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Molecular characterization of rabies virus in trade dogs from Plateau state, Nigeria

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Rabies is one of the most dreaded diseases known to mankind and it is endemic in Nigeria. This study was aimed at the detection and molecular characterisation of the rabies virus antigen in trade dogs in Plateau State. A cross-sectional study was performed to determine the prevalence of rabies antigen in the brain tissues of slaughtered dogs, from February to June 2018 using a direct fluorescent antibody test and mouse inoculation technique. Direct Fluorescent Antibody Test, Polymerase Chain Reaction (PCR), sequencing and phylogenetic analysis of the identified rabies virus were also undertaken. Phylogenetic analysis was done using molecular evolutionary genetic analysis (MEGA) 10 to determine their evolutionary relationship with other rabies virus sequences deposited in Genbank. Results indicated, out of 450 dog brain samples tested 14(3.1%) were positive for rabies virus antigen. Zoographic data obtained showed 66.3% of dogs were female, and the dogs were kept mainly for security (n=54) and breeding (n=28). Fourteen positive samples were used to inoculate 70 adult mice, only 8 samples were positive and 2 samples were also positive by RT PCR. Phylogenetic analysis of the nucleotide sequences showed that the two rabies virus sequences in this study, sequence MZ148311 had very close identity with EU038106 (99.8% homology) and sequence MZ148312 had perfect identity with EU038087 (100% homology) was most closely related to lyssavirus that has been reported to be circulating previously in Plateau and Nigeria, belonging to the Africa 2 lineage.

Keywords: Characterisation, Detection, Plateau State, Rabies antigen, Trade dogs

Introduction

Rabies is one of the most widespread viral zoonotic diseases known to mankind and a global health challenge, accounting for over 61,000 human deaths annually in Africa and Asia (WHO, 2013). Annually, rabies kills about 7,000 people in Central Africa, 2,000 in North Africa, 6,000 in Southern Africa and 6,000 in West Africa (Hampson *et al.,* 2015). It has an almost 100% case fatality rate; although incurable but a 100% preventable viral zoonotic disease that causes acute encephalomyelitis in all warm-blooded mammals (Sherikar *et al.,* 2011).

The World Health Organization (WHO), the World Organization for Animal Health (WOAH), the Food and Agriculture Organization of the United Nations (FAO) and the Global Alliance for Rabies Control (GARC) have unveiled plans to end human deaths from dog-transmitted rabies by 2030 (WHO, 2017). The rabies virus is an enveloped, neutrophilic, non-segmented, single-stranded, "bullet" shaped, negative-sense RNA virus in the genus *Lyssavirus*, family *Rhabdoviridae* (Nel, 2005).

Rabies virus antigen has been detected in some apparently healthy slaughtered dogs in Plateau State (Konzing *et al.,* 2015; Kia *et al.,* 2018). It has also been established that dogs imported into Plateau State were sourced from some Northern States of Nigeria, Chad and Niger Republics and transported to the popular dog markets at Angwan Kare dog market (Jos South LGA), Dawaki and Amper dog markets (Kanke LGA) in Plateau State (Konzing *et al.,* 2015). Plateau State is also known to have some of the most flourishing dog markets in Nigeria where the purchase and slaughter of dogs take place. Some of the dogs were usually purchased from far northern states and neighbouring countries like Chad and Niger with no vaccination records (Sabo *et al*., 2008; Konzing *et al* 2015). There is the possibility of some of the dogs escaping from the dog markets into the surrounding community and transmitting rabies to both animals and man (Konzing *et al* 2015). The close association between man, dogs and the slaughtering of dogs for meat by some people in the State poses a great public health risk (Sabo *et al*., 2008).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a technique used to study the molecular pathogenesis and diagnosis of a variety of viral diseases, including rabies (OIE, 2018). PCR amplifies a specific segment of a viral genome and since primers are selected from conserved regions of the genome, most assays amplify a fragment of the nucleoprotein N gene (Tordo et al., 1996; Dzikwi et al., 2017). In generic approaches intended to detect all lyssaviruses, either hemi-nested or fully nested amplifications are used and have applications for both antemortem (saliva and cerebrospinal fluid) and postmortem samples (brain tissues) (Fooks *et al.,* 2009). Some of these diagnostic procedures are also applied for further viral characterisation, including sequencing (Kumar *et al.,* 2018). Also, strain-specific RT-PCR has been developed to distinguish various rabies virus strains in a particular region (Nadin-Davis *et al.,* 1993). The aim of this study was to detect rabies virus antigens from trade dogs for molecular characterization.

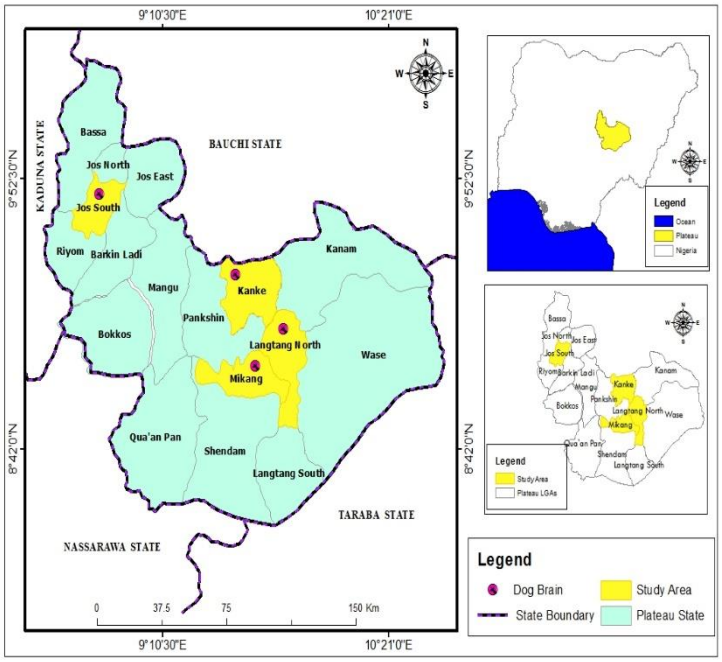

Figure 1: Map of Plateau State showing the study areas (source: GIS and Remote Sensing Software Arc Map 10.1 Department of Geography, Ahmadu Bello University, Zaria)

Materials and Methods

Study area

Plateau State (Figure 1) is surrounded by four states namely: Bauchi State to the North East, Kaduna State to the North West, Nasarawa State to the South West and Taraba State to the South East with an area of 26,889 square kilometres.

The population of the State is made up of urban and rural dwellers; the rural dwellers are predominantly peasant farmers who are also involved in

socioeconomic activities like animal rearing. The urban dwellers are mostly civil servants, industrialists and traders (Konzing, 2021). The State has over forty ethno-linguistic groups which include; "Berom", "Mwaghavul", "Ngas", "Taroh" "Goemai" and "Hausa" among others (Konzing, 2021). Four local government areas (LGAs) namely: Jos South, Kanke, Langtang North and Mikang LGAs of Plateau State were selected based on documented information from previous studies (Sabo *et al.,* 2008; Konzing *et al.,* 2015).

Approval of Ethical Clearance

Ethical clearance was obtained from number ABUCAUC/2020/57 of Ahmadu Bello University Zaria and number AEC/02/79/19 of the National Veterinary Research Institute Vom.

Study design

A cross-sectional study based on a convenience sampling technique was used to collect brain tissue samples from slaughtered dogs for rabies antigen detection by Direct Fluorescent Antibody Test (DFAT). The sample collection was based on the willingness of the dog owners to participate in the study. Dog brain samples were collected from the three senatorial zones of the State; zone 1 (Jos South LGA), zone 2 (Kanke LGA) and Zone 3 (Langtang North LGA and Mikang LGA) (Etikan *et al.,* 2016). Sample collection was from February to June 2018 in Plateau State, intending to use rabies virus antigen-positive samples for molecular analysis. A total of 450 dog brain samples were collected from slaughtered dogs meant for human consumption and information on age, sex, breed and source was obtained. The rabies-positive samples were inoculated into suckling mice and the positive samples were also analysed by Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Nucleoprotein Gene Sequencing.

Sample size determination

The sample size for dog brains was determined using the formula by Thrusfield (2007):

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where n = sample size

Z = 1.96 standard normal value for desired confidence (normal distribution table)

P = prevalence rate of rabies virus antigen (42%) (Sabo *et al.,* 2008)

d = desired absolute precision (0.05)

Hence,

$$n = \frac{1.96^2 \times 0.42 (1- 0.42}{0.05^2} = \frac{3.8416 \times 0.42 \times 0.58}{0.0025} = 374$$

n = 374 samples were taken to increase precision, the sample size was increased by 20% to 450 samples.

Sample collection for dog brain

The dog brain samples were collected from freshly slaughtered dogs using the technique described by Barrat (1996). A clean sterile straw was inserted through the occipital foramen to the direction of one of the eyes with the straw passing through the hindbrain, hippocampus, cerebrum and cerebellum. The procedure was repeated twice to ensure that enough brain tissue was extracted from the head of the freshly slaughtered dog, and then the portion of the straw containing the brain sample was cut off with a pair of sterile scissors into a labelled anticoagulant free plain sample bottle.

Laboratory analysis

Direct fluorescent antibody test (DFAT)

Direct Fluorescent Antibody Test (DFAT) was performed at the Viral Zoonoses Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. This was performed on brain tissue samples collected from slaughtered dogs based on the technique described by CDC (2006).

Mouse inoculation test (MIT)

The presumptive positive tissue obtained from DFAT was used to inoculate 70 suckling mice at the Rabies Laboratory of the National Veterinary Research Institute (NVRI) Vom. The procedure was carried out as described by Lembo *et al.* (2006).

Detection of nucleoprotein gene of Lyssaviruses using reverse transcription polymerase chain reaction

A combination of JW12, JW6 and JW10 primers was used in this study to target the conserved partial region of the N gene (Table 1) (Hall, 1999). The primers were synthesised by Macrogen (Netherlands, Europe), reconstituted with 10 mM Tris-HCl (pH 8.0) and 100 mM EDTA buffer (Promega; WI, USA), and stored frozen until utilised. The amplification of the N gene was performed using a C1000 thermal cycler (BIO-RAD, Germany) (Hall, 1999).

Table 1: Oligonucleotide primers for RT-PCR amplification of rabies virus and rabies-related viruses

Primer	Sequence (5´ to 3´)	F/R	Position in the genome
JW12	ATGTAACACC(C/T) CTACAATTG	F	55-73
JW6 (M)	CAATTCGCACACATTTTGTG	R	660-641
JW6 (M)	CAGTTAGCGCACATCTTATG	R	660-641
JW10 (DLE2)	GTCATCAAAGTGTG(A/G) TGCTC	R	636-617
JW10 (ME1)	GTCATCAATGTGTG(A/G) TGTTC	R	636-617
JW10 (P)	GTCATTAGAGTATGGTGTTC	R	636-617

D (Duvenhage virus), P (Pasteur virus), L (Lagos bat virus), M (Mokola virus), E1 (European bat lyssavirus 1), E2 (European bat lyssavirus 2), F (Forward), R (Reverse) (Tordor *et al.,* 1986).

Viral RNA extraction

The RNA was extracted using Qiagen extraction kit (Germany) according to the Manufacturer's instructions and eluted in a final 50 µL elution buffer.

Reverse transcription polymerase chain reaction

Reverse transcription was performed with 1 µg of the total viral RNA. The RNA was heat-denatured at 94^0C for 1 minute and annealed with 1.0 µL (10 pmol) of JW12 and then immediately snap-cooled on ice for 5 min. This was immediately followed by reverse transcription, performed at 45 ^{0}C for 90 min in a 20 µL reaction.

Gene AMP Gold RNA PCR reagent kit (Applied Biosystem, USA) containing 0.4 µL (50 U/µL) Reverse transcriptase, 0.04 µL (20 U/µL) ribonuclease inhibitor, 2.2 µL (10 mM) of deoxynucleoside triphosphate (dNTP) mixture, 2.5 µL (100 mM) dithiothreitol (DTT), and 5× RT-PCR reaction buffer was used and the procedure was carried out according to the manufacturer's guidelines.

Polymerase chain reaction

The initial amplification of all the cDNA was performed using the primers JW12 JW6 (M) and JW6 (DPL), targeting the full-length N gene (Heaton *et al.,* 1997). A lyophilised anti-rabies vaccine (low egg passage Flurry Strain) from the National Veterinary Research Institute (NVRI) Vom served as the positive control in the PCR and a non-template blank served as the negative control.

PCR was performed using Gene AMP Gold RNA PCR reagent kit (Applied Biosystem, USA) in a 25 µL reaction mixture containing 4 µL (100µM) of the cDNA, 1.5µL (25 mM) $MgCl_2$, 1.0 µL (10 mM dNTP) mixture, 1.0 µL (10 pmol) each of JW12, JW6 (DPL) and JW6 (M), 0.125 µL (5U/µL) Taq polymerase and 9.875 µL nuclease-free water to make up to 25 µL reaction volume was used and the procedure was carried out according to the manufacturer's guidelines.

Electrophoresis and gel image documentation

The amplicons were detected and analysed by ethidi um bromide-stained 2% agarose gel electrophoresis and visualised under UV transillumination after electrophoresis at 120 volts and 400mA for 35 min (Labnet, Power Station 300, USA), with a 100 bp DNA ladder as the molecular weight marker (Promega, USA).

PCR product purification and sequencing

The DNA sequencing reaction was performed by Macrogen Europe, Netherlands using both forward and reverse primers at 5µM concentration together with the purified DNA amplicon. The sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City) on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City)

Phylogenetic analysis

A contig sequence of each rabies virus was obtained after the alignment of the forward and reverse sequences using algorithms in BioEdit Sequence Alignment Editor Version 7.2.6.1 software (Hall, 1999). The edited sequence was used for a Mega blast search [https:// blast.ncbi.nlm.nih.gov/Blast.cgi] for highly similar nucleotide sequences in the Genbank® database at the region of overlap of the two (Hall, 1999). Contig sequences were trimmed in both 5/ and 3/axes to 620 for MZ148312 and 502 for MZ148311 bp for the partial N regions, respectively.

The sequences were aligned using the Multiple Sequence Comparison by Log Expectation (MUSCLE) option provided in the Molecular Evolutionary Genetics Software Version 10 (MEGA 10) (Kumar *et al.* 2018). Partial N nucleotide sequences of lyssaviruses from this study, and representative rabies viruses from other parts of Nigeria, neighbouring countries, Africa, and other continents (Genbank) were included in the analysis.

The phylogenetic tree was rooted using RD 126 (EU038087) and RD 121 (EU038106) lyssaviruses and other distant related lyssaviruses. The evolutionary history was inferred using the Neighbor-Joining method (Kumar *et al.* 2018). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Heaton *et al.,* 1997). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Heaton *et al.,* 1997).

The evolutionary distances were computed using the Kimura 2-parameter method, and poisson correction method are in the units of the number base substitutions per site (Heaton *et al.,* 1997). This analysis involved 52 nucleotide seque nces; codon positions included were 1st+2nd+3rd + Non-coding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 1437 positions in the final dataset. Evolutionary analyses were conducted in MEGA 10 (Kumar *et al.* 2018).

Data analysis

The prevalence of rabies antigen detection was calculated as a proportion of the total number of dog brains examined and confirmed positive. The source, age, sex and breed of dogs were recorded and tabulated. Chi-square was used to test for association between zoographic variables and the direct Fluorescent Antibody Test (DFAT) status of the dog brain tissues. Phylogenetic analysis was done using MEGA 10 to determine the evolutionary relationship between the rabies virus sequences obtained with other rabies virus sequences deposited in the National Centre for Biotechnology Information (NCBI) Genbank.

Table 2: Distribution of rabies antigen-positive dog brain tissues by zoographic variables in Plateau State, Nigeria (n = 450)

Variable	Frequency (%)	Positive samples (%)
Sex		
Males	199 (44.2)	6 (3.0)
Females	251 (55.8)	8 (3.2)
Location		
Northern zone	364 (80.9)	12 (3.3)
Central zone	59 (13.1)	2 (3.3)
Southern zone	27 (6)	0 (0.0)
Source of dog		
Unknown	216 (48)	5 (2.3)
Katsina State	63 (14)	2 (3.1)
Plateau State	21 (4.7)	0 (0.0)
Sokoto State	25 (5.6)	1 (4.0)
Kano State	88 (19.6)	4 (4.5)
Bauchi State	3 (0.7)	0 (0.0)
Borno State	6 (1.3)	0 (0.0)
Niger Republic	10 (2.2)	0 (0.0)
Kaduna State	4 (0.9)	1 (25)
Cameroon Republic	1 (0.2)	0 (0.0)
Zamfara State	10 (2.2)	1 (10)
FCT Abuja	1 (0.2)	0 (0.0)
Gombe State	1 (0.2)	0 (0.0)
Jigawa State	1 (0.2)	0 (0.0)
Age		
Adult	448(99.6)	14(3.1)
Young adult	2(0.4)	0(0.0)
Breed		
Local dog	447(99.3)	14(3.1)
Cross breed	3(0.7)	0(0.0)
DAT		
Positive	14(3.1)	14(3.1)
Negative	436(96.9)	0(0.0)

Results

A total of 450 dog brain samples were collected, out of which 199 (44.2%) were from male dogs and 251 (55.8%) from female dogs. Six (3.0%) of the male dogs and 8 (3.2%) of the female dogs tested positive for rabies. Three hundred and sixty-four (80.9%) samples were collected from the Northern Zone of Plateau State out of which 12(3.3%) were positive, 59 (13.1%) samples were collected from the Central Zone out of which 2 (3.3%) were positive and 27 (6%) samples were collected from the southern zone of the state of which none was positive (Table 2) (Plate I).

The results also showed that 216 (48%) of the dogs

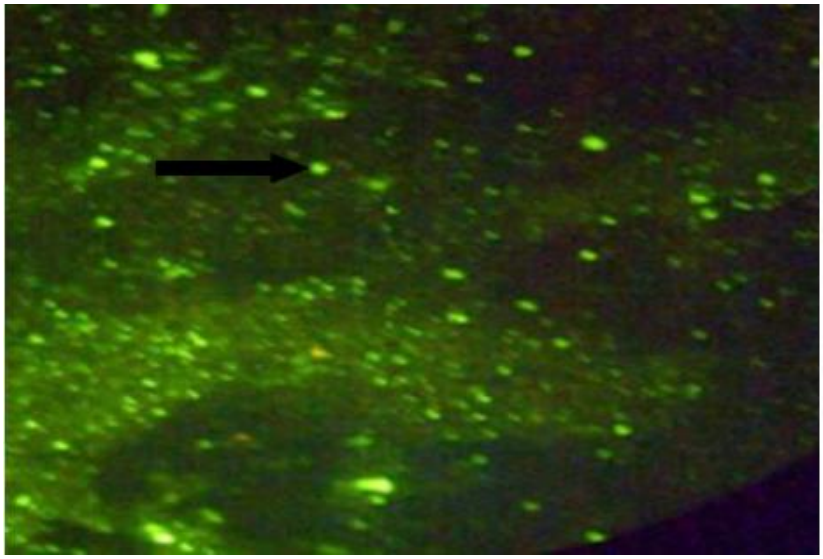

Plate I: A Direct Fluorescent Antibody Test (DFAT) rabies antigen positive slide from sample number 444 from this study

sampled from the dog markets were from unknown locations (i.e. the butchers did not know the State or Country of origin), out of which 5 were positive for rabies. Two out of the 63 (14%) dogs transported from Katsina State were positive for rabies. Distribution according to age indicated 448 (99.6%) of the dogs were adults out of which 14 (3.1%) tested positive for rabies. Only 2 (0.4%) of the dogs were young adults i.e. between 6 months to 1 year old (Table 2). Four hundred and forty-seven (99.3%) of the dogs sampled were local dogs and 3 (0.7%) of them were crossbreed (Table 2). There was no statistically significant association between zoographic variables (sex,

Table 3: Association between zoographic variables and direct fluorescent antibody test status of dogs sampled in Plateau State, Nigeria (n = 450)

Variable	Positive (%)	Negative (%)	Total	χ^2	*P* – value
Sex					
Male	6 (42.9)	193 (44.3)	199	0.011	0.917
Female	8 (57.1)	243 (55.7)	251		
Location					
Northern zone	12 (85.7)	352 (80.7)	364	0.354	1.000
Central zone	2 (14.3)	57 (13.1)	59		
Southern zone	0 (0.0)	27 (6.2)	27		
Source					
Unknown	5 (35.7)	211 (45.4)	216	16.488	0.379
Katsina State	2 (143)	61 (14)	63		
Plateau State	0	21 (4.8)	21		
Sokoto State	1 (7.1)	24 (5.5)	25		
Kano State	4 (28.6)	84 (19.3)	88		
Bauchi State	0	3 (0.70)	3		
Borno State	0	6 (1.4)	6		
Niger Republic	0	10 (2.3)	10		
Kaduna State	1 (7.1)	3 (0.7)	4		
Cameroon Republic	0	1 (0.2)	1		
Zamfara State	1 (7.1)	9 (2.1)	10		
FCT Abuja	0	1 (0.2)	1		
Gombe State	0	1(0.2)	1		
Jigawa State	0	1(0.2)	1		
Age					
Adult	14 (100)	434 (99.3)	448		1.000
Young adult	0 (0.0)	2 (0.7)	2		
Breed					
Local dog	14 (100)	433 (99.3)	447		1.000
Crossed breed	0 (0.0)	3 (0.7)	3		

age, breed, source and location of the dogs) and Direct Fluorescent Antibody Test (DFAT) status of the dog brain sample at $P < 0.05$ (Table 3).

Fourteen rabies-positive samples earlier obtained by DFAT were used for MIT, but 8 samples were positive. The remaining 6 samples were again used for MIT but the result was still negative. This was further confirmed by DFAT on the brain tissues of the dead mice which were all positive. The remaining 6 DFAT-positive samples were later re-inoculated into another set of 30 suckling mice but no mortality was recorded (Table 4).

Out of the 14 DFAT-positive samples, 2 (14.3%) showed high-quality amplicons at the expected rabies standard band size of 600bp (Plate II). The PCR products of the external primers (primers JW6 and JW12) of the isolates are presented on Plate II.

Two isolates yielded amplicons of the expected size (600bp) of the N gene region analysed. None of the remaining 12 samples was amplified in the hemi-nested PCR (Table 4). The gel documentation of the PCR products of the two positive samples is shown on Plate II.

The obtained contig sequences were deposited in Genbank and accession numbers were assigned as MZ148311 and MZ148312 which further indicated they belonged to the Africa 2 lineage. The two isolates from this study, with accession number MZ148311 had a very close identity (99.8% sequence homology) with a Plateau isolate EU038106 while MZ148312 also had perfect similarity (100% sequence homology) with another Plateau isolate with accession number EU038087. The identified isolates also clustered with Taraba isolate MKI44831 [99%], KX874612 (99%), KF022180 (99%), as well as EU718771 (98.6%), EU718735 (98.2%) and EU718736 (98.6%) from Chad Republic (Figure 2).

Table 4: Distribution of dog brain samples tested by various diagnostic methods and those found positive, in Plateau State, Nigeria

Diagnostic Method	Number of samples	Number of positive	Percentage of positive (%)	Positive sample numbers
DFAT	450	14	3.11	199, 175, 444, 120, 425, 370, 219, 173, 446, 162, 188, 001, 140 and 291
MIT	14	8	57.14	175, 162, 446, 370, 219, 188, 173 and 120
RT-PCR	14	2	14.29	370 and 219

DFAT = Direct Fluorescent Antibody Test
MIT = Mouse Inoculation Test
RT-PCR = Reverse Transcription Polymerase Chain Reaction

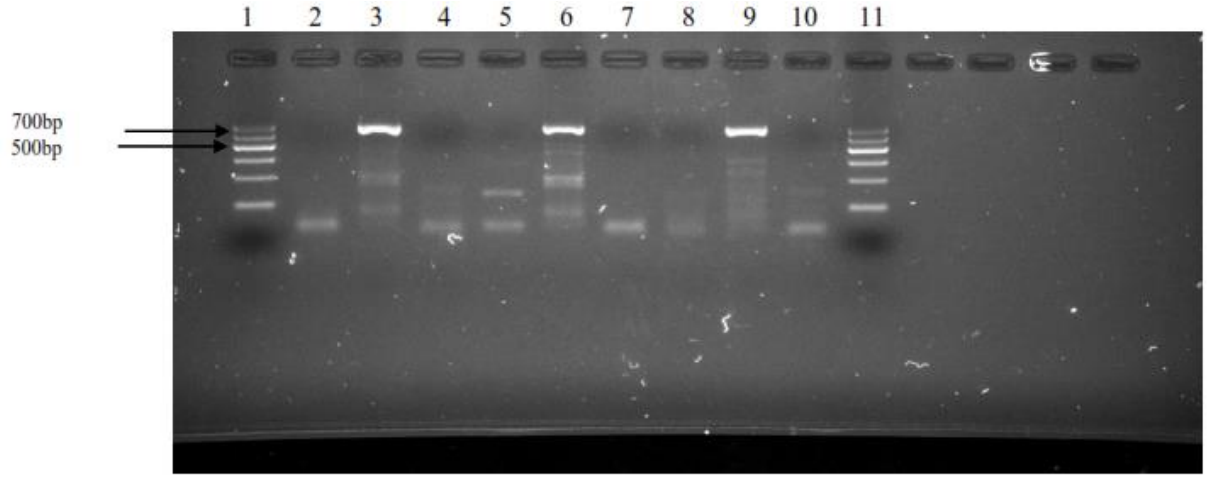

Plate II: Agarose gel showing amplicons of rabies antigen following RT-PCR and electrophoresis
Lanes 1 and 11 are molecular weight markers 100 base pairs (bp) ladder
Lanes 2 to 8 are the brain tissues of dogs
Lanes 3 [sample 370] and 6 (sample 219) are positive for the rabies virus antigen
Lane 9 is a positive control (anti-rabies vaccine from NVRI Vom) while Lane 10 is a negative control (Nuclease free water)

Discussion

Some studies have been conducted by different researchers to determine the prevalence of rabies virus antigen in the brain tissues of slaughtered dogs in Plateau State and some states in Nigeria and the results have shown that the rabies virus is present even in dogs that appeared to be apparently healthy (Mshelbwala *et al.,* 2013; Konzing *et al.,* 2015; Kia *et al.,* 2018). Studies earlier conducted on rabies antigen detection in Nigeria include those by Konzing *et al.* (2015) in Plateau State, Aliyu *et al.* (2010) in Adamawa State and Isek (2013) in Cross-Rivers State. Despite the fact that several studies have been conducted on rabies antigen detection and in some cases molecular characterisation of the virus reported cases of rabies persist in Plateau State and some parts of Nigeria, which pose a serious public health danger (Kia *et al.,* 2018; Eze *et al.,* 2020).

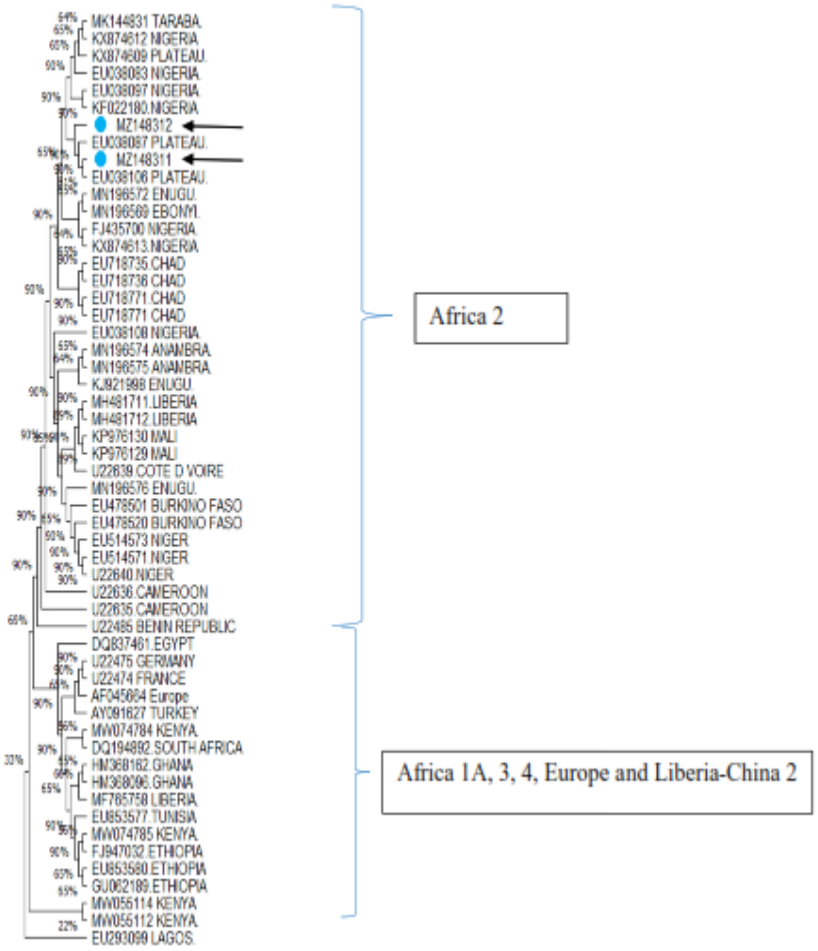

Figure 2: Phylogenetic tree constructed by neighbour-joining method for the partial N gene sequences are indicated by blue colour circles, the bootstrap consensus tree inferred from 1000 replicates

The prevalence of 3.1% obtained from this study is lower than the prevalence of 3.4% in Cross River State (2013) and the 4.9% obtained previously in Plateau State by Konzing *et al.* (2015). But the result also is at variance with the work of Sabo *et al.* (2008) in Plateau State which recorded a prevalence of 42%. The decrease in the prevalence of this study compared to previous studies on rabies may be due to increased vaccination against rabies and increased awareness campaigns on rabies prevention and control. Dogs sourced from Plateau State 21 (4.7%) to the dog market were not as high in number as compared to the ones sourced from Katsina State 63 (14%) and Kano State 88 (19.6%) which further buttresses the fact that the majority of the dogs slaughtered at the dog markets did not originate from Plateau State (Sabo *et al.,* 2008). It was also observed from this study that there was no statistically significant association between zoographic variables and brain tissue samples detection for rabies antigen ($P < 0.05$) which is in agreement with the work of Mshelbwala *et al.* (2013) in Abia State, suggesting that rabies virus antigen detection is not dependent on the zoographic variables of the slaughtered dogs. The majority 364 (80.9%) of the brain samples were collected from the northern zone at the dog market in Bukuru, Jos South Local Government Area (LGA), which is consistent with the study done by Konzing *et al.* (2015), that 146 (71.9%) brain samples collected from the northern zone were the highest among the three zones. The reason for this is that the slaughter of dogs was centralised at the dog market ("Kasuwan Kare") along Bukuru expressway in Jos South LGA, with over 30 dogs being slaughtered on a daily basis except for Tuesdays and Sundays when very few dogs were slaughtered.

The slaughter of dogs at the Central Zone was mainly on Thursdays for Dawaki mark*et al*so known as "Calabar" market and Saturdays for "Amper" market (Sabo *et al.,* 2008). In the Southern Zone, the slaughter of the dogs was done at the houses of the dog butchers with about 5 dogs slaughtered by each butcher on a daily basis. There was no centralised dog market like the Northern Zone and the slaughter was done based on the availability of the dogs. The detection of only two positive samples by RT-PCR out of 14 DFAT-positive samples was very low this could be due to the fact that the integrity of the viral RNA was probably compromised during transportation of the samples or due to the fast-degrading nature of the viral RNA occasioned by a periodic power failure during storage. Repeated freezing and thawing of the samples during DFAT procedure could be another factor for recording low RT-PCR positive samples Kia *et al.* (2018). Similar findings were also reported by Garba, (2015) that out of 13 DFAT-positive rabies samples, only 3 (23.1%) were RT-PCR positive. Kia *et al.* (2018), also reported that out of 92 DFAT-positive rabies samples only 4 (4.3%) were RT-PCR positive.

Phylogenetic analysis showed that the lyssavirus nucleotide sequences obtained in this study were of the Africa lineage 2 which is consistent with previous studies and buttresses the fact that rabies viruses within the same geographical location have a common origin (Talbi *et al*., 2009; Eze *et al*., 2020). Also based on sequence analysis, rabies viruses in Africa have been delineated into four lineages namely; Africa 1, 2, 3 and 4 (David *et al.,* 2007). The Africa 2 lineage has a wide distribution within western and central Africa, with little overlap with the Africa 1 lineage in Central African Republic (CAR) and Nigeria (Talbi *et al.,* 2009). The two RABV variants in this study were homogenous and closely related (99.8% sequence homology and 100% sequence homology), suggesting a common origin distinct to the group. The result is consistent with the findings of a previous study that reported 99% nucleotide similarity within a series of rabies lyssaviruses from Katsina, Lagos and Plateau States of Nigeria (Ogo *et al.,* 2011). This observation further supports the idea that rabies viruses from dogs in Nigeria belong to a single genetic lineage and a single major variant is maintained in this host (Ogunkoya *et al.,* 2006; Dzikwi *et al.,* 2017).

However, some of the variants from the same locality tend to group together, which could be attributed to a single and local rabies virus variant in that specific geographic zone (Paez *et al.,* 2003). Based on interaction with the dog handlers, the dogs were purchased from some far northern states of Nigeria and neighbouring countries like Chad and Niger Republics then transported to the dog markets in Plateau State with no record of anti-rabies vaccination thereby posing serious public health risk to Plateau State and Nigeria at large (Konzing *et al.,* 2015) Kia *et al.,* 2018). This further demonstrates how long-distance transmission of rabies is enhanced by human-mediated dog movements (Sabo *et al.,* 2008).

The occurrence and continuous circulation of multiple closely related rabies virus sequences in Africa are not limited to Nigeria, as a rabies virus from Mali (KP976130 and KP976129) clustered with two rabies viruses from Liberia (MH481711 and MH481712) and Cote d' Voire (U22639) (Olarinmoye *et al.,* 2019). Also, rabies virus isolates from Niger (U22640, EU514571 and EU514573) clustered with two rabies virus isolates from Burkina Faso (EU478520 and EU478501) and a rabies virus from Nigeria (MN196576) (Olarinmoye *et al.,* 2019).

The results of this study show the unrestricted movement of dogs within Africa and a possible transfer of the rabies virus from neighbouring countries into Nigeria. There was a cluster of isolates from Chad that was sandwiched by Nigerian isolates in the Africa 2 lineage. This may imply that there is an unrestricted movement of dogs within the region (Adeyemi & Zessin 2000; Kia *et al*., 2018). High sequence homology demonstrated by Chadian isolates, EU718771 (98.6%), EU718735 (98.2%) and EU718736 (98.6%) to the two isolates MZ148311 and MZ148312 in this study and the apparent monophyletic character of these two isolates is of epidemiologic significance. This confirms that the possible transfer of rabies virus among the two neighbouring West African countries may be due to indiscriminate transboundary and uncontrolled movement of dogs between Nigeria and Chad as previously suggested (Kia *et al*., 2018).

In conclusion, a prevalence of 3.1% rabies virus antigen was detected in the brain tissues of dogs slaughtered

for human consumption in this study. Rabies virus is

olates MZ148311 from this study had 99.8% sequence homology with EU038106 from Plateau State and isolateMZ148312 had 100% sequence homology with EU038087 from Plateau State and clustered with the Africa 2 lineage. There should be mass vaccination of dogs against rabies and public health awareness on the dangers of rabies, particularly in rural areas where awareness of rabies is inadequate.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Adeyemi I & Zessin K (2000). Retrospective dog rabies vaccination evaluation at the University of Ibadan, Nigeria (1988-1992). *Veterinarski Arhiv,* **70**(5): 223-230.

Aliyu TB, De N, Yenda EN & Lynn M (2010). Prevalence of rabies virus antigens in apparently healthy dogs in Yola, Nigeria. *Researcher,* **2**(2): 1553-9865

Barrat J (1996). Simple Technique for the Collection and Shipment of Brain Specimens for Rabies Diagnosis. In: *Laboratory Techniques in Rab*ies (FX Meslin, MM Kaplan, H Koprowski), fourth edition. World Health Organization, Geneva, Switzerland. Pp 1-16.

CDC (2006). Standard Operating Procedure for the Rapid Fluorescent Focus Inhibition Test (RIFFT) for determining rabies virus Neutralizing Antibody. Pp 1-92.

David D, Hughes GJ, Yakobson BA, Davidson I, Hikmat U, Aylan O, Kuzmin IV & Ruprecht CE (2007). Identification of novel canine rabies virus clades in the Middle East and North Africa. *Journal of General Virology,* **88**(3): 967-980.

Dzikwi AA, Umoh JU & Kwaga JKP (2017). Phylogenetic analysis of rabies virus isolates from dogs in Plateau State, Nigeria. *Journal of Biology Research,* **3**(2): 10-14.

Etikan I, Musa AS & Alkassim SY (2016). Comparison of convenience sampling and purposive Sampling. *American Journal of Theoretical and Applied Statistics,* **5**(1): 1-4.

Eze UU, Ngoepe EC, Anene MB, Ezeokonkwo CR, Nwosuh IC & Sabeta TC (2020). Molecular detection of rabies Lyssaviruses from dogs in South Eastern Nigeria: Evidence of transboundary transmission of rabies in West Africa. *Viruses*, **12** (134): 1-13.

Fooks AR, Johnson N, Freulling CM, Wakeley PR, Banyard AC, McElhinney LM, Marston, DA, Dastjerdi A, Wright E, Weiss RA & Muller T (2009). Emerging technologies for detection of rabies virus: challenges and hopes in the 21st century. *PloS Neglected Tropical Diseases,* **3**(9): 1371.

Garba A (2015). Epidemiology of Rabies in Niger State, Nigeria. Ahmadu Bello University, Zaria, Nigeria. Ph.D Dissertation, Department of Veterinary Public Health and Preventive Medicine, Pp 74 -80.

Hall TA (1999). Bioedit, a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* **41**(41): 95–98.

Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ & Cleaveland S (2015). Estimating the global burden of endemic canine rabies. Public Library of Science (PLoS) *Neglected Tropical Diseases,* **9**(4): e0003709.

Heaton PR, Johnstone P, McElhinney LM, Cowley R, O'Sullivan E & Whitby JE (1997). Heminested PCR assay for detection of six genotypes of rabies and rabies related-viruses. *Journal of Clinical Microbiology,* **35**(11): 2762– 2766.

Isek IT (2013). Epidemiological studies of canine rabies in Ogoja, Cross River State. M.Sc Thesis*,* Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Pp 31-58

Kia GSN, Hung Y, Zhou M, Zhou Z, Gnanadurai CW, Leysona CM, Umoh JU, Kazeem HM, Ehizibolo DO, Kwaga JKP, Nwosuh CI & Fu ZF (2018). Molecular characterisation of a rabies virus isolated from trade dogs in Plateau State, Nigeria. *Sokoto Journal of Veterinary Sciences,* **16**(1): 54-62.

Konzing L, Umoh JU & Dzikwi AA (2015). Trade dog – dog meat processors interface in rabies transmission. *International Journal of Applied Research,* **1**(11): 83-91.

Konzing L (2021). Epidemiological Studies of Canine Rabies and Molecular Characterisation of Rabies Virus from Plateau State, Nigeria. Ph.D Thesis, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria. Pp 66-87.

Kumar S, Stecher G, Li M, Knyaz C & Tamura K (2018). MEGA 10: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* **35**(6): 1547-1549.

Lembo T, Niezgoda M, Velasco-Villa A, Cleavland S, Ernest E & Rupprecht CE (2006). Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerging Infectious Diseases,* **12**(2): 310-368.

Mshelbwala PP, Ogunkoya AB & Maikai BV (2013). Detection of rabies antigen in the saliva and brains of apparently healthy dogs slaughtered for human consumption and its public health implications in Abia State, Nigeria. *ISRN Veterinary Science,* doi.10.1155/2013/468043.

Nadin-Davis SA, Casey GA & Wandeler A (1993). Identification of regional variants of the rabies virus within the Canadian province of Ontario. *Journal of General Virology,* **74**(5): 829–837.

Nel LH (2005). Vaccines for lyssaviruses other than rabies. *Expert Review of Vaccines* **4**(4): 533-540.

OIE (2018). Rabies Infection with Rabies Virus and Other Lyssaviruses). *Terrestrial Manual,* https//www.oie.int/fileadmin/home_standards/tahm2.0.1.17_RABIES.pdf, retrieved 26-06-2021.

Ogo MF, Nel L & Sabeta CT (2011). Phylogenetic evidence of the public and veterinary health threat of dog rabies in Nigeria. *Nigerian Veterinary Journal*, **32**(1): 40–44.

Ogunkoya AB, Beran GW & Wiktor TJ (2006). Antigenic analysis of some Nigerian street rabies virus using monoclonal antibodies. *Nigerian Veterinary Journal,* **27**(2): 41–53.

Olarinmoye AO, Kamara V, Jomah ND, Olugasa BO, Ishola OO, Kamara A & Luka PD (2019). Molecular detection of rabies virus strain with N-gene that clustered with China lineage 2 co-circulating with Africa lineages in Monrovia, Liberia: First reported case in Africa. *Epidemiogy and Infection*, **147**(2): 85-90.

Paez A, Nunez C, Grarcia C & Bushel J (2003). Molecular epizootics in Columbia: Evidence for human and dog rabies associated with bats. *Journal of General Virology,* **84**(4): 795–802.

Sabo G, Umoh JU, Sackey AKB, Ahmad A & Okolocha EC (2008). The role of dog trade in the epidemiology of rabies. *A Review of Veterinary Clinical Practice Bulletin,* **1**(1): 63-72.

Sherikar AT, Bachil VN & Thapliyal DC, (2011). *Textbook of Elements of Veterinary Public Health.* Indian Council of Agricultural Research. Pp 301-305.

Talbi C, Holmes EC, Benedictis P, Faye O, Gamatie E, ND, Diarra A, Elmamy BO, Sow A, Adjogoua EV and Sangare O. (2009). Evolutionary history and dynamics of dog rabies virus in western and central Africa. *Journal of General Virology,* **90**(4): 783–791.

Thrusfield M, (2007). *Veterinary Epidemiology*. Third edition. Pp 434-440.

Tordor N, Poch O, Ermine A & Keith G (1986). Primary structure of leader RNA and nucleoproteins genes of the rabies genome: segmented homology with VSV. *Nucleic Acids Research,* **14**(6): 2671-2683.

Tordo N, Sacramento D & Bourhy H (1996). The polymerase chain reaction technique for diagnosis, typing and epidemiological studies. In: Meslin FX, Kaplan MM, Koprowski, H, *Laboratory techniques in rabies*. Geneva, World Health Organization. Pp 157– 170.

WHO (2013). WHO expert consultation on rabies, sec ond report. Geneva, Switzerland. **931** 1-88. http://apps.who.int/iris/bitstream/10665/85346/1/9789241209823_eng.pdf, retrieved 26-06-2021.

WHO (2017). Towards a Rabies-free World as Unparalleled Global Initiative gets underway Global Partners Announce Plan to End Human Deaths from Dog-transmitted Rabies by 2030 Geneva. https://www.who.int/neglected_diseases/news/WRD_2017_press_release/en/, retrieved 29-12-2019.