wjpmr, 2018,4(7), 38-46

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

MOLECULAR CHARACTERISATION OF HOOKWORM DETECTED FROM PEASANT FARMERS IN SELECTED LOCAL GOVERNMENT AREAS OF KADUNA STATE, NIGERIA

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Article Received on15/05/2018 **Article Revised on 05/06/2018** **Article Accepted on 26/06/2018**

ABSTRACT

This study aimed at detecting the human hookworm species among peasant farmers in selected Local Government Areas (LGAs) of Kaduna State, Nigeria. A cross sectional, descriptive study was carried out between November 2014 and October 2015. One thousand two hundred eligible peasant farmers were enrolled in the study. One LGA was selected from each of the three Senatorial Zones of Kaduna State by simple random sampling method. The results obtained showed the overall prevalence of hookworm infection in the study population using microscopy technique to be 18.7%. In respect to LGA, prevalence of hookworm infection was 24.0% in Jema'a, 17.3% in Chikun and 14.8% in Zaria LGA. The prevalence of hookworm infection was found to be significantly associated with the studied LGAs ($P< 0.002$). The result of the electrophoresis of 15 amplicons obtained from 15 positive stool samples from the three selected LGAs showed a corresponding base pair bands of 310bp which corresponds with the base pair of the *N. americanus* positive control. The result of the sequencing and basic local alignment search tool (BLAST) obtained showed that Sequence of Sample No. 5 from Zaria LGA had 95% identity with that of *N. americanus*, sequence of Sample No. 10 from Chikun LGA had 99% identity with that of *N. americanus* and finally sequence of Sample No.15 from Jema'a LGA also gave a similar correlation of 97% as that of *N. americanus.* The result of the specie specific identification of the two human hookworm species (i.e. *N. americanus* and *A. duodenale*) obtained shows that *N. americanus* was predominant in the study area (Kaduna State, Nigeria) during the study. The results of this study indicated an overall prevalence rate of hookworm infection of 18.7% among peasant farmers in Kaduna State. This indicates a potential risk of severe anaemia among individuals particularly the vulnerable groups such as pregnant women and children of school age who participate in farming. In conclusion, this study revealed the transmission of human hookworm among peasant farmers in Kaduna State to be at alarming rate. Thus, major prevention and control measures should be adopted to avoid further spread of the infection; and more so, there is a need for prompt treatment of the infected persons as well as creating a law that will prohibit indiscriminate defeacation on farm lands.

KEYWORDS: Hookworm, Peasant farmers, Molecular Characterisation, Kaduna State, Nigeria.

INTRODUCTION

Hookworms are nematodes belonging to the family *Ancylostomatidae*, super-family *Strongyloidea.* Human hookworm infection is a soil-transmitted infection caused by *Necator americanus* and *Ancylostoma duodenale.* It is the leading cause of anaemia and protein malnutrition, afflicting an estimated 740 million people in the developing nations of the tropics (Cheesebrough, 2005; CDC, 2013).

Hookworm infection is spread by faecal contamination of the soil; infection occurs when infective third-stage filariform larvae (L_3) penetrate the hands, feet, arms or legs, especially when a person walk with bare-feet (Paniker and Jayaram, 2007) Signs of advanced severe infection includes anaemia and protein deficiency, including emaciation, cardiac failure and abdominal distension (Ayoya *et al*, 2006; Drisdelle, 2006). Peasant farmers are vulnerable group in acquiring the infection. The infection has been noted to be more common in families who are involved in agricultural pursuits (Damen et al., 2007). Hookworm infection which is common among farmers causes severe anaemia leading to high morbidity and mortality and consequently causing low productivity and food insecurity in Nigeria.

The role played by farmers in the economic development of Nigeria after petroleum cannot be over emphasized. Therefore, this study determined the prevalence of hookworm infection among peasant farmers in Kaduna State and also the molecular characterisation of the hookworm detected using PCR technique, sequencing, BLAST and phylogenetic analysis.

METHODOLOGY

Study Area

The study was carried out in some selected LGAs of Kaduna state. The state is located between latitude 90° and 140° north of the equator and longitude 70° and 100° east of the Greenwich meridian, it occupies a landmass of about 70,210 square kilometres on the map of Nigeria. The topography is that of an undulating plateau that forms part of the rich tourist attractions in areas like Kufena in Zaria, Kagoro, Kwoi, and Gwantu. According to the National Population Commission (2006) census figure, Kaduna state has a population of over 6 million people. Kaduna State has 23 Local Government Areas and 3 Senatorial Zones (North, Central and Southern) Senatorial Zones.

The main occupations of majority of the people in the study area are peasant farming, petty trading, mining and few white collar jobs. The state has two distinct seasons (dry and wet or raining seasons). The former takes place from November to March while the latter occurs between April and October.

Study design

A cross sectional, descriptive study was carried out between November 2014 and October 2015. One thousand two hundred eligible peasant farmers were enrolled in the study. The study was carried out in some selected LGAs of Kaduna State, Nigeria by simple random sampling method.

Study population

The population studied comprised of voluntary consented peasant farmers in Jema'a, Chikun and Zaria Local Government Areas in Southern, Central and Northern zones of Kaduna State respectively, where farming activities are very high involving the vulnerable groups (women and children between ages 10 and 15 years). The age considered in this study was 10 years and above including males and females peasant farmers. Ethical clearance was obtained from Kaduna State Ministry of Health. A feasibility study of the selected LGAs was carried out with the co-operation of the district heads; sensitization lecture was given to the people in all the study areas.

Sample Collection and Analysis

Sample Size: Using a reported 36% prevalence of intestinal parasitic contamination of vegetables in Jos, Plateau State, Nigeria by Damen *et al* (2007), the sample size was calculated using the formular of Israel (1992);

$$n = \frac{Z^2pq}{d^2}$$

Where n = number of samples to be collected
Z = standard normal distribution at 95% confidence limit = 1. 96
P = prevalence rate of infection of previous study = 36% = 0.36
q = 1- p
d = absolute desired precision = 0.05%

Therefore,

$$n=\frac{1.96^2 x 0.36 x 0.64}{0.05^2}$$

$$n=\frac{0.8851046}{0.0025}$$

n= 354.04184
n= 354 and approximated to 400 samples.

The calculated sample size was 354 but was estimated to four hundred (400) stool samples; these were collected from each Senatorial zone of which a total of one thousand two hundred (1,200) stool samples were collected from the three senatorial zones of the state and used for the study.

Inclusion and Exclusion Criteria

Children aged 10 years and above who participates actively or assist their parents in farming were included in the study and all consenting farmers.

Children below 10 years of age were excluded from the study, non- consenting parents together with their children as well as all civil servants, business or traders and those who do not depend on farming as their main source of income and livelihood were excluded.

A total of 1,200 samples were collected, comprising 400 samples from each Local Government of the three senatorial zones of the state. Specimens were collected using clean, leak-proof, and transparent, screw capped stool containers which were labelled and packed in insulated iceboxes and transported to the laboratories in Microbiology Department Ahmadu Bello University Zaria. The samples were analyzed starting with Macroscopy i.e. checking for colour, consistency and constituents of the stool. Microscopy was carried out using Direct Wet Mount and Formal-Ether Concentration Techniques; Using an applicator stick, about 1g (pea-size) of the stool was taken from a mixed specimen into about 10 ml of normal saline (physiological saline) it was emulsified and sieved through gauze using glass funnel into a pointed end glass centrifuge tube then washed twice by centrifuging at 3000rpm for 5 minutes, the supernatant was discarded and the deposit resuspended and transferred into a screw-cap centrifuge tube then 7ml of 10% formal saline was added then followed by 3ml of ether, it was covered and shake vigorously for 20 seconds, it was centrifuged at 3000rpm for 3 minutes. After centrifuging, the parasite's ova were sedimented to the bottom of the tube and the faecal – debris was collected in a layer between the ether and formal saline, using the applicator stick , other 3 layers were removed leaving only the sediment at the bottom of the tube which were suspended and a drop of it placed at the centre of a clean grease free slide and covered with cover

slip carefully avoiding air bubbles and examined systematically under x10 and x40 objectives respectively (Cheesbrough, 2015). The results were recorded as Scanty 1-3 (+) per preparation, few 4-10 (++), Moderate 11-20 (+++) and Heavy 21-40 (++++) (Cheesbrough, 2010). Remaining Samples were preserved using 95% ethanol and kept in the refrigerator at -4^{o}C for further molecular studies.

Data Analysis

The results obtained were analysed using Statistical Package for Social Science (SPSS) Version 22. Pearson chi-square test was used to measure the association between variables. Statistical significance was indicated by a two-tailed test at 95% confidence intervals and $P \leq 0.05$ was considered significant.

Molecular Analysis

DNA Extraction

The PowerSoil DNA Kit was used according to manufacturer's instructions, in the DNA Laboratory, Faculty of Veterinary Medicine Ahmadu Bello University Zaria, Nigeria. Genomic DNA was extracted directly from microscopically positive fecal samples. Approximately 0.3 g of fecal sample was added into a Power Bead Tube and incubated at 70°C for 10 minutes with the presence of cell lysis and disruption agent provided by the manufacturer. Subsequently, the fecal sample was subjected to homogenization and lysis procedure for complete cell lysis by mechanical shaking using Vortex Machine (J.P.SELECTA,7001721).The extraction of the DNA was carried out using the cold centrifuge machine (SUNON WEALTH ELEC MACH TGL-16G). Final elution of DNA was performed in 50 mL of elution buffer. The extracted DNAs were stored at –20°C until required for polymerase chain reaction (PCR) amplification after the DNA quantification using spectrophotometer (Shimadzu1700) to determine the average concentration and purity of the DNA extracted (Gallagher and Wiley 2008).

Designed Primers

Table 1: Primers Sequences used for the two step Semi- nested PCR designed by Gasser, *et al*., 2009.

PCR run	Hookworm spp	Primer sequence	Base pair size
First PCR	**N. americanus** **A. duodenale**	NC1 (5′-ACG TCT GGTTCA GGG TTC TT-3′) NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′)	
Second PCR	**N. americanus** **A. duodenale**	NA (5′-ATG TGC ACG TTA TTC ACT-3′) NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) AD1 (5′-CGA CTT TAG AAC GTTTCG GC-3′ NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′)	310 420

SEMI-NESTED PCR ASSAY (PROTOCOL)

A two-step semi-nested PCR was used for DNA amplification of hookworm species. For the first amplification, forward primer NC1 (5'-ACG TCT GGT TCA GGG TTC TT-3') and reverse primer NC2 (5'-TTAGTT TCT TTT CCT CCG CT-3') was used to amplify approximately 310-basepair and 420-basepair regions of internal transcribed spacer 2 and 28S ribosomal RNA region of *N. americanus* and *Ancylostoma spp*.

The PCR was conducted in a 50 mL volume with the final mixture containing 10x PCR buffer, 2.5 Mm dNTPs, 25mM MgCl2, 10 pmol of each primer, 5 units of Taq polymerase, and 6 mL of DNA template.

The sample was heated to 94°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds (denaturing), 55°C for 30 seconds (annealing), and 72°C for 30 seconds (extension), and a final extension at 72°C for 7minutes.

Negative control samples without DNA (DNase/RNase free water) and samples containing *N. americanus* and *Ancylostoma spp*. genomic DNA (positive control) was included in each PCR run. Subsequently, samples that produced fragment approximately 310 and/or 420 base pairs in the first PCR were subjected to a second amplification. Amplification was conducted by using forward primer NA (5¢-ATGTGCACGTTATTCACT-3¢) for *N. americanus*18 and AD1 (5¢-CGA CTT TAG AAC GTTTCG GC-3¢) for *Ancylostoma spp* and NC2 as a common reverse primer.

The secondary amplification reagent concentrations were similar to those of the first round of PCR except that 6 mL of primary PCR product was added instead of DNA. The cycling conditions for the second round amplification was 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute (denaturing), 55°C for 1 minute (annealing), and 72°C for 1 minute (extension), and a final extension at 72°C for 7 minutes. In both amplifications, samples were incubated in the Thermal Cycler.

Electrophoresis

The PCR products (amplicons) were optimized and 5 amplicons were selected from each LGA by simple random sampling, thereby making a total of 15 amplicons altogether. The 15 amplicons were loaded into their respective wells and ran at the same time with the molecular ladder (marker) and controls in 1.5% agarose gel for 40 minutes at 120 Amp voltage.

The amplicon's bands were viewed under gel dock. The best 3 amplicons were selected, one from each of the

three LGA. These were cut out, purified and sequenced using CEQ2000 DNA Analysis system sequencer (beckman coulter). Basic Local Alignment Search Tool (BLAST) was carried out to check the identity of the isolates from NCBI Gene Bank as well as the means to determine the Hookworm specie and finally the Phylogenetic tree was constructed using the Neighbour–joining and boostrab analysis method.

Data Analysis

The results obtained were analyzed using Statistical Package for Social Science (SPSS) Version 22. Pearson chi-square test was used to determine the association between variables. Statistical significance was indicated by a two-tailed test at 95% confidence intervals, $P \leq 0.05$ was considered significant.

RESULTS

Out of the 1,200 stool samples examined, the overall prevalence of hookworm was 18.7% (224/1200). In respect to LGA, prevalence of hookworm infection was 96 (24.0%) in Jema'a, 69 (17.3%) in Chikun and 59 (14.8%) in Zaria Local Government Area respectively (**Table 2**).

Table 2: Prevalence of Hookworm Infection In three Local Government Areas of Kaduna State (Using Stool Microscopy).

Local Govt Area	Number Examined	Number Positive (%)	Number Negative (%)	p-value
Jema'a	400	96 (24.0)	304 (76.0)	0.002
Chikun	400	69 (17.3)	331 (82.8)	
Zaria	400	59 (14.8)	341 (85.3)	
Total	**1, 200**	**224 (18.7)**	**976 (81.3%)**	

The result of the electrophoresis of 15 amplicons obtained from 15 positive stool samples from the three selected LGAs showed a corresponding base pair bands of 310bp which corresponds with the base pair of the N. americanus positive control. Hence, further investigation was carried out by selecting one strongly positive sample from each of the three LGA for sequencing, BLAST and phylogenetic analysis.

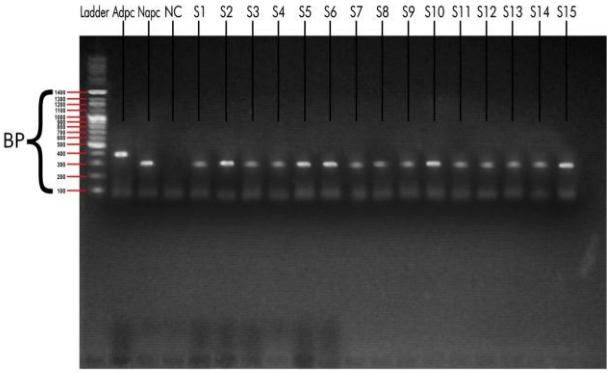

Plate 1: PCR gel Photo of Amplicons of Hookworm isolated from stool samples of peasant farmers showing the BP of CO1 Gene.

Key:
BP – Base pair, Adpc – *Ancylostoma duodenale* Positive control,
Napc – *Necator americanus* positive control, NC – Negative control, S - Sample

The result of the sequencing and basic local alignment search tool (BLAST) obtained showed that Sequence of Sample No. 5 from Zaria LGA had 95% identity with that of *N. americanus* obtained from Togo by Hu *et al*., (2003). Sequence of Sample No. 10 from Chikun LGA had 99% identity with that of *N. americanus* obtained from Togo by Hu *et al*., (2003) and finally Sequence of Sample No.15 from Jema'a LGA also gave a similar correlation of 97% as that of *N. americanus* from Togo obtained by Hu *et al*., (2003).

The result of the specie specific identification of the two human hookworm species (i.e. *N. americanus* and *A. duodenale*) obtained shows that *N. americanus* is predominant in the study area (Kaduna State Nigeria).

Table 3: Result of Nucleotide Sequences submitted to NCBI Gene Bank (submission ID: 2109788).

Sample No	LGA	Accession No	Max Score	Total Score	Query cover (%)	E Value	% identity	Decription of organim	Country
5	Zaria	M H 311534	676	676	100	0.0	95	*N. americanus*	Nigeria
10	Chikun	M H 311535	839	839	100	0.0	99	*N. americanus*	Nigeria
15	Jema'a	M H 311533	835	835	99	0.0	97	*N. americanus*	Nigeria

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
JLGA            ATACCCACAA AAAAACCGTA ACAAATACAG TTCATACTAA CAAACTTATA
ZLGA            ACAAAAAAAC CGTAACAAAT ACAGTTCATA CAAACAAACT TATATGTTCT
CLGA            ATACCCACAA AAAAACCGTA ACAAATACAG TTCATACTAA CAAACTTATA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
JLGA            TGTTCTAAAG AAATAGATCT ACTACGCAAA TTTTTTGTCG TACATATAAA
ZLGA            AAAGAAATAG ATCTACTACG CAAATTTTTT GTCGTACATA TAAAATTAAT
CLGA            TGTTCTAAAG AAATAGATCT ACTACGCAAA TAAATGTCGT ACATATAAAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        110        120        130        140        150
JLGA            ATTAATACCA CCTAAAATAG AACTTAAACC AGCACAATGC AAACTAAAAA
ZLGA            ACCACCTAAA ATAGAACTCA AACCAGCACA ATGCAAACTA AAAATAGCTA
CLGA            TTAATACCAC CTAAAATAGA ACTTAAACCA GCACAATGCA AACTAAAAAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        160        170        180        190        200
JLGA            TAGCTAAATC AACACTTCTA CCTGGATGTC CTAACGTACT TAAAGGTGGA
ZLGA            AATCGACACT TCTACCTGGA TGCCCTAACG TACTTAAAGG TGGATAAACA
CLGA            AGCTAAATCA ACACTTCTAC CTGGATGTCC TAACGTACTT AAAGGTGGAT

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        210        220        230        240        250
JLGA            TAAACAGTCC AACTAGTCCC ACAACCTATA TCGACAAAAC ATGAATCTAA
ZLGA            GTTCAACTAG TTCCACAACC TATATCAACA AAACATGAAT CTAAAATCAA
CLGA            AAACAGTCCA ACTAGTCCCA CAACCTATAT CGACAAAACA TGAATCTAAA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        260        270        280        290        300
JLGA            AATCAAAAAC ATAGCAGTGG GTAATAATCA AAAACTTAAA TTATTTAAAC
ZLGA            AAACATAGCA GTAGGTAATA ATCAAAAACT TAAATTATTT AAACGAGGAA
CLGA            ATCAAAAACA TAGCAGTGGG TAATAATCAA AAACTTAAAT TATTTAAACG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        310        320        330        340        350
JLGA            GAGGAAAACT TATATCCGGA GCCCCCAACA TTAAAGGTAA CATTCAATTA
ZLGA            AACTTATATC CGGAGCCCCC AACATTAAAG GTAACACTTC AATTACCAAA
CLGA            AGGAAAACTT ATATCCGGAG CCCCCAACAT TAAAGGTAAC ATTCAATTAC

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        360        370        380        390        400
JLGA            CCAAAACCAC CAATTATTCT AGGTATTACC ATAAAAAAAA TTATTAAAAT
ZLGA            ACCACCAATT ATACTAGGGT ATAACCATAA AAAAAATTAT TAAAATAGCA
CLGA            CAAAACCACC AATTATTCTA GGTATTACCA TAAAAAAAAT TATTAAAATA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                        410        420        430        440        450
JLGA            AGCATGTGCC GTAATAACAG AATTATATAA CTGACCATTC CCCAACAACA
ZLGA            .......... .......... .......... .......... ..........
CLGA            GCATGTGCCG TAATAACAGA ATTATATAAC TGACCATTCC CCAACAACAA
                ....|....| ....|....| ....|
                        460        470
JLGA            AACCAGGCTT AGCCAACTCC AAATA
ZLGA            .......... .......... .....
CLGA            ACCAGGCTTA GCCAACTCCA AACG.
```

Fig. 1: Showing sequence alignment of the three Hookworm Isolates Alignment: C:\Users\USER\ Desktop\ All hookworm Seq WEB. Fas.

The result of the Phylogenetic analysis generated, gave a cladogram which indicated that the *N. americanus* obtained from the study area are all from the same genetic origin, even though that of Jema'a LGA and Chikun LGA tends to be more closely related than that of Zaria LGA. There is also a close genetic relationship between the isolates (*N. americanus*) from this study area (Kaduna, Nigeria) and those of Togo and China.

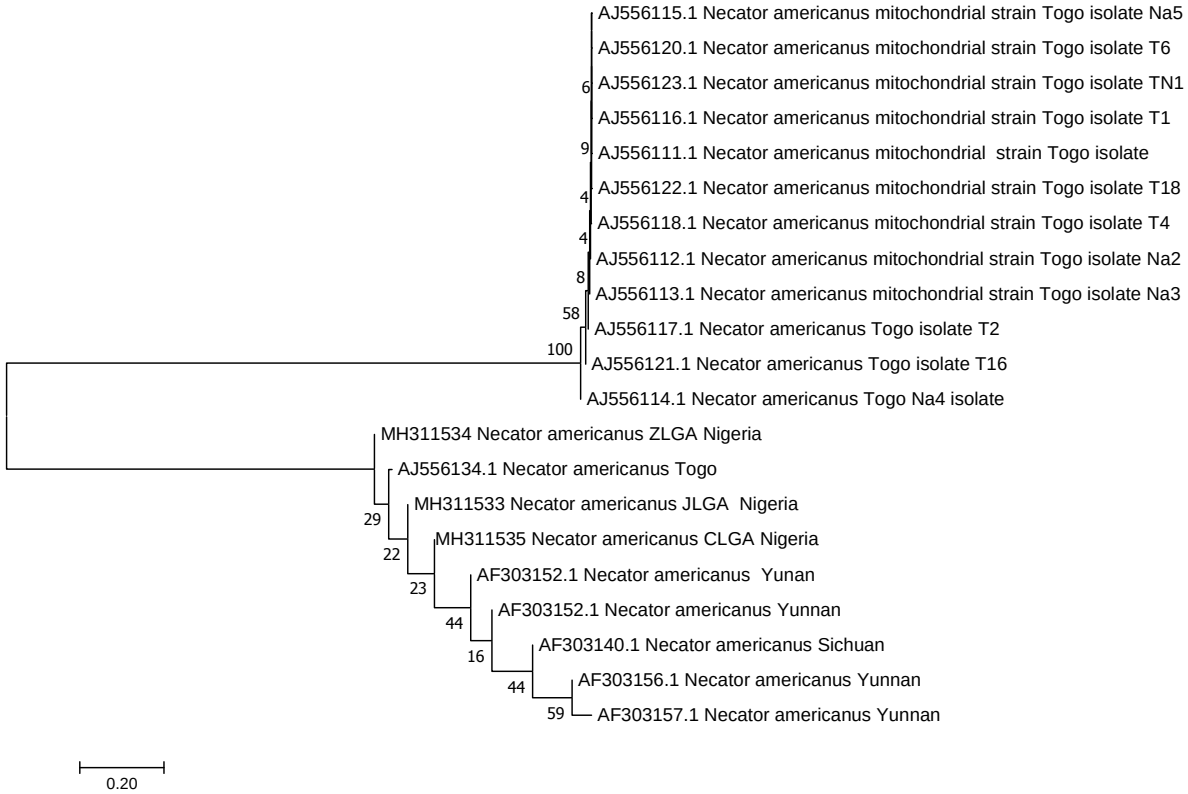

Fig. 2: Phylogenetic Tree of the Hookworm Isolates Obtained From Peasant Farmers.

The Phylogenetic Tree Was Constructed Using The Neighbour – Joining Method and Boostrap analysis.

DISCUSSION

The result of microscopy, showed higher prevalence of Hookworm infection in Jema'a LGA of the Southern Senatorial Zone followed by Chikun LGA of the Central Senatorial Zone and Zaria LGA of Northern Senatorial Zone had the least with a statistical significant difference. The overall prevalence of hookworm infection in the study area (Kaduna State) was 18.7%.

The differences may be due to the topography of the location, the climatic condition, type of soil and the tradition as well as the agricultural cultural practices of the people and their environmental sanitary attributes.

It was also noted that most of the farmers in Zaria LGA have the privileged of using pipe borne water and bore holes as their source of drinking water while most of the farmers in Jema'a and Chikun uses mostly water from the rivers and wells which are more exposed to all kinds of pollution or faecal contamination. They also had lower number of toilet facilities and many of them undergo backyard farming barefooted (Brooker *et al*, 2004; Thomas, 2009). Hence, these could be the reasons for their differences in prevalence rate.

The result of the electrophoresis of 15 amplicons obtained from 15 positive stool samples from the three selected LGAs showed a corresponding base pair bands of 310bp which was identical with the base pair of the *N. americanus* positive control. Hence, further investigation was carried out by selecting one strongly positive sample from each of the three LGA and all were positive for *N. americanus* infection which indicated that *N. americanus* is dominant over *A. duodenale* in the study area.

The result of the sequencing and basic local alignment search tool (BLAST) obtained showed that Sequence of Sample No. 5 from Zaria Local Government Area had 95% identity with that of *N.americanus* obtained from Togo by Hu *et al*., (2003). Sequence of Sample No. 10 from Chikun Local Government Area had 99% identity with that of *N.americanus* obtained from Togo by HU *et al*., (2003) and finally Sequence of Sample No.15 from Jema'a Local Government Area also gave a similar identity of 97% as that of *N. americanus* from Togo obtained by Hu *et al*., (2003) indicating that all isolates are from the same genetic origin.

The result of the specie specific identification of the two human hookworm species (i.e. *N. americanus* and *A. duodenale*) obtained revealed that *N. americanus* is predominant in the study area (Kaduna State, Nigeria). However, i strongly believe that there could be presence of mixed infection of *A. duodenale* missed out during the selection of samples for molecular study. In addition, the migration of people from Europe and other endemic areas of *N. americanus* during Slave trade Missionary era and even up till today to Sahara and Sub- Saharan Africa could have contributed to the transmission of the *N. americanus* to Kaduna State, Nigeria and other West African countries such as Togo.

Furthermore, considering the fact that climatic condition play a vital role in the growth, replication and transmission of hookworm and that *N. americanus* is a worldwide parasite but strive better in the warm region of China, Sahara and Sub- Saharan Africa, it is possible that after being transferred to these region became highly proliferative due to conducive environmental factors suitable for them, hence dominate the presence of *A. duodenale.*

The result of the Phylogenetic analysis generated, gave a cladogram which indicated that the *N. americanus* obtained from the study area are all from the same genetic origin, even though that of Jema'a LGA and Chikun LGA tends to be more closely related than that of Zaria LGA and this could be due to the fact that Jema'a LGA is geographically closer to Chikun LGA thus share very similar climatic factors also genetic mutation as a result of little changes in environmental factors could be the result of such little differences.

It is also obvious that there is close genetic relationship between the hookworm isolates (*N. americanus*) from Nigeria and that of Togo, this could be due to the fact that Nigeria has a close border with Togo hence easy migration by infected individuals from Togo to Nigeria and verse versa, on the other hand, the close genetic relationship between the hookworm isolates (*N. americanus*) from Nigeria and that of China could also be due to movement or migration of Chinese engineers who came to Nigeria and constructed rail way lines particularly in the study area (Kaduna State).

The result of this work agree with the work of many researchers who observed that *N. americanus* is endemic in many Tropical and Subtropical regions of the world, including parts of Africa, India, China, South-east Asia, the South-west Pacific Islands, South and central America, the Caribbean Island and Southern USA (e.g. Liu *et al*., 1999; Behnke *et al*., 2000; Gandhi *et al*., 2001).

CONCLUSION

The results of this study indicated an overall prevalence rate of hookworm infection of 18.7% among peasant farmers in Kaduna State. This indicates a potential risk of severe anaemia among individuals particularly the vulnerable groups such as pregnant women and children of school age who participate in farming.

Further studies by molecular detection using the PCR and gel- electrophoresis revealed that *N. americanus* is the main hookworm specie common in the study area. However, there is high possibility of getting some *A. duodenale* or mix infection of *N. americanus* and *A. duodenale* if higher number of samples are used for molecular detection.

The sequencing and basic alignment search tool (BLAST) further confirmed that hookworm was isolated and all were *N. americanus* species. Therefore, it can be concluded that *N. americanus* is the major hookworm specie common in the study area since the result of the Phylogenetic analysis generated, gave a cladogram identical to that of the gene bank obtained from Togo and China as well as "one clade" (Jema'a LGA, Chikun LGA and Zaria LGA) which confirmed that the *N. americanus* obtained from the study area are all from the same genetic origin.

It is very important to note that farming is an occupation that is practiced all over the whole world; there is no nation that can survive without agriculture. Before the discovery of petroleum in Nigeria, agriculture was the number one source of income in the Nigerian economy. Now considering the Nigerian economic recession, the Government is calling on all Nigerians to turn to agriculture which I concur.

Finally, from both past and present studies there is overwhelming evidence that hookworm infection is now a public health problem; there is also good evidence from pilot studies that the problem is amenable to solution.

RECOMMENDATIONS

Based on the findings in this study, the following recommendations were made

1. There should be awareness campaign programmes in respect to the indiscriminate defecation on farm land and the use of untreated faeces as manure on farms.
2. Indiscriminate stooling should be discouraged especially the practice of defaecation on farm land, backyard and bush should be prohibited; provision of toilet facilities will help in reducing the spread of Hookworm infection. Untreated human excreta, animal dung or raw sewage should not be used as fertilizer in agriculture unless decomposed to destroy the ova and larvae of hookworm by heat generated or ammonium sulphate should be added to the fresh faeces to strength of 12% to destroy the hookworm embryo within 24 hours. People should not walk bare-footed especially in known infected areas and the use of personnel

protective equipment by farmers should be encouraged.

3. Pipe-borne water and bore-hole should be made available to the populace to replace rivers and other bad sources of water supply and should be treated.
4. Mass treatment of infected farmers and the general public is highly recommended, especially in the rural areas where peasant farming is being practiced. It is also recommended that if the patient is suffering from severe anaemia ferrous sulphate (200mg) be administered three times daily at the same time with antihelmintic treatment; this should be continued until haemoglobin values return to normal.
5. Ground-itch, blisters and wounds were found to be common among farmers in the study area which corresponds to the clinical pathogenic characteristics of *N. americanus*. Hence, blisters and wounds caused by allergic reaction should be cleaned and properly treated by health personnels.
6. The Government of Kaduna State should enforced the law on environmental sanitation and ensure proper disposal of sewage and faecal matter in both rural and urban areas of the state.
7. A similar study should be carried out in the same study area and the number of positive samples used for specie specific molecular identification be increased to see if there could be *A. duodenale* or mix infection of *N. americanus* and *A. duodenale.*

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