



OCCURRENCE AND MORPHOMETRICS OF *Schistosoma bovis* IN CATTLE IN ENUGU STATE SOUTH-EAST NIGERIA

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AUTHORS' CONTRIBUTIONS

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ABSTRACT

Schistosomes are digenetic trematode that causes the debilitating disease schistosomiasis, which poses serious public health and veterinary concerns worldwide especially in the tropics and subtropics. Environmental changes due to natural or human activities can have impact on the epidemiology and distribution of human and animal schistosomiasis and may lead to interspecies hybridization events. This study ascertained the occurrence and morphometric characteristics of *Schistosoma bovis* in Enugu State. Microscopy of faecal and warm water incubation of intestinal mesenteric veins of cattle from different abattoirs was used to demonstrate eggs and extract adults of *Schistosoma species*. Morphotypes of adult and egg morphometry of *Schistosoma bovis* species were recorded. An over all *Schistosoma bovis* prevalence of 50.64% was recorded. Proportion of infection varied according to slaughterhouse. Gariki slaughterhouse had highest infection of 31(77.5%), followed by 23(57.5%) in New artisan, 18(45%) in Ogbete slaughterhouse, and 9(22.5%) in Mammy market slaughterhouse. For adult worms, the male mean body length was 4.6 ± 1.3 mm, while the female mean body length was 7.8 ± 0.07 mm, the female length was significantly ($p < 0.05$) longer than the male while there was no significant difference ($p < 0.05$) between the male and female body width. There was significant difference between the male and female oral sucker width. *Schistosoma bovis* eggs seen were spindle shaped possessing a terminal spine. The morphometric characteristics of eggs did not vary according to location ($p < 0.05$). Prevalence of *Schistosoma specie* in the cattle could be as a result extensive grazing and drinking from rivers used by human which suggests possibilities of hybridization and zoonotic schistosomes.

Keywords: Schistosomes; morphometrics; cattle; health.

1. INTRODUCTION

Schistosomes are dioecious trematode, which cause schistosomiasis, a chronic illness that infects both animals and humans, posing significant veterinary and public health concerns globally, notably in subtropical and tropical regions. About 220 million people are affected by schistosomiasis in 78 developing nations, with an average of 200,000 deaths annually. Africa is

home to over 95% of affected persons [1]. The burden of human schistosomiasis is well reported, contrary to significantly less known infections in wildlife and domestic livestock, despite the high number of infections and species (host and *Schistosoma*) involved. The parasite *Schistosoma* originated in Asia, *S. japonicum* infection in humans was reported in 5 countries; China, Indonesia, Malaysia Cambodia and the Philippines. *Schistosoma haematobium* is endemic

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in 52 countries; Eastern Mediterranean, Turkey, Middle East, India and African Countries and *S. mansoni* infection reported in 53 countries from Arabian Peninsula to Brazil, Suriname, Certain Caribbean Islands and Venezuela, *S. mansoni* and *S. haematobium* are reported to be endemic in 40 Countries. Around 2-5 million years ago, the parent species in Africa developed into present *S. mansoni* and *S. haematobium* [2]. It emerged in Egypt via infected baboons and slaves from the Land of Punt, arguably as early as the fifth Dynasty of ancient Egypt [2]. Recent approach for tackling infections caused by species of the *S. haematobium* group, include the sampling of livestock and understanding zoonotic species, necessitated by the identification of hybrids involving human- and animal-infecting species of the *S. haematobium* group, such as *S. haematobium* and *S. bovis*, and more recently *S. mattheei*. Development of water resources projects, growing populations, migration, competing goals in the health sector and focal spread of disease and infection have all increased the distribution levels of schistosomiasis [3,2]. *Schistosoma bovis* infects cattle and cause bovine Schistosomiasis in livestock. Animals can be affected by 19 *Schistosoma* species, five of which have received special attention due to the severity of disease found in domestic ruminant animals in Africa (*S. bovis*, *S. curassoni*, and *S. mattheei*) and Asia (*S. mekongi*, and *S. japonicum*) [4]. In terms of schistosomiasis in animals, it is predicted that 165 million cattle are affected globally [5]. While human schistosomiasis is closely studied in most nations, bovine schistosomiasis is rarely studied. The repartition, prevalence, intensity, and transmission dynamics of animal diseases, are not properly documented.

Natural occurrences or anthropogenic activities, notably agricultural activities, can have a considerable impact on the incidence and spread of schistosomiasis, and also contribute to interspecies hybridization occurrences [6].

Introggressive hybridization develops as hybrid species frequently backcross with one or both parental types, serving as a channel for genetic recombination between species. Hybridization amongst parasites also happens and has the potential to have significant evolutionary consequences [6]. Several research findings suggest that interspecies crosses among parasite can result in lineages with increased infectivity compared to parental species, potentially resulting in atypical pathologies and a broader host spectrum [7,8], and thus new epidemiological dynamics [9]. Hybridization and introgression occurrences in parasites are thus anticipated to

represent significant obstacles to parasitic disease prevention, control, and treatment [6].

Nigeria is one of the countries in the world where schistosomiasis is endemic [1]. Nigeria has the greatest schistosomiasis burden in Africa [10], with schistosomiasis endemic in all 36 states [11]. Communities located near riverbanks are particularly vulnerable to transmission foci. Farming, fishing, swimming, washing kitchen utensils or clothing, bathing, drinking, and getting water from contaminated water bodies are common water contact activities in the communities surrounded with streams or River in Enugu state. Furthermore, animal farming is a popular occupation among the locals, allowing cattle to mingle with people along the riverbanks of the rivers that surround these towns. Since *Schistosoma* has previously been described as endemic in the country [11-13], we predict that the hybridization of *Schistosoma* and zoonotic transmission may be occurring in Nigeria in the shadows. This research was conducted to determine the occurrence and morphometric characteristics of *Schistosoma bovis* in cattle reared in Enugu State.

2. METHODS

2.1 Study Area

The research was conducted in four major abattoirs in Enugu State. The slaughterhouses include: Ogbete main market slaughterhouse, Enugu North local government area, Afor Awkwunano (Gariki) Slaughterhouse, Enugu South local government area, and new artisan slaughterhouse and Eke Agbani Slaughterhouse, in Nkanu West local government area. The average temperature in Enugu is cooler to mild temperature of 60 °F (15.5°C) in the cold months and gets up to 80°F (26.67°C) in the warmer months, making it ideal for outdoor recreation with friends and family or simply for personal leisure. Enugu has good soil-land and climate conditions throughout the year due to its elevation of approximately 223 meters (732 ft.) above sea level, and the soil is effectively drained during raining season. The average temperature of Enugu State in February is roughly 30.64 °C (87.15F), while the coldest temperatures occur in November, hitting 15.86 °C. Normal rainfall amounts range from 0.16 cubic centimetres (0.0098 cu in) in February to 35.7 cubic centimetres (2.18 cu in) in July. The majority of Enugu's population lacks access to potable water, requiring farmers to rely on natural water bodies, ponds, rivers, and other water sources infected with schistosome parasite developmental stages for livestock rearing.

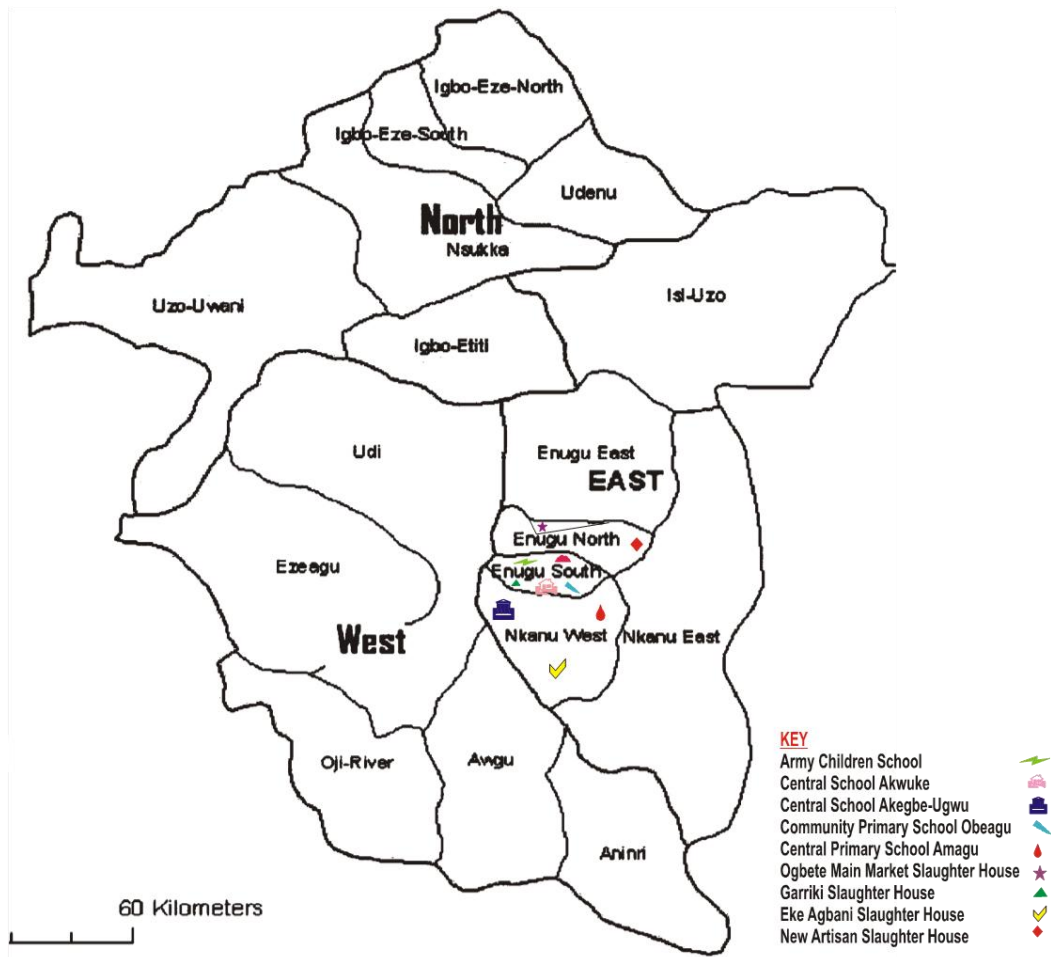


Fig. 1. Map of Enugu state showing the studied local government areas

2.2 Study Design

Randomized Sampling was used to collect faecal and intestinal specimens from a subset of the population. The cattle population were grouped according to slaughterhouses where the faecal or intestinal specimens were collected. A total of 160 cattle were randomly sampled in four public abattoirs. The sampling lasted for three months.

2.3 Extraction of Eggs and Adult *Schistosoma* from Cattle Intestinal Specimen

Warm cattle intestine was collected immediately after slaughter from the butcher between 7 am and 10 am weekly in each abattoir. Blood was washed off from the intestine surface by deepening in clean lukewarm water (37°C). The intestine was then placed in a cellophane bag, and transported to Applied Biology and Biotechnology Laboratory Enugu. Thereafter, the intestine was placed in a flat petri dish. The blood vessels were then teased and torn apart with the aid of

lancets and laboratory scissor to open it apart. This was done several times along the intestine to rupture the vessels as much as possible and later the intestine was incubated in warm water at room temperature for one hour to allow the worms to come out of the vessels. Adult *Schistosoma* that crawled out were picked up with the aid of forceps. The worms were suspended in a normal saline and later fixed in alcohol formalin glacial acetic acid, stained with hydrochloric carmine, cleared with methyl silicate and mounted on a histological slide with Canada balm and later examined with $\times 40$ magnification. The microscope eyepiece was fitted with calibrated reticles and stage micrometer. The unit of the stage micrometer was 10 microns. Every measurement was calculated using a calibration factor of 4.

2.4 Extraction of Eggs from Faecal Sample of Cattle

A piece of filter paper was placed on a table and an applicator stick was used to pick the stool sample

from the sample tube and placed on the filter paper, the stool sample was strained with an applicator stick and wire screen for removing the particulate material. A cardboard with a hole at the centre was placed on a glass slide, stool sample was picked with the applicator and filled into the hole made at the centre of the cardboard, and afterwards the cardboard was removed. The stool was then covered in cellophane that had been soaked in a solution of 3% malachite green and 100 mls of glycerol. The cellophane and applicator stick was again used to spread the stool to reduce the thickness of the smear and the slide viewed under microscope.

2.5 Morphological and Morphometric Characterization of Eggs and Adult Worms

Egg morphometry was differentiated using microscopy, and phylogenetic characteristics were compared, and matched as described by Moné et al. [14]. The presence of egg, adult worm or both in cattle signifies infection.

2.6 Data Analysis

Morphological and morphometric data were analysed using one-way analysis of variance (ANOVA). Significant values were defined as p value < 0.05. The number of cattle infected per slaughterhouse was presented in percentages.

3. RESULTS

3.1 Overall Occurrence of *Schistosoma bovis* According to Slaughterhouses Studied

The overall *Schistosoma bovis* prevalence was 50.64% and total mean infection of 20.3 (12.66%) (Table 1). Proportion of infection per slaughterhouse shows that 31(77.5%) cattle were infected in Gariki slaughterhouse, 23(57.5%) in New artisan slaughterhouse, 18(45%) cattle were infected in Ogbete slaughterhouse, and 9(22.5%) in Mammy market slaughterhouse (Table 1).

3.2 Morphometry of Adult Male and Female *Schistosoma bovis* Extracted from Cattle Intestine in the Different Slaughterhouses

The morphometry of the adult *Schistosoma* species extracted from cattle in various slaughterhouses are shown in Table 2. A total of 30 adult *Schistosoma bovis* were extracted from the mesenteric vein of cattle in the different slaughterhouses. The mean of, body length, body width, length and width of Oral and

ventral suckers of both male and female *Schistosoma bovis* species were measured (Table 2). For the male *Schistosoma bovis*, the mean body length was 4.6 ± 1.3 mm, while the largest body mean width was 0.3 ± 0.08 mm. The mean oral sucker width was 0.2 ± 0.02 mm, the mean ventral sucker length was 0.2 ± 0.05 mm, and while mean ventral sucker width was 0.30 ± 0.03 mm. While for the female, the mean body length was 7.8 ± 0.07 mm, largest body width was 0.2 ± 0.06 mm, mean oral sucker length 0.04 ± 0.005 mm, while the mean oral sucker width was 0.06 ± 0.001 mm. The mean ventral sucker length was 0.002 ± 0.001 and the mean ventral sucker width 0.2 ± 0.003 mm (Table 2). The female length was significantly ($p < 0.05$) longer than the male while there was no statistically significant difference ($p = 0.1175$) between the male and female width. There was also statistically significant difference ($p = 0.01431$) between the male and female oral sucker (Table 2).

3.3 Morphometric Characteristics of *Schistosoma* Eggs Obtained in Cattle from Ogbete, Gariki, New artisan and Mammy Slaughterhouses

The morphometry of *Schistosoma* eggs found in cattle from the four slaughterhouses are shown in Table 3. The total mean length of *Schistosoma bovis* eggs found in various abattoirs was 89.6 ± 0.04 μ m. Egg from Gariki slaughterhouse was longest and measure 90.3 μ m followed by the egg found in the cattle from Ogbete which had length of 89.8 μ m, while egg length of 89.2 μ m was measured in Mammy slaughterhouse and then the egg found in New artisan slaughterhouse measured 89.0 μ m in length (Table 3). The width of *Schistosoma* eggs was 59.4 μ m. The *Schistosoma* eggs found in Mammy market slaughterhouse had the highest width of 59.5 μ m, followed by the eggs found in the cattle from Ogbete and Gariki slaughterhouses with the width of 59.4 μ m and then the eggs found in the cattle from New artisan slaughterhouse with width of 59.3 μ m. For the spine length of *Schistosoma* eggs found in cattle from different markets, the *Schistosoma* eggs found in cattle from Ogbete market slaughter presented the highest length of 10.0 μ m. This was followed by *Schistosoma* eggs found in cattle from New artisan slaughterhouse with a length of 9.7 μ m, *Schistosoma* eggs found in cattle from Mammy market slaughterhouse 9.6 μ m and *Schistosoma* eggs found in the cattle from Gariki slaughter house 9.5 μ m (Table 3). The *bovine Schistosoma* eggs were spindle shaped and had a terminal spine with overall width, length and spine length of 59.4 ± 11 μ m, $89.3.0 \pm 0.21$ μ m, and 9.7 ± 2.1 μ m respectively (Table 4).

Table 1. Overall occurrence of *Schistosoma bovis* in cattle according to slaughterhouses

Slaughterhouse	Number sampled	Number infected	%
Ogbete	40	18	45%
Gariki	40	31	77.5%
New artisan	40	23	57.5%
Mammy market	40	9	22.5%
Total	160	81	50.64%
Mean		20.3	12.66%

Table 2. Morphometric characteristics of adult male and female *Schistosoma bovis* extracted from cattle intestine in the different slaughterhouses

Measured characteristics	Male	Female P value
	Mean ± SD	Mean ± SD
Body length (mm)	4.6 ± 1.3	7.8±0.7 (p=0.0015)
Largest body width (mm)	0.3 ± 0.8	0.2 ± 0.06 (p =0.1175)
Oral sucker length (mm)	0.2 ±0.02	0.04 ± 0.005(p=0.01431)
Oral sucker width (mm)	0.2± 0.007	0.06 ± 0.001
Ventral sucker length (mm)	0.2 ± 0.005	0.02 ± 0.01
Ventral sucker width (mm)	0.30 ± 0.003	0.2 ± 0.03

Table 3. Morphometry of *Schistosoma* eggs obtained in cattle from Ogbete, Gariki, New Artisan and Mammi Slaughterhouse

Egg features	Sources of egg				Total	Mean	±SEM
	OSH	GSH	NASH	MMSH			
Egg length (µm)	89.8	90.3	89.0	89.2	358.3	89.6	±0.04
Egg width(µm)	59.4	59.4	59.3	59.5	237.6	59.4	±0.02
Spine length (µm)	10.0	9.5	9.7	9.6	38.8	9.7	±0.1

Key: OSH: Ogbete Slaughterhouse, GHS: Gariki Slaughterhouse, NASH: New artisan Slaughterhouse, MMSH: Mammi market slaughterhouse. SEM: Standard error of mean.

Table 4. Morphometry of the eggs of *Schistosoma bovis* from the sampled cattle

Characteristics	Mean
Shape	Spindle shaped
Type of spine	Terminal
Width	59.4 ± 11
Length	89.6 ± 0.21
Length of spine	9.7 ± 2.1

4. DISCUSSION

The study revealed the occurrence and varied characteristics of *Schistosoma bovis*, in some cattle sampled in slaughterhouses in Enugu State. *Schistosoma* infection was found in some cattle samples. The study found an overall *Schistosoma bovis* prevalence of 50.6% during the study period. This finding was above the prevalence earlier observed by a previous report by Squire et al. [15] which found a prevalence of 21.7% *Schistosoma bovis* infection in South Ghana. The *Schistosoma* infection observed in this study may not be unconnected with

the practice of livestock grazing near along riverbanks in schistosomiasis-infected areas on a regular basis. The cattle march into such rivers to drink and urinate thereby getting exposed to infective stages of *Schistosoma* species and also seeding the water ways with eggs of the parasite [16].

The availability of multipurpose dams as well as other water bodies and animal-water contact locations determine *Schistosoma* distribution. The stream and river networks that run across Enugu State are particularly relevant in this case because they provide habitat for intermediate snail hosts [17,18]. Additionally, the weather conditions in Enugu state positively influence the proliferation of *Schistosoma*. Kouadio et al. [19] found that weather conditions, the existence of lakes and rivers, and livestock management strategies all influence the occurrence of infections by trematode. In traditional system of livestock production, livestock drink water from dams and rivers, dropping feces carrying parasite eggs and thereby continuing the life cycle of the parasite. A study conducted in Tanzania's southern highlands

revealed that system adopted in the management of livestock determine the prevalence of infections caused by trematode [20]. Another study discovered that trematode were prevalent in areas that adopted the traditional systems in livestock management, moderate in large-scale dairy systems, and low in small-scale dairy systems [21].

Our findings suggest the presence of *Schistosoma* species in same sites, infecting the same hosts by passing through biological barriers and perhaps causing mixed illnesses [22]. Wide genetic diversity in *Schistosoma* sp. has been seen in Mali and Nigeria [23], this may be as a result of varying amounts of contact amongst coexisting *Schistosoma* species in the area [24]. The presence of distinct *Schistosoma* species in the host can change pathogenicity and clinical outcomes (Brouwer et al. 2003), offering new challenges to existing control techniques and instruments by increasing the potential of transmission and reducing therapeutic effectiveness [25,26].

Our study also demonstrated morphometric variations of adult *Schistosoma* extracted from cattle intestine and the characteristics of eggs collected from cattle samples in various slaughterhouses. Adult *Schistosoma bovis* observed in this study is slightly smaller than other *Schistosoma species* reported elsewhere. Taylor [27] reported that adult male *Schistosoma bovis* as approximately 9-22mm in length and 1-2 mm in width while the female measure 12-28 mm long and 1-2 mm in width contrary to the findings of this report. The female length was significantly ($p < 0.05$) longer than the male while there was no statistically significant difference ($p = 0.1175$) between the male and female width. There was also statistically significant difference ($p = 0.01431$) between the male and female oral sucker. *Schistosoma haematobium* eggs are voided through the urine and usually have a typical round to oval shape with a terminal spine. The eggs measure between 100–156 μm long and 40–50 μm wide with usual length between 115–135 μm long [28]. A previous report also suggests that the European hybrid eggs have a mean length of 106.5 μm , a width of 42.8 μm with a spine length of 10.4 μm . The current results show that the eggs observed in this study were spindle shaped and measure $89.6 \pm 0.21 \mu\text{m}$ in length and $59.4 \pm 11\mu\text{m}$, more similar to *S. haematobium* eggs. This is also consistent with the introgression levels that show a predominance of *S. haematobium*-type genetic material. However, sometimes eggs appeared intermediate with spindle or diamond shapes, which are characteristic of *S. bovis* eggs (usually bigger and measuring between 170–223.9 μm long and 55–66.0 μm wide). In addition, *S. bovis* eggs are released in

the feces of infected animals, due to *S. bovis* locating around the mesenteric vessels [28].

The variations in the morphology of eggs of same species isolated from cattle indicate inherent heterogeneity within the same strain [29,26]. Data from epidemiology investigations confirm this assumption [30]. The variability identified in strains of *Schistosoma* demonstrates intraspecific variation as well as the impact of regional origin on species of *Schistosoma* [31]. Other isolates of normal *S. bovis* had eggs that were longer and narrower than those found in this study. In reality, they would overlap with a significant proportion of 'normal' *S. bovis* eggs. The width and length of *Schistosoma* eggs from the same geographical location differ slightly.

The slight differences in shapes and sizes of the eggs, as noted in the present study are a problem in distinguishing different species of *Schistosoma* eggs through microscopic examinations. This presents an issue in identifying the species responsible for infection [32]. According to Weerakoon et al. [33], additional laboratory tests, including DNA analysis, are required for precise characterization of the parasite.

5. CONCLUSION

This research was conducted to establish the occurrence and morphometrics of Schistosomes in cattle reared and slaughtered in Enugu state. *Schistosoma* infection is endemic in the study area. The study showed the existence of *Schistosoma bovis* in some of the cattle sampled. The cattle rearing system is critical in maintaining Schistosomiasis transmission. *Schistosoma* distribution is also determined by the availability of multipurpose stream and river networks across Enugu State, which provide habitat for intermediate snail hosts. The slight difference in shape and size of *Schistosoma* eggs is a problem in distinguishing between the different *Schistosoma* species through microscopic examinations. The presence of close morphometrics among demonstrated eggs from different slaughterhouses indicates possibilities of hybrid and zoonotic Schistosomes in Enugu State.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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