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Research Article

Prevalence of Tick Infestations and Molecular Identification of Tick-Borne Pathogens among Dogs in Okpokwu L.G.A, Benue State, Nigeria

Adulugba O.A.[1*], Atsuwe T.S.[2], Abah E.A.[3]

[1,3]Dept. of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo, Nigeria
[2]Dept. of Zoology, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria

**Corresponding Author: adulugbaode@gmail.com*

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Abstract— This study aimed to determine the prevalence of tick infestation and identify tick-borne pathogens in dogs in Okpokwu LGA, Benue State, Nigeria. Samples were collected from 120 asymptomatic dogs, including ticks and blood. Tick identification was performed using a stereomicroscope and standard taxonomic keys. Thin blood films were prepared, and DNA was extracted for examination using microscopy and Polymerase Chain Reaction (PCR). Out of the 120 dogs examined, 76 (63.3%) were infested with various tick species. A total of 484 adult ticks were collected, represented by four species: *Rhipicephalus sanguineus* (415, 85.7%), Rhipicephalus *Boophilus annulatus* (36, 7.4%), Rhipicephalus *(Boophilus) decoloratus* (22, 4.5%), and *Hyalomma truncatum* (11, 2.3%). There was a significant difference ($p < 0.005$) in abundance among the four tick species. Blood screening revealed that 5 (4.2%) dogs were positive for tick-borne pathogens by microscopy, and 11 (9.2%) by PCR. Specifically, 4 (3.33%) dogs were positive for Babesia canis vogeli by microscopy, and 9 (7.5%) by PCR. Additionally, 1 (0.83%) dog was positive for *Ehrlichia canis* by microscopy, and 2 (2.5%) by PCR. *Anaplasma spp.* and *Theileria spp.* were not detected. The prevalence of tick-borne pathogens was higher in male dogs (7, 13.0%) compared to female dogs (4, 6.1%). Adult dogs (8, 14.5%) were more prone to infections than younger dogs (1, 3.8%). There was a significant association ($p < 0.05$) between age and prevalence of tick-borne infections. PCR showed high sensitivity (77.2%), specificity (93.6%), positive predictive value (85.0%), and negative predictive value (90.2%) at a 95% confidence interval. The findings suggest that the prevalence of tick infestation and tick-borne pathogens in Okpokwu LGA is significantly influenced by favorable environmental conditions and climate. Effective control and preventive strategies are necessary to reduce tick infestations and tick-borne pathogens in Nigeria.

Keywords— Infestations, Molecular Identification, Tick-Borne Pathogens, Dogs, Prevalence of tick, taxonomic keys

1. Introduction

Ticks are obligate ectoparasites that belong to the arachnid class within the phylum Arthropoda. They subsist by feeding on their hosts' blood and tissue fluids [1]. Free-roaming dogs are especially prone to tick infestations, which are influenced by environmental and climatic factors. These parasites can transmit various diseases to dogs and humans, some of which are zoonotic [2]-[3]. Ticks find their hosts by detecting breath, moisture, vibrations, body heat, or odors and then attach themselves to suitable hosts [4]. Numerous tick species, such as Haemaphysalis, Ixodes, Boophilus, Dermacentor, and Amblyomma, can infest dogs and transmit tick-borne pathogens. In Nigeria, the brown dog tick (Rhipicephalus sanguineus) is the most prevalent and widely distributed species, leading to significant tick infestations [1],[3],[5].

Tick bites can result in irritation, redness, swelling, itching, and self-trauma. A severe consequence of tick bites is the release of neurotoxins from tick saliva, which can cause tick paralysis, systemic illness, and hypersensitive reactions [3],[6].

The domestic dog (Canis lupus familiaris), a member of the genus Canis (canines), is the closest animal companion to humans [2]. Dogs are valuable not only as pets and companions but also for hunting, security, and providing employment and income in developing countries [5]. In Benue State, particularly in rural areas, most dogs are free-roaming, with only a few kenneled [1]. As a result, many dogs are exposed to ticks, leading to infestations.

Ticks are vectors of several canine pathogens. In Nigeria, tick-borne pathogens are a leading cause of morbidity and mortality in dogs, with some pathogens also posing zoonotic risks. These pathogens include protozoa such as Babesia spp., Theileria, and Hepatozoon canis, as well as bacteria like Anaplasma, Ehrlichia, and Rickettsia [7]-[11]. Clinical signs of tick-borne diseases include fever, anemia, weight loss,

weakness, lethargy, splenomegaly, generalized lymphadenopathy, hemoglobinuria, collapse due to erythrolysis, hypoxic injury, systemic inflammation, thrombocytopenia, and pigmenturia [12]-[14]. Some of these diseases are significant public health concerns [9].

Numerous epidemiological studies on tick-borne pathogens in dogs have been conducted in Nigeria. These diseases lead to productivity losses and economic impacts due to morbidity and mortality. While diagnosis is often based on physical examination, clinical signs, and blood smears using microscopy and serology, few studies utilize molecular techniques [1].

Despite numerous reports from other parts of Nigeria, there is limited information on the prevalence of ticks and tick-borne diseases in dogs in Benue State. Therefore, understanding the occurrence and distribution of tick-borne pathogens in dog populations and their tick vectors is crucial for effective control. This study was conducted to investigate the prevalence of tick infestations and the molecular identification of tick-borne pathogens among dogs in Okpokwu LGA, Benue State, Nigeria.

Besides Section 1, which provides the background and rationale for this work, the rest of the article is organized as follows: Section 2 reviews the related literature on this research topic; Section 3 details the methodology, including materials and methods; Section 4 presents the results, analyzes them, and provides a discussion; and the final Section 5 concludes the research and outlines future directions.

2. Related Work

Sumbria and Singla [15] explained that ticks are obligate haematophagous arthropods. They consist of a capitulum (head) and a flattened, oval-shaped body (idiosoma), and thrive well in conducive environments. Hot and humid climatic conditions favor the growth and development of parasites. Higher temperatures accelerate the development rates of larvae, nymphs, and adults, depending on the stage and species. Adetayo et al. [16] also stated that tick infestation in dogs is a major concern for dog owners and the general public. Approximately 840 known species of ticks have been identified [17].

Abdulkareem et al. [18] and Rwang et al. [19], in their separate studies, highlighted that Ixodid ticks infesting dogs include species such as Haemaphysalis, Rhipicephalus, Ixodes, Boophilus, Dermacentor, and Amblyomma. These species occur at varying prevalence levels in different parts of the world. Among the different species of ticks infesting dogs, the brown dog tick (Rhipicephalus sanguineus) is the most dominant and abundant species worldwide.

According to Arong et al. [17], the domestic dog (Canis lupus familiaris) lives with humans and is considered man's best animal friend. Dogs are kept for various purposes such as pets, companions, hunting, security, herding, sources of income, and even as a source of animal protein among some ethnic groups. Dogs are highly prone to tick attacks. Modu et al. [3] reported that the predilection sites for ticks on dogs include the head, neck, inguinal region, ear, interdigital region, thoracic region, thigh, tail, scrotal region in males, and perineum and breast regions in females. Previous studies have reported a significant increase in tick infestations among dogs [20], [16].

Singla et al. [21] also reported that ticks are important vectors of diseases, capable of transmitting a range of zoonotic diseases to dogs and humans. Sumbria et al. [22] identified some zoonotic pathogens transmitted by ticks, including encephalitis, Lyme disease, rickettsiosis, and ehrlichiosis, which are emerging as global health threats.

Leisewitz [23] noted that microscopy (blood smear) is an important tool for diagnosing and distinguishing large from small parasites. Happi et al. [9] highlighted that while both microscopy and molecular methods are important, microscopy shows low positive results compared to the PCR technique. Kamani et al. [24] reported that the presence of the DNA of Piroplasmida (Babesia spp, Hepatozoon spp, Theileria spp) and Anaplasmataceae (Anaplasma spp, Ehrlichia spp) has been detected using molecular diagnostic tools. Karshima et al. [25] emphasized that in Nigeria, studies based on molecular diagnosis such as highly sensitive polymerase chain reactions (PCR) techniques are inadequate. Therefore, the importance of molecular surveillance and control strategies is highly needed.

3. Theoretical Framework

Ticks are blood-feeding arachnids belonging to the phylum Arthropoda, subsisting on the blood and tissue fluids of their hosts. Free-roaming dogs are particularly vulnerable to these parasites, with infestations influenced by both environmental and climatic factors. These ectoparasites are capable of transmitting a range of diseases to both dogs and humans, including zoonotic illnesses. Ticks locate their hosts by sensing breath, moisture, vibrations, body heat, or odors, after which they attach and begin feeding. Various tick species such as Haemaphysalis, Ixodes, Boophilus, Dermacentor, and Amblyomma are known to infest dogs and transmit pathogens. In Nigeria, the brown dog tick (Rhipicephalus sanguineus) is the most widespread, causing significant infestations.

Tick bites can cause a range of local reactions, including irritation, redness, swelling, itching, and self-inflicted trauma. More severe reactions can occur due to neurotoxins in tick saliva, leading to conditions such as tick paralysis, systemic illnesses, and allergic reactions.

Domestic dogs (Canis lupus familiaris) are closely associated with humans and serve various roles, including companionship, hunting, security, and sources of employment and income, especially in developing regions. In Benue State, many dogs are free-roaming, which increases their exposure to ticks and subsequent infestations.

Ticks transmit numerous pathogens to dogs, contributing to high morbidity and mortality rates, with some pathogens also posing risks to humans. These include protozoa like Babesia spp., Theileria, and Hepatozoon canis, and bacteria such as Anaplasma, Ehrlichia, and Rickettsia. Dogs infected with these pathogens may exhibit symptoms such as fever, anemia, weight loss, weakness, lethargy, splenomegaly, generalized lymphadenopathy, hemoglobinuria, erythrolysis-related collapse, hypoxic injury, systemic inflammation, thrombocytopenia, and pigmenturia. Some of these tick-borne diseases are also significant public health issues.

In Nigeria, many epidemiological studies have focused on tick-borne pathogens in dogs. These pathogens cause substantial productivity losses and economic impacts due to morbidity and mortality. Diagnosis typically relies on physical examination, clinical signs, and blood smears analyzed using microscopy and serology, though molecular techniques are less commonly employed. Despite numerous studies from various regions of Nigeria, there is scant data specifically addressing the prevalence of ticks and tick-borne diseases in dogs within Benue State.

Understanding the prevalence and distribution of tick-borne pathogens in dog populations, and the ticks that transmit them, is essential for effective control measures. This study aims to investigate the prevalence of tick infestations and to identify tick-borne pathogens at the molecular level among dogs in Okpokwu LGA, Benue State, Nigeria.

4. Experimental Method/Procedure/Design

4.1 Study Area

This research was conducted in the Okpokwu Local Government Area (LGA) of Benue State, located in the north-central region of Nigeria. The LGA's headquarters are in Okpoga. It lies in the Savannah zone, between latitudes 7° 20' N and 7° 30' S, and longitudes 8° 12' W and 8° 20' E, with annual rainfall averaging about 1650 mm from April to October. The LGA has an estimated population of 203,561 residents. The vegetation in the southern part of the state is a mix of grasses and forests, providing a suitable habitat for various rare animal species. Consequently, the state holds significant potential for the development of viable forest and wildlife reserves.

4.2 Study Design

The study involved selected households with dogs in four randomly chosen council wards: Ichama, Ojigo, Okpoga, and Ugbokolo within the Okpokwu LGA. A total of 120 dogs were examined for the presence of ticks and blood samples were collected to detect tick-borne pathogens from September 2023 to February 2024.

4.3 Sample Collection

A total of 120 dogs were screened for ticks. Ticks were removed using forceps or by hand following standard procedures and preserved in 2% formalin for identification [5]. Blood samples (2 ml each) were collected from the dogs via the cephalic vein into ethylene diamine tetraacetic acid (EDTA) bottles and onto 3MM Whatman filter papers (Whatman International Ltd., Maidstone, England). The blood-spotted filter papers were allowed to air dry, then transferred into individual plastic bags, labeled, and stored at room temperature in a desiccator containing silica gel. All samples were transported to the Zoology Department Laboratory at Joseph Sarwuan Tarka University, Makurdi for tick identification, microscopy, and molecular analysis.

4.4 Identification of Ticks

Ticks collected from the dogs were examined under a stereomicroscope and identified morphologically using standard taxonomic keys [1].

4.5 Microscopic Examination and Molecular Analysis of Blood Samples

Thin smears were prepared from the blood samples, air-dried, and fixed in methanol for 3–5 minutes. The slides were stained with 10% Giemsa for 20-30 minutes and examined under an oil immersion lens (×100) to detect intra-erythrocytic piroplasms (intra-erythrocytic merozoites of Babesia) and morulae of Ehrlichia canis.

4.6 DNA Extraction

Parasite DNA was extracted from the blood samples using a paper punch to remove a circular piece of the dry blood spot. The circular piece was divided into four pieces with a scalpel blade and transferred into a 1.5 ml microcentrifuge tube. The parasite DNA was then extracted using the tissue protocol of the Zymo -DNATM Miniprep Plus Kit (Zymo, Irvine, California, USA) with some modifications to the initial steps. The purity and concentration of all extracted DNA samples were tested using a Nano-drop at 260-280 nm. DNA samples were stored at -20°C for further processing [26]. The samples were screened using primers for Anaplasma/Ehrlichia spp. and Babesia/Theileria spp.

4.7 PCR Amplification

Those that turned positive were further subjected to species-specific PCR for species identification.The Babesia/Theileria PCR was performed with primers RLB-F2 (5'-GAC ACAGGG AGG TAG TGA CAA G-3')and RLB-R2 (biotin-5'-CTA AGA ATT TCA CCT CTG ACA GT-3') amplifying a fragment of 320–520 bp from the 18S rRNA gene. The Ehrlichia/Anaplasma PCR was performed with primers Ehr-R (5'-biotin-CGG GAT CCC GAG TTT GCC GGGACT TYT TCT-3') and Ehr-F (50-GGA ATT CAG AGTTGG ATC MTG GYT CAG-30) amplifying a fragment of 320 –500 bp from the 16SrRNA gene. The conditions for the PCR included an initial step of 3 min at 42°C, 10 min at 94°C, 10 cycles of 94°C (20 s)–67°C (30 s)–72°C (30 s), with lowering of the annealing step after every second cycle by 2°C (touchdown PCR). The reaction was then followed by 40 cycles of denaturation at 94°C for 30s, annealing at 57°C for 30s, and extension at 72°C for 30s. PCR products were visualized under UV light in a 1.5% ethidium bromide-stained agarose gel after gel electrophoresis as described by [9]and [10].

4.8 Ethical Considerations

Ethical approval for the study was obtained from the Department of Agriculture (Animal section) of Okpokwu LGA, Benue State. All dogs were sampled with the owner's consent and handled according to standard procedures.

4.9 Statistical Analysis

The collected data were analyzed using SPSS version 20. Simple percentage and chi-squared tests were used to analyze the data, with P-values of < 0.05 considered significant. The diagnostic performance of PCR was compared with microscopy by calculating sensitivity, specificity, positive predictive value, and negative predictive value using the following formulas:

$$Sensitivity = \frac{Number\ of\ true\ positive}{Number\ of\ true\ positives + Number\ of\ false\ Negatives}$$

$$Specificity = \frac{Number\ of\ true\ Negatives}{Number\ of\ true\ Negatives + Number\ of\ false\ positives}$$

$$Positive\ predictive\ value = \frac{Number\ of\ true\ positive}{Number\ of\ true\ positives + Number\ of\ false\ positive}$$

$$Negative\ predictive\ value = \frac{Number\ of\ true\ negative}{Number\ of\ false\ negative + Number\ of\ true\ Negatives}$$

5. Results and Discussion

The prevalence of tick infestation among dogs in the selected council wards in Okpokwu L.G.A of Benue State is detailed in Table 1. Out of 120 dogs examined, 76 (63.3%) were found to be infested with ticks. Ojigo and Ichama wards had the highest infestation rates at 80.0% and 70.0%, respectively, while the lowest infestation rate was recorded in Okpoga at 46.7%. There was no significant difference between the locations and the prevalence of tick infestation (X^2 = 5.47, df = 3, P < 0.05).

Tick infestations and the pathogens they carry remain a significant health concern for dog populations in Nigeria. This study revealed a high prevalence of 63.3% tick infestation in dogs in Okpokwu LGA. This finding is lower than the 80.0% and 79.0% reported in Jalingo LGA of Taraba State [5] and Wamba LGA of Nasarawa State [4], respectively. However, it is higher than the 58.1% [3] and 56% [27] reported in Kano State and Ogun State, respectively. The variations may be due to favorable environmental conditions and breeding sites for ticks in the area. Poor attention given to the dogs by the owners and the seasonal activity of ticks in the environment also contributes to the high infestation rates. In this area, dogs are owned but roam freely, increasing their exposure to ticks and making infestation endemic.

Table 1: Prevalence of Tick Infestations among Dogs in Okpokwu L.G.A

Location	No of the dogs examined	Total no of dogs infested (%)
Ichama	30	21 (70.0)
Ojigo	30	24 (80.0)
Okpoga	30	14 (46.7)
Ugbokolo	30	17 (57.0)
Total	120	76 (63.3)

The study identified four tick species, with Rhipicephalus sanguineus being the most prevalent 415(85.7%), followed by Rhipicephalus Boophilus annulatus 36(7.4%), Rhipicephalus (Boophilus) decoloratus 22(4.5%), and Hyalomma truncatum 11(2.3%). The difference in occurrences and abundance among the four tick species was significant (X^2 = 5.32, P > 0.05). These findings align with previous studies reporting high prevalence rates for Rhipicephalus sanguineus, contrasting with reports that found Boophilus species to be more prevalent. This discrepancy may be due to climatic factors, such as warmer temperatures and higher humidity in the study area.

In this study, four species of ticks were identified as shown in Table 2. Rhipicephalus sanguineus was the most prevalent, accounting for 415 ticks (85.7%), followed by Rhipicephalus Boophilus annulatus with 36 ticks (7.4%), Rhipicephalus (Boophilus) decoloratus with 22 ticks (4.5%), and Hyalomma truncatum with 11 ticks (2.3%). These results are consistent with previous studies by researchers [1], [12], and [4], who reported prevalence rates of 80.5%, 99.9%, and 88.9% for Rhipicephalus sanguineus, respectively. However, this finding contrasts with the reports by [28] and [3], which indicated higher prevalence rates for Boophilus at 88.0% and 91.6%, respectively. The differences may be attributed to climatic factors, as the study area experiences warmer temperatures and higher humidity year-round compared to other regions.

Table 2: Identified Tick Species Infesting Dogs in Okpokwu LGA of Benue State

Species	No. of ticks collected
Rhipicephalus sanguineus	415(85.7)
Rhipicephalus (Boophilus) decoloratus	22(4.5)
Rhipicephalus (Boophilus) annulatus	36(7.4)
Hyalomma truncatum	11(2.3)
Total	484(100)

The overall prevalence of tick-borne pathogens among dogs sampled from the four council wards in Okpokwu L.G.A. is detailed in Table 3. Out of 120 dogs, 11 (9.2%) tested positive for infections. The highest prevalence was observed in Ojigo with 5 cases (4.2%), followed by Ichama with 3 cases (2.5%), and the lowest in Okpoga with 1 case (0.83%). There was no significant difference in infection rates among the different locations (P>0.05).

The prevalence of Babesia canis vogeli infection among dogs was 7.5%, followed by Ehrlichia canis at 1.7%. Anaplasma spp and Theileria spp were not detected in any dogs. Babesia canis infection was highest in Ojigo with 4 cases (13.3%) and lowest in Okpoga with 1 case (3.3%). Ehrlichia canis was found in 1 dog each from Ojigo and Ichama, with no cases in Okpoga and Ugbokolo. There was no significant difference in infection rates among the locations (P>0.05).

Tick-borne pathogens remain a significant health concern for dogs in tropical regions. Despite the high prevalence of tick infestation in the study area, the overall prevalence of tick-borne pathogens was relatively low. Out of 120 asymptomatic dogs examined, 11 (9.2%) tested positive for tick-borne pathogens, with 9 (7.5%) cases of Babesia canis vogeli and 2 (1.7%) cases of Ehrlichia canis. Anaplasma spp and Theileria spp were not detected by microscopy or PCR. These findings are lower than those reported by Peter et al. [2], who found 29.2% of dogs positive for haemoparasites, including 22.9% for canine babesiosis and 4.1% for canine ehrlichiosis.

This study's findings showed a lower prevalence of E. canis and a higher prevalence of B. vogeli compared to the work of [29], which reported 13% for E. canis and 1.09% for B. vogeli. However, the prevalence of Babesia and Ehrlichia in this study was higher than the 2.8% reported by [9] in Maiduguri, Nigeria. E. canis DNA was found in 22.9% of tested pet animals without exclusion criteria [19]. In the northern parts of Nigeria, E. canis prevalence was documented at 12.7%, with this study focusing on dogs with tick infestations or symptoms of tick-borne diseases [7]. Romanowsky stain revealed a 3% prevalence of Ehrlichia canis in dogs in Makurdi Metropolis [13]. The low prevalence of Ehrlichia canis in this study could be due to the difficulty in detecting Ehrlichia morulae in blood samples.

The prevalence of Babesia canis (B. canis vogeli) infection recorded in this study was relatively lower than the reports of [30], [31], and [12], who documented infection rates of 10.2%, 57.1%, and 10.8% in Markurdi, Gwagwalada Area Council, and six Area Councils of the Federal Capital Territory, Abuja, respectively. However, this study's result was higher than the 6.6% canine babesiosis reported from four states in Nigeria using sensitive molecular techniques by [8]. The findings were similar to the 7.3% reported by [32] in Abeokuta. These differences may be attributed to variations in locations, environmental conditions, climate, tick abundance, vegetation, host availability, and diagnostic methods. As observed by [12], increased susceptibility to tick bites and continuous exposure to other potential risk factors could explain these differences. Ehrlichia canis and Babesia vogeli, two of the most common tick-borne pathogens in dogs, infect blood cells and cause Canine Monocytic Ehrlichiosis and Babesiosis, resulting in significant morbidity and mortality in tropical regions [31].

Theileria spp and Anaplasma spp were not detected in the current study. This contrasts with the findings of [11], who reported Hepatozoon canis (6%) as the most prevalent blood parasite, followed by Babesia rossi (4%), and single positive samples each for Babesia vogeli (0.6%) and Anaplasma platys (0.6%). Similarly, [8] and [14] reported canine theileriosis prevalence of 6.4% and 4% in Nigeria, and 6.86% and 10.78% among dogs in the district of Lahore, respectively. The variations in the prevalence of tick-borne pathogens could be due to different diagnostic methods and the level of care provided to dogs. [2] suggested that low parasitemia might not be detected by microscopic examination of blood smears, indicating that the absence of parasites in a blood smear does not confirm the absence of infection. [33] proposed that more sensitive diagnostic methods might detect more positive cases.

Table 3: Prevalence of Tick-Borne Pathogen among Dogs in Relation to Location in Okpokwu L.G.A

Location	No examined	Babesia (%)	Anaplasma (%)	Theileria (%)	Ehrlichia (%)	Total Positive (%)
Ichama	30	2 (6.7)	00	00	1 (3.3)	3. (2.5)
Ojigo	30	4 (13.3)	00	00	1 (3.3)	5 (4.2)
Okpoga	30	1 (3.3)	00	00	0 (00)	1 (0.83)
Ugbokolo	30	2 (6.7)	00	00	0 (00)	2 (1.7)
Total	120	9 (7.5)	00	00	2 (1.7)	11 (9.2)

($P<0.005$)

Table 4 presents the prevalence of tick-borne pathogens among dogs in relation to sex. Male dogs had a higher infection rate of 7 (13.0%) compared to female dogs at 4 (6.1%). In terms of age, adult dogs (14.5%) were more prone to tick-borne infections than puppies (3.8%). There was a significant association ($P<0.05$) between age and the prevalence of tick-borne infections in dogs in Okpokwu L.G.A.

This study revealed a higher prevalence of infections in male dogs (13.0%) compared to female dogs (6.1%). This may be due to differences in environmental exposure, roaming, and sexual behaviors, and inadequate care given to male dogs compared to female dogs. These findings align with [12], who reported that male dogs were 1.24 times more prone to Babesia infection than female dogs, suggesting that the sex of the animal may influence the occurrence of tick-borne parasitic diseases. However, this study's findings contrast with [34] and [31], who reported a higher prevalence of haemoprotozoa in female dogs than in male dogs, attributing this to reduced immunity in female dogs due to stress from pregnancy, lactation, and improper animal management, making them more prone to infections.

The results indicated a higher prevalence (14.5%) in adult dogs (over 1 year old) compared to younger ones (3.8%) (under 1 year old). The higher prevalence of infection among older animals could be due to increased exposure to tick bites, which aligns with previous reports. However, the current study's findings contradict [35], who recorded higher disease prevalence in younger dogs (81.54%) compared to older ones (46.81%). Similarly, [36] reported that younger dogs were more susceptible to Babesia canis infection than adult dogs, reasoning that their underdeveloped immune systems make them more prone to canine babesiosis. [31] observed that the presence of maternal immunity in younger dogs helps them resist infections with blood parasites.

Table 4: Tick-Borne Pathogen Infections Based on Age, Class, and Sex of Dogs

Age class (months)	No male examined	No male positive (%)	No female examined	No female Positive (%)	Total No examined	Total No infected (%)
Puppy(Less than 6)	11	1 (9.1)	15	0 (0.0)	26	1 (3.8)
Juvenile (7-12)	17	1 (5.9)	22	1 (4.5)	39	2 (5.1)
Adult (Above 12)	26	5 (19.2)	29	3 (10.3)	55	8 (14.5)
Total	54	7 (13)	66	4 (6.1)	120	11 (9.2)

(P<0.005)

Table 5 presents the comparison of true positive cases for Babesia canis vogeli and Ehrlichia canis in infected dogs (n = 120) using both microscopy and PCR methods. Detection rates were 3.33% and 7.5% for Babesia canis vogeli, and 0.83% and 2.5% for Ehrlichia canis, respectively. Anaplasma spp. and Theileria spp. were not detected. The findings were statistically significant (p> 0.05).

The results indicated higher positive rates with PCR compared to microscopy. This aligns with studies by [9] and [37], which reported higher positive results using PCR (36.2% and 26.8%) compared to microscopy (10.3% and 10.7%). PCR confirmed all microscopically positive samples and identified five additional samples not detected by microscopy. PCR detected two cases of Ehrlichia canis compared to one case detected by microscopy. This suggests that false positives and false negatives may occur with microscopic examination.

[38] noted that microscopic examination for piroplasma identification can lead to technical issues and false morphological diagnoses. [36] highlighted the importance of identifying typical intracellular inclusions or morulae within monocytes or lymphocytes in peripheral blood smears for diagnosing E. canis. However, morulae are difficult to detect microscopically, making this method less sensitive and specific for detecting chronic cases, low parasitemia, or bacteremia with tick-borne heamoparasites.

Table 5: Microscopy and PCR Comparison of True Positive Cases in Samples (n=120)

Test	Microscopy (%)	PCR (%)
Babesia canis vogeli	(3.33)	9(7.5%)
Anaplasma spp	0(00)	0(00)
Theileria spp	0(00)	0(00)
Ehrlichia canis	1(0.83)	2 (2.5)
Total	5 (4.2)	11(9.2)

(p> 0.05).

Table 6 presents the diagnostic parameters of microscopy for detecting Babesia using PCR as the reference test. PCR showed the highest sensitivity with an estimated value of 77.2%, specificity at 93.6%, a Positive Predictive Value (PPV) of 85.0%, and a Negative Predictive Value (NPV) of 90.2%, all recorded at the upper limits of a 95% confidence interval.

The implications of misdiagnosis are severe, as the life of the infected dog is threatened. Misdiagnosed dogs may be denied appropriate treatment or preventive therapy, potentially leading to drug resistance. The microscopy used in this study demonstrated less effectiveness, sensitivity, and specificity in detecting tick-borne pathogens with low parasitemia compared to PCR. [26] stated that PCR has become an important tool to support conventional diagnostic methods. [9] noted that microscopy and clinical examinations tend to underdiagnose the prevalence of tick-borne pathogens and argued that PCR should be adopted for a better understanding of the epidemiology of blood-borne infectious agents in dogs.

PCR demonstrated higher sensitivity and specificity in identifying tick-borne hemiparasites compared to microscopic diagnostic methods. This is likely because PCR methods are gene-specific, with primers developed for particular species, indicating that PCR has a higher probability of detecting true negative samples. However, major challenges in using molecular techniques to detect pathogens in Nigeria include the scarcity of molecular laboratories, the availability of necessary facilities, high costs, and technical expertise [11].

Table 6. Diagnostic Parameters of Microscopy with PCR Test as Reference Test.

Parameters	Estimated value (%)	95% confidence interval	
		Lower limit limit (%)	Upper Limit (%)
Sensitivity	84.6	69.8	77.2
Specificity	94.6	92.6	93.6
(PPV)	91.2	78.7	85.0
(NPV)	90.8	89.5	90.2

Positive predictive value (PPV), Negative Predictive Valve (NPV)

The PCR agarose-gel electrophoresis results, displayed in Plate 1, show the primary reaction of Theileria/Babesia positive samples. These samples were amplified at 385 base pairs, as indicated by the thick white band between the 300 and 400 base pair markers. The DNA ladder is visible in lanes 1, 2, and 3, which indicate uninfected (negative) dog samples. Lanes 4, 5, and 6 show positive samples, while lane 7 serves as the positive and negative control.

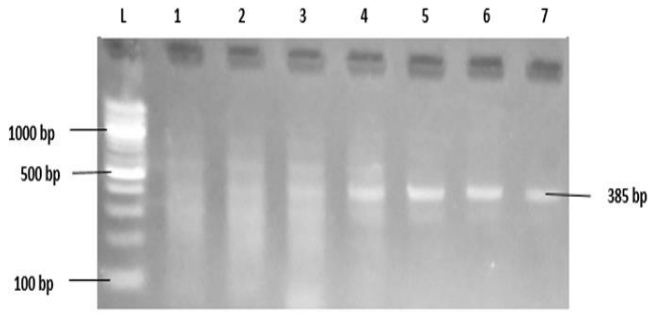

Plate 1: Agarose-gel electrophoresis of PCR Primary reaction of Theileria/Babesia

The PCR agarose-gel electrophoresis results for Babesia canis vogeli positive samples, shown in Plate 2, reveal amplification at 278 base pairs, indicated by the thick white band between the 200 and 300 base pair markers. Lanes 1 through 9 display positive samples for Babesia canis vogeli, while lane 10 contains the positive and negative controls.

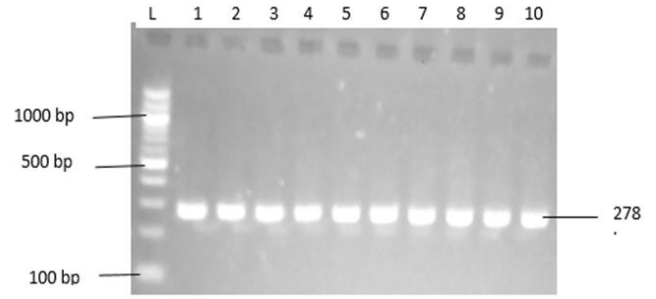

Plate 2. Agarose-gel electrophoresis of PCR Secondary reaction of *Babesia canis vogeli*.

6. Conclusion and Future Scope

6.1 Conclusion

This study revealed an overall tick infestation prevalence of 63.3% among dogs in Okpokwu L.G.A., Benue State. Four tick species were identified: Rhipicephalus sanguineus was the most prevalent, accounting for 85.7% (415 ticks), followed by Rhipicephalus (Boophilus) annulatus at 7.4% (36 ticks), Rhipicephalus (Boophilus) decoloratus at 4.5% (22 ticks), and Hyalomma truncatum at 2.3% (11 ticks). Rhipicephalus sanguineus was the dominant species, likely due to favorable environmental conditions that support its year-round survival and development.

The study found a prevalence of 4.2% and 9.2% for tick-borne pathogens among asymptomatic dogs in Okpokwu L.G.A. using microscopy and Polymerase Chain Reaction (PCR), respectively. The higher prevalence in males compared to females may be attributed to differences in environmental exposure, roaming, and sexual behaviors.

PCR proved more effective than microscopy in detecting tick-borne pathogens, providing molecular evidence of Babesia canis vogeli and Ehrlichia canis in the study area. PCR results indicated an overall prevalence of 7.5% for Babesia canis vogeli and 2.5% for Ehrlichia canis. The reliability of PCR as a diagnostic method highlights its value in identifying these diseases. Effective preventive and control measures for tick infestations and tick-borne pathogens, along with continuous monitoring of dogs and public awareness campaigns, are strongly recommended.

6.2 Future Scope

i. Expanded Geographical Studies: Future research could extend to other regions within Benue State and beyond to assess tick infestation and tick-borne pathogen prevalence in diverse ecological zones. Comparative studies across different locations can help understand environmental and climatic factors influencing tick distribution and pathogen transmission.
ii. Longitudinal Studies: Conducting longitudinal studies to monitor seasonal variations and long-term trends in tick infestations and tick-borne diseases would provide valuable insights into the dynamics of tick populations and the effectiveness of control measures over time.
iii. Host Behavior and Ecology: Further investigation into the behavioral and ecological factors contributing to the higher prevalence of ticks and tick-borne pathogens in male dogs compared to females could lead to targeted interventions. Studies focusing on dog roaming patterns, habitat preferences, and interactions with wildlife reservoirs would be beneficial.
iv. Genetic and Molecular Analysis: Advanced genetic and molecular analyses of tick populations and tick-borne pathogens can reveal genetic diversity, resistance patterns, and the potential emergence of new strains. Whole-genome sequencing and phylogenetic studies could enhance our understanding of pathogen evolution and spread.
v. Alternative Diagnostic Methods: Exploring and validating other diagnostic techniques, such as next-generation sequencing (NGS) and multiplex PCR, could offer more comprehensive and rapid detection of multiple pathogens, improving diagnostic accuracy and reducing time to treatment.
vi. Integrated Tick Management Programs: Developing and implementing integrated tick management programs that combine chemical, biological, and environmental control methods. Research should focus on the efficacy and sustainability of these integrated approaches, considering the potential impacts on non-target species and the environment.
vii. Vaccine Development: Investigating the potential for developing vaccines against common tick species, like Rhipicephalus sanguineus, and tick-borne pathogens, such as Babesia canis vogeli and Ehrlichia canis. Vaccine research could lead to proactive prevention strategies, reducing reliance on chemical control methods.
viii. Public Health and Awareness Campaigns: Enhancing public health initiatives and awareness campaigns to educate dog owners and communities about tick prevention, control measures, and the importance of regular veterinary check-ups. Research on the effectiveness of various educational approaches can help design more impactful outreach programs.
ix. Impact of Climate Change: Studying the impact of climate change on tick distribution and the prevalence of

tick-borne diseases. Predictive modeling and scenario analysis can help anticipate changes in tick ecology and inform adaptive management strategies.

x. One Health Approach: Promoting a One Health approach that integrates veterinary, medical, and environmental sciences to address the complex interactions between humans, animals, and their environments in the context of tick-borne diseases. Collaborative research efforts can lead to holistic solutions for managing tick infestations and reducing disease transmission risks.

Data Availability (Size 10 Bold)
Data will be available upon request

Conflict of Interest
Authors declare that they do not have any conflict of interest.

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Authors' Contributions
Adulugba, O.A. drafted the manuscript and conducted the laboratory tests. Atsuwe T.S developed the methodology, and Abah, E. A edited the manuscript. All authors approved the final version of the manuscript.

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AUTHORS PROFILE

Dr. Abel Ode Adulugba, holds a B.Sc in Biology (2002), M.Sc. Zoology (pure and Applied Parasitology) (2010), both from Benue State University, Makurdi, and Ph.D Parasitology (2023) From Joseph Sarwuan Tarka University Makurdi (formally Federal University of Agriculture Makurdi). He also has a Professional Diploma in Education (PDE) (2016), from the Apa College of Education Aidogodo, Okpoga. Presently, he is a Lecturer in the Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo. Also, the Ag. Director, Directorate of Research and Development (DR &D) unit of the institution. He has several journal publications both national and international to his credit. Also, he attended several workshops, and national and international conferences. Research areas of interest include Biological Sciences, Parasitology and Public Health, Entomology. Entrepreneurship related to sciences. He is a member of the Parasitology and Public Health Society of Nigeria (PPSN)and the Entomological Society of Nigeria (ESN). He is happily married with children.

Dr. Simon Terese Atsuwe holds a B.Sc in Zoology (2010), M.Sc in Parasitology (2016), and Ph.D Parasitology(2023) all from the Federal University of Agriculture Makurdi, now Joseph Sarwuan Tarka University Makurdi. He also has a Post Graduate Diploma in Education (2022), from the University of Ilorin. Presently, he is a biology teacher at Fevosun College, Makurdi. He has several journal publications both national and international to his credit and has attended several workshops and conferences. His area of interest in research includes Tick-Borne Diseases, Molecular Biology, Vector-Borne Diseases, Veterinary Parasitology, and Entomology. He is a member of the Institute of strategic management Nigeria (ISMN), Parasitology and Public Health Society of Nigeria (PPSN), Entomological Society of Nigeria (ESN), Zoological Society of Nigeria (ZSN), and Teachers Registration Council of Nigeria (TRCN). He is happily married with children.

Abah Emmanuel Ada holds a B.Sc in Biological Sciences in 2006 from the Benue State University, Makurdi, and an M.Sc parasitology from Joseph Sarwuan Tarka University (Formerly University of Agriculture, Makurdi). Presently, he is a Lecturer in the Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo. He has national and International Journals to his credit. Also, he attended several workshops and national and international conferences. Research areas of interest include Biological Sciences, Parasitology and Public Health, and Entomology. Entrepreneurship related to sciences. He is a member of the Parasitology and Public Health Society of Nigeria (PPSN)and the Entomological Society of Nigeria (ESN). He is happily married with children.

