RESEARCH ARTICLE ———————

MOLECULAR DETECTION OF *TRYPANOSOMA EVANSI* USING ROTAT 1.2 (VSG) GENES IN CATTLES FROM BORNO AND GOMBE STATES, NIGERIA

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ABSTRACT

Trypanosoma evansi (*T. evansi*) causes surra, an important haemoprotozoan disease of economic importance that affects a wide range of hosts. The research was designed to detect *T. evansi* using a molecular method in cattle from Gombe and Borno States, Nigeria. Blood samples from 455 apparently healthy cattle were collected from the month of May to October, 2018 in different Local Government areas. Microscopy detection revealed an overall prevalence rate of 11.8% in Gombe State and 9.0% in Borno State during the study period. Out of the 455 blood samples collected, 50 randomly selected samples, with 25 each from Borno and Gombe states, were subjected to molecular analysis for identification of *T. evansi*. Twenty-one positive samples of *T. evansi* were detected in 50 cattle using PCR, targeting the RoTat 1.2 variable surface glycoproteins (VSG) gene. PCR-positive samples were furtherly phylogenetically analysed. Sequence analysis of the Rotat 1.2 VSG gene showed high variability between the isolates from this study and the isolates earlier deposited in the GenBank. Microscopic examination exhibited a very low sensitivity, whereas PCR using RoTat 1.2 VSG gene showed more sensitivity in the diagnosis of *T. evansi* from cattle. Conclusively, the phylogenetic results obtained from the Neighbor joining technique suggest that the isolate of *T. evansi* in this study is not related to the isolates from Egypt, Kenya, Sudan and India.

Keyword: Cattle, Nigeria, RoTat 1.2 Gene, *Trypanosoma evansi*

INTRODUCTION

Trypanosoma evansi (*T. evansi*) is the most common pathogenic trypanosome worldwide and causes the sickness known as "surra" (Luckins, 1988; Cadioli et al., 2006). It is pathogenic to numerous species of both domestic and wild animals and has a broad host range (Franke et al., 1994a; Herrera et al., 2002). Biting flies, belonging to species such as *Tabanus*, *Stomoxys*, *Haematopota*, *Lyperosia*, and *Chrysops spp.* are the main mechanical method of transmission (Fraser, 1909; Nieschulz, 1926; 1927a; Nieschulz, 1927b; Luckins, 1999) of the disease. The most significant host in Africa is camels (Dia et al., 1997), however, cattle have also been identified as the second most highly vulnerable animals (Mahmoud and Gray, 1980). Experimental research conducted in Nigeria has confirmed that *T. evansi* can infect donkeys, cattle, sheep, and goats; however, the disease has a long course in these animals (Ilemobade, 1971; Audu et al., 1999; Shehu et al., 2006). There are considerable differences in the severity of syndromes caused by *T. evansi* infection in different geographical areas of its occurrence, depending on the virulence of the strain, the susceptibility of the host (Sawitri et al., 2022) and the presence of other concurrent diseases. Although *T. evansi* is primarily a parasite of camels, there is a likelihood that animals herded together with camels may become infected with *T. evansi* and serve as a reservoir (Aregawi et al., 2019). As such, there is the need to have a better understanding of disease susceptibility of various animal species with respect to *T. evansi* infection.

Light microscopy of various preparations (wet mount, thin and thick blood films, and buffy coat technique) is the most widely used and accessible diagnostic test for trypanosomosis. *T. evansi* in stained blood smears under the light microscope is described as having a large size body (25–35 μm), small and subterminal kinetoplast, thin posterior extremity, large undulating membrane, central nucleus, and free flagellum, according to Desquesnes et al. (2013). However, it is time-consuming, unsuitable for use in field conditions, and has a low sensitivity and specificity (Eisler et al., 2004; Kumar et al., 2012). There has been a number of advancements in trypanosomosis diagnosis recently, leading to a better understanding of the disease's location and mode of transmission- a must for any effective treatment (Magez et al., 2021). These developments have made it possible to accurately identify and detect several Trypanosoma species (Traub et al., 2005). Although enzyme-based immunosorbent assay (ELISA) was thought to have improved pathogen determination sensitivity, antigen detection with monoclonal antibody (mAb)-based ELISA is highly unreliable because immune active agents in the blood can't tell the difference between active infections and past infections (Geysen et al., 2003). More sensitivity and reliability were available than with earlier approaches with the introduction of polymerase chain reaction (PCR), a revolutionary technique for direct parasite detection and identification. PCR analysis (Masiga et al., 1992; Ngomtcho et al., 2017; Weber et al., 2019) and Trypanosoma species-specific DNA probes (Habeeb et al., 2021) have greatly improved the identification and comprehension of trypanosome diversity, particularly in light of the high prevalence of mixed trypanosome infections that are found in the field (Solano et al., 1995; Woolhouse et al., 1996). There is a paucity of information on the disease pattern in different livestock species in Africa, including Nigeria. In Nigeria, extensive work has been done on animal trypanosomosis (Shehu et al., 2006; Karshima et al., 2016; Dabo and Maigari 2018; Umeakuana et al., 2019; Weber et al., 2019), but little has been done on infection due to *T. evansi,* especially in cattle within the study area. Since there is a considerable difference in the severity of syndromes caused by *T. evansi* infection in different geographical areas of its occurrence, it is desirable to evaluate the prevalence of cattle infected with this strain of *T. evansi* in cattle in the study area.

MATERIALS AND METHODS

Ethical approval

All applicable national and institutional guidelines for the care and use of animals were followed. All procedures performed in the studied animals were following the ethical standards of the Faculty of Veterinary Medicine Committee on Animal Use and Care, where the study was conducted.

Study areas and sampling points

This study was carried out in Borno and Gombe States of Northeastern Nigeria (Figure 1). Borno state lies between longitude 120º 8E and latitude 10º 23N and the climate, ecology and vegetation is Sahelian, semi-arid and savannah with flooded pastures towards Lake Chad and mountainous areas in the Southeast. The State shares international borders with Niger, Cameroun and Chad Republics, which have similar relative humidity during the dry season and rainy season (Ishaku and and Majid, 2010).

Sampling was conducted in three urban and rural areas—Konduga, Jere, and Maiduguri—selected from among the 27 Local Government Areas (LGAs) of Borno State. Similarly, Dukku, Gombe, and Yamaltu Deba were sampled from 11 LGAs of Gombe State, Nigeria.

Study design

This study was carried out in two phases, via: Phase I: Microscopic examination of thin blood smears for identification of *Trypanosoma* parasite, Phase II: Molecular detection and characterization of Rotat 1.2 (VSG) genes of *T. evansi* by using polymerase chain reaction (PCR).

Study animals and sample collection

This study was conducted from May to October, 2018. A non-probability convenience sampling method was used with emphasis on areas having large populations of cattle. A total number of 455 randomly selected apparently heathy cattle from selected livestock markets and farms were examined for *T. evansi* regardless of age and sexes. Blood samples were collected through jugular vein in vacutainer tubes containing ethylene di-amine tetraacetic acid (EDTA), and then divided into two parts. One was for microscopic examination and the remaining sample was preserved at -20 ºC for DNA extraction of trypanosomes for PCR.

Microscopic detection for *Trypanosoma*

Thin blood smears were prepared from all the samples collected for parasitological examination, the thin blood smears were air dried, fixed in methanol, stained with a 10% solution of Giemsa, and then observed under the light microscope using 400X and 1000X magnification objectives, according to Zajac et al. (2021). Blood sample

Figure 1 Map of Nigeria showing the 36 states, including Gombe and Borno States (blue star), Nigeria

Source: https://www.nigeriagalleria.com/Nigeria/States_Nigeria/Borno/ - Search Images (bing.com)

of cattle from the study areas were considered positive for *T. evansi* when stained blood smears revealed the presence of *Trypanosoma* parasite. Photomicrographs of *T. evansi* parasites were taken using Amscope digital camera for microscope version (3.0 China).

Isolation of trypanosome DNA

DNA extraction

From the 455 blood samples collected, a total of 50 randomly selected samples, with 25 each from Borno and Gombe States were subjected to molecular analysis for detection of *T. evansi* using standard technique according to Kyari et al. (2021). Briefly, genomic DNA was extracted from the whole blood samples using AccuPrep® Genomic DNA Extraction kit (K-3032, Bioneer, Germany), according to manufacturer's instructions. DNA quantity and purity were measured using a spectrophotometer (BioPhotometer plus, Eppendorf, Hamburg, Germany) and stored at - 20 °C until further use.

PCR amplification

Forward and reverse primer sequences used at an amplification fragment of 250bp (base pair) are shown below:

F5ʹ-GCGGGGTGTTTAAAGCAATA-3ʹ,
R5ʹ-ATTAGTGCTGCGTGTGTTCG-3ʹ
(Invitrogen®, USA)
(Barghash et al., 2016).

In whole reaction quantity of 25 μl having 50 ng of template DNA and 12.5μl of standard commercial PCR Mastermix (AccuPrep®, Germany), the PCR amplification reaction was prepared. A concentration of 10 pmol/μl was used for the primers (RoTat 1.2VSG gene). This was followed by PCR amplification in a Biometra thermocycler. The following circling conditions were used: 94°C/3min (1 cycle) for denaturation, annealing at 57°C/1 min, 72°C/1 min (40 cycles) for polymerization and 72°C/5 min for final extension (Barghash et al., 2016). Subsequently, the outcome of every sample was electrophoresed via 2% agarose, and for visualization of amplified

DNA and comparison with standard DNA 100bp (Bioneer, AccuPrep®, USA), the voltage was set at 60V. Using a transilluminator, the amplicons were examined for expected size.

PCR products sequencing

The products of PCR were cleaned up, decontaminated and sent for direct sequencing in both directions (Bioneer, South Korea). All procedures involved were carried out according to standard protocols. The Basic Local Alignment Search Tool (BLAST) programs were used to align and compare the sequences obtained with others stored in the GenBank. A phylogenetic tree was constructed using the neighbour-joining (NJ) algorithms, version 3.6a 2.1 of Bioedit Software (Hall, 2013). The produced DNA sequences were flung into the GenBank databases in order to locate homologous sequences that had been documented. Also, the Maximum Parsimony (MP) method was used to infer the outcome of phylogeny from present study and other isolates from previous studies.

Data analysis

Prevalence, chi-square and odds ratio were used for the analyses. Contingency coefficient was used to determine association between the statuses of infection. The data were analyzed using JMP, version 11 software (SAS institute Inc. Cary, NC). Results from the analyses were considered significant at a p-value of less than 0.05.

RESULTS

Microscopic identification of *T. evansi* in cattle from Gombe and Borno States, Nigeria

It was revealed that microscopic identification of *T. evansi* had higher prevalence in Gombe (11.8%) than Borno State (9.0%) (Table 1).

Table 1 Microscopic identification of *T. evansi* in cattle from Gombe and Borno States, Nigeria

Study area	Sex of cattle	No of cattle examined	No of cattle infected (%)	Prevalence (%) 95% CI (LL - UL)	χ^2	p- value	Odd ratio
Gombe	Male	66	10 (15.2)	6.9 (0.0381 – 0.1231)	1.301	0.2540	1.6883
	Female	78	7 (9.0)	4.9 (0.0237 – 0.0969)			
Borno	Male	174	11 (6.3)	3.5 (0.0199 – 0.0623)	3.455	0.0631	1.9628
	Female	137	17 (12.4)	5.5 (0.0344 – 0.0858)			

CI = Confidence interval; LL = Lower limit; UL = Upper limit; χ^2 = Chi-square LGA = Local Government Area

Additionally, the results of *T. evansi* prevalence based on the LGAs from samples in Gombe State showed that the prevalence was higher in Yamaltu Deba (6.9%), followed by Dukku (2.8%) and Gombe (2.1%). The association between the statuses of infection in the Local Government Areas of Gombe State was not significant (Chi-square test = 2.39; p = 0.3034 with a contingency coefficient of 0.128, indicating weak association). However, the prevalence of *T. evansi* based on the LGAs in Borno State showed higher prevalence rate in Konduga (4.2%), followed by MMC (3.2%) and Jere (1.6%) LGAs. The association between the statuses of infection in the local government areas of Borno State was not significant (Chi-square test = 3.556; p = 0.1689 with a contingency coefficient of 0.128, indicating weak association). Also, the PCR technique revealed higher (42.0%) prevalence rate for *T. evansi* than the microscopy technique (12.0%) (Table 2).

Table 2 Comparison between microscopic and molecular (PCR) detection rate of *T. evansi* in cattle from Gombe and Borno States, Nigeria

Technique	No. of samples analyzed	*T. evansi* positive sample (% prevalence)
Microscopy	50	06 (12.0%)
PCR	50	21 (42.0%)

Keys: PCR = polymerase chain reaction, No.= number, *T. evansi* =*Trypanosoma evansi*, % = percentage

The phylogenetic tree created from the 5 *T. evansi* RoTat 1.2 gene isolates obtained from cattle in Borno and Gombe states using the neighbor joining method at 1000 bootstrap replicates indicated that 4 of the isolates (No 27, No 45, No 33 and No 39) formed a major clade with 2 subclades. However, in one of the subclades, one of the *T. evansi* isolates (No 39) further diverged from the other isolates, while one of the remaining isolates (No 50) distanced itself from all the other four isolates to form a major clade that looks like an outgroup (Figure 2).
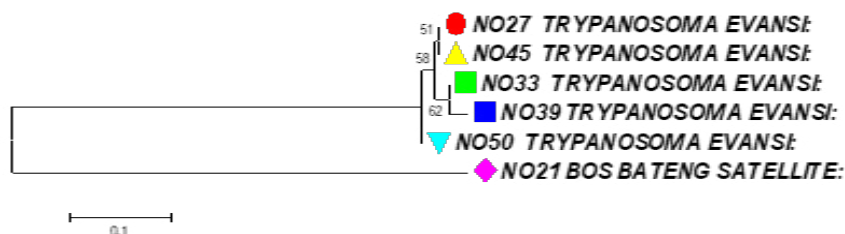
Figure 2 Phylogenic Tree of 5 *T. evansi* Partial RoTat 1.2 Gene Sequences obtained from cattle examined in Borno and Gombe States. Neighbor-joining was used to Inferred the tree, and 1000 Bootstrap Replicates were used.

The evolutionary divergence estimate calculated using the pairwise distance estimation method at four decimal places between the *T. evansi* partial RoTat 1.2 gene sequence from cattle in the studied areas is reported here. Using No 27 as reference isolate indicated that *T. evansi* No 27 and No 45 were the same at 1 standard error, while the other 3 appeared to be evolutionary different from each other. At 2 standard errors, isolates No 39 and No 45 appeared to be the same (0.0164) as seen in Table 3.

Table 3 Pairwise distance estimation (at 4 decimal places) between the *T. evansi* partial RoTat 1.2 gene sequences from cattle in Nigeria's Borno and Gombe States

	1	2	3	4	5	6
NO27__TRYPANOSOMA_EVANSI:		0.0112	0.0161	0.0000	0.0082	0.1476
NO33__TRYPANOSOMA_EVANSI:	0.0164		0.0113	0.0112	0.0081	0.1456
NO39_TRYPANOSOMA_EVANSI:	0.0332	0.0164		0.0161	0.0140	0.1554
NO45__TRYPANOSOMA_EVANSI:	0.0000	0.0164	0.0332		0.0082	0.1476
NO50__TRYPANOSOMA_EVANSI:	0.0082	0.0082	0.0248	0.0082		0.1418
NO21_BOS_BATENG_SATELLITE:	0.8615	0.8615	0.9146	0.8615	0.8363	

Note: Values on top represent the standard errors, while the bottom diagonal values are the actual variance estimates

The evolutionary divergence estimation of the 5 isolates obtained from this study and other geographically dispersed isolates calculated, using the pairwise distance estimation at 3 decimal places for the incomplete RoTat 1.2 *T. evansi* gene sequences, indicated that three of the isolates from this study (isolates No 27, No 33 and No 45) appeared to be the same at 1 standard error (Table 4). On the other hand, two isolates from Egypt and one from Kenya (isolates No JX888091, KF726106 and AF317914, respectively) with an estimation of 1.303 and 1.719 for isolates reported in this study are related to those from Egypt and Kenya, respectively.

The phylogenetic tree created from the 5 isolates based on this research and other isolates from geographically distant locations retrieved from the GeneBank reveals that all the isolates from this study distanced themselves from all other isolates obtained from other geographical locales to form a major clade. It is important to note that the Asian isolates from India appeared to distance evolutionarily and diverged themselves from the other African isolates (Figure 3).
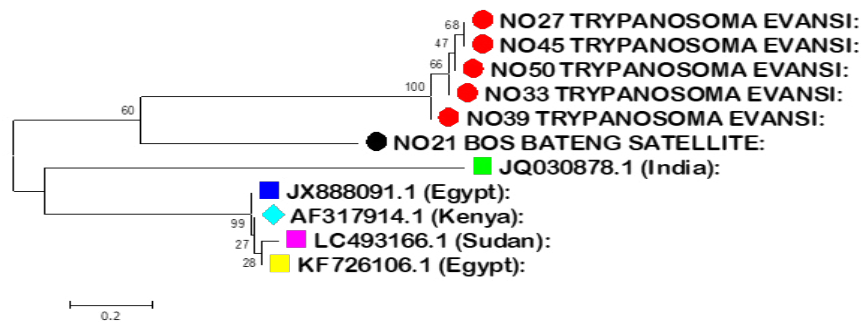
Figure 3 Nigerian RoTat 1.2 *T. evansi* Isolates Phylogenetic Tree obtained in present study were compared with other geographically dispersed *T. evansi* RoTat 1.2 isolates. The phylogentic tree was generated with 1000 Bootstarp Replicates and the Neighbor-joining technique.

Table 4 Pairwise distance estimation (at 3 decimal places) between the *T. evansi* partial RoTat 1.2 gene sequences from cattle in Borno and Gombe States compared with other geographically dispersed isolates

	1	2	3	4	5	6	7	8	9	10	11
NO21_BOS_BATENG_SATELLITE:		0.352	0.358	0.331	0.352	0.323	0.562	0.660	0.748	0.660	0.660
NO27_TRYPANOSOMA_EVANSI:	1.303		0.016	0.020	0.000	0.011	1.036	0.558	0.651	0.558	0.558
NO33_TRYPANOSOMA_EVANSI:	1.303	0.023		0.012	0.016	0.011	0.952	0.491	0.568	0.491	0.491
NO39_TRYPANOSOMA_EVANSI:	1.242	0.034	0.011		0.020	0.017	0.851	0.491	0.568	0.491	0.491
NO45_TRYPANOSOMA_EVANSI:	1.303	0.000	0.023	0.034		0.011	1.036	0.558	0.651	0.558	0.558
NO50_TRYPANOSOMA_EVANSI:	1.242	0.011	0.011	0.023	0.011		0.952	0.491	0.568	0.491	0.491
JQ030878.1_(India):	1.615	2.392	2.159	1.982	2.392	2.159		0.508	0.508	0.508	0.508

	1	2	3	4	5	6	7	8	9	10	11
JX888091.1_(Egypt):	1.719	1.615	1.524	1.524	1.615	1.524	1.524		0.011	0.000	0.000
LC493166.1_(Sudan):	1.839	1.719	1.615	1.615	1.719	1.615	1.524	0.011		0.011	0.011
AF317914.1_(Kenya):	1.719	1.615	1.524	1.524	1.615	1.524	1.524	0.000	0.011		0.000
KF726106.1_(Egypt):	1.719	1.615	1.524	1.524	1.615	1.524	1.524	0.000	0.011	0.000	

Note: Values on top represent the standard errors, while the bottom diagonal values are the actual variance estimate.

DISCUSSION AND CONCLUSION

Molecular diagnostic techniques represent essential tools for the detection of parasitic infection because of their high sensitivity, and are widely spread in the detection of surra globally because they are rapid, accurate and reliable (Sengupta et al., 2010).

Nonetheless, microscopy and PCR may exhibit extremely similar sensitivity in early stages of the infection. Conversely, during the chronic phase of infection, microscopic examination exhibits very low sensitivity, whereas PCR is 2-3 times more sensitive (Desquesnes and Davila, 2002). Out of the 50 samples tested using PCR amplification with RoTat 1.2, it is likely that the 21 *T. evansi* positively tested cattle were most likely infected during the active stage of illness. This finding may suggest that enough parasites were circulating in the blood of the infected cattle, allowing for their detection in both blood smear examination and PCR assay (Desquesnes et al., 2013). Compared with microscopic blood examination, the PCR technique is suggested for application in the diagnosis of trypanosomosis due to its greater sensitivity. Further molecular characterization and phylogenetic analysis of *T. evansi* isolates in naturally infected cattle in Gombe and Borno States showed PCR- RoTat 1.2 that amplified at 205 bp, followed by sequence analysis of this fragment. The choice of this gene is in accordance with a previous study by Claes et al. (2004) who reported a higher sensitivity by using the RoTat 1.2 in the PCR assay for diagnosis of *T. evansi* in bovine species compared to other related gene primers. Thus, this study confirms the greater sensitivity of PCR- RoTat 1.2 gene as a diagnostic primer tool for *T. evansi,* even at a very low infection. PCR used in this study for the detection of the presence of *T. evansi* RoTat 1.2 gene at genus and species levels, as expected, formed a major clade compared to other dispersed geographical isolates. More so, the results of the phylogenetic analysis in this study have also shown that all putative species/genus identified using BLAST searches of the GenBank clustered together (dendogram), indicating very close genetic relatedness. The RoTat 1.2 gene used here is a reliable marker for phylogeny, as it has been previously used to infer phylogenetic affinities and establish evolution and divergence of *T. evansi* in different species of bovine (Barghash et al., 2014). The phylogenetic tree constructed from the 5 *T. evansi* RoTat 1.2 gene isolates obtained from cattle in Borno and Gombe states using the neighbor joining method at 1000 bootstrap replicate indicated that 4 of the isolates formed a major clade with 2 subclades. However, in one of the subclades, one of the *T. evansi* isolate further diverged from the others, while one of the remaining isolates distanced itself from all the other four to form a major clade that looked like an outgroup. This finding is similar to previous reports by Barghash et al. (2016) in Camels from Egypt who observed variation between *T. evansi* isolates in their study and those earlier deposited in the GeneBank.

Moreover, evolutionary divergence calculated using the pairwise distance estimation method at four decimal places between the partial RoTat 1.2 gene sequence of *T. evansi* from cattle in Borno and Gombe states, using No. 27 as a reference isolate, indicated that *T. evansi* at 27 and 45 are the same at one standard error. In contrast, the other 3 appeared to be evolutionary different from each other. At two standard errors, isolates No 39 and No 45 appeared to be the same. This finding supported the reports

of Elhaig et al. (2016) and Elata et al. (2019) who also reported *T. evansi* in cattle and donkeys in Egypt and Sudan, respectively, using molecular phylogenecity. The phylogenetic tree created from the 5 isolates obtained, and compared with other isolates from other geographically dispersed locations in the GeneBank revealed that all the isolates from this study distanced themselves from all other isolates obtained from other geographical locales to form a major clade. It is important to note that the Asian isolates from India appeared to distance evolutionarily and diverged itself from the other African isolates. This agrees with the result of Urakawa et al. (2001) in Kenya who obtained similar isolates that claded closely with the isolate from this study. The estimation of evolutionary divergence of the 5 isolates here and other geographically dispersed isolates calculated using the pairwise distance estimation at 3 decimal places for the partial RoTat 1.2 gene sequences of *T. evansi* indicated that 3 of the isolates from this study (isolates No. 27, No. 33 and No. 45) appeared to be the same at 1 standard error, while two isolates from Egypt (Elhaig et al., 2016) and one from Kenya (Urakawa et al., 2001) were close to the isolates obtained in this study. A partial sequence of RoTat 1.2 VSG gene was reported to be identical to the *T. evansi* sequences reported from India and Kenya, but the varied similarity was seen when aligned with other isolates of *T. evansi* in Africa, which supported the findings of *T. evansi* sequences in Egypt by Witola et al. (2005). Using Maximum Parsimony (MP) method, the outcome of phylogenies in the present study suggested that the isolates of *T. evansi* from Kenya, Egypt, India and Sudan were more diverse than other isolates. This finding agrees with the reports of Elhaig et al. (2016) and Elata et al. (2019). A strong tree clustering of all known *T. evansi* stations that are circulating worldwide and that were retrieved from GenBank with values at pertinent nodes was shown by phylogenetic analysis. The length of the horizontal lines on the phylogenetic tree, which represented the evolutionary relationship between the sequences, was proportionate to the estimated genetic distance between the sequences.

In conclusion, RoTat 1.2 VSG molecular technique proved to be more sensitive and reliable as a diagnostic tool for *T. evansi* in cattle examined in Nigeria's states of Borno and Gombe. *T. evansi* detected in this study was found to be unrelated to some isolates from Africa and Asia in the GeneBank. It is, therefore, recommended that, since the gene used in this study is a protein coding gene, there is the need to further translate the nucleotide sequences obtained from this study into amino acids. There is also the need to look for the presence of tandem repeat sequences, which are useful for vaccine production.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Concept and Design -AMB; Supervision - OE; AWM; MMB; Sample collection –JD; SMT; HIA; HMBM; Literature review and analysis and or interpretation of data- LA; Writing and Critical review- HA

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MOLEKULARNA DIJAGNOSTIKA *TRYPANOSOME EVANSI* KORIŠTENJEM (VSG) GENA KOD GOVEDA U DRŽAVAMA BORNO I GOMBE U NIGERIJI

SAŽETAK

Trypanosoma evansi (*T. evansi*) uzrokuje suru, važno hematoprotozoarno oboljenje od ekonomskog značaja koje ima širok spektar domaćina. Kod goveda smo proveli istraživanje sa ciljem dijagnosticiranja *T. evansi* korištenjem molekularne metode u državama Gombe i Borno, u Nigeriji. Prikupljeni su uzorci krvi od 455 naizgled zdravih goveda od mjeseca maja do oktobra, 2018. godine sa različitih područja vladinih uprava. Mikroskopiranjem je ustanovljena prevalenca od 11.8% za državu Gombe i 9.0% za državu Borno za vrijeme trajanja studije. Od prikupljenih 455 krvnih uzoraka, 50 randomiziranih uzoraka, po 25 iz država Borno i Gombe, su podvrgnuti molekularnoj analizi u svrhu identifikacije *T. evansi*. Kod 50 goveda je otkriven dvadeset i jedan uzorak pozitivan na *T. evansi* korištenjem PCR metode sa RoTat 1.2 genom za varijabilne površinske glikoproteine (VSG) kao metom. PCR-pozitivni uzorci su dalje analizirani filogenetički. Sekvencijska analiza Rotat 1.2 VSG gena je pokazala visoku varijabilnost između izolata iz ovog istraživanja i izolata ranije deponiranih u GenBank. Mikroskopski pregled je pokazao veoma nisku senzitivnost, dok je PCR uz korištenje RoTat 1.2 VSG gena pokazao veću senzitivnost u dijagnosticiranju *T. evansi* kod goveda. U zaključku, filogenetski rezultati dobiveni tehnikom pridruživanja pokazuju da izolat *T. evansi* iz našeg istraživanja nije povezan s izolatima iz Egipta, Kenije, Sudana i Indije.

Ključne riječi: Goveda, Nigerija, RoTat 1.2 gen, *Trypanosoma evansi*