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Association between Intensity of Dead and Live Schistosoma Eggs and Urinary Tract Morbidity in School Children and Adults in Itilima District, Tanzania

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ABSTRACT

Urinary schistosomiasis caused by *Schistosoma haematobium* has been associated with urinary tract morbidity in areas where the disease is endemic. However, knowledge of the impact of the parasite's dead and live eggs on the pathogenesis of urinary tract morbidity is scanty. This study examined the relationship between the intensity of dead and live *S. haematobium* eggs and the development of urinary tract morbidity among schoolchildren and adults in Itilima District, Tanzania. This was a cross-sectional study where a total of 682 urine samples were examined for *S. haematobium* infection. Dead and live *S. haematobium* eggs were identified using vital stains (1% neutral red and 0.4% trypan blue). Haematuria was examined using a urinalysis reagent strip, and its presence was confirmed microscopically following standard procedures. These analyses were followed by ultrasound examination to determine the association between the intensity of dead and live *S. haematobium* eggs with urinary tract morbidity. 112 urine samples were examined for dead and live *S. haematobium* eggs. The prevalence of dead and live *S. haematobium* eggs was 12.5% (14/112) and 77.7% (87/112), respectively. The prevalence of bladder wall thickening was 67.7% (44/112), and this was significantly associated with low intensities of dead *S. haematobium* eggs ($\chi 2$ = 9.652, p = 0.047). Haematuria was microscopically confirmed in 80.4% of infected participants, and its severity increased significantly with an increase in the intensity of live *S. haematobium* eggs ($\chi 2$ = 0.367, p = 0.024), suggesting the presence of active transmission of the parasite in Itilima District. Control interventions through health education are necessary to enhance public well-being in the Itilima District.

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1.0 Introduction

Human schistosomiasis remains a major public health problem in most resource-poor countries, with 200 million people being infected each year (Colley *et al.,* 2017 & Dejon-Agobé *et al.,* 2019). Several *Schistosoma* species are known to infect humans, including *Schistosoma haematobium,* which is the sole causative agent of urinary schistosomiasis (Fu *et al*., 2012). *S. haematobium* infection has been associated with most of the urinary tract morbidity in areas where the parasite is endemic (Zhong *et al.,* 2013, Botelho *et al.,* 2015).

During infection, it is the *S. haematobium* eggs that are important pathogenic agents and hence responsible for most of the morbidities associated with schistosomiasis (Sarvel *et al.,* 2006, Skelly 2013, Forson et al. 2019). The pathological impacts of *S. haematobium* begin when its eggs move from blood vessels through the tissues of the urinary bladder and finally into its lumen (Ross *et al.,* 2007, Skelly 2013). During the movements, some of *S. haematobium* eggs are trapped in tissues, especially the bladder and ureter, resulting in heavy egg deposits that are found in advanced (chronic) stages of infection (Zhong *et al.,* 2013, Poturalski et al. 2017). The miracidium larva inside *the S. haematobium* egg secretes soluble antigens in the host's blood. The antigens then stimulate the host's immune cell responses as an attempt to get rid of the infection (Skelly 2013). The host's immune cells, which include T and B lymphocytes, macrophages, mast and plasma cells, fibroblasts, and eosinophils, migrate to surround the deposited *S. haematobium* eggs (Gryseels *et al.,* 2006). This leads to the granulomatous reactions and finally fibrosis that cause death of the trapped *S. haematobium* eggs (Fried *et al.,* 2011, Skelly 2013). As a result, the normal bladder epithelium undergoes change to a form that does not normally occur in the bladder tissue (metaplasia) from transitional epithelium to squamous epithelium. Subsequently, squamous epithelium undergoes abnormal growth (dysplasia), which can develop into squamous cell carcinoma (SCC) (Zhong *et al.,* 2013).

Furthermore, severe fibrosis can lead to the thickening of the urinary bladder wall and ureter (Dejon-Agobé *et al.,* 2019). The narrowing of the ureter lumen as a result of wall thickening causes poor urine drainage from the kidney, leading to pressure fluid build-up and dilation of the urinary system, a condition known as hydronephrosis (Onile *et al.,* 2016, Elmadani *et al.,* 2013). Therefore, the severity of these events and the ensuing disease depends largely on the number of *S. haematobium* eggs (both dead and live) and the individual's immune responses (Gryseels *et al.,* 2006, Skelly 2013). While much is known on the possible impact of urinary schistosomiasis intensity and severity of urinary tract morbidity (Onile et al. 2016, Dejon-Agobé *et al.,* 2019), it is not clear to what extent the burden of dead or live *S. haematobium* eggs is responsible for part or all of the damage that occurs in the urinary system.

Another pathological outcome of S. *haematobium* infection is the presence of blood in urine (haematuria). Technically, haematuria, which is defined as the presence of three or more red blood cells per high-power field of urine sediments, is the most important sign of urinary schistosomiasis (Lee *et al.,* 2013). The effect of haematuria varies from insignificant health impact to potentially life-threatening when associated with urinary tract morbidity, such as bladder cancer (Bignall & Dixon 2018). The detection of haematuria depends on whether it can be recognised as gross (visible), also known as macroscopic haematuria (O'Connor *et al.,* 2021). On the other hand, the detection may be possible using a routine urinalysis reagent strip (URS) and is termed as non-visible or microscopic haematuria (Davis *et al.,* 2012, Bolenz *et al.,* 2018). The detection of *S. haematobium* viable eggs in a urine sample indicates the active infection and transmission in the area (Khadra *et al.,* 2000, Knopp et al. 2018). The severity of haematuria among individuals infected with urinary schistosomiasis varies with the intensity of the parasite's eggs excreted in urine (Bignall & Dixon 2018, Knopp *et al.,* 2018). Most studies have reported the urinary tract morbidity linked with the excretion of *S. haematobium* eggs detected in urine samples from various countries (Onile *et al.,* 2016;

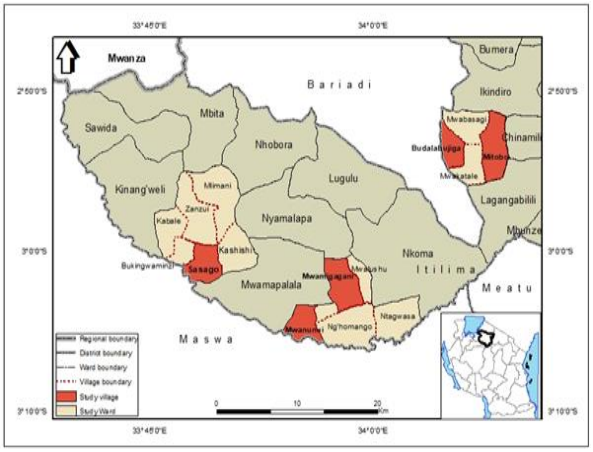
Knopp *et al.,* 2018 & Wiegand *et al.,* 2021). However, it is not known whether these morbidities are related to the intensity of dead or live *S. haematobium* eggs excreted by infected individuals. Therefore, the aim of this study was to determine the association between the burden of dead *S. haematobium* eggs and levels of bladder wall thickening. In addition, the study aimed to determine the intensity of live *S. haematobium* eggs and how they influence the severity of haematuria among school children and adults in the study area.

2.0 Materials and Methods

2.1 Study Area

The study was carried out in the Itilima District, Simiyu Region, located in northwestern Tanzania (Fig. 1). The district lies at an estimated altitude of 1,272 m above sea level, with an area of 2,647.7 sq. km and an estimated population of 313,900 people, according to the Tanzania Population and Housing Census of 2012. The climate is generally of a subtropical type with an average rainfall of 700-950 mm per year. The hot and dry season occurs from June to September, and the average temperature ranges from 29°C during the daytime to 19°C at night.

Figure 1
Map of Itilima District Indicating Studied Villages



2.2 Study Population

The study included schoolchildren aged 9+ years and adults (aged 18+ years) residing in five villages in Itilima District. The villages that were selected were potentially endemic for urinary schistosomiasis based on health records from the Itilima District Medical Office.

2.3 Sample Size Estimation

The study's sample size was estimated using the formula $n = Z2PQ/e2$ (Singh & Masuku 2014), where n is the number of expected study participants, Z is the score for a given confidence interval, p is a known or estimated prevalence of 44.9% for schistosomiasis-related urinary tract morbidity in northwestern Tanzania (Rambau *et al.,* 2013), $Q = (1-p)$, and e is the permissible error of estimation. The desired confidence level was 95% with a permissible estimation error of 5%. Therefore, based on the formula, the minimum sample size was n=1.962*0.449 (1-0.449)/0.052 =380.

2.4 Study Design and Sampling Procedures

A cross-sectional study was carried out employing multistage sampling procedures. The purposive sampling was used to select villages with expected high prevalence of urinary schistosomiasis. School children and adults were selected using a simple random selection method to ensure an equal probability for each member of the targeted population to be included in the study (Singh & Masuku 2014). After the selection of study villages, a meeting was held with local leaders and primary school administration and committee. The study objectives and data collection procedures were explained to them by the researcher before commencement of urine collection. Adult participants 'were invited by the village chairperson/executive officer from different sub-villages by blowing a whistle one day before the sampling date. People who responded to the previous call and were willing to participate in the study were gathered in the open ground. The objectives of the study and sampling protocol were explained to them, and they were informed that participation was voluntary and that there was no reward for participating or coercion for withdrawing. The consent form was given to each adult participant for them to read. For illiterate participants, the form was read for them by the research assistant. People were given the chance

to ask any question(s) related to the study. Willing participants were asked to sign the consent form, and for those who could not read or write, a thumbprint was used for consenting. Each participant was provided with urine collection materials, and only those who brought back containers with urine samples were registered. The study also involved school children from respective villages aged 9+ years, whereby the permission to access children was obtained from school administration and committees. The children were also informed about the purpose of the study, and those who agreed to participate were asked for assent by filling and signing a form before being enrolled into the study.

2.5 Ethical Considerations and Confidentiality

The study was approved by the Medical Research Coordination Committee (MRCC) of the National Institute for Medical Research (NIMR), Tanzania, through ethical clearance certificate No. NIMR/HQR.8a/Vol.IX/3546. The confidentiality was maintained by excluding the participants' identifiers information.

2.6 Eligibility Criteria

2.6.1 Inclusion Criteria

School children aged 9+ years old and adults who had been living in the study area for one year consecutively had not received praziquantel treatment within six months before the study period and consented (Hein *et al.,* 2015).

2.6.2 Exclusion Criteria

Excluded were school children less than 9 years of age, recent immigrants to the study area, and those who did not give informed consent. Other excluded groups included pregnant women and those who had received praziquantel within six months prior to the study period (Poggensee *et al.,* 2000).

2.7 Data Collection

2.7.1 Collection and Examination of Urine Samples

Each participant was provided with a 50 mL wide-mouthed plastic urine container and requested to collect approximately 20 mL of urine. To increase the chances for yield of *S. haematobium* eggs, participants were asked to collect urine between 10 am and 2 pm (Geleta *et al.,* 2015). Urine samples were tested for haematuria using urinary reagent strips, and the presence of *S. haematobium* eggs was examined using the filtration method. The urine sample was shaken to mix it well, then 10 mL was drawn into a syringe. The syringe was placed onto a filter membrane fixed inside the microfilter holder, and the urine was pushed and filtered. The filter was removed from the holder by forceps and placed onto the slide and observed under a light compound microscope at 400x magnification for identification. The *S. haematobium* eggs were identified based on an oval shape and a terminal spine (Utzinger et al. 2015) and recorded as the number of *S. haematobium* eggs per 10 mL of urine.

2.7.2 Ultrasound Examination

All participants testing positive for *S. haematobium* were subjected to ultrasound examination (SonoScape diagnostic portable ultrasound machine, A6T/A6/A5) to assess the health status of urinary system organs, i.e., the bladder, ureters, and kidneys. Each participant was provided with three to four glasses of drinking water depending on their age and instructed to wait until the urinary bladder was full of liquid before being examined by ultrasound (Barda *et al.,* 201; *Garcia et al.,* 2018). Ultrasound examination was performed following standard procedures, and pathologies were assessed and recorded as recommended (WHO 1996). All *S. haematobium*-positive subjects were treated with a single dose of praziquantel free of charge.

2.7.3 Examination for Dead and Live Schistosoma haematobium Eggs

The examination for dead and live *S. haematobium* eggs was performed using vital stains 0.4% trypan blue and 1% neutral red, respectively, following guidelines in Forson *et al.* (2019) as detailed below:

i. Two slides of positive samples were placed into the Petri dishes containing wet cotton wool.
ii. 500 µL of urine suspension was pipetted and placed into each plastic vial. Then 10 µL

of Trypan blue (0.4%) was added to one of the vials containing urine suspension, and 10 µL of neutral red (1%) was added to another vial.

iii. About 50 µL of each stained urine suspension was sucked and placed onto a microscope slide, then incubated into petri dishes containing wet cotton wool. The dead and live *S. haematobium* eggs would retain 0.4% trypan blue stain and 1% neutral red, respectively.

iv. Each slide was then examined at 400× magnification to observe for stain retention in *S. haematobium* eggs. As explained elsewhere (Forson *et al.,* 2019), dead *S. haematobium* eggs would retain 0.4% trypan blue stain and produce a blue colour, while live eggs would not. On the other hand, live *S. haematobium* eggs (viable) would retain 1% neutral red stain to give a red colour, while dead ones would not. All slides with stained urine suspension were observed by two trained laboratory technicians for data quality control. Based on these criteria, dead and live *S. haematobium* eggs were identified, categorised, and counted accordingly.

2.7.4 Urine Examination for Haematuria

2.7.4.1 Urinalysis by Reagent Strips

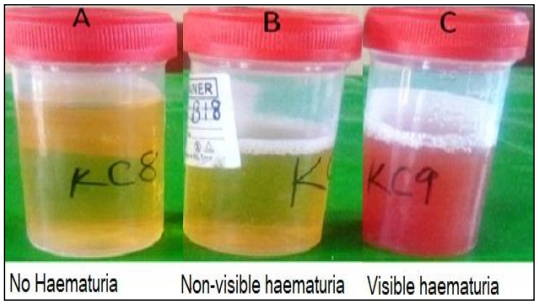
The microhematuria was tested by using urinalysis reagent strips (URS). The level of haematuria was categorised and recorded as non-hemolyzed trace, hemolyzed trace, small, moderate, and large according to the colour label on the canister and the strips.

2.7.4.1 Microscopic Examination of Haematuria

Haematuria-positive urine samples (through URS) were examined under a microscope for confirmation following standard procedures and guidelines (Sharp *et al.,* 2013 & Price *et al.,* 2014). About 10 mL of urine was poured into a conical flask and allowed to stand for approximately one hour to enable sedimentation. The supernatant was disposed of while keeping approximately 3 mL containing urine sediments in suspension. About 50 µL of urine suspension was drawn and placed onto a microscope slide, then examined for haematuria under 400× magnification. The absence of red blood traces or the presence of less than three red blood cells (RBCs) per high power field (≤ 3 RBCs/HPF) was considered as haematuria negative or no haematuria (NH) (Sharp *et al.,* 2013). The presence of three or more RBCs per high-power field (≥ 3 RBC/HPF) was considered positive for haematuria, and this was categorised as non-visible haematuria (NVH). The visible red colour in urine suspensions by the naked eye was categorised as visible haematuria (VH) (Figure 2). However, visible haematuria was also confirmed by observing urine sediments under a microscope to confirm whether the red colour of the sample was due to the presence of RBCs or not. RBCs were identified by their characteristics of a biconcave or doughnut shape without nuclei and the presence of a central pale area.

Figure 2

Categories of Haematuria As Observed Among Study Participants at Itilima District, Tanzania in 2021



2.8 Data Analysis

Data were entered and analysed in IBM SPSS (version 20.0). The descriptive analysis and frequency tables were used to obtain the number and percentage rates of different variables. The live and dead *S. haematobium* eggs were categorised as ultra-light intensity (1-5 eggs/50 µL of urine suspension), very light intensity (6-10 eggs/50 µL), and light intensity (11-49 eggs/50 µL) (Knopp *et al.,* 2018). The absence of red blood traces or the presence of less than three red blood cells (RBCs) per high power field (≤ 3 RBCs/HPF) was considered as haematuria negative or no haematuria (NH) (Sharp *et al.,* 2013 & Price *et al.,* 2014). The presence of three or more RBCs per

high power field (≥ 3 RBC/HPF) was considered positive for haematuria, and this was categorised as non-visible haematuria (NVH). The visible red colour in urine suspensions by the naked eye was categorised as visible haematuria (VH) (Sharp et al. 2013 & Price *et al.,* 2014). The chi-square test was used to determine the strength of association between infection intensity of dead *S. haematobium* eggs and bladder wall thickening (categorical variables) and the relationship between live *S. haematobium* eggs and the severity of haematuria (both categorical variables). Data analysis was performed at a 95% confidence level, and a p-value of ≤ 0.05 was considered statistically significant.

3.0 Results

3.1 Prevalence and Intensity of Dead and Live Schistosoma haematobium Eggs

Urine samples from 112 (16.4%) participants, who tested positive for *S. haematobium,* were examined for dead and live *S. haematobium* eggs. The prevalence of dead *S. haematobium* eggs was found in 14 (12.5%) of schistosomiasis-positive individuals. Of these, 12 (10.7%) had ultra-light intensity of dead *S. haematobium* eggs (1-5 eggs/50 µL of urine suspension), while 2 (1.8%) had light intensity (i.e., 11-49 eggs/50 µL of urine suspension). For dead *S. haematobium* eggs, the very light intensity category was missing (i.e., 6-10 eggs/50 µL of urine suspension). On the other hand, live *S. haematobium* eggs were present in 87 (77.7%) of schistosomiasis-positive individuals. Of these, 54 (48.2%) had ultra-light intensity (1-5 eggs/50 µL), 16 (14.3%) had very light intensity (6-10 eggs/50 µL), and 17 (15.2%) had light intensity (11-49 eggs/50 µL of urine suspension). About 9 (8%) of infected participants had both live and dead *S. haematobium* eggs in urine suspension, although these were excluded from analysis as this was out of the scope of the study.

3.2 Association between Dead Schistosoma haematobium Eggs and Urinary Tract Morbidity

A total of 65 (63.7%) of the 102 schistosomiasis-positive individuals had one or more lesions in the urinary system. Among those with dead *S. haematobium* eggs, 2 of them (15.4%) had no morbidity, 12 (76.9%) had moderate morbidity, while 1 (7.7%) had severe urinary tract morbidity. The bladder wall thickening was present in 44 (67.7%) individuals, and the morbidity was significantly associated with 1-5 eggs/50 µL intensity of dead *S. haematobium* eggs (χ^2 = 9.652, p = 0.047). For participants lacking dead *S. haematobium* eggs, 34 (39.3%) had no urinary tract morbidity, 37 (41.6%) were diagnosed with moderate morbidity, while 17 (19.1%) had severe morbidity. The severity of urinary tract morbidity was significantly associated with < 1-5 eggs/50 µL intensity of dead *S. haematobium* eggs detected in a urine sample ($\chi 2$ = 13.485, p = 0.009, Table 1).

Table 1
Association between Intensity of Dead S. haematobium Eggs and Urinary Tract Morbidity

Examined pathology	Observation	Dead *S. haematobium* eggs N (%)			Total N (%)	p-value
		0-eggs	-1-5eggs	11<eggs		
Bladder shape	Normal	59(85.5)	8(11.6)	2(2.9)	69(67.6)	0.560
	Distorted	30(90.9)	3(10.1)	0(0.0)	33(32.4)	
Wall irregularities	None	67(89.3)	6(8.0)	2(2.7)	75(73.5)	0.235
	Multifocal/diffuse	22(81.5)	5(18.5)	0(0.0)	27(26.5)	
Wall thickening	None	53(91.4)	3(5.2)	2(3.4)	58(56.9)	0.047
	Focal	8(66.7)	4(33.3)	0(0.0)	12(11.8)	
	Multifocal/diffuse	28(87.5)	4(12.5)	0(0.0)	32(31.4)	
Bladder mass	None	79(87.8)	9(10)	2(2.2)	90(88.2)	0.651
	Single	6(75)	2(25)	0(0.0)	8(7.8)	
	Multiple	4(100)	0(0.0)	0(0.0)	4(3.9)	
Right ureter	Not visualized	85(86.7)	11(11.2)	2(2.0)	98(96.1)	0.738
	Dilated,	4(100)	0(0.0)	0(0.0)	4(3.9)	
Left ureter	Not visualized	84(86.6)	11(11.3)	2(2.1)	97(95.1)	0.681
	Dilated	5(100)	0(0.0)	0(0.0)	5(4.9)	
Right renal pelvis	Not dilated	85(87.6)	10(10.3)	2(2.1)	97(95.1)	0.760
	Moderate dilated	4(80)	1(20)	0(0.0)	5(4.9)	
Left renal pelvis	Not dilated	83(86.5)	11(11.5)	2(2.1)	96(94.1)	0.628
	Moderate dilated	6(100)	0(0.0)	0(0.0)	6(5.9)	

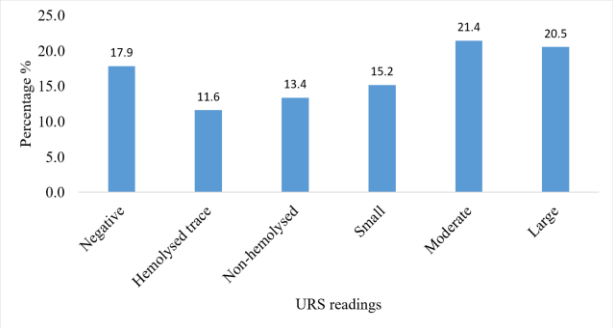
3.3 Red Blood cells in Urine (Haematuria)

3.3.1 Urinalysis Examination for Haematuria

Under urinalysis reagent strips examination, 92 (82.1%) of the participants were positive for haematuria at different levels, while 20 (17.9% were haematuria negative (Fig 3).

Figure 3

Frequency of URS Readings among Study Participants at Itilima District, Tanzania in 2021

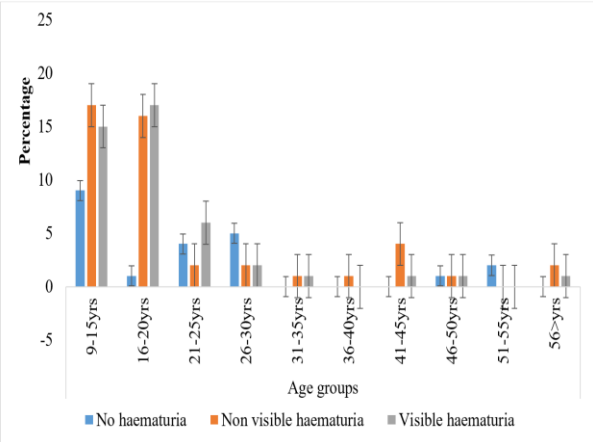


3.3.2 Microscopic Confirmation for Haematuria

Microscopic examination of haematuria in the urine samples of *S. haematobium*-infected participants confirmed that about 22 (19.6%) of them were haematuria negative, about 46 (41.1%) had non-visible haematuria (NVH), while 44 (39.3%) had visible haematuria (VH). The level of haematuria was significantly higher among study participants with lower age compared to higher age groups (χ^2 = 9.500, p = 0.009, Fig. 4).

Figure 4

Variation of Levels of Haematuria with Age Groups in Itilima District, Tanzania in 2021

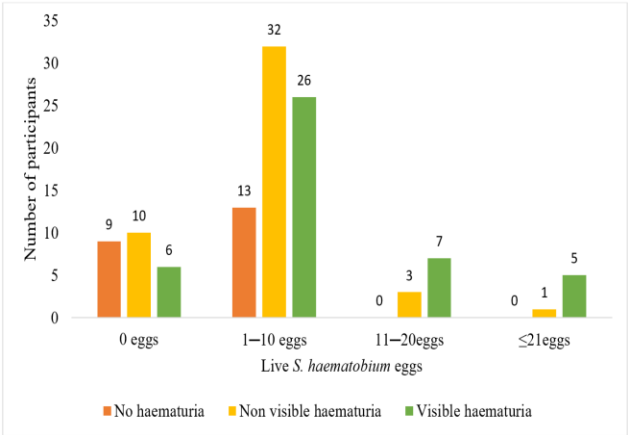


3.3.3 Relationship between Live Schistosoma haematobium Eggs Counts and Severity of Haematuria

A total of 87 (77.7%) participants were detected with live *S. haematobium* eggs, 14 (16.1%) of them were confirmed to be haematuria negative, 36 (41.4%) had non-visible haematuria (NVH), while 37 (42.5%) presented with visible haematuria (VH). Among 25 participants lacking live *S. haematobium* eggs, 7 (28%) of them had no haematuria (NH), 12 (48%) had NVH, while 6 (24%) presented with VH. The severity of haematuria increased significantly with the intensity of live *S. haematobium* eggs (χ^2 = 0.367, p = 0.024) (Fig 5).

Figure 5

Egg Counts of Live Schistosoma haematobium and Severity of Haematuria in Itilima District, Tanzania in 2021



4.0 Discussion

The present study investigated the association of dead or live *S. haematobium* eggs on urinary tract morbidity among communities in Itilima District, Tanzania. The presence of participants who harboured dead *S. haematobium eggs* indicated the existence of chronic schistosomiasis infection in the area. On the other hand, the high prevalence of live *S. haematobium* eggs suggested the presence of active urinary schistosomiasis transmission in the community. It was observed further that the intensity of dead and live *S. haematobium* eggs was low among participants. This can be explained by the low infection intensity found during the

filtration process. Secondly, dead *S. haematobium* eggs trapped in bladder tissues are usually surrounded by host immune cells, resulting in granulomatous formation. Such a situation may hinder the free movement of dead *S. haematobium* eggs across the tissue to the bladder lumen, leading to fewer dead eggs in the expelled urine (Fried *et al.,* 2011).

The urinalysis reagent strip (URS) results for red blood cells (haematuria) in urine samples differed from that of microscope examination. The URS had lower haematuria-negative readings compared to microscopically confirmed results (i.e., 17.9% for URS compared to 19.6% for microscopic confirmation). This is likely because URS has a high sensitivity of 97.8% and moderate specificity of 58.8% compared to 95.83% sensitivity and 93.33% specificity for microscopic examination (Cohen & Brown 2003, Robinson *et al.,* 2009). Thus, URS can detect a minute amount of haematuria, which is less than three red blood cells per high power field (i.e., 1-2 RBCs/HPF), a condition considered as negative under microscope examination (Sharp *et al.,* 2013 & Price *et al.,* 2014); therefore, fewer negative haematuria readings are reported under URS. In addition, URS readings may be misleading because the technique cannot differentiate between myoglobin, haemoglobin, and red blood cells. Hence, examination under URS often presents fewer haematuria-negative results compared to microscopic examination as reported elsewhere (Cohen & Brown 2003 & McDonald *et al.,* 2006). Furthermore, some of the haematuria-negative readings under URS were confirmed to be haematuria-positive under microscopic examination. This was possibly caused by a light infection of *S. haematobium,* as reported by Knopp *et al.* (2018), that URS sensitivity is low in light intensity for urinary schistosomiasis. The URS results for haematuria should not, therefore, be used as a stand-alone indicator of morbidity in the bladder. Instead, microscopic confirmation should be considered for concluding about haematuria status in schistosomiasis endemic communities, as suggested by other investigators (Bignall & Dixon 2018).

To the best of our knowledge, this is the first study to investigate and report on the association between the intensity of dead and live *S. haematobium* eggs and urinary tract morbidity in the study area. One of the morbidities, bladder wall thickening, was observed mostly among participants infected with *S. haematobium. The current observation* is in line with the findings reported by Elmadani *et al.* (2013) in Sudan. Using ultrasound examination, the authors observed that the majority (90.4%) of individuals infected with urinary schistosomiasis had bladder wall thickening. In the present study, bladder wall thickening was significantly higher in individuals with low intensities of dead *S. haematobium* eggs in urine suspension. This can be explained by the fact that most of the dead *S. haematobium* eggs remain trapped in the urinary bladder tissue and thus cannot be detected in a urine sample. In addition, the higher prevalence of bladder mass observed among participants with low intensities of dead *S. haematobium* suggests the existence of chronic parasite infection in Itilima District as recommended elsewhere (Shiff *et al.,* 2006 & Mohamed 2012). The single to multiple bladder masses increase the severity of morbidity as they reduce the size of the lumen of the urinary bladder. Such a condition can lead to high frequency of urination, dysuria, urine retention, and eventually the development of squamous cell carcinoma of the urinary bladder (Shiff *et al.,* 2006).

Furthermore, moderate to severe lesions were observed among urinary schistosomiasis-infected individuals without dead *S. haematobium* eggs; this finding agrees with observations done by Dejon-Agobé *et al.* (2019) in Gabon. The authors reported that people with low intensity of *S. haematobium* may have urinary tract morbidity while excreting few or no eggs on the day of investigation. Therefore, the severity of urinary tract morbidity is independent of Schistosoma eggs detected in the urine sample. On the other hand, negative urinary tract morbidity was observed in 15.4% of participants with dead *S. haematobium* eggs. This suggests that harbouring dead *S. haematobium* eggs in urine does not indicate a damaged urinary tract system, as Wiegand *et al.* (2021) suggested. The authors concluded that individuals with a heavy *S. haematobium* burden do not always present with urinary tract morbidity. The fact that

urinary tract morbidity was detected in participants with dead *S. haematobium* eggs and those lacking them indicates that bladder lesions are possibly caused by the parasite's eggs accumulated in the bladder mucosa rather than those excreted in urine, as Neal (2004) suggested. The bladder wall thickening is not always associated with the number of dead *S. haematobium* eggs counted in urine samples from infected individuals. However, the detection of dead *S. haematobium* in the urine sample of an infected individual indicates the chronic infection with the parasite, which requires further investigation in the internal organs of the urinary system. Therefore, it is necessary to assess the viability of *S. haematobium* eggs for proper decision-making in controlling urinary schistosomiasis and its related morbidities in endemic areas.

Moreover, haematuria was common among people infected with live *S. haematobium,* indicating perhaps that this was a sign of active schistosomiasis infection, as also postulated in other studies (Wiegand *et al.,* 2021). Microscopic confirmation of negative haematuria in 19.6% of people infected with *S. haematobium* suggests that haematuria readings should not be taken as the criterion for the absence of parasite infection. Instead, microscopic examination of *S. haematobium* eggs through urine filtration should be considered for the conclusion of the parasite's infection in individuals (Tetteh-Quarcoo *et al.,* 2019). The severity of haematuria in the present study was significantly associated with an increase in the number of live *S. haematobium* eggs. This suggests that active movement of live *S. haematobium* eggs across the bladder injures bladder wall tissues, releasing red blood cells (haematuria). The absence of live *S. haematobium* eggs in some urine samples with NVH and VH may have been caused by light infection intensity, as suggested by other researchers (Knopp *et al.,* 2018). For instance, Knopp *et al.* (2018) pointed out that it is difficult to detect *S. haematobium* eggs and haematuria in areas with a light intensity of infection by using the urine filtration technique and URS. Therefore, variation in the number of dead and live *S. haematobium* eggs should not be considered as the sole criterion for assessing the severity of morbidity in the urinary tract system. Thus, other techniques, including ultrasound examination, are necessary to detect and confirm urinary tract morbidity in areas where schistosomiasis is endemic.

5.0 Conclusion

The severity of bladder wall thickening was independent of dead *S. haematobium* egg intensity, suggesting that the abundance of dead eggs in urine samples is possibly not an indicator of the urinary bladder pathology. However, the severity of haematuria varied with live *S. haematobium* egg intensity, indicating active transmission of the parasite in the community.

6.0 Recommendations

The ultrasound examination should be considered a necessary procedure for individuals infected with urinary schistosomiasis regardless of dead or live *S. haematobium* eggs detected in urine samples. The prevention and control of schistosomiasis-related urinary tract morbidity should involve all members of the community in endemic areas.

7.0 The Limitations of the Study

The study The present study focused on both dead and live *S. haematobium* eggs; thus, all laboratory analyses were supposed to be done in the field settings. The present study did not involve soil and water analysis and thus could not make strong conclusions on the environmental factors associated with schistosomiasis transmission in the study area. In addition, the study's objectives relied on the presence of parasite eggs in the urine of individuals; thus, *S. haematobium*-negative subjects were excluded from ultrasound analysis for examination of urinary tract morbidities. The study could not, therefore, provide a thorough explanation of the causative agents of other urinary tract morbidities.

8.0 Abbreviation

URS—Urinary reagent strip

RBCs—red blood cells
NH- No haematuria
NVH- Non-visible haematuria
VH- Visible haematuria
HPF—high power field

9.0 Author Contributions

CY, the main author of the article, was involved in proposal writing, study design, data collection, data processing and analysis, and preparation of the manuscript. PFR was involved in data collection, data processing, analysis, and preparation of the manuscript. SMK was involved in the preparation of the study design, data processing, and preparation of the manuscript. All authors read and approved the final manuscript.

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12.0 Declaration of Conflicting Interests

All authors declare that they have no competing interests.

13.0 References

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