## Research Paper

# Morphometric analysis of schistosome eggs recovered from human urines in communities along the shoreline of Oyan River Dam in Ogun State, Nigeria

A. A. Bayegun[1] [iD], O. O. Omitola[1], C. U. Umunnakwe[1], F. A. Akande[2],

O. P. Akinwale[3], H. O. Mogaji[4], K. O. Ademolu[1], V. P. Gyang[3], S. N. Odoemene[5],

J. R. Stothard[6] and U. F. 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, Ekiti State, Nigeria; [5]Adeleke University, Ede, Osun State, Nigeria and [6]Department of Tropical Disease Biology, Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

## 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 River 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 1984 *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 166 μm, 66.8 μm, respectively; the second represented 32.2% of the eggs and are more elongated, with a mean length of 198 μm, and width of 71.3 μm. Our results revealed significant variations in sizes of the schistosome eggs recovered (length: $t = -35.374$, degrees of freedom (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 *Schistosoma 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 bona fide *S. bovis* infection in cattle and vector snails in the presumptive areas of hybridization.

## Introduction

Human schistosomiasis is one of the most prominent neglected tropical diseases (NTDs) in Africa (Hotez & 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 (World Health Organization, 2022). There are six species of the genus *Schistosoma* infecting humans worldwide, with four common species in Africa: *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma guineensis* and *Schistosoma intercalatum*. *Schistosoma 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 (World Health Organization, 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 (Centers for Disease Control, 2021; World Health Organization, 2021).

Largely, schistosomiasis is a focal disease (Global Schistosomiasis Alliance, 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

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

activities support the transmission cycle of the parasites, with infestation of water bodies 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 of praziquantel to children between ages 5 and 15 years in endemic communities (World Health Organization, 2022), with possible complementary provision of WASH interventions to promote behavioural change (Grimes *et al.*, 2015). However, in endemic communities, with infested surface water bodies, where both humans and livestock interact, there are growing concerns on the hybridization of closely related species of humans (*S. haematobium*) and cattle (*Schistosoma 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 & 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 a 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 & Kamath, 2009), with the disease being endemic across all 36 States in the country (Federal Ministry of Health, 2019). Around certain transmission foci, precisely, communities situated along the banks of Oyan-dam in the south-western 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 such as fishing, farming, bathing, swimming, drinking, washing clothes or kitchen utensils and fetching of water from infested surface water bodies are common (Akinwale *et al.*, 2010; Ekpo *et al.*, 2017). Furthermore, livestock farming is one of the most common occupations 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 & Alohuntade, 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 the Oyan River Dam in the south-western part of Nigeria, as part of an ongoing effort towards detection of possible hybridization cases.
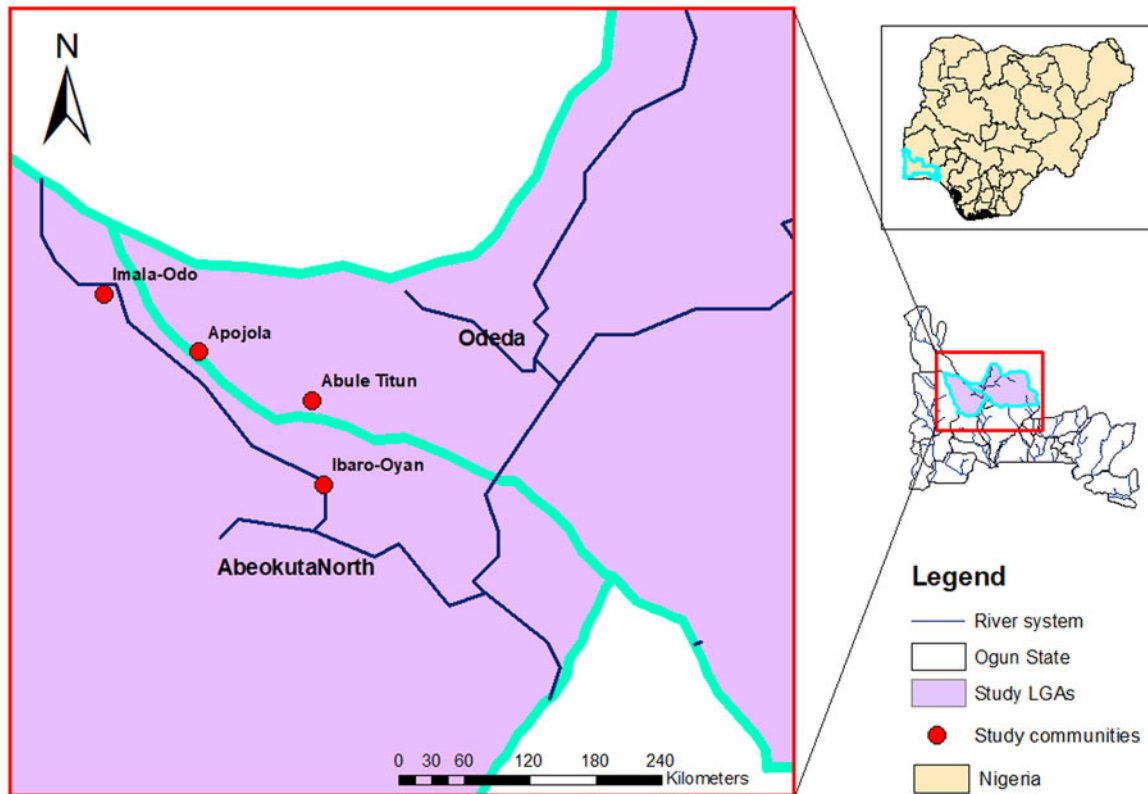
**Fig. 2.** Map of Ogun State showing the local government areas (LGAs) and study communities and river system.

## Methods

### 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 south-western region of the country (fig. 2). It covers a total area of 16,409.26 sq. km between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E. The State has 20 local government areas (LGAs) 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 1990s, 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 schisto-somiasis since 1991 (Ekpo et al., 2017), with predominant occupations being 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 water bodies, and more importantly shared interaction between cattle with humans along the shoreline of the dam that surrounds these communities (fig. 1).

### Sample size determination and recruitment of study participants

The sample size for this study was determined using the formula described by Thrusfield (2005), $n_s = (1.96/d)^2 \cdot (p(1-p)$ 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, that is, an average of 96 persons per community. The communities were compact, and invitations to participate were sent to all residents through a local mobilizer. 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 infants and preschoolers (1–4 years), 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, ster-ile 30 ml universal containers and preserved with 70% ethanol. Collections were made between 10.00 and 14.00 h as recommended (Ekpo et al., 2010) and transported to the laboratory in ice boxes. Urine samples were processed using sedimentation techniques, and examined under the microscope for the presence of S. haema-tobium eggs. A total of 219 (57.0%) of the samples examined were positive for S. haematobium eggs, and separated for subsequent screening using the 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 (Pitchford, 1965; Boon *et al.*, 2017). Atypical schistosomes were identified by their spindle egg-shape (Moné *et al.*, 2015; Boon *et al.*, 2017). 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 logarithmic 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 an independent sample *t*-test, and statistical difference was set at 95% confidence interval (*P*-value < 0.05). The corresponding dataset and other supporting files have been attached as supplementary files.

## 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 the 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).

### Morphotypes of Schistosoma *eggs across the study areas*

A total of 1984 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 (figs 3–5). 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

**Table 1.** Demographic characteristics and infection status of study participants.

| | Communities | | | | | | | | | |
| | Imala-Odo | | Abule Titun | | Apojola | | Ibaro-Oyan | | Total | |
| | Number examined (NE) (%) | Number infected (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 | |

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

| Communities | Number of eggs examined | Round-to-oval shape | | | Spindle shape | | |
|---|---|---|---|---|---|---|---|
| | | 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 and spined eggs * atypical and spined eggs; df, 3; Chi-square, 7.735; *P*-value, 0.05 | | | | | | | |
| typical and spineless eggs * atypical and spineless eggs; df, 3; Chi-square, 22; *P*-value, 0.000. | | | | | | | |

df, degrees of freedom; *, crosstabulation



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



**Fig. 5.** A spineless *Schistosoma* egg.
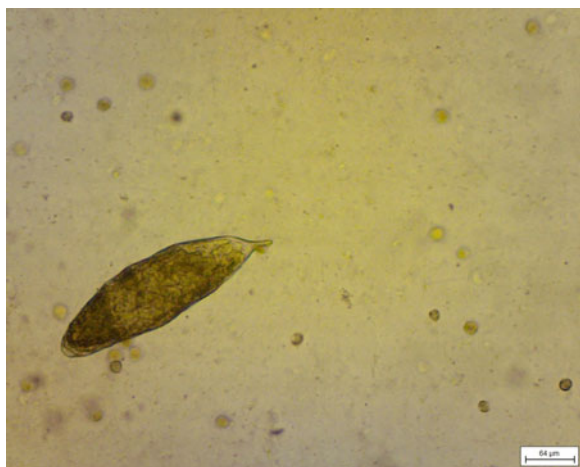


**Fig. 4.** An atypical spindle-shaped *Schistosoma* egg.

($P = 0.05$). However, the 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 ($P = 0.00$).

### Morphometrics of Schistosoma *eggs across the study areas*

Two major egg morphotypes were identified across the 1984 eggs that were analysed. The first morphotype represented 67.8% of the eggs, with a round-to-oval shape, mean length and width of $166 \pm 18$ µm and $66.8 \pm 9$ µm, respectively (fig. 6). Also, the second morphotype represented 32.2% of the eggs and are more elongated, with a mean length and width of $198 \pm 18$ µm and $71.3 \pm 8$ µm, respectively (fig. 7). The dimensions of each morphotype were significantly different, both across the communities surveyed, and when grouped (for length: $t = -35.374$, degrees of freedom (df) = 1982, $P = 0.000$; for weight: $t = -10.431$, df = 1982, $P = 0.000$) The mean length, mean width and length/width ratio of the eggs for both morphotypes are shown in table 3.
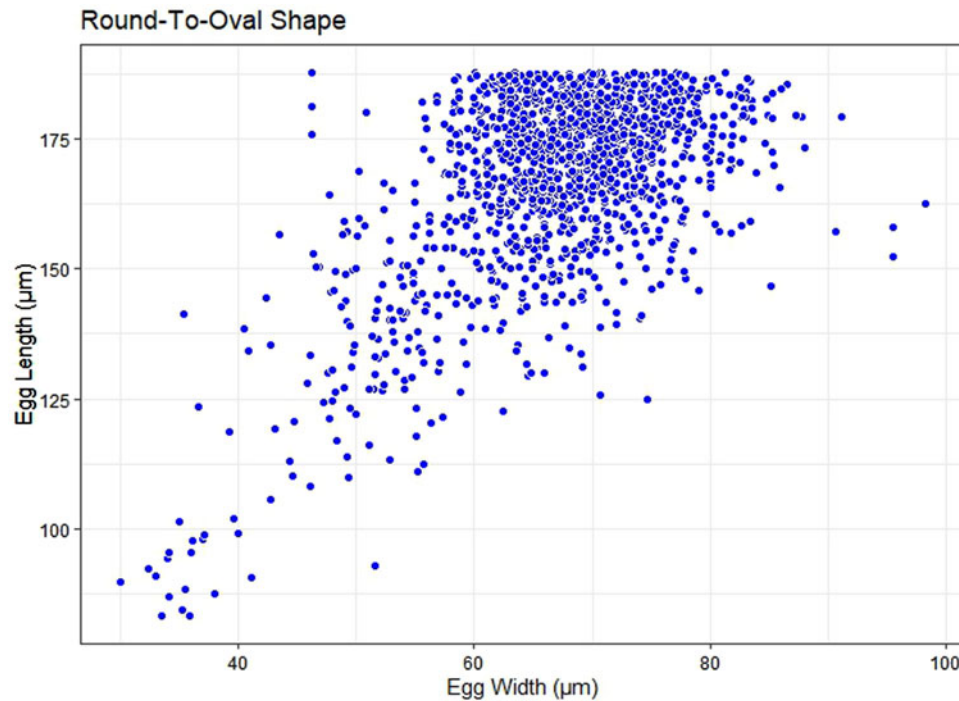
## Round-To-Oval Shape



**Fig. 6.** Scatterplot distribution of typical *Schistosoma haematobium* egg recovered from human urine. Length is within 83–187 µm (Pitchford, 1965).
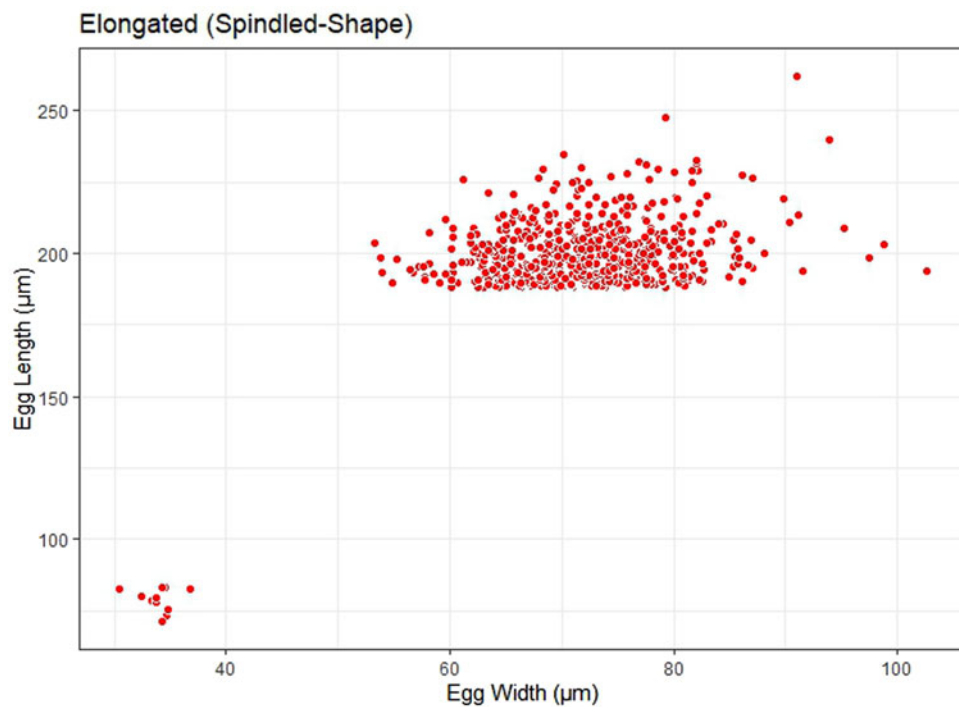
## Elongated (Spindled-Shape)



**Fig. 7.** Scatterplot distribution of atypical *Schistosoma* egg recovered from human urine. Length exceeds 83–187 µm (Pitchford, 1965).

## Discussion

Human urinary schistosomiasis caused by *S. haematobium* is one of the most important NTDs in Sub-Saharan Africa, with a wide geographical spread in Africa, the Middle East and Corsica (World Health Organization, 2022). Nigeria remains 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 communities. This prevalence falls within the World Health Organization hyperendemic thresholds (World Health Organization, 2022), and conforms with earlier reports, highlighting unabated transmission of schistosomiasis across the study area, despite ongoing

**Table 3.** The morphometrics of *Schistosoma* eggs recovered across the study areas (length of typical *Schistosoma haematobium* egg: 83–187 μm; Pitchford, 1965).

| | Number of eggs examined | Mean ± standard deviation | | |
| --- | --- | --- | --- | --- |
| | | 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*, degrees of freedom (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 |

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 throughout 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 the Republic of Benin have reported the hybridization of *S. haematobium* that infects humans and *S. bovis* that infects cattle (Huyse *et al.*, 2009, 2013; Léger *et al.*, 2016; Soentjens *et al.*, 2016; Savassi *et al.*, 2020, 2021). These countries share the Niger river and its tributaries, with Nigeria, where the possibility of migrating snails infected with hybrid schistosomes (*S. haematobium* x *S. bovis*) is likely to become established in the Nigerian rivers system. Although snails' migratory patterns can be influenced by flood, their powers of dispersal are often limited, compared to humans and cattle, who are more mobile species with greater dispersal tendencies, necessary to the 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 whether hybridization cases already exist in the country.

Currently, there are no studies reporting the spatial co-distribution of *S. haematobium* and *S. bovis*. However, *S. haematobium* has been largely reported among humans in Nigeria (Ekpo *et al.*, 2017; Otuneme *et al.*, 2019; Ejike *et al.*, 2021), 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). *Schistosoma 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 the 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 remaining 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 the Republic of 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 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 *S. haematobium* x *S. bovis* with DNA sequencing of eggs from both morphotypes. Molecular analyses are currently being planned; however, the findings reported here provide preliminary evidence on the morphotypes and morphometrics of eggs recovered from human urine in the south-western 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 bona fide *S. bovis* infection in cattle and vector snails in the presumptive areas of hybridization.

**Ethical statement.** This study received ethical approval from the Health Ethics Review board of Ogun State Hospital, Ijaiye, with the approval number SHA/RES/VOL.4/154. A pre-survey contact/advocacy meeting was organized 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. 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.

## References

**Akande F and Alohuntade M** (2021) Diagnosis of bovine gastrointestinal parasites: comparison of different techniques and different solutions. *Annals of Parasitology* **67**(3), 407–416.

**Akinwale OP, Ajayi MB, Akande DO, Gyang PV, Adeleke MA, Adeneye AK, Adebayo MO and Dike AA** (2010) Urinary schistosomiasis around Oyan Reservoir, Nigeria: twenty years after the first outbreak. *Iranian Journal of Public Health* **39**(1), 92–95.

**Almeda J, Ascaso C, Marçal GA, Corachan M, Southgate VR and Rollinson D** (1996) Morphometric variability of *Schistosoma intercalatum* eggs: a diagnostic dilemma. *Journal of Helminthology* **70**(2), 97–102.

**Boon NAM, Fannes W, Rombouts S, Polman K, Volckaert FAM and Huyse T** (2017) Detecting hybridization in African schistosome species: does egg morphology complement molecular species identification? *Parasitology* **144** (7), 954–964.

**Centers for Disease Control** (2021) *Schistosomiasis. Laboratory identification of parasites of public health concern.* Available at https://www.cdc.gov/dpdx/ schistosomiasis/index.html (accessed 20 September 2021).

**Ejike CU, Oluwole AS, Omitola OO, Bayegun AA, Shoneye IY, Akeredolu-Ale BI, Idowu OA, Mafiana CF and Ekpo UF** (2021) Schisto and Ladders version 2: a health educational board game to support compliance with school-based mass drug administration with praziquantel – a pilot study. *International Health* **13**(3), 281–290.

**Ekpo UF, Laja-Deile A, Oluwole AS, Sam-Wobo SO and Mafiana CF** (2010) Urinary schistosomiasis among preschool children in a rural community near Abeokuta, Nigeria. *Parasites & Vectors* **3**(1), 58.

**Ekpo UF, Odeyemi OM, Sam-Wobo SO, Onunkwor OB, Mogaji HO, Oluwole AS, Abdussalam HO and Stothard JR** (2017) Female genital schistosomiasis (FGS) in Ogun State, Nigeria: a pilot survey on genital symptoms and clinical findings. *Parasitology. Open* **3**(1), e10.

**Federal Ministry of Health** (2019) *Neglected Tropical Diseases Master Plan 2015–2020.* Available at https://espen.afro.who.int/system/files/content/ resources/NIGERIA_NTD_Master_Plan_2015_2020.pdf (accessed 15 February 2022).

**Global Schistosomiasis Alliance** (2020) *Accelerating progress for schistosomiasis control and elimination post-2020.* Available at https://www.eliminateschisto.org/sites/gsa/files/content/attachments/2019-06-26/Final_GSA_ Accelerating%20Progress%20For%20Schistosomiasis%20Control%20and %20Elimination%20Post%202020%20Meeting%20Report_0.pdf (accessed 12 March 2022).

**Grimes JET, Croll D, Harrison WE, Utzinger J, Freeman MC and Templeton MR** (2015) The roles of water, sanitation and hygiene in reducing schistosomiasis: a review. *Parasites & Vectors* **8**(1), 156.

**Hambali IU, Adamu NB, Ahmed MI, Bokko P, Mbaya AW, Tijjani AO, Biu AA, Jesse FFA and Ambali A** (2016) Sero-prevalence of *Schistosoma* species in cattle in Maiduguri metropolis and Jere local government areas of Borno State, Nigeria. *Journal of Advanced Veterinary and Animal Research* **3**(1), 56–61.

**Hotez PJ and Kamath A** (2009) Neglected tropical diseases in Sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases* **3**(8), e412.

**Huyse T, Webster BL, Geldof S, Stothard JR, Diaw OT, Polman K and Rollinson D** (2009) Bidirectional introgressive hybridization between cattle and human schistosome species. *PLoS Pathogens* **5**(9), e1000571.

**Huyse T, Van Den Broeck F, Hellemans B, Volckaert FAM and Polman K** (2013) Hybridisation between the two major African schistosome species of humans. *International Journal for Parasitology* **43**(8), 687–689.

**Kincaid-Smith J, Boissier J, Allienne J-F, Oleaga A, Djuikwo-Teukeng F and Toulza EA** (2016) Genome wide comparison to identify markers to differentiate the sex of larval stages of *Schistosoma haematobium*, *Schistosoma bovis* and their respective hybrids. *PLoS Neglected Tropical Diseases* **10**(11), e0005138.

**Kincaid-Smith J, Tracey A, de Carvalho AR, et al.** (2021) Morphological and genomic characterisation of the *Schistosoma* hybrid infecting humans in Europe reveals admixture between *Schistosoma haematobium* and *Schistosoma bovis*. *PLoS Neglected Tropical Diseases* **15**(12),

**Léger E and Webster JP** (2017) Hybridizations within the genus *Schistosoma*: implications for evolution, epidemiology, and control. *Parasitology* **144**(1), 65–80.

**Léger E, Garba A, Hamidou AA, Webster BL, Pennance T, Rollinson D and Webster JP** (2016) Introgressed animal schistosomes *Schistosoma curassoni* and *S. bovis* naturally infecting humans. *Emerging Infectious Diseases* **22** (12), 2212–2214.

**Moné H, Mouahid G and Morand S** (1999) The distribution of *Schistosoma bovis* Sonsino, 1876 in relation to intermediate host mollusc–parasite relationships. *Advances in Parasitology* **44**(1), 99–138.

**Moné H, Minguez S, Ibikounlé M, Allienne J-F, Massougbodji A and Mouahid G** (2012) Natural interactions between *S. haematobium* and *S. guineensis* in the Republic of Benin. *The Scientific World Journal* **793420**, 8.

**Moné H, Holtfreter MC, Allienne J-F, et al.** (2015) Introgressive hybridizations of *Schistosoma haematobium* by *Schistosoma bovis* at the origin of the first case report of schistosomiasis in Corsica (France, Europe). *Parasitology Research* **114**(11), 4127–4133.

**Olamiju FO, Nebe OJ, Mogaji H, et al.** (2022) Schistosomiasis outbreak during COVID-19 pandemic in Takum, Northeast Nigeria: analysis of infection status and associated risk factors. *PLoS One* **17**(1), e0262524.

**Onyekwere AM, Rey O, Allienne JF, Nwanchor MC, Alo M, Uwa C and Boissier J** (2022) Population genetic structure and hybridization of *Schistosoma haematobium* in Nigeria. *Pathogens (Basel, Switzerland)* **11**(4), 425.

**Otuneme OG, Obebe OO, Sajobi TT, Akinleye WA and Faloye TG** (2019) Prevalence of schistosomiasis in a neglected community, south-western Nigeria at two points in time, spaced three years apart. *African Health Sciences* **19**(1), 1338–1345.

**Pitchford R** (1965) Differences in the egg morphology and certain biological characteristics of some African and Middle Eastern schistosomes, genus *Schistosoma*, with terminal-spined eggs. *Bulletin of the World Health Organization* **32**(1), 105–120.

**Platt RN, McDew-White M, Le Clec'h W, et al.** (2019) Ancient hybridization and adaptive introgression of an invadolysin gene in schistosome parasites. *Molecular Biology and Evolution* **36**(10), 2127–2142.

**Savassi BAES, Mouahid G, Lasica C, Mahaman SK, Garcia A, Courtin D, Allienne JF, Ibikounlé M and Moné H** (2020) Cattle as natural host for *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858 x *Schistosoma bovis* Sonsino, 1876 interactions, with new cercarial emergence and genetic patterns. *Parasitology Research* **19**(7), 2189–2205.

**Savassi BAES, Dobigny G, Etougbétché JR, Avocegan TT, Quinsou FT, Gauthier P, Ibikounlé M, Moné H and Mouahid G** (2021) *Mastomys natalensis* (Smith, 1834) as a natural host for *Schistosoma haematobium* (Bilharz, 1852) Weinland, 1858 x *Schistosoma bovis* Sonsino, 1876 introgressive hybrids. *Parasitology Research* **120**(5), 1755–1770.

**Soentjens P, Cnops L, Huyse T, Yansouni C, De Vos D, Bottieau E, Clerinx J and Van Esbroeck M** (2016) Diagnosis and clinical management of *Schistosoma haematobium*–*Schistosoma bovis* hybrid infection in a cluster of travelers returning from Mali. *Clinical Infectious Diseases* **63**(12), 1626–1629.

**Taylor MG** (1970) Hybridisation experiments on five species of African schistosomes. *Journal of Helminthology* **44**(3), 253–314.

**Thrusfield M** (2005) *Veterinary epidemiology*. pp. 117–198 2nd edn, Oxford, Blackwell Science.

**Touassem R** (1987) Egg polymorphism of *Schistosoma bovis*. *Veterinary Parasitology* **23**(3–4), 185–191.

**World Health Organization** (2021) Schistosomiasis. Available at https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (accessed 20 September 2021).

**World Health Organization** (2022) *Guideline on control and elimination of human schistosomiasis*. Geneva: World Health Organization. Available at https://www.who.int/publications/i/item/9789240041608 (accessed 15 March 2022).