Epidemiological Assessment of Urinary Schistosomiasis among School-aged Children in Selected Local Government Areas in Gwandu Emirate of Kebbi State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Urinary schistosomiasis remains a public health problem in the tropics. The study examines the prevalence of urinary schistosomiasis among school-aged children in Bunza, Koko and Aliero Local Government Areas (LGAs) in Gwandu Emirate of Kebbi State, Nigeria. Four hundred and seventy-four (474) urine samples were examined for ova of *Schistosoma haematobium* using sedimentation technique and chemical reagent strip for haematobium detection. The overall prevalence of *S. haematobium* recorded was 126 (26.58%). The distribution of infection by location revealed that Bunza had highest infection (35.1%), followed by Koko (28.00%) and Aliero (17.1%). Bunza showed Odd ratio (OR) of 2.02 and 95% CI (1.33 – 3.08) with statistical significance of P < 0.0014

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which indicates that location plays a significant factor in prevalence of infection. The prevalence of infection in relation to gender showed that males had the highest percentage 37.13%, Chi-square revealed that there was difference between the males and female infection rate (P < 0.0001) while 16.03% was observed among the females. The age bracket 9 – 12 years old 47.05% were the most affected with the infection, while children 5 – 8 years bracket with 15.08% had the least infection rate. Chi-square shows that there was difference between the ages and infection rate of (P<0.0001). Blood in urine (haematuria) was significantly associated with infection rate. Water-contact activities recorded revealed that there was association between water contact and infection rate (P-value < 0.0001). With the high prevalence of infection in the study areas, there should be a concerted effort by stakeholders in the state, not just to establish a comprehensive data on the prevalence of the infection, but also to take decisive action in its control.

1. INTRODUCTION

Schistosomiasis is a parasitic infection caused by species of blood flukes of the genius *Schistosoma* with an estimated 200 million people affected worldwide [1]. It is one of the neglected tropical diseases, with Nigeria leading in Africa [2]. While the distribution of infection has changed, 80 – 85% of disease is currently found in sub-Saharan Africa, and the number of people infected is not on the decline [1]. The impact of schistosomiasis has been under-estimated compared to that of malaria and tuberculosis [3]. Schistosomiasis was first described in 1851 by Theodor Bilharz, after whom the disease was initially named (bilharziasis) [4]. However, it remains endemic in 75 developing countries (600 million people at risk) and infects about 300 million people (120 million with symptoms, 20 million suffering the sheer consequences of the disease and about 100 thousand deaths) [5].

There are five species of *Schistosoma* identified [1] out of which *Schistosoma mansoni, Schistosoma japonicum,* and *Schistosoma haematobium* are most commonly found in sub-Saharan Africa. *S. mansoni* is the most widespread; *S. haemotobium* is concentrated in Africa and the Middle East, while *S. japonicum* is primarily found in Asia. *S. mansoni* and *S. japonicum* cause chronic hepatic and intestinal fibrosis while *S. haematobium* affects the urinary tracts and kidney as well as the reproductive systems [1]. There are four types of schistosomiasis: intestinal schistosomiasis caused by *S. mansoni, S. japonicum,* and *S. intercalatum*; urinary schistosomiasis caused by *S. haematobium* [6].

Schistosoma haematobium causing urinary schistosomiasis which is the focus of this study is a very serious environmental health problem in many tropical and sub-tropical countries, with school-age children usually being the most affected group [5]. In Nigeria, series of studies has shown variation in the endemicities of the disease from meso-endemic proportions to relatively hyper-endemic level in other parts. Nigeria is still one of the countries known to be endemic for urinary schistosomiasis as established by Anosike et al. [7].

Water-contact activities and traditional agricultural practices are reported as factors responsible for the distribution of the disease and its snail vectors [8]. It is estimated that 180 million people worldwide live in endemic areas and 90 million are infected with the parasite; most of these live in sub-Saharan Africa [9]. Approximately 70 million people suffer from hydronephrosis (an accumulation of urine in kidney due to obstruction of ureter). It is also estimated that 150, 000 people die each year as a result of renal failure and unknown significant number from bladder and other genital urinary cancers [9].

The disease is associated with rivers, streams, ponds and water development projects, where the snail intermediate hosts breed and people make various water contacts with these bodies of water for the domestic, economic and recreational purposes [10]. In Nigeria, especially in the rural communities, insufficient knowledge of the etiology of the disease, poor sanitary habits and health education, as well as lack of safe drinking water are the main causes of its endemicity [11].

The public health importance of schistosomiasis has been documented. The important target

organs for *Schistosoma haematobium* eggs are in the urogenital tract – the urinary bladder, ureter, vagina (female genital schistosomiasis) - and kidneys, where they induce histopathological changes. The clinical manifestation of urinary schistosomiasis includes haematuria, anaemia and cysts, and if left untreated could lead to squamous cell cancer of the bladder. *S. haematobium* has been reported to cause genital lesions in 30% of women who are infected and these lesions may increase the risk of HIV transmission [12]. There is, therefore, the need to undertake this study in order to document the prevalence of the infection in the state which would in turn help concerned stakeholders make informed decisions regarding its control.

2. METHODOLOGY

2.1 Study Area

The study areas are Bunza, Aliero and Koko/Besse Local Government Areas all in Gwandu Emirate of Kebbi State, Nigeria. Bunza has the coordinates of 12.0916° N, 4.0108° E. Aliero's coordinates are 12.2900° N, 4.4671° E while Koko/Besse has the coordinates of 11.4458° N, 4.4388° E [13]. Most of the people are agrarian, farming in vegetable, cereals as well as fishing.

2.2 Study Design

Gwandu Emirate is made of ten local government areas. Random sampling was used to select the three local governments in the study. A letter of introduction was obtained from the Department of Biological Sciences (Zoology unit), Kebbi State University of Science and Technology to seek permission from the Ministry of Education before the commencement of the study. Also, consent was obtained from various community heads where the study was carried out.

2.3 Sample Collection

Fresh urine samples were collected between the hours of 10 am – 2 pm in a pre-numbered plastic screw-capped bottles mainly from school aged-children because of their high risk of schistosoma infection. This age group gives a reliable reflection of the general situation of the disease in an area [14]. Four schools were randomly selected in each of the three LGAs. Children were selected randomly from each of the schools

and screened for urinary schistosomiasis giving a total of 474 urine samples.

2.4 Snails Samples Collection and Identification

Bulinus globosus is an intermediate host of *S. haematobium*; it is a species of tropical fresh water snail. The snails were collected over a period of two months using a scoop net as designed by [15]. This was usually done in the evenings within the hours of 4pm to 6pm twice in a week. A total of five scoopings were usually carried out per site per visit. Rubber boot and hand gloves were used during the scooping operations to prevent contact with infected water. The snails *Bulinus spp* were identified for its sinistral shell; it's very large body whorl and a small spire.

2.5 Data Collection

A brief health lecture was given to the Head Teachers of the schools about schistosomiasis, the children were also enlightened about the possible means of contacting the disease, the hazards associated with the infection and how it can be prevented before the collection of the urine. A structured questionnaire was also administered to obtain information on demographic data and risk factors of acquiring urinary schistosomiasis. Information such as sex, age, parent occupation, source of water etc. were also requested.

2.6 Sample Size Determination

Sample size was determined using standard formular described by [16].

$$N = \frac{t^2 \times P(1-P)}{(mt)^2}$$

Where

n = required sample size
t = confidence interval at 95% (standard value of 1.96)
P = assumed prevalence of *S. haematobium* infection
m = margin of error at 5%

$$N = \frac{(1.96)^2 \times 0.5(1-0.5)}{(0.045)^2}$$
$$= \frac{3.8416 \times 0.25}{0.002025}$$
= 474.2716 (approximately 474)

2.7 Analysis of Urine for Blood and Protein

Urine samples were collected in the study areas between 10.00 am and 2.00 pm. The samples were immediately analyzed for the presence of blood (haematuria) and protein (proteinuria) using the reagent strip method comi – 9 Macherey – Nagel. The analysis was carried out before the preservation so as not to interfere with the chemical nature of the urine. The strip was dipped into the urine and the results were checked by comparing the changes with the range of colours on the pack as instructed by the manufacturers.

2.8 Urine Analysis for *Schistosoma* Eggs

After the heamaturia and proteinuria test, each urine sample was immediately preserved with 1 ml of 10% formal saline to prevent the eggs from hatching into miracidia before examination. It was further placed in black polyethylene bags during transportation to Federal Medical Centre, Birnin Kebbi, Nigeria, for parasitological analysis. The urine samples collected were processed using sedimentation technique as described by [14]. 10 mls of urine sample was taken and centrifuged at 3000 rpm for 5 minutes, after which it was allowed to stand for 30 minutes. The supernatant was discarded while the sediment was pipetted onto a grease-free glass slide and covered with a cover slip. The slide was then examined under the light microscope using X10 and X40 objectives. Urine samples containing terminal spine eggs characteristics of *S. haematobium* were counted each for positive sample and the result expressed as eggs/10 mls of urine.

2.9 Statistical Analysis

The data obtained from the questionnaires were analyzed using Statistical Package for Social Science (SPSS) version 25. Chi square was used to determine the differences within ages and sex of subjects, and possible association. A two-by-two contingency table of [17] was used to calculate the sensitivity and specificity of the screening test. Odd ratios were determined to assess risk factors associated with Schistosomiasis among school aged children in the study area. Associations were considered significant at P<0.05.

3. RESULTS

3.1 Prevalence of *S. haematobium* with Respect to Community

Out of the 474 pupils screened in the study area for Schistosoma infection, 126 (26.58%) were found positive. With respect to location, the infection was significantly related to the community in question. Peak prevalence was recorded in Bunza 57 (36.01%), than in Koko 42 (28.00%) and Aliero 27 (17.01%) as shown in Table 1. Odds ratio value of 2.02 and P-value of 0.0014 for Bunza shows very significant association between the community and the prevalence of the infection while odds ratio value of 0.45 and 1.0 for Aliero and Koko respectively shows no significant effects between the communities and the infection.

3.2 Age-specific Prevalence of *S. haematobium*

Table 2 shows prevalence of the parasites in relation to age of the pupils Ages 9 – 12 group had the highest prevalence of 75 (47.05%), followed by those with the age of 13 and above 26 (16.05%). The prevalence of the infection is lower among the pupils between the age of 5 - 8 years with 25 pupils infected out of 158 and prevalence rate of 15.08%. Odd ratio value of 0.419 obtained for 5 – 8 years and 0.426 for 13 years and above showed negative associations between the age group and the infection while odds ratio value of 4.69 for 9-12 years shows positive association between the age group and the infection. There is statistically a strong significant association between age group (9 – 12 years) and the infection (P< 0.0001).

Table 1. Prevalence of *S. haematobium* stratified by local government areas

Community	Number examined	Number infected	Percentage (%)	Chi-square	OR	P-value	95% CL
Aliero	158	27	17.01	10.26	0.45	0.0014**	0.28-0.73
Bunza	158	57	36.01	10.23	2.02	0.0014**	1.327-3.076
Koko	158	42	28.00	0.00	1.0	1.00ns	0.649-1.541
Total	474	126	26.58				

X^2 = 10.26, 2.02, 0.00; OR = 0.45, 2.02,1.0; (P<0.05)

Table 2. Prevalence of *S. haematobium* in relation to age of the pupils

Age	Number examined	Number infected	Percentage	Chi-square	OR	P-value	95% CL
5 – 8	158	25	15.08	11.925	0.419	0.0006***	0.26-0.68
9 – 12	158	75	46.05	51.382	4.695	<0.0001***	3.05
≥ 13	158	26	16.05	11.687	0.426	0.0006***	
Total	474	126	26.58				

X^2 = 11.93, 51.38, 11.69; OR = 0.42, 4.70,0.43; (P<0.05)

3.3 Gender-specific Prevalence of *S. haematobium* among the Pupils

Table 3 depicts gender-specific prevalence of the infection among the pupils. The prevalence was higher among males 88 (37.13%) compared to their female counterpart 38 (16.03%). The result of this study shows that there is a positive association between gender and the prevalence of the infection (OR = 3.09) and association is strongly significant (P<0.0001).

3.4 Prevalence of *S. haematobium* in Relation to Environmental Factor

Prevalence of *S. haematobium* in relation to presence of water body in the community is shown in Table 4. In this study, presence of water body plays a role in transmission of the disease. The prevalence is higher among pupils who have more rivers in their communities (40.93%) than those who have few rivers (5.70%). Odds ratio value of 11.46 and P-value of 0.0001 shows intimate association between the presence of water body and prevalence of urinary schistosomiasis.

3.5 Prevalence of the Infection Based on Socio-Demographic Factors of the Pupils

The effect of socio-demographic variables on the prevalence of urinary schistosomiasis in the study population is presented in Table 5. A noticeable difference in prevalence was observed among various occupations, where prevalence varied from 14.05% - 33.67%. The prevalence of *S. haematobium* among pupils whose parents' occupation is farming/fishing was 67 (33.67%) out of 199. This is followed by pupils whose parents are artisan (30.43%). Prevalence of the parasites was low among pupils whose parents' occupation are civil service and trading/business with total infected cases of 17 (14.05%) and 21 (24.71%) respectively. An odds

ratio value of 1.86 showed that there is intimate association between farming/fishing and the prevalence of the infection and the association is statistically significant (P = 0.004).

Level of education showed no significant influence (OR = 1.30, P =0.28) on prevalence despite higher infection rate among illiterates (28.38%) than in those who had formal education (23.39%).

In this study, water contact plays significant role in the transmission of urinary schistosomiasis. The prevalence is highest among pupils who indicated that they go for swimming or fishing 113 (43.63%) out of 259, than those who do not go for swimming or fishing 13 (6.05%) as shown in Table 5. An odds ratio value of 12.03 shows positive association between the prevalence and the infection, and association is strongly significant (P<0.0001).

3.6 Prevalence of the Infection Based on Water Contact Activities

A total of six water contact activities were recorded. These are bathing, swimming, washing, fishing, fetching water, and irrigation. Differences in prevalence was observed among these various water contact activities where prevalence varied between 39.58% - 16.04% (Table 6). Highest prevalence of the infection was recorded among pupils that engaged in swimming (39.58%) while low prevalence was recorded among pupils whose water contact activity is fetching (16.04). The result of this study revealed significant association between water contact and the prevalence of urinary schistosomiasis (P = 0.0122).

3.7 Prevalence of Haematuria and Proteinuria among Affected Pupils

Reagent strips were used to determine the presence of blood in urine (haematuria) and presence of protein (proteinuria). 474 samples of

urine were analyzed out of which 350 representing 73.83% were positive for haematuria while 280 representing 59.07 was positive for proteinuria (Table 7). The specificity and sensitivity of haematuria and proteinurai in the diagnosis of urinary schistosomiasis are shown in Tables 9 and 10. The sensitivity and specificity of haematuria were 75% and 32% respectively, while sensitivity and specificity of proteinuria were 57.5% and 32% respectively.

3.8 Distribution of *S. haematobium* Snail Vector

The *Bulinus spp* is an intermediate host in the transmission of urinary schistosomiasis which is a water and snail borne disease. The distribution of the snail vector in the study area shows percentage of (50%) in Bunza and the other areas with (16.66%) for Aliero and (33.33%) for Koko.

Table 3. Prevalence of *S. haematobium* infection based on gender of the pupils

Gender	Number examined	Number infected	Percentage	Chi-square	OR	P-value	95% CL
Male	237	88	37.13	25.96	3.09	<0.0001***	2.00-
Female	237	38	16.03				4.78
Total	474	126	26.58				

$X^2 = 25.96$; OR = 3.09; (P<0.05)

Table 4. Prevalence of *S. haematobium* in relation to presence of water body

Presence of river	Number examined	Number infected	Percentage	Chi-square	OR	P-value	95% CL
Yes	281	115	40.93	70.95	11.46	<0.0001***	5.96-22.03
No	193	11	5.70				
Total	474	126	26.58				

$X^2 = 70.95$; OR = 11.46; (P<0.05)

Table 5. Prevalence of *S. haematobium* in relation to socio-demographic variables of the pupils

Variables	Number examine	Number infected (%)	Chi-square	OR	P-value	95% CL
Parent's occupation						
Farming/fishing	199	67(33.67)	8.21	1.86	0.004	1.23-2.81
Civil servant	121	17(14.05)	12.23	0.37	0.0005	0.21-0.64
Trading	85	21(24.71)	0.09	0.89	0.77	0.52-1.53
Artisan	69	21(30.43)	0.40	1.25	0.53	0.71-2.19
Total	474	126(26.25)	1.15	1.30	0.28	0.84-2.00
Parent's education						
Illiterate	303	86(28.38)				
Literate	171	40(23.39)				
Total	474	126(26.58)				

$X^2 = 8.21, 12.23, 0.09, 0.40,1.15$; OR = 1.86, 0.37, 0.89, 1.25, 1.30, (P<0.05)

Table 6. Prevalence of the infection in relation to water contact activities

Water contact Act.	No. examined	No. infected	Percentage	Chi-square	P-value
Bathing	77	18	23.37	14.609	0.0122
Swimming	96	38	39.58		
Washing	63	12	19.04		
Fishing	71	13	16.04		
Fetching	81	12	16.90		
Irrigation	86	23	26.74		
Total	474	126	26.58		

$X^2 = 14.61$, (P<0.05)

Table 7. The prevalence of haematuria and proteinuria in the study area

Haematuria			
Study area	**No Ex**	**No +ve**	**% Prevalence**
Bunza	158	140	88
Aliero	158	120	76
Koko	158	90	56
Total	474	350	22.0
Proteinuria			
Study area	**No Ex**	**No +ve**	**% Prevalence**
Bunza	158	113	71
Aliero	158	105	66
Koko	158	62	39
Total	474	280	17.6

Table 8. Distribution of snail species vector in study areas

No. of snail species identified				
Study area	***Bulinus spp***	***Biomphalaria spp***	***Oncomelania spp***	**%**
Aliero	20	-	-	16.68
Bunza	30	-	-	50
Koko	20	-	-	33.33
Total	60			

Table 9. Sensitivity and specificity of haematuria in the diagnosis of *S. haematobium* infection

Haematuria	Infected	Not infected	Total
+ve	300 (True +ve)	50 (False +ve)	350
-ve	100 (False –ve)	24 (True –ve)	124
Total	400	74	474

$$Sensitivity = \frac{True+ve}{True+ve\ plus\ False-ve} \ X \ \frac{100}{1} = \frac{300}{400} \ X \ \frac{100}{1} = 75\%$$

$$Specificity = \frac{True-ve}{True-ve\ plus\ False+ve} \ X \ \frac{100}{1} = \frac{24}{74} \ X \ \frac{100}{1} = 32\%$$

Table 10. Sensitivity and specificity of proteinuria in the diagnosis of *S. haematobium* infection

Proteinuria	Infected	Not infected	Total
+ve	230 (True +ve)	50 (False +ve)	280
-ve	170 (False –ve)	24 (True –ve)	194
Total	400	74	474

$$Sensitivity = \frac{True+ve}{True+ve\ plus\ False-ve} \ X \ \frac{100}{1} = \frac{230}{400} \ X \ \frac{100}{1} = 57.5\%$$

$$Specificity = \frac{True-ve}{True-ve\ plus\ False+ve} \ X \ \frac{100}{1} = \frac{24}{74} \ X \ \frac{100}{1} = 32\%$$

4. DISCUSSION

As one of the neglected transmitted diseases (NTDs), urinary schistosomiasis has remained a significant cause of mortality and morbidity in many tropical and subtropical populations of the world. This study shows high prevalence of the infection in Bunza followed by Koko, and Aliero having the least prevalence rate. The high prevalence in Bunza might be due to presence of water bodies which result in high water-contact activities in the surrounding streams and rivers that may be harbouring snails-shedding cercaria. Other factors such as poor sanitation, inadequate and indiscriminate disposal of human waste (stool and urine) can be contributing factors. Prevalence in Koko was also a bit high; this could be as a result of the period when the research was carried out being in the rainy season. This could have enhanced the water-contact activities water bodies. Aliero had lower infection prevalence due to lack of water bodies. This pattern of infection of individuals in different locations in the study areas was similar to those recorded by [18,19,5].

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Prevalence studies in relation to age showed 9 - 12 years age group has the highest prevalence than the ages between 5 – 8 years and 13 years above. Possible reasons for these findings may be probably because this age group spends more time in infested streams in one or more water contact activities, such as playing, swimming, washing cloth, bathing etc. As a result, they can get infected by the infective stage of the parasite, cercariae. This age group also contaminates the streams with their urine and faeces which may contain the eggs of the parasite. So, they act as sources of the infective stages of the disease. Urinary schistosomiasis is age-dependent as individual in young age range were not highly infected. This finding is consistent with those of [20].

With regard to gender related infection, the study shows that males were more infected by the disease with 88 (37.13%) compared with the females 38 (16.03%). Odd ratio of 3.09 which shows that there is association with infection and also it is statistically significant (P<0.001) association between sex and infection. The high prevalence recorded in males may be due to high water-contact activities by males as they are more involved in farm work than females. Also, males partake in other water-contact activities such as fishing, swimming and bathing, while females do not participate in these activities due to social-cultural norms. In addition, girls mature earlier and they are socially more restricted in public places than males who swim naked or half-naked. As a result, males get more exposed to cercariae-infested water bodies and get infected by the parasite. This finding is in agreement with the work of other researchers such as [20,21,22]. There is a statistically strong significant association between prevalence of infection and gender. The higher prevalence among males than among females is consistent with earlier reports in Nigeria [23,24].

Environmental factor such as water body also influences high prevalence among pupils who have rivers in their communities with (40.93%) than those who do not have access to river (5.70%). It shows that there is an intimate association between the presence of water body and prevalence of urinary with odd ratio of 6.80. This shows that individuals whose sources of water supply were from water bodies such as streams and rivers were more infected. This observation agrees with records of [21,25,22,26]. The high prevalence rate could be due to the fact that the infected individuals were exposed to

river or streams harbouring the infective stage i.e. the cercariae.

In this study, it was observed that there was disparity among the various occupations where prevalence varied from 14.05% - 33.67%. The prevalence of urinary schistosomiasis was 67 (33.67%) for pupils whose parents are farmers, and those whose parents are civil servant was 17 (14.05%), trading/Artisan with total infected cases of 21 (24.71%) and 21 (30.43%) respectively. An odd ratio value of 1.86 shows that there is intimate association between farming/fishing and prevalence of the infection and association is statistically significant, but there was no association between the prevalence with civil servant and trading/Artisan. The higher infection rates in farming/fishing could be attributed to their frequent contact with water bodies as farmers use the water from infested streams or rivers for their recreational, occupational and domestic activities, and the fishermen are also in contact with water bodies. These categories of people therefore have greater exposure to infective stages of the parasite.

In relation to the distribution of snail vectors, the percentage was higher in Bunza. The higher percentage of the snail vector in Bunza may be perhaps due to the presence of more water bodies than the two other study areas. Other species of snail intermediate host where not seen around the study area.

Haematuria is the most common sign of urinary schistosomiasis and it has been estimated that infected individuals lose between 2.6 mm and 12.6 mm of blood per day [27]. This study reveals that out of the 474 people tested positive for *Schistosoma haematobium*, 350 (73.8%) were having haematuria while 250 (59.07%) had proteinuria. From these findings, gross haematuria (appearance of urine in blood) showed that there is an association between blood in urine appearance and infectivity. Haematuria acts as a good indicator of urinary schistosomiasis infection and as preliminary field survey tool in epidemiological studies [28].

5. CONCLUSION AND RECOMMENDA-TIONS

The study shows that urinary schistosomiasis is a common infection in the study area. Infection is significantly higher among the males compared to the females. Also, the children of

farmers/fishermen had highest prevalence of infection compared to those from other occupational groups. The prevalence was common among younger children than the older ones. Water-contact activities also have a significant role in the transmission of the disease.

In the light of the findings of this study, and for the prevalence of urinary schistosomiasis to be reduced in the study areas, the following are therefore recommended:

1) Further studies should be carried out in other local government areas of Kebbi State to establish comprehensive data on the prevalence of the disease.
2) Public awareness campaign programmes on schistosomiasis should be initiated in the areas.
3) Vulnerable groups such as fishermen, farmers, irrigation workers and communities with high prevalence should have access to regular screening and treatment for schistosomiasis and prevention measures promoted within their respective working environments.
4) Policy makers should recognize the disease as a focal public health problem and should be willing to promote and support control when and where necessary.
5) Provision of portable water to the masses to reduce contact/exposure to water bodies which may become infested with infective stages of the parasite.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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