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Two cases of urinary schistosomiasis with unusual egg presentations: Dra 1 repeat sequence not detected

Henry Gabriel Bishop, Helen Ileigo Inabo, Elijah Ekah Ella, Mohammed Bello

ABSTRACT

Introduction: *Schistosoma haematobium* is the primary cause of urinary schistosomiasis in man. It is rare to find other human schistosome species in urine because they are located in the intestines, or those of animal origin. Mixed infections of human and animal species of schistosomes may occur in cattle breeding areas like Nigeria.

Case Report: During a prevalence study on urinary schistosomiasis, two teenage boys from different local government areas (LGAs) of Kaduna State, Nigeria had mixed urinary *Schistosoma* infections. Their urine samples were centrifuged at 3000 rpm (revolutions per minute) for 5 minutes. Microscopic examination of the urine sediments revealed highly polymorphic eggs (or morphotypes). After subjecting the genomic DNA for detection of *S. haematobium* Dra 1 tandem repeat sequence by polymerase chain reaction (PCR), it was not amplified. However, there was amplification in a classical urinary schistosomiasis caused by *S. haematobium* (which served as positive control).

Conclusion: Unusual egg presentations in urinary schistosomiasis may present a dilemma in making diagnostic conclusion. Hence, these two cases suggest the possibility of human–animal *Schistosoma* hybrids circulating in the area, especially *S. haematobium–S. bovis* hybrids.

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INTRODUCTION

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes of the genus *Schistosoma* [1]. The disease is most prevalent among people in rural and some urban areas of tropical and sub-tropical countries, especially those communities that lack access to safe drinking water and good sanitation [1–3]. Schistosomiasis is regarded as one of the neglected tropical diseases affecting man [4]. In Africa, the distribution of *S. haematobium* is sympatric with species infecting domestic cattle and wild ungulates, including *S. bovis*, *S. intercalatum* and *other* species that are closely related [5, 6]. Members of *S. haematobium* group are known to develop in the same *Bulinus* snails (as common intermediate hosts), producing almost indistinguishable cercariae [5, 7].

Children are mostly unaware of the risk of transmission of schistosomiasis via cercariae-infested water bodies and hence more infections occur [2]. There had been

reports of hybrid schistosomes in some West African countries like Mali, Senegal, Benin, and Cameroon. These countries together with Nigeria share the River Niger and its tributaries. In Nigeria, control of schistosomiasis had been focused on preventing human contact with cercarial-infested water bodies, but there is lesser attention on the possible role of livestock as reservoir hosts [8]. In cattle-raising areas, as humans undertake agricultural, recreational, or fishing activities in the water bodies, cattle also come to take a drink.

Recently, researchers have given close attention to understand the genetic diversity, polymorphism, and hybridization among phylogenetically related human and animal *Schistosoma* species. Continued intra- and inter-breeding among *Schistosoma* species will pose threats to successful diagnosis, treatment, and vaccine production. There is lack of information on diversity of *Schistosoma* species and possible hybridization among closely related species from Nigeria.

CASE REPORT

During a prevalence study on urinary schistosomiasis in Kaduna State, Nigeria, we came across two unique forms of mixed urinary *Schistosoma* species infections among two junior secondary school teenage boys, 13 years old from Giwa LGA and 14 years old from Kaduna South LGA, Nigeria. Twenty (20) mL urine sample was collected from each of them. The 13 years old boy had visible hematuria, but the other boy did not experience any of the symptoms like painful urination or visible hematuria.

There was mixed *Schistosoma* infections in urine samples of the two cases. Urine sample of the 13 years old boy had terminal-spined eggs characteristic of *S. haematobium,* and other spineless oval eggs (Figure 1). While urine sample from the 14 years old boy contained both terminal-spined eggs that were characteristic of *S. haematobium* and sub-terminal-spined eggs suggestive of hybrid types as shown in Figure 2.

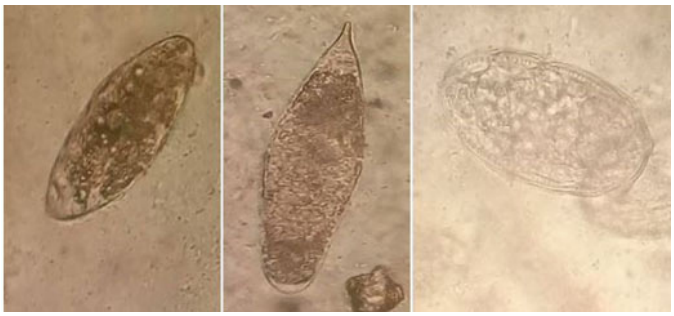

Figure 1: Egg (left) is elliptical but spineless. Egg (middle) is elongated, nearly spindle-shaped with terminal spine. Egg (right) is oval with a bump-like inconspicuous sub-terminal spine.

After subjecting the extracted genomic DNA from the mixed infections to PCR analysis, there was no amplification for the Dra 1 sequence of *S. haematobium* from both cases as shown on Lane 2 (from the 13 years old boy in Giwa LGA) and Lane 3 (from the 14 years old boy in Kaduna South LGA) in Figure 3. However, from the positive control, the 121bp Dra 1 tandem repeat sequence was amplified.

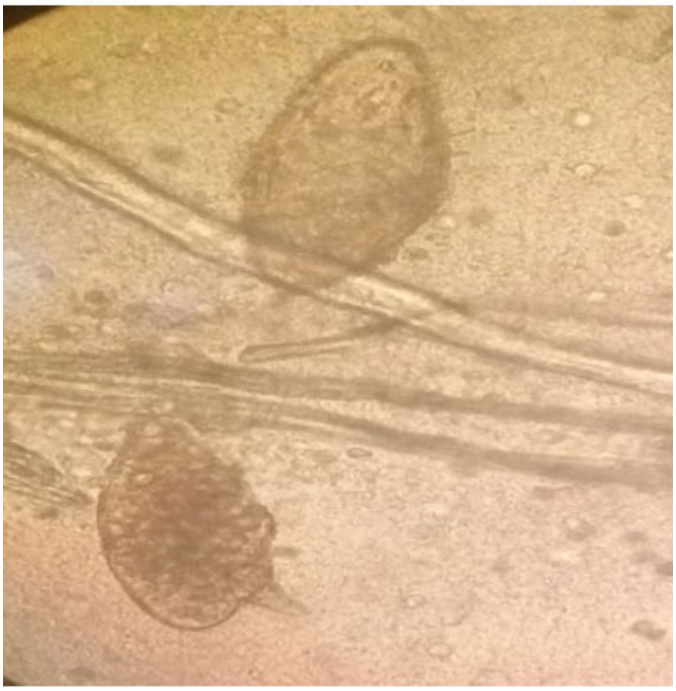

Figure 2: The egg above is elliptical terminal-spined. The egg below is elliptical with sub-terminal-spine.

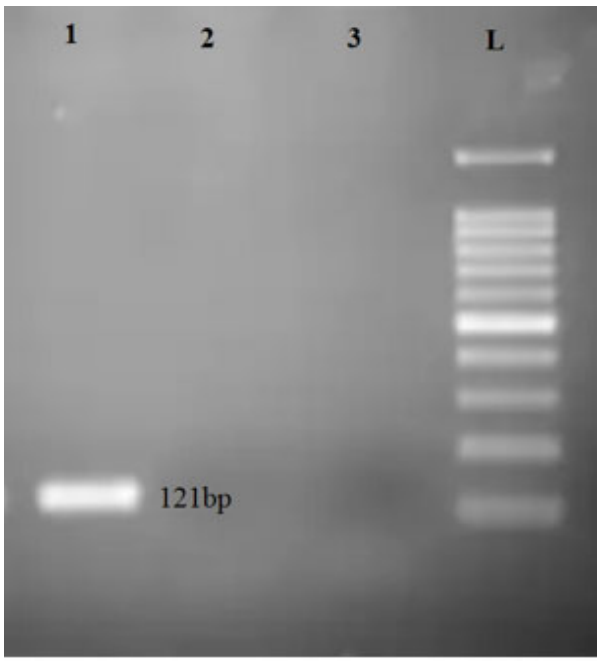

Figure 3: Lane L is a 100bp ladder marker. Lane 1 is a positive control from classical urinary schistosomiasis. Lane 2 is a mixed infections case from 13 years old boy. Lane 3 is a mixed infections case from 14 years old boy.

DISCUSSION

The urine samples were subjected to urinalysis (using SG11100-Uric 11V, Guilin Zhonghui Technology Co., Ltd, China), and both contained microhematuria, proteins, leukocytes, and ketones. Each urine sample was gently agitated to mix and divided into two equal volumes of 10 mL and subjected to centrifugation at 3000 revolutions per minute (rpm). One of the sediments was examined under 10× and 40× objectives of the light microscope for detection of eggs of *Schistosoma*. The other sediment was used for genomic DNA extraction using phenol-chloroform method. The sediment was transferred onto a sterile test tube containing 200 µL nuclease-free-water and heated at 100°C for 10 minutes [6]. The mixture was vortexed briefly. A total of 250 µL of the mixture was added to 250 µL of buffer-saturated phenol/chloroform/isoamylalcohol (pH 7.5–7.8), and 25 µL of 3 M sodium acetate (pH 5) and vortexed for 1 minute. After 15 minutes incubation on ice, it was centrifuged for 10 minutes at 12,000 rpm at room temperature (26°C). The aqueous layer was transferred to a 1.5 mL Eppendorf tube containing 250 µL isopropanol and incubated for 5 minutes on ice. The mixture was centrifuged at 12,000 rpm at 4°C for 5 minutes. Supernatant was discarded and the pellet was re-suspended in 50 µL of 70% ethanol, vortexed and centrifuged at 13,000 rpm for 2 minutes at room temperature (30°C). The supernatant was discarded and the residual ethanol was eliminated by a quick centrifuge spin. The pellet was air-dried in a half-open tube for about 30 minutes and suspended in 20 µL of TE (10 mM TrisCl, 1 mM EDTA, pH 8), vortexed and stored at –20°C for polymerase chain reaction [7, 9]. The genomic DNA was electrophoresed and viewed under UV-light.

The Dra 1 primers were designed for amplification of 121bp tandem repeat sequence of *S. haematobium*, which was previously described by Hamburger et al. [10] and validated by Ibironke et al. [11] and used for *S. haematobium* DNA detection on filtered urine [12]. Dra 1 forward and reverse primers and other consumables were obtained from Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa and prepared according to manufacturers' instructions.

The polymerase chain reaction (PCR) was performed in a 25 µL reaction unit containing 12.5 µL of OneTaq Quick-Load 2X Mastermix with Standard Buffer (New England Biolab Inc.), 0.5 µL DRA1F (0.01 umol forward primer, 5′-GATCTCACCTATCAGACGAAAC-3′), 0.5 µL DRA1R (0.01 umol reverse primer, 5′-TCACAACGATACGACCAAC-3′), 5 µL *S. haematobium* genomic DNA (as template) and 6.5 µL nuclease-free water. The PCR cycling conditions included initial denaturation at 95°C for 5 minutes, followed by 33 cycles at 95°C for 30 seconds (denaturation), annealing at 47°C for 60 seconds and extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. After which samples were kept on hold at 4°C. A positive control of extracted genomic DNA from urine with *S. haematobium* was used. The PCR products were visualized on 2% agarose gel.

Though the 14 years old boy did not present with any overt symptoms of the disease, urinalysis indicated evidence of microhematuria. Furthermore, both of them had proteins, leukocytes, and ketones in their urine samples. These urinary indices by application of urinalysis can serve as useful indicators of urinary schistosomiasis. Classically, urinary schistosomiasis is caused by *S. haematobium*, and recovered eggs were oval with terminal spines. The detection of elongated, nearly spindle-shaped eggs with terminal spines and elliptical spineless eggs are not commonly reported in cases of urinary schistosomiasis. Similarly, mixed terminal-spined eggs and sub-terminal-spined eggs are uncommon in Nigeria. These two cases of urinary schistosomiasis are rare occurrences of mixed infections. Majority of prevalent *Schistosoma* mixed infections are between *S. haematobium* and *S. mansoni*, which are human parasites [13, 14]. Study by Ojo et al. [15] in South West Nigeria suggested the possibility of interspecific interactions between *S. haematobium* and *S. mansoni*, as distinctive eggs of the two species were found regardless of predilection sites. Typically, terminal-spined eggs and lateral-spined eggs are discharged through urine and stool respectively, and common in co-infections. Both eggs with bump-like inconspicuous sub-terminal spines (Figure 1) and the elliptical egg with sub-terminal spines are much more of morphotypes of *S. bovis*. Such variations in egg morphotypes had been previously reported in Europe by Kincaid-Smith et al. [16].

Abnormal routing of predilection sites by *Schistosoma* species had been reported [17]. During heavy *Schistosoma* infections, the eggs can be carried to any location in the body, capable of causing tissue damage [18]. Hybridization had also been reported among mammalian schistosomes that are phylogenetically related in regions where distinctive snail hosts co-exist [7, 19]. Cattle rearing methods like transhumance and nomadism are responsible for movement of domestic livestock, permitting animal contact with multiple potential sites for schistosomiasis transmission [20].

Hybrids of *Schistosoma* species that infect man exist. Those species that infect animals and man can also hybridize [21]. A major concern is on the epidemiology and zoonotic transmission of human–animal schistosome hybrids [20]. Hybrid species of *S. haematobium* (which lives mainly in the venous plexus of human bladder) and *S. bovis* (mainly in mesenteric veins of animal intestine) had been reported in humans [22] in some West African countries, especially in Senegal [23, 24], Mali [25], and recently becoming endemic in South Europe [16, 26]. There is no comprehensive information on *Schistosoma* species genomic diversity in Nigeria yet, compared with data from neighboring countries.

Giwa and Kaduna South LGAs are cattle-breeding areas where these rare cases of mixed urinary

schistosomiasis were found in Northern Nigeria. In cattle breeding areas, both humans and the cattle share the same rivers. *Bulinus* snails serve as intermediate host for both *S. haematobium* and *S. bovis*. Within the snail or human host, hybridization can occur. Hybrid species of *Schistosoma* discharge morphologically highly polymorphic eggs [16].

There was no amplification of Dra 1 tandem sequence of *S. haematobium* in both cases of mixed urinary *Schistosoma* infections. This directly implied that the species in these cases where not classically *S. haematobium*, but probably some hybrid species causing urinary schistosomiasis, and may be circulating in Nigeria. Zoonotic schistosomiasis can undermine control efforts on schistosomiasis, especially in Africa.

CONCLUSION

In this report, the two cases of mixed urinary schistosomiasis from Kaduna State in Nigeria had microhematuria, proteinuria, leukocyturia, and ketonuria as detected by urinalysis. Such indices can be of diagnostic importance in urinary schistosomiasis. Microscopic examination of the cases revealed rare occurrences of mixed infections of egg morphotypes of *Schistosoma* species (other than *S. haematobium*), suggestive of hybridization phenomenon.

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Author Contributions

Henry Gabriel Bishop – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Helen Ileigo Inabo – Conception of the work, Design of the work, Interpretation of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Mohammed Bello – Design of the work, Interpretation of data, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Authors declare no conflict of interest.

Data Availability
All relevant data are within the paper and its Supporting Information files.