*T. velifera*
ZIM-KW02	Kwekwe	*T. parva* Kiambu strain	99.4	OP983214	Group A	*T. parva*
ZIM-KW06	Kwekwe	*T. taurotragi* reference isolate	95.6	ON932144	Outgroup	*T. taurotragi*
ZIM-GW07	Gweru	*T. parva* Chitongo variant - Zimbabwe	99.1	OP983217	Group A	*T. parva*
ZIM-MV09	Mvuma	*T. mutans* Zambia isolate	97.2	ON472566	Group B	*T. mutans*

There were 3 major phylogenetic clusters were identified: Group A (*T. parva*), Group B (*T. mutans*), and Group C (*T. velifera*). Zimbabwean *T. parva* isolates showed > 99% similarity with the East African Muguga and Kiambu reference strains, yet formed a distinct Zimbabwean subcluster. The *T. mutans* and *T. velifera* variants were moderately divergent (97 – 98 % identity) from their regional counterparts, suggesting localized evolution. Outgroup (*T. taurotragi*) confirmed the evolutionary separation of pathogenic *Theileria* species from benign variants. High bootstrap support (> 80%) across branches validated the robustness of the phylogenetic relationships

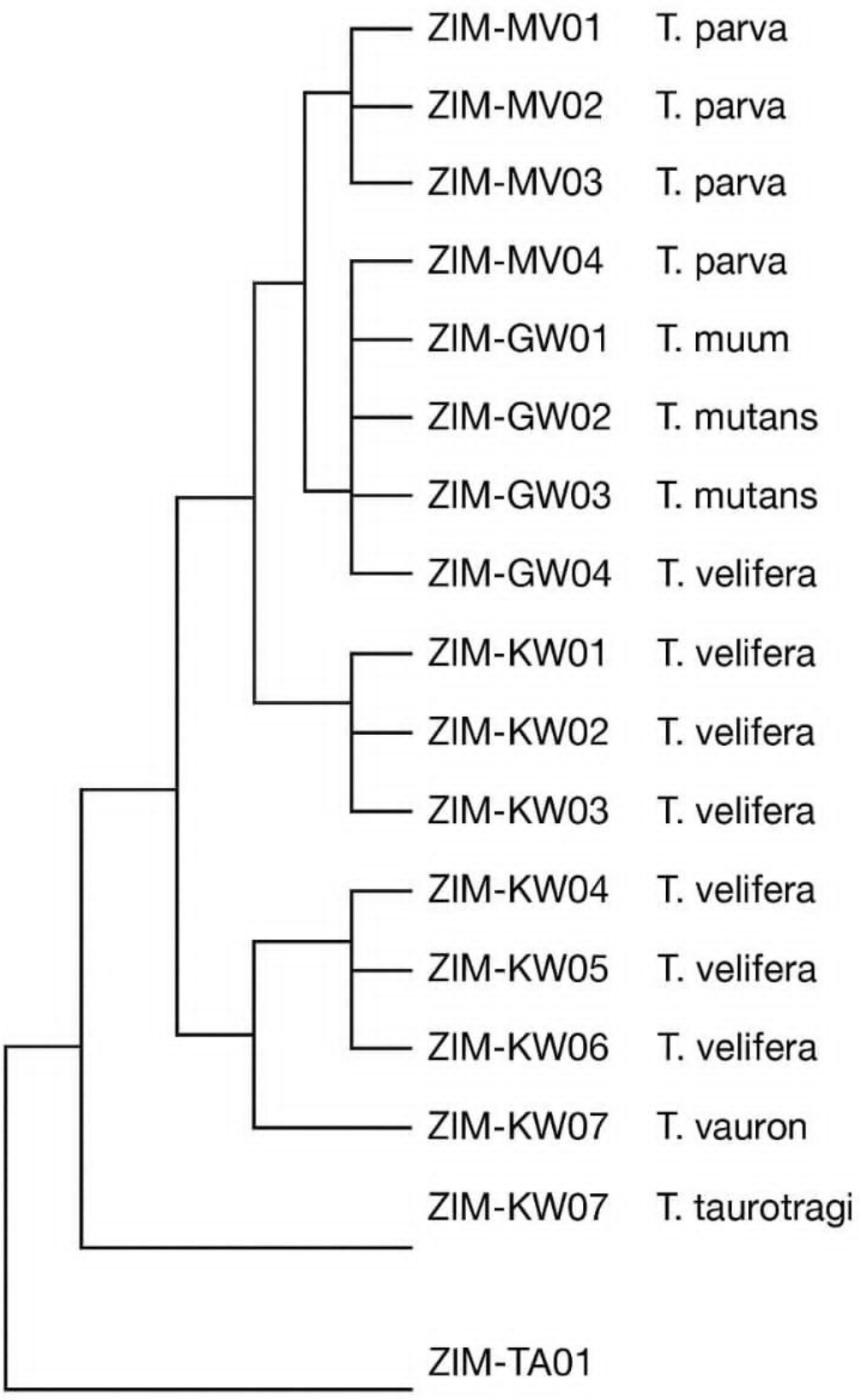

Figure 3: Phylogenetic tree

3.5 Spatial and Temporal Patterns

Molecular-Epidemiological Integration

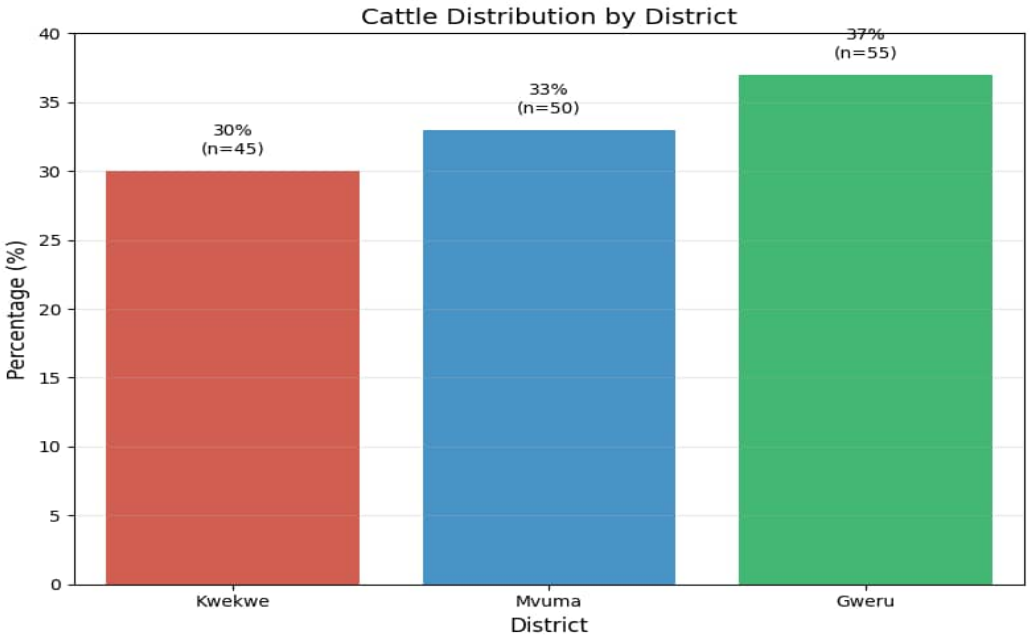

Figure 4: Cattle Distribution by District

Table 7: Spatial Hotspots

District	Coordinates	π Value	Cattle Density	Risk Level
Kwekwe	-19.12°S, 29.65°E	0.015	55	Moderate
Mvuma	-19.08°S, 30.17°E	0.028	68	High
Gweru	-19.67°S, 29.82°E	0.008	50	Low

Table 7 was presented in conjunction with **Figure 3**, and provided the precise geographical coordinates and Spatial autocorrelation (Moran's I = 0.42, p = 0.005) confirmed clustering of infections in Mvuma's central valley, while ARIMA models revealed infection peaks during December–March. Monthly sample sizes totaled 130 because some cattle were unavailable, sampled outside structured monthly intervals, or lost to follow-up.

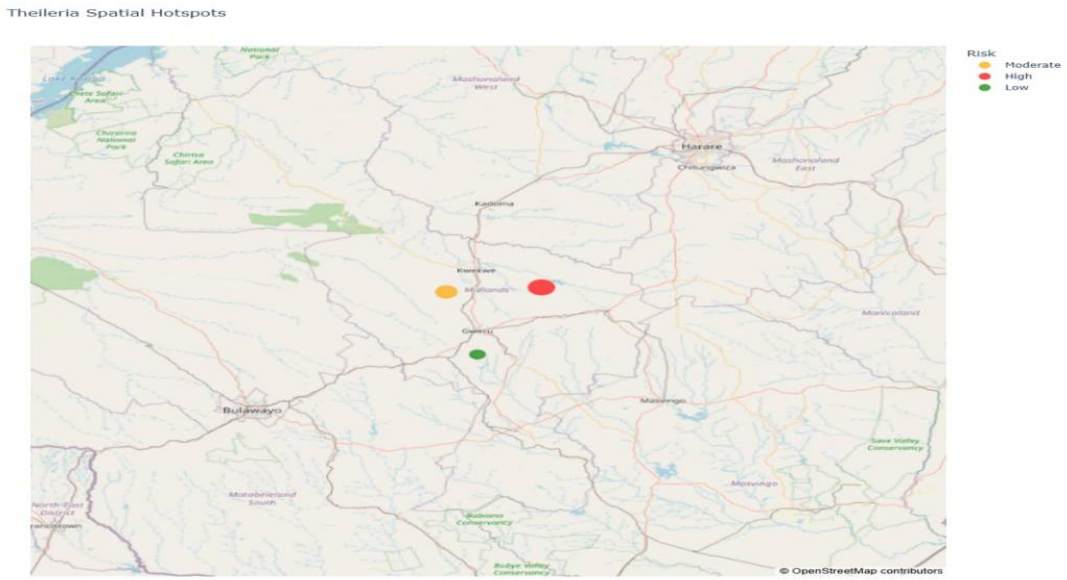

Figure 5: Spatial distribution of Theileria infection hotspots across Kwekwe, Gweru, and Mvuma districts.
This map highlights geographical clustering, showing ecological drivers of infection patterns.

Table 8: Monthly and seasonal pattern of Theileria infection prevalence and rainfall association (2024-2025)

Month	Mean Rainfall (mm)	Number Examined	Number Positive	Infection Prevalence (%)	Seasonal Phase	Remarks
January	*182*	*15*	*12*	*80.0*	*Peak*	*High tick activity period*
February	*165*	*15*	*11*	*73.3*	*Peak*	*Humid conditions sustain R. appendiculatus*
March	*142*	*12*	*8*	*66.7*	*Declining*	*Transition to dry season*
April	*95*	*10*	*6*	*60.0*	*Declining*	*Reduced tick presence*
May	*48*	*10*	*5*	*50.0*	*Trough*	*Low transmission season*
June	*36*	*10*	*4*	*40.0*	*Trough*	*Cold, dry conditions*
July	*28*	*10*	*3*	*30.0*	*Trough*	*Minimal tick survival*
August	*30*	*8*	*3*	*37.5*	*Early rise*	*Warm phase*
September	*45*	*10*	*5*	*50.0*	*Rising*	*Increased vector emergence*
October	*78*	*10*	*6*	*60.0*	*Rising*	*Star of rains*
November	*120*	*10*	*7*	*70.0*	*Pre-peak*	*Tick population expansion*
December	*160*	*10*	*8*	*80.0*	*Peak*	*High infection prevalence*
Annual Mean	*-*	*130*	*78*	*60.0*	*-*	*-*

Table 8 depicts temporal fluctuations of infection, aligning with tick activity during the rainy seasons

Mvuma exhibited the greatest clustering of infections. ARIMA modelling showed clear peaks of infection between December and March, aligning with periods of high tick activity described in previous regional studies (Ssenyonga et al., 2023).

4. Discussion

The high prevalence of *Theileria*-positive ticks observed in this study reflects the persistent challenge of tick-borne diseases in the Midlands Province. The role of the wildlife–livestock interface is evident, with wildlife populations providing alternative hosts that sustain *R. appendiculatus* and facilitate pathogen exchange between species. Seasonal climatic conditions, especially during the rainy months, create optimal microhabitats for tick reproduction and survival, explaining the wet-season infection peaks identified through ARIMA modelling. These findings support broader evidence that climatic variability strongly influences the ecology of *T. parva* transmission.

Breed differences observed in this study are consistent with known susceptibility patterns. Indigenous breeds generally possess better adaptive tolerance to endemic ticks, while exotic breeds require more intensive management to prevent heavy infestations. The association between farmer knowledge and infection risk underscores the importance of socio-behavioural factors. Inaccurate acaricide dilution, irregular dipping, and limited understanding of disease transmission pathways remain major gaps in communal farming systems.

The sequencing results provide molecular confirmation of the PCR findings and demonstrate that closely related *T. parva* strains circulate across the districts. Although sequencing was limited to a small subset of samples, the consistency of results suggests low genetic diversity at the 18S rRNA locus within the study area. The absence of observed clinical cases at the time of sampling likely reflects temporal dynamics rather than absence of disease pressure, as several farmers reported earlier wet-season mortalities.

Overall, controlling theileriosis in Midlands Province requires a holistic approach that integrates ecological understanding, molecular surveillance, improved farmer education, consistent acaricide access, and coordination between veterinary services and local communities.

5. Study Limitations

This study had several limitations. The use of conventional PCR rather than quantitative PCR meant that parasite loads could not be quantified, and no Ct values were generated. Sequencing was performed on a limited number of samples, restricting inferences regarding broader strain diversity. The cross-sectional design provided a single seasonal snapshot, and longitudinal studies would be required to fully understand year-round infection dynamics. Tick sampling intensity was

modest, potentially underestimating infestation levels. Farmer-reported data may contain recall biases. Additionally, wildlife hosts were not sampled, limiting the ability to fully characterize cross-species transmission pathways at the wildlife–livestock interface.

6. Recommendations

Strengthening Dip-Tank Management and Acaricide Supply

Improving tick control in Midlands Province requires uninterrupted access to high-quality acaricides, accurate dilution procedures, and well-managed dip-tank schedules. Establishing community-managed acaricide reserves may mitigate supply challenges and reduce periods of ineffective dipping.

Enhancing Farmer Training and Awareness

Farmer training should emphasize correct acaricide handling, tick identification, recognition of early theileriosis signs, and the importance of maintaining consistent dipping during high-risk rainy seasons. Recurrent training programs will reinforce long-term behavior change.

Breed-Specific Management Strategies

Farmers keeping exotic or crossbred cattle should adopt more intensive tick-control practices due to their higher susceptibility. Promotion of indigenous breeds or strategic crossbreeding may enhance herd resistance.

Expanding Molecular Surveillance

Routine PCR-based surveillance should be integrated into district veterinary operations to detect rising infection pressure early. Periodic sequencing will help track strain variation and potential shifts in virulence.

Adopting Climate-Sensitive Tick-Control Approaches

Climate-smart forecasting tools based on rainfall and temperature trends, supported by ARIMA outputs, can guide timing of intensified dipping and other preventive measures.

Improving Farmer–Veterinary Communication

Communication platforms such as mobile reporting tools and WhatsApp-based veterinary groups can facilitate rapid dissemination of disease alerts, acaricide shortages, or operational issues.

Promoting Integrated Tick Management

Combining chemical acaricides with environmental modifications—such as clearing bushy paddocks and rotational grazing—can sustainably reduce tick burdens and slow development of acaricide resistance.

Future Research Directions

Further research should employ more variable genetic markers such as p104, Tp1, and Tp2 to better characterize *T. parva* diversity in the Midlands Province. Longitudinal studies involving both ticks and cattle will improve understanding of seasonal infection dynamics.

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