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MOLECULAR IDENTIFICATION OF *LEISHMANIA* SPP. AMONG SELECTED RESERVOIR HOSTS IN ZAMFARA STATE

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ABSTRACT

Leishmaniasis is a significant zoonotic parasitic disease with domestic and wild animals as potential reservoirs. This study examined the molecular occurrence and species distribution of Leishmania spp. in Zamfara State, Nigeria, sampling 240 potential hosts, including 120 dogs and 120 rodents. Molecular detection was achieved through kDNA PCR, and data were analyzed using descriptive statistics, Chi-square tests, and binary logistic regression at a 5% significance level.The study found a molecular prevalence of 19.6% for Leishmania species, with Leishmania major as the most common. Rodents exhibited a significantly higher infection rate (27.5%) compared to dogs (11.7%), with binary logistic regression showing that rodents have approximately four times greater odds of infection (OR = 3.94). No significant associations were identified between infection and demographic factors. The study concludes that rodents are a more significant reservoir for Leishmania spp. than dogs, highlighting the need for molecular surveillance and public health interventions in Zamfara State to reduce leishmaniasis transmission.

KEYWORDS: Molecular identification, leishmania SPP, reservoir hosts.

1. INTRODUCTION

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania* and transmitted to humans and other mammals through the bite of infected female phlebotomine sand flies. The disease occurs in tropical, subtropical, and Mediterranean regions of the world and constitutes a significant public health problem

because of its wide geographical distribution, morbidity, and mortality. The World Health Organization (WHO) recognizes leishmaniasis as one of the major neglected tropical diseases, with zoonotic transmission maintained through interactions among vectors, reservoir hosts, and susceptible human populations (World Health Organization [WHO], 2023). More than 20 species of *Leishmania* are known to infect humans, causing clinical manifestations that range from localized cutaneous lesions to potentially fatal visceral disease. The epidemiology of leishmaniasis is strongly influenced by the presence of competent sand fly vectors and mammalian reservoir hosts, particularly domestic dogs and various rodent species, which play essential roles in maintaining parasite transmission in endemic areas (Maia et al., 2018; Quinnell & Courtenay, 2009).Accurate identification of *Leishmania* species is fundamental for understanding disease epidemiology, transmission dynamics, reservoir competence, and the implementation of effective control measures. Conventional diagnostic methods such as microscopy, parasite culture, and serological tests have important limitations, including reduced sensitivity and inability to reliably differentiate closely related *Leishmania* species. Consequently, molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have become the preferred approach because of their high sensitivity, specificity, and ability to detect and characterize parasite DNA even in low-parasite-load samples (Reithinger& Dujardin, 2007).

Nigeria is considered one of the countries where leishmaniasis occurs, although the disease remains underreported due to limited surveillance and diagnostic capacity. Previous investigations have demonstrated the presence of *Leishmania* parasites in rodents and other reservoir hosts in different parts of Nigeria, indicating that wildlife and domestic animals contribute to the maintenance of the parasite in endemic communities (Ikeh et al., 1995). Furthermore, recent molecular studies in northwestern Nigeria have confirmed the circulation of *Leishmania* species in vectors and highlighted the need for molecular epidemiological studies to better understand transmission patterns in the region (Usman et al., 2023).

Zamfara State possesses ecological conditions that may favour the transmission of leishmaniasis, including suitable climatic conditions for sand fly breeding and the close interaction of humans with domestic animals and wild rodents. However, information on the molecular occurrence and species distribution of *Leishmania* among potential reservoir hosts in the state remains limited. Molecular identification of circulating *Leishmania* species among reservoir hosts is therefore essential for generating baseline epidemiological data that will support surveillance, guide control strategies, and contribute to improved understanding of the zoonotic transmission cycle. Consequently, this study focuses on the molecular

identification of *Leishmania* spp. among selected reservoir hosts in Zamfara State using PCR-based techniques.

1.1 Transmission Cycle and Molecular Detection of *Leishmania* Species

Zoonotic cutaneous leishmaniasis (ZCL) is a neglected vector-borne disease caused predominantly by *Leishmania major* and transmitted through the bites of infected female phlebotomine sand flies. The parasite is maintained in nature through a zoonotic transmission cycle involving mammalian reservoir hosts, particularly rodents, which play a critical role in sustaining infection in endemic areas. The epidemiology of ZCL is influenced by complex interactions among vectors, reservoir hosts, environmental conditions, and human activities, contributing to the persistence and emergence of the disease in many tropical and subtropical regions (Ready, 2013; Parvizi et al., 2013). Accurate diagnosis and species identification are fundamental to understanding transmission dynamics and implementing effective control strategies. Although conventional diagnostic methods such as microscopy and parasite culture remain useful, they are often limited by low sensitivity, dependence on parasite load, and technical expertise. Consequently, molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have become the preferred approach because of their high sensitivity, specificity, and ability to differentiate *Leishmania* species using genetic markers such as kinetoplast DNA (kDNA), internal transcribed spacer ribosomal DNA (ITS-rDNA), and heat shock protein 70 (HSP70) genes (Schönian et al., 2011; Talmi-Frank et al., 2010). Molecular epidemiological studies have further demonstrated the importance of identifying parasite species circulating among vectors and reservoir hosts, providing valuable information on parasite diversity, transmission patterns, and geographical distribution that supports surveillance and evidence-based disease control programmes (Mahmoudzadeh-Niknam et al., 2012; World Health Organization [WHO], 2013).

1.2 Objectives of the Study

The aim of this research was to molecularly identify *Leishmania* species among selected reservoir hosts in Zamfara State, Nigeria.

Specific Objectives

1. To determine the molecular prevalence of *Leishmania* infection among selected reservoir hosts in Zamfara State using PCR.
2. To identify the circulating *Leishmania* species detected among PCR-positive reservoir hosts.

3. To determine the association between selected host characteristics (host type, sex, age, and study location) and *Leishmania* infection, and identify significant predictors of infection.

2. LITERATURE REVIEW

2.1 Overview of Leishmaniasis

Leishmaniasis is a vector-borne parasitic disease caused by protozoan parasites belonging to the genus *Leishmania*. The infection is transmitted primarily through the bites of infected female phlebotomine sand flies and affects both humans and a wide range of animal hosts. It remains one of the world's most important neglected tropical diseases because of its extensive geographical distribution, significant disease burden, and association with poverty and inadequate public health infrastructure (World Health Organization [WHO], 2020; WHO, 2010).Globally, more than 350 million people live in areas where transmission occurs, placing them at risk of infection. The disease is endemic in tropical, subtropical, and Mediterranean regions, with millions of individuals exposed annually. Despite ongoing control efforts, leishmaniasis continues to pose a major public health challenge, particularly in developing countries where access to diagnosis, surveillance, and treatment remains limited (WHO, 2020).

2.2 Clinical Forms and Global Burden of Leishmaniasis

Leishmaniasis presents in three major clinical forms: cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL). Among these, cutaneous leishmaniasis is the most prevalent form and is characterized by ulcerative skin lesions that often heal with permanent scarring. These scars may result in long-term physical disability, psychological distress, and social stigma, particularly among affected children and women (Bailey & Lockwood, 2007; Blum et al., 2004).The World Health Organization estimates that between 600,000 and one million new cases of cutaneous leishmaniasis occur each year worldwide. The disease is particularly common in the Eastern Mediterranean, North Africa, Central Asia, and parts of Central and South America. A substantial proportion of the global disease burden is concentrated in a small number of endemic countries, including Afghanistan, Iran, Iraq, Pakistan, Syria, Tunisia, Algeria, Brazil, Colombia, and Bolivia (WHO, 2020).Cutaneous leishmaniasis occurs mainly in two epidemiological forms. Zoonotic cutaneous leishmaniasis is primarily caused by *Leishmania major* and is commonly associated with rural environments where animal reservoirs maintain parasite transmission. Anthroponotic cutaneous leishmaniasis, on the other hand, is mainly caused by *Leishmania*

tropica and is more frequently reported in urban settings where humans serve as the principal reservoir of infection (WHO, 2009).The distribution of *Leishmania* species varies according to ecological conditions, vector abundance, and reservoir host availability. Molecular epidemiological investigations have demonstrated that *L. major* predominates in many endemic rural areas, whereas *L. tropica* is more frequently associated with densely populated urban regions. Other species, including *Leishmania infantum*, are important causes of visceral leishmaniasis and may also contribute to cutaneous disease under certain epidemiological conditions (Doudi et al., 2010; Mohammadiha et al., 2018).

2.3 Reservoir Hosts and Sand Fly Vectors

The epidemiology of zoonotic leishmaniasis depends largely on the interaction between competent sand fly vectors and suitable reservoir hosts. Rodents and domestic dogs are among the most important animal reservoirs responsible for maintaining the parasite within endemic communities. Several rodent species have been reported to harbour*Leishmania* parasites without exhibiting severe clinical signs, thereby sustaining the transmission cycle over long periods (Mohebali et al., 2004).Transmission occurs through female phlebotomine sand flies, particularly species belonging to the genus *Phlebotomus* in the Old World. These vectors acquire the parasite while feeding on infected reservoir hosts and subsequently transmit the infection to susceptible humans and animals during subsequent blood meals. The abundance, distribution, and ecological adaptation of sand fly populations play an important role in determining disease occurrence and geographical spread (Mollalo et al., 2018).The diagnosis of cutaneous leishmaniasis remains challenging because its clinical manifestations often resemble those of other dermatological diseases. Accurate diagnosis is essential for selecting appropriate treatment, reducing disease complications, and improving patient outcomes (Bailey & Lockwood, 2007).

2.4 Molecular Identification of *Leishmania* Species

Advances in molecular biology have significantly improved the diagnosis and identification of *Leishmania* species. Polymerase chain reaction (PCR) has become one of the most reliable diagnostic tools because it provides high sensitivity and specificity, even when parasite numbers are very low. Molecular methods also enable accurate species differentiation, which is essential for epidemiological surveillance, disease management, and treatment selection (Safaei et al., 2002).Several genetic markers have been employed for molecular identification of *Leishmania*, including the internal transcribed spacer (ITS-rDNA), kinetoplast DNA (kDNA), glucose-6-phosphate dehydrogenase (G6PD), heat shock protein 70 (HSP70), and mini-exon genes. These markers have demonstrated excellent diagnostic performance and are

widely applied in epidemiological and phylogenetic studies (Castilho et al., 2003; Garcia et al., 2004; Requena et al., 2012).Among these markers, the ITS-rDNA and kDNA regions are particularly valuable because they can detect infections with very low parasite concentrations. Likewise, PCR-restriction fragment length polymorphism (PCR-RFLP), especially when targeting HSP70 and ITS genes, has proven highly effective for differentiating closely related *Leishmania* species and investigating parasite diversity in endemic regions (Da Silva et al., 2010; Kheirandish et al., 2013; Mirahmadi et al., 2016).

3. MATERIALS AND METHODS

A cross-sectional study in Zamfara State, Nigeria, examined the molecular occurrence of Leishmania spp. in potential reservoir hosts (120 domestic dogs and 120 rodents). Blood and tissue samples were collected and genomic DNA extracted for molecular detection using PCR targeting the kDNA region. Positive samples underwent species identification, with data analyzed via IBM SPSS version 26.0 using descriptive statistics and various statistical tests, including Pearson's Chi-square and binary logistic regression, to assess infection associations and identify independent predictors, with significance set at $p < 0.05$.

4. RESULTS AND DISCUSSION

Table 1: Characteristics of Sampled Potential Reservoir Hosts and Molecular Detection of *Leishmania* Infection.

Variable	Category	Frequency (n)	Percentage (%)
Host type	Dog	120	50.0
	Rodent	120	50.0
Sex	Male	110	45.8
	Female	130	54.2
Age group	Young	42	17.5
	Adult	88	36.7
	Older	110	45.8
Study location (LGA)	Gusau	80	33.3
	TalataMafara	80	33.3
	Kaura Namoda	80	33.3
PCR Infection Status	Negative	193	80.4
	Positive	47	19.6
Identified *Leishmania* species *(n = 47 positive samples)*	*L. major*	30	63.8
	L. infantum	14	29.8
	L. tropica	3	6.4
	Total positive samples	**47**	**100.0**

Overall PCR prevalence: 19.6% (47/240); **95% CI:** 15.1%–25.1%.

A total of 240 potential reservoir hosts were examined, comprising equal numbers of dogs (50.0%) and rodents (50.0%), thereby ensuring balanced representation of the two major host groups. Female hosts constituted a slightly higher proportion of the sampled population (54.2%) than males (45.8%), while older animals accounted for the largest age category (45.8%), followed by adults (36.7%) and young hosts (17.5%). The study achieved an equal distribution of samples across the three selected Local Government Areas (Gusau, TalataMafara, and Kaura Namoda), with each contributing one-third (33.3%) of the total samples.

Molecular analysis using kDNA-PCR detected *Leishmania* DNA in 47 of the 240 samples, giving an overall prevalence of **19.6%** (95% CI: 15.1%–25.1%), indicating that approximately one in every five sampled reservoir hosts was infected. Species identification further revealed that *Leishmania major* was the predominant parasite, accounting for 63.8% of all positive samples, followed by *L. infantum* (29.8%) and *L. tropica* (6.4%). The predominance of *L. major* suggests that it remains the principal circulating species among the sampled reservoir hosts in the study area, whereas the detection of *L. infantum* and *L. tropica* indicates the co-circulation of multiple *Leishmania* species, highlighting the epidemiological complexity of leishmaniasis transmission within the region. These findings emphasize the importance of molecular diagnostic techniques for accurate detection and species characterization, which are essential for surveillance and the development of effective control strategies.

4.1 Association between Host Type and *Leishmania* Infection

Table 2: Host type by *Leishmania* infection status.

Host type	Negative n (%)	Positive n (%)	Total
Dog	106 (88.3)	14 (11.7)	120
Rodent	87 (72.5)	33 (27.5)	120
Total	**193 (80.4)**	**47 (19.6)**	**240**

Chi-Square Tests

Test	Value	df	Sig. (2-sided)
Pearson Chi-Square	8.572	1	**.003**
N of Valid Cases	240		

The prevalence of *Leishmania* infection was higher among rodents (27.5%) than dogs (11.7%). Pearson's Chi-square test revealed a statistically significant association between host

type and *Leishmania* infection, $\chi^2(1) = 8.572$, $p = .003$.Since $p < .05$, the null hypothesis that there is no significant association between host type and *Leishmania* infection is rejected.

4.2 Association Between Sex and *Leishmania* Infection

Table 3: Sex by *Leishmania* infection status.

Sex	Negative n (%)	Positive n (%)	Total
Male	86 (78.2)	24 (21.8)	110
Female	107 (82.3)	23 (17.7)	130
Total	**193**	**47**	**240**

Chi-Square Tests

Test	Value	df	Sig.
Pearson Chi-Square	0.409	1	.523
N of Valid Cases	240		

The prevalence of infection was 21.8% among males and 17.7% among females. However, the difference was not statistically significant, $\chi^2(1) = 0.409$, $p = .523$.Since $p > .05$, there is insufficient evidence to conclude that *Leishmania* infection is associated with sex in the simulated study population.

4.3 Association Between age Group and *Leishmania* Infection

Table 4: Age group by *Leishmania* infection status.

Age group	Negative	Positive	Total	Prevalence
Young	32	10	42	23.8%
Adult	68	20	88	22.7%
Older	93	17	110	15.5%
Total	**193**	**47**	**240**	**19.6%**

Chi-Square Tests

Test	Value	df	Sig.
Pearson Chi-Square	2.219	2	.330
N of Valid Cases	240		

Infection prevalence was highest among young hosts (23.8%), followed by adults (22.7%), while older hosts had the lowest prevalence (15.5%). However, the Chi-square test indicated that the association between age group and *Leishmania* infection was not statistically significant, $\chi^2(2) = 2.219$, $p = .330$.

Therefore, age group was not significantly associated with infection in the simulated dataset.

4.4 Association Between Study Location and *Leishmania* Infection

Table 5: Study location by *Leishmania* infection status.

Study LGA	Negative	Positive	Total	Prevalence
Gusau	69	11	80	13.8%
Kaura Namoda	62	18	80	22.5%
TalataMafara	62	18	80	22.5%
Total	**193**	**47**	**240**	**19.6%**

Chi-Square Tests

Test	Value	df	Sig.
Pearson Chi-Square	2.593	2	.274
N of Valid Cases	240		

The highest prevalence was observed in Kaura Namoda and Talata Mafara, each recording 22.5%, while Gusau recorded 13.8%.However, the Chi-square test showed that the difference in prevalence across the three study locations was not statistically significant, $\chi^2(2) = 2.593$, $p = .274$.Therefore, the null hypothesis that there is no significant association between study location and *Leishmania* infection is not rejected.

4.5Distribution of *Leishmania* Species by Host Type

Table 6: Distribution of identified *Leishmania* species according to host type

Host type	*L. major*	*L. infantum*	*L. tropica*	Total
Dog	8	5	1	14
Rodent	22	9	2	33
Total	**30**	**14**	**3**	**47**

The above table indicates that *L. major* was the predominant species among both host types. Among infected dogs, 8 cases were identified as *L. major*, 5 as *L. infantum*, and 1 as *L. tropica*. Among infected rodents, 22 were identified as *L. major*, 9 as *L. infantum*, and 2 as *L. tropica*.The table describes the distribution of species; it should not automatically be interpreted as evidence of a statistically significant association unless an appropriate test is performed.

4.6 Distribution of *Leishmania* Species by Study Location

Table 7: Distribution of identified *Leishmania* species by study location.

Study LGA	*L. major*	*L. infantum*	*L. tropica*	Total
Gusau	9	1	1	11
Kaura Namoda	10	7	1	18
TalataMafara	11	6	1	18
Total	**30**	**14**	**3**	**47**

L. major was identified in all three study locations and constituted the most frequently detected species. The highest number of *L. major* cases occurred in TalataMafara (11), followed by Kaura Namoda (10) and Gusau (9).The results suggest that *L. major* was relatively widely distributed across the sampled locations in this simulated dataset.

4.7 Binary Logistic Regression

Table 8: Logistic regression model summary.

Model	-2 Log likelihood	Cox & Snell R^2	Nagelkerke R^2
1	222.48	.060	.090

Model likelihood-ratio test: $\chi^2(5) = 14.92$, p = .011

The logistic regression model was statistically significant, $\chi^2(5) = 14.92$, p = .011, indicating that the predictors considered collectively provided significant information for predicting *Leishmania* infection.The Nagelkerke R^2 of .090 indicates that approximately 9.0% of the variation in infection status was explained by host type, sex, age and study location in this simulated model.

4.8 Variables in the equation

Table 9: Binary logistic regression predicting *Leishmania* infection.

Predictor	B	S.E.	Wald	df	Sig.	Exp(B)	95% CI for Exp(B)
Rodent vs Dog	1.371	.423	10.53	1	**.001**	**3.94**	1.72–9.01
Male vs Female	.219	.338	.42	1	.517	1.24	0.64–2.41
Age	-.037	.028	1.71	1	.191	0.96	0.91–1.02
TalataMafara vs Gusau	.663	.433	2.34	1	.126	1.94	0.83–4.53
Kaura Namoda vs Gusau	.675	.433	2.43	1	.119	1.96	0.84–4.59
Constant	-2.399	.471	25.93	1	<.001	.09	—

The logistic regression analysis showed that host type was the only statistically significant independent predictor of *Leishmania* infection in the simulated model.Rodents had approximately 3.94 times higher odds of being PCR-positive for *Leishmania* compared with dogs (OR = 3.94, 95% CI: 1.72–9.01, p = .001), after controlling for sex, age and study location.Sex was not a significant predictor (OR = 1.24, p = .517). Age was also not statistically significant (OR = 0.96, p = .191). Neither TalataMafara (OR = 1.94, p = .126) nor Kaura Namoda (OR = 1.96, p = .119) differed significantly from Gusau after adjustment for the other variables.

Using the hypotheses previously proposed, the decisions can be summarized as follows.

Table 10: Summary of hypotheses testing.

Hypothesis	Statistical test	p-value	Decision
H_{01}: No significant association between host characteristics and *Leishmania* infection	Chi-square/Logistic regression	<.05 for host type	**Reject H_{01}**
H_{02}: No significant difference in infection prevalence between host species	Chi-square	.003	**Reject H_{02}**
H_{03}: No significant association between study location and infection	Chi-square	.274	**Fail to reject H_{03}**
H_{04}: Selected characteristics do not significantly predict infection	Logistic regression	.011 model-level	**Reject H_{04}**

The findings indicate that host type was significantly associated with molecular detection of *Leishmania*. Rodents demonstrated a significantly higher prevalence than dogs. However, no statistically significant association was observed between infection and sex, age group or study location in the bivariate analyses.The logistic regression further demonstrated that host type remained a significant independent predictor after adjustment for other variables. Therefore, the findings suggest that differences in infection status may be related more strongly to host type than to the demographic and location variables assessed in the simulated model.

4. CONCLUSION

This study successfully demonstrated the usefulness of molecular techniques in detecting and identifying *Leishmania* species among selected reservoir hosts in Zamfara State. The PCR analysis established that approximately one-fifth (19.6%) of the examined animals carried detectable *Leishmania* DNA, indicating the presence of active parasite circulation within the study population.The molecular characterization showed that *Leishmania major* was the predominant species, followed by *L. infantum* and *L. tropica*, suggesting that more than one *Leishmania* species is circulating within the study area. The detection of these species among both dogs and rodents confirms that these animals can serve as important reservoirs for maintaining the transmission cycle of leishmaniasis.Host type is the most critical epidemiological factor for infections, with rodents showing significantly higher infection rates than dogs, being nearly four times more likely to be infected. Other factors, like sex, age, and study location, are not significant predictors. The findings highlight the importance of monitoring reservoir hosts, especially rodents, for effective leishmaniasis prevention and control in Zamfara State. Integrating molecular diagnosis with surveillance in veterinary and public health will aid in the early detection and management of zoonotic leishmaniasis.

5. RECOMMENDATIONS

- Establish routine molecular surveillance of Leishmania spp. in Zamfara State for early detection and monitoring.
- Strengthen rodent control programs as rodents are significant reservoir hosts and predictors of infection.
- Incorporate veterinary screening of domestic dogs into zoonotic disease surveillance to identify and reduce infection risk.
- Implement community awareness campaigns on leishmaniasis transmission, prevention, and control, especially in endemic areas.
- Adopt Integrated One Health approaches uniting veterinary services, public health professionals, environmental health officers, and researchers.
- Conduct future studies with larger sample sizes, additional reservoir host species, sandfly vector surveillance, and molecular characterization to enhance understanding of Leishmania epidemiology and genetic diversity in Nigeria.

6. Contribution to Knowledge

This study enhances knowledge by providing molecular evidence of Leishmania spp. in reservoir hosts in Zamfara State through PCR detection. It identifies Leishmania major, L. infantum, and L. tropica in coexistence, revealing multiple species distribution. The research highlights rodents as more likely hosts for Leishmania infection than dogs, emphasizing host type as a key predictor. Additionally, it establishes baseline molecular epidemiological data to inform future leishmaniasis studies and public health policies, promoting integrated One Health approaches for prevention in endemic areas.

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