Morphometric analysis of schistosome eggs recovered from human urines in communities along the shore-line of Oyan-dam in Ogun State, Nigeria

Adedotun Ayodeji Bayegun[1*], Olaitan Olamide Omitola[1], Uche Cynthia Umunnakwe[1], Adedayo Foluke Akande[2], Olaoluwa P. Akinwale[3], Hammed Oladeji Mogaji[4], Kehinde O. Ademolu[1], Pam Vincent Gyang[3], Nnayere Simon Odoemene[5], Russell John Stothard[6], Uwem Friday Ekpo[1*]

[1]Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Nigeria; [2]Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria; [3]Molecular Parasitology Research Laboratory, Public Health and Epidemiology Department, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria; [4]Department of Animal and Environmental Biology, Federal University, Oye-Ekiti, Nigeria; [5]Adeleke University, Ede, Osun State, Nigeria; [6]Department of Tropical Disease Biology, Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

*Corresponding authors: dbayegun@gmail.com, ufekpo@hotmail.com

Abstract

There are growing concerns that communities characterized with surface water, where both humans and livestock interact for agricultural, domestic, cultural, and recreational purposes, are likely to support hybridization between schistosome species infecting humans and livestock. This study therefore investigated the morphometrics of schistosome eggs recovered from human urine samples in four schistosomiasis endemic communities (Imala-Odo, Abule-Titun, Apojula and Ibaro-Oyan) along the banks of Oyan dam in Ogun State, Nigeria. Recovered eggs were counted, photographed, and measured with IC Measure™ for Total Length, Maximum Width, and a ratio of egg shape. A total of 1,984 *Schistosoma* eggs were analysed. Two major egg morphotypes were identified; The first represented 67.8% of the eggs, with the typical round to oval shape and mean length and width of 166µm, 66.8µm, respectively. The second morphotype represented 32.2% of the eggs and are more elongated, with a mean length of 198µm, and width of 71.3µm. Our results revealed significant variations in sizes of the schistosome eggs recovered (length: t=-35.374, df=1982, p=0.000; weight: t=-10.431, df=1982, p=0.000), with the atypical shaped eggs appearing more elongated than expected. These eggs might represent individuals with some degree of contribution from *S. bovis* or possibly other Schistosoma species known to be present in Nigeria. Hence, this observation calls for further molecular studies to establish the genetic information about the miracidia from both atypical and typical eggs. It is also important to establish the presence of bonafide *S. bovis* infection in cattle and vector snails in the presumptive areas of hybridization.

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Introduction

Human schistosomiasis is one of the most prominent neglected tropical diseases (NTDs) in Africa (Hotez and Kamath, 2009). The disease is caused by water-borne, snail-transmitted trematode parasites of the genus *Schistosoma*, and is widely distributed in 78 countries with about 206 million cases and 2.5 million disability adjusted life years (WHO, 2022). There are six species of the genus Schistosoma infecting humans worldwide, with four common species in Africa; *S. haematobium, S. mansoni, S. guineensis* and *S. intercalatum. S. haematobium* is responsible for most of the morbidity in Africa, with the adult parasite inhabiting the vesicular and pelvic venous plexus of the bladder and causing urogenital schistosomiasis (WHO, 2021). The pathologies associated with this species is dependent on the severity of infection, migration of the worms and inflammatory responses to the presence of the eggs (WHO, 2021; CDC, 2021).

Largely, schistosomiasis is a focal disease (GSA, 2020), which thrives in rural and marginalized urban populations that share proximities with surface waterbodies containing the appropriate susceptible species of freshwater snail vectors, and are characterized by inadequate or poor access to water, sanitation and hygiene (WASH) facilities (Olamiju *et al*., 2022). In such areas, contact activities with surface waterbodies ranges from bathing, washing of clothes, swimming and playing are not uncommon (Olamiju *et al*., 2022). These activities support the transmission cycle of the parasites, with infestation of waterbodies through open urination or defecation by an infected resident, and subsequent infection of other residents through other domestic contact purposes. Control and elimination for schistosomiasis have therefore focused on mass administration (MDA) of praziquantel to children between ages 5 and 15 years in endemic communities (WHO, 2022), with possible complementary provision of WASH interventions to promote behavioural change (Grimes *et al*., 2015). However, in endemic communities, with infested surface waterbodies, where both humans and livestock interact,

there are growing concerns on the hybridization of closely related species of humans (*S. haematobium*) and cattle (*S. bovis*) (Léger *et al*., 2016). Hybrids are commonly identified based on discordance between nuclear and mitochondrial markers (Onyekwere et al., 2022). The emergence of hybrid lineages has raised significant concerns for schistosomiasis control and elimination effort (Léger and Webster, 2017), although the extent to which hybridization is actually occurring at present is subject to debate (Platt *et al*., 2019). We therefore hypothesize that the presence of atypical characteristics (morphology and morphotype) of *Schistosoma* eggs may serve as potential indicator in the detection of possible hybridization cases, since different egg morphotypes have been observed among patients infected with *S. haematobium* × *S. bovis* hybrids (Moné *et al*. 2012, 2015; Soentjens *et al*. 2016; Boon *et al.* 2017).

Nigeria has the highest burden of schistosomiasis in Africa (Hotez and Kamath, 2009), with the disease been endemic across all 36 States in the country (FMOH, 2019). Around certain transmission foci, precisely, communities situated along the banks of Oyan-dam in the southwestern part of the country, prevalence can reach as high as 90% (Akinwale *et al*., 2010). The communities (Abule-Titun, Apojola, Ibaro and Imala-Odo) have remained highly endemic since 1991 despite ongoing interventions (Ekpo *et al*., 2017). Predominant water contact activities like fishing, farming, bathing, swimming, drinking, washing clothes or kitchen utensils and fetching of water from infested surface waterbodies are common (Akinwale *et al*., 2010; Ekpo *et al*., 2017). Furthermore, livestock farming is one of the most common occupation of the populace, and this allows interaction between cattle with humans along the banks of the dam that surrounds these communities (Fig 1). Since *S. bovis* has been previously reported as the predominant livestock species in the country (Hambali *et al*., 2016; Akande and Alohutade, 2021), we therefore hypothesize that *Schistosoma* hybridization and zoonotic transmission may be ongoing in Nigeria in obscurity. This present study therefore aims to

characterize the morphology of *Schistosoma* eggs recovered from urine samples of humans living along the banks of Oyan-dam in the southwestern part of Nigeria, as part of an ongoing effort towards detection of possible hybridization cases.

Fig 1. Sharing of common water source by humans and cattle at Apojola community.

Methods

Ethical Statements and Consideration

This study received ethical approval from Health ethics review board of Ogun State Hospital, Ijaiye, with the approval number SHA/RES/VOL.4/154. Pre-survey contact/advocacy meeting was made to study communities to obtain consents from the community leaders after explaining the objectives of the research to them. Communities willing to participate in the study completed written consent forms. Subsequently, children and adults were also informed about the study through community meetings organized by the consenting representatives. Formal written consents were provided by all participants above 16 years of age. However, for children below 16 years of age, an assent form was completed by each child, in addition to a consent form completed by their parents/caregiver. Only participants with completed consent and assent forms were recruited in the research.

Study communities

This study was conducted in four communities along the shoreline of Oyan River Dam in Ogun State, Nigeria. Ogun State is one of the 36 States in the Federal Republic of Nigeria, located in the southwestern region of the country (Fig 2). It covers a total area of 16,409.26 sq. km between latitude 6.2^{o}N and 7.8^{o}N and longitude 3.0^{o}E and 5.0^{o}E. The State has 20 Local Government Areas (LGA) comprising 236 political wards. The greater proportion of the State lies in the tropical rainforest zone, while the far northern part has features of the Guinea Savannah. In the early 90s, the Oyan River Dam was established, with its shorelines around

four major communities; Imala-Odo and Ibaro Oyan in Abeokuta North LGA, and Apojola and Abule-titun in Odeda LGA (Fig 2). These communities have remained endemic for schistosomiasis since 1991 (Ekpo *et al*., 2017), with predominant occupations been fishing and livestock farming (Akinwale *et al*., 2010). This in addition to poor access to improved WASH facilities have also promoted water contact activities such as bathing, swimming, drinking, washing clothes or kitchen utensils and fetching of water from surface waterbodies, and more importantly shared interaction between cattle with humans along the shoreline of the dam that surrounds these communities (Fig 1).

Fig 2: Map of Ogun State showing the local government areas (LGAs) and study communities and river system

Sample size determination and recruitment of study participants

The sample size for this study was determined using the formula described by (Thrusfield, 2005), $\boldsymbol{n_s} = (\frac{\mathbf{1.96}}{\boldsymbol{d}})^2 . (p(1-p)$ where n_s is the sample size, p is the existing prevalence in the study area, and d is the degree of accuracy. In determining the sample size, a prevalence of 47% (Ekpo *et al*., 2017), and a degree of accuracy of 5% was considered at 95% level of confidence. The minimum sample size determined therefore was 383 i.e., an average of 96 persons per community. The communities were compact, and invitations to participate were sent to all residents through a local mobiliser. Recruitment and collection of samples was done at a central location and only residents who consented to the study procedures were enrolled into the study.

Sample collection and examination

Urine samples were collected from consenting residents comprising of infants and preschoolers (1-4years), school-aged children (5-15 years) and those above 16-64 years across study

communities between October and November, 2019. Age of participants were validated using birth-cards to avoid information bias. Three-hundred and eighty-four samples were collected in dark, sterile 30ml universal containers and preserved with 70% ethanol. Collections were made between 10.00 and 14.00 hours as recommended (Ekpo *et al*., 2010) and transported to the laboratory in iceboxes. Urine samples were processed using sedimentation techniques, and examined under the microscope for the presence of *S. haematobium* eggs. A total of 219 (57.0%) of the samples examined were positive for *S. haematobium* eggs, and seperated for subsequent screening using morphometric method. Participants who were positive with *S. haematobium* eggs were treated with praziquantel.

Morphometric analysis of *S haematobium* eggs

A total of 1984 eggs were recovered from urine sediment of infected participants. Eggs were picked at random and examined using morphometric methods. Microphotographs of the *Schistosoma* eggs and ova were taken using an AmScope MD130 1.3MP Digital Microscope (United Scope LLC., CA, USA) and the IC Measure™ (The Imaging Source Europe GmbH, Bremen, Germany) computer software was used to measure the total length (including the terminal spine) and the maximum width. The egg length/width ratio was subsequently computed. Qualitative characteristics such as unusual morphology was noted, and the presence or absence of the terminal spine was also recorded. The eggs were classified as typical if they have a round-to-oval shape or atypical if they have spindle/elongated shape (Boon *et al*., 2017; Pitchford, 1965). Atypical schistosomes were identified by their spindle egg-shape (Boon *et al*., 2017; Moné *et al*., 2015). A total of 639 (32.2%) eggs were characterized with atypical shape.

Data analysis

Data collected were analysed using SPSS version 23.0 for Windows. Descriptive statistics and differences in proportions were tested using the Chi-square statistics, either for trend or independence, as appropriate. The number of eggs counted was transformed using lograthmic function (log (n+1)), to normalize the distribution of the residual values for statistical analyses. Morphometric data were exported from the IC Measure™ into Microsoft Excel for analysis. Differences between means between the egg morphotypes across the study communities were tested using independent sample t-test, and statistical difference was set at 95% confidence interval (p-value < 0.05).

Results

Demographic characteristics of study participants and infection status

A total of 384 participants were recruited, 198 (51.6%) males and 186 (48.4%) females, between the age group 1-4 years (112; 29.2%), 5-15 years (190; 49.5%) and 16-64 years (82; 21.4%). An overall prevalence of 219 (57.0%) was recorded, with majority of the infection among the participants from Ibaro-Oyan with a prevalence of 62.4%. Also, the prevalence of infection was higher in the males (31.8%) and in the 5-15 years age group (26.0%) (Table 1).

184 ***Table 1: Demographic characteristics and infection status of study participants***

	Communities									
	Imala-Odo		Abule Titun		Apojola		Ibaro-Oyan		Total	
	NE (%)	NI (%)	NE (%)	NI (%)	NE (%)	NI (%)	NE (%)	NI (%)	NE (%)	NI (%)
Sex										
Male	55 (58.5)	34 (36.2)	51 (58.6)	29 (33.3)	61 (51.7)	39 (33.1)	31 (36.5)	20 (23.5)	198 (51.6)	122 (31.8)
Female	39 (41.5)	21 (22.3)	36 (41.4)	18 (20.7)	57 (48.3)	25 (21.2)	54 (63.5)	33 (38.8)	186 (48.4)	97 (25.3)
Total	94 (100.0)	55 (58.5)	87 (100.00	47 (54.0)	118 (100.0)	64 (54.2)	85 (100.0)	53 (62.4)	384 (100.0)	219 (57.0)
p-value		0.525		0.663		0.042		0.819		0.064
Age group (in years)										
1-4	29 (30.9)	17 (18.1)	22 (25.3)	11 (12.6)	32 (27.1)	20 (16.9)	29 (34.1)	18 (21.2)	112 (29.2)	66 (17.2)
5-15	53 (56.4)	29 (30.9)	49 (56.3)	27 (31.0)	50 (42.4)	20 (16.9)	38 (44.7)	24 (28.2)	190 (49.5)	100 (26.0)
16 -64	12 (12.8)	9 (9.6)	16 (18.4)	9 (10.3)	36 (30.5)	24 (20.3)	18 (21.2)	11 (12.9)	82 (21.4)	53 (13.8)
Total	94 (100.0)	55 (58.5)	87 (100.0)	47 (54.0)	118 (100.0)	64 (54.2)	85 (100.0)	53 (62.4)	384 (100.0)	219 (57.0)
p-value		0.436		0.906		0.027		0.988		0.165

185 **NE: Number Examined; NI: Number Infected**

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Morphotypes of *Schistosoma* eggs across the study areas

A total of 1,984 schistosome eggs from 219 (57.0%) infected individuals were photographed and measured. This comprised 605 (30.5%), 313 (15.8%), 775 (39.1%) and 291 (14.7%) eggs from Imala-Odo, Abule-titun, Apojola and Ibaro-oyan, respectively (Table 2). Three egg morphotypes were recorded in this study (Fig 3, 4 and 5). The majority of the schistosome eggs were of the typical round-to-oval shape (1345, 67.8%), and atypical elongated or spindle-shape (639, 32.2%). By location, 417 (68.9%), 192 (61.3%), 523 (67.5%) and 213 (73.2%) of the eggs recovered from Imala-Odo, Abule-titun, Apojola and Ibaro-oyan were round-to-oval shape, while 188 (31.1%), 121 (38.7%), 252 (32.5%) and 78 (26.8 %) were spindle-shaped respectively (Table 2). There was a significant variation in egg morphotypes across the study area ($p = 0.017$). About 98.7% and 99.2% of the typical and atypical eggs were with spines. There was also a significant variation in the presence of spined eggs across the study area ($p = 0.05$). However, majority of the spineless *Schistosoma* eggs (17, 77%) were of the typical round to oval shape, and (5, 23%) with the spindle shape. The typical-shaped but spineless eggs were recovered from Imala-odo, (8, 1.3 %), Apojola (5, 0.6 %) and Ibaro-oyan (4, 1.4 %), while the spineless spindle shaped eggs were recovered from Abule-titun (5, 1.6 %). There was also a significant variation in the presence of spineless eggs across the study area ($p = 0.00$).

Fig 3. A typical Schistosoma haematobium egg with the terminal spine

Fig. 4. An atypical spindle-shaped Schistosoma egg

Fig. 5. A spineless Schistosoma egg

Table 2: The morphotypes of *Schistosoma* eggs across the study areas

	Egg Morphotypes						
		Round-to-oval shape			Spindle shape		
Communities	**NEE**	**Total**	**Spine**	**Spineless**	**Total**	**Spine**	**Spineless**
Imala-Odo	605	417 (68.9)	409(98.1)	8(1.9)	188 (31.1)	188(100)	-
Abule-Titun	313	192 (61.3)	192(100)	-	121 (38.7)	116(95.9)	5(4.1)
Apojola	775	523 (67.5)	518(99)	5(1.0)	252 (32.5)	252(100)	-
Ibaro-oyan	291	213 (73.2)	209(98.1)	4(1.9)	78 (26.8)	78(100)	-
Total	1984	1345 (67.8)	1328(98.7)	17(1.3)	639 (32.2)	634(99.2)	5(0.8)
Typical eggs * Atypical eggs: Df: 3, Chi-square: 10.246, p-value: 0.017							
Typical & spined eggs * Atypical & spined eggs : Df: 3, Chi-square: 7.735, p-value: 0.05							
p-value; typical & spineless eggs * atypical & spineless eggs : Df: 3, Chi-square: 22, p-value: 0.000							

NEE; Number of eggs examined ; Df; Degree of freedom

Morphometrics of *Schistosoma* eggs across the study areas

Two major egg morphotypes were identified across the 1,984 eggs that were analyzed. The first morphotype represented 67.8% of the eggs, with a round to oval shape, mean length and width of 166±18µm and 66.8±9µm, respectively (Figure 6). Also, the second morphotype represented 32.2% of the eggs and are more elongated, with a mean length and width of 198±18µm and 71.3±8µm, respectively (Figure 7). The dimensions of each morphotypes were significantly different, both across the communities surveyed, and when grouped (for length: t=-35.374, df=1982, p=0.000; for weight: t=-10.431, df=1982, p=0.000) The mean length, mean width and length too width ratio of the eggs for both morphotypes are shown in Table 3.

Figure 6: Scatterplot distribution of typical Schistosoma haematobium egg recovered from human urine. Length is within 83-187 µm (Pitchford, 1965)

222 ***Figure 7: Scatterplot distribution of atypical Schistosoma egg recovered from human urine.***

223 ***Length exceeds 83-187 µm (Pitchford, 1965)***

224 **Table 3: The morphometrics of *Schistosoma* eggs recovered across the study areas**

225 **(Length of typical *S. haematobium egg*: 83-187 µm; Pitchford, 1965)**

		Mean ± Standard Deviation		
	NEE	**Length (µm)**	**Width (µm)**	**Length/width ratio (µm)**
Imala Odo				
Round to oval	417	164.58±20.55	65.51±9.61	2.54±0.29
Spindle (atypical)	188	198.63±18.89	70.68±7.79	2.82±0.25
t, df, p-value		-19.954, 603, 0.000	-6.475, 603, 0.000	-11.557, 603, 0.000
Abule Titun				
Round to oval	192	172.18±12.60	69.21±8.06	2.52±0.31
Spindle (atypical)	121	201.47±10.90	74.23±7.14	2.74±0.28
t, df, p-value		-21.076, 311, 0.000	-5.609, 311, 0.000	-6.304, 311, 0.000
Apojola				
Round to oval	523	165.57±19.46	65.86±9.43	2.54±0.28
Spindle (atypical)	252	196.73±23.57	69.64±9.18	2.84±0.29
t, df, p-value		-19.458, 773, 0.000	-5.270, 703, 0.000	-13.719, 703, 0.000
Ibaro-Oyan				
Round to oval	213	169.59±13.62	69.58±6.33	2.45±0.25
Spindle (atypical)	78	197.65±6.71	73.22±7.84	2.72±0.26
t, df, p-value		-17.421, 289, 0.000	-4.072, 289, 0.000	-8.201, 289, 0.000
Total				
Round to oval	1345	166.84±18.36	66.82±9.03	2.52±0.29
Spindle (atypical)	639	198.30±18.82	71.25±8.44	2.80±0.28
t, df, p-value		-35.374, 1982, 0.000	-10.431, 1982, 0.000	-20.497, 1982, 0.000

226 **NEE: Number of eggs examined**

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Discussion

Human urinary schistosomiasis caused by *S. haematobium* is one of the most important neglected tropical diseases in sub-Saharan Africa, with a wide geographic spread in Africa, Middle East and Corsica (WHO, 2022). Nigeria remained the most endemic country in Africa, and this present study reports a prevalence of 57.0% (range: 47.1% - 63.8%) across the four study communites. This prevalence falls within the WHO hyperendemic thresholds (WHO, 2022), and conforms with earlier reports, highlighting unabated transmission of schistosomiasis across the study area, despite ongoing chemotherapy interventions (Akinwale *et al*., 2010; Ekpo *et al*., 2017). For over a decade, control efforts have been focused on humans, with little attention given to schistosomiasis in livestock. *Schistosoma bovis*, a zoonotic species has an extensive geographical distribution also in Africa, and have been reported in Benin, Togo, Burkina Faso, Niger, and Nigeria (Moné *et al.* 1999; Savassi *et al*., 2020), Countries such as Mali, Senegal, Niger and Benin Republic have reported the hybridization of *S. haematobium* that infects humans and *S. bovis* that infects cattle (Huyse *et al*., 2009, 2013; Soentjens *et al*., 2016; Leger *et al*., 2016; Savassi *et al*., 2020, 2021). These countries share Niger river and its tributaries, with Nigeria, where the possibility of migrating snails infected with hybrid schistosomes (*Schistosoma haematobium* x *Schistosoma bovis*) is likely to become established in Nigerian rivers system. Although snails' migratory patterns can be influenced by flood, however their power of dispersal are often limited, compared to humans and cattle, who are more mobile species with greater dispersal tendencies, necessary to spread *Schistosoma* hybrids. Our study therefore marks as the first attempt to investigate the morphotypes and morphometrics of *Schistosoma* eggs collected from human samples in Nigeria, as part of an ongoing effort to detect if hybridization cases already exist in the country.

Currently, there are no studies reporting the spatial co-distribution of *S. haematobium* and *bovis*. However, *S. haematobium* has been largely reported among humans in Nigeria (Ekpo *et al*., 2017; Otuneme *et al*., 2019; Ejike *et al.*, 2020), and there are scanty but emerging reports on *S. bovis* among livestock (Hambali *et al*., 2016). Although the eggs of both species are terminally-spined, they differ in several other aspects. For instance, *S. haematobium* eggs are deposited in urine, and are round-to-oval in shape with a length ranging from 83 to 187 μm (Pitchford, 1965). In contrast, *S. bovis* eggs are deposited in stool and are spindle-shaped, consisting of a broad middle portion and drawn-out rod-like ends, one bearing a well differentiated spine, the other evenly rounded (Taylor, 1970; Touassem, 1987). *S. bovis* eggs are also larger than those of *S. haematobium*, with a length ranging from 90 to 220 μm (Pitchford, 1965), and as high as 300 μm (Taylor, 1970; Touassem, 1987). On the other hand, we found 22 spineless eggs with miracidia, representing approximately 1% of all eggs examined, with majority of them having the typical *S. haematobium* shape and size range. It is therefore valuable to further unravel in future studies, the potentials and contributions of these eggs in the *S. haematobium* and *S. bovis* hybridization pathway. Nevertheless, about 67% of the egg sizes reported in our study (166·84 ± 18.36 μm × 66.82 ± 9.03 μm) corresponds very well with *S. haematobium* egg sizes (Pitchford, 1965). In addition, the mean egg length/width ratio of 2.5 recorded for these eggs also corresponds with that reported by Boon *et al*. (2017). However, the remainder one-third of the eggs had an intermediate shape between the typical *S. haematobium* and *S. bovis* ova. These eggs are more elongated in shape. These findings conform with the report of Savassi *et al* (2020) in Benin, and Moné *et al*. (2015) in France. The sizes of the atypical eggs (198.30 ± 18.82 μm × 71.25 ± 8.44 μm) are significantly longer and bigger than reported for *S. haematobium*. Also, the 2.8 mean egg length/width ratio recorded is significantly higher than those of *S haematobium* and approaches the ratio reported for *S.*

bovis by Boon *et al*. (2017). These dimensions for the intermediate eggs further suggest that these eggs have a shape resembling *S. bovis.*

The egg shape was taken using digital microscopes and sizes were measured using the IC Measure™ in the laboratory (off-field). Obviously, these morphological methods are time-consuming and hence would be impractical in the context of the high-throughput screening of samples, especially in the field. Besides, microscopic and morphological methods are best suited for rapid detection of eggs and identification of species, mostly for programmatic purposes (Kincaid-Smith *et al*., 2021). However, egg size and shape (length/width ratio) could be highly variable within a single patient, hence impeding impede species identification (Almeda *et al*. 1996). As such, morphometric analysis of eggs cannot be solely used as a tool in establishing the presence of hybrid schistosomes (Boon *et al*., 2017). It should therefore be noted that this present study did not use molecular methods to verify species status. Hence, some of these measurements could include hybrid rather than pure *S. haematobium* or *S. bovis* eggs. It is therefore important to validate our hypothesis of a possible hybridization between *Schistosoma haematobium* x *Schistosoma bovis* with DNA sequencing of eggs from both morphotypes. Molecular analyses are currently being planned, however the findings reported here provides preliminary evidence on the morphotypes and morphometrics of eggs recovered from human urine in the southwestern region of Nigeria, which sheds light on an important but understudied area, hence calling for more research efforts in other parts of the country.

Conclusion

Our results revealed significant variations in sizes of the schistosome eggs recovered, with the atypical shaped eggs appearing more elongated than expected. These eggs might represent individuals with some degree of contribution from *S. bovis* or possibly other Schistosoma

species known to be present in Nigeria. Hence, this observation calls for further molecular studies to establish the genetic information about the miracidia from both atypical and typical eggs. It is also important to establish the presence of bonafide *S. bovis* infection in cattle and vector snails in the presumptive areas of hybridization.

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Conflict of Interest

The author(s) declare none

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008**.**

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