Research Article

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Journal of Infectious Diseases and Treatment

2018
Vol.4 No.2:1

DOI: 10.21767/2472-1093.100042

Disparities in Sanitary Conditions/Habits and Helminthiasis Prevalence between School-Age-Children Living in Fishing and Farming Communities in Ghana: A Cross Sectional Study

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Received date: February 06, 2018; **Accepted date:** February 28, 2018; **Published date:** March 02, 2018

Citation: Tandoh MA, Mills-Robertson FC, Wilson MD, Anderson AK. Disparities of Sanitary Conditions/Habits and Helminthiasis Prevalence between School-Age-Children Living in Fishing and Farming Communities in Ghana: A Cross Sectional Study. J Infec Dis Treat. 2018, Vol.4 No. 2:1.

Abstract

Background: Helminthiasis is a sub group of the neglected tropical diseases that is still persisting in most countries in sub-Saharan Africa and is a major cause of chronic morbidity with associated poor intellectual and physical growth in children. Children are the most affected group, probably, due to their poor hygienic practices and poor play habits. This study determined the prevalence of helminth infections and disparities in the hygienic, sanitary conditions and health behaviors among school-age children living in two selected communities in Ghana.

Methods and findings: This was a cross-sectional study conducted in fishing (n=84) and farming (n=80) communities in the Kwahu Afram Plains South District in the Eastern Region of Ghana among school-age children using structured questionnaires and laboratory analysis.

One hundred and sixty-four (164) pupils (2 schools each from fishing and farming communities) participated in the study comprising 50.6% males and 49.4% females, as well as their parents or primary caregivers. About 9.6% Soil-transmitted helminths (STH) were observed in the farming communities with none recorded in the fishing communities (p=0.007). Conversely, 33.8% of *S. haematobium* infection occurred in the fishing compared to 1.2% in the farming community (p<0.0001). About 48.8% of all children obtained their drinking water from the Afram River compared to 51.2% from boreholes. Overall, 31.7% of all children lived close to a water source, with 48.8% versus 13.8% being in the farming and fishing communities respectively (p<0.0001). Handwashing after toilet use was reported by 61.0% participants with 86.9% versus 33.8% of children within the farming and fishing communities engaging in this practice respectively (p<0.0001).

Conclusions: Poor hygienic practices and sanitary conditions were more prevalent in the fishing communities than in the farming communities. *S. haematobium* infection was significantly higher among the fishing communities while STH infections solely occurred among children in the farming communities. The best predictors for schistosome infections were swimming in the river, water storage method, farming activities, and source of drinking water.

Keywords: Sanitation; Helminthiasis; School-age children; Hygiene; Ghana

Abbreviations: STH: Soil-Transmitted Helminths; SAC: School-Age Children; CI: Confidence Interval; OR: Odds Ratio; UNICEF: United Nations International Children's Emergency Fund; WHO: World Health Organization.

Background

According to the World Health Organization, over 610 million school-age children (SAC) are at high-risk of soil-transmitted helminth (STH) and schistosome-associated morbidities [1], thus, children are considered as a high risk group for helminth infections (helminthiasis) due to their poor hygienic practices and poor play habits such as having direct contact with infested fresh water bodies through swimming [2,3]. The most common helminths of humans which causes intestinal helminthiasis include hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworm (*Ascaris lumbricoidedes*), and the whipworm (*Trichuris trichiura*) while those that cause urinary and intestinal schistosomiasis include schistosomes (*Schistosoma haematobium*, *S. japonicum*, *S.*

mansoni and *S. mekongi*) [4]. In Ghana, the most common helminth is *Schistosoma haematobium* [5], with an estimated national prevalence rate of 70.9% [6].

Infections with these helminths (STH and schistosomes) in children lead to increased morbidities through their adverse effects on their nutritional status [7], leading to impaired growth and anemia [8], as well as impaired cognitive process [9].

The main strategy for the control of STH and schistosomes has been through the use of periodic deworming drug treatment (anthelmintics), using albendazole or mebendazole for STH and praziquantel for schistosomiasis [10]. However, the long-term control of these helminths is still a public health concern as there are some challenges with the periodic deworming interventions. For instance, the availability of the deworming drugs is not sustainable as most of the control programs depend on drug donors [1]. This has led to the observation of increased helminth infection rates similar to their pretreatment levels by the 18th to 24th months after the cessation of periodic deworming programs [11,12]. Furthermore, drug resistance from the periodic deworming is a major public health concern as there is evidence of this occurring in some livestock [13,14].

The World Health Organization together with UNICEF (2009), have therefore provided guidelines on the improvement of sanitation, water and hygiene (WASH) as a more sustainable approach of controlling helminth infections [1]. This approach includes access to hardware such as toilet facilities, clean water in addition to healthy behaviors such as handwashing at critical times such as after the use of toilet. The WASH approach has been shown to reduce re-infection rates of helminth infections [15-17]. However, the existing literature on the subject does not cover existing disparities of helminthiasis and sanitary conditions between the two distinct helminth-endemic communities (fishing and farming) in Ghana in order to identify the specific potential determinants of helminth infections between these communities. Thus the rationale of this cross-sectional study which aimed to determine the prevalence of schistosomiasis (due to *S. haematobium* and/or *S. mansoni*) and soil-transmitted helminthiasis (due to *A. lumbricoides*, *T. trichiura* and/or the hookworms) among the SAC within two distinct communities (farming and fishing) in the Kwahu Afram Plains South District in the Eastern Region of Ghana, and to elucidate the disparities and associations of infection and hygienic practices, sanitary conditions and health behaviors among SAC in the two communities. These helminths were investigated because they commonly occur among SAC in Ghana [6,18,19]. Findings from this study will inform public health experts and policy makers to plan and implement interventions which will be tailored to suit the specific needs of the two unique communities and potentially reduce re-infection rates.

Materials and Methods

Study site

This study was conducted in the Kwahu Afram Plains South District in the Eastern Region of Ghana with Tease as its district capital. It has a total land area of approximately 3,095 sqkm. The land is undulating and rises to between 60 meters to 120 meters above sea level. The district falls within the savannah transitional zone and the savannah woodland which is made up of fire resistant trees. The district is drained to the south by the Afram River and the Volta River to the East. These rivers flow continuously throughout the year and are used for domestic as well as agricultural purposes (**Figure 1**) [20].

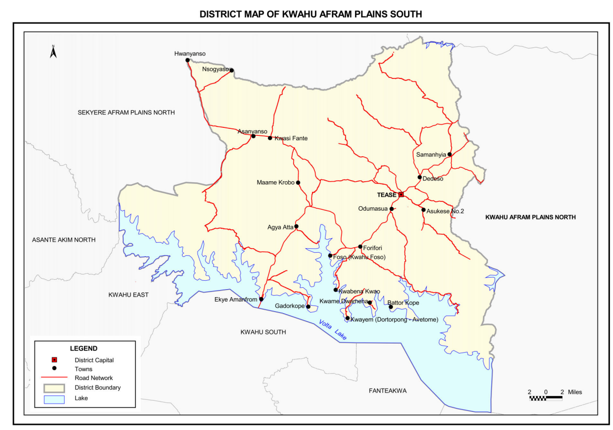

Figure 1 District Map of Kwahu Afram Plains South.

Study design and subjects

This was a cross-sectional study conducted during the summer (May-June 2017). A total of 164 pupils participated in the study together with their parents or primary guardian (caregiver). Two main communities; fishing (n=84) and farming (n=80) were used for this study. Pupils were recruited from four randomly selected schools (two schools from the fishing and two schools from the farming communities) in the Kwahu Afram Plains South District of Ghana). Although participants were selected from their respective schools, the primary focus of this study was based on which communities those schools were located and the communities in which the participants lived.

The estimated sample size for the study was 192, based on 80% power and 0.05 alpha and 95% confidence interval in hemoglobin level, to detect an odds ratio of 2.5 between infected versus uninfected children based on information from other studies. However, the number of pupils in the randomly selected schools within the proposed age range (9-12) were not adequate resulting in a relatively smaller sample size of 164.

Inclusion and exclusion criteria

Children who were living in the selected communities (farming and fishing communities of the Kwahu Afram Plains South District) and who were within the ages 9-12 years of the randomly selected schools (2 schools in each community) were included in the study. Parents/guardians of eligible children had to be 25 years or older to prevent minors (older siblings) standing in as caregivers of the children to participate in the study for accurate information on the index child.

Children who did not live in the randomly selected communities, as well as those who were out of the age range of 9-12 years were excluded from the study. Children who self-reported to have sickle cell anaemia and malaria, as well as those who had obvious physical ailments such as goitre and elephantiasis, and those who were already taking nutritional supplements at the time of enrolment were excluded from the study.

Participant recruitment

To select the study sites, a random selection of the district was done to choose either the south or north district of the Kwahu Afram Plains, and a list of all schools within the fishing and farming communities in the selected district (Kwahu Afram Plain South District) was obtained from the Student Health and Education Program (SHEP) coordinator. Two (2) schools each from the farming and fishing communities were randomly selected through a balloting process. Participants (SAC) were then recruited from the two (2) randomly selected primary schools within each of the two selected communities. Data were collected on sociodemographic, parasitic (urine and stool analysis for STH and schistosome infections) as well as hygienic and sanitary conditions.

Questionnaire development

The WHO recommended child form for parasitological nutritional survey was modified and used for data collection [21]. Our questionnaire comprised of two parts: one part for parents; consisting of 33 questions of 3 sections (sociodemographic, water and sanitation, and knowledge in helminth infection); and a second part for the children comprising of 17 questions. A similar version of this questionnaire had been validated and used in a previous study among school-age children in Ghana [19].

Data Collection Procedure

Administration of children and parent questionnaires

Trained research assistants and teachers asked questions in the participants' local language in the filling of both the parent/caregiver and child questionnaires. The parents/caregivers were questioned first, followed by the children.

Demographic information

Participants and their caregivers were assisted to complete the questionnaire in an in-person interview format. Demographic information including age, sex, ethnicity, number of children in the household, sanitation and hygiene knowledge and practices, source of drinking water, parental employment, and educational status among others were obtained through interviews with the participants and their caregivers using well-structured questionnaire [21].

Parasitic infections assessment and treatment

The participants (children) were provided with labelled plastic stool and urine containers during the time of their registration into the study. They were then given directions on how much urine and stool were to be deposited into each container such that the urine did not have to exceed a certain mark on the urine container provided. They were also given directions on how to safely use the plastic scoop attached to the lid of the stool container to scoop the fecal sample and gently deposit it within the stool container. Stool and urine samples of study participants were collected at a designated point in the various schools by trained research assistants to assess the presence of intestinal helminths (Hookworm, *Ascaris lumbricoides*, *S. mansoni* and *Trichuris trichuria*) and urinary schistosomiasis (due to *S. haematbium* infection). Participants who could provide their urine and stool specimen on the same day were encouraged to do so.

The stool and urine samples were stored on ice (in chest) and transported to the Holy Spirit Health Centre Laboratory within the district for analysis. One gram of fresh fecal sample was examined microscopically for the eggs of intestinal worms using the Kato-Katz technique [22]. The estimation of intensity of infection for each species was recorded as eggs per gram (epg) of stool. The fecal egg count was repeated 2 to 3 times to ensure the accurate measurement of helminths. The stool and urine analysis were done by a Laboratory technician. In order to detect the presence of hookworms in the stool, the slides were read within 30–60 min of preparation. The type and number of eggs were recorded on a recording form alongside the sample number. Finally, the number of eggs counted on each fecal sample was multiplied by a factor of 24 to get the number of eggs per gram (epg). The epg determined was used to classify the infection as either light (1-999), moderate (1000-9,999) or heavy (≥ 10,000) intensity for Trichuris; and light (1-1,999), moderate (2,000-3,999) or heavy (≥ 4000) intensity for Hookworm infections [23].

For urine samples, a urine reagent strip (Combi ten) was used to analyse 163 of the urine samples of the subjects to test for the presence of blood. Furthermore, the urine filtration method was used to analyse the urine samples by filtering 10 mL of urine to extract and count the eggs of the worms microscopically in order to estimate the intensity of the infection. Visual measures of hematuria (macrohematuria) was also performed on the urine by trained personnel [24]. The intensity of *S. haematobium* was classified as either low (</=50 eggs/10 ml urine) or high (>50 eggs/10 ml) [23].

Statistical Analysis

All the data collected in this study were entered into Microsoft Excel (2016) and exported into IBM SPSS Version 24 for Windows for all statistical analysis. Descriptive statistics of means, median, standard deviations and ranges were determined for continuous variables (Index child age, caregiver age, among others). Proportions were determined for categorical variables (community type, education level, marital status, number of children in household, toilet facility at home, communal eating among others). This was followed by a bivariate analysis using the Chi square ($\chi 2$) and the Fischer Exact tests where appropriate to determine significant differences between the categorical variables (community types, toilet facilities, handwashing habits among others). Univariate and multivariate logistic regression analyses were also conducted to examine the association between the independent variables and the dependent variables. The level of statistical significance was defined at $p<0.05$.

Results

Characteristics of the study participants

The age of the children ranged between 9 and 12 years old with no significant difference in mean age between the communities (p=0.880). The mean age of the parents/caregivers was 40.36 ± 10.11 years (farming) and 37.09 ± 10.20 years (fishing) communities (p=0.41) (**Table 1**). Randomly selected schools were Trebu Primary (28.0%), St. Michael Primary (20.7%), Kwasi Fante Primary (27.4%) and Asanyansu Primary (23.8%) (**Table 1**).

By gender, participants were observed to be 50.6% males and 49.4% females with 55.5% of caregivers being the real mothers of the participants. A significantly higher proportion of mothers were from the fishing communities 59.3% (p=0.028). About 84.8% of the children came from a household where both mother and father were present with majority (73.8%) of the parents/guardians being crop farmers. About 43.3% of the parents/guardians had had no formal education while only 10.4% had completed high school (**Table 1**). Over half (57.35%) of the participants were from the Ewe tribe, with a significantly greater proportion of them (93.8%) being in the fishing communities (p<0.0001), while overall, 34.1% were from the Northern tribe of Ghana (**Table 1**).

Table 1 Characteristics of Children and Adult Caregivers by Communities.

Variable	Overall n (%)	Farming n (%)	Fishing n (%)	p.value
Schools in Communities:				
Trebu Primary	46 (28.0%)	0 (0.0%)	46 (57.5%)	<0.0001
St. Michaels Primary	34 (20.7%)	0 (0.0%)	34 (42.5%)	
Kwasi Fante Primary	45 (27.4%)	45 (53.6%)	0 (0.0%)	
Asanyansu Primary	39 (23.8%)	39 (46.4%)	0 (0.0%)	
Adult Interviewed:				
Father	32 (19.5%)	10 (11.9%)	22 (27.5%)	0.028
Mother	91 (55.5%)	54 (59.3%)	37 (46.3%)	
Male Guardian	9 (5.5%)	6 (7.1%)	3 (3.8%)	
Female Guardian	32 (19.5%)	14 (16.7%)	18 (22.5%)	
Tribe:				
Northerners	56 (34.1%)	56 (66.7%)	0 (0.0%)	<0.0001
Ewes	94 (57.35%)	19 (34.1%)	75 (93.8%)	
*Others	14 (8.5%)	9 (10.7%)	5 (6.3%)	
Marital Status:				
Married	139 (84.8%)	61 (72.6%)	78 (97.5%)	<0.0001
^Not married	25 (15.2%)	23 (27.4%)	2 (2.5%)	
Parent/Guardian Highest Education:				
Never attended school	71 (43.3%)	48 (57.1%)	23 (28.7%)	0.001

Elementary school	36 (22.0%)	16 (19.0%)	20 (25.0%)	
Junior High school	40 (24.4%)	22 (14.3%)	28 (35.0%)	
Senior High school	17 (10.4%)	08 (9.5%)	09 (11.3%)	
Parent/Guardian Job Title:				
Crop Farmer	121 (73.8%)	69 (82.1%)	52 (65.0%)	0.008
Fish farmer	9 (5.5%)	0 (0.0%)	9 (11.3%)	
Business Person	18 (11.0%)	6 (7.1%)	12 (15.0%)	
Skilled Worker	8 (4.9%)	5 (6.0%)	3 (3.8%)	
Service Provider	8 (4.9%)	4 (4.8%)	4 (5.0%)	
Number of Children in Household:				
1-4	57 (36.1%)	28 (33.3%)	29 (39.2%)	0.574
5-8	68 (43.0%)	36 (42.9%)	32 (43.2%)	
>8	33 (20.9%)	20 (23.8%)	13 (17.6%)	
Index Child Gender:				
Male	83 (50.6%)	43 (51.2%)	40 (50.0%)	0.879
Female	81 (49.4)	41 (48.8%)	40 (50.0%)	
Index Child Age:				
9 yrs	49 (30.2%)	23 (28.0%)	26 (32.5%)	0.162
10 yrs	34 (21.0%)	17 (20.7%)	17 (21.3%)	
11 yrs	22 (13.6%)	16 (19.5%)	6 (7.5%)	
12 yrs	57 (35.2%)	26 (31.7%)	31 (38.8%)	

Mean Characteristics of Participants by Communities			
Variables	Communities		p. value
	Farming	Fishing	
	(mean ± SD)	(mean ± SD)	
Caregiver Age	40.36 ± 10.11	37.09 ± 10.20	0.41
Index Child Age	10.55 ± 1.21	10.53 ± 1.30	0.88

*Others constitute the 'Akans' and the 'Gas'; ^Not married include adults who are 'never married', 'widowed' or 'separated'.

Sanitary conditions of the study population

Overall, 48.8% of the study population obtained their drinking water from the Afram River, whilst 51.2% of them obtained theirs from boreholes. All the participants who had borehole as their drinking water source were from the farming communities whilst those who obtained their drinking water from Afram River were from the fishing communities. Similarly, 50.6% of all the participants versus 49.4% obtained their bathing water from the borehole and Afram River, respectively. About 31.7% of the participants lived close to a water source, and of these, a significantly higher proportion of them were in the farming communities (48.8%) compared to only 13.8% in the fishing communities (p<0.001) (**Figure 2**).

Also, a majority of all the children (83.0%) who did not have toilet facilities at home engaged in open defecation and of these, 59.1% were in the farming communities compared to 100% of those in the fishing communities (p<0.0001). Also, 40.9% of those in the farming communities reported using public latrine, compared to none from the fishing communities.

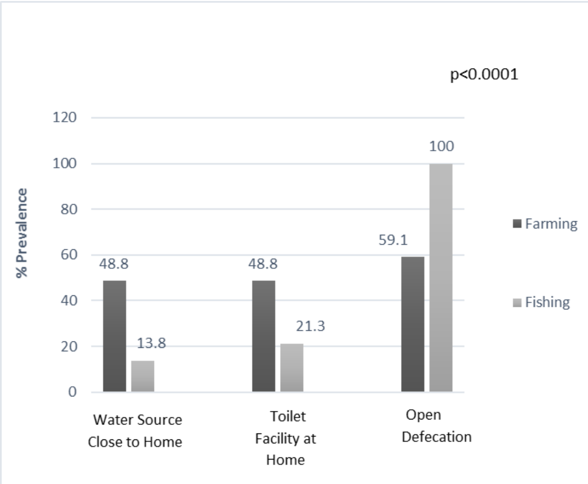

Figure 2 Sanitary Conditions by Community Type.

Prevalence of STH and Schistosoma haemtobium infections

Figure 3 shows the prevalence of Soil-Transmitted Helminths (STH) and Schistosomiasis among the participants. Out of 163 children participants who provided their stool samples, 8 of them had the eggs of STH in their stools, of which 3 were eggs of Trichuris trichiura (whipworm) and 5 were eggs of Hookworm. All the 8 children with the STH infection lived within the farming communities. No eggs of Ascaris nor *S. mansoni* were detected in the stool samples. Of the 164 children who provided their urine, 28 of them had *Schistosoma haematobium* eggs in their urine. No visible macrohematuria was observed in the urine samples, however, a urinary reagent strip analysis showed that 26 children had microhematuria (**Figure 3**).

As shown in **Table 3**, the prevalence of STH in the farming community was only 9.6% with no case found in the fishing communities (p=0.007). One (1) child had a light intensity of Trichiuris, two (2) had moderate infection of Trichiuris, whilst five (5) of children had a light intensity of Hookworm infection. However, due to the lower prevalence of SHT cases, the major focus of the discussion of our results is based on the statistical analysis of *Schistosoma haematobium* infections (**Table 3**). A significantly higher percentage (33.8%) of *S. haematobium* infection occurred in participants in the fishing communities, whilst only one (1) child in the farming community (1.2%) had the infection (p<0.0001). The intensity of *S. haematobium* was heavy amongst 18 of the infected children in the fishing communities compared to none in the farming communities. Those with light intensity infections in the fishing communities were nine (9) compared to only one (1) in the farming community, (p=0.357) (**Table 3**). Microhematuria was observed in 32.5% of children from the fishing communities compared to none in the farming communities (p<0.0001) (**Table 3**). Of the children who had urinary schistosomiasis, 9.9% of their parents were crop farmers, 55.6% were fish farmers and 32.4% were in a non-farming occupation (p<0.0001). No significant differences were observed in the prevalence of *S. haematobium* infection by age of the child. More females 18.5% than males 15.7% were infected with *S. haematobium*, but this was not significantly different (p=0.681).

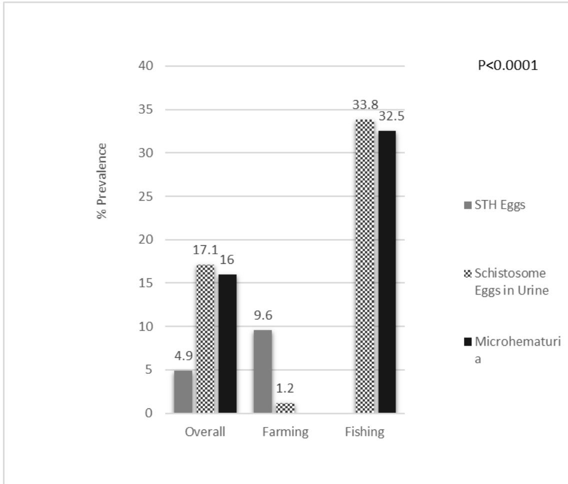

Figure 3 Helminth Prevalence.

Hygienic practices and habits of study participants

Among the variables that were considered for hygienic practices, waste disposal method, water treatment method, occasions of handwashing, frequency of handwashing, both adult and index child practice of handwashing after toilet use, type of hand washing agent used, hand washed with soap and water before eating, engaging in pica behavior, engaging in farming activities, swimming in river, and fingernails biting were all significantly different between the farming and fishing communities (**Table 2**). Overall, 59.1% disposed their waste in the bush, with a significantly higher proportion (85.0%) of this practice occurring in the fishing communities compared to 34.5% in the farming communities (p<0.0001). Overall, only 26.2% of the participants used the public refuse damp as their site of waste disposal (**Table 2**). Also, 51.8% of the household of the total study participants stored their water in containers (plastic/rubber bowls, buckets, gallons or water jar), with 48.2% storing their water in covered tanks. The habit of always treating drinking water was practiced by 13.4%, whilst 83.5% did not treat their drinking water at all (**Table 2**). The most common water treatment method among those who treated their water was the sieving method which was used by 37.9% of total households. This was significantly practiced among the fishing communities, (63.6%) compared to the farming communities (22.2%) (p=0.030). The use of Naphthalene balls was also commonly practiced by 34.5% of all households of participants. Other water treatment methods include the use of palm fruit fiber, tank cleaning and use of dettol disinfectant (**Table 2**).

Table 2 Participant Hygienic Practices (N=164).

Variables		Communities		p. value
	Overall n (%)	Farming n (%)	Fishing n (%)	
Waste Disposal Method:				
Public refuse dump	43 (26.2%)	38 (45.2%)	5 (6.3%)	<0.0001
Dug out pit	24 (14.6%)	17 (20.2%)	7 (8.8%)	
Bush	97 (59.1%)	29 (34.5%)	68 (85.0%)	
Water Storage Method:				
Container	85 (51.8%)	43(51.2%)	42 (52.5%)	0.877
Tank	79 (48.2%)	41 (48.8%)	38 (47.5%)	
Treat Drinking Water:				
Always	22 (13.4%)	13 (15.5%)	9 (11.3%)	0.658
Sometimes	5 (3.0%)	3 (3.6%)	2 (2.5%)	
No	137 (83.5%)	68 (81.0%)	69 (86.3%)	
Treatment Method:				
Use of Palm fruit fiber	2 (6.9%)	2 (11.1%)	0 (0.0%)	0.03
Tank cleaning	2 (6.9%)	2 (11.1%)	0 (0.0%)	
Sieving	11 (37.9%)	4 (22.2%)	7 (63.6%)	
Use of Alum	3 (10.3%)	0 (0.0%)	3 (27.3%)	
Use of Dettol Disinfectant	1 (3.4%)	0 (0.0%)	1 (9.1%)	
Use of Naphthalene balls	10 (34.5%)	10 (55.6%)	0 (0.0%)	
Frequent Handwashing at Home:				
Yes	80 (48.8%)	58 (69.0%)	22 (27.5%)	<0.0001
Sometimes	70 (42.7%)	26 (31.0%)	44 (55.0%)	
No	14 (8.5%)	0 (0.0%)	14 (17.5%)	
Occasions of Handwashing:				
Before/After eating	43 (28.5%)	21 (25.0%)	22 (32.8%)	0.025
After Toilet	12 (7.9%)	11 (13.1%)	1 (1.5%)	
After Work	16 (10.6%)	6 (7.1%)	10 (14.9%)	
Before eating and after toilet	80 (53.0%)	46 (54.8%)	34 (50.7%)	
Adult Practice of Handwashing After Toilet use:				
Yes	148 (90.2%)	81 (96.4%)	67 (83.8%)	0.008
No	16 (9.8%)	3 (3.6%)	13 (16.3%)	
Index Child Practice of Handwashing After Toilet Use:				
Yes	100 (61.0%)	73 (86.9%)	27 (33.8%)	<0.0001
No	64 (39.0%)	11 (13.1%)	53 (66.3%)	
Wash hands with Soap and Water Before Eating:				

Yes	79 (48.2%)	57 (67.9%)	22 (27.5%)	<0.0001
No	85 (51.8)	27 (32.1%)	58 (72.5%)	
Hand Washing Agents Used:				
Water only	33 (20.2%)	7 (8.3%)	26 (78.8%)	<0.0001
Water and soap	129 (79.1%)	77 (91.7%)	52 (65.8%)	
Ash	1 (0.6%)	0 (0.0%)	1 (1.30%)	
Communal Eating:				
Yes	146 (89.0%)	73 (86.9%)	73 (91.3%)	0.457
No	18 (11.0%)	11 (13.1%)	7 (8.8%)	
Engage in Pica Behavior:				
Yes	40 (24.4%)	26 (31.0%)	14 (17.5%)	0.048
No	124 (75.6%)	58 (69.0%)	66 (82.5%)	
Wash Fruits Before Eating:				
Yes	121 (73.8%)	66 (78.6%)	55 (68.8%)	0.161
No	16 (9.8%)	18 (21.4%)	25 (31.3%)	
Engage in Farming Activities:				
Yes	129 (78.9%)	78 (92.9%)	51 (63.8%)	<0.0001
No	35 (21.3%)	6 (7.1%)	29 (36.3%)	
Index Child Barefooted Outside:				
Yes	130(79.3%)	68 (81.0%)	62 (77.5%)	0.701
No	34 (20.7%)	16 (19.0%)	18 (22.5%)	
Swim in River:				
Yes	103 (62.8%)	40 (47.6%)	63 (78.8%)	<0.0001
No	61 (37.2%)	44 (52.4%)	17 (21.3%)	
Fingernails Biting:				
Yes	94 (57.3%)	39 (46.4%)	55 (68.8%)	0.005
No	70 (42.7%)	45 (53.6%)	25 (31.3%)	

The practice of frequent hand washing at home was reportedly practiced by 48.8%, with a significantly higher proportion of this practice occurring in the farming communities (69.0%) than the fishing communities (22.5%) (p<0.0001). Overall, the occasion during which hands were mostly washed was 'before eating and after toilet use', practiced by 53.0% of all participants, with a significantly higher proportion of this occurring in the farming communities (54.8%) than the fishing communities, (50.7%) (p=0.025). Also, a majority (90.2%) of all the adults (caregivers) reported that they washed their hands after toilet use, and this practice among caregivers was significantly higher among those in the farming communities (96.4%) than their counterparts in the fishing communities (83.8%) (p=0.008) (**Table 2**). Conversely, a lower percentage (61.0%) of all children reported washing their hands after toilet use, with this practice being significantly higher among children in the farming communities (86.9%), compared to their counterparts in the fishing communities which was just 33.8% (p<0.0001) (**Table 2**).

Generally, the most common washing agent reported being used for handwashing was 'water and soap' (79.1%), with this practice being significantly higher in the farming communities (91.7%) compared to the fishing communities (65.8%) (p<0.0001). Whilst the use of 'only water' was reportedly practiced by 20.2% of the total households of participants, it was disproportionately higher in the fishing communities (78.8%) compared to 8.3% of the farming communities (p<0.0001) (**Table 2**). About 48.2% of the children reported washing their hands with soap and water before eating which was significantly practiced in the farming communities (67.9%) compared to 27.5% in the fishing communities (p<0.0001). Also, 24.4% of all children who participated in the study engaged in pica behavior (geophagia). This behavior was significantly higher among children in the farming communities (31.0%) compared to those in the fishing communities (17.5%) (p=0.048). About 57.3% of all children also reported the habit of fingernail biting with (68.8%) and (46.4%) of them in fishing versus farming communities, respectively involved in this practice (p=0.005) (**Table 2**). Whilst majority of all the children (62.8%) swam in the river, a significantly higher proportion of them (78.8%) were from the fishing communities compared to 47.6% of those in the farming communities (p<0.001). Similarly, 79.3% of all the children walked barefooted with no significant difference between the two communities (**Table 2**).

Health knowledge and practices towards helminth infections among study participants

As shown in **Table 3**, only 15.2% of the parents/caregivers of the participating children reported having very good knowledge about helminths. The habit of deworming the index child was also reported by 52.4% of all the parents/caregivers. Also, about 14.0% of all the caregivers reported that the index child always complained of abdominal pains, with 8.5% of them seeing blood in their stool (**Table 3**). Overall, 12.8% of all the caregivers reported seeing blood in the index child's urine with 18.8% of these within the fishing communities compared to 7.1% of in the farming communities (p=0.035). About 56.7% were reported to have experienced some fever in the past 6 months prior to this study, with significantly higher proportions (70%) occurring in the fishing communities compared to 44.0% of children in the farming communities (p=0.001) (**Table 3**).

Table 3 Helminth Infections and Participant Health Knowledge and Practices (N=164).

Variable	Communities			p. value
	Overall N (%)	Farming n (%)	Fishing n (%)	
Caregiver's Knowledge about Helminths:				
Very well	25 (15.2%)	12 (14.3%)	13 (16.3%)	0.859
Not adequate	69 (42.1%)	37 (44.0%)	32 (40.0%)	
No	70 (42.7%)	35 (41.7%)	35 (43.8%)	
Caregiver Deworms Index Child:				
Yes	86 (52.4%)	46 (54.8%)	40 (50.0%)	0.639
No	78 (47.6%)	38 (45.2%)	35 (50.0%)	
Frequency of Abdominal Pains in Index Child:				
Always	23 (14.0%)	13 (15.5%)	10 (12.5%)	0.381
Sometimes	86 (52.4%)	47 (56.0%)	39 (48.8%)	
Never	55 (33.5%)	24 (28.6%)	31 (38.8%)	
Blood in Index Child's Stool:				
Never	150 (91.5%)	76 (90.5%)	74 (92.5%)	0.782
Sometimes	14 (8.5%)	8 (9.5%)	6 (7.5%)	
Blood in Index Child's Urine:				
Never	143 (87.2%)	78 (92.9%)	65 (81.3%)	0.035
Sometimes	21 (12.8%)	6 (7.1%)	15 (18.8%)	
Index Child's Experience of Fever in 6 Months:				
Yes	93 (56.7%)	37 (44.0%)	56 (70.0%)	0.001
No	71 (43.3%)	47 (56.0%)	24 (30.0%)	
Helminth Eggs in Index Child's Stool:				
Yes	8 (4.9%)	8 (9.6%)	0 (0.0%)	0.007
No	155 (95.1%)	75 (90.4%)	80 (100%)	
S. hematobium Eggs in Index Child's Urine:				

Yes	28 (17.1%)	1 (1.2%)	27 (33.8%)	<0.0001
No	136 (82.9%)	83 (98.8%)	53 (66.3%)	
S. hematobium Intensity:				
Light	10 (35.7%)	1 (100%)	9 (33.3%)	0.357
Heavy	18 (64.3%)	0 (0.0%)	18 (66.7%)	
Microscopic Blood in Index Child's Urine:				
Yes	26 (16.06)	0 (0.0%)	26 (32.5%)	<0.0001
No	137 (84.0%)	83 (100%)	54 (67.5%)	

Predictors of childhood *S. haematobium* infection

Table 4 shows the predictors of childhood *S. haematobium* infection in the Afram Plain South District of the Eastern Region of Ghana. Our univariate analysis shows that swimming in the Afram River and engaging in the practice of fingernails biting were associated with *S. haematobium* infection while using borehole water as both drinking and bathing water source, practicing frequent handwashing and having a toilet facility at home were protective against the infection. In the multivariate analysis adjusting for confounders, drinking water source, engagement in farming activities swimming in the river and water storage remained statistically significant predictors of *S. haematobium* infection. Borehole as drinking water source was 97% protective compared to the Afram River as drinking water source and using container for water storage was 84% protective compared to using tank against *S. haematobium* infection among the children studied. As expected, children who reported to swimming in the Afram River were more than 5 times (OR=5.27; 95% CI: 1.15–24.25, p=0.033) more likely to have *S. haematobium* infection (**Table 4**).

Table 4 Predictors of Childhood *S. haematobium* Infection in the Kwahu Afram Plains South District.

Variable	n	Unadjusted OR (95%CI)	p-value	Adjusted OR (95% CI)	p-value
Drinking Water Source:					
Borehole	84	0.024 (0.003-0.179)	<0.0001	0.033 (0.004-0.268)	0.001
Afram River	80	1			
Bathing Water Source:					
Borehole	83	0.024 (0.003-0.185)	<0.0001	-------	-------
Afram River	81	1			
Fingernails Biting:					
Yes	94	4.211 (1.513-11.725)	0.006	-------	-------
No	70	1			
Toilet Facility at Home:					
Yes	58	0.177 (0.051-0.614)	0.006	-------	-------
No	106	1			
Engage in Farming Activities:					
Yes	129	0.183 (0.076-0.438)	<0.0001	0.267 (0.080-0.884)	0.031
No	35	1			
Swim in River:					
Yes	103	4.329 (1.424-13.162)	0.01	5.272 (1.147-24.245)	0.033
No	61	1		1	
Water Storage:					

Container	85	0.248 (0.099-0.622)	0.003	0.156 (0.052-0.471)	0.001
Tank	79	1		1	
Frequent Handwashing at Home:					
Yes	80	0.278(0.071-1.094)	0.067	-------	-------
Sometimes	70	0.741 (0.205-2.682)	0.648		
No	14	1			
Shoes Worn to School:					
Yes	140	0.429 (0.158-1.159)	0.095	-------	-------
No	24				
The multivariate analysis was adjusted for 'Index Child Age' and 'Community type'. Hosmer and Lemeshow Test: Chi-Square=1.974, p-value=0.982.					

Discussion

In this study we evaluated the prevalence and predictors of helminth infections among school age children in farming versus fishing communities in Ghana. There were similarities in index child, caregiver and household characteristics irrespective of community. Significant disparities in hygienic practices and sanitation resources were found between the communities while significant differences in the source of drinking and bathing water, availability of toilet facilities at home (**Figure 2**), handwashing practices, waste disposal methods and water treatment methods were also found. There were also significant differences in pica practices and habitual fingernails biting between children from farming communities versus those from the fishing communities (**Table 2**). Most importantly, the type of helminth infection differed by the community type (**Table 3**).

The burden of diseases associated with STH and schistosomes is higher in places where there is inadequate coverage of sanitation [25]. Thus, it is difficult to eliminate helminth infections in areas where there is poor access to water and sanitation. Almost half of all the caregivers (43.3%) had no formal education, which is likely to contribute to the helminth infection rate of their children by virtue of their lack of knowledge, practices and beliefs [26]. Significant disparities existed between the two communities in terms of their proximity to drinking and bathing water source, toilet facility at home, waste disposal methods, water treatment methods, handwashing frequency (after toilet use and before eating), washing agent used for handwashing, pica practice, farming activities, swimming in river and fingernail-biting habits. These disparities could be attributed to the close proximity or otherwise of the subjects to a fresh water source which may propagate the transmission of schistosomiasis in the fishing communities as the fresh water source is used for occupational activities such as fishing [27] or domestic activities like cooking, laundry, bathing, drinking etc. [28].

Overall, children in the fishing communities were observed to engage in unhealthier hygienic practices such as not washing hands after toilet use, not washing hands with soap and water before eating, not washing fruits before eating, using only water as a hand washing agent, engaging in more pica behavior, swimming in the Afram River and finger nails biting than those in the farming communities (**Table 2**). All these poor hygienic practices have been associated with increased rates of helminth infections in other studies [27,29,30].

This study showed a relatively low overall prevalence (4.9%) of STH (Hookwowm and Trichiuris) in the study communities, whilst the prevalence of urinary schistomiasis (*S. haematobium*) was 17.1%. The generally low prevalence of helminth infection observed could be attributed firstly to a relatively smaller sample size (164) than anticipated and secondly to the regular national deworming program which has been integrated with the School Health Education Program (SHEP), which is run together by the Ghana Education and the Ghana Health Services' Neglected Tropical Disease Control Program (NTDCP) and other Non-Governmental Organizations with the primary aim of providing periodic dewormers for schistosome and STH control among school-age children [31]. Furthermore, the observed prevalence for STH infection (4.9%) in our study was lower than the 12.5% reported in a recent study among 200 pupils in Elmina, a fishing community in the Central Region of Ghana [29]. In that study, only stools were examined, but not the urine of the pupils, therefore the presence of urinary schistosomiasis due to *S. haematobium* infection could not be investigated, which was rather highly prevalent in our study (17.1%). These observations suggest that using combined biomarkers may be an effective means of assessing the true prevalence and different species of helminth among children.

The higher prevalence *S. haematobium* observed in the fishing communities confirms an increased risk of schistosomiasis with close contact with fresh water bodies [30,32]. On the contrary, the relatively low *schistosoma haematobium* infection observed among the farming communities could be attributed to improved source of drinking water (borehole) in those communities compared to the Afram River source in the fishing communities. In another study among school-age children in the Amakwa Afram Plains

District of Ghana, the most common STH infection found was hookworm (STH) [27], with prevalence rates higher (14.0%) than was observed in our current study. Similarly, another study in the Kintampo North Municipality in the Brong Ahafo Region of Ghana, also observed even higher prevalence rate of 39.1% for hookworm infection among school-age children [18]. This comparatively lower prevalence (4.9%) observed in our study could be due to the recent deworming program that had taken place in the Kwahu Afram Plains South District 10 months (August 2016) prior to our study (Interview of headmaster and district nurse), suggesting lower reinfection rates of STH such as hookworm, Trichuris and Ascaris.

In the southern part of Ghana, another study observed very high prevalence (83.9%) of *S. haematobium* among 354 children. This was much higher than what we observed (17.1%) in the current study. This prevalence suggests pockets of high prevalence of helminth infections (both STH and schistosome) in Ghana which are likely not covered by the periodic deworming program or the lack of knowledge or awareness and appropriate practices by parents/caregivers. The situation calls for evaluation of the reach of the national deworming program and awareness of helminth infection among parents/caregivers of children to inform appropriate interventions to eradicate these infections.

Others have asserted that the current diagnostic techniques used in the screening for helminths are usually not sensitive enough to detect light infections, which could be contributing to the underestimation of the helminthiasis burden [33]. It is, therefore, also possible that the relatively low prevalence of especially STH observed in the current study may be due to either missing out on the detection of light infections in the children or the collection of a single stool sample from our participants, which could have been improved with the collection of multiple stool samples [34]. Nevertheless, over the years, the prevalence of helminth infections (both STH and schistosomiasis) has declined in Ghana. A recent evaluation of the ongoing national deworming programs among school-age children in the Volta Region of Ghana reported low prevalence rates of helminths infection at 1.2% Ascariasis, 0.91% hookworm infection, 10.3% urinary schistosomiasis and with only 1 infection due to *S. mansoni* [35]. The national prevalence of schistosomiasis in Ghana has also been gradually declining from 72.5% in 2003 to 70.9% in 2010 [6]. These observed declining trend of helminth infections could be attributed to the nationwide deworming intervention programs.

In the latter part of the 20th century, the London-based Partnership for Child Development evaluated the delivery of deworming drugs to children in Ghana, and they also observed that deworming children was cost effective and was beneficial to their health and educational development [36]. Such interventions could also have contributed to the decreasing trend of infections reported in various other studies in different parts of Ghana [6,28,35,37].

It is common practice for children to play in water bodies and void their urine during such times. This potentially leads to the deposition of schistosome eggs from infected children into water bodies which propagates the transmission cycle of helminth infections [1]. With a majority of children in the fishing communities in this study (78.8%) admitting to swimming in a fresh water body (Afram River) compared to only 47.6% of those in the farming communities suggests that those in the fishing communities have more contact with fresh water body than those in the farming communities which is further away from the Afram River (over an hour drive). Frequent contact with fresh water bodies is likely to result in high transmission or prevalent rates of helminth infections even in places where there are improved latrines [32].

Several studies have also shown that the closeness of human settlements to fresh water bodies and close contact with intermediate freshwater snail hosts create the right environment for disease transmission and high prevalence of helminth infections [30,32]. Thus, it is not very surprising that with the exception of one child who lived in the farming community, the remaining 27 cases of urinary schistosomiasis in this study were among children who lived in the fishing communities. It is likely the single urinary schistosome-infected child from the farming community might have been infected during a visit to families or friends in the fishing community or may have been a regular swimmer in the Afram River. Similarly, in another study, urinary schistosomiasis symptoms were found to be strongly associated with Lake Volta in Ghana where 43% of the children had blood in their urine (microhematuria; a symptom of urinary schistosomiasis), while almost 90% of children who attended school close to the shore of the lake had blood in their urine [27].

In this study, a majority of the total households who did not have toilet facility in their homes engaged in open defecation (83.0%). Similar findings in a study in Cote d'Ivoire observed that open defecation was a common practice among study participants, with only one out of five households having latrines. Thus, open defecation was reported as a determinant for helminths [38]. However it has been argued that having access to a safe source of water and good sanitation does not always translate into low infection rates of helminths, because it has been shown that the provision of latrines is not always used purposefully by some communities due to the poor maintenance of the facilities [39]. This could lead to children preferring to engage in the practice of open defecation than using poorly maintained latrines. For instance, 55% of some Kenyan school children complained of dirty latrines whilst 64% and 66% reported strong odor of latrines and uncomfortable latrines respectively, as responses to their impressions of latrines [40].

According to data from the Ghana Demographic and Health Survey (2014) [41], children who lived in households that had improved and unshared toilets (59% nationally) as well as 43% in urban centers were more likely to have their stools safely disposed than those in households without improved toilet facilities (30% nationally) and (37% rural areas). Since a greater proportion of the children within the farming communities in our study had access to toilet facilities at home than those in the fishing communities (**Figure 2**), it possibly contributed to their overall low prevalence of helminth infections observed, since the availability and use of sanitation facilities has been shown to significantly reduce STH infections [15]. So potentially, the safe disposal of stools among the farming community children protected them from high helminth infections. Sanitation and behavior change, and the prevention of contact with waterbodies or reservoirs/irrigation canals harboring the fresh water snails (intermediate host) are also strategies for helminth control interventions [6]. Several studies have also asserted that having access to clean water, whilst improving on sanitary conditions are very important in the prevention of re-infection with helminths after they have been treated [42,43].

It is imperative that both adults and children in helminth-endemic areas are given health education to influence behaviors such as a reduction in the direct contact with infested fresh water bodies to reduce helminth transmission as was done in Zanzibar [44,45]. In that study, 42.7% of all caregivers of children said they did not have any knowledge about helminth infections. In some settings, it is not regarded as a health problem at all by those who are infected with helminths in the community [6]. In the Ghana Partnership for Child Development (GPCD) intervention study in the Volta Region of Ghana, they reported that most parents in Ghana did not perceive worm infections as a major health problem for children [26]. Only 4.1% of the parents reported that worms were a health problem for their children. They rather reported headache, fever and malaria as common health problems. Even though our study did not specifically ask caregivers detailed questions on helminth infections, the results in the Tanzania study showed that parents were able to report on symptoms of STH and schistosome. Also, more than 90% of the Tanzanian parents reported blood in urine as a symptom of schistosomiasis. Thus, more parents seemed more knowledgeable about helminth infections in Tanzania [26].

In our study, only 52.4% of the caregivers reported to personally deworm their children and this was similar in both communities (farming versus fishing). Treatment for schistosomiasis can, however, have problems with adherence because of the likelihood of people alternating between the orthodox medication provided by healthcare workers and those they personally obtain from local herbalists [6].The existing problems of treatment adherence has also been attributed to other factors such as the level of knowledge of the infection status of the population, their educational level, the availability of transportation, as well as their perceived quality or benefit of treatment [39].

Personal hygiene should be one of the focuses of health education in helminth endemic communities [46]. Although the focus of our study was not on the sanitation, water, and hygiene (WASH) conditions within the school setting, it has already been reported by UNICEF that only 46% of schools have regular water supply and only 37% have toilets in priority countries [47]. The higher frequency of handwashing observed among the farming communities in our study (**Table 2**) may be due to the constant supply of safe portable water obtained through the borehole in the farming communities, in contrast to the water source (Afram River) for those in the fishing communities.

The transmission of helminth eggs/larvae can also be through the oral-fecal route, thus contaminated hands could increase infection rate. Surprisingly, in a study conducted by Dankwa et al., a significantly higher percentage of children who reported washing their hands after toilet use (10%) were found to be positive for STH compared to those who did not (2%) (p=0.004), whilst of those who reported of washing their hands before eating (89.5%), 12.0% of them were still positive for STH infection but this was not statistically different from those who said they washed their hands sometimes or they did not wash their hands at all [29]. This observed high prevalence could be attributable to other factors other than handwashing, as 35% of their total study population also engaged in the habit of sucking their fingers. Similarly, fingernail biting was practiced by about 57.3% of children in our study, with a significantly higher proportion of them living within the fishing communities compared to those in the farming communities, which could potentially have increased the helminth infection rates.

With a greater proportion of adults in the farming communities washing their hands after toilet use compared to those in the fishing community, could suggest the break in transmission from mothers (majority of the caregivers) to their children during cooking and serving of their foods in the former. Also, with a greater proportion of children within the farming communities engaging in handwashing than those in the fishing communities, could potentially reduce the contamination of foods and other surfaces with helminth eggs/larvae and ultimately contribute to low infection rates observed in the farming communities (**Table 2**). A majority of households reported using both soap and water (79.1%) for handwashing, with a significant proportion occurring among the farming communities. Conversely, in a study in Kenya among 1,106 pupils in 39 public primary schools, majority of them reported the unavailability of soap for handwashing [48].

Pica behavior (geophagia) in our study was practiced by a quarter of the participating children, with majority of them from the farming communities. This could be attributed to a significantly higher proportion of the farming community children having more contact with the soil through farming activities than those in the fishing communities (which may have increased their chances of soil eating habits). Thus, this must have resulted in the observed STH infections (hookworm and Trichiuris) only among the farming community children. Similarly, lower levels (13.0%) of geophagia was practiced by pupils in one study in the Nyanza Province of Kenya [40]. In another study, in the same District of Kenya, 51% of the pupils reported soil eating behavior at home or in school [15].

Walking bare footed was not significantly different between the two communities, and was not a predictor of helminth infection, which is in contrast to other studies where they observed that infection with hookworm was higher in pupils who walked barefooted [29]. Since the majority of children in our study walked bare footed, they stood at a risk of contracting a soil-transmitted helminth (STH) infection which could occur through the direct penetration of the skin from contaminated soil. So the low levels of STH observed could be attributable to less contamination of the soil though open defecation, especially in the farming areas.

The best predictors for schistosome infections among the children in our study were swimming in the river, water storage method, farming activities, drinking and bathing water source. Children who swam in the Afram River were more likely to get schistosomiasis compared to those who did not (OR: 5.272, CI: 1.147-24.245, p=0.033). Similarly, one main factor that was significantly associated with *S. mansoni* infection among some Ethiopian school children was swimming [49]. Close contact with fresh water bodies have also been implicated in high rates of helminth infections in children living close to the Volta Lake in Ghana [27]. This could potentially expose children to schistosome eggs or larvae as they have long contact with the water either through swimming or for occupational activities. This could also explain why children whose water source for either drinking or bathing were from the borehole, compared to those whose source were from the Afram River (farming versus fishing communities) were less likely to be infected with schistosome infections (OR=0.033, CI: 0.004-0.268, p=0.001). Hence, a reduction of exposure to environmental conditions that predisposes individuals to helminth eggs or larvae will potentially be more protective [27]. It is therefore important that after deworming interventions, the use of clean water and adequate sanitation are implemented for the reduction of re-infection rates [50].

Also, those who stored their water in containers (plastic/rubber bowls, buckets, gallons or water jar), were less likely to get schistosomiasis compared to those who stored their water in tanks (OR: 0.156, CI: 0.052-0.471, p=0.001). This could be attributed to the convenience of being able to wash the smaller containers frequently than the larger tanks (**Table 4**), because the use and safe storage of water in the home is very important in improving the health benefits of people in the community [25].

Limitations of the Study

Even though this study did not specifically assess the detailed knowledge of caregivers and children on the topic of helminth infections and associated risk factors or symptoms, it thoroughly assessed their hygienic practices and sanitation conditions which have a direct impact on transmission rates. Secondly, although the study placed much emphasis of sanitary conditions and hygienic practices in their homes without assessing such factors in the schools, the prevailing conditions in most high risk schools has already been established [40,47]. Thus, we see the hygienic practices and sanitary condition in their home environment as equally likely to impact their health and helminth infection compared to whatever factors they are likely to be exposed to in school. Another limitation is our lack of assessment on frequency of swimming in the Afram River to examine how it influenced helminth infection.

Conclusions of the Study

Findings from this study shows the current disparities that still exist between fishing and farming communities in rural Ghana in relation to helminth infections (due to STH and schistosomes), as well as the knowledge, attitudes and practices of both adults and children towards it. Overall, *S. haematobium* infection was the most prevalent helminth infection observed among children in this study (17.1%), whilst STH (hookworm and Trichiuris) infection was 4.9%, no infections due to Ascaris or *S. mansoni* were observed.

S. haematobium infection was significantly high in the fishing (33.8%) than the farming (1.2%) communities. This observation could be attributed to children in the fishing communities engaging in more activities in and around the Afram River that potentially exposed them to more schistosome parasites. There were general disparities in sanitation resources and hygienic practices between the farming and fishing communities. There is the need for awareness creation about helminth infection and their transmission in relation to poor hygienic practices and sanitary conditions among both children and adults in helminth endemic areas especially in fishing communities. Future studies should include the assessment of sanitation and hygienic practices in the school environment to determine pertaining conditions and awareness.

Declarations

Ethical approval

Approval to conduct this study was obtained from the Human Subjects Institutional Review Board of the University of Georgia (STUDY00004580), the Ethical Committee Board at the Kwame Nkrumah University of Science and Technology, Ghana (CHRPE/RC/182/17), the Regional Director of Ghana Education Service and the Regional Director of Health Services of the Eastern Region, Ghana. The Head teachers of the selected schools that participated also gave permission to conduct the study with pupils in their respective schools. Also, the parents/caregivers of the participants provided their consent for their index child to participate in the study whilst the children gave their assent for the study.

Consent for publication

The consent and assent from parents and children were respectively sought for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This material is based upon work supported by the United States Agency for International Development, as part of the Feed the Future initiative, under the CGIAR Fund, award number BFS-G-11-00002, and the predecessor fund the Food Security and Crises Mitigation II grant, award numberEEM-G-00-04-00013 and the 2017 Innovative and Interdisciplinary Research Grant from the University of Georgia Graduate School.

Authors' contribution

AKA: Responsible for proposal development, data collection, data entry and analysis, in addition to the writing and review of the manuscript.

MAT: Proposal development, data collection, entry and analysis, as well as manuscript writing and review.

FCMR: Study implementation, data interpretation and review of draft manuscript.

MDW: Proposal concept development and reviewing of draft manuscript.

All authors read and approved the final versions of the manuscript.

Acknowledgements

We would like to acknowledge the headmasters, teachers and pupils of Trebu District Assembly Primary School, St. Mark School, Kwasi Fante District Assembly School and Asanyansu Primary School for cooperating with us in the data collection. A big thanks also goes to the Regional Education Office, Koforidua, Eastern Region, as well as the Director of Ghana Education Service, Afram Plains South District, the Regional Director of Ghana Health Service, Koforidua, Eastern Region, and the District Director of Health Service of Afram Plains South for giving us the permission to carry out this study in the district. We also acknowledge the Staff of The Holy Spirit Health Center, especially, Reverend Father Andrew Evans Okwan, Sister Mary Nkrumah and Heriuta Acheaa, for all the assistance they gave during our data collection and analysis in their facility. We would also like to acknowledge the KNUST Staff (Dr. Charles Apprey, Mr. Frank Agyemang Bonsu and Mr. Nat Ato Yawson) and the Laboratory Technicians of the Clinical Analysis Lab (CAnLab) of KNUST for assisting with the data collection and laboratory assessments.

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