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Research Article

Molecular Identification of *Trypanosoma evansi* Isolated from Camels (*Camelus dromedarius*) in Sokoto and Its Environs, Nigeria

Adama Musa Abdullahi[1], *Tasiu Mallam Hamisu[2], Umar Muhammed Chafe[3], Aminu Ibrahim Daneji[3] and Musibau Olayinka Alayande[4]

[1]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto, Nigeria
[4]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Usmanu Danfodio University, Sokoto, Nigeria
***Corresponding Author's email*: tasiugln@unimaid.edu.ng; Phone: +2348068064657**

ABSTRACT
Trypanosoma evansi is a significant pathogen affecting camels, with limited molecular epidemiological data available in Sokoto and its environs, Nigeria. This study aimed to identify *T. evansi* using Polymerase Chain Reaction (PCR) technique in one-humped camels (*Camelus dromedarius*) across key camel-rearing zones in Sokoto State. A total of 400 camels were selected using convenience sampling technique from abattoirs, markets, and households across four Local Government Areas (LGAs). Blood samples were collected and screened microscopically using Giemsa stain, with 54 positive samples subjected DNA extraction, measuring DNA purity and its concentration, then PCR, targeting three genes: ESAG 6/7, ITS-1, and RoTAT 1.2. Finally, PCR products were analyzed via agarose gel electrophoresis. Results demonstrated that 83.33% (46/54) of the samples were positive for ESAG 6/7 gene, 96.30% (52/54) for the ITS-1 gene, and 96.30% (52/54) for the RoTAT 1.2 gene, which indicate very high positivity rates for the three target genes of *T. evansi* among camel population. The high positivity rates suggest that majority of the tested camel population is infected with *T. evansi* associated with these genes It is recommended that routine molecular surveillance of *T. evansi* be implemented in camel populations to facilitate early detection and improve control strategies for trypanosomosis. Further studies should focus on molecular characterization of *T. evansi* so as to inform a targeted intervention strategy against the parasite in the region.

Keywords: Camels; ESAG 6/7; PCR; RoTAT 1.2; *T. evansi*; ITS-1

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INTRODUCTION

Trypanosoma evansi, the causative agent of surra, is a protozoan parasite with a profound impact on camel health, productivity, and the livelihoods of communities dependent on camel husbandry across Africa, Asia, and the Middle East. The disease is transmitted primarily by biting flies and is characterized by chronic wasting, anemia, reproductive failure, and, in severe cases, death, leading to substantial economic losses in affected regions (Selim *et al.,* 2022; Khan *et al.,* 2023; Kim *et al.,* 2024). Infection by *T. evansi* is widespread in camel-rearing areas, with prevalence rates varying significantly depending on the diagnostic method and region. For instance, studies in Egypt and Saudi Arabia have reported prevalence rates ranging from

10% to over 45% using molecular techniques, which are notably higher than those detected by traditional microscopy (Elhaig *et al.,* 2013; Malki and Hussien, 2022). Similarly, in Kenya and Algeria, molecular and serological methods have consistently demonstrated higher infection rates than parasitological techniques (Njiru *et al*, 2004; Boushaki *et al.,* 2019). Prevalence rates of *T. evansi* in camels are highly variable across Nigeria, ranging from 6% to over 45% depending on region and diagnostic method, with molecular techniques consistently revealing higher rates than microscopy or serology (Kyari *et al.,* 2021; Mamman *et al.,* 2021; Mamman *et al.,* 2023). The parasite is also present in cattle and vectors, though at lower rates, and mixed infections are common (Takeet *et al.,* 2013; Odeniran *et al.,* 2019).

The economic consequences of surra are severe, including reduced productivity, increased mortality, and reproductive losses, as evidenced by high rates of abortion and neonatal death in infected herds (Khan *et al.,* 2023). The disease remains a significant constraint to livestock production and rural livelihoods in Nigeria (Danyayam and Abdullahi, 2025).

The advent of molecular diagnostic tools, particularly polymerase chain reaction (PCR), has revolutionized the detection and characterization of *T. evansi*. PCR assays targeting genes such as ESAG 6/7, ITS-1, and RoTAT 1.2 have demonstrated superior sensitivity and specificity compared to conventional parasitological methods (Amer *et al,* 2011; Elhaig *et al.,* 2013; Metwally *et al.,* 2021; Malki and Hussien, 2022). Given the high prevalence and economic impact of *T. evansi*, routine molecular surveillance is essential for early detection and effective control of trypanosomiasis in camel populations. In Nigeria, particularly in Sokoto State and its environs, camels (*Camelus dromedarius*) are vital for transportation, meat, and milk production, yet the molecular epidemiology of *T. evansi* in these populations remains poorly understood.

MATERIALS AND METHODS

Determination of Sample Size and Sample Collection

A total of 400 one-humped camels (*Camelus dromedarius*) were selected using the formula by Thrushfield (2005), which calculated a minimum sample size of 332 based on an expected prevalence of 31.5% (Argungu *et al.,* 2015). This was rounded up to 400 in order to increase precision. Sampling spanned four purposively chosen camel-rearing LGAs in Sokoto State: Sokoto abattoir (100 camels), Tambuwal households (100), Ilella market (100), and Goronyo market (100). Convenience sampling was employed due to herd availability and owner consent. Blood samples (10 mL) were collected via venipuncture into EDTA vacutainer tubes and stored at -20°C until Polymerase Chain Reaction (PCR) analysis.

Molecular Detection of *T. evansi*

DNA Extraction, Concentration and Purity

Out of a total of 400 samples collected, 54 microscopy-positive cases (4 from Goronyo, 21 Sokoto, 13 Tambuwal, 16 Ilella) underwent DNA extraction using manufacturer's protocol (Qiagen, Germany), where, the blood samples were lysed with proteinase K, DNA was precipitated with ethanol, purified using spin columns, and eluted for Polymerase Chain Reaction (PCR) analysis. The concentration (A260) and purity (A260/280) of the extracted DNA were measured using nanodrop Bio spectrophotometer (Shimadzu, Japan).

Polymerase Chain Reaction

Three genes ESAG 6/7, ITS-1, and RoTAT 1.2 were amplified using PCR. Reaction mixture containing 12.5 µL Top Taq master mix, 1 µL each of forward and reverse primers (Table 1), 5.5 µL nuclease-free water, and 5 µL DNA template was used. The thermocycling conditions included initial denaturation at 94°C for 3 minutes, 35 cycles of 94°C (30 s), 58°C (30 s), 72°C (1 min), and final extension at 72°C for 5 minutes. The PCR products were electrophoresed on 1.5% agarose gels (90V, 50 minutes) in 1X TAE buffer, stained with ethidium bromide, and visualized using a Bio-Rad GelDoc system (Bio-Rad, USA).

Table 1. Primer sequences used for PCR amplification of ESAG 6/7, ITS-1, and RoTAT 1.2 genes of *T. evansi* in one-humped camels across key camel-rearing zones in Sokoto State, Nigeria

Target genes	Primer sequence	Product size
ESAG F	5'-CAT TCC AGC AGG AGT TGG AGG-3'	740bp (Isobe *et al*., 2003)
ESAG R	5'-TTG TTC ACT CAC TCT CTT TGA CAG-3'	
RoTAT F	5'-GCC ACC ACG GCG AAA GAC-3'	488bp (Urakawa *et al.*, 2001)
RoTAT R	5'-TAA TCAGTGTGGTGT GC-3'	
ITS-1 F	5'-CGCCCGAAAGTTCACC3-'	480bp (Salim *et al*., 2011)
ITS-1 R	5'- GCGTTCAAAGATTGGGCAAT-3'	

Legend: F= Forward; R=Reverse

RESULTS

DNA Quality and Purity

The DNA concentrations of the samples ranged from 76 ng/µl to 172 ng/µl, while the purity ratios varied between 1.78 and 2.01, indicating that the DNA was of sufficient quality and purity for downstream molecular analyses. For example, sample Sok2 had a concentration of 162 ng/µl and a purity of 1.82; Gry9 had 94 ng/µl and 2.01; Tbw4 had 112 ng/µl and 1.94; and Ile7 had 151 ng/µl and 1.99. These results confirm that the DNA samples were suitable for PCR amplification.

Molecular Detection of *T. evansi* Target Genes

ESAG 6/7 Gene

PCR amplification targeting the ESAG 6/7 gene of *T. evansi* was performed on all 54 microscopy-positive samples. Following agarose gel electrophoresis, 46 out of 54 samples (83.33%) exhibited the expected 740 bp band, confirming the presence of the ESAG 6/7 gene in the majority of the samples (Figure 1).

ITS-1 Gene

The Internal Transcribed Spacer 1 (ITS-1) region was amplified using specific primers, yielding an expected product size of approximately 480 bp. Analysis of the PCR products on a 1.5% agarose gel revealed that 52 of the 54 samples (96.30%) produced the expected band (Figure 2).

RoTAT 1.2 VSG Gene

PCR amplification of the RoTAT 1.2 Variant Surface Glycoprotein (VSG) gene was also conducted on the 54 samples. The expected PCR product size was 488 bp. After electrophoresis and visualization, 52 samples (96.30%) were positive (Figure 3).

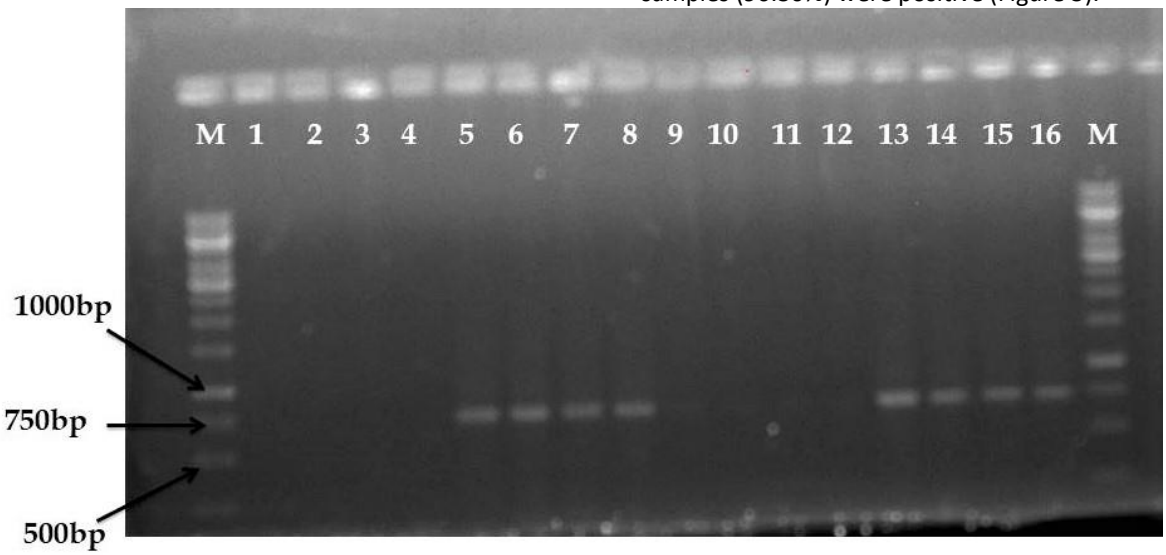

Figure 1: PCR product bands (Lanes 1-16) of *T. evansi* ESAG gene from samples collected from camels in Sokoto
The expected amplicon size is 740 bp. Lanes 5,6,7,8,13,14,15 and 16 are positive. M = Molecular marker, bp = base pair

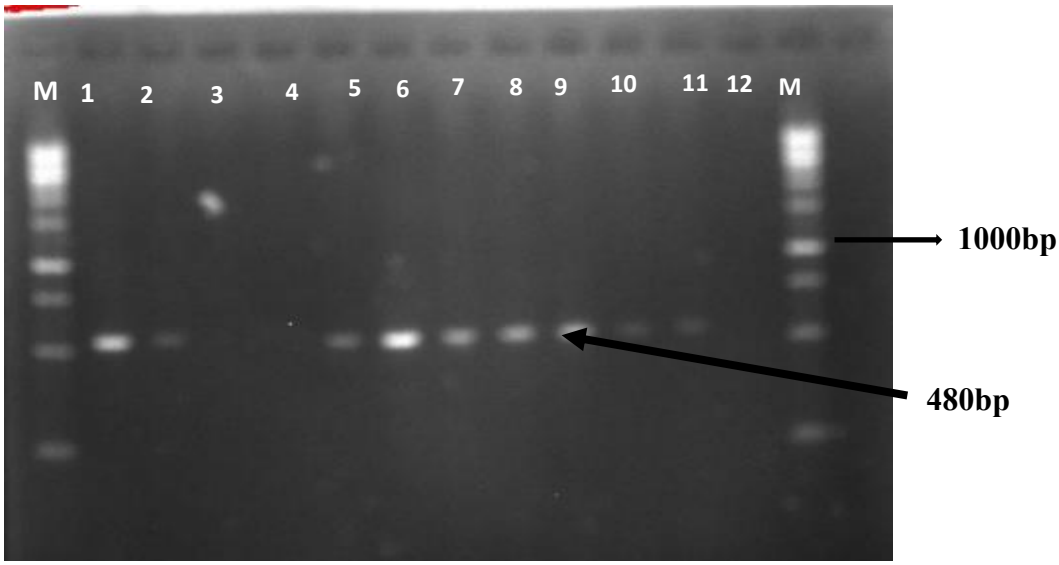

Figure 2: PCR product bands (Lanes 1-12) of *T. evansi* ITS-1 gene from samples collected from camels in Sokoto
The expected amplicon size is 480 bp. Lanes 1,2,5,6,7,8,9, 10 and 11 are positive. M = Molecular marker, bp = base pair.

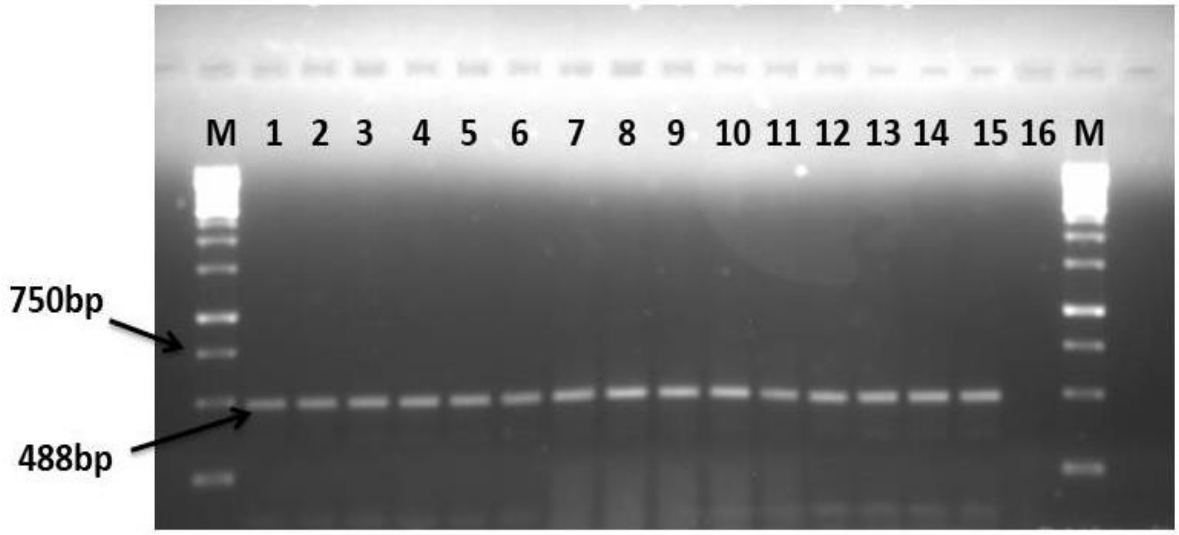

Figure 3: PCR product bands (Lanes 1-16) of *T. evansi* RoTAT 1.2 gene from samples collected from camels in Sokoto
The expected amplicon size is 488 bp. Lanes 1,2,3,4,5,6,7,8,9,10,11,12,13,14 and 15 are positive. M = Molecular marker, bp = base pair

DISCUSSION

Trypanosoma evansi is a protozoan parasite responsible for surra, a disease causing significant economic losses in livestock across Africa and Asia. Accurate and sensitive detection of *T. evansi* is essential for effective disease control, given the parasite's genetic diversity and the limitations of traditional diagnostic methods. Molecular techniques targeting specific genetic markers such as the ESAG 6/7 gene, the Internal Transcribed Spacer 1 (ITS-1) region, and the RoTAT 1.2 Variant Surface Glycoprotein (VSG) gene, have been developed to improve diagnostic accuracy and epidemiological surveillance (Witola *et al.,* 2005; Salim *et al*., 2011; Verma *et al*., 2023). The present study aimed to evaluate the prevalence and genetic diversity of *T. evansi* in microscopy-positive samples by amplifying these three genetic targets using PCR.

The PCR result targeting the ESAG 6/7 gene confirmed the presence of this gene in 83.33% of microscopy-positive samples. The ESAG 6/7 genes encode transferrin receptors, which are crucial for iron uptake and parasite survival. Studies have shown significant genetic variability in ESAG6/7 among *T. evansi* isolates, with diversity in the

transferrin-binding regions correlating with the parasite's broad host range and potentially influencing pathogenicity and virulence (Witola *et al.,* 2005). For example, research from Thailand found both conserved and variable regions in ESAG6/7, with some genetic variants associated with higher virulence (Witola *et al.,* 2005). This genetic diversity may explain why not all microscopy-positive samples were PCR-positive, as primer mismatches or gene absence/variation could affect amplification.

In this study, the ITS-1 region showed a high positivity rate (96.30%), consistent with its established use as a sensitive and broad-range marker for trypanosome detection (Salim *et al.,* 2011; Behour and Fattah, 2023). ITS-1 PCR is widely used for multi-species detection and has been effective in large-scale epidemiological studies, such as those in Sudan and Egypt, where it reliably identified *T. evansi* as the predominant species infecting camels (Salim *et al.,* 2011; Behour and Fattah, 2023). The high detection rate in these results aligns with these findings, supporting ITS-1 as a robust diagnostic target for *T. evansi* and related trypanosomes.

Detection of RoTAT 1.2 VSG gene also yielded a high positivity rate (96.30%). The RoTAT 1.2 VSG gene is a widely used marker for *T. evansi* diagnosis due to its specificity and prevalence in most *T. evansi* strains (Ngaira *et al.,* 2004; Tong *et al.,* 2018; Kumar *et al.,* 2021; Verma *et al.,* 2023). However, studies from Kenya, Sudan, and Egypt have reported the absence of the RoTAT 1.2 gene in some *T. evansi* isolates, indicating genetic diversity and the existence of non-RoTat 1.2 variants (Ngaira *et al*., 2004; Salim *et al*., 2011; Behour and Fattah, 2023). This genetic variability can lead to false negatives in RoTat 1.2-based assays, as seen in some field studies where a subset of *T. evansi* isolates lacked the gene entirely (Ngaira *et al.,* 2004; Salim *et al*., 2011; Behour and Fattah, 2023). The high detection rate in this study suggests that the majority of circulating strains in the sample set possess the RoTat 1.2 gene, but the few negative cases may represent such genetic variants.

CONCLUSION

The high detection rates for ITS-1 and RoTAT 1.2 VSG genes support their continued use in *T. evansi* diagnostics, but genetic variability, especially in ESAG 6/7 and RoTAT 1.2, necessitates the use of multiple molecular markers for comprehensive surveillance and accurate diagnosis, a strategy increasingly relevant for Nigeria and other endemic regions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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