An extensive burden of giardiasis associated with intestinal schistosomiasis and anaemia in school children on the shoreline of Lake Albert, Uganda

Hajri Al-Shehri[a,b], Michelle C. Stanton[b], James E. LaCourse[b], Aaron Atuhaire[c], Moses Arinaitwe[c], Aida Wamboko[c], Moses Adriko[c], Narcis B. Kabatereine[c,d] & J. Russell Stothard[b,*]

[a] *Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool, L3 5QA UK*; [b] *Ministry of Health, Asir District, Kingdom of Saudi Arabia*; [c] V*ector Control Division, Ministry of Health, Kampala, Uganda;* [d] *Schistosomiasis Control Initiative, School of Public Health, Imperial College London, London W2 1PG, UK*

* Corresponding author. Tel: +44 (151) 705 3724. E-mail: russell.stothard@lstmed.ac.uk

Abstract

Background: Water-borne parasitic diseases associated with poverty still blight the lives of African school children. In Uganda, intestinal schistosomiasis is still common along the shoreline of Lake Albert, despite ongoing control, and co-infection with giardiasis and malaria is poorly known. To shed light on putative interactions between diseases, a prospective cross-sectional parasitological survey was undertaken across 5 primary schools.

Methods: Stool samples from total of 254 school children, aged 5-10 years, were examined by microscopy and rapid diagnostic tests (RDTs), with additional real-time PCR assays for detection of *Giardia* DNA. A finger-prick blood sample was also taken from each child and tested for malaria and haemoblobin levels measured. Assocations between diseases and anaemia were assessed.

Results: Intestinal schistosomiasis (46.5%), giardiasis (41.6%) and malaria (56.2%) were common, and a quarter of children were anaemic (<115 g/L). Up to 87.0% of children were excreting *Giardia* DNA and the prevalence of heavy infection by real-time PCR (Ct ≤ 19) was 19.5%, being positively associated with light, moderate and heavy egg-patent schistosomiasis, as well as, with anaemia.

Conclusion: In this setting, an extensive burden of giardiasis was revealed with heavy intensity infections associated with egg-patent intestinal schistosomiasis and anaemia. To improve child health, greater attention on giardiasis is needed along with exploring joined-up actions across diseases that promote better water hygiene and sanitation measures.

Keywords: *Schistosoma*, *Giardia*, *Plasmodium*, epidemiology, co-infection, control

Introduction

In impoverished regions of sub-Saharan Africa water-borne parasitic diseases in addition to malaria pose a significant threat to the well-being of children. In parts of Uganda, for example, intestinal schistosomiasis is hyper-endemic along the shorelines of the Great Lakes and is particularly rife around Lake Albert[1-3]. Local aetiological factors include favourable freshwater snail habitats, poor local sanitation and hygiene, as well as, extensive levels of daily water contact by shoreline communities[4-6]. While an extensive burden of malaria is known[7-10], the occurrence and extent of another waterborne, poverty-related disease, giardiasis, has yet to be investigated. In 2003, a national control programme against schistosomiasis and intestinal worms was launched at Pakwach (see Figure 1)[11]. Since then there have been substantive actions to deliver praziquantel and albendazole by mass drug administration (MDA) to at-risk children attending primary schools and adults in endemic areas[2]. Owing to high levels of re-infection[12], an extensive burden of intestinal schistosomiasis remains and children with hepatosplenic and gastrointestinal disease can be found[13].

Giardiasis is caused by *Giardia* spp., a binucleate flagellated protozoan, and is associated with ingestion of infectious cysts on foods or in drinking water, as contaminated by faecal waste from humans and(or) animals carrying patent or cryptic infections[14, 15]. In low and middle income countries, prevalence of human giardiasis can vary widely, typically from 0-30%, and depending on which diagnostic is applied, can be almost universal in certain populations[16,17]. Although infections with *Giardia* spp. may be acute and self-limiting, chronic disease, such as persistent diarrhoea, leads to malabsorption and weight loss[18]. Co-infection with other poverty-related diseases, such as soil-transmitted helminthiasis occurs, and with ascariasis, immunomodulation of the gut mucosal surface favours chronic giardiasis[19, 20]. Across Uganda, formal reporting and surveillance of giardiasis is very scant[21-23]. While the disease is zoonotic in livestock and an important anthropozoonosis of the mountain gorilla[24,25], the burden of giardiasis in school children is largely unknown, as are any associations with intestinal schistosomiasis.

The aim of this present investigation was to conduct a prospective cross-sectional parasitological survey to ascertain current levels of intestinal schistosomiasis, giardiasis and malaria among schoolchildren (5-10 years of age) on the shoreline of Lake Albert, Uganda. Associations between diseases were assessed and as three of the five schools first surveyed for intestinal schistosomiasis in 2002-2003, a comparison through time was made.

<insert Figure 1 near here please>

Materials and methods

Study location

In June 2015 in Buliisa District, Western Uganda, a prospective cross-sectional parasitological survey, using parasitological methods augmented with rapid diagnostic tests (RDTs), was undertaken across 5 primary schools. Three schools were selected on the basis that a baseline assessment had been undertaken in 2002-2003 and historical data were available from: Biiso (GPS co-ordinates: 01° 45.516 N, 031° 25.236 E), Walakuba (GPS 01° 50.323 N 031° 22.740 E) and Runga (GPS 01° 43.828 N 031° 18.603 E) (26, 27). Two additional schools were selected Bugoigo (01° 54.004 N 031° 24.750 E) and Busingiro (GPS 01° 44.090 N 031° 26.855 E) on the basis that the former school was sited very close to the shoreline while the latter school was furthest away from lake (~25 kilometres). Busingiro was accessible on a daily basis from Bugoigo, where the field team was encamped, and intestinal schistosomiasis was presumed to be minimal there allowing associations between diseases in the putative absence of *Schistosoma mansoni* to be assessed (see Figure 1).

Examination of children

In liason with the headmaster and class teachers, a pre-target enrollment of 60 children per school, of balanced gender, randomly recuited from classes primary 1 to 3, was performed. A finger-prick blood was collected from each child and tested for malaria by RDT (SD BIOLINE Malaria Ag P.f/Pan test, SD Diagnostics, Korea) and haemoglobin levels by HemoCue® portable hemoglobin photometer (HemoCue, CA 92630, USA). Children were considered anaemic if haemoglobin levels were below 115 g/L[28].

Examination of stool specimens

Each child was requested to provide a stool sample on two consecutive days. All stool samples received on the first day were tested for faecal occult blood (FOB) (Mission Test, Acon Laboratories, SanDiego, CA). In Bugoigo and Runga schools, all children were tested with Quik-Chek tests for *Giardia* and *Cryptosporidium* (GIARDIA/CRYPTOSPORIDIUM QUIK-CHEK, Alere, UK). The Quik-Chek RDT is typically used to measure clinically relevant giardiasis with a level of detection of 6000 cysts/ml of faeces. Owing to a limited supply of 100 Quick-Check tests alone, only children from these two schools were tested while a more general detection of giardiasis was undertaken by recourse to real-time PCR assays. Duplicate faecal smears were prepared from each stool sample from consecutive days using Kato-Katz thick smear technique (41.7 mg) and examined by compound light microscopy

and helminth egg tallies were recorded according to eggs per gram of stool (light 1-99, medium 100-399, heavy 400+). A later 10% re-read of slides was performed as quality control. After filtration through a metal mesh (212µm pore size), small faecal pellet-sized sample from each child was preserved in absolute ethanol for later DNA extraction and real-time PCR analysis.

Real-time PCR analysis

After transfer to the UK and each sample was spiked with Phocine Herpes Virus to act as an internal control for extraction and performance of real-time PCR assays. Genomic DNA was extracted and detection of *Giardia* DNA was performed using the Taqman® assay following primer, probes and protocols of Verweij *et al.*[29]. Assays were performed in a Chromo-4 with Opticon monitor™ Version 3.1. (Biorad, UK) and levels of infection categorised according to Ct values: no infection (Ct $\geq$40), light (30$\leq$Ct$\leq$39), moderate (20$\leq$Ct$\leq$29), and heavy (Ct$\leq$19) intensity infections[29].

Statistical analysis

All data collected in the field were recorded on pro-forma data sheets then tabulated in Microsoft Excel with double entry cross-check. Analysis was performed with R statistical package v 2·10·1 (The R Foundation for Statistical Computing, Vienna, Austria) and SPSS software (v 24.0, SPSS Inc., IBM, USA). For percentage values, 95% confidence intervals (CI_{95}) were estimated using the exact method (30). With an extensive burden of giardiasis revealed by real-time PCR, greater focus was placed on exploring association of heavy infections with *Giardia* inclusive of: gender, age, anaemia and infection intensity of other parasitic diseases. A univariate logistic regression analysis was performed to study the association between infection status and the risk factors under consideration. The level of significance of $p < 0.05$ was set for multivariable analysis; the model was interpreted using adjusted ORs and 95% CIs. The prevalence of intestinal schistosomiasis at Runga, Walakuba and Biiso between 2002 and 2015 was compared by a 1-tailed Z-test [30].

Ethical approval

The Ugandan Council for Science and Technology and the Liverpool School of Tropical Medicine granted approval for this study. Each child was examined by the project nurse (AA) and praziquantel (40 mg/Kg, Merck, Germany) and albendazole (400 mg, GSK, UK) treatment was administered to all participants irrespective of their infection status. Children with a positive malaria RDT were each offered a take-home, 3-day course of coartem (Lonart®, Cipla, India) with the first and third administrations directly overseen by the project nurse.

Results

General characteristics

A total of 274 children were originally recruited, however, owing to instances of failure to produce stool, a final data set was composed of 255 children, mean age of 6.8 years (95% CI=6.6, 6.9) with a balanced male (49.4%) to female (50.5%) ratio.

<insert Table 1 near here please>

Prevalence of disease

Table 1 illustrates the prevalence of infection across the five schools, egg-patent intestinal schistosomiasis of 46.5%, giardiasis and malaria by RDT of 41.6% and 56.2%, respectively. The prevalence of egg-patent intestinal schistosomiasis at Walakuba and Runga was 82.0% and 86.8%, with the majority of children having heavy intensity infections. Overall, prevalence of *Giardia* by RDT was 41.6% but by real-time PCR increased to 87.9% which was graded into light (27.6%), medium (40.8%) and heavy (19.5%) categories upon comparison with Ct values. Just over half of the children were positive for malaria by RDT, with triple co-infection, in just under a fifth of the population. A quarter of children were judged anaemic and FOB was found in 11.0% of stool samples.

<insert Table 2 near here please>

Associations with giardiasis

Factors potentially associated with the presence of heavy *Giardia* infection as ascertained by real-time PCR were analysed are shown in Table 2. The prevalence of heavy *Giardia* infection was strongly associated with Runga school [OR, 5.7 (95% CI, 2.18–17.0)] while both age and gender had no a noticeable impact. The positive association [OR, 2.3 (95%CI, 0.89,5.1)] with faecal occult blood (FOB) was marginal but all egg-patent intestinal schistosomiasis infection intensities were positively associated some 3 to 4 fold. There was a significant association between anaemic children and heavy giardiasis [OR, 2.2 (95%CI, 1.41,4.3)], Figure 2.

<insert Figure 2 near here please>

Intestinal schistosomiasis through time

In 2002, the prevalence and the arithmetic mean intensity of children with intestinal schistostosomiasis at Runga, Walakuba and Biiso in 2002 versus 2015 was 95.2% (631 epg) *v* 86.7% (1723 epg), 90.4%

(1026 epg) *v* 80.3% (814 epg) and 17.2% (52 epg) *v* 19.6% 198 (epg) respectively. The declines (approximately 10%) in prevalence at Runga and Walakuba were statistically significant (p<0.05) while the small incline at Biiso was not (p>0.3).

Discussion

In this survey across 5 primary schools, an extensive burden of parasitic diseases, inclusive of co-infections, has been revealed, see Table 1. Triple infections of intestinal schistosomaisis, giardiasis and malaria affected just under a fifth of all sampled children. Although all were sufficiently fit to attend school, more general markers of ill-health, such as anaemia (25.7%) and faecal occult blood (11.0%), revealed an underlying morbidity, as previously seen in this area[31, 32]. Along the lakeshore, access to tap water is still very poor which no doubt helps both intestinal schistosomiasis and giardiasis flourish[17, 33-35]. Moreover, a concurrent malacological survey of the shoreline was undertaken and found numerous snails shedding schistosome cercariae[36], as several times before[5,37]. Clearly, there is a pressing need to establish and sustain safe water sources for both drinking and washing on the immediate shoreline as a foundation step towards promoting well-being[38].

The burden of intestinal schistosomiasis appears greatest at Runga and Walakuba schools, as it once was upon comparison with other locations along this lake[26,27]. Despite best efforts of control with school-based delivery by MDA of praziquantel, the resultant declines in egg-patent prevalence (~10%) were small although statistically significant. Whilst this is movement in the right direction, rather worryingly, there were large numbers of children with heavy intensity infections, Table 1. Looking to the future, a more aggressive treatment strategy is needed gain and sustain control, such as exploring biannual treatment cycles in both children and adults, as well as, tackling the burden of infection in preschool-aged children[36, 39].

The pervasive nature of giardiasis in this lakeshore setting is of concern and warrants greater attention in future. Use of the Quik-Chek RDT revealed that, whilst no cryptosporidiosis was encountered, a considerable number of children at Runga and Bugoigo were patently infected with clinically relevant *Giardia.* This was later confirmed by real-time PCR which, by having greater diagnostic sensitivity[29, 40-42], found a larger proportion of children with some evidence of infection. This points towards very high levels of exposure perhaps by the daily ingestion of cysts from lake water from unsafe water sources.

Whilst there was a strong association of heavy *Giardia* infection and egg-patent intestinal schistosomiasis its precise aetiology is yet to be determined. It is reasonable to speculate that egg-

induced lesions, which perforate and immunomodulate the mucosal surface[31], likely alter the bowel environment to become more conducive for colonisation of ingested *Giardia* cysts. Similarly, while it is difficult to disentangle precisely the relative role each disease is playing in the current context of intestinal morbidity, there was a clear association of heavy giardiasis with anaemia [OR, 2.2 (95%CI, 1.41,4.39)]. Amongst other causual factors, this could be due to underlying nutritional deficiencies, mediated by general intestinal inflammation, inclusive of chronic blood loss by faecal occult blood[9, 32]. Nevertheless, if anaemia is to be tackled effectively here, from a public health perspective it is important to tackle all three diseases as best possible simultaneously.

Figure legends

Figure 1. Inset: sketch map of Uganda and study area denoted A. Outline map of the 5 schools inspected. The primary schools of Biiso and Busingiro are located on top of the escarpment whereas Bugoigo, Walakuba and Runga on the lakeshore plain. Once part of Masindi District, the schools are now located within Buliisa District after administrative areas were revised. The northern village of Packwack was where the national control programme against bilharzia and intestinal worms was launched in 2003.
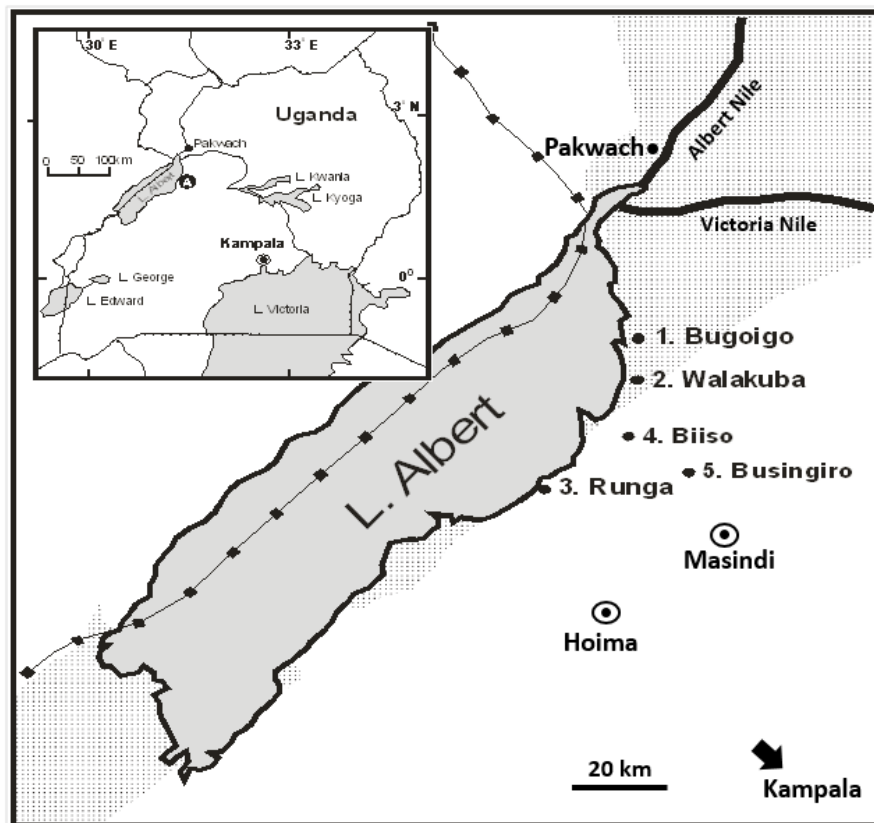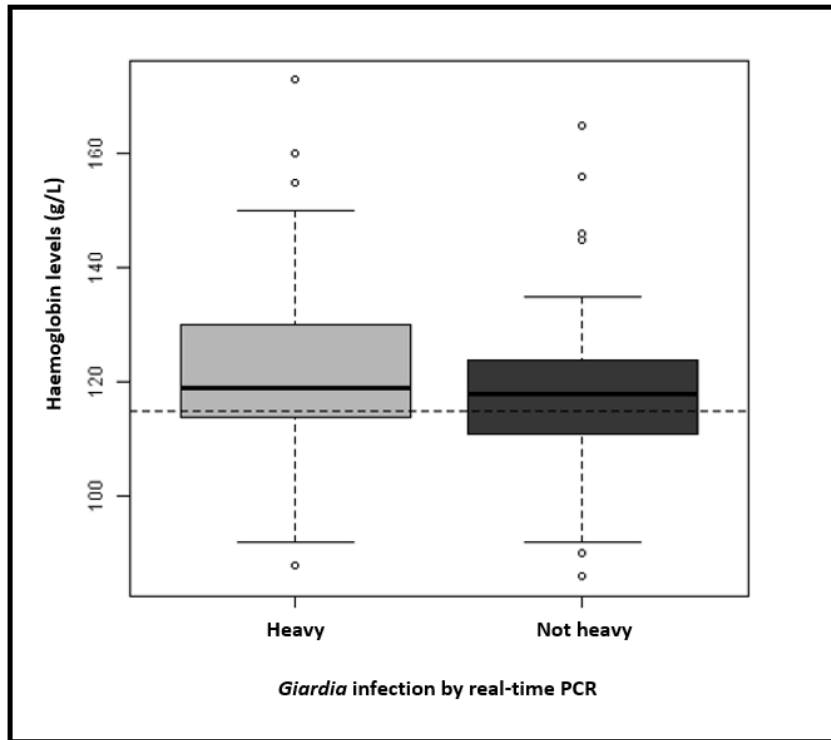
Figure 1

Figure 2. Box plot of haemoglobin levels and heavy infection status with *Giardia* as measured by real-time PCR.

Figure 2

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Table 1. Prevalence of infection by school and across the total of 254 children aged between 5 and 10 years of age.

School Locations		Bugoigo (*n*=60) %	Walukuba (*n*=56) %	Runga (*n*=54) %	Biso (*n*=51) %	Busingiro (*n*=50) %	Total (*n*=271) %
Prevalence							
S. mansoni (Kato-Katz)							
	Negative	63.3 (38/60)	18.0 (9/50)	13.2 (7/53)	80.3 (41/51)	92.0 (46/50)	53.4 (141/264)
	Light	26.6 (16/60)	10.0 (5/50)	13.2 (7/53)	5.8 (3/51)	2.0 (1/50)	12.1 (32/264)
	Moderate	5.0 (3/60)	26.0 (13/50)	11.3 (6/53)	9.8 (5/51)	4.0 (2/50)	10.9 (29/264)
	Heavy	5.0 (3/60)	46.0 (23/50)	62.2 (33/53)	3.9 (2/51)	2.0 (1/50)	23.4 (62/264)
Giardia (QUIK CHEK[a])							
	Negative	63.0 (29/46)	ND	54.0 (27/50)	ND	ND	58.3 (56/96)
	Positive	36.9 (17/46)	ND	46.0 (23/50)	ND	ND	41.6 (40/96)
Giardia (TaqMan®)							
	Negative	5.4 (3/55)	12.0 (6/50)	5.8 (3/51)	16.0 (8/50)	27.0 (13/48)	12.9 (33/254)
	Light	47.2 (26/55)	12.0 (6/50)	1.9 (1/51)	28.0 (14/50)	45.8 (22/48)	27.1 (69/254)
	Moderate	36.3 (20/55)	54.0 (27/50)	50.9 (26/51)	38.0 (19/50)	22.9 (11/48)	40.5 (103/254)
	Heavy	10.9 (6/55)	22.0 (11/50)	41.1 (21/51)	18.0 (9/50)	4.1 (2/48)	19.2 (49/254)
Malaria (RDTs)							
	Negative	48.2 (28/58)	64.8 (35/54)	38.8 (21/54)	31.3 (16/51)	34.0 (17/50)	43.8 (117/267)
	Positive	51.7 (30/58)	35.1 (19/54)	61.1 (33/54)	68.6 (35/51)	66.0 (33/50)	56.1 (150/267)
Co-infection*							
	Negative	85.0 (51/60)	80.0 (45/56)	51.8 (28/54)	86.2 (44/51)	98.0 (49/50)	80.0 (217/271)
	Positive	15.0 (9/60)	19.6 (11/56)	48.1 (26/54)	13.7 (7/51)	2.0 (1/50)	19.9 (54/271)
Anaemia							
	(<115Hb)						
		36.6 (22/60)	33.9 (19/56)	44.4 (24/54)	5.8 (3/51)	ND	30.7 (68/221)

[a] QUIK CHEK *GIARDIA/CRYPTOSPORIDIUM CHEK®* test detects *Giardia* cyst antigens and *Cryptosporidium* oocyst antigens
* Co-infection of all three parasitic infections (GIA-Giardia, SCH-Schistosomiasis and MAL-Malaria)

Table 2. Adjusted risk factors associated with heavy *Giardia* infection by real-time PCR

Heavy *Giardia* infection*	Positive (n=49)	Negative (n=205)	OR	[95% CI]
	%	%		
School				
Bugoigo	10.9	89.1	-	-
Runga	41.2	58.8	5.7	[2.2, 17.0]
Walukuba	22.0	78.0	2.3	[0.8, 7.2]
Biiso	18.0	82.0	1.8	[0.6, 5.7]
Busingiro	4.2	95.8	0.4	[0.1, 1.6]
Age group				
5-6	19.3	80.7	-	-
7-8	20.8	79.2	1.1	[0.56, 2.1]
9-10	15.4	84.6	0.7	[0.26, 1.9]
Gender				
Female	15.9	84.0	-	-
Male	23.0	77.0	1.5	[0.84, 2.9]
Faecal occult blood (FOB)				
Negative	17.7	82.3	-	-
Positive	32.1	67.8	2.2	[0.89, 5.1]
Kato-Katz intensity				
Negative	11.2	88.8	-	-
Light	33.3	66.6	3.9	[1.54, 10.0]
Moderate	26.9	73.0	2.9	[1.01, 7.9]
Heavy	28.1	71.9	3.1	[1.41, 6.8]
Anaemia+				
Negative	16.0	84.0	-	-
Positive	30.0	70.0	2.2	[1.14, 4.3]

* Heavy *Giardia* infection categorised by real-time PCR (Ct value ≤19) versus negative, light and moderate infection
+ Hb less than 115 g/L