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Short communication

Emergence and genetic diversity of zoonotic *Onchocerca* species among human populations in Taraba State, Nigeria

Danlami E. Akafyi[1*], Iliya S. Ndams[1,2], Ishaya H. Nock[1], Gloria Chechet[3], Alfons Renz[4], Pierre Kamtsap[4], Ibrahim M. A. Moussa[5] and Sørge Kelm[5]

[1] Department of Zoology, Ahmadu Bello University, Zaria, Nigeria
[2] Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria, Nigeria
[3] Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria
[4] Institute of Evolution and Ecology, Department of Comparative Zoology, University of Tübingen, Germany
[5] Centre for Biomolecular Interactions Bremen (CBIB), University of Bremen, Bremen, Germany

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***Corresponding author:**
Danlami E. Akafyi
dakafyippp@gmail.com

Abstract

In the present study, the genetic diversity of *Onchocerca* (*O.*) species was investigated by amplifying a fragment of the 16S rRNA gene from the parasite obtained in some endemic areas in Taraba State, Nigeria. Three local government areas were selected: two onchocerciasis-endemic and one non-onchocerciasis-endemic regions. A total of 211 skin snips using a sterile sclera punch were obtained from consenting participants, males and females residing within the areas for at least ten years or since birth, by convenience sampling methods. The emerged microfilariae were examined microscopically. Nine microfilaria-positive skin snips were identified and preserved in RNALater®. DNA was extracted from recovered microfilariae and residual skin snip specimens and was tested by standard Polymerase Chain Reaction (PCR) using primers targeting the 16S gene. Six PCR-positive samples were sequenced and analyzed. Two sequences varied with those from other regions, suggesting a likely diversity of *O. volvulus* populations in the study area. A novel finding of *O. ochengi*, the pathogenic cattle parasite, was identified in some samples, suggesting a potential zoonotic species in humans. Further investigation on the extent of emerging zoonotic onchocerciasis by *O. ochengi* in the light of cattle, *Simulium* vectors (blackflies), and environmental and human overlap in the study area is required.
Keywords: Genetic diversity, 16S gene, *Onchocerca* spp., *Onchocerca ochengi*, Zoonosis

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Introduction

Onchocerciasis is called river blindness because the blood-feeding black flies that transmit the ultimately blinding disease are prevalent throughout many major river basins in Africa in lush, fertile land alongside the rivers they breed (Crump et al., 2012). An estimated 120 million people are worldwide at risk of onchocerciasis. The number of people infected with *Onchocerca volvulus* is 26 million, with dermal microfilariae in 31 sub-Saharan African countries (WHO, 2017). Nigeria ranks among those countries with the highest disease burden worldwide (WHO, 2017), accounting for about a third of the global prevalence, with an estimated 8 million Nigerians infected cases with *O. volvulus*.

Nigeria has about 50 million persons in 40,000 communities at risk, with over 6.5 million suffering from severe itching or dermatitis, and approximately 500,000 are blind (Griswold et al., 2018). The annual treatment regimen with ivermectin in some areas has achieved high onchocerciasis control (Higazi et al., 2013; Cruz-Ortiz et al., 2012). Indeed, several field studies have shown a reduction in the transmission of infection (Winnen et al., 2002). However, in some parts of Nigeria, there are still places where the disease's active transmission continues, and the prevalence of *O. volvulus* is still high (Federal Ministry of Health, 2017).

About one-third of the *O. filariidae* cases are in Africa. *Simulium damnosum* sibling species are the main vectors of *O. volvulus*, *O. ochengi* (parasite of cattle), and *O. ramachandrini* (parasite of warthogs) (Krueger et al., 2007). Reliable and rapid taxonomic identification of parasitic disease agents is essential for

their correct diagnosis; hence, molecular detection of species diversity has become increasingly popular (Alhassan et al., 2014). The close similarity of *Onchocerca* species necessitates the exact identification of the developmental stages as a condition for assessing transmission indices in endemic areas (Renz et al., 1994).

In Northern Cameroon, which borders the Taraba State of Nigeria, the prevalence of microfilariae is presented by *O. ochengi* in cattle reaches 66–71% (Trees et al., 2000; Awadzi et al., 2004). However, since *O. ochengi* is closely related to *O. volvulus*, the routinely used O-150-target is commonly used as a diagnostic marker for *O. volvulus* clusters. *Onchocerca* species have the problem of discriminating the exert species (Krueger et al., 2007). However, molecular phylogenetic analysis of three ribosomal DNA gene fragments [12S, 16S rRNA genes, and NADH dehydrogenase subunit 5 (ND5)] from five *Onchocerca* species in Mali has shown potential for use in species separation and segregation in diversity studies (Morales-Hojas et al., 2006).

Also, developing DNA probes specific for *O. volvulus* and *O. ochengi* has become an additional tool in population genetics studies and for discriminating among *Onchocerca* species specifically (Danladi et al., 2018; Eisenbarth et al., 2016). Almost all vectors of human onchocerciasis are also partially zoophilic. Thus, they can transmit infective larvae of animal *Onchocerca* species from cattle, warthogs, and antelopes (Achukwi et al., 2000; Adler, 2005) and other zoonotic species. *O. volvulus* populations may be misdiagnosed during surveillance studies, affecting the accuracy of epidemiological data and improper interpretation of human onchocerciasis (Weil et al., 2000).

Understanding the *O. volvulus* population will be crucial to achieving elimination goals. For instance, if parasites are found in a given region following repeated rounds of mass drug administration (MDA), it would be informative to know whether they represent a sentinel population that has evaded treatment or parasites that have been reintroduced from other areas (Choi et al., 2016). A multi-site study evaluating five Community Directed Treatment with Ivermectin (CDTI) projects in Nigeria and Cameroon reported that over one-quarter of the community's age-eligible people were non-compliant. This category of people might be a reservoir for continued transmission of onchocerciasis in some endemic areas (Wanji et al., 2015; Senyonjo et al., 2016). Early studies of *O. volvulus* found little genetic diversity between parasites isolated from some locations in Africa (Akogun, 1999; Crawford et al., 2019).

No report on *O. ochengi* isolated from human population samples has been documented. Nonetheless, Uni and coworkers in Japan reported zoonotic filariasis caused by *O. dewittei* japonica in a resident of Hiroshima Prefecture, Honshu, Japan (Uni et al., 2010). The study indicated that this human case was caused by *O. dewittei* japonica suggests that zoonotic onchocerciasis can occur in rural areas in Japan where wild boar, *Simulium* vectors, and human populations overlap. *O. lupi* has also been reported to infect dogs, cats, and humans in the United States (Noma et al., 2014).

The present study aimed to investigate the threat and potential of emerging zoonotic onchocerciasis caused by *O. ochengi*, which is likely to occur in the study area due to predisposing factors like the overlaps among cattle, *Simulium* vectors, and humans in Taraba state, Nigeria.

Materials and methods

The study area is characteristic of the southern Guinea Savanna, with annual mean temperatures ranging between 27 to 37°C, relative humidity between 62-97%, and an average rainfall of 1800 mm (Figure 1). The physical features surrounding the study area are mountains about 1000 m in height (Federal Ministry of Health, 2017). The onchocerciasis endemicity extends across the international border to Northern Cameroon. The rivers and tributaries of streams traversing the communities and draining into the river Benue provide numerous breeding sites for the black flies in the study area (Akogun, 1999).

The collection of samples was carried out in 2019. Briefly, superficial skin biopsies (skin snips) were taken from the iliac crest, as previously described (Noma et al., 2014). A total of 211 consenting participants from Gashaka (n=61), Yorro (n=74), and Lau (n=76) local government areas, following Rapid Epidemiological Assessment (REA) guidelines (Thiele et al., 2016).

Each snip from individual participants was immediately placed into the 1.5 mL microcentrifuge tubes with approximately 100 μL physiological saline and incubated for 12–24 hours (Lloyd et al., 2015). The emerging microfilariae were examined microscopically at 40–400× magnification. Then, the microfilariae and the corresponding residual skin snips were transferred to a 1.5 mL Eppendorf tube containing 200 μL of RNAlater® preservative (Sigma-Aldrich, Munich, Germany) and stored at -20°C in Parasitology and Entomology Laboratory, Ahmadu Bello University Zaria, Nigeria, until DNA extraction, PCR amplification and sequencing which was conducted in Centre for Biomolecular Interactions Bremen (CBIB), University of Bremen, Germany.

Before extraction of genomic DNA from the residual skin snips and free-floating MF in RNALater preservative, 400 μL of a 1× phosphate-buffered saline solution containing 0.05% Tween-20 (PBS-Tween) were added to each microcentrifuge tube contents. The genomic DNA was extracted from the samples (individual microfilaria) using the QIAampo DNA Investigator Kit (Qiagen, Hilden, Germany) according to the Manufacturer's instructions, using a 6–12 hours digest at 56°C with mixing to ensure complete lysis. Purified DNA was eluted from QIAamp MinElute columns with 60 μL Buffer ATE after a 10-minute incubation at room temperature and quantified with a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Amplification of ribosomal DNA (rDNA) targeting a fragment of the 16S gene (465 bp), with a set of

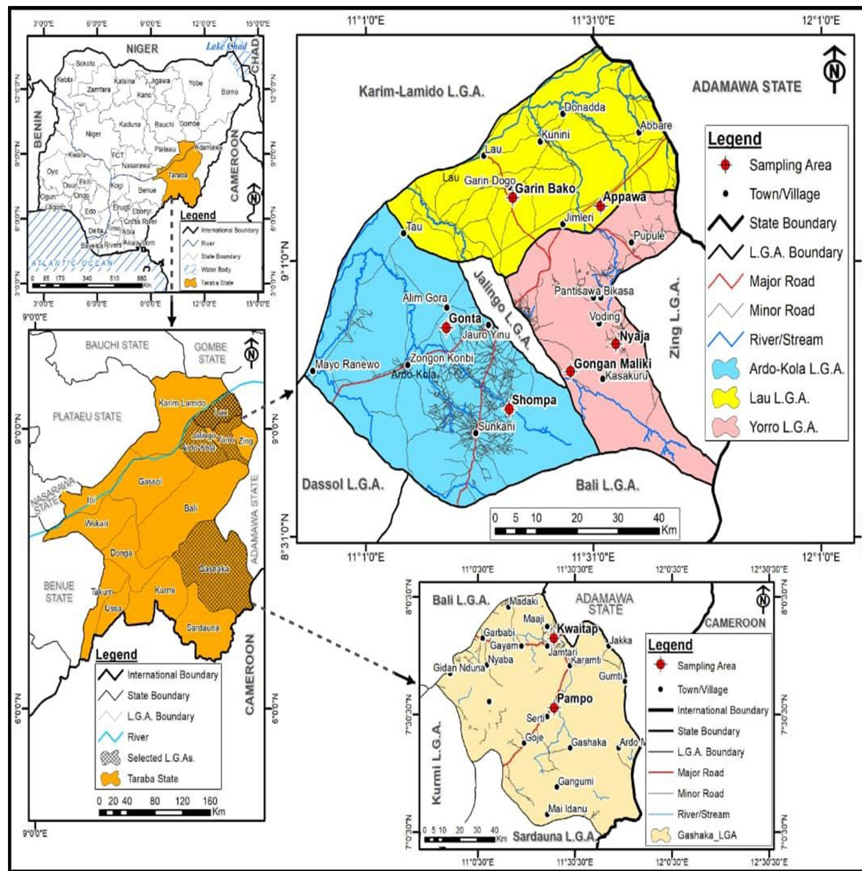

Figure 1: Map of study site in Taraba state, Nigeria, Prepared by Map Gallery, Geography Department, Ahmadu Bello University Zaria, Nigeria (2020).

Table 1: Distribution of microfilariae in the study population.

Local Government Area	Sample site	Coordinates	No. of samples	No. of positive (%)
Gashaka*	Kwaitp	7°49' 49.40"N 11° 24' 05.90"E	43	3(7.0%)
	Pompa	7°30' 15.84"N 11° 21'45.54" E	20	0
Yorro*	Nyaja	8°45' 50.65"N 11° 35'20.45"E	41	2(4.9%)
	Gogon Malaki	8°35' 20.65"N 11° 30'15.40"E	43	4(9.3%)
Lau**	Appawa	9°20' 10.00"N 11° 31'15.30"E	31	0
Total			211	9(4.2%)

*Onchocerciasis endemic Local Government Area.

**Non-Onchocerciasis endemic Local Government Area.

primers (Forward: 5'-TGG CAG CCT TAG CGT GAT G-3', Reverse-: 5'-CAA GAT AAA CCG CTC TGT CTC AC-3') for *Onchocerca* species identity was used (Eisenbarth et al., 2013). In brief, 1 μL was added to a total reaction volume of 25 μL consisting of 1.5 mM MgCl2, 0.2 mM dNTP, 50 pmol forward and reverse primer, 1× Green Dream Taq buffer, 1 U Taq polymerase (Quigen, Hilden, Germany). The cycling conditions were initial denaturation for 4 min at 94°C, denaturation at 94°C for 30 s, annealing at 52.5°C for the 30s, and elongation at 72°C for 90 s for 35 cycles, and a final elongation of 7 min at 72°C and held at a temperature of 4°C till further electrophoresis. The PCR products were resolved in a 3% TBE agarose gel electrophoresis process, stained with Stain G (SERVA Electrophoresis, Heidelberg, Germany). Amplicons were visualized in a gel documentation system consisting of a UV Benchtop Imaging System Transilluminator (Hero®, Munich, Germany).

The amplicons were excised and purified using GeneJET Gel extraction and purification kit as described by the Manufacturer's instructions (Thermo Scientific, Bremen, Germany). Sanger sequencing was carried out in Microsynth Sequencing Laboratories Göttingen, Germany. The sequenced samples were trimmed using Geneious and Basic Local Alignment Search Tools (BLAST) algorithms for similarity search of 16S rRNA *Onchocerca* species in the NCBI

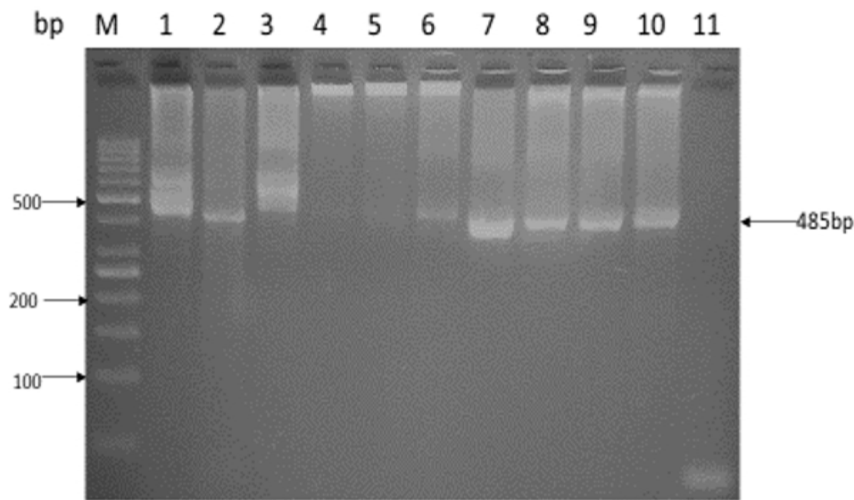

Figure 2: Electrophoresis gel picture of the 16S gene. Lane M: 50 bp ladder; Lane 1: *O. volvulus* 5619526. (MN717178); Lane 2: *O. volvulus* 5619597 (MN717179); Lane 3: *O. volvulus* 5619596 (OQ619373); Lane 7: *O. ochengi* 5657519(MN718152); Lane 8: *O. ochengi* 5657518 (MN718151); Lane 9: *O. ochengi* 5657520 (MN718153); Lane 10: *O. volvulus* AY462903 positive control kindly provided by Prof. Alfon Renz, University of Tubingen, Germany, and Lane 11 is negative control template).

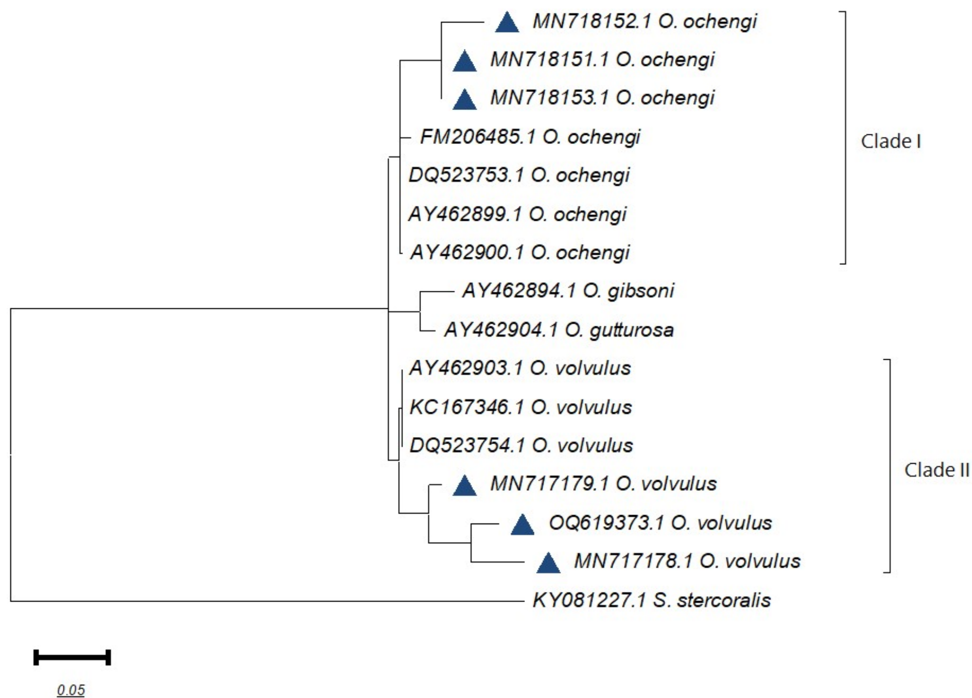

Figure 3: Maximum likelihood construction of a phylogenetic tree of the characterized *O. volvulus* and *O. ochengi* compared to previously published sequences. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. Evolutionary analyses were conducted in MEGA11. In cluster 1: Sequence in from study area MN718151; MN718152; MN718153; AY462897; AY462903; FM206485.1; DQ523753.1 and AY462900.1 from GenBank of *O. ochengi*. In cluster II: Sequence from study area; MN717178; MN717179; OQ619373; AY462902; DQ523754.1; KC167346.1; from GenBank of *O. volvulus*. AY462894 and AY462904 are in-group species, while KY081227 is an out-group.

(http://www.ncbi.nlm.nih.gov/BLAST). Sequences were edited in Bioedit (http://www.mbio.ncsu.edu/BioEdit) and aligned with sequences retrieved from published 16S sequences of *O. volvulus* (n= 3), *O. ochengi* (n=3), *O. gutturosa* (n=1), *O. gibsoni* (n=1), and *Strongyloides stercolaris* (n=1) from other regions and an out-group sequence in the GenBank for comparison and validation of inter and intra-specific variability in MEGA11 algorithms using Maximum likelihood trees construction and p-distance model p = nd/n. Where p = proportion of nucleotide sites, nd= the number of nucleotide differences, and n = the total

Table 2: Pairwise nucleotide distance in a p-distance model of 16S *O. volvulus* and *O. ochengi* sequences between this study and some in-group species.

Species	Pairwise distance															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1. AY462903-*O. volvulus*																
2. DQ523754-*O. volvulus*	0.000															
3. KC167346-*O. volvulus*	0.000	0.000														
4. OQ619373-*O. volvulus**	0.035	0.020	0.006													
5. MN717178-*O. volvulus**	0.050	0.018	0.006	0.036												
6. MN717179-*O. volvulus**	0.009	0.006	0.006	0.011	0.009											
7. MN718152-*O. ochengi*	0.049	0.027	0.017	0.065	0.067	0.019										
8. AY462894-*O. gibsoni*	0.039	0.036	0.036	0.078	0.089	0.047	0.073									
9. AY462904-*O. gutturosa*	0.031	0.032	0.030	0.070	0.085	0.041	0.071	0.026								
10. KY081227-*S. stercoralis*	0.392	0.365	0.372	0.403	0.421	0.381	0.405	0.404	0.403							
11. FM206485-*O. ochengi***	0.019	0.012	0.011	0.046	0.056	0.021	0.033	0.045	0.035	0.395						
12. DQ523753-*O. ochengi***	0.010	0.010	0.011	0.031	0.029	0.017	0.016	0.038	0.032	0.367	0.002					
13. AY462900-*O. ochengi***	0.014	0.012	0.013	0.048	0.063	0.023	0.040	0.039	0.029	0.394	0.008	0.002				
14. MN718151-*O. ochengi*	0.015	0.015	0.012	0.017	0.015	0.013	0.002	0.042	0.036	0.370	0.005	0.004	0.005			
15. MN718153-*O. ochengi*	0.015	0.015	0.012	0.017	0.015	0.013	0.004	0.042	0.034	0.374	0.004	0.004	0.005	0.000		
16. AY462899-*O. ochengi*	0.012	0.010	0.011	0.046	0.061	0.021	0.038	0.038	0.028	0.392	0.006	0.000	0.001	0.004	0.004	

* *O. volvulus* from samples of the current study area.

** *O. ochengi* from samples of the current study area.

number of nucleotides compared (Kumar et al., 2018).

Results

This study recorded a microfilariae prevalence of 4.3% (9/211) from Gashaka (n=3) and Yorro (n=6). Lau LGA is a non-endemic area (Table 1). The microfilariae of *O. volvulus* are differentiated by not being sheathed and possessing nuclei that do not extend to the tip of the tail.

A gel documentation system depicts amplification from skin snips using 16S primers from the study area (Figure 2). Sequences obtained from this study were deposited in the GenBank with accession numbers MN718151 (G44 *O. ochengi*), MN718152 (*O. ochengi*), MN718153 (*O. ochengi*), MN717178 (Y07 *O. volvulus*), MN717179 (Y02 *O. volvulus*) and OQ619373 (G49 *O. volvulus*).

In concatenated analyses of 16S data sets (Figure 3), high bootstrap support was achieved for the clade comprising nucleotide sequence data from the study and GenBank of some in-group species: *O. volvulus*, *O. ochengi*, *O. gibsoni*, *O. gutturosa*, and an outgroup species *Strongyloides stercolaris*. They were all placed as basal species of the genus with high bootstrap support, and their relationship was resolved. *O. volvulus* sequence data in this study were identical to that of the reference sequence in cluster 1, while two sequence data of *O. volvulus* in this study are distinct and clearly showed diversity to sequence data of *O. volvulus* from the GenBank as shown in cluster 1. In the second cluster, the three *O. ochengi* sequences in this study and the reference sequence data were similar (Figure 3).

Next, we conducted pairwise nucleotide sequence comparisons of the protein-coding sequences of 790 positions in the final dataset between the three *O. volvulus* and *O. ochengi* each from this study and the published sequences of some in-group species and an outgroup species (Table 2). The *O. volvulus* sequence (AY462902) varied from 0.03% to 0.05% with sequences of *O. volvulus* from GenBank, indicating the likely diversity of the sequences obtained from the study area. Similarly, the pairwise nucleotide diversities between *O. ochengi* from this study and *O. ochengi* (AY462897) from GenBank were very similar. However, the variation with other in-group individual species ranges from 0.15 to 0.53% (Table 2).

We evaluated the phylogenetic relationship of *O. ochengi* in this study with other deposited sequences in the GenBank by constructing a maximum likelihood (ML) for comparison (Figure 3). The *O. ochengi* sequences from the study area cluster with the *O. ochengi* sequence obtained from the GenBank.

Discussion

The microfilaria prevalence of 4.2% reported in this study corresponds with previous parasitological examination findings in the same study area (Krueger et al., 2007; Idowu et al., 2013; Thiele et al., 2016; Danladi et al., 2018), indicating continual transmission in those endemic communities. However, Onchocerciasis transmission assessment after 15 years of the Mass Drug Administration in six endemic villages in Central Nigeria showed a 1.08% prevalence (Evans et al., 2014). In Kaduna State, Northwest Nigeria, after 15 to 17 years of ivermectin treatment, microfilariae could not be detected in the skin snips, and the prevalence had fallen to 0% in 27 communities investigated (Tekle et al., 2012). Notwithstanding, there are still areas with high prevalence in Nigeria (Federal Ministry of Health, 2017). For instance, Onchocerciasis distribution in the upper Imo River basin in South East Nige-

ria, reported by Dozie et al. (2005). showed a 26.8% prevalence, higher than the present study.

In the current study, the 16S rRNA gene sequences obtained were aligned with GenBank reference sequences and showed a close relationship to *O. volvulus*. The study by Choi et al. (2016) analyzed the whole genomes of 27 adult *O. volvulus* from Ecuador, Uganda, and some regions of West Africa and pointed to greater levels of genetic diversity than reported in this study (Higazi et al., 2001). A similar finding by Thiele et al. (2016) reported that some species are nearer to *O. volvulus*, as observed in this study (Table 2). As reported by various workers (Dozie et al., 2005; Evans et al., 2014; Thiele et al., 2016), they suggest the high diversity of African species. According to (Krueger et al., 2007), the human parasite *O. volvulus* might have evolved from an ancestral bovine parasite in Africa. The study further observed likely genetic diversity within the populations of *O. volvulus*. The *O. volvulus* sequence (AY462902) varied from 0.03% to 0.05% with sequences of *O. volvulus* from GenBank, indicating the likely diversity of the sequences obtained from the study area. Further genetic analyses of the parasite in human populations in other regions in Nigeria are required to discriminate the extent of the parasite diversity.

The current study recorded a preliminary occurrence of *O. ochengi* in human skin biopsies. The patients were healthy farmers born and residing in Kwaitap village in Gashaka LGA, Taraba State, Nigeria. Skin snipes were obtained from the upper hip of the patients; there were neither nodules nor any clinical manifestation associated with filariasis observed among the participants. The study site is characterized by the abundance of cattle populations that cohabitate grazing among the human population. The study sites have high cattle populations, which might serve as reservoirs for bovine parasites (*O. ochengi*). Almost all vectors of human onchocerciasis are zoophilic, thus transmitting both animal and human *Onchocerca* species. However, the status of the animal parasite is still unknown in Nigeria.

Although intensive studies have been conducted in Cameroon (Wahl et al., 1994; Weil et al., 2000), there is no report of *O. ochengi* being isolated from human populations yet. Nonetheless, in Japan, zoonotic filariasis is caused by *O. dewittei* japonica has been described in a resident of Hiroshima Prefecture, Honshu, Japan (Uni et al., 2010), and Onchocerca lupi has been reported to infect dogs, cats, and humans in the United States (Roe et al., 2020). Against this background, this study proposes a further investigation into the threat and potential of emerging zoonotic onchocerciasis (*O. ochengi*) due to cattle, *Simulium* vectors, and humans overlapping in the study area.

In conclusion, two sequences showed variation with those from other regions, suggesting a likely diversity of *O. volvulus* populations in the study area. The study revealed a preliminary case of potential zoonosis of *O. ochengi* in the study area's human sample.

Article Information

Ethics approval and consent to participate. Approval was obtained from the ethics committee from Ahmadu Bello University Zaria, Committee for Human Subject Research (ABU-CUHSR/Zoology/2020/006). Informed consent was obtained from all individual participants included in the study. All participants were informed and consented to the publication of this article.

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Conflict of Interest. The authors have no conflicts of interest to declare relevant to the article's content.

Authors contributions. DEA: experimental design, performing the study, and writing of the manuscript; IHN, GC, IHAM, PK, AR, SK: experimental design and writing of the manuscript. ISN, GC, AR: performing the study; IMAM: Performed the experiments; analyzed the data; Wrote the paper. PK, AR: data analysis. AR: Contributed reagents/materials/analysis tools; Wrote the paper.

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References

Achukwi, M.D., Harnett, W., Renz, A., 2000. *Onchocerca ochengi* transmission dynamics and the correlation of *O. ochengi* microfilaria density in cattle with the transmission potential. Veterinary Research 31, 611–621. 10.1051/vetres:2000144.

Adler, P.H., 2005. Blackflies, the Simuliidae, in: Marquardt, W.H. (Ed.), Biology of Disease Vectors. 2 ed.. Academic Press, pp. 127–140.

Akogun, O.B., 1999. Onchocerciasis in Taraba State, Nigeria: Clinical-epidemiological study of at-risk males in Bakundi District. International Journal of Medical Microbiology 289, 371–379. 10.1016/s0934-8840(99)80077-2.

Alhassan, A., Makepeace, B.L., LaCourse, E.J., Osei-Atweneboana, M.Y., Carlow, C.K.S., 2014. A simple isothermal DNA amplification method to screen black flies for *Onchocerca volvulus* infection. Plos One 9, e108927. 10.1371/journal.pone.0108927.

Awadzi, K., Attah, S.K., Addy, E.T., Opoku, N.O., Quartey, B.T., Lazdins-Helds, J.K., Ahmed, K., Boatin, B.A., Boakye, D.A., Edwards, G., 2004. Thirty-month follow-up of sub-optimal responders to multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. Annals of Tropical Medicine and Parasitology 98, 359–370. 10.1179/000349804225003442.

Choi, Y.J., Tyagi, R., McNulty, S.N., Rosa, B.A., Ozersky, P., Martin, J., Hallsworth-Pepin, K., Unnasch, T.R., Norice, C.T., Nutman, T.B., Weil, G.J., Fischer, P.U., Mitreva, M., 2016. Genomic diversity in *Onchocerca volvulus* and its *Wolbachia* endosymbiont. Nature Microbiology 2, 16207. 10.1038/nmicrobiol.2016.207.

Crawford, K.E., Hedtke, S.M., Doyle, S.R., Kuesel, A.C., Armoo, S., Osei-Atweneboana, M., Grant, W.N., 2019. Utility of the *Onchocerca volvulus* mitochondrial genome for delineation of parasite transmission zones. BioRxiv 10.1101/732446.

Crump, A., Morel, C.M., Omura, S., 2012. The onchocerciasis chronicle: from the beginning to the end? Trends in Parasitology 28, 280–288. 10.1016/j.pt.2012.04.005.

Cruz-Ortiz, N., Gonzalez, R.J., Lindblade, K.A., Richards, F.O., Sauerbrey, M., Zea-Flores, G., Dominguez, A., Oliva, O., Catú, E., Rizzo, N., 2012. Elimination of Onchocerca volvulus transmission in the Huehuetenango focus of Guatemala. Journal of Parasitology Research 2012, 638429. 10.1155/2012/638429.

Danladi, T., Elkanah, S.O., Wahedi, J.A., Elkanah, D.S., 2018. Distribution and prevalence of human onchocerciasis in Ardo-Kola and Yorro local government areas, Taraba State, Nigeria. Adamawa State University Journal of Scientific Research 6, 222–229.

Dozie, I.N.S., Onwuliri, C.O.E., Nwoke, B.E.B., Onwuliri, V.A., 2005. Clinical and parasitological aspects of onchocercal skin diseases in Nigeria. Tropical Doctor 35, 142–144. 10.1258/0049475054620626.

Eisenbarth, A., Achukwi, M.D., Renz, A., 2016. Ongoing transmission of Onchocerca volvulus after 25 years of annual ivermectin mass treatments in the Vina du Nord River Valley, in north Cameroon. PLoS Neglected Tropical Diseases 10, e0004392. 10.1371/journal.pntd.0004392.

Eisenbarth, A., Ekale, D., Hildebrandt, J., Achukwi, M.D., Streit, A., Renz, A., 2013. Molecular evidence of 'siisa form', a new genotype related to *Onchocerca ochengi* in cattle from north Cameroon. Acta Tropica 127, 261–265. 10.1016/j.actatropica.2013.05.011.

Evans, D.S., Alphonsus, K., Umaru, J., Eigege, A., Miri, E., Mafuyai, H., Gonzales-Peralta, C., Adamani, W., Pede, E., Umbugadu, C., Saka, Y., Okoeguale, B., Richards, F.O., 2014. Status of onchocerciasis transmission after more than a decade of mass drug administration for onchocerciasis and lymphatic filariasis elimination in central Nigeria: Challenges in coordinating the stop MDA decision. PLoS Neglected Tropical Diseases 8, e3113. 10.1371/journal.pntd.0003113.

Federal Ministry of Health, 2017. Nigeria Onchocerciasis Elimination Plan. Hamful Press Ltd.

Griswold, E., Unnasch, T., Eberhard, M., Nwoke, B.E.B., Morales, Z., Muheki Tukahebwa, E., Kebede, B., Anagbogu, I., Katabarwa, M., Habomugisha, P., Tadesse, Z., Miri, E.S., Evans, D., Cohn, D., Elhassan, E., Richards, F., 2018. The role of national committees in eliminating onchocerciasis. International Health 10, i60–i70. 10.1093/inthealth/ihx048.

Higazi, T.B., Katholi, C.R., Mahmoud, B.M., Baraka, O.Z., Mukhtar, M.M., Qubati, Y.A., Unnasch, T.R., 2001. *Onchocerca volvulus*: Genetic diversity of parasite isolates from Sudan. Experimental Parasitology 97, 24–34. 10.1006/expr.2000.4589.

Higazi, T.B., Zarroug, I.M.A., Mohamed, H.A., Elmubark, W.A., Deran, T.C.M., Aziz, N., Katabarwa, M., Hassan, H.K., Unnasch, T.R., Mackenzie, C.D., Richards, F., Hashim, K., 2013. Interruption of Onchocerca volvulus transmission in the Abu Hamed focus, Sudan. The American Journal of Tropical Medicine and Hygiene 89, 51–57. 10.4269/ajtmh.13-0112.

Idowu, E., Adeneye, A., Adeleke, M., Sulyman, M., Mafe, M., 2013. Endemicity of onchocerciasis in some communities in Taraba State, Nigeria, following long term treatment with ivermectin. The Zoologist 11, 21–28. URL: https://www.ajol.info/index.php/tzool/article/view/107273.

Krueger, A., Fischer, P., Morales-Hojas, R., 2007. Molecular phylogeny of the filaria genus *Onchocerca* with special emphasis on afrotropical human and bovine parasites. Acta Tropica 101, 1–14. 10.1016/j.actatropica.2006.11.004.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35, 1547–1549. 10.1093/molbev/msy096.

Lloyd, M.M., Gilbert, R., Taha, N.T., Weil, G.J., Meite, A., Kouakou, I.M.M., Fischer, P.U., 2015. Conventional parasitology and DNA-based diagnostic methods for onchocerciasis elimination programmes. Acta Tropica 146, 114–118. 10.1016/j.actatropica.2015.03.019.

Morales-Hojas, R., Cheke, R.A., Post, R.J., 2006. Molecular systematics of five onchocerca species (Nematoda: *Filarioidea*) including the human parasite, *O. volvulus*, suggest sympatric speciation. Journal of Helminthology 80, 281–290. URL: https://www.ncbi.nlm.nih.gov/pubmed/16923273.

Noma, M., Zouré, H.G.M., Tekle, A.H., Enyong, P.A.I., Nwoke, B.E.B., Remme, J.H.F., 2014. The geographic distribution of onchocerciasis in the 20 participating countries of the African Programme for Onchocerciasis Control: (1) Priority areas for ivermectin treatment. Parasites & Vectors 7, 325. 10.1186/1756-3305-7-325.

Renz, A., Enyong, P., Wahl, G., 1994. Cattle, worms, and zooprophylaxis. Parasite 1, 4–6.

Roe, C.C., Yaglom, H., Howard, A., Urbanz, J., Verocai, G.G., Andrews, L., Harrison, V., Barnes, R., Lyons, T., Bowers, J.R., Engelthaler, D.M., 2020. Coyotes as reservoirs for *Onchocerca lupi*, United States, 2015-2018. Emerging Infectious Diseases 26, 2989–2993. 10.3201/eid2612.190136.

Senyonjo, L., Oye, J., Bakajika, D., Biholong, B., Tekle, A., Boakye, D., Schmidt, E., Elhassan, E., 2016. Factors associated with ivermectin non-compliance and its potential role in sustaining *Onchocerca volvulus* transmission in the west region of Cameroon. PLoS Neglected Tropical Diseases 10, e0004905. 10.1371/journal.pntd.0004905.

Tekle, A.H., Elhassan, E., Isiyaku, S., Amazigo, U.V., Bush, S., Noma, M., Cousens, S., Abiose, A., Remme, J.H., 2012. Impact of long-term treatment of onchocerciasis with ivermectin in Kaduna State, Nigeria: First evidence of the potential for elimination in the operational area of the african programme for onchocerciasis control. Parasites & Vectors 5, 28. 10.1186/1756-3305-5-28.

Thiele, E.A., Cama, V.A., Lakwo, T., Mekasha, S., Abanyie, F., Sleshi, M., Kebede, A., Cantey, P.T., 2016. Detection of *Onchocerca volvulus* in skin snips by microscopy and real-time polymerase chain reaction: Implications for monitoring and evaluation activities. The American Journal of Tropical Medicine and Hygiene 94, 906–911. 10.4269/ajtmh.15-0695.

Trees, A.J., Graham, S.P., Renz, A., Bianco, A.E., Tanya, V., 2000. *Onchocerca ochengi* infections in cattle as a model for human onchocerciasis: Recent developments. Parasitology 120 Suppl, S133–42. 10.1017/s0031182099005788.

Uni, S., Boda, T., Daisaku, K., Ikura, Y., Maruyama, H., Hasegawa, H., Fukuda, M., Takaoka, H., Bain, O., 2010. Zoonotic filariasis caused by *Onchocerca dewittei japonica* in a resident of Hiroshima Prefecture, Honshu, Japan. Parasitology International 59, 477–480. 10.1016/j.parint.2010.05.006.

Wahl, G., Enyong, P., Schibel, J.M., Ngosso, A., Tubbesing, H., Moyou, R., Renz, A., 1994. Transmission of *Onchocerca volvulus*, *O. ochengi* and type D filariae by *Simulium damnosum* s.l. in north Cameroon and its influence on the epidemiology of human onchocerciasis. Parasite 1, S7–S10. 10.1051/parasite/199401s1007.

Wanji, S., Kengne-Ouafo, J.A., Esum, M.E., Chounna, P.W.N., Adzemye, B.F., Eyong, J.E.E., Jato, I., Datchoua-Poutcheu, F.R., Abong, R.A., Enyong, P., Taylor, D.W., 2015. Relationship between oral declaration on adherence to ivermectin treatment and parasitological indicators of onchocerciasis in an area of persistent transmission despite a decade of mass drug administration in Cameroon. Parasites & Vectors 8, 667. 10.1186/s13071-015-1283-6.

Weil, G.J., Steel, C., Liftis, F., Li, B.W., Mearns, G., Lobos, E., Nutman, T.B., 2000. A rapid-format antibody card test for diagnosis of onchocerciasis. The Journal of Infectious Diseases 182, 1796–1799. 10.1086/317629.

WHO, 2017. Integrating neglected tropical diseases. Fourth World Health Organization report on neglected tropical diseases. Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva 27.:226-231. URL: https://www.who.int/publications/i/item/9789241565448.

Winnen, M., Plaisier, A.P., Alley, E.S., Nagelkerke, N.J.D., van Oortmarssen, G., Boatin, B.A., Habbema, J.D.F., 2002. Can ivermectin mass treatments eliminate onchocerciasis in Africa? Bulletin of the World Health Organization 80, 384–391. URL: https://www.ncbi.nlm.nih.gov/pubmed/12077614.