

Parasitological, Serological and Molecular Survey of Camel Trypanosomosis (Surra) in Northern Nigeria

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Research Article

Keywords: Trypanosoma evansi, Surra, camels, Northern Nigeria, polymerase chain reaction

Posted Date: June 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-641740/v1>

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Abstract

Surra, a parasitic disease caused by *Trypanosoma evansi* and transmitted non-cyclically by biting flies significantly affects the health, productivity and market value of camels thereby constituting a major constraint to food safety, security and economy. This is the first study on the prevalence of surra in Northern Nigeria, using a broad variety of diagnostic tests along the parasitological-serological-molecular continuum hence, emphasizing it as a major enzootic risk for camels in Nigeria. In this cross-sectional study, 600 blood samples were collected from camels at major abattoirs in Northwestern Nigeria and evaluated for the prevalence of surra due to *T. evansi* using parasitological (Giemsa staining), serological (CATT/*T. evansi*) and molecular (VSG-PCR and sequencing) methods. The overall prevalence of surra recorded in this study was 5.3%, 11.5% and 22.5% using Giemsa stained blood smears, CATT/*T. evansi* and VSG-PCR respectively. However, higher prevalence of 6.0%, 13.7% and 26.7% by Giemsa stained blood smears, CATT/*T. evansi* and VSG-PCR was recorded in Katsina state when compared with Kano state. A significantly ($p < 0.05$) higher prevalence by VSG-PCR was observed when compared with both parasitological and serological methods used. Although age and body condition scores were associated ($p < 0.05$) with surra prevalence in sampled camels, no seasonal association ($p > 0.05$) was however recorded. Sequencing of the VSG region of *Trypanosoma* spp. Further confirmed the presence of *T. evansi* as the aetiological agent of surra from the sampled camels. Findings from this study call for the implementation of adequate control measures aimed at reducing the impact of *T. evansi* infections on camel production in Nigeria.

Background

Trypanosomosis in camels also referred to as 'Surra' is a vector-borne disease (VBD) that constitutes a major threat to farm animals in tropical and sub-tropical countries including Nigeria. The disease is caused by *Trypanosoma evansi* which belongs to the sub-genus *Trypanozoon*. Microscopically, the organism is long and slender with a prominent undulating membrane, and long free flagellum (Getachew, 2005). The disease is endemic in Africa, Asia and South America and it is transmitted mechanically by the bites of haematophagous flies; *Tabanids*, *Stomoxys* and *Hippoboscids* (Eyop and Matios, 2013).

Trypanosomosis in camels is considered the most serious animal protozoan disease in African countries that depend on camels as an important source of food and income for millions of herders (Kamidi *et al.*, 2017). The disease causes production losses, anaemia, weight loss and abortion in a range of domestic species in Africa, Asia and South America. Camel trypanosomosis is usually asymptomatic but can be fatal when not properly diagnosed, and treatment initiated early in the course of the disease (Desquesnes *et al.*, 2013).

Laboratory diagnosis of Surra is often recommended for the confirmation of infection (Hassan-Kadle *et al.*, 2019). Although the standard trypanosome detection methods (STDMs) have been used over the years in the diagnosis of animal Trypanosomiasis, neither the parasitological nor the serological methods are sensitive and specific enough to differentiate between the various species of *Trypanosoma* in animals. Thus, various genetic and molecular methods have been developed to overcome the limitation of the STDMs (Barghash *et al.*, 2016). To date, the molecular diagnostic techniques for the diagnosis of *Trypanosoma* infection offer better results. Furthermore, the increased sensitivity and specificity of the molecular techniques lies in the ability to detect all the stages of infection and the low levels of *Trypanosoma* spp. DNA in blood and tissue samples from animals. To this end, several primers have been developed for the amplification and sequencing of different target genes or regions such as ribosomal DNA, internal transcribed spacer region (ITS), Kinetoplast DNA and Variable Surface Glycoprotein (VSG) genes of the parasites (El-Wathig *et al.*, 2016; Tehseen *et al.*, 2017).

This study sought to (1) determine the prevalence of camel trypanosomosis in northern Nigeria using parasitological, serological and molecular methods and (2) to assess the risk factors for camel trypanosomosis in the study areas. Also, genetic characterization and the phylogenetic relationship of sequences in this study will be compared to sequences in the GenBank.

Materials And Methods

Ethical approval

All experimental protocols and animal work were approved by the Animal Use and Care Committee of the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with the certification ID: NVRI AEC REF No: ACE/02/88/20. All experiments were conducted under strict adherence to the principle of laboratory animal care.

Study area

The study was conducted at the central abattoirs of Katsina (latitudes 11°08'N and 13°22'N and longitude 6°52'E and 9°20'E) and Kano (latitude 12°40'N and 10°31'N and longitude 7°40'E and 9°30'E) states located in the Northwestern region of Nigeria. The climate of the two study areas is composed of two major seasons, the dry and wet seasons. The wet season starts from May to ends in September or early October. While the dry season begins in October and ends in April or early May. The mean annual rainfall is about 690mm while the mean annual temperature ranges between a maximum of 33°C and a minimum of 19°C. The vegetation is mainly savanna; climatically define as Sudan savanna, which is characterized by the presence of scattered trees and shrubs in open grassland (Wakawa et al., 2016). The choice of Katsina and Kano States in the Northwestern region was based on (1) the presence of camel international markets in these two states and (2) both states are the major trans-Sahara animal trade routes to Nigeria.

Sample size determination

The sample size was determined based on the prevalence of 27% (Enwezor and Anthony, 2005) with a 95% confidence level and 5% precision as recommended by Thrusfield (1995). A total of 600 camels (*Camelus dromedarius*) were examined in this survey with 300 camels sampled from each of the two selected states, also exactly 150 each examined during the dry and wet seasons respectively.

Sampling

This study was conducted on apparently healthy camels brought for slaughter in the main abattoirs in Kano and Katsina cities, Northwestern Nigeria. Within the two states, specific abattoirs were chosen purposively where a high number of camels are largely slaughter for human consumption and also transported to other locations within Nigeria. On every sampling day, apparently healthy camel (n = 600) was selected from the population across the abattoirs and seasons, with the total number of 135male and 165female in Kano state and 103male and 197female in Katsina state being sampled. The camels were examined before slaughter to determine their ages according to their dentition as described by Johnson, (2003). Furthermore, the camels were categorized into; good, fair or poor based on the body conditions score according to Salah (2016).

Blood samples from camels were collected by jugular venipuncture for laboratory diagnosis. About 3ml of blood from each camel were collected into plain tubes and then kept under room temperature (25°C) until visible cloth retraction was seen. The clothed samples were then centrifuged at 1500rpm for 5min, and the serum was aliquotted

and stored at -80°C until serological analysis was performed. Four (4) ml of blood was placed into a tube containing anticoagulants for parasitological and molecular analysis. In the field the blood samples were kept in a cold box packed with ice and immediately transported to the Parasitology laboratory of the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria where they were preserved at -80°C till further analysis.

Parasitological examination

For the parasitological diagnosis of the presence of *Trypanosoma* spp. from the blood samples collected, microscopic examination of thin smears of bloodstained with Giemsa stain was carried out (OIE, 2012). Briefly, blood samples were processed for microscopic examination according to standard procedures (Soulsby, 1982). Stained blood smears were examined under the microscope using the oil immersion objective (×100) for the detection of *Trypanosoma* spp. A minimum of 50 microscopic fields were examined before the result was determined.

Serological examination

Commercially available Card Agglutination Test (CATT/ *T.evansi*) kits were purchased from the Laboratory of Serology, Institute of Tropical Medicine, Antwerp, Belgium and used in this study. Serum samples were tested with Card Agglutination Test for Trypanosomiasis (CATT/ *T.evansi*) following the instruction of the manufacturer with slight modifications (Ibrahim *et al.*, 2011). Briefly, one drop test serum was diluted 1:4 in CATT-Buffer. The mixture was then pipetted onto a plastic-coated test card. One drop of CATT reagent was added and the reaction mixture was spread out using a clean stirring rod. The reaction mixture was allowed to react on the card with manual rotation for 5min. Positive reactions were interpreted based on the appearance of blue granular agglutinations visible to the naked eye. Positive and negative controls were included in each reaction run.

Molecular characterization

DNA was extracted from the whole blood collected from each of the sampled animals using quick-DNA miniprep kit (Zymo Research) according to the instructions of the manufacturer. The eluted DNA was then stored at -20°C until PCR analysis. Published primers, TE-FOR-(5'-TGCAGACGACCTGACGCTACT-3') and TE-REV-(5'-CTCCTAGAAGCTTCGGTGTCTCT-3') for the amplification of the 227bp fragments of *T. evansi* (Wuyts *et al.*, 1994) was used in this study. The PCR amplification of the samples were performed in a 25µl reaction that contained 2.5µl of genomic DNA extract, 0.5µl of 20µM primer (TE-FOR and TE-REV), 12.5µl of one Taq® Quick-Load® 2x Master Mix with Standard Buffer (New England Bio Labs) and the volume made up with 9.0µl Nuclease Free Water (Promega®). This was taken to a pre-set and pre-heated Applied Biosystem® 9700 PCR Machine for amplification. The reaction conditions were: initial denaturation at 95°C for 4minutes followed by 30cycles of denaturation at 95°C for 1minutes, annealing at 60°C for 1minutes, extension at 72°C for 1min and final extension at 72°C for 10minutes. The PCR products were visualized in 1.5% Agarose gel stained with ethidium bromide. The gel was observed for the appropriate size DNA band under a UV trans-illuminator.

Positive amplicons were sent to a commercial sequencing company (Macrogen Europe, Netherlands) for sequencing in the forward direction. Sequences obtained were manually edited and compared with the sequences available at the GenBank database using the Basic Local Alignment Sequence Techniques (BLAST) programme and databases of the NCBI (National Centre for Biotechnology Information, Bethesda, MD, USA) (www.blast.ncbi.nlm.nih.gov/blast.cgi). The obtained sequences reported in this study were deposited in the GenBank with the accession number (MZ394796 and MZ394795) for *T.evansi* isolated from Katsina and

MZ394797 for *T.evansi* isolated from Kano. Phylogenetic analysis was constructed by comparing identified sequence in this study with the related sequences from GeneBank using the maximum likelihood (ML) method with the distance algorithms available in the Molecular Evolutionary Genetics Analysis package (MEGA 5).

Data analysis

Data generated during the study were entered into excel sheet and analyzed using the R statistical software (R core Team. 2013). The association between prevalence and risk factors was assessed using the chi-square test. The level of significance was set at $p \leq 0.05$.

Results

The overall prevalence of *T. evansi* in the study area was 5.3%, 11.5% and 21.5% by microscopy, serology and PCR, respectively (Table 1). However, using the three methods explored in this study, a state-wise comparison indicated a higher prevalence of Surra in Katsina state when compared with Kano state (Table 1). Female camels and those between 1-3 years of age were mostly affected with *T. evansi* when compared with male and other age groups. In Katsina state camels with good body condition were not infected with *T.evansi*, but infection with *T.evansi* was recorded among camels with good body condition in Kano state. (Table 1).

There was a significant difference ($p<0.05$) between the *T. evansi* prevalence detected by the three diagnostic methods used in this study. PCR was the most sensitive followed by serology where as microscopic detection was the least sensitive (Table 2). Furthermore, the Agarose gel electrophoresis of the amplified PCR products from positive samples revealed a 227-bp fragment (Figure 2). Regardless of the diagnostic method used, *T. evansi* prevalence was associated with body condition score and the age of the animals. Furthermore, the sex of the camels was associated with the prevalence of *T. evansi* based on serology and PCR methods employed. However, there was no association between the prevalence of *T. evansi* in camels and seasons within the study area (Table 2).

The maximum likelihood phylogenetic tree generated a topology showing the *T. evansi* from Nigeria forming a cluster with high bootstrap with *T. evansi* from Iran (GenBank: MF188845.1) as well as *T.evansi* isolated from Dog in India (GenBank: MG600142.1). Other *Trypanosoma* spp. form distinct clades according to their sequence similarities (Figure 3).

Table 1. Prevalence of Surra in Northern Nigeria

KATSINA STATE									
Variables	Number positive /number examined based on:								
	Microscopy			Serology			PCR		
Body condition score	Wet season	Dry season	Total (%)	Wet season	Dry season	Total (%)	Wet season	Dry season	Total (%)
Poor	3/29	6/103	9/132 (6.8)	6/29	14/103	20/132 (15.2)	24/29	15/103	39/132 (29.5)
Fair	7/85	1/26	8/111 (7.2)	14/85	2/26	16/111 (14.4)	18/85	18/26	36/111 (32.4)
Good	0/36	1/21	1/57 (1.8)	5/36	1/21	6/57 (10.5)	4/36	1/21	5/57 (8.8)
Age group									
1-3	4/62	2/47	6/109 (5.5)	12/62	4/47	16/109 (14.7)	26/62	10/47	36/109 (33.0)
4-6	6/79	5/78	11/157 (7.0)	11/79	9/78	20/157 (12.7)	19/79	20/78	39/157 (24.8)
7-9	0/8	1/22	1/30 (3.3)	2/8	3/22	5/30 (16.7)	1/8	4/22	5/30 (16.7)
10-12	0/1	0/3	0/4 (0)	0/1	0/3	0/4 (0)	0/1	0/3	0/3 (0)
Sex									
Male	5/54	2/49	7/103 (6.8)	7/54	4/49	11/103 (10.7)	11/54	9/49	20/103 (19.4)
Female	5/96	6/101	11/197 (5.6)	18/96	12/101	30/197 (15.2)	35/96	25/101	60/197 (30.4)
Total	10/150	8/150	18/300 (6.0)	25/150	16/150	41/300 (13.7)	46/50	34/150	80/300 (26.7)
KANO STATE									
Body condition score									
Poor	1/85	8/103	9/188 (4.8)	5/85	16/103	21/188 (11.2)	12/85	20/103	32/188 (17.0)
Fair	0/17	1/34	1/51(2.0)	1/17	2/34	3/51 (5.9)	1/17	5/34	6/51 (11.8)
Good	4/48	0/13	4/61(6.6)	4/48	0/13	4/61 (6.6)	9/48	2/13	11/61 (18.0)
Age group									
1-3	1/65	3/58	4/123 (3.3)	4/65	10/58	14/123 (11.4)	8/65	13/58	21/123 (17.1)

4-6	4/84	6/86	10/170 (5.9)	6/84	8/86	14/170 (8.2)	14/84	13/86	27/170 (15.9)
7-9	0/1	0/5	0/6 (0)	0/1	0/5	0/6(0)	0/1	1/5	1/6 (16.7)
10-12	0/0	0/1	0/1 (0)	0/0	0/1	0/1 (0)	0/0	0/1	0/1 (0)
Sex									
Male	2/65	4/70	6/135 (4.4)	3/65	8/70	11/135 (8.1)	8/65	10/70	18/135 (13.3)
Female	3/85	5/80	8/165 (4.8)	7/85	10/80	17/165 (10.3)	14/85	17/80	31/165 (18.8)
Total	5/150	9/150	14/300 (4.7)	10/150	18/150	28/300 (9.3)	22/150	27/150	49/300 (16.3)
Overall	15/300	17/300	32/600 (5.3)	35/300	34/300	69/600 (11.5)	68/300	61/300	129/600 (21.5)

Table 2. Risk factor associated with Surra Northern Nigeria

Variables	Number positive /number examined (%)				
	Microscopy	Serology	PCR	χ^2	P
Body condition score					
Poor	18/320 (5.6) ^a	41/320 (12.8) ^a	71/320 (22.2) ^a	597	0.00*
Fair	9/162 (5.6) ^b	19/162 (11.7) ^b	42/162 (25.9) ^b	4900	0.001*
Good	5/118 (4.2) ^c	10/118 (8.5) ^c	16/118 (13.6) ^c	7.1	0.028*
Age group					
1-3	10/232 (4.3) ^a	30/232 (12.9) ^a	57/232 (24.6) ^a	506	0.02*
4-6	21/327 (6.4) ^b	35/327 (10.7) ^b	66/327 (20.2) ^b	266	0.00*
7-9	1/36 (2.8) ^c	5/36 (13.9) ^c	6/36 (16.7) ^c	99.9	0.01*
10-12	0/5 (0) ^d	0/5 (0) ^d	0/5 (0) ^d	NR	NR
Sex					
Male	13/238 (5.5) ^a	22/238 (9.2) ^a	38/238 (16.0) ^a	163.9	0.03*
Female	19/362 (5.2) ^a	47/362 (13.0) ^b	91/362 (25.1) ^b	610	0.00*
Season					
Dry	17/300 (5.7) ^a	35/300 (11.7) ^a	61/300 (20.3) ^a	311	0.00*
Wet	15/300 (5.0) ^a	35/300 (11.7) ^a	68/300 (22.7) ^a	457	0.01*

NR=Null result.

Values with asterisk (*) are statistically different across the row. Figures with different superscript along the columns are significantly different for the variable.

Discussion

Camels will continue to constitute an important part of the lives and livelihood of subsistent farmers in Nigeria, both as draught animals and a source of protein. However, diseases such *T.evansi* infection causes a setback to camel productivity and wellbeing with a net socio-economic consequence to the farmers. In this study low to high prevalence (5.3% – 21.5%) of *T.evansi* was detected in northern Nigeria depending on the diagnostic method used. The relatively low prevalence recorded in this study is consistent with earlier reports from Nigeria (Mbaya *et al.*, 2010; Argungu *et al.*, 2015; Wakili *et al.*, 2016) and other parts of the world (Dia *et al.*, 1997; Tehseen *et al.*, 2015; Olani *et al.*, 2016; Bala *et al.*, 2018; Hassan-Kadle *et al.*, 2019).

The low prevalence recorded by the microscopic examination method could be due to its low sensitivity. However, the CATT/*T. evansi* serological assay detected higher cases of *T. evansi* infections from the sampled camels than the microscopy. The disadvantage though is the inability of this test to differentiate between active infection and

antibodies from treated animals. It has been reported that following treatment, antibodies from the treated animal remain in blood circulation up to nearly four weeks, thus such animals will be detected as positive cases (Olaho-Mukani *et al.*, 1996; Thammasart *et al.*, 2001; Singh and Chaudhri, 2002; Aregawi *et al.*, 2015; Birhanu *et al.*, 2015; Tehseen *et al.*, 2015; Mohamoud, 2017). Therefore, this fact necessitates getting a reliable history of the animal regarding recent anti-trypanosome treatment before sample collection to make CATT/*T.evansi* and all other antibody detecting tests more reliable.

The PCR diagnosis gave a higher prevalence than the other two methods used in this study. This finding attests to the ability of this method to detect and amplify low levels of parasite DNA in blood circulation. Unlike the blood smear examination by microscopy where a high level of parasitaemia, as well as the morphology of the parasite, is required to detect positive sample, the PCR is reputed to be sensitive at detecting low parasitaemia (Abdel-Rady, 2006). However, some factors such as the presence of PCR inhibitors during DNA extraction have been reported to limit the sensitivity of PCR (Shyma *et al.*, 2013). Generally, the prevalence recorded by PCR was significantly higher than the results from the other methods used in this study. This could be due to the fact that PCR (i) is an accurate, more sensitive and specific method in the diagnosis of trypanosomes infected camels than the parasitological method. It also overcomes the problem of non-specific reaction as in the case of the serological method (ii) can also detect low parasitaemic camels in mild as well as chronic cases (Abdel-Rady, 2006). The results from this study agree with the work of Nahla *et al.* (2011) who reported a higher prevalence (90.0%) of Surra using molecular technique (PCR) than both serological (CATT/*T.evansi*) (47.6%) and the Parasitological (3.7%) techniques, respectively. Conversely, Tehseen *et al.* (2015) reported a prevalence of 0.7%, 47.7% and 30.5% through parasitological, serological and molecular techniques. This is not surprising because in serological techniques antibodies can remain in circulation for several months after treatment, thus given a false positive result.

A sex-wise comparison indicated that female camels sampled in this study had a higher prevalence of Surra than the male camels. This might be attributed to stressor other sex-related physiological conditions including pregnancy and/or lactation which may reduce disease resistance in female camels and render them more susceptible to infections (Bhutto *et al.*, 2009). There was no relation between Surra prevalence and the season throughout this study. This finding is at variance with previous reports (Löhr *et al.*, 1985; Singh and Joshi, 1991; Kashiwazaki *et al.*, 1998; Jindal *et al.*, 2005; Desquesnes *et al.*, 2013). Even though the prevalence of *T.evansi* among camels herd is strongly depended on the size of the vector population, wet season and swampy nature of an area (Mohammed, 1999). However, we believe that at the time of sampling chronic infection may be rampant among the camels herds and therefore may not be strictly influenced by seasonal factors like vector abundance as earlier suggested by some researchers (Batra *et al.*, 1994; Soodan *et al.*, 1995). Furthermore, younger camels examined in this study were more predisposed to *T. evansi* infection than older camels and this agrees with the findings of Mbaya *et al.* (2010) and Kassa *et al.* (2011) respectively. Age susceptibility and lack of premunity have been suggested to account for the higher incidence of *T.evansi* in young camels (Soulsby, 1982; Njiru *et al.*, 2002).

In this study, camels in poor or fair body conditions had a higher prevalence of Surra when compared with those in good body condition as previously reported by Eyop *et al.* (2013) but contrary to the report of Idehen *et al.* (2018). The body condition score is related to the plan of nutrition of the animals, hence, their ability to mount resistance to infections including Surra. Animals with poor body conditions are malnourished and therefore susceptible to disease conditions however, there is often a relationship between the season and body condition score of camels. The extensive system of camel husbandry and management practiced by farmers in the study area is supported by the readily available pasture for camels during the rainy season compared to the dry season. There is a need for more feed supplements to the camels especially during the dry season when draught is intense and food scarcity is

obvious, thereby meeting the high demand in the supply of safe and sustainable animal protein to humans. Also, adequate veterinary care should be provided to the camels to alleviate the effects of different animal diseases including Surra.

Conclusion

This study reports the prevalence of camel Trypanosomiasis in northern Nigeria using three diagnostic methods. Based on our knowledge, this is the first report of *T.evansi* infection in camels using three diagnostic methods along the parasitological-serological-molecular continuum, especially the use of PCR and sequencing to confirm the diagnosis. Taken together, Surra is prevalent in camels in northern Nigeria and constitutes a constraint to camel productivity in the area. Adequate control measures aimed at reducing the impact of trypanosomes on camel production in the study area is recommended.

Declarations

Competing of interest

None exist

Consent for publication

Not applicable

Ethics approval

All experimental protocols and animal work were approved by the Animal Use and Care Committee of the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria with the certification ID: NVRI AEC REF No: ACE/02/88/20. All experiments were conducted under strict adherence to the principle of laboratory animal care.

Funding

No funding was received for this research

Availability of data and material

Not applicable

Authors Contribution

MSA and DAD collected samples, carried out laboratory work, analyzed data and prepared the manuscript. **JAY, DGA,** Were involved in conceiving the project, the study design and in reviewing the manuscript. **KJ** carried out the phylogenetic analysis and contributed to data analysis. **RRC and OOO** managed the technical aspect of the studies and finalizing the manuscript. **TDA, PJG** contributed in the molecular and Parasitological analysis of the samples. All authors read and approved the final manuscript.

Acknowledgement

We acknowledge the Nigerian Institute for Trypanosomiasis Research (NITR) and National Veterinary Research Institute (NVRI) both in Vom, Plateau state, Nigeria for helping out in the field and laboratory diagnosis respectively.

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Figures

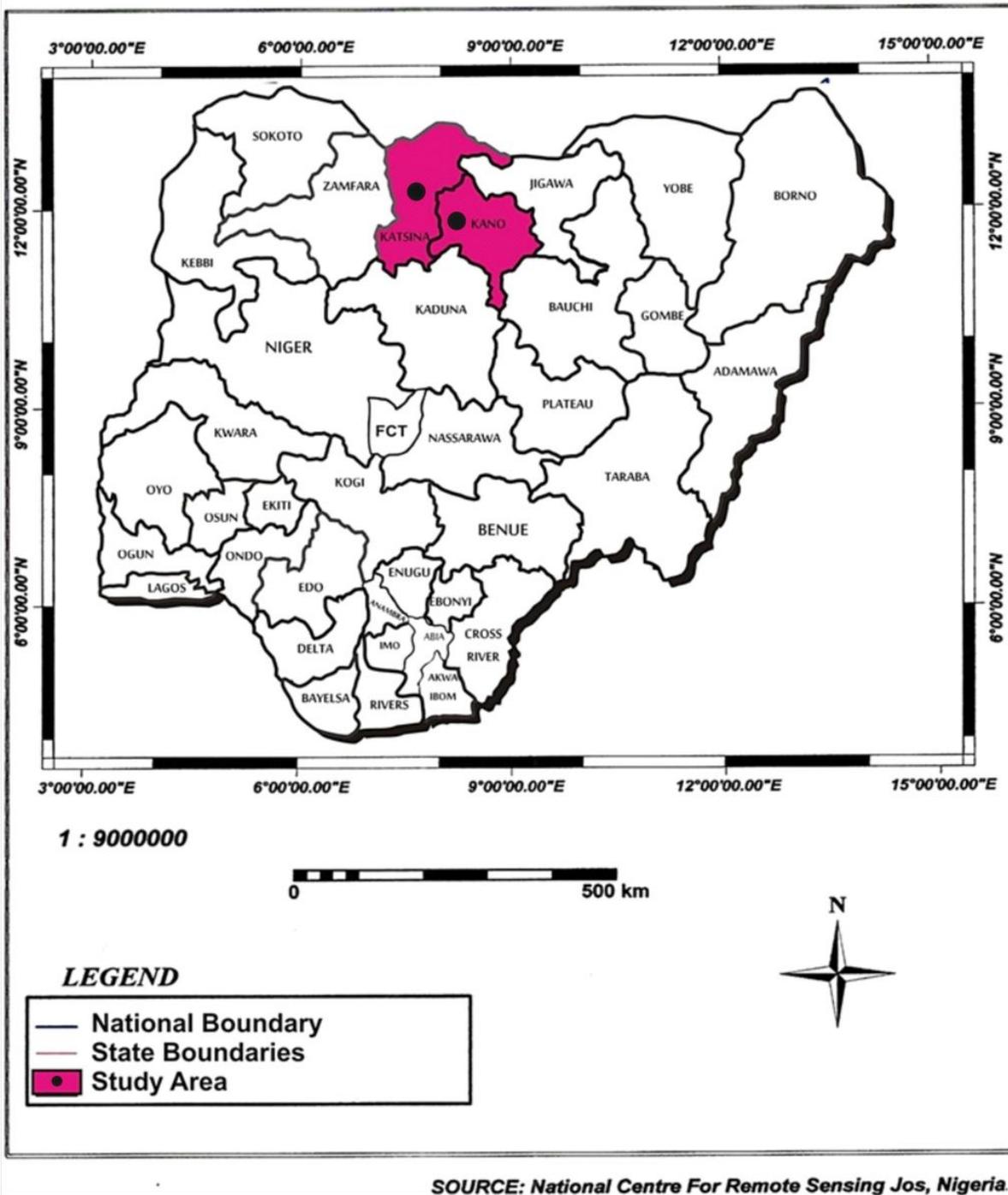


Figure 1

Map of Nigeria, West Africa showing the sampling sites

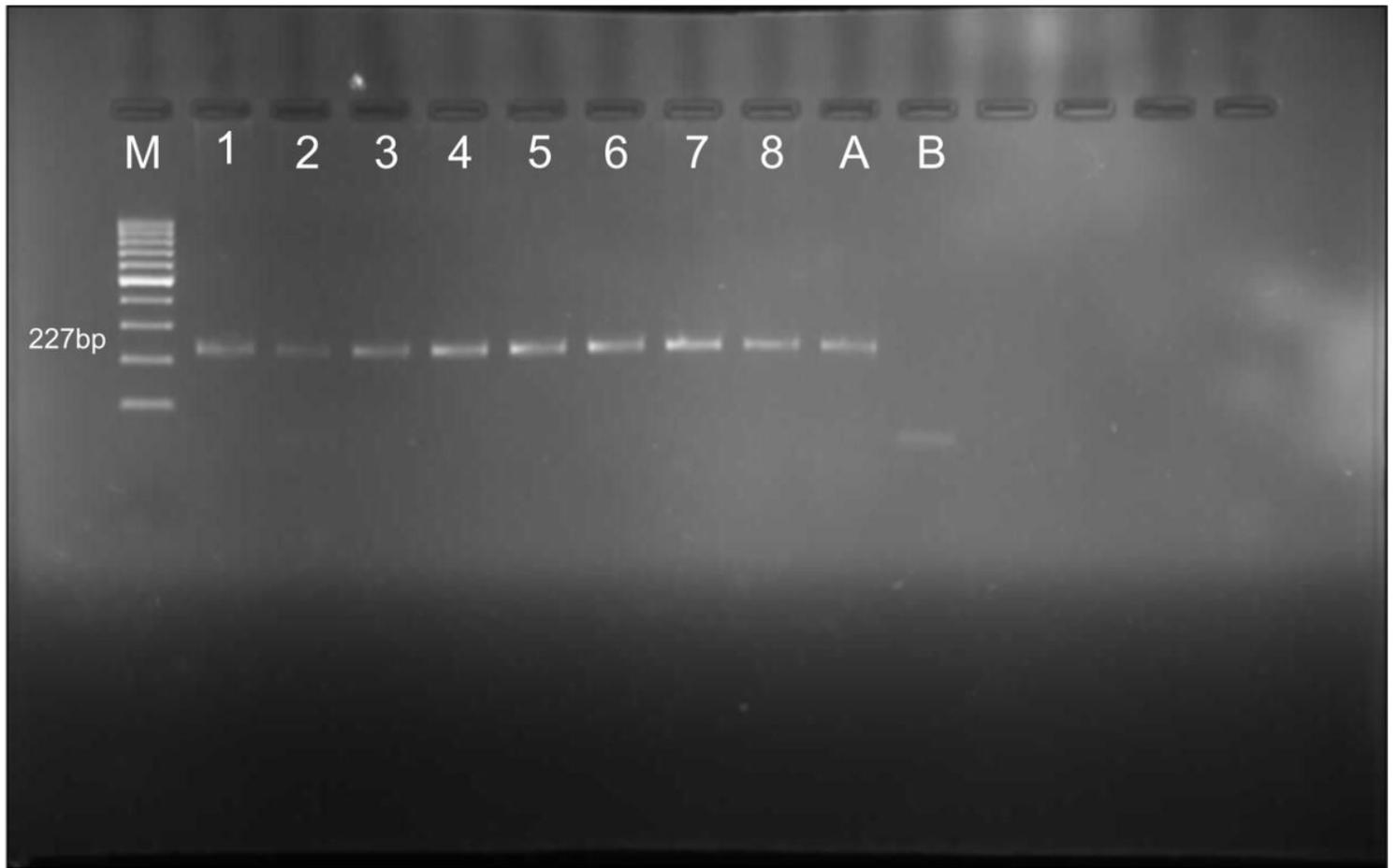


Figure 2

Agarose gel picture showing PCR amplification of 227 bp of *T.evansi* from camels in Nigeria. (Lane M: 100bp ladder; 1- 8: positive samples; Lane A: Positive control; Lane B: Negative control).

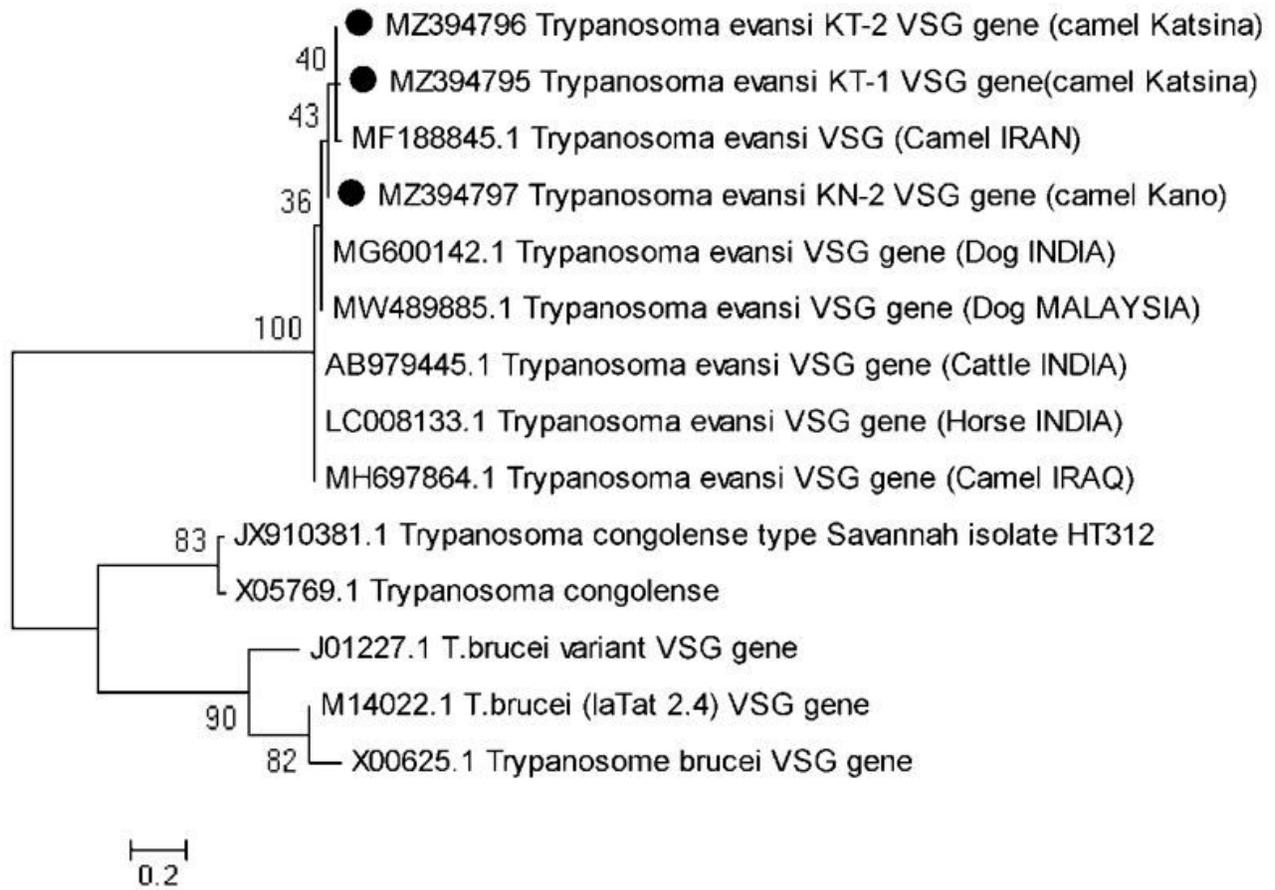


Figure 3

Phylogenetic relationships of *T. evansi* detected in this study with trypanosome sequences from different parts of the world in GenBank. Evolutionary history inferred by maximum likelihood method with *Trypanosoma brucei* as an out-group.