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DETECTION AND SEQUENCE ANALYSIS OF *TOXOPLASMA GONDII* B1 GENE IN TISSUES OF SOME BIRD SPECIES IN PLATEAU STATE, NIGERIA

Ishaku B. Shalangwa[1,2], Beatty-Viv Maikai[2], Jacob K. P. Kwaga[2], Oluyinka O. Okubanjo[3], Pam D. Luka[4], Joshua Kamani[5], Kenneth O. Ikejiofor[6], Helen Gyang[4], Adedapo O. Adedeji[7]

[1]Department of Veterinary Laboratory Technology, Federal College of Animal Health and Production Technology, Vom, Plateau State
[2]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria
[3]Department of Veterinary Parasitology, Ahmadu Bello University, Zaria
[4]Biotechnology Division, National Veterinary Research Institute Vom, Plateau State
[5]Parasitology Department, National Veterinary Research Institute Vom, Plateau State
[6]Department of Animal Health, Federal College of Animal Health and Production Technology, Vom
[7]Department of Crop Production and Environmental Biology, Faculty of Agriculture, University of Ibadan, Oyo State Nigeria

drshalangs2014@gmail.com

ABSTRACT

***Toxoplasma gondii* is a single-cell parasite capable of infecting almost all homeotherms posing a grave public health risk globally. There is limited available literature on the *T. gondii* strains circulating in bird species in the Plateau State, of Nigeria. Consequently, this study was carried out to identify and confirm *T. gondii* infection and also determine the relationship of the DNA sequences with those of bird species in other parts of the world. To achieve this, brain and heart tissues of 25 bird species were sampled and a nested polymerase chain reaction (nPCR) and sequence analyses of the B1 gene were carried out. The DNA of *T. gondii* was identified in the heart and brain tissues of 7/7 (100.0 %) of wild bird species, and 15/18 (83.3 %) of domestic local chickens (*Gallus gallus domesticus*) sampled. The evolutionary relationship among the *T. gondii* sequences in this study using phylogenetic tree constructed by maximum likelihood method showed the sequences shared a common ancestor with the Type I RH strain (GenBank: AF179871). The *T. gondii* sequences were in a cluster distinct from other sequences in the GenBank. Calculations of genetic differentiation and genetic diversity indices undertaken and collated revealed three haplotypes with higher haplotype diversity within the *T. gondii* sequences obtained from wild birds (0.667) compared with the sequences from local chickens (0.333). A 97–100 % homology among the aligned sequences of *T. gondii* in the study shows that only one strain type exists in all of the samples. This study has established the occurrence of *T. gondii* infection in asymptomatic bird species in the study area and portrays them as carriers, and potential sources of human infection.**

Key words: bird species; carriers; diversity; haplotype; nPCR; parasite; sequencing

INTRODUCTION

Toxoplasma gondii is a single-cell intracellular parasite that belongs to the phylum Alveolata and subphylum Api-

complexa. The parasite is of public health and veterinary importance causing disease in diverse animal species [9]. Based on genotypic investigations, the organism is divided into three primary clonal lineages named as type I, type II, and type III [29]. The use of single and multi-locus markers has however revealed greater diversity and dispersion of the organism and also indicated wider genetic variability than previously reported [2, 24, 34, 42, 43]. These include the atypical or recombinant strains, which do not belong to any archetypal genotypes like I, II or III. On the basis of human exposure and infection, the most predominant clonal lineage is the type II. The atypical isolates of type I or I-like are more likely to produce serious toxoplasmic retinochoroiditis in humans, as these affect immune-compromised patients with serious acute and dispersed toxoplasmosis [12, 25].

In humans, the postnatal infection of *T. gondii* occurs by ingesting either raw or undercooked meat, or by ingesting oocysts from contaminated soil, food and water, while congenital infections occur via transplacental transmission of tachyzoites [3, 27, 46]. In addition, human exposure to *T. gondii* infection is possible because they hunt, and consume raw, or undercooked meat of animals from different species of wildlife [32]. Following exposure, the infection in humans varies greatly, from asymptomatic, to severe acute toxoplasmosis marked by abortion, and/or neonatal deaths [12, 37]. Susceptibility to infection is higher in neonates, and immunocompromised persons, resulting in severe neurological disorders such as, hydrocephalus, ocular, or congenital abnormalities [4, 20].

The susceptibility to *T. gondii* infection varies among different avian species, with over 38 species spanning eight orders, being natural hosts of the parasite [16]. Upon exposure, subclinical *T. gondii* infections occur in birds, and as revealed by W i l s o n et al. [50], wild birds may serve as important intermediate hosts, because of their high dispersal capabilities. This is further accentuated by the presence of *T. gondii* seropositive animals in felid-free areas, such as the Arctic and oceanic islands being attributed to migratory birds infected else-where that arrives and enters the local food chain [15]. The clonal types and atypical strains of *T. gondii* have also been reported in wild birds [4, 49]. Because of their feeding style in close proximity to contaminated soil, ground feeding birds, both domestic, and wild have been considered as the best indicators of environmental oocyst contamination [38]. Although clinically resistant, wild birds, chickens raised in backyard, and commercial free-range systems, may harbour viable *T. gondii* [18, 19]. Thus, ingestion of infected chicken meat may be a source of infection for humans and other animals [17].

Antibody based evidence of exposure to *T. gondii* infection has been reported in several animal species in some parts of Nigeria, with only a few studies available, on direct detection of the organism in tissues of domestic local chickens and wild birds. Molecular detection of *T. gondii,* and the analysis of the genetic structure of the parasite circulating in tissues of bird species in Plateau State, Nigeria has also not been established [20, 40, 48]. Since *T. gondii* infection in ground feeding birds reflect environmental contamination, the focus of this current study was to confirm exposure to *T. gondii* infection in domestic local chickens and wild birds in Plateau State, Nigeria using molecular techniques and ascertain the genetic structure and diversity of the *T. gondii* circulating in their tissues. In addition, the study was also undertaken to compare the sequences of the *T. gondii* B1 gene in the study area, with those from other parts of the world.

MATERIALS AND METHODS

Location of study

This study was conducted in Plateau State, Nigeria. It is the twelfth largest state situated roughly in the mid of Nigeria with elevated hills at its boundaries surrounding the capital city of Jos. There are three major political divisions: northern, central, and southern Plateau. The locations specify latitudes as 9.2422°N and 10.1153°N, and longitudes as, 8.6957°E, and 9.5210°E which are, 1, 280 m above sea level. Also, the state has about 26,899 square kilometres of land mass [31]. The average temperature ranges are between 18 °C and 22 °C, which gives the state a temperate climate at the higher altitudes, even though it is located in the tropical zone [33]. The location is warmest between the months of March and April, while it sees a lowering in temperatures between December and February. The Shere hills comprise of some of the Plateau's undulating hills, and rock formation, ranging to the east side of Jos city. The hills have a vegetation of guinea savanna, and is located at 9°57N, 9°3E,|9.95°N, and 9.05°E [33].

Ethical consideration

Ethical permit and approval was obtained from the National Veterinary Research Institute (AEC/02/48b/18**)** Vom, and from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2022/013), before the commencement of the study. All relevant international, national and/or institutional guidelines for the care, and use of animals were observed during this study.

Target population

The study population comprised of avian species in Plateau State, Nigeria, while the target population were domestic local chickens (*Gallus gallus domesticus*), and wild birds.

Collection of brain and heart tissues from free-range chickens and wild birds

A non-probability purposive sampling technique was employed. Eighteen (18) domestic local chickens comprising of six each from the three political zones were obtained from live bird markets. Information on the age, sex, and source of domestic local chickens were recorded.

Seven (7) resident, non-palearctic, non-endangered wild birds classified as of least concern by the International Union for Conservation of Nature (IUCN; www.iucnredlist.org/) were sampled from Shere hills. Further identification of wild birds by an expert ornithologist regarding the age, sex, taxonomic order was recorded according to the guide provided by Borrow and Demey [11] and Svenson [44]. The brain and heart tissues were harvested as described by AVMA [8] based on previous studies that reported the utilization of these samples for the isolation and characterization of *T. gondii* [20]. The tissues were kept refrigerated at -20 ^{o}C until used.

DNA extraction and quantification

The DNA was extracted from the brain and heart tissues of domestic local chickens and wild birds using DNeasy Blood and Tissue kit (Qiagen, Hilden Germany) at the Biotechnology Division of the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Approximately 1 gram of pooled brain and heart tissues of each wild bird and local chicken were homogenized with a mortar and pestle and placed in a 1.5 ml microcentrifuge tube. The DNA extraction was performed using the instructions provided by the manufacturer's protocol. The quantification of DNA was performed with a Thermo Scientific™ Nano Drop 2000C spectrophotometer and the optical density was determined 260 nm. Purity of extracted DNA was estimated using the ratio of absorbance (OD_{260}) at 260 nm to absorbance (OD_{280}) at 280 nm (A260:A280).

Molecular detection of *T. gondii* in brain and heart tissues of wild birds

The presence of *T. gondii* specific DNA in tissues of wild birds and local chickens were determined using a nested polymerase chain reaction (nPCR) that amplifies the B1 region as demonstrated previously by Hohlfeld et al. [28].

First PCR amplification for B1 gene

Table 1 lists primer sequences targeting a 197 base pairs region of B1 gene for first PCR amplification designed to detect the DNA of *T. gondii.* A total volume of 50 µl was prepared for the PCR amplification reaction, which contains a template DNA of 10 µl, 10X PCR buffer (without Mg_2^+) of 5 µl, dNTP mix (2.5 mM concentration each) of 4.0 µl, 25 mM $MgCl_2$ of 4 µl, 0.3 µl external Forward Primer of concentration 50 µM, 0.3 µl external Reverse Primer of concentration 50 µM, FastStart Taq enzyme (5 $U.\mu l^{-1}$) of 0.2 µl and 26.2 µl of nuclease free H_2O to make up the final volume to 50 µl. Reactions were carried out using the following PCR steps: Initial denaturation step at 94 °C for 5 min, then 35 cycles of: Denaturation for 30 sec at 95 °C; annealing for 30 sec at 46 °C , extension for 30 sec at 72 °C and the final extension step of 10 min at 72 °C.

Table 1. Primer sequence, annealing temperatures and amplicon size

Primer sequence	Annealing Temp (°C)	Target region	Amplicon size	Reference
Tox 1F -5'GGAACTGCATCCGTTCATGAG3' Tox 1R -5'TCTTTAAAGCGTTCGTGGTC3'	46	B1 gene	197 bp	Burg et al. [13]
Tox 2F -5'TGCATAGGTTGCAGTCACTG3' Tox 2R -5' GGCGACCAATGTGCGAATAGACC3'	53.5	B1 gene (nested)	97 bp	Burg et al. [13]

Second Nested PCR

The nested PCR amplifies a 97 bp region within the B1 gene. The PCR product obtained from the first PCR was used as the template and primers used are as stated in Table 1. The products of first round of PCR was then subjected to a second PCR run containing 5 µl first round PCR product, 5.0 µl of 10 x PCR buffer (Mg), 4.0 µl dNTPs (2.5 mM each), 4.0 µl 25 mM $MgCl_2$, 0.30 µl nested forward Primer (50 µM each), 0.30 µl nested reverse Primer (50 µM each), 0.20 µl FastStart Taq (5 U.µl), and 31.2 µl H_2O to obtain a final volume of 50.0 µl. Cycling conditions were set at: denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 95 °C, 30 sec; annealing at 53.5 °C for 30 sec and extension at 72 °C for 30 sec. During each amplification, DNA of *T. gondii* from tachyzoites of the RH strain kindly provided by Prof Shen Jilong of the Institute of Zoonoses, Anhui Medical University, China, together with double distilled water were used as positive and negative controls, respectively.

Gel electrophoresis

Amplicons were resolved on 2.5 % agarose in TBE buffer stained with ethidium bromide (0.3 $\mu g.ml^{-1}$) according to the method suggested by Green and Sambrook [23]. Electrophoresis was carried out at 90 V for 60 min alongside a 100 kb size ladder (New England Biolabs, USA) to view the 197 bp of B1 gene and a 50 kb ladder as a molecular weight marker for the 97 bp nested PCR products. The DNA bands were viewed under a UV trans-illuminator.

Sequencing and sequence analyses

Ten (10) positive amplicons of the nPCR out of twenty-five (25) were randomly selected and nine (9) were successfully sequenced using PCR primers in both forward and reverse directions at a commercially based sequencing company (Inqaba Biotec, South Africa). Editing of sequences were manually done using Chromas 2.23 (Technelysium, edited with the aid of Helensvale, Australia) and compared for similarities to sequences using Basic Local Alignment Search Tool (BLAST) program in the GenBank hosted by the National Centre for Biotechnology Information (NCBI). Sequences obtained in this study were submitted to the GenBank database and the accession numbers (MW795324− MW79533) are publicly available.

Partial sequences of the B1 gene from *T. gondii,* from various countries were retrieved from GenBank and used for the multiple sequence alignment with the nine sequences obtained in this study using ClustalW in BioEdit [26]. Inter- and intra-sequence pairwise identities were performed with pairwise gap deletions using SDT v1.2 software [39]. A phylogenetic tree was constructed based on the Jukes-Cantor model to show the evolutionary relationships among the *T. gondii* sequences using the maximum likelihood method in MEGA v.6.06 software [45]. The values of Bootstrap replicates were set to 1,000 while a strain of *Neospora caninum* (AY94117) was used as an out-group.

Using the homologous 130 bp section of the B1 gene that is common to both sets of hosts (local chickens and wild birds), comparative analyses of some parameters were estimated and obtained for population structure as follows: Haplotype diversity (Hd), average nucleotide diversity (π), the statistics of population estimation for mutation based on the number of segregating sites (θ-W), number of polymorphic or segregating sites (S), the average number of differences in nucleotides between sequences (k) and the statistics of population estimation for mutation based on the total number of mutations (θ-Eta). The above parameters were obtained using DnaSP v5.10.01 software [35]. It was also used to calculate the Tajima's D, Fu and Li's F* and D*, and Fu's F to confirm deviation of the *T. gondii* populations from neutrality assuming a constant population size, with zero recombination and migration [41]. A negative value of Tajima's D statistics indicates excessive low-frequency polymorphism triggered by population expansions, background selection, or genetic hitchhiking. The opposite positive values of Tajima's D statistic suggest basic levels of low and high-frequency polymorphisms, which indicates reducing population size or balancing selection.

RESULTS

In this study, tissues (brain and heart) from wild birds (n = 7) and local chickens (n = 18) were screened for DNA of *T. gondii* using the B1 gene as target with the second product of amplification showing the expected size of amplicon to be 97 base pairs. The DNA of *T. gondii* was found in all the seven (100.0 %) wild birds which compris-

es the Northern-red Bishop (*Euplectes franciscanus*), Yellow-fronted canary (*Crithagra mozambica*), Red-cheeked cordon bleu (*Uraeginthus bengalus*), Common bulbul (*Pycnonotus barbatus*), Speckled mouse bird (*Colius striatus*), Scarlet-chested sunbird (*Chalcomitra senegalensis*), and Green-headed sunbird (*Cyanomitra verticalis*). *T. gondii* DNA was detected in 15 out of 18 (83.3 %) domestic local chickens (*Gallus gallus domesticus*) (Table 2 and 3).

The study showed that the *T. gondii* B1 gene sequences populations were homogenous. The similarities ranged from 97.18 % to 100 % (Table 4). Within the *T. gondii* B1 genes obtained from wild birds (GenBank Accession numbers MW795325, MW795327, and MW795329), similarities ranged from 98.31 to 100 % (Table 4). The sequences shared a common ancestor with the Type I RH strain (AF179871) from USA, as shown in Figure 1 and 2. Using colour-coded distance matrices, all the sequences from this study appear in dark brown squares which, as indicated on the key, indicate over 95 % similarities (Figure 1). When compared with the others from the database, it gave the blue squares (Figure 1). This confirms that the sequences from this study are somewhat homogenous and distinct.

The entire *T. gondii* B1 gene fragment in this study clustered together within the same clade, thus suggesting that they possess a common origin, irrespective of their host or location (Figure 2). From the total *T. gondii* sequences obtained (n = 9), only three haplotypes were observed (Table 5). However, haplotype numbers for *T. gondii* sequences obtained from both domestic local chicken (n = 6) and wild birds (n = 3) were both 2, indicating higher genetic variation within the *T. gondii* sequences from the wild birds. Similarly, Table 5 shows that there was higher haplotype diversity within the *T. gondii* sequences obtained from wild birds (0.667) as compared to sequences from domestic local chickens (0.333). Additionally, distances between the genes were calculated, and the highest π values, were obtained from *T. gondii* sequences obtained from wild birds (0.00939) than those obtained from domestic local chicken (0.00635). Negative values were obtained from Fu, and Li's D, F statistical tests for the total population isolated from local chicken, which did not deviate from zero statistically ($P > 0.10$), as shown in Table 5.

Table 2. Characteristics of adult wild birds sampled and their respective nPCR results

Sample code	Taxonomic order	Common names (Biological names)	Sex	nPCR
WB 8	Passeriformes	Northern-red Bishop (Eupiectes franciscanus)	U	+
WB 10	Passeriformes	Yellow-fronted canary (Crithagra mozambica)	U	+
WB 42	Passeriformes	Red-cheeked cordon bleu (Uraeginthus bengalus)	M	+
WB 75	Passeriformes	Common bulbul (Pycnonotus barbatus)	U	+
WB 72	Coliiformes	Speckled mousebird (Colius striatus)	U	+
WB 74	Passeriformes	Scarlet-chested sunbird (Chalcomitra senegalensis)	M	+
WB 26	Passeriformes	Green-headed sunbird (Cyanomitra verticalis)	M	+

M = male; F = female; U = undefined

Table 3. Characteristics of domestic local chickens (*Gallus gallus domesticus*) sampled and their respective nPCR results

Sample code	Location	Sex	Age	nPCR
JN 5	Jos North	Female	1 yr	+
JN 14	Jos North	Male	2 yr	-
JSG 10	Jos South	Male	2 yr	+
JSG 16	Jos South	Female	11/2yr	+
JEF 5	Jos East	Female	21/2yr	+
JEMJ 15	Jos East	Female	11/2yr	+
SH 18	Shendam	Female	1 yr	+
SDK 7	Shendam	Female	1yr	+
MM 18	Mangu	Female	11/2yr	-
MM 19	Mangu	Female	1 yr	-
KK 19	Kanke	Male	1 yr	+
KK 8	Kanke	Female	1 yr	+
BKD 22	Bokkos	Male	3 yr	+
BKD 16	Bokkos	Female	11/2yr	+
BKD 22	Bokkos	Male	1 yr	+
QK 22	Quanpan	Female	11/2yr	+
LNG 16	Lantang North	Male	1 yr	+
LNG 22	Langtang North	Female	11/2yr	+

Table 4. Pair-wise sequence identities percentage among the nine partial glycerol-3-phosphate dehydrogenases (B1) gene from *Toxoplasma gondii* from local chickens and wild birds in Plateau State, Nigeria.

Accession numbers	MW795325	MW795326	MW795324	MW795327	MW795328	MW795329	MW795330	MW795331	MW795332
MW795324			-						
MW795325	-		100.00						
MW795326	100.00	-	100.00						
MW795327	98.59	99.05	98.59	-					
MW795328	100.00	100.00	100.00	99.07	-				
MW795329	100.00	100.00	100.00	98.31	100.00	-			
MW795330	100.00	100.00	100.00	99.07	100.00	100.00	-		
MW795331	97.18	98.11	97.18	98.15	98.15	98.15	98.15	-	
MW795332	100.00	100.00	100.00	99.23	100.00	100.00	100.00	98.15	-

Table 5. Genetic variability determinants and neutrality tests on the B1 gene from *Toxoplasma gondii* populations obtained from local chickens and wild birds in Plateau state, Nigeria

Population	N	H	S	Hd	Eta	Π	K	θ-W	θ-Eta	Tajima's D	Fu and Li's D	Fu and Li's F
All isolates (n = 9)	200	3	2	0.417	3	0.00901	0.6389	0.01555	1.1038	-1.6091	-1.6827	-1.8457
Local chickens (n = 6)	130	2	2	0.333	2	0.00635	0.6667	0.00834	0.8759	-1.132	-1.1553	-1.1951
Wild birds (n = 3)	200	2	1	0.667	1	0.00939	0.6667	0.00939	0.6667	NS	NS	NS

N – Number of nucleotide sites; h – Haplotype number; S – Total number of variable or segregation sites; Hd – Haplotype diversity; Eta – Total number of mutations; π – Nucleotide diversity; K – Average number of nucleotide differences between sequences; θ-W – Waterson's estimate of population mutation rate based on the total number of segregating sites; θ-Eta – Waterson's estimate of population mutation rate based on the total number of mutations

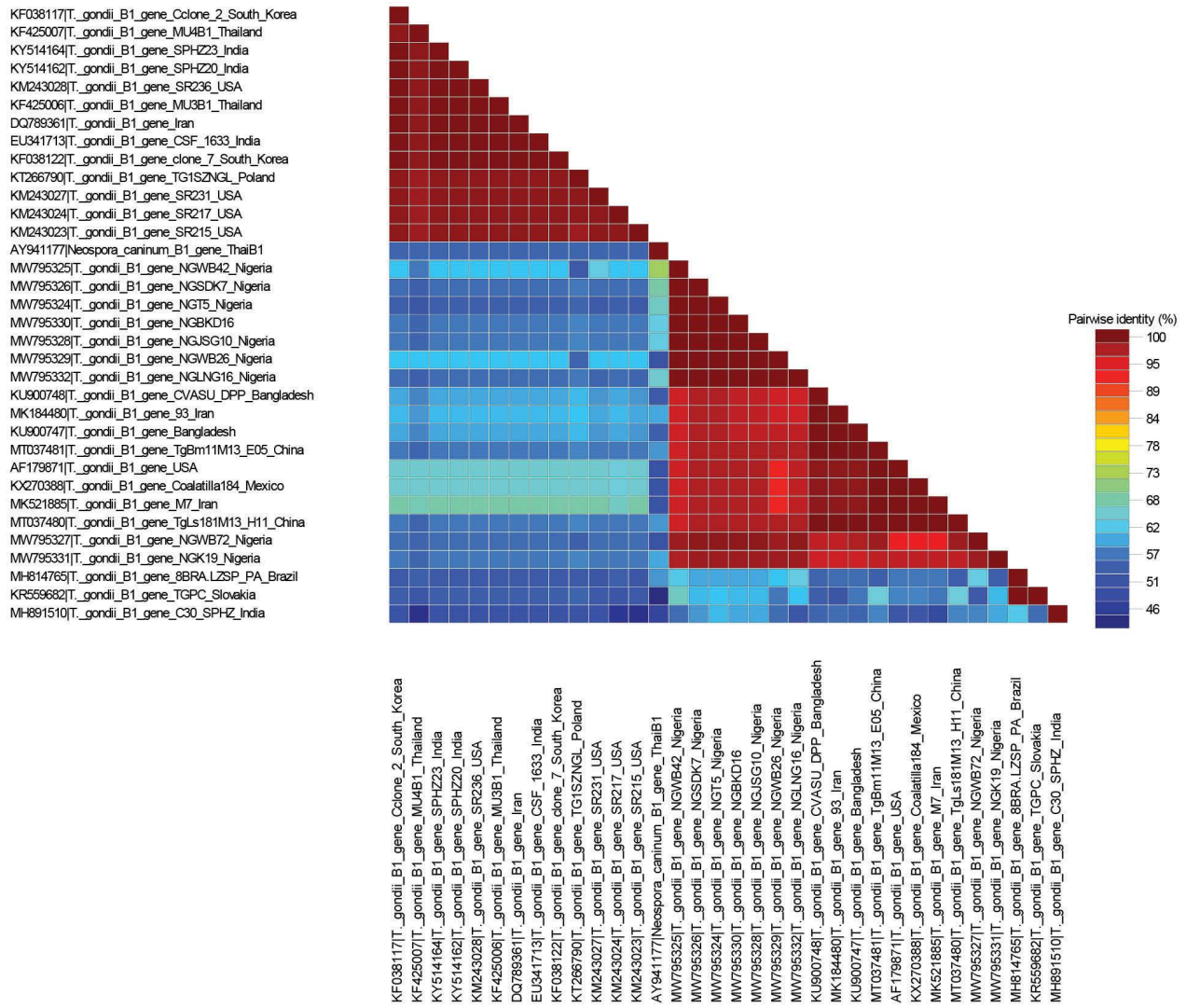

Fig. 1. Pairwise sequence identities of partial glycerol-3-phosphate dehydrogenase (G3PD) encoded by (B1) gene compared with other isolates from GenBank from *Toxoplasma gondii* found in local chickens and wild birds in Plateau State, Nigeria.

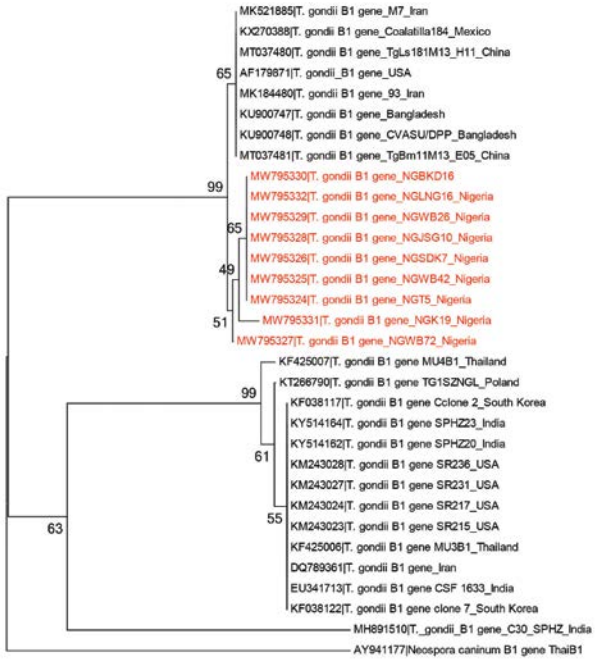

Fig. 2. Phylogenetic analyses of *T. gondii* sequences obtained from local chickens and wild birds in Plateau State, Nigeria with selected sequences in GenBank.

The phylogenetic tree was produced in MEGA v.6.06 software, using the maximum likelihood inference based on the Jukes-Cantor model T a m u r a et al. [45]. The values for percentage bootstrap support (1,000 iterations) are mentioned at the branch nodes. The tree is rooted with *Neospora caninum* (GenBank: AY941177) as an outgroup. The number of nucleotide substitutions occurring per site is shown in the scale bar.

DISCUSSION

The high detection rate of *T. gondii* DNA in tissues of local domestic chickens and resident, non-palearctic wild birds in this current study confirms asymptomatic infection within this biome. Comparatively, the high detection of *T. gondii* DNA presence in tissues of local domestic chickens in this study are close to the report of A i g n e r et al. [1] in Brazil in which *T. gondii* DNA detection rates ranged between 80.8 % and 84.6 % in brain and heart tissues of chickens sampled. This high detection rate of *T. gondii* DNA presence in tissues of local domestic chickens and wild birds could be attributed to a possible high exposure to the organism due to a considerable environmental contamination. The findings also portray avian species in the study area as potential carriers and source of human *T. gondii* infection. N z e l u et al. [40] in a study conducted in Benue State, Nigeria reported a relatively lower detection rate of 30.6 % of *T. gondii* DNA in tissues of free-range chicken while M o s e et al. [38] in a similar study in Kenya, Eastern Africa reported a relatively higher detection rate of 79.0 %. Lower prevalence of 42.0 % have been reported in Brazil [22]. The differences in the detection rates of *T. gondii* in tissues of free-range chickens in the present study and that reported in other studies may be attributed to the differences in infection rates/occurrence of the disease in the different study areas influenced by the differences in the climatic conditions and possibly cat abundance. In addition, the differences may be accounted by differences in the type of avian tissues sampled and the molecular techniques employed in the different studies.

Direct genome sequencing of regions identifies a complete and greater varied genetic diversity than other methods of analysis, including RFLP [42]. It was observed that the *T. gondii* B1 gene fragment sequences in this study were homogenous populations as there was a high sequence identity (97−100 %) within the samples. Among the *T. gondii* B1 genes obtained from wild birds (GenBank: MW795325, MW795327, and MW795329), similarities ranged from 98 to 100 %. Thus, there was no distinction in pattern regarding the similarities of *T. gondii* isolated with regards to their host of origin or source of isolation. Interestingly, all the sequences of *T. gondii* B1 gene derived from this study clustered together in the same clade, thus suggesting that they are homogenous with a common origin, irrespective of their host. Even though the isolates in this study, shared a common descent with Type I RH strain (AF179871), there seems to be a high genetic diversity and variability existing between the strains in this study with those from other regions of the world. Consequently, it means that the sequences of *T. gondii* B1 gene in this study are somewhat unique compared to other geographic locations. Studies such as the one reported by V e l m u r u g a n et al. [48] revealed an exceptionally atypical strain of *T. gondii* from Nigerian (isolate Tg-CkNg1) and Ghana among 19 isolates genotyped using 10 PCR-RFLP markers. *T. gondii* with a type II genetic background and with limited genetic diversity were reported to be common in in tissues of chickens, pigs and pregnant women in Benue State, Nigeria [40]. Similarly, studies in eared doves in Brazil revealed a prevalence of 22.3 % with five genotypes identified including one that had not been previously identified [14]. The result of the sequence analysis and evolutionary phylogeny in this study alludes to the possible divergence of the strains existing in this part of the world from the clonal strains.

Haplotype diversity and haplotype number analyses revealed that from the total *T. gondii* sequences (n = 9), only three haplotypes were observed. This may suggest a relatively genetically stable (relative to our sample size) *T. gondii* strain circulating in this area, in contrast, to the findings by Martínez-Flores et al. [36] and Nzelu et al. [40] who reported 13 and 9 haplotypes in the sequences of B1 gene and 529 bp repeat element from naturally infected sheep in Mexico and chickens, pigs and humans in Benue, Nigeria respectively. The haplotype numbers for *T. gondii* sequences obtained from both local chicken (n = 6) and wild birds (n = 3) were both 2, indicating higher genetic variation within the *T. gondii* sequences obtained from the wild birds. Similarly, there was higher haplotype diversity within the *T. gondii* sequences obtained from wild birds (0.667) compared with sequences from local chicken (0.333). This further confirms previous reports that agricultural animals sustain less diverse *T. gondii* genotypes than wild animals [20].

The nucleotide polymorphism occurring within the isolates was evaluated using Tajima's D statistical test [41]. Except for the sequences from wild birds, the statistical analysis of Tajima's D, Fu and Li's D, and Fu and Li's F showed negative values for the total population and isolates from local chicken did not significantly differ from zero ($P > 0.10$). These results indicate that increase in population, background selection or genetic drift is responsible for more low-frequency polymorphisms.

Similar research to this present one has been undertaken involving the detection of *T. gondii* in tissues from five different continents in pigs from North India (Asia), wild bats from Colombia (North America insular regions), cats from Iran (Asia) and wild birds from China (Asia), sheep from Mexico (greater North America), swine from Burkina Faso (Africa) as well as wild cats from the Czech Republic (Europe). All these studies utilized the B1 gene in the detection of the organism [5, 7, 8, 10, 30, 36, 47]. In 10 out of 19 studies on *T. gondii* detection in chickens, the B1 gene was targeted [19], highlighting the significance of this gene in the molecular detection of the organism. Additionally, since the B1 gene is diagnostic, it could reveal inherent characteristics (including genetic diversity) present within the organism even though its function is yet unknown.

CONCLUSIONS

The current study has revealed a high rate of occurrence of *T. gondii* DNA in tissues of domestic local chickens and wild birds and confirmed exposure to infection in this biome. The findings highlight the importance of avian species as asymptomatic intermediate hosts in Plateau State, Nigeria. Humans are therefore at risk of acquiring infection through handling and or eating undercooked infected domestic local chickens and handling of wild bird meats in the study area. The distinct *T. gondii* sequences of *T. gondii* detected, and the higher haplotype diversity in the sequences in the wild birds than those from domestic local chickens indicate high genetic variability in the *T. gondii* sequences circulating in the area. There is a need for a multidisciplinary approach towards understanding the interactions between the different hosts and the environment that may enhance the spread of the organism in the area. In addition, a 'one health' concept will be necessary towards planning effective control and prevention strategy. Subsequent studies that can use a larger or more elaborate sample size and techniques to obtain more information about the *T. gondii* circulating in tissues of birds in the area is recommended.

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CONFLICT OF INTEREST

The authors wish to declare that no conflict of interest exists regarding the publication of this paper.

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