



RESEARCH ARTICLE

PREVALENCE, INTENSITY OF INFECTION RATE AND THE ASSOCIATED FACTORS OF
SCHISTOSOMA MANSONI IN STUDENT CHILDREN AT WALAME DON BOSCO
ELEMENTARY SCHOOL, DILLA, ETHIOPIA

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ABSTRACT

Background and Study aim: *Schistosoma mansoni* is one of the three major species which cause schistosomiasis in humans. Female worms of *S. mansoni* deposit 190 to 300 eggs daily. Each egg of *S. mansoni* bears a prominent lateral spine. The key objective of this study was to identify and confirm which species of genus *Schistosoma* is causing schistosomiasis in student children of Walame Don Bosco elementary school including in those of kindergarten within the same school compound.

Patients and Methods: Fresh stool samples of 500 student children were examined under a compound light microscope for the suspected parasitic species of genus *Schistosoma*. Fresh stool samples of 10 students were examined daily from Monday through Friday.

Result: Out of 500 student children examined 102 were found positive for *S. mansoni*.

Conclusion: The possibility that all the hazards mentioned above and the undesirable immunologic reactions against the antigens released from the eggs, of *S. mansoni*, trapped in tissues of different types & locations of the patient's body are the potential risks, i.e., morbidity/complications imposed to happen later in life, on the becoming adult stage population of today's student children infected with *S. mansoni* in Walame region. Therefore, publically coordinated strong preventive measures must be devised to eradicate *S. mansoni* infection from the study area.

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INTRODUCTION

The parasitic disease schistosomiasis is caused in humans mostly by three species of blood flukes (trematodes) of the genus *Schistosoma*. These three species are:- *S. mansoni*, *S. haematobium*, and *S. japonicum* (Collins *et al.*, 2011; Bogitsh *et al.*, 2005; Doughty, 1996; Burke *et al.*, 2009; Wikimedia. Schistosomiasis. Wikimedia Foundation Inc. 2016). Less prevalent species, such as *S. mekongi*, *S. intercalatum*, and *Trichobilharzia ocellata* may also cause human schistosomiasis. Female worms of *S. mansoni* deposit 190 to 300 eggs daily each bearing a prominent lateral spine (Elbaz and Esmat, 2013; Clerinx and Soentjens, 2015). Schistosomiasis is the 3rd most devastating tropical disease in the world, being a major source of morbidity and mortality for developing tropical countries (Zeibig, 1997; Paniker, 2007; Dailey, 1996). Schistosomiasis is due to immunologic reactions to *Schistosoma* eggs trapped in tissues (Cook, 1996; Ahmed, 2015; Media Centre, 2016; Borda and Rea, 2006). Antigens released from the egg stimulate a granulomatous reaction

involving T cells, macrophages, and eosinophils that results in observable symptoms and signs of schistosomiasis. *S. mansoni* is named after Sir Patrick Manson, who first identified it in Formosa (now Taiwan). On the other hand, the term schistosomiasis is equivalently or synonymously referred to as bilharziasis derived from the fact that "schistosomiasis" was discovered by Theodore Bilharz, a German surgeon was working in Cairo, who first identified the etiological agent *Schistosoma haematobium* in 1851 (Ahmed, 2015). The key objective of this study is to identify and confirm which species of genus *Schistosoma* is causing schistosomiasis in student children of Walame Don Bosco elementary school including in those of kindergarten within the same school compound.

MATERIALS AND METHODS

Student children of Walame Don Bosco elementary school including those of kindergarten within the same school compound were the sites of sample taking and Walame was the specific study area because it was suspected to be the only source of schistosomiasis in children who didn't come from other places and who didn't go to & live in other places either. The compound of Walame Don Bosco elementary school does

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have a regular kindergarten (KG) that consisted of KG1, KG2, and KG3 classes. The total sample size of 500 student children were examined for the suspected parasitic species of genus *Schistosoma* that infect humans. Fresh stool sample was taken from every student of each class, 10 students per day, beginning from KG1, KG2, KG3, grade 1, grade 2, grade 3, grade 4, grade 5, grade 6, and grade 7 from Monday through Friday. The statistics selected, being relevant to interpret and analyse the results of this research activity were:- percentage, and pie chart. The fresh stool sample of each student was examined with a compound light microscope at three stages:

- Direct Wet Mount,
- Concentration Technique, and
- Permanently Stained Preparation.

Procedure

Direct Wet Mount:

- About 2.5 ml of fresh stool sample was taken in a small vial from each of 500 students of the school and kindergarten. Immediately after that, 0.85% NaCl solution in distilled water warmed to 37⁰ C was added to each vial of fresh stool sample taken.
- Then, 1 drop of 0.85% warm (37⁰ C) aqueous NaCl was placed on a clean slide.
- Next, about 1 drop of the stool specimen (from that of any single student) was added to the slide and mixed with the drop of NaCl solution.
- The saline wet mount was covered with a coverslip and examined under a suitable objective lens. This procedure was to allow determining the motility and gross morphology of adult schistosomes if they are found by chance (because the author of this study & some lab technicians of clinics in the study region, had seen them once in copula in fresh stool samples) as well as to clear adhering debris from the surface of the eggs. The stool specimen of any particular student child who was positive for the suspected parasite was preserved in 10% formalin to be used in the stages of Concentration Technique and Examination of Permanently Stained Preparation. The stool specimen of each student child was prepared, observed, and preserved exactly in this way.

Concentration technique

- Involved concentrating the number of the diagnostic stage (eggs) of the suspected parasites in the stool specimen that was collected and preserved in 10% formalin. These concentrated & preserved specimens were part of the complete examination and allowed the detection of small numbers of the parasites that could have been difficult. In order to concentrate the number of the diagnostic stage of the parasite, the speed and concentration time were set at 500xg for 5 minutes.
- Then, the diagnostic stage of the parasites, particularly the eggs were expected to sediment at the bottom of the centrifuge tube of the centrifuged specimen and the stool debris was discarded.

Examination of permanently stained preparations

- Detection and identification of the diagnostic stage of schistosome parasites preserved in 10% formalin,

would depend on the examination of a permanently stained smears under the oil immersion objective lens.

- These stained slides would provide a permanent record of the suspected schistosome parasites of man.
- The identifications in the stages (steps) of Direct Wet Mount and Concentration Technique would be tentative until confirmed by the permanently stained slide.
- The staining was with Safranin.
- About 3 drops of Safranin solution was added to the stool specimen suspension preserved in formalin in a bottle of about 50 ml and waited for about 6 hours to get the diagnostic stage of the schistosome parasite stained.
- A drop of Yetwin Mounting Medium melted at 65⁰ C was placed on a clean slide, then on this drop of mounting medium, a drop of the stool specimen preserved in formalin & stained with Safranin was added and mixed well with the tip of a needle. Next, the specimen was covered with a coverslip and left on a table for about 24 hours to let the mounting medium solidify & harden.
- Thereafter, the specimen in the hardened mounting medium was examined under the oil immersion objective lens to check the presence of the suspected schistosome parasite (i.e., the egg which is the diagnostic stage) of man. The stages of the pathogen found (a pair of adults in copula & egg) were healed using Adobe Photoshop CS6 and colored with the color balance.

RESULTS

Out of 500 student children examined for *Schistosoma mansoni* 102 were found positive.

Table 1. The number of student children examined for *Schistosoma mansoni* in Walame Don Bosco Elementary School, Oct. 2015 to Jun. 2016

Total number of student children examined for <i>Schistosoma mansoni</i>	Number of student children positive for <i>Schistosoma mansoni</i>	Infection rate in percentage
500	102	20.4%

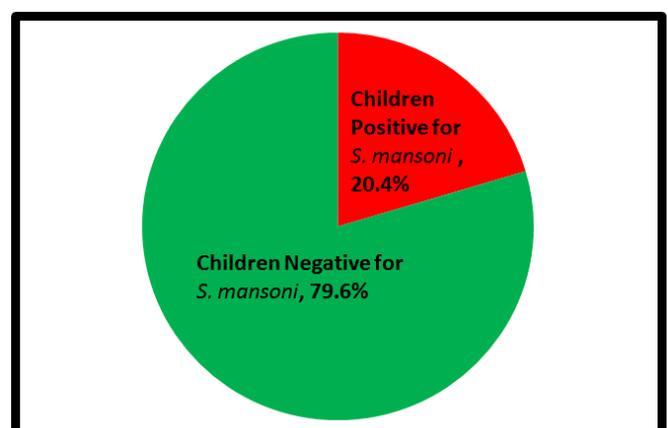


Figure 1. The statistic of pie chart depicting the number of student children examined for species of *Schistosoma* where 20.4% were positive for *Schistosoma mansoni* and 79.6% were negative at Walame Don Bosco Elementary School, Dilla, Geddo Zone, SNNPR, Ethiopia, 2016

Treatment

The drug of choice and available for treating the 102 student children positive for *S. mansoni* in the study area was *albendazole*. The drug was administered following the prescription and supervision by an authorized medical doctor (i.e., Dr. Corazon B. JACA, FMA). 400 mg 1 tablet single dose on empty stomach was prescribed against *S. mansoni* for each infected student child. Yes, in the sub-Saharan Africa in the endemic areas of schistosomiasis, the recommended drug of choice against schistosomiasis is paziquantel. However, here in my study area *albendazole* has practically proved to be curative against the local genomic strain of *S. mansoni* infection. The recovery of the infected children was confirmed by repeated re-examinations of fresh stool samples after treatment.

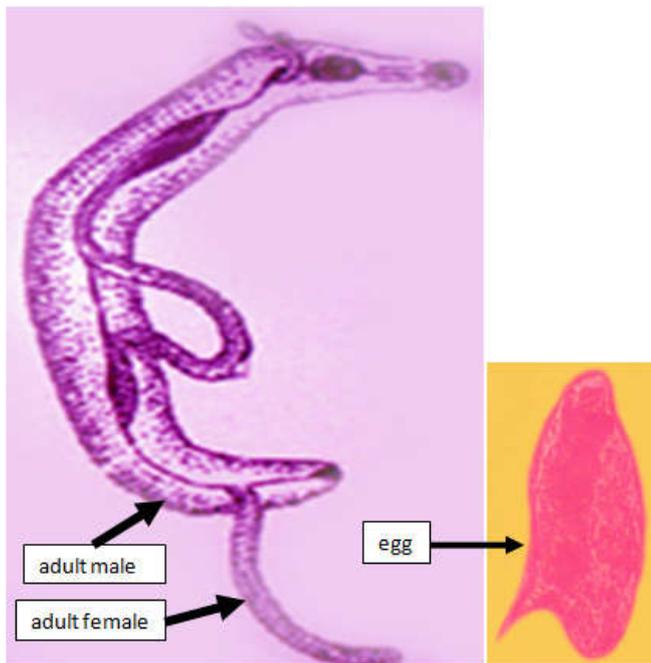


Figure 1. *Schistosoma mansoni*, adult female in the ventral groove of the adult male and the egg

The coupled adult male & female were isolated from fresh stool sample of only one of the 102 student children infected with *S. mansoni*. The actual diagnostic stage found in the fresh stool samples of all 102 infected student children was egg.

DISCUSSION

Infection by *S. mansoni* requires contact with the skin-penetrating cercariae in fresh water where they emerge from snails that are intermediate hosts (Rose *et al.*, 2014). The eggs laid by *S. mansoni* and lodged in liver do secrete toxins that can damage the invaded tissues, the most vulnerable ones being the hepatocytes (Wilson *et al.*, 2007). Schistosomiasis is the third most devastating tropical disease in the world, being responsible for morbidity and mortality for developing tropical countries like Ethiopia where *S. mansoni* is one of the common causative pathogens. The overall prevalence rate of *S. mansoni* in a Ugandan Lake Victoria fishing community was 88.6% (Tukahebwa *et al.*, 2013). Of the world's 207 million estimated cases of schistosomiasis, 93% occur in sub-Saharan Africa and the United Republic of Tanzania is the second country that has

the highest burden of schistosomiasis in the region, Nigeria being the first (Mazigo *et al.*, 2012). Adult worms digest erythrocytes and although most of their energy is obtained by glucose metabolism, egg production is dependent on fatty acids being derived from the host (Clley *et al.*, 2014). In endemic areas, infection is normally acquired early in life, and children bear the highest burden of the infection whereas complications are more commonly observed in adults (Mutengo *et al.*, 2014). According to van der Werf and others, close to 280,000 people worldwide die of schistosomiasis-related complications every year. Although the infection rates of *S. mansoni* in endemic areas of Africa were far more than what was observed in this study the infection rate of 20.4% at Walame area was still very high & threatening. The main source of infection with *S. mansoni* for student children of Walame Don Bosco Elementary School is their recreational activity of swimming in Walame fresh water streams that are infested with infective cercariae of *S. mansoni*.

Although most human schistosomiasis is caused by *S. haematobium*, *S. mansoni*, and *S. japonicum* whereas less prevalent species that infect humans include *S. mekongi*, *S. intercalatum*, and *Trichobilharzia ocellata*, the only species of *Schistosoma* which is invading the population of student children of Walame Elementary School and the young children of kindergarten is *S. mansoni*. The harms (damages) humans suffer from schistosomiasis is due to the immunologic reactions against *Schistosoma* eggs that are trapped in tissues of different types and locations of patient's body. In this case, the antigens released from the egg of *S. mansoni* stimulate a granulomatous reaction involving T cells, macrophages, and eosinophils that results in clinical disease. The intensity of damage or the degree of harm to the victim of *S. mansoni* infection depends on the number and location of the eggs trapped in tissues. Such granulomatous reactions are already known to be initially inflammatory reactions that are readily reversible but in the later stages of the disease, the pathology is associated with collagen deposition and fibrosis, resulting in organ damage that can be only partially reversible. Although schistosomiasis-related mortality is low compared with mortality caused by human immunodeficiency virus (HIV) or malaria, chronic morbidity is high and often underreported (Mutengo *et al.*, 2014).

The fact that the eggs of *S. mansoni* can end up (i.e., trapped) in the:

- skin,
- brain,
- muscle,
- adrenal glands,
- eyes,
- female genital region and also the truth that the trapped eggs do form granulomas in the,
- uterus,
- fallopian tube, and
- ovaries,

has been fully known in science. Schistosomes live as long as 40 years, in their human hosts; adult male and female worms live much of this time in copula (Clley *et al.*, 2014). The potential complications/chronic morbidity that can happen in the adult stage (of the present student children infected who are

between 7 to 12 years of age), being associated with *S. mansoni* infection include:

- Gastrointestinal bleeding,
- Gastrointestinal obstruction,
- Malnutrition,
- Schistosomal nephropathy,
- Renal failure,
- Pyelonephritis,
- Hematuria,
- Hemospermia,
- Squamous cell bladder cancer,
- Sepsis,
- Pulmonary hypertension,
- Cor pulmonale,
- Neuroschistosomiasis-transverse myelitis, paralysis, and cerebral microinfarcts,
- Infertility,
- Severe anemia,
- Low-birth-weight babies,
- Spontaneous abortion,
- Higher risk for ectopic pregnancy complications from vulva or fallopian granuloma, and
- Carcinoma of the liver, bladder, or gall bladder (Rose *et al.*, 2014; Wilson *et al.*, 2007; Tukahebwa *et al.*, 2013; Mazigo *et al.*, 2012; Clley *et al.*, 2014; Mutengo *et al.*, 2014).

In addition to its damageful pathogenicity, *S. mansoni* is also capable to evade the immunity of human host using many tools, including tegument, antioxidant proteins, and defenses against host membrane attack complex. That is true because the tegument coats the worm and acts as a physical barrier to host antibodies and complement system of attack. Although human host immune defenses are capable of producing the oxidant termed superoxide that has a dynamic detrimental effect on individual worms of *S. mansoni*, this pathogen is able to produce a number of antioxidant proteins that block the effect of superoxide. Antioxidant molecules protect human health being generated/produced by the immune system of a person including the supplemental antioxidants derived from diet such as fresh or frozen fruits, vegetables and eggs. However, here in human schistosomiasis the role of antioxidant protein molecules produced by *S. mansoni* is opposite, serving as virulence factors of *S. mansoni*. In other words, the:

- Tegument of *S. mansoni*,
- Antioxidant proteins produced by *S. mansoni*,
- Defenses by *S. mansoni* against human host immune response of membrane attack complex, &
- Antigens released from eggs of *S. mansoni* trapped in tissues are the virulence factors of *S. mansoni*.

In conclusion, the possibility that all the hazards mentioned above and the undesirable immunologic reactions against the antigens released from the eggs, of *S. mansoni*, trapped in tissues of different types & locations of the patient's body are the potential risks, i.e., morbidity/complications imposed to happen later in life, on the adult stage population of today's student children infected with *S. mansoni* in Walame region. Therefore, publically coordinated strong preventive measures must be devised to eradicate *S. mansoni* infection from the study area.

Conflict of interest

I confirm that I don't have any competitive conflict of interest with any body.

Financial support

The financial support, covering the cost of medicine used to treat the infected children was provided by Dilla University. Assigning an authorized medical doctor for prescription & clinical supervision was performed by Dilla Don Bosco.

Ethics

Ethical permission/clearance to perform the research work for the well-being of human subjects was obtained from:- Dilla University, the Office of Gedeo-Zone Administration, and the Directors of the schools involved in the study. The demand for the continuity of this study project and participation by the participant student children & their parents was unusually high.

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