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Molecular Characterization and Detection of Infection in Vector Snails of Urinary Schistosomiasis around Erinle and Eko Ende Dams in South West Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AOH and OPA conceived the concept and designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AOJA supervised the study and edited the manuscript. Author MAA performed the statistical analysis. Authors PVG and AOH performed the molecular assay and managed the analyses of the study. Authors AOH and MAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The prevalence of the schistosome cercariae in snail intermediate hosts has been known as one of the valuable predictors of the level of schistosomiasis transmission in different localities.

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This study was undertaken to determine molecular characterization and detection of infection in vectors snails of urinary schistosomiasis around Erinle and Eko-Ende Dams, South western Nigeria.
Study Design: Epidemiological survey.
Place and Duration of Study: Medical Microbiology and Parasitology Department, Obafemi Awolowo College of Health Science, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria between January 2010 and November 2012.
Methodology: The snails collected from communities around Erinle and Eko-Ende dams were identified using standard morphological keys. The infectivity of the *Bulinus* species by Schistosome was determined through cercaria shedding and Polymerase Chain Reaction (PCR) of amplification of Dra 1 gene repeats of *S. haematobium* while snail characterization was done using PCR-RFLP.
Results: Of the 277 snails screened, 78 (28.28%) were positive for cercaria shedding while 108 (38.98%) were positive for PCR screening. There was significant difference in the infectivity status determined by cercaria shedding and the PCR technique (p=0.05). All the snails characterized by PCR-RFLP were *Bulinus truncatus* showing the species is involved in the transmission of urinary schistosomiasis in the study area.
The relatively high prevalence of schistosome infection in snail intermediate hosts around the two dams suggests active transmission of urinary schistosomiasis and underscores the need for integrated control in tackling the menace of the disease at the study area.

Keywords: Bulinus; Schistosoma haematobium; cercaria shedding; PCR; Nigeria.

1. INTRODUCTION

Schistosomiasis transmission is affected by a variety of factors, including rates of human contact with infested water bodies, the breeding of intermediate host snails within water bodies, and the rate of successful entry and development of schistosome stages in both molluscan and human host [1]. Most intermediate host snails are known to be adapted to a broad range of environmental conditions like temperature, rainfall and salt concentrations [1,2]. They are also found in both standing and flowing water bodies. Water current velocity has a profound limiting effect on snail survival. According to [3], a drag current of between 0.36 m/s and 0.94m/s dislodges all snails and flushes them downstream. Impoundment of a river to create a man-made lake eliminates the controlling effect of current velocity and makes the resultant habitat more favourable to snail growth and propagation.

Freshwater snails belonging to the genus *Bulinus* act as intermediate hosts in the life cycle of the widespread of schistosomiasis In Africa, Madagascar and adjacent regions [4-6]. The relationship and interaction between schistosomes and snails is very specific and compatibility may differ over quite small geographical ranges [7]. As a result, there is need for a stout system of identification and classification to supplement the traditional approaches [4], when considering the host compatibility with *Schistosoma haematobium*. The nominal Bulinid species list indicates that only a few act as intermediate hosts of *S. haematobium*. Past review of [8] reviewed the relationship between the schistosome parasites and their intermediate hosts and reported a wide range of compatibility spectra [8] Recently [6], has posited that the use of PCR –RFLP for snail characterization is cheap and could be adapted in monitoring snail intermediate hosts involved in localized transmission of urinary schistosomiasis in Nigeria.

The methods for estimating schistosomiasis transmission potential focus on monitoring the components of the human-snail-water interface, where transmission occurs [9]. Surveillance techniques include monitoring schistosome egg output [10], water contact activities [11], snail infection rates, [12,13] and, quantity of cercariae in the water [14]. Measurement of infection rates in field populations of snails is a basic part of studies on the epidemiology of schistosomiasis [15]. The information obtained can facilitate planning of schistosomiasis control, the assessment of its outcomes and used for mathematical modeling of schistosome transmission for a specific area [16]. Snail infection rates are routinely measured only by determining the rate of snails shedding cercariae [17], bearing in mind that prepatent infection can last for several weeks with only a proportion of snails reaching the stage of cercarial shedding. It will variably exceed patent infection rates over time [18].

Though some attempts have been made in different parts of Nigeria to document the snail intermediate host infectivity with schistosome

infection, the information on snail intermediate hosts involved in the transmission of schistosomiasis and their infectivity status in many communities still remain understudied. With the exception of the report of [6] which studied few snail samples from two (Ilee and Oree) around Erinle dam, the large scale monitoring of the snail composition and transmittion of snails intermediate hosts at Erinle and Eko-Ende dams is still awaiting documentation. Information gathered from the residents around the two dams indicates that the urinary schistosomiasis ('Atosi Aja' in local language) is still a serious health problem in the study area. Designing effect control strategies to halt the transmission involves in-depth understanding of all the factors involve and promoting transmission of the disease in the study area. This study therefore utilized cercaria shedding, Polymerase Chain Reaction amplification of Dra1 gene repeats and PCR-RFLP to determine the species composition and infective status of the snail intermediate hosts of urinary schistosomiasis in Erinle and Eko-Ende dams, Southwestern Nigeria.

2. MATERIALS AND METHODS

2.1 The Study Area

The five communities around Erinle dam and Eko-Ende reservoir were studied. They are Ilie, Oba, Oke-Ore and Eko-Ende and Eko-Ajala respectively [Fig. 1]. The goegraphical coordinates of the area lies between Latitude 7°44' and 7°57' N and Longitude 4°26' and 4°41 East of the Greenwich Meridian. The Erinle Dam (Owalla Dam), is an extension of the old Ede Dam on Erinle River and Eko-Ende Dam on Otin River [19]. The study area is in tropical rainforest climate [19]. The mean annual rainfall is about 1400 mm with the wet season covering March to October with its beginning and end marked by torrential rains and thunderstorms. Temperatures are generally high and almost uniform throughout the year [20].

2.2 Ethical Clearance

Ethical clearance and approval was obtained from Osun state Ministry of Health and Ethical Committee of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State. Informed consent was also sought and obtained from the communities around the study area.

2.3 Snail Sampling and Morphological Identification of Snails

Sampling involved scooping with a Hairston drag (70 x 20 x 20 cm) scooper and manual search for snails attached to boats, bamboo fish traps, submerged stones, sticks and vegetation stands at human contact site in the reservoirs according to [21].

Snails were collected from identified human water contact sites in the reservoirs with the aid of a snail identification field guide. A number of several snails with similar morphological characteristics to the genus, *Bulinus,* were collected from the study site. A snail identification guide was used to separate *Bulinus* from other genus as described by [22-25], and confirmed at Molecular Parasitology Laboratory of Nigeria Institute of Medical Research Lagos.

2.4 Extraction of Genomic DNA from Snails

DNA was extracted from each snail using CTAB extraction buffer containing 2-mercaptoethanol, hexadecyltrimethyl-ammonium bromide (CTAB) (solid), *tris*(hydroxylmethyl) amino-methane, ethylenediaminetetraacetic acid, disodium salt solution (EDTA), and sodium chloride as previously described by [25] and [6]. Each snail was removed from the 70% ethanol and soaked in TE (10 mM Tris HCl and 1 mM EDTA) overnight so as to get rid of the remaining ethanol. Tissue from each of the snails was placed in a sterile 1.5 ml Eppendorf tube, 500 µl of CTAB solution added and the tissue was grinded followed by the addition of 10 µl of proteinase K solution (20 mg/ml) and incubated at 55℃ for 1 hr, with occasional gentle mixing. Genomic DNA was extracted from the CTAB buffer by adding an equal volume of chloroform and isoamyl alcohol (24:1) to each tube. The organic and aqueous layers were gently mixed for 5 min and spun at 13,000 rpm for 20 min. The upper aqueous layer was removed into another sterile Eppendorf tube and an equal volume of 100% ethanol was added, mixed and incubated at −20℃ overnight in order to enhance DNA precipitation. Resulting solution was spun at 13,000 rpm for 20 min and the pellet was washed with 70% ethanol and spun for another 20 min. The supernatant was removed and the pellets were dried at room temperature. When completely dried, the pellet was re-suspended in 25 µl of water and stored at 4℃ until used.

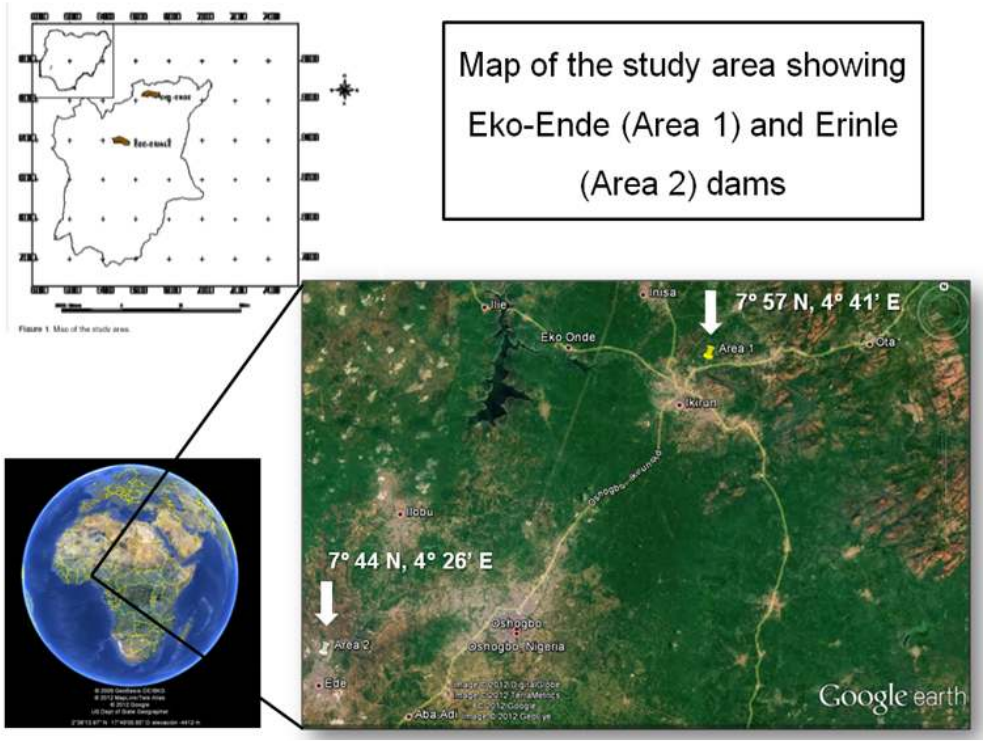


Fig. 1. Map of the study area showing the dams
Source: (Adediji and Ajibade, 2008) [19]

2.5 Molecular Identification of Snails

Snail identification was performed by PCR amplification of the snails" ribosomal Internal.

Transcribed Spacer (ITS) region using forward primer *Ettsl* 5'TGCTTAAGTTCAGCGGGT3' and reverse primer *Etts2* 5'TAACAAGGTTTCCGTAGGTGAA3' as described by [6] and [26]. The denaturation and renaturation of ETTS was performed according to [26]. The amplifications was carried out in a final volume of 25 µl in a mixture containing 2.5x of the PCR buffer, 1 ul of $MgCl_2$, 0.5 ul of dNTPs, 0.5 µl of Etts forward and reverse primers (Promega, Biomers .net GmbH, Germany), 0.5 µl of genomic DNA from snails, 0.3 µl Taq polymerase and 19.2 µl of DD water. PCR conditions consisted of 95℃ for 4 minutes followed by 95℃ for 15 seconds, 48℃ for 15 seconds, 72° for 60 seconds for 4 cycles each. Continued at 95℃ for 15 seconds for 41 cycles, 53℃ for 45 seconds of 41 clycles, 72℃ for 1 minute for 41 cycles, the reaction was completed by incubation at 72℃ for 3 minutes and maintained at 10℃ at infinity after completion. The amplicons were confirmed by visualization on 1.5% agarose gel and the amplified products were digested with *RsaI*, a 6-base cutting restriction enzyme, following the method of [26]. The digests were visualized on 1.5% agarose gel followed by photo documentation using Gel Documentation and analysis System (Clinx Science Instruments, USA).

2.6 Restriction Fragment Length Polymorphism (PCR-RFLP)

The denaturation and renaturation of Etts was performed according to [6,26]. The enzymatic digestion of the amplicons was carried out in a total volume of 15 µl in a solution consisted of 0.75 µl of RSA 1 (Restriction Enzyme, Promega, Biomers .net GmbH, Germany), 1.5 µl of Buffer, 0.5 µl of BSA (Bovine Serum Albumin), 10 µl of DNA (Amplicons) and 2.25 µl duoble distilled water reaction mixture. Master mixed was made and then 5 ml was transferred into each microtitre well, followed by 10 µl of the amplicons. The content was properly covered to prevent evaporation. This was Incubated at 37℃ for 30 minute. The digests were electrophorezed on 1.5% agar rose gel in 0.5 TBE buffer run at 100 volts for 50 minutes to confirmed the product of the digestion.

The gel was visualized with ultraviolet transilluminator. Photo documentation was performed with Gel Documentation and Analysis System (Clinx Science Instruments, USA).

2.7 Cercaria Shedding

Each of the morphologically identified Bulinus snail was placed in petri-dish containing distilled water and exposed to artificial light in the laboratory for 2 hours as described by [27]. The snails were observed under dissecting microscope for cercaria and the cercaria were picked and stored at -800. Both shedding and non shedding Bulinus species were kept in 70% ethanol and preserved for molecular analysis.

2.8 Molecular Screening of Snails for *Schistosoma* Infection

PCR amplifications were carried out in a total volume of 25 µl in a solution containing 2.5× of PCR buffer, 1× of $MgCl_{2,}$ 0.5 Mm of dNTPS, 1 µl of 25 ρmol/ µl of each of 5'GATCTCACCTATCAGACGAAAC3' and 5'TCACAACGATACGACCAAC 3'primers, 1 unit of Taq polymerase (Promega, Biomers, Germany) and 1 µl of genomic DNA template. The thermal cycles were put at 90℃ for 5minutes for 1 cycle, 95℃ for 30 seconds for thirty-five cycles, 72℃ for 30 seconds for thirty-five cycle, 72℃ for 7minutes for thirty-five cycle, and 4℃ at infinity for each cycle as earlier described by [6]. 10 µl of the PCR products were separated in 3% ethidium bromide stained agarose gel prepared with 1× TBE buffer. The gel was run at 100 V for 50 minutes and visualized with ultraviolet transilluminator. All the PCR amplifications were performed with the Thermal Cycler (Techno Thermal iCycler) and the amplified products were visualized on 1.5% agarose gel. Photo documentation was performed with Gel Documentation and Analysis System (Clinx Science Instruments, USA).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Morphological identification of the snails around Erinle and Eko-Ende dams

The morphological identification of the snails showed that several species of snails were encountered at the two dams; ranging from discoid shaped species to globose, oviate and other shaped snails. The Bulinus species which are the only species so far implicated as intermediate hosts were identified, having globose shape and the aperture opened to the left. The characteristic opening bulinid shaped snails encountered measured 6-9 mm. The snails are dark in colour. The shells are more than 8 mm high with mean of 8.6 mm. Mesocone of the first lateral teeth were simple, triangular and deeply separated from the endocone. Shells were relatively strong, columella often strongly reflexed other snails were discarded since they had not been implicated in the transmission of *S. haematobium.*

3.1.2 Molecular identification of the snails

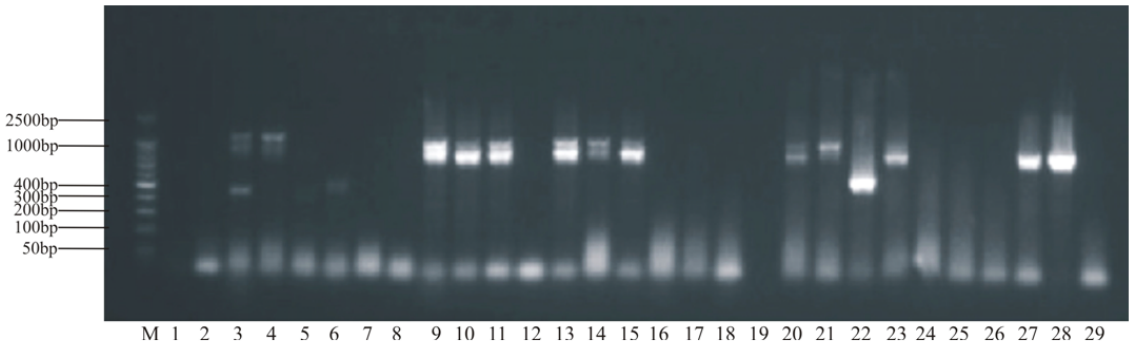
Snails were morphologically identified as Bulinus species across the communities around the two dams. The snails were pooled based on the dams since they were picked from the dams at different locations. The PCR amplifications of the Internal Transcribed Spacer (ITS) region of the genomic DNA of the snails and electrophoretic migration of the PCR amplicons produced bands of different sizes while amplification was not successful for some snails (Fig. 2). The repeated PCR assays of the samples that did not show amplifications still revealed the same pattern. However, only the samples that showed bands had their PCR amplicons digested. The digestion of the PCR amplicons with Rsa1 enzyme (PCR-RFLP) showed that all the snails collected at the two dams were *Bulinus truncatus* (Fig. 3 and Table 1).

3.1.3 The infection status of the snail intermediate hosts in the study areas

A total of two hundred and seventy-seven Bulinus snails which comprised 193 from Erinle dam and 84 from Eko-Ende were exposed for cercaria shedding and PCR amplification of Dra 1 repeat genes of schistosome infection. Fifty seven snails from Erinle (29.53%) shedded bifurcated cercariae while 22 (26.19%) from Eko-Ende dam shedded bifurcated schishtosome cercariae. However, 75 snails (38.86%) and 33 (39.28%) snails were positive for Dra1 genes from Erinle and Eko-Ende dams respectively (Table 2 and Fig. 4). There was no significant difference (P=0.06) in snail infectivity between the two dams but the difference in infectivity status obtained in snails by cercaria shedding and PCR -Dra1 was statistically significant (P=0.04).

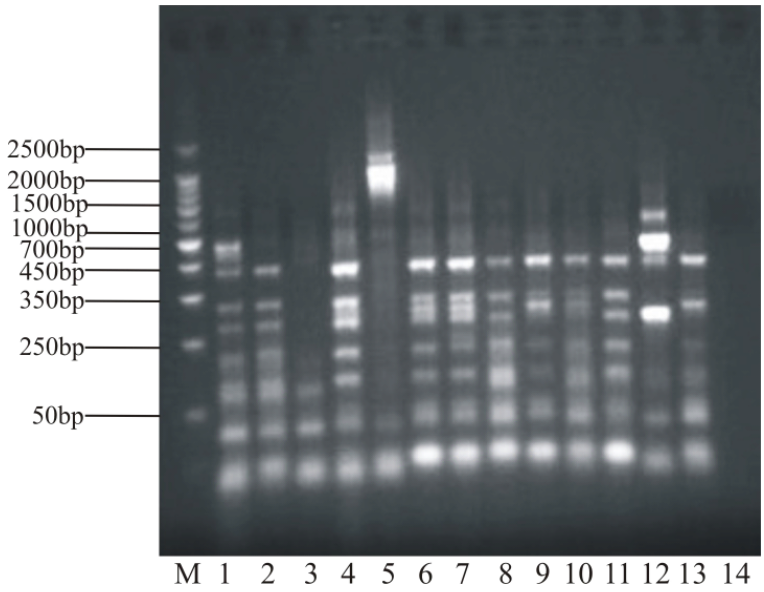
Table 1. Molecular id entification of snail intermediate hosts using PCR-RFLP

Dams	Snails identified RFLP	Bulinus globosus	Bulinus truncatus	Physa specoes	Other
Erinle	52	0	52	0	0
EKO-	24	0	24	0	0
Total	76	0	76	0	0



Agarose gel stained with ethidium bromide showing the different bands obtained from amplification of the ITS region of the snails. Lanes – M: size marker (Promega ladder 50 – 2500bp); 2-21, 23-26 snails under investigation, 27-28: (previously confirmed snail species through sequencing) *Bulinus truncatus and Bulinus globusus respectively*; 1*:* negative control and 22 *Physa acuta*.

Fig. 2. Agarose gel elector ophoresis of ITS region amplicons



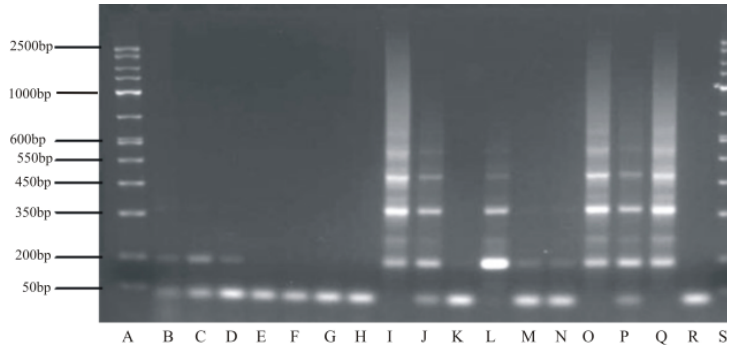
Gel showing species-specific banding patterns obtained from digestion of the 1.3kbp PCR product containing the ITS region using *Rsa1*. Lanes – M: size marker (Promega ladder 50-2500bp); 2 - 4: (previously confirmed snail species through sequencing) *Bulinus globossus, Bulinus truncatus and Physa acuta respectively*; 5-13(Snails under investigation) 14: negative control.

Fig. 3. Gel electrophoresis of the amplicons digested eith RSAI restrication enzyme

Table 2. Infectivity status of the *Bulinus snails* at *Erinle* and *Eko-Ende Dams*

Dams	No screened by light exposure and Dra 1	Number (%) positive for cercaria shedding	Number (%) positive for Dra1 gene (PCR)
Erinle	193	57 (30.0%)	75 (38.9%)
eko-ende	84	22 (26.2%)	33 (39.3%)
Total	277	79 (28.6%)	108 (39.0)

Key: Numbers outside parentheses are total positive
( ) Numbers in parentheses are pecentages positive



Agarose gel stained with ethidium bromide showing the infection status of snails. Lanes – A and S: size marker (Bioneer ladder 100bp); B,C,D, M and N infected snails with weak bands; E, F, G, H, K - uninfected snail; I, J, L, O,and P infected snails with stronge bands of Dra1 gene repeats; Q -DNA from a previously confirmed infected *Bulinus truncatus* as positive control; R - negative control.

Fig. 4. Agarose gel electrophoresis of DRAl generepats of snail infection status

3.2 Discussion

The identification of the snails morphologically showed Bulinus species and by PCR-RFLP and snail DNA sequencing revealed that *B. truncatus* is the species involved in the transmission of *S. haematobium* at the study area [6] This is consistent with most of the studies on snails in Nigeria where *B. truncatus* has been implicated as the main intermediate host of *S. haematobium* with sympatric existence of *B. globosus* in some areas [28]. Brown [29], reported a similar observation that freshwater pulmonate snails of the genus Bulinus are the intermediate hosts for *S. haematobium* and occur commonly throughout much of Africa and adjacent regions. Also Duwa and Oyeyi [30], in their study revealed the presence of a sparse population of *B. globosus* with only a few (4.3%) shedding cercaria of schistosome type from Jakara Dam. Kariuki [31], identified *Bulinus* (*africanus*) *nasutus* and a few numbers of *Bulinus forskalii* and *Melanoides tuberculata* in the Msambweni area, Coast Province, Kenya as intermediate host of *S. haematobium* using morphological characteristic features. The absence of Physa and *B. globusus* at the study area was contrary to the recent report of [6] in which sympatric existence of *B. truncatus, B. globusus* and *Physa acuta* were observed in some rivers in Southwestern Nigeria. The analysis profiles for *B. truncatus* closely match those obtained by [6] for the same species. The PCR-RFLP technique is a rapid and cost-effective method of assessing Bulinus species distribution in the study area and it would be useful for identifying snails from a developing country like Nigeria. The use of sequencing for snail species determination in Erinle and Eko-Ende Dams Osun State was very effective, but expensive, slow and may probably not be recommended in limited resource settings like Nigeria.

The high number of bifurcated cercaria shedded by the snails and the number of snails positive for Dra1 Schistosome infection (over 25%) in this study attest to the active transmission of *S. haematobium* at the two dams. The infectivity of the snails may be a reflection of frequent contamination of the dams with infected human excreta (urine). [31] reported a similar observation in the Msambweni area of the Kwale

District in Kenya, an area endemic for *S. haematobium*. The molecular technique showed higher infectivity than cercaria shedding. This is possible as the PCR could also detect the pre-patent stage of the infection in snails.

Recently, [6], demonstrated that *B*. *truncatus* could be infected by both *S*. *haematobium* and *S*. *bovis* which could only be determined by advance molecular technique. Since Dra1 has high sensitivity of identifying *S*. *haematobium* where bovis are not common, all the cercaria shed and identified in the present study were identified as the *S*. *haematobium* cercaria. This study, to the best of our knowledge is the first report on pre-patent schistosomal infection in snails in Erinle and Eko-Ende dams. The PCR results also enabled analysis of the temporal and quantitative relationships between populations of snails having pre-patent and patent infections at transmission sites as earlier posited by [32]. This is the first report on species composition and infectivity status of the snail intermediate hosts responsible for local transmission of urinary schistosomiasis in these communities and one of the earlier reports in the use of molecular approaches in characterization of the local intermediate hosts in Nigeria.

4. CONCLUSION

The results confirmed the allopatric existence of *B*. *truncatus* at the study area. There was an active transmission of urinary schistosomiasis at the two dams as the cercaria shedding and polymerase chain reaction indicated high snail infectivity. The high prevalence of schistosome infection in snail intermediate hosts around the two dams implies active transmission of urinary schistosomiasis at the study area. There is therefore need for integrated control which must encompasses snail control in stemming the menace of schistosomiasis at the study area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. King CH. Epidemiology of schistosomiasis: Determinants of transmission of infection. Mahmoud AAF, ed. Schistosomiasis. London: Imperial College Press. 2001; 115–132.
2. Ofoezie IE, Bolton P, Imevbore AMA, Christensen NO. Schistosomiasis and other helminth infections in irrigation schemes in Sokoto, Katsina and Kebbi States of Nigeria. The Nig. J. of Parasitol. 1996;17:31–37.
3. Boelee E, Laamrani H. Environmental control of schistosomiasis through community participation in a Moroccan oasis. Tropical. 2004;9(9):997.
4. Richards A Kane, Russell Stothard J, Aidan M Emery, David Rollinson. Molecular characterization of freshwater snails in the genus *Bulinus*: A role for barcodes? Parasites & Vectors. 2008;1:15. DOI: 10.1186/1756-3305-1-15
5. Akinwale OP, Ajayi MB, Akande DO, Adeleke MA, Gyang PV, Adeneye AK, Dike AA. Prevalence of *Schistosoma haematobium* infection in a neglected community, South Western Nigeria. International J. of Health Res. 2009;2(2): 149-155.
6. Akinwale OP, Kane RA, Rollinson JR, Stothard JR, Ajayi MB, Akande DO, Ogungbemi MO, Duker C, Gyang PV, Adeleke MA. Molecular approaches to the identification of *Bulinus* species in south-west Nigeria and observations on natural snail intections with schitosomes. Journ. of Helminthol. Cambridge University Press; 2010.
DOI: 10.1017/S0022149X10000568
7. Rollinson D, Stothard JR, Southgate VR. Interactions between intermediate snail hosts of the genus *Bulinus* and schistosomes of the *Schistosoma haematobium* group. Parasitol. 2001;123: 245-260.
8. Frandsen F. Studies of the relationships between *Schitosoma* and their inter-mediate hosts. II The genus Bulinus and *Schistosoma haematobium* from Sudan, Zaire and Zambian. Journal of Helminthology London. 1979;(53):205-212.

9. Vercruysse J, Shaw DJ, de Bont J. Index of potential contamination for schistosomiasis. Trends Parasitol. 2001; 17:256–261.
10. Barbour AD. The importance of age and water contact patterns in relation to *Schistosoma haematobium* infection. Trans R Soc Trop Med Hyg. 1985;79:151–153.
11. Chandiwana SK, Woolhouse ME. Heterogeneities in water contact patterns and the epidemiology of *Schistosoma haematobium.* Parasitology. 1991;103: 363–370.
12. Sturrock RF, Klumpp RK, Ouma JH, Butterworth AE, Fulford AJ, Kariuki HC, Thiongo FW, Koech D. Observations on the effects of different chemotherapy strategies on the transmission of *Schistosoma mansoni* in Machakos District, Kenya, measured by long-term snail sampling and cercariometry. Parasitology. 1994;109:443–453.
13. Sturrock RF, Diaw OT, Talla I, Niang M, Piau JP, Capron A. Seasonality in the transmission of schistosomiasis and in populations of its snail intermediate hosts in and around a sugar irrigation scheme at Richard Toll, Senegal. Parasitology. 2001;123(Suppl):S77–S89.
14. Ouma JH, Sturrock RF, Klumpp RK, Kariuki HC. A comparative evaluation of snail sampling and cercariometry to detect *Schistosoma mansoni* transmission in a large-scale, longitudinal field-study in Machakos, Kenya. Parasitology. 1989;99: 349–355.
15. Sturrock RF. The schistosomes and their intermediate hosts. Mahmoud AAF, ed. Schistosomiasis. London: Imperial College Press. 2001;7–83.
16. Barbour AD. Modeling the transmission of schistosomiasis: An introductory view. Am J Trop Med Hyg. 1996;55(Suppl):135–143.
17. Anderson RM, May RM. Prevalence of schistosome infections within molluscan populations: Observed patterns and theoretical predictions. Parasitology. 1979; 79:63–94.
18. Woolhouse ME, Chandiwana SK. Spatial and temporal heterogeneity in the population dynamics of *Bulinus globosus* and *Biomphalaria pfeifferi* and in the epidemiology of their infection with schistosomes. Parasitology. 1989;98: 21–34.
19. Adediji A, Ajibade LT. The change detection of major dams in Osun State, Nigeria using remote sensing (RS) and GIS techniques. Journ. of Geog. and Region. Plan. 2008;1(6):110-115.
20. Iloeje N. Regional Geography of Nigeria. 2nd Edition University Press Oxford. 1978;75.
21. Ofoezie IE, Imevbore AMA, Balogun MO, Ogunkoya OO, Asaolu SO. A study of an outbreak of schistosomiasis in two resettlement villages near Abeokuta, Ogun state Nigeria. J. of Helminthol. London. 1991;65:95-105.
22. Mandahl-Barth G. Intermediate hosts of Schistosoma, African Biomphalaria and Bulinus: II. Bulinus, Bulletin of World Health Organization Geneva. 1957;17: 1-65.
23. Danish Bilharziasis Laboratory. A field guide to African freshwater snails. I West African species: Teaching notes, Charlottenlund, DBL. 1973;29.
24. Brown, DS. Freshwater snails of Africa and their medical importance, London. Taylor & Francis. 1980;487.
25. Ude EA, Akinwale OP, Ukaga CN, Ajayi MB, Akande DO, Gyang PV, Adeleke MA, Dike AA. Prevalence of Urinary schistosomiasis in Umuowele, Agulu community, Anambra State, Nigeria. Intern. Journ. of Health Research. 2009; 2(4):347-353.
26. Stothard JR, Hughes S, Rollinson D. Variation within the internal transcribed spacer (ITS) of ribosomal DNA genes of intermediate snail hosts within the genus *Bulinus* (Gastropoda: Planorbidae). Acta Trop. 1996;61:19-29.
27. Frandsen F, Christensen NO. An introductory guide to the identification of cercariae from freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. Acta Tropic Basel. 1984;41: 181-202.
28. Emejulu AC, Alabaronye FF, Ezenwaje HMG, Okafor FC. Investigation into the prevalence of urinary schistosomiasis in the Agulu Lake Area of Anambra State, Nigeria. J Helminthol. 1994;68:119-123.
29. Brown DS. Freshwater snails of Africa and their medical importance. In: (2nd edition), Taylor and Francis, London. 1994;609-615.
30. Duwa MR, Oyeyi TI. The role of Jakara dam in the transmission of

schistosomiasis. Bayero J. of Pure and Applied Scien. 2009;2(1):58–63.

31. Kariuki HC, Clennon JA, Brady MS, Kitron U, Sturrock RF, Ouma JH, Ndzovu STM, Mungai P, Hoffman O, Hamburger J, Pellegrini C, Muchiri EM, King CH. Distribution patterns and cercarial shedding of *Bulinus nasutus* and other snails in the Msambweni area, coast province, Kenya. Am. Journ. of Trop. Med. and Hyg. 2004;70:449–456.

32. Hamburger Joseph, Orit Hoffman H, Curtis Kariuki, Eric M, Muchiri John H, Ouma Davy K, Koechi Robert F, Sturrock, Charles H King. 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. Am. J. Trop. Med. Hyg. 2004;71(6): 765-773.

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