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Research Article

Molecular Epidemiology of *Babesia* and *Theileria* spp among Slaughtered Cattle in Abeokuta, Southwestern Nigeria

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ABSTRACT

Cattle are vulnerable to tick-borne pathogens like *Babesia* and *Theileria*, which pose health threats and zoonotic risks. This study examined piroplasm infections, tick infestations, and demographics among cattle in Abeokuta, Ogun State, Nigeria. From October 2018 to February 2021, 300 slaughtered cattle were analyzed. Blood samples were assessed by microscopy and PCR, while tick identification relied on morphology. Demographic data, including breed, sex, and body condition, along with tick presence and location, were recorded. Findings showed 73.8% were White Fulani cattle. Tick infestation affected 89.3%, with piroplasm prevalence at 13.3% via microscopy and 65.7% through PCR. Most ticks were found near the anal and udder areas, with female cattle showing higher infection rates. Significant associations were identified between piroplasm infection and factors like sex, breed, body condition, and tick presence. Infection rates were high in White Fulani cattle ($p = 0.032$), those with poor body conditions ($p = 0.007$), and tick-infested cattle ($p = 0.010$). No significant relationship was found between tick sex and infection status. All cattle infested with *Amblyomma variegatum* or co-infested with *A. hebraeum* and *Boophilus microplus* tested positive. Cattle with partially or fully engorged ticks were also infected. PCR showed greater sensitivity but less specificity than microscopy. These findings highlight a significant burden of tick infestations and piroplasm infections, indicating endemicity and the urgent need for enhanced surveillance and targeted tick control strategies.

Keywords: *Babesia;* Cattle; Public Health; *Theileria;* Ticks

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INTRODUCTION

Cattle (*Bos taurus*) are large ruminants consisting of three major subspecies: *B. t. primigenius*, *B. t. indicus* (Zebu), and *B. t. taurus* (Taurine) (Wilson & Reeder, 2005; ITIS, 2018). In West Africa, indigenous Taurines, Zebu breeds, and crossbreds are commonly raised (Adebambo, 2012). In Nigeria, cattle contribute about 45% of the national animal protein intake (Lawal-Adebowale, 2012) and play important roles in dairy production, traction, hides, and cultural practices.

Predominantly managed by Fulani pastoralists, Nigerian cattle are raised under transhumant systems influenced by seasonal forage availability, vector avoidance, and sociocultural traditions (Iyayi & Taiwo, 2003; Omoike *et al*., 2014). However, this mobility increases exposure to ectoparasites—particularly ticks—during the rainy season (June–October), when infestations peak (Bayer & Maina, 2002).

Tick species such as *Rhipicephalus decoloratus*, *Amblyomma variegatum*, and *Hyalomma truncatum* are widely distributed throughout

Nigeria and serve as vectors for several veterinary and zoonotic pathogens, including *Babesia*, *Theileria*, *Anaplasma*, and *Ehrlichia* spp. (Jongejan & Uilenberg, 2004; Lorusso *et al*., 2016). Among these, *Boophilus microplus*, *A. variegatum*, and *H. dromedarii* are particularly significant due to their effects on livestock productivity (Horak *et al*., 2002; Kamani *et al*., 2017; Akande & Fagbemi, 2018).

Tick-borne haemoprotozoa such as *Babesia* and *Theileria* spp. are responsible for babesiosis and theileriosis, respectively—diseases characterized by fever, anaemia, jaundice, and haemoglobinuria (Mehlhorn & Schein, 1984; Anosa, 1988). Economically significant *Babesia* species include *B. bigemina*, *B. bovis*, *B. divergens*, and *B. major*, while *T. annulat*a and *T. parva* cause more severe infections that are often fatal in naïve animals (M'ghirbi *et al*., 2008; Lacrosse, 2017). Although natural immunity can reduce disease severity, antigenic diversity and life cycle complexity hinder effective vaccine development (Bishop *et al*., 2004). Recovered animals may remain chronically infected and serve as reservoirs (Purnell, 1981).

Due to the subclinical nature of many infections, accurate diagnosis is essential. While microscopy is still widely used, its sensitivity remains limited (Altay *et al*., 2008). Molecular diagnostics, particularly polymerase chain reaction (PCR), provide enhanced accuracy and are increasingly important in surveillance programs (Amorim *et al*., 2014; Opara *et al*., 2016).

This study examines the prevalence and molecular diversity of *Theileria* and *Babesia* spp. in slaughtered cattle in Abeokuta, Nigeria. It further investigates the associations with tick infestation and host demographic factors to inform control strategies for bovine piroplasmosis.

MATERIALS AND METHODS

Study Area and Location

The study was conducted in Abeokuta, the capital of Ogun State (Figure 1) South-West Nigeria, situated between latitudes 6.2°Nand 7.8° N and longitudes 3.0°E and 5.0°E (Akande *et al*., 2010) in the tsetse fly-infested forest climate, which is below the seventh parallel in the west and the sixth parallel in the east, with an annual rainfall between 1800mm and 3000mm, approximately 600,000km 2 to 700,000km 2 (Jahnke, 1982). The rainy season is split into two parts (April-July and September-October) while the dry season begins in November and runs through March (Sam-Wobo *et al*., 2016). Abeokuta metropolis has only two Local Government Areas (LGAs); Abeokuta South Local Government Area and Abeokuta North LGA, with Odeda LGA covering the city's outskirts, are accessed via the Abeokuta/Ibadan expressway. Each local government had at least one abattoir with a stationed veterinarian. Three abattoirs, one from each LGA, were chosen using the number of slaughters per day as criterium; Lafenwa (Abeokuta North LGA), Gbangba (Abeokuta south LGA) and Obantoko (Odeda LGA).

Consents

Permission was obtained from the Ogun State Veterinary Services, and abattoir heads were selected. Before sampling, verbal informed consent was also secured from cattle owners and butchers.

Animal Selection and Examination

Three hundred (300) cattle were randomly selected from three LGAs, irrespective of age, sex, or size. Physical examinations documented sex (via external genitalia), breed (based on hump, horn, coat color, and height per (Adebambo, 2012), age (determined by dental eruption patterns; Parish & Karisch, 2013), and body condition score (BCS) on a 9-point scale (Eversole *et al*., 2005). Animals were categorized as young (<6 months) or adult (≥6 months), with BCS classified as thin (1–4), optimal (5–7), or heavy (8–9).

Sample Collection and Processing

About 5mls of venous blood samples were collected in EDTA tubes post-slaughter, labeled, and transported to the Veterinary Parasitology Laboratory at the College of Veterinary Medicine. Thick and thin smears were stained and examined microscopically for piroplasms (Cheesbrough, 2006). Positive reference samples (*Theileria* spp., *Babesia* spp.) were obtained from Akande Laboratory, FUNAAB.

Ticks were manually removed using forceps (Okello-Onen *et al*., 1999), stored in 70% ethanol at 4°C (Levin & Schumacher, 2016), and subsequently cleaned and sorted by species, sex, life stage, and engorgement (Rodríguez *et al*., 2014). Morphological identification employed standard taxonomic keys (Horak *et al*., 2002; Walker *et al*., 2003, 2014).

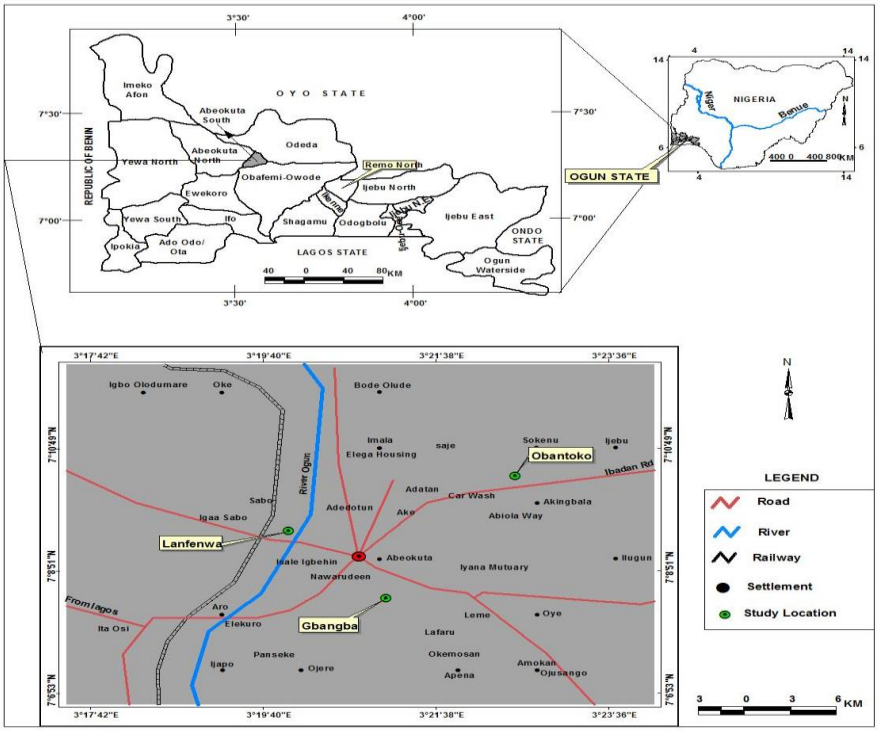

Figure 1: Top Right: Map of Nigeria showing Ogun State. Top Left: Map of Ogun State showing Abeokuta. Bottom: Map of Abeokuta showing the study sites: Lafenwa, Gbangba, and Obantoko

DNA Extraction and PCR Amplification

According to the manufacturer's protocol, DNA was extracted from 100 µl of whole blood using a commercial kit (NIMR, Lagos). The quality of DNA was confirmed by electrophoresis and Nanodrop. PCR targeted the 18S rRNA gene using primers Pyro A/B (Gubbels *et al*., 1999). Each 20 µl reaction included FIREPol® Master Mix (Solis BioDyne), primers, DNA template, and DNA-free water. Thermal cycling involved an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (94°C), annealing (58°C), and extension (72°C), each lasting 1 minute, with a final extension at 72°C for 6 minutes. Products were visualized on ethidium bromide-stained agarose gels.

Sequencing and Analysis

PCR products were purified using the QIAquick® PCR Purification Kit and sequenced bidirectionally on the ABI® 3730xl (Applied Biosystems) at Epoch Life Science Inc., USA. The sequence quality was verified using ABI 5.2 software; consensus sequences were generated in BioEdit and compared against GenBank entries using BLASTn.

Statistical Analysis

Data was analyzed using SPSS version 20.0. Descriptive statistics summarized frequencies. Chi-square tests assessed associations between infection status and categorical variables. Statistical significance was set at $p < 0.05$.

Results and Discussion

Demographic and Epidemiological Characteristics of Cattle and Tick Populations

In this study, 300 cattle were sampled across three abattoirs in Abeokuta, with the majority (72%) coming from the Lafenwa abattoir (Table 1). The cattle population was primarily female (92.3%), and most animals had body condition scores ranging from optimal to heavy. White Fulani (Bunaji) cattle represented 78.3% of the sample, emphasizing their prominence in the local market. This aligns with previous reports on the breed's broad distribution and commercial value, particularly for beef and milk production (Alphonsus *et al*., 2012).

Approximately 89.3% of the cattle were infested with ticks, most commonly around the anal region (40.7%) and udder region (32.7%). The identified tick species included *Boophilus microplus* (60.0%),

Amblyomma hebraeum, and *A. variegatum*. Most of the recovered ticks were female (80%), exhibiting varying levels of engorgement: fully engorged (31.8%) and partially engorged (37.1%) (Table 2).

PCR analysis showed a piroplasm prevalence of 65.7% in cattle, significantly higher than the 13.3% recorded with microscopy (Fig. 2).

Table 1: Demographic Characteristics of Cattle Population

Variable	**Frequency (%) N = 300**	**Number positive for Piroplasm**		***p-value***
		Microscopy **(%)**	PCR **(%)**	
Sex				
Female	277 (92.3)	40 (14.4)	191 (69.0)	0.04*[a]
Male	23 (7.7)	0 (0.0)	6 (26.1)	0.01*[b]
Body condition score (BCS)				
Thin (1-4)	38 (12.7)	3 (7.9)	35 (92.1)	0.38[a]
Optimum (5-7)	158 (52.7)	26 (16.5)	125 (79.1)	0.01 *[b]
Heavy (8-9)	104 (34.6)	11 (10.6)	37 (35.6)	
Cattle Breed				
Bos taurus taurus (Taurines)	0			
Bos taurus indicus (Zebus)	300 (100.0)	40 (13.3)	197 (65.7)	
White Fulani	235 (78.3)	20 (8.5)	170 (72.3)	0.07[a]
Chad	16 (5.3)	7 (43.8)	5 (31.3)	0.03*[b]
Sokoto Gudali	5 (1.7)	0 (0.0)	0 (0.0)	
Crossbreed	44 (14.7)	13 (29.5)	22 (50.0)	
Cattle Age				
1-3	131 (43.7)	23 (17.6)	92 (70.2)	0.68[a]
4-6	98 (32.7)	12 (12.2)	59 (60.2)	0.07[b]
7-9	66 (22.0)	4 (6.1)	46 (69.7)	
>9	5 (1.6)	1 (20.0)	0 (0.0)	
Tick Infestation				
Present	268 (89.3)	37 (13.8)	180 (67.2)	0.71[a]
Amblyomma spp	45 (16.8)	3 (8.1)	24 (13.3)	0.01*[b]
Boophilus spp	170 (63.4)	5 (13.5)	120 (66.7)	0.00*[ab]
Both species	53 (19.8)	29 (78.4)	36 (20.0)	0.09[ba]
Absent	32 (10.7)	3 (9.4)	17 (53.1)	
Site preference of ticks on cattle				
Anal	122 (40.7)	9 (24.3)	89 (49.4)	0.00*[a]
Udder	98 (32.7)	12 (32.4)	57 (31.7)	0.16[b]
Groin	10 (3.3)	4 (10.8)	7 (3.9)	
Abdomen	25 (8.3)	8 (21.6)	19 (10.6)	
Limbs	13 (4.3)	4 (10.8)	8 (4.4)	
Abattoir				
Lafenwa	216 (72.0)	31 (14.4)	145 (67.1)	0.591[a]
Gbangba	46 (15.3)	4 (8.7)	28 (54.3)	0.391[b]
Obantoko	38 (12.7)	5 (13.2)	24 (63.2)	

Note: *p-value $\leq$ 0.05 where differences between categories are statistically significant, [a] is p-value using microscopy technique, [b] is p-value using PCR technique, [ab] is p-value using microscopy technique for the various tick species, [ba] is p-value using PCR technique for the various tick species, prevalence of piroplasm using microscopy was 13.3% (40 of 300), prevalence of piroplasm using PCR was 65.7% (197 of 300)

Table 2: Characteristics of Ticks Collected in the Study

Variables	Frequency (%) N = 966	Number positive for Piroplasm N = 203		*p- value*
Tick Species		*Theileria* spp **(%) N=176**	*Babesia* spp **(%) N=27**	
A. variegatum	194 (20.1)	45 (25.6)	7 (25.9)	
A. hebraeum	192 (19.9)	24 (13.6)	0 (0.0)	
B. microplus	580 (60.0)	107 (60.8)	20 (74.1)	
Feeding Status				
Not engorged	300 (31.1)	52 (29.6)	0 (0.0)	
Partially engorged	358 (37.1)	24 (13.6)	4 (14.8)	
Fully engorged	308 (31.8)	100 (56.8)	23 (85.2)	
Sex of Ticks				
Females	773 (80.0)	107 (60.8)	25 (92.6)	
Males	193 (20.0)	69 (39.2)	2 (7.4)	

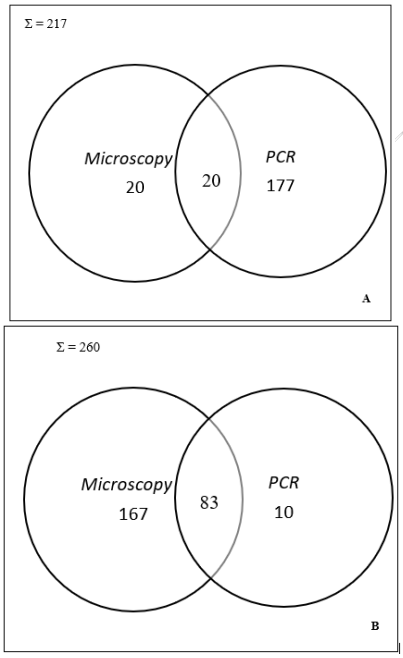

Figure 2: Comparing the prevalence of piroplasm infection in the cattle population using microscopy and PCR. A- Positive cases; B- Negative cases

Among the PCR-positive cases, 93.9% were infected with *Theileria* spp., 3.1% with *Babesia* spp., and 3% had co-infections. Of the 40 samples that tested positive by microscopy, 20 were confirmed by PCR, while 83 of the 260 microscopy-negative samples were also negative via PCR. This discrepancy highlights the superior sensitivity of molecular diagnostics in detecting subclinical infections.

Plate 1 shows an electropherogram highlighting the various band sizes at approximately 400 bp for *Babesia spp*. (Gandy *et al*., 2024) using universal primers PIRO-A/PIRO-B, which target a fragment of the 18S rRNA gene. Table 3 presents the sequencing results, supporting the detection of mostly *Babesia* spp. in tick samples and *Theileria* spp. in blood samples. The species of *Theileria* characterized in this study include: *Theileria mutans* (MT898574 and KX882747), *Theileria* spp. Yokoyama (LC467609), and uncultured *Theileria* spp. (LC553509). *Theileria mutans* were the most prevalent of the *Theileria* spp. at 50.0%. The characterized species of *Babesia* are *Babesia vulpes* (MW056071) and *Babesia* sp.

(MG264372) (Table 3). *Babesia vulpes* (75.0%) was the most prevalent (Table 3).

Theileria mutans is a benign form of *Theileria* (Agina *et al*., 2020) and may shed further light on the pathogenesis observed in cattle throughout this study. *Babesia vulpes*, a *Babesia* found in dogs, may have been introduced by *Amblyomma variegatum*. This three-host tick has an indiscriminate host range, including wild and domesticated animals such as dogs (Walker *et al*., 2014). Dogs are common in the bush, especially as hunting companions, which may explain how ticks encounter them. Some pastoralists also keep dogs that may accompany them during their transhumant sojourn. A life stage (larvae or nymph) may have fed on the dogs, dropped off to molt, and the adult may eventually end up on cattle (Walker *et al*., 2014). Reports have shown that *Amblyomma* spp. and *Boophilus* spp. can vector *Babesia* spp. (Tomassone *et al*., 2005; Kamani *et al*., 2011). However, the role of *Amblyomma* spp. in transmitting *Babesia* spp is unknown, *Boophilus* spp. will successfully transmit *Babesia* spp. However, the development and infectivity of *Babesia vulpes* in cattle have yet to be reported.

Tick Infection and Transmission Dynamics

Of the 966 ticks collected, 21% tested positive for piroplasm DNA, with *Theileria* spp. accounting for 85.2% of infections and *Babesia* spp. for 14.8% (Table 2). Infected ticks were sourced from 71 cattle, including 55 carrying *Theileria*-positive ticks, five with *Babesia*-positive ticks, and 11 harboring both. Notably, all cattle infested with *A. variegatum* or the combination of *B. microplus* and *A. hebraeum* were PCR-positive for piroplasm infection. This supports prior evidence of the high vectorial capacity of these tick species in transmitting *Theileria* and *Babesia* parasites (Walker *et al*., 2003; Madder *et al*., 2010).

Engorgement status appeared to correlate with infection, as all 27 cattle infested with partially or fully engorged ticks tested positive for piroplasms. This observation supports the life-cycle-based understanding of pathogen transmission in ticks. For instance, *B. microplus* acquires *Babesia* spp. during feeding, it can be transmitted in subsequent life stages, while *Theileria* spp. is acquired during larval or nymphal feeding and transmitted by the next nymphal or adult stage (Madder *et al*., 2010).

Interestingly, 21% of PCR-positive cattle showed no visible tick infestation at sampling, suggesting prior exposure. Since engorged female ticks often detach to oviposit, their absence does not rule out previous contact with infected vectors nor indicate resistance to infection.

Diagnostic Performance of PCR vs. Microscopy

Using microscopy as the gold standard, PCR demonstrated a sensitivity of 66.7% but a low specificity of 31.9%, resulting in an overall accuracy of 35.5% (Table 4).

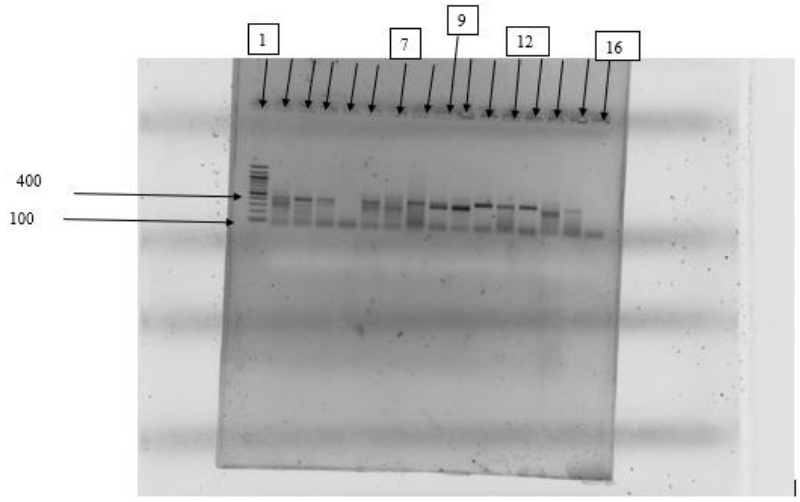

Plate 1: Agarose gel showing DNA bands of the different parasites; 1- Molecular Ladder; 2- Positive Control for *Theileria* spp; 3-4, Blood samples positive for *Theileria* spp; 5- Blood sample negative for any parasite; 6 and 8-Tick samples positive for *Theileria* spp; 7- Tick Sample negative for any parasite; 9 Positive control for *Babesia* spp; 10- Blood sample positive for *Babesia* spp; 11-13- Tick samples positive for *Theileria* spp; 14-Sample positive for *Babesia* spp;15- Sample positive for *Theileria* spp; 16-Negative control

Table 3: Characterization and Speciation

Sample Origin	Specie Identified	Percentage Homology (%)	Accession Number
Blood	*Theileria* sp Yokoyama	72.45	LC467609
Blood	*Theileria mutans*	90.91	MT898574
Blood	Uncultured *Theileria* sp	83.53	LC553509
Blood	*Theileria mutans*	84.71	KX882747
Blood	*Babesia* sp	81.61	MG264372
Tick	*Babesia vulpes*	87.99	MW056071
Tick	*Babesia vulpes*	87.50	MW056071
Tick	*Babesia vulpes*	85.58	MW056071

Table 4: Sensitivity and Specificity of PCR Technique in the Detection of Piroplasm in Cattle Blood using Microscopy as Gold Standard

Statistic	Value	95% CI
Sensitivity	66.67%	47.19% - 82.71%
Specificity	31.92%	26.30% - 37.96%
Positive Likelihood Ratio	0.98	0.75 - 1.28
Negative Likelihood Ratio	1.04	0.61 - 1.79
Disease prevalence (*)	13.3%	7.09% - 14.44%
Positive Predictive Value (*)	10.1%	7.97% - 12.85%
Negative Predictive Value (*)	89.25%	82.92% - 93.42%
Accuracy (*)	35.52%	30.01% - 41.32%

The positive predictive value was 10.15%, while the negative predictive value was 89.25%. These findings emphasize the higher sensitivity of PCR in detecting latent and chronic infections, whereas microscopy may overlook cases of low parasitemia. Nevertheless, the dependence on microscopy in low-resource settings is justifiable due to its affordability and ease of operation.

The discrepancy in detection emphasizes a critical public health concern—cattle with undetectable parasitemia under microscopy may serve as silent reservoirs, facilitating ongoing transmission within the herd and environment.

Implications for Disease Control and Public Health

This study highlights the significant burden of tick infestation and piroplasm infections in cattle in the Abeokuta area, revealing a tick prevalence of 89.3% and a piroplasm prevalence of 65.7% via PCR. Given the unregulated movement and communal grazing of cattle following market acquisition, these findings indicate a high risk of disease transmission to other animals and potentially to humans, especially pastoralists, due to the zoonotic potential of these haemoparasites.

Although previous claims about *Bos taurus indicus's* resistance to tick infestations exist (Mattioli *et al.,* 2000; Piper *et al.,* 2009), this study provides emerging evidence to the contrary in the Nigerian context. Factors such as indiscriminate crossbreeding, insufficient veterinary oversight, and vectors' ecological adaptability contribute to these vulnerabilities.

CONCLUSION

This research constitutes the first molecular survey of piroplasms and their tick vectors in cattle in Abeokuta. This study confirms the high prevalence of piroplasm infection (65.7%) and tick infestation (89.3%) among slaughtered cattle in Abeokuta, indicating endemicity and the existence of asymptomatic carriers that act as reservoirs for both susceptible animals and tick vectors. The findings support earlier reports that theileriosis is more prevalent than babesiosis in the region. The brief window between cattle purchase and slaughter, during which animals graze freely, presents a significant risk of transmission to other livestock and pastoralists due to the zoonotic potential of these pathogens. Additionally, the study emphasizes the superior sensitivity of PCR as a reliable diagnostic tool for detecting piroplasm infections. It highlights the urgent need for integrated tick control programs, molecular surveillance, and a One Health approach to protect animal and human populations.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Fadiji Oyinkansola S. and Sammy Sam-Wobo designed the study. The work was conducted as a thesis for her Ph.D. program, which Sam-Wobo supervised. Akande F.A. is the tick expert consulted at every stage of this work. She also proofread all the drafts of these manuscripts and made impactful suggestions. Ayodele Babalola extracted and drafted the first version of this manuscript from the thesis, while Sunday Olaniyan worked with Fadiji Oyinkansola in the molecular laboratory, troubleshooting protocols until its eventual completion.

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