

Molecular Identification of Mitochondrial Cox 1 Gene in *Bulinus* Snail Vectors of Urinary Schistosomiasis Isolated from Rice Farms in Kaduna State, Nigeria.

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ABSTRACT

Urinary schistosomiasis is an important and the most prevalent water-based human parasitic diseases encountered among rural farming populations particularly, rice farmers. *Bulinus* snails are intermediate hosts that transmit *Schistosoma haematobium* infection. Molecular identification of the mitochondrial *Cox1* gene of cercariae isolated from *Bulinus* snails recovered from some selected rice farms in Kaduna State, Nigeria was carried out using multiplex polymerase chain reaction. A total of twelve (12) *Bulinus* snails, three (3) from each geographical zone were randomly collected, identified and exposed for cercarial shedding. Isolated DNA from the cercariae were subjected to PCR amplification of the *Dra1* repeat fragment to confirm their infectivity status. Differential diagnosis of the cercariae was carried out using the mitochondrial *Cox1* gene as a diagnostic molecular marker of the *Schistosoma* species. Microscopic examination revealed a total of 9 (75.0%) of the *Bulinus* snails shed cercariae. Giwa and Kajuru Local Government Areas each had a prevalence of 3 (100%), Igabi 2 (66.7%) and Kachia had the least, 1 (33.3%). All the snails 12 (100%) revealed the *Dra1* repeat fragments amplified at the 121 bp implying that they were all infected with schistosomes. The mitochondrial *Cox1* gene revealed the cercariae of both *Schistosoma haematobium* and *Schistosoma bovis* presumably implying the possibility of hybridization and introgression of the parasite which is an indicator of eventual potential evolution of new species of schistosomes in the study area. Further research using specific nuclear marker profiles for their identification should be explored.

Keywords: Urinary schistosomiasis, *Bulinus*, Rice farms, *Cox1* gene, Nigeria

INTRODUCTION

Urinary schistosomiasis is a human disease, which is caused by the trematode *Schistosoma haematobium*. Various species of freshwater snails belonging to the genus *Bulinus* act as intermediate hosts of this aetiological agent in humans and its closely-related animal infective species (Rollinson *et al.*, 2001; Kane *et al.*, 2008). They are found around sources of water such as streams, slow flowing rivers, ponds and irrigation canals. Disease transmission is further aided via active penetration of the skin by the cercariae during various human water- contact activities for recreational, domestic, occupational and agricultural purposes (WHO, 2015). The cultivation of rice in flooded fields and swampy areas can facilitate the transmission of urinary schistosomiasis by providing a suitable environment for the survival of the intermediate host (*Bulinus* snail).

In Kaduna State, Nigeria, approximately 80% of the population relies on agriculture, specifically rice cultivation. Because rice farming often necessitates irrigation from natural or artificial water bodies, farmers frequently work in direct contact with these sources. This exposure poses a significant risk for urinary schistosomiasis, a disease that can severely impair labour output and overall agricultural productivity. Reports have established that both natural (McManus *et al.*, 2011) and artificial water bodies (Duwa and Oyeyi, 2009) serve as transmission foci of the parasite. Examining cercariae or infected *Bulinus* snails for estimating the transmission of *S. haematobium* is always confounded by the need to differentially identify it from its closely- related animal infective *Schistosoma* species (*S. bovis*, *S. mattheei*, *S. curassoni*, *S. intercalatum* and *S. magrebowiei*) since they develop in the same intermediate host. The implications of these observations are unclear for human disease, but these populations of hybrid schistosomes could prove problematic if they can replace existing species and parasite strains or extend intermediate host ranges. It was observed by Hamburger *et al.* (2004) that PCR can enable detection of snail infection from its very earliest stages and can identify the entire population of infected snails, regardless of whether they eventually shed cercariae. The use of Polymerase Chain Reaction (PCR) has been adopted for detection of a tandemly repeated DNA sequence termed *Dra1* in the genome of *S. haematobium* (Hamburger *et al.*, 2001; Ibiroke *et al.*, 2011). Differential tools for the accurate monitoring of infected snails using internal transcribed spacer and mitochondrial *cox1* genes are needed. The aim of this study therefore, was molecular identification of mitochondrial *cox 1* gene in *Bulinus* snail vectors of urinary schistosomiasis isolated from rice farms in Kaduna state, Nigeria.

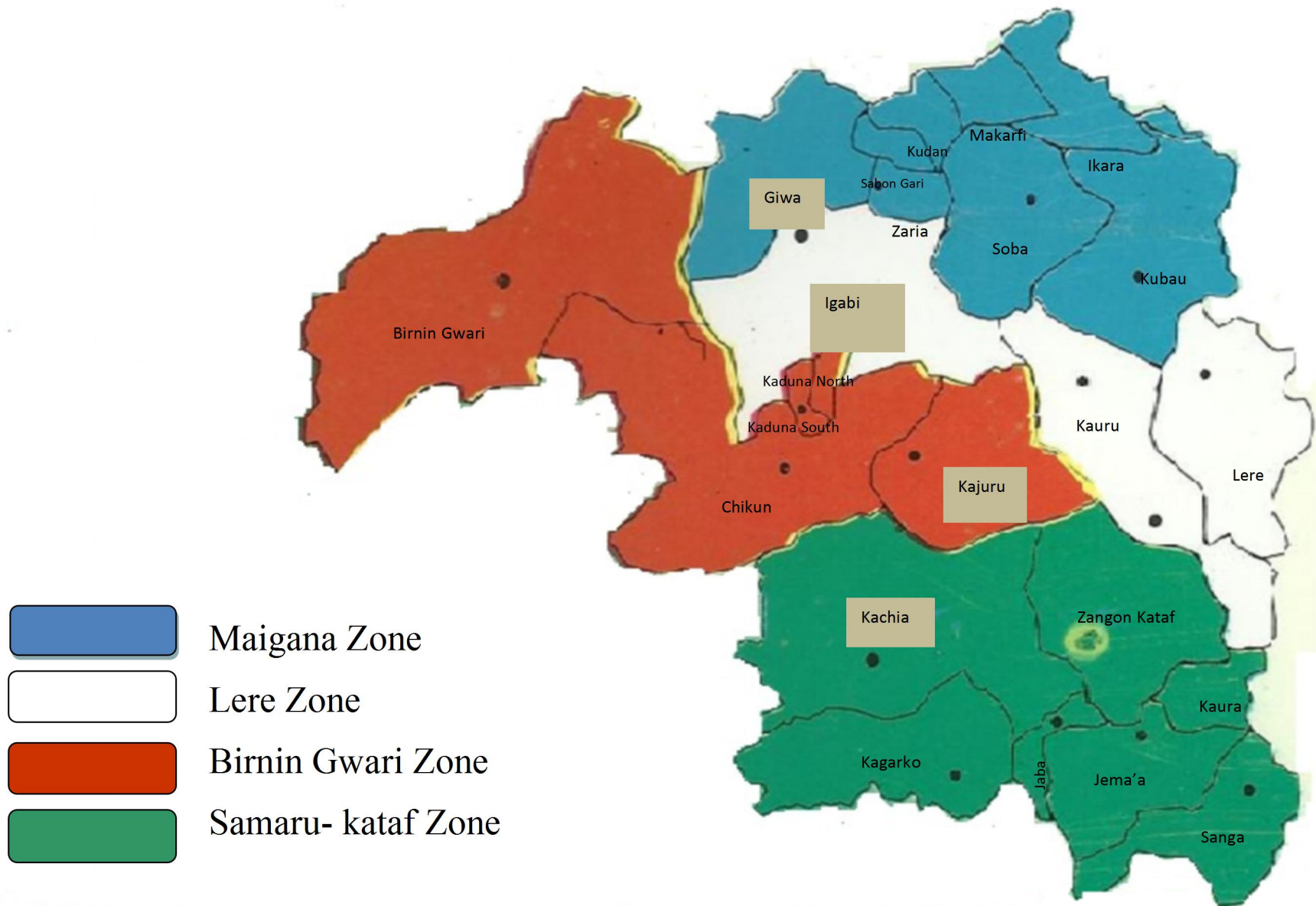


Fig. 1: Map of Kaduna State showing the sampling locations

Source: Kaduna State Agricultural Development Program (KADP)

Collection of *Bulinus* snails

Adult *Bulinus* snail samples were collected using a pair of long forceps from the rice farms and also from ponds, dams and other water bodies which were linked to the rice farms and served as snails' habitats from the respective study sites in Kaduna State, Nigeria. They were kept in a glass beaker containing dechlorinated water or rainwater, with some stones and snail food (*Daucus carota* L. (carrot leaf) and *Tridax procumbens* (Coatbuttons)). Morphological identification was done at the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

The *Bulinus* snails were then exposed to sunlight to allow shedding of cercariae and viewed under the microscope. The harvested cercariae were preserved in 70% ethanol and kept at room temperature for future use.

Genomic DNA extraction from cercariae

The preserved tissues from each of the *Bulinus* snail containing the cercariae placed in sterile 1.5 ml Eppendorf tubes and preserved in 70% ethanol were spun for one minute at 10,000 x g. The supernatant was removed and the residue was re-suspended in phosphate buffer saline (PBS). Four hundred (400 µl) of tissue lysate buffer was placed in an Eppendorf tube and 10 µl of proteinase K was added. It was vortexed and incubated at 65 °C on a heat block (Lab-line® Incorporated) and vortexed again at intervals of 20 minutes for one hour thirty minutes (1 hour 30 minutes). To this mixture, 400 µl of phenol-chloroform was added. This was then vortexed and spun for 10 minutes at 10,000 x g. The supernatant was separated into new Eppendorf tubes and 400 µl of chloroform was again added. This was then mixed and centrifuged at 10,000 x g for 1 minute. The upper aqueous layer was removed into new sterile Eppendorf tubes and 1000 µl of absolute ethanol plus 40 µl of three molar (3 M) sodium acetate was added, mixed and incubated at – 20 °C overnight in order to enhance DNA precipitation. The resulting solution was spun for 10 minutes at 12,000 X g and the pellets were washed with 400 µl of 70% ethanol and spun for another 5 minutes. The supernatant was then removed and the pellets were dried at room temperature. When completely dry, the pellet was re-suspended in 50 µl of deionized water and stored at – 20 °C. The extracted DNA was validated by checking for its purity on agarose gel electrophoresis and later excised for polymerase chain reaction.

Polymerase Chain Reaction amplification and visualization

PCR amplification targeting the schistosome *Dra1* repeat was performed on genomic DNA extracted from the snails' cercariae. The reaction used the forward primer 5'-GATCTCACCTATCAGACGAAAC-3' and the reverse primer 5'-TCACAACGATACGACCAAC-3', as previously described by Hamburger et al. (2001) and Akinwale et al. (2011), producing an expected amplicon of 121 base pairs (bp). Detection of the *Cox 1* genes from the cercariae was carried out using a 20 bp universal forward primer for both *S. haematobium* and *S. bovis* (*Shb*) 5' TTTTTTGGTCATCCTGAGGTGTAT 3' and species-specific reverse primers were used to produce amplicons of different sizes, *S. haematobium* (*sh*) of size 543 base pairs (bp) 5' TGATAATCAATGACCCTGCAATAA 3' and *S. bovis* (*sb*) of size 306 base pairs (bp) 5' CACAGGATCAGACAAACGAGTACC 3' (Webster et al., 2010). Snails' identification was performed by PCR amplification of the snails' ribosomal internal transcribed spacer

(ITS) region using forward primer *Etts1* 5' TGCTTAAGTTCAGCGGGT 3' and reverse primer *Etts2* 5' TAACAAGGTTTCCGTAGGTGAA 3' (Ten Hove *et al.*, 2008).

A multiplex PCR was performed on the *Bulinus* snails, and amplification was carried out using a thermal cycler (MJ Research, Inc., USA). The primers were reconstituted inside a PCR hood to prepare a working solution. A 10 µM working solution was obtained by adding 90 µl of nuclease-free water to 5 µl each of the forward and reverse primers. The samples were subsequently optimized to determine the optimum PCR conditions.

The total reaction volume for the PCR was 20 µl. The multiplex PCR PreMix (AccuPower®, Bioneer) contained 1 U of Taq DNA polymerase, pyrophosphatase and pyrophosphate, 250 µM each of dNTPs (dATP, dCTP, dGTP, and dTTP), and 1× reaction buffer with 2 mM MgCl₂, including stabilizer and tracking dye. The PreMix was reconstituted with 14 µl of nuclease-free water, followed by the addition of 2 µl each of the *Dra1* repeat primers, *Etts1* and *Etts2* primers, *Cox1* gene primers, and the DNA template (genomic DNA). For the negative control, all primers and nuclease-free water were added except the DNA template.

The reaction mixture was vortexed for 30 seconds and placed in the thermal cycler under the following conditions: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes. The PCR conditions used for the amplification of the *Cox1* gene were similar, except that the annealing temperature was adjusted to 52 °C. Amplification was confirmed by visualization on a 2% agarose gel, followed by photodocumentation using a gel documentation and analysis system (Bio-Rad, Italy).

Extraction and purification of PCR amplicons

The DNA fragments (PCR amplicons) obtained above were excised from the agarose gel and weighed in a 1.5 ml microtube. To every 100 mg was added 300 µl of gel buffer (Buffer GB -an agarose gel solubilisation and DNA binding buffer, which is used in DNA extraction from agarose gel or DNA clean-up procedures). The mixture was placed on a heat block at 50 °C for 8 minutes with mixing by tapping the tubes bottom every 2-3 minutes for complete melting. Exactly 1 gel volume (300 µl) Isopropanol was added to the mixture and mixed. To DNA mini columns with collection tubes, 750 µl of the mixture was transferred and centrifuged at 11,000 rpm for 30-60 seconds. The flow through was discarded and the columns were put back into the collection tube where 750 µl of the DNA wash buffer was added and centrifuged at 11,000 rpm for 1 minute at room temperature. The flow through was discarded and the empty columns were centrifuged at 11,000 rpm for 1 minute to remove residual ethanol. The columns were placed in clean 1.5 ml microcentrifuge tubes and 25 µl of elution buffer was added, incubated at room temperature for 1 hour and centrifuged at 11,000 rpm for 1 minute to elute the DNA which was stored at – 20 °C.

RESULTS AND DISCUSSION

Infection status of *Bulinus* snails

Bulinus snails collected from Giwa and Kajuru Local Government Areas each had a prevalence of 100% (3/3) based on the microscopic morphological appearance of the cercariae with Kachia having the least of 33.3% (1/3). However, they all had 100% (3/3) *Dra1* repeat fragment amplified at the 121 bp by PCR, implying that they were all infected with schistosomes as shown in Table 1.

Table 1: Infectivity status of *Bulinus* snails in the different study locations

Locations	Number examined	Microscopic identification of cercariae number (%) positive	<i>Dra1</i> repeat fragment number (%) positive
Giwa	3	3 (100)	3 (100)
Igabi	3	2 (66.7)	3 (100)
Kajuru	3	3 (100)	3 (100)
Kachia	3	1 (33.3)	3 (100)
Total	12	9 (75.0)	12 (100)

The infectivity status of the snails indicated by amplification of the *Dra1* repeat implies that all the snails collected from Giwa, Igabi, Kajuru and Kachia local government areas respectively were infected with schistosomes as shown in Plate I.

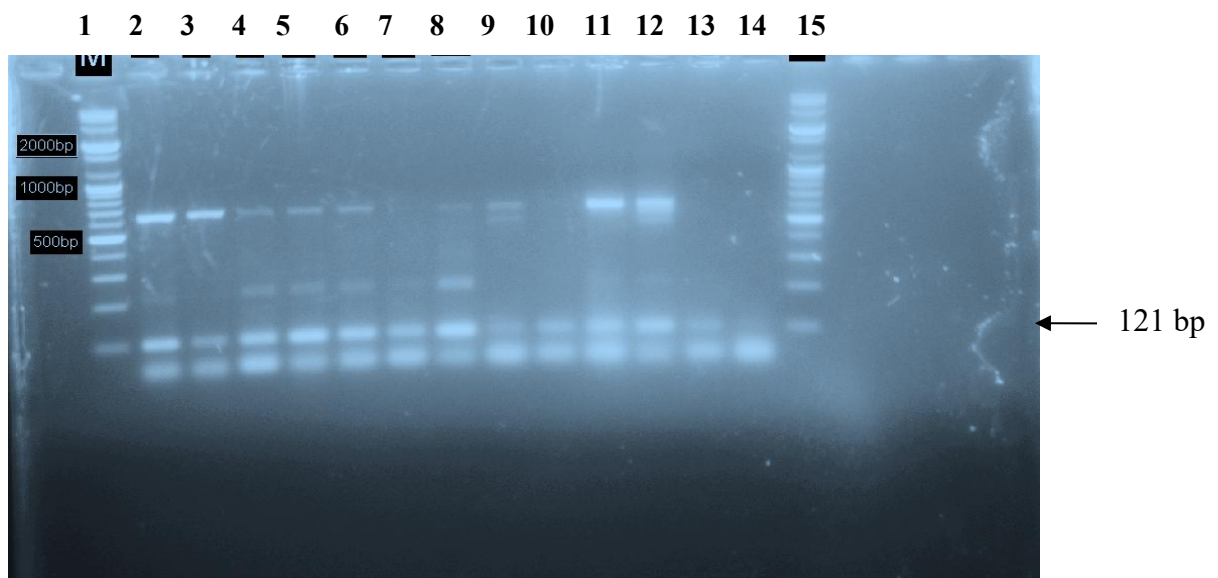


Plate I: Multiplex PCR amplicons of ITS region of *Bulinus* snails and *Dra1* fragments.

Key:

Lanes 1 & 15: 100bp DNA marker

Lanes 2, 3, 4, 5, 6, 9, 11 and 12: Amplicons of *Bulinus* snails' DNA.

Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13: Amplicons of *Dra1* fragments at expected 121 bp

Lanes 14: Negative control

Plate II represents the multiplex PCR amplification of the mitochondrial *Cox1* genes of cercariae from the *Bulinus* snails which produced bands of different sizes indicating the presence of both *Schistosoma haematobium* and *Schistosoma bovis*.

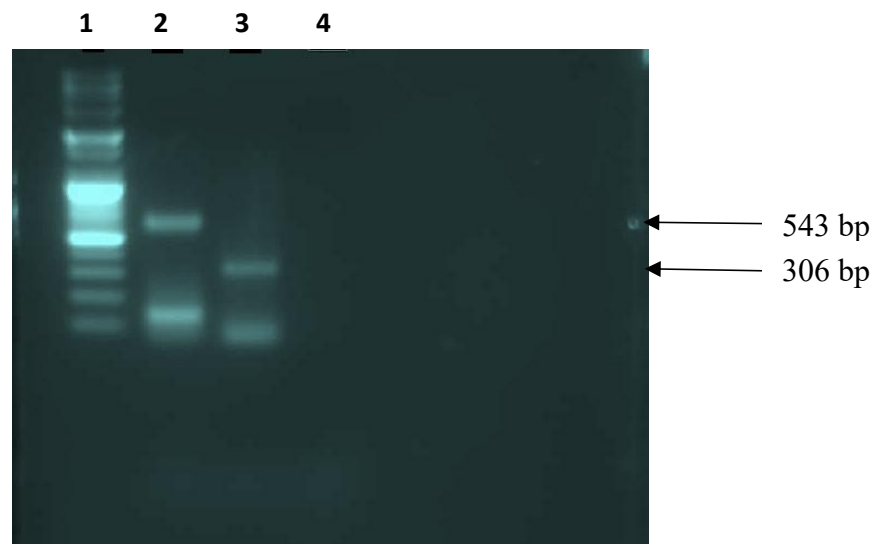


Plate II: Amplicons of the *mt Cox1* genes of *S. haematobium* and *S. bovis* in *Bulinus* snails.

Key:

Lane 1: 100bp DNA marker

Lane 2: Amplicon of *S. haematobium* at expected 543 bp band

Lane 3: Amplicon of *S. bovis* at expected 306 bp band

Lane 4 - Negative control

Plate III represents the multiplex PCR amplification of the mitochondrial *Cox1* gene of the cercariae from *Bulinus* snails which produced only bands of *Schistosoma bovis*.

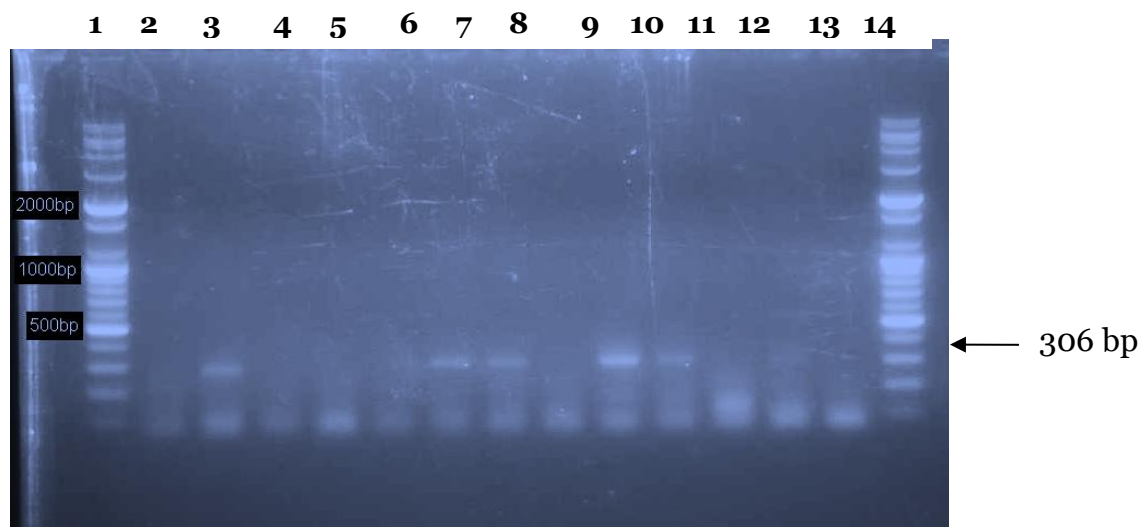


Plate III: Amplicons of the *mt Cox1* genes of *S. bovis*

Key:

Lanes 1 & 15: 100bp DNA marker

Lanes 3, 7, 8, 10 and 11: Amplicons of *mt Cox1* genes of *S. bovis* at expected 306 bp band

Lane 14: Negative control

Bulinus snails collected from the respective study locations were shown by PCR amplification of the *Dra1* repeat fragments to be infected, an observation which implied that, all the *Bulinus* snails in this study were shedding the bifurcated cercariae indicating their potential infectivity. The infectivity of the snails may be as a result of contamination of the rice farms or water bodies with infected human urine. The finding in this study was similar to observations made by Ajayi *et al.* (2015) and Hassan *et al.* (2016). In a study by Awua *et al.* (2019), the *Dra1* repeat fragments were also observed in farmers who actively participated in rice cultivation. This could probably have been due to the presence of these *Bulinus* snails in the rice fields.

The molecular detection of the *Dra1* repeat fragment by PCR showed potentially high infectivity in the snails. *Bulinus* snails which did not harbour any cercariae by microscopic examination were found to have the *Dra1* repeat fragment as well as detected by the PCR indicating the pre-patent stage of the infection in the snails. This is in consonance with findings of Ajayi *et al.* (2017) who reported the presence of the *Dra1* repeat fragments in all twenty-three (23) snails which were not shedding cercariae. This also concurred with findings of Hassan *et al.* (2016) who revealed higher prevalence rates of the *Dra1* repeat fragments in snails than by cercarial shedding. Establishing

infection in snails using the *Dra1* repeat fragment is a very useful tool in determining whether or not a snail has been exposed to and infected with schistosomes (Akinwale *et al.* 2011).

Differentiation of cercariae using mitochondrial *Cox1* genes

The identification of the transmission foci of *S. haematobium* and *S. bovis* in most epidemiological studies is usually complex due to the fact that the cercariae of both of these widely distributed species are difficult to distinguish as they are produced in the same bulinid snails. In the transmission loci, the snails' species exposed to the schistosome may not reveal which of the parasites is responsible for infection. From this study, the differential diagnosis employing the use of *Cox1* gene as a molecular marker in species identification revealed that the bulinid snails from these rice farms were infected with both *S. haematobium* and *S. bovis* as detected by the multiplex rapid diagnostic PCR (RD-PCR).

This finding is of great epidemiologic importance because, owing to the fact that these two parasites occur in sympatry in the study locations, there could be a possibility of hybridization of the parasites if they infect the farmers, and this could be debilitating to them if not checked. Evidence of hybridization between *S. haematobium* and *S. bovis* has been reported in about 88% of children surveyed in areas of suspected species overlap excreting hybrid miracidia in Senegal (Webster *et al.*, 2013). Ajakaye *et al.* (2024) also reported natural interspecific hybridization between the human and bovine parasites in Ondo and Jigawa States. *S. haematobium* – *S. bovis* hybrid has been widely studied across Africa and these hybrids may have grave consequential roles in the elimination of disease transmission, burden and morbidity. This is especially evident in Nigeria where nomadic herders migrate with their livestock across the different regions of the country with the possibility of infected animals excreting the *S. bovis* eggs along their paths and in open water body fields as they migrate.

These hybrids usually result from male and females of each species readily pairing to produce viable offsprings. If this is however the case, the rice farmers will potentially be at great risk of infection with these hybrids where the hybridization may eventually influence the phenotypic characteristics, disease epidemiology, transmission, morbidity and drug efficacy, consequently leading to a resistance in praziquantel with the evolution of new species of parasites (Detwiler and Criscione, 2010).

The hybridization can be facilitated by the natural progression in farming population (both human and livestock) movements and expansion as well as changes in snail distribution which create areas of sympatric distribution. A previous study by Huyse *et al.* (2009) reported *S. haematobium* / *S. bovis* hybrids which were sampled from humans from several widely dispersed villages along the Senegal river basin. A similar study by Bremond *et al.* (1993) also reported the possibility of the hybridization. Enudi *et al.* (2024) and Ajakaye and Enabulele (2025) also reported cases of *S. bovis* infecting humans in different parts of Nigeria. This is not surprising, and is inevitable as the creation of water bodies for agriculture especially rice fields can lead to increased prevalence and distribution of the intermediate host; the *Bulinus* snail, as well as human and animal movements to these areas, thereby increasing chances of sympatric transmission of these species. This sympatric association is known to be widespread

throughout much of Africa. Advances in parasite identification can help elucidate transmission patterns, intermediate host specificity and interactions between closely related parasites. Understanding and monitoring of such inter-species interactions will be essential for the optimization and evaluation of control strategies across such potential hybrid areas which may eventually turn out to be dreadful.

CONCLUSION

In conclusion, this study observed the presence of potentially infective parasites of *Bulinus* snails isolated from rice farms in some parts of Kaduna State, Nigeria. The *Dra1* repeat fragments were detected in *Bulinus* snail intermediate hosts that were not shedding cercariae as detected by PCR which suggest its usefulness and value for the identification of *S. haematobium* infections and *S. bovis* in low transmission settings. The mitochondrial *Cox1* genes employed in this study was able to differentiate between *S. haematobium* and *S. bovis* in parts of Kaduna State, Nigeria. Further analysis on the genetic variation of *S. haematobium* using the *Cox1* gene as a mitochondrial molecular marker should be investigated. Molecular tools like, specific nuclear marker profiles should be used to investigate the *Bulinus* snails in the study area for their proper identification as this will serve as a baseline for future research.

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CONFLICTS OF INTEREST

The authors declare there is no conflict of interest.

REFERENCES

- Ajakaye, O.G. and Enabulele, E.E. (2025). *Schistosoma bovis* infecting humans in Nigeria. *American Journal of Tropical Medicine and Hygiene*, 112(3): 713. [Doi:10.4269/ajtmh.24-0714](https://doi.org/10.4269/ajtmh.24-0714).
- Ajakaye, O.G., Enabulele, E.E., Balogun, J., Oyeyemi, O.T. and Grigg, M.E. (2024). Extant interspecific hybridization among trematodes within the *Schistosoma haematobium* species complex in Nigeria. *PLOS Neglected Tropical Diseases*, 18(4): e0011472. <https://doi.org/10.371/journal.pntd.0011472>
- Ajayi, J.B., Sam-Wobo, S.O. and Agbeyangi, A.O. (2015). Prevalence of Urinary Schistosomiasis in parts of Ogun State, Nigeria. *Journal of Natural Sciences Research*, 5(5):62-68.
- Ajayi, J.B., Sam-Wobo, S.O. and Agbeyangi, A.O. (2017). Molecular identification of *Bulinus* species in Ogun State, South-West Nigeria and observations on

- snail infections with schistosome. *International Journal of Horticulture, Agriculture and Food Science*, **1**(1):15-20.
- Akinwale, O.P. Kane, R.A, Rollingson, D., Stothard, J.R., Ajayi, M.B., Akande, D.O., Ogungbemi, M.O., Duker, C., Gyang, P.V. and Adeleke, M.A. (2011). Molecular approaches to the identification of *Bulinus* species in South-West Nigeria and observations on natural snail infections with schistosomes. *Journal of Helminthology*, **85**(3):283-293.
- Awua, Y., Inabo, H.I., Asuke, U.A. and Okubanjo, O.O. (2019). Evaluation of Polymerase Chain Reaction Technique in Confirming Urinary Schistosomiasis among Rice farmers in parts of Kaduna State, Nigeria. *Nigerian Journal of Parasitology*, **40**(1):82-86.
- Bremond, P., Sellin, B., Sellin, E., Nameoua, B., Labbo, R., Théron, A. and Combes, C. (1993). Evidence for the introgression of the human parasite *Schistosoma haematobium* by genes from *Schistosoma bovis* in Niger. *Comptes Rendus De L'Academie Des Sciences Iii Sciences De La Vie- Life Sciences*. **316**:667-670.
- Detwiler, J.T. and Criscione, C.D. (2010). An infectious topic in reticulate evolution: Introgression and hybridization in animal parasites. *Genes*, **1**:102-123.
- Duwa, M.R. and Oyeyi, T.I. (2009). The role of Jakara dam in the transmission of schistosomiasis. *Bayero Journal of Pure Applied Science*, **2**:58-63.
- Enudi, A.O. Nmorsi, O.P.G. and Egwunyenga, A.O. (2024). Human schistosomiasis due to *Schistosoma bovis* in Nigeria. *American Journal of Tropical Medicine and Hygiene*, **111**: 1230-1236.
- Hamburger, J., He, N., Abbasi, I., Ramzy, R.M. and Jourdane, J. (2001) Polymerase chain reaction assay based on a highly repeated sequence of *Schistosoma haematobium*: a potential tool for monitoring schistosome-infested water. *American Journal of Tropical Medicine and Hygiene*, **65**:907-911.
- Hamburger, J., Hoffman, O., Kariuki, H.C., Muchiri, E.M., Ouma, J.H., Koech, K.K., Sturrock, R.F. and King, C.H. (2004). Large-scale-polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: a new tool for studying the dynamics of snail infection. *American Journal of Tropical Medicine and Hygiene*, **71**:765-773.
- Hassan, A.O., Amoo, A.O.J., Akinwale, O.P., Adeleke, M.A. and Gyang, P.V. (2016). Molecular characterization and detection of infection in vector snails of urinary schistosomiasis around Erinle and Eko Ende dams in South West Nigeria. *British Microbiology Research Journal*, **14**(1):1-10.
<http://dx.doi.org/10.4314/njpar.v40i1.13>

- Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T., Polman, K. and Rollinson, D. (2009). Bidirectional introgressive hybridisation between a cattle and human schistosome species. *PLoS Pathogens*, **5**(9): e1000571.
- Ibironke, O.A., Phillips, A.E., Garba, A., Lamine, S.M. and Shiff, C. (2011). Diagnosis of *Schistosoma haematobium* by detection of specific DNA fragments from filtered urine samples. *American Journal of Tropical Medicine and Hygiene*, **84**:998–1001.
- Kane, R.A., Stothard, J.R., Emery, A.M. and Rollinson, D. (2008). Molecular characterisation of freshwater snails in the genus *Bulinus*: A role for barcodes? *Parasites and Vectors*, **1**(1): 15. Doi: 10.1186/1756-3305-1-15.
- McManus, D.P., Gray, D.J., Ross, A.G., Williams, G.M. and He, H.B. (2011). Schistosomiasis research in the dongting lake region and its impact on local and national treatment and control in China. *Neglected Tropical Disease*, **5**:1053-1053.
- Rollinson, D., Stothard, J.R. and Southgate, V.R. (2001). Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. *Parasitology*, **123**:245-260.
- Ten Hove, R.J., Verweij, J.J., Vereecken, K., Polman, K., Dieye, L. and van Lieshout, L. (2008). Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *Schistosoma haematobium* infection in stool samples collected in Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **102**(2): 179-185.
- Webster, B.L., Diaw, O.T., Seye, M.M., Webster, J.P. and Rollinson, D. (2013). Introgressive hybridization of *Schistosoma haematobium* group species in Senegal: Species barrier breakdown between ruminants and human schistosomes. *PLoS Neglected Tropical Diseases*, **7**: e2110. Doi: 10.1371/Journal.pntd.0002110.
- Webster, B.L., Rollinson, D., Stothard, J.R. and Huyse, T. (2010). Rapid diagnostic multiplex PCR (RD-PCR) to discriminate *Schistosoma haematobium* and *S. bovis*. *Journal of helminthology*, **84**, 107–114.
- World Health Organization (WHO) (2015). Epidemiological situation- Strategy- Partnership. <http://www.int/schistosomiasis/en>