Schistosoma mansoni infection as a Predictor of Low Aerobic Capacity in Ugandan Children.

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ABSTRACT

Using the 20-meter shuttle run test (20mSRT) as a morbidity metric, we assessed if *Schistosoma mansoni* infection was associated with decreased aerobic capacity in Ugandan children across a range of altitudes, either at low (~600m) or high (~1000m) altitudes. A total of 305 children were recruited from six schools within the Buliisa district, Lake Albert, Uganda. A subset (n=96) of these had been previously assessed and treated for schistosomiasis +/- malaria two weeks prior. Fitness scores on the 20mSRT were translated into VO2max using a standardised equation. Unadjusted and multivariable-adjusted analyses were performed using VO2max as the primary outcome.

Analysis of fitness scores from 304 children, inclusive of the subset follow-up cohort, revealed a median VO2max of 45.4 mL kg^{-1} min^{-1} (IQR 42.9 - 48.0 mL kg^{-1} min^{-1}). Children residing at high altitudes demonstrated increased aerobic capacities (46.3 vs 44.8 $mLkg^{-1}$ min^{-1}, P = 0.031). The prevalence of stunting, wasting, *S. mansoni* egg patent infection, malaria, giardiasis, anemia and fecal occult blood were 36.7%, 16.1%, 44.3%, 65.2%, 21.4%, 50.6%, and 41.2%, respectively. Median VO2max was elevated in those previously treated, compared with those newly recruited (46.3 mL kg^{-1} min^{-1} vs 44 mL kg^{-1} min^{-1}, P < 0.001). Multivariable-adjusted analysis revealed a strong negative association between *S. mansoni* egg patent infection and VO2max at low altitude (beta coefficient -3.96, 95% CI -6.56, -1.37, P = 0.004). This is the first study to document a negative association between *S. mansoni* infection and aerobic capacity at low altitudes using the 20mSRT.

INTRODUCTION

Intestinal schistosomiasis, as caused by infection with *Schistosoma mansoni*, is an important contributor towards chronic morbidity in African children as measured by various methodologies.[1, 2, 3, 4, 5, 6, 7, 8, 9, 10] However, its impact upon diminished exercise tolerance is not well explored. By contrast, the functional consequence of *Schistosoma haematobium*-associated anemia has been assessed by the 20m shuttle run test (20mSRT) and validated to provide an accurate correlate of aerobic capacity, the VO2max (measured in mL kg^{-1} min^{-1}).[11]

The pathophysiological pathway underlying decreased physical fitness in children with either form of schistosomiasis is complex, hinging upon immuno-pathological lesions and generalised inflammatory responses.[12, 13, 14] Anemia is a cause of decreased oxygen carrying capacity and has been associated with both heavy and light *Schistosoma* infections in childhood.[1, 2, 4, 9, 15, 16, 17, 18, 19, 20, 21, 22] The predominant underlying mechanism seems to be anemia of inflammation, involving pro-inflammatory cytokines including TNF-alpha and Interleukin-6.[23, 24, 25] Other mechanisms include ulcerative passage of eggs through the intestinal wall causing extracorporeal blood loss, splenic sequestration and autoimmune hemolysis.[17, 26, 27]

Lake Albert in Western Uganda provides the optimum habitat for *Biomphalaria* snails, the intermediate host for *S. mansoni,* making it a hub for *S. mansoni* transmission. Previous studies have identified egg patent *S. mansoni* infection prevalences of up to 82% amongst children aged 5-10 years living in the region.[28] Since 2004, the control of schistosomiasis-related morbidity in Uganda has been centered upon the targeted, periodic distribution of praziquantel therapy to school-aged children aged over 4 years and selected ‘at risk’ adult populations,.[29] Proxy markers of morbidity have since been evaluated, including faecal occult blood, anemia, and faecal calprotectin testing, quality of life questionnaires, biometry, clinical palpation and measurement, portable ultrasonography and fitness tests.[12, 30, 31]

Previous studies investigating the relationship of *S. mansoni* infection with physical fitness as measured by the 20mSRT have been inconclusive, limited by small sample sizes,

and have not compared or incorporated altitudinal effects.[7, 8] Altitude acclimatization with an associated increase in red blood cell volume may occur at altitudes as low as ~1000m.[32] This study aimed to determine whether *S. mansoni* infection was associated with decreased aerobic capacity in Ugandan children living at low (~600m) or high (~1000m) altitudes. It was hypothesised that *S. mansoni* infection would correlate with decreased aerobic capacity in Ugandan children and that this association would be less pronounced in children living at high altitude.

METHODS

Ethics Statement & Eligibility Criteria

Ethical approval was obtained from the London School of Hygiene & Tropical Medicine (LSHTM) Ethics Committee (LSHTM number 12034), Liverpool School of Tropical Medicine (LSTM) Masters Review Panel (M09-17), and the Vector Control Division, Ministry of Health, Uganda (VCDREC-082). Children were considered eligible for enrolment if they were aged 7-15 years, medically fit, had resided in a *S. mansoni*-endemic area for at least two years, and could provide child assent.

Study Setting & Population

This study was carried out in six *S. mansoni*-endemic schools within the Buliisa district of Lake Albert in Western Uganda: Biiso (latitude 41.4199, longitude 1.7606), Busingiro (latitude 31.4475, longitude 1.7354), Bugoigo Islamic (latitude 31.4122, longitude 1.9000), Bugoigo Primary (latitude 31.4167, longitude 1.9089), Nyamukuta (latitude 31.4000, longitude 1.8683), and Walukuba (latitude 31.3831, longitude 1.8425). Epidemiological data previously collected within this region provided a useful foundation and thereby influenced the selection of schools for our study.[28, 33, 34] Buliisa is bordered by Nebbi (north), Masindi (east), Hoima (south), and the Democratic Republic of Congo (west). Biiso and Busingiro lay at altitudes of 1004m and 1062m respectively. The remainder of the schools lay adjacent to Lake Albert with an altitude of 616m. The geographical proximities of the schools to the lake

shoreline are <1km for Bugoigo and Walukuba, and approximately 9km and 14km for Biiso, and Busingiro, respectively. Egg patent *S. mansoni* infection prevalences among children aged 5-10 years in the villages of Bugoigo, Walukuba, Biiso, and Busingiro have been previously identified to be 36.7%, 82.0%, 19.7% and 8.0% respectively.[28] No transmission of *Schistosoma haematobium* has been documented on parasitological surveys in the field of study.[28, 35]

The study involved 305 schoolchildren aged 7 to 15 years. Of the 305 schoolchildren, a total of 96 children from Biiso, Busingiro, and Bugoigo Islamic schools were followed up from two weeks prior. The team had performed an identical armoury of parasitological diagnostic tests, 20m-shuttle run testing, and had administered praziquantel, albendazole and, if malaria-positive, artemether-lumefantrine therapy.

The study team was comprised of members from LSHTM, LSTM and the Vector Control Division, Ministry of Health, Uganda. Subjects were enrolled following random selection from the P2 to P6 class registers of each school over a 9-day period in June 2017. For each village, community mobilisers assisted with community sensitisation prior to data collection. Three of the six schools sampled had been recently sensitised by the preceding LSTM team. Head teacher consent and written child assent were obtained. The information sheets were translated into the local Alur dialect and distributed. The rationale for the study was explained using a local translator.

Forty to sixty children were sampled per day. The principal investigator, a qualified medical practitioner, assessed each child's general health prior to study participation. Each child was assigned a unique study identification number which was written on a wristband to be worn by the child during testing. They were asked a brief series of questions related to their demographics, medical background, and previous praziquantel administration using LSHTM Open Data Kit software on a tablet device (http://opendatakit.lshtm.ac.uk/odk/). The frequency of mass drug administration with praziquantel at each school was recorded following head teacher questioning.

Anthropometric Assessment

Assessment for stunting was performed using validated charts based on height-for-age (HFA) Z-score: 'stunted' was defined as ≤ 2 to > 3 S.D. below the mean, and 'severely stunted' was defined as ≤ 3 S.D. below the mean.[36] Calibrated measurements of weight and height were obtained by trained field workers using standardised scales and a standardised stadiometer, respectively. The height values obtained were for only a subset of the new participants and were converted to HFA Z-scores according to a standardised reference.[37] Body mass index (BMI) was calculated for each child for whom height and weight were obtained and converted to BMI-for-age (BFA) Z-scores according to a standardised reference.[37] Results were recorded on the standardised data collection form.

20-meter Shuttle Run Test

Each participant undertook a 20-meter Shuttle Run Test (20mSRT).[11] The test was performed in the school grounds on a clear and level playing field during school hours to maximise convenience and minimise disruption to the school day program. Six to twelve children were tested at any one time. For every four children, one observer was ascribed to ensure adequate monitoring of their performance. Careful instructions were given using a local translator and a brief demonstration of the test was performed by the principal investigator prior to testing. All children were kept well hydrated, and water and sugary snacks were made available.

Materials used included two pre-measured 20-meter ropes, markers, a microphone, a portable speaker, and a tablet device with a relevant application for the 20mSRT (Bleep Fitness Test, Aspectica Ltd). Coloured bibs were worn by the study participants for ease of identification. Each fitness score was then translated into VO2max (mL kg^{-1} min^{-1}) using a validated reference.[11]

Field-Based Parasitological Diagnostic Testing & Treatment

A single urine specimen was obtained from each child and tested for the presence of urine circulating cathodic antigen (urine-CCA; Rapid Medical Diagnostics, Pretoria, South Africa). Urine-CCA has the advantage of detecting light intensity infections which may be missed using the traditional Kato-Katz technique.[38] The test band reaction intensity was semi-quantitatively graded as negative (-), trace positive (tr), single positive (+), double positive (++), and triple positive (+++).

The presence of *S. mansoni* infection was determined by duplicate Kato-Katz thick fecal smears (each 41.7mg) prepared by trained field technicians in accordance with Katz et al.[39] Kato-Katz examination indicates infection with mature, egg-shedding worms. The technique was employed to provide further information into the level of egg excretion, which is likely a proxy marker of bowel morbidity in addition to infection. Microscopy with a natural light source was used for in-field interpretation on the day of testing. *S. mansoni* egg counts and the number of eggs per gram (epg) of stool based upon the mean of the two specimens were documented. Each fecal specimen was tested for the presence of *Giardia duodenalis* infection using the Giardia/Cryptosporidium Quik Chek test (TECHLAB®, Inc.), and human hemoglobin and transferrin using the Transferrin/FOB Combo Rapid Test Cassette (Hangzhou AllTest Biotech co. Ltd.).

Capillary blood sampling was used to determine the total hemoglobin level (HemoCue 201+, Angelholm, Sweden) and screen for malaria infection (Standard Diagnostics BIOLINE Malaria Ag P.f./Pan, Alere, TM.). Follow-up children were not screened for malaria, given the likelihood of persistent antigenemia following recent testing.

Of the new participants, those who tested positive for schistosomiasis on urine-CCA and/or malaria were administered standardised therapy for schistosomiasis and/or malaria, respectively in keeping with national guidelines. All participants were administered albendazole therapy. Of the follow-up participants, only those who tested positive for urine-CCA were administered praziquantel therapy, given their recent treatment by the preceding

team. No children were identified as being unwell or required referral to the local Level 2 health care facility.

Data Management & Statistical Analysis

All data collected was de-identified, entered into Microsoft Excel (Version 16.13.1) or LSHTM Open Data Kit software, and stored on an encrypted USB device. Data analysis was performed using STATA 14.2 on those for whom 20mSRT data was obtained. Separate analyses of the entire cohort and of the follow-up participants were conducted. Descriptive analyses with stratifications by school and altitude (low: ~600m, high: ~1000m) were performed. Wilcoxan Rank Sum, Kruskall Wallis, Spearman's correlation, Chi-squared tests, paired T test, and ANOVA were used to identify differences between schools and altitudes. Linear regression was employed to determine the unadjusted associations between independent covariates and the dependent variable, VO2max (continuous). Independent covariates of interest included egg patent *S. mansoni* infection (dichotomous), malaria infection (dichotomous), fecal occult blood (ordinal), anemia (dichotomous), stunting based on validated charts (dichotomous) and HFA Z-score ≤ 2 S.D. below the mean (dichotomous), and wasting defined by BFA Z-score ≤ 2 S.D. below the mean (dichotomous).[29, 36, 37] Anemia was defined according to standardised cut-offs for age: < 11.5g dL^{-1} (5 - 11y), < 12.0g dL^{-1} (12 - 14y) and adjusted for altitude using the equation 'Hb (g dL^{-1}) – 0.2g dL^{-1}' for an altitude approximating 1000m.[40] Logistic regression was used to examine the unadjusted associations between the aforementioned covariates and dependent variables of fecal occult blood, anemia and stunting (by validated charts). Multivariable-adjusted linear regression was performed using VO2max as the dependent variable and multivariable-adjusted logistic regression analyses were undertaken using anemia, fecal occult blood and stunting each as the dependent variable. Model selection was performed using a stepwise procedure, followed by Akaike's Information Criterion (AIC) as the model selection criterion. The model which minimised the AIC was selected. All analyses were stratified by gender and altitude.

RESULTS

Participation

Six schools within the Buliisa district were consecutively sampled: Biiso (n = 48), Busingiro (n = 46), Bugoigo Islamic (n = 48), Bugoigo Primary (n = 61), Nyamukuta (n = 61), and Walukuba (n = 40). Of the 305 children who participated, 304 completed the 20mSRT and were included within the final analysis. Only one child did not complete the 20mSRT due to a minor foot injury. Five children did not provide fecal samples and seven children did not provide urine for testing. Malaria, capillary hemoglobin, and fecal occult blood were limited by resource availability given the diversion of their use by the local clinic. Of 104 children sampled at baseline, 96 children completed the 20mSRT at follow-up (92.3%) and were included within the final analysis. The main reason for lack of follow-up was absence from school on the day of testing (Table 1). The remaining 208 children included within the final analysis were those newly recruited to the study.

Descriptive Analyses

The age, gender, and parasitology distributions were similar between schools, with the exception of malaria (P = 0.003, Table 1, Figure 1). The prevalence of *S. mansoni* may have been confounded by the variable distances of the schools from the lake. The prevalence of *P. falciparum* malaria was significantly higher at 1000m compared with 600m altitudes (P = 0.015, Table 2, Figure 1). Prevalence of *S. mansoni* by urine-CCA was highest (80.5%), followed by P. falciparum (65.2%), *S. mansoni* by egg patency (44.3%), and *Giardia duodenalis* infection (21.3%). All of the schools studied had received mass drug administration with praziquantel within the preceding twelve months. Overall, 34.5% of children were classified as anemic (n = 86/249) and 41.2% of children had fecal occult blood in the stool. There were no differences in prevalence of anemia or fecal occult blood and median hemoglobin between schools (Table 1).

Anthropometrics & Nutritional Status

Acute and chronic malnutrition were identified within all schools. Overall, 36.7% of children were stunted according to a height-for-age Z-score ≤ 2 S.D. below the mean (n = 79/215) and 16.7% were stunted according to validated charts (n = 49/293). Of the latter, 1% were severely stunted based on a height-for-age score ≤3 S.D. below the mean (n = 3/293, Table 1).

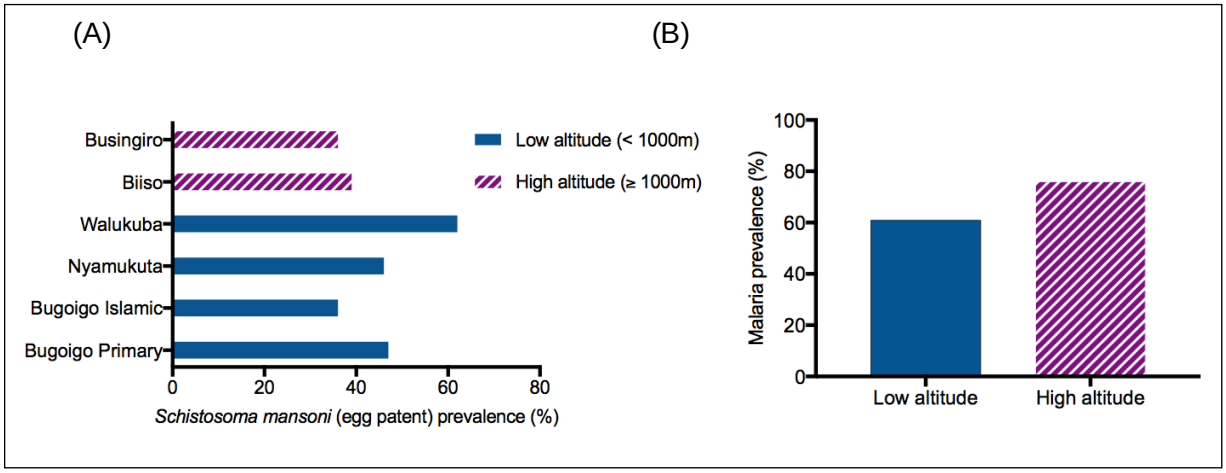


Figure 1: (A) Prevalence of egg patent S. mansoni *infection according to school and altitude. (B) Prevalence of malaria infection according to altitude.*

Performance in the 20mSRT

Careful instructions and a test demonstration were provided prior to shuttle run testing. Overall, the 20mSRT was well understood with very few false starts and trips observed. If either occurred, a rest period was provided and testing was recommenced. Overall, median VO2max was 45.4 mL kg^{-1} min^{-1} (IQR 42.9 – 48 mL kg^{-1} min^{-1}) with higher values obtained by males compared with females (47.5 mL kg^{-1} min^{-1} vs 43.9 mL kg^{-1} min^{-1}, $P < 0.001$, Table 1). Those children living at high altitude demonstrated a higher median VO2max compared with those residing at low altitude (46.3 mL kg^{-1} min^{-1} vs 44.8 mL kg^{-1} min^{-1}, $P = 0.031$, S1 Table).

When compared with a Canadian cohort, males demonstrated lower VO2max for all ages.[11] Females demonstrated a lower VO2max up until the age of 12 years, after which an upward trend was observed. Figure 2 illustrates the differences between the Canadian and study cohorts by age and gender, and incorporates data from a Kenyan cohort for comparison.[2] Outliers at the ages of 7 years (n = 3) and 15 years (n = 3) were excluded (S3 Table).

TABLE 1: Demographic, Hematologic, Immunochemical, Parasitological & 20m-Shuttle Run Test Findings in Villages of the Buliisa District.

Parameter	Total (n = 304)	Biiso (n = 48)	Bugoigo Islamic (n = 48)	Bugoigo Primary (n = 61)	Busingiro (n = 46)	Nyamukuta (n = 61)	Walukuba (n = 40)	P Value*
DEMOGRAPHY								
Median age in years (interquartile range)	11 (10-12.5)	11.5 (10-12.5)	11 (9-12)	11 (10-13)	11 (9-12)	10 (10-12)	12 (10-13)	0.091
% Female (n)	49.7 (151/304)	50.0 (24/48)	50.0 (24/48)	49.2 (30/61)	47.8 (22/46)	50.8 (31/61)	50.0 (20/40)	1.000
ANTHROPOMETRY								
Median height in centimeters (interquartile range)	134 (128.5-140.5)	130.5 (126.4-137.5)	133.5 (127.7-142.2)	135.2 (127.6-141.7)	134 (129.6-139)	136 (131.0-139.5)	140.5 (131.1-145.2)	0.186
% Stunted by HFA Z-score (n)**	36.7 (79/215)	41.2 (14/34)	51.4 (19/37)	43.2 (19/44)	17.2 (5/29)	29.4 (15/51)	35.0 (7/20)	0.064
% Stunted by validated charts (n)***	16.7 (49/293)	20.8 (10/48)	20.5 (9/44)	17.2 (10/58)	13.3 (6/45)	13.8 (8/58)	15.0 (6/40)	0.333
% Stunted (n)	15.7 (46/293)	20.8 (10/48)	20.5 (9/44)	15.5 (9/58)	13.3 (6/45)	13.8 (8/58)	10.0 (4/40)	
% Severely stunted (n)	1.0 (3/293)	0.0 (0/48)	0.0 (0/44)	1.7 (1/58)	0.0 (0/45)	0.0 (0/58)	5.0 (2/40)	
Median body mass index (interquartile range)	16.1 (14.8-17.3)	14.8 (13.2-16.3)	N/A	16.0 (14.7-17.2)	N/A	16.2 (15.2-17.5)	N/A	0.652
% Wasted (n)****	11.8 (8/68)	0.0 (0/2)	N/A	11.1 (4/36)	N/A	13.3 (4/30)	N/A	0.838
HAEMATOLOGY								
Median hemoglobin in g dL^{-1} (interquartile range)#	12.0 (11.4-12.7)	12.0 (11.4-12.6)	12.2 (11.4-12.8)	11.8 (11.2-12.4)	12.1 (11.3-12.8)	12.3 (11.5-13)	12 (11.5-12.5)	0.274
% Anemic (n)*****#	34.5 (86/249)	41.0 (16/39)	33.3 (12/36)	44 (22/50)	35.1 (13/37)	21.2 (11/52)	34.3 (12/35)	0.232
IMMUNOCHEMICAL								
% Fecal occult blood test positive	41.2 (61/148)	46.9 (15/32)	44.0 (11/25)	32.0 (8/25)	27.3 (6/22)	48.2 (13/27)	47.1 (8/17)	0.489
PARASITOLOGY								
Schistosomiasis								
% *S. mansoni* infection by urine-CCA (n)~	80.5 (231/287)	82.6 (38/46)	75.0 (33/44)	87.7 (50/57)	79.1 (34/43)	79.0 (45/57)	77.5 (31/40)	0.663
% Egg patent *S. mansoni* infection (n)~~	44.3 (127/288)	39.1 (18/46)	36.4 (16/44)	46.6 (27/58)	35.7 (15/42)	45.8 (27/59)	61.5 (24/39)	0.163

Mean epg (95% confidence interval))~~	449.5 (330.1-568.9)	215.2 (108.1)	568.6 (156.2-981.1)	430.4 (170.9-690.0)	505.8 (202.6-809.1)	505.8 (202.6-809.1)	656.3 (284.5-1028.1)	0.241
S. mansoni intensity~~								
% Negative (n)	55.9 (161/288)	60.9 (28/46)	63.6 (28/44)	53.5 (31/58)	64.3 (27/42)	54.2 (32/59)	38.5 (15/39)	
% Light (n)	10.1 (29/288)	4.4 (2/46)	9.1 (4/44)	13.8 (8/58)	7.1 (3/42)	10.2 (6/59)	15.4 (6/39)	
% Medium (n)	11.8 (34/288)	13.0 (6/46)	6.8 (3/44)	8.6 (5/58)	14.3 (6/42)	13.6 (8/59)	15.4 (6/39)	
% Heavy (n)	22.2 (64/288)	21.7 (10/46)	20.5 (9/44)	24.1 (14/58)	14.3 (6/42)	22.0 (13/59)	30.8 (12/39)	
Malaria								
% Malaria (n)^	65.2 (122/187)	88.9 (24/27)	82.6 (19/23)	65.9 (29/44)	63.0 (17/27)	43.2 (16/37)	58.6 (17/29)	**0.003**
% P. falciparum (n)	65.2 (122/187)	88.4 (24/27)	82.6 (19/23)	65.9 (29/44)	63.0 (17/27)	43.2 (16/37)	58.6 (17/29)	**0.008**
% Mixed (n)	11.2 (21/187)	18.5 (5/27)	8.7 (2/23)	15.9 (7/44)	7.4 (2/27)	8.1 (3/37)	6.9 (2/29)	0.648
Giardiasis								
% Giardia duodenalis infection (n)^^	21.4 (63/294)	14.9 (7/47)	14.9 (7/47)	18.6 (11/59)	25 (11/44)	23.3 (14/60)	35.1 (13/37)	0.193
20m-SHUTTLE RUN TEST								
Median VO2max in mL kg^{-1} min^{-1} (interquartile range)	45.4 (42.9-48.0)	45.7 (43.9-47.9)	46.0 (43.6-48.9)	45.4 (43.0-47.5)	47.0 (42.9-49.5)	45.4 (43.8-47.5)	42.1 (40.8-45.0)	**< 0.001**
Males	47.5 (43.9-49.0)	47.5 (45.5-49.2)	48.4 (45.9-50.4)	46.3 (44.8-49)	48.0 (46.4-50.0)	47.25 (43.8-49.7)	43.2 (41.7-46.4)	**0.005**
Females	43.9 (41.5-46.3)	44.6 (42.9-45.7)	43.9 (41.8-46)	43.9 (41.5-47.0)	43.9 (42.9-47.5)	44.8 (42.9-46.3)	41.5 (39.9-43.8)	0.100

*Indicates significance of differences among the villages by Kruskal-Wallis or Chi-squared analysis, paired T test or ANOVA. Statistically significant differences (P ≤ 0.05) indicated in **bold**. **As defined by height-for-age Z-scores ≤ 2 S.D. below mean.[37] ***According to validated stunting charts based on height-for-age Z-score: 'stunted' (≤ 2 - > 3 S.D. below mean), 'severely stunted' (≤ 3 S.D. below mean).[36] ****As defined by BMI-for-age Z-scores ≤ 2 S.D. below mean.[37] *****As per standardised hemoglobin cut-offs for age: < 11.5 g/dL (5 - 11y), < 12.0 g/dL (12 - 14y). #Hemoglobin adjusted for altitude. [40] ~As per urine-cathodic circulating antigen testing. ~~As per dual Kato-Katz examination. Intensity defined by epg: 1 - 99 = light; 100 - 399 = medium, ≥ 400 = heavy.[29] ^As per malaria rapid diagnostic testing. ^^As per Giardia/Cryptosporidium Quik Chek test.

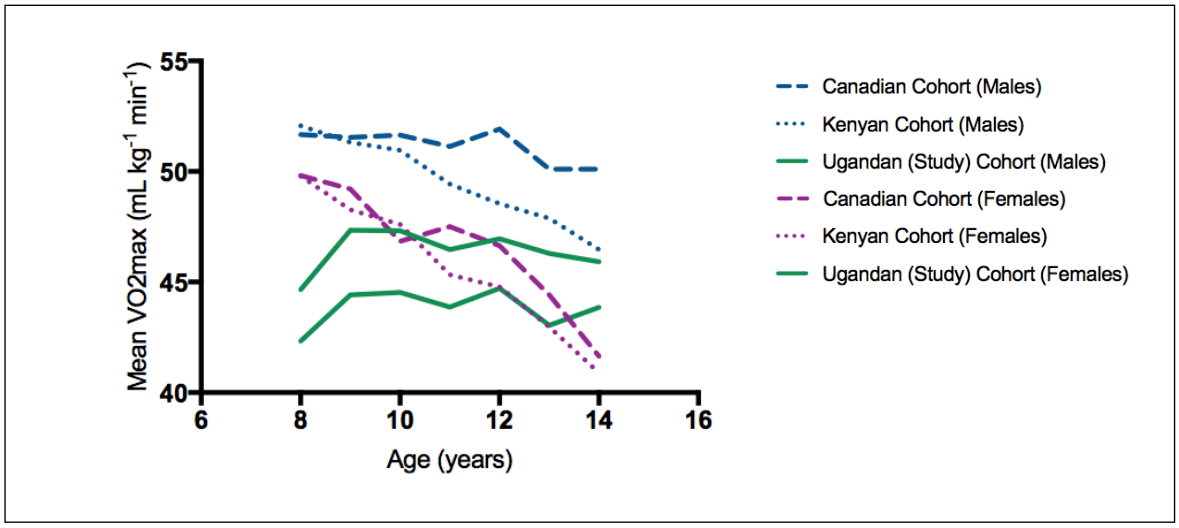


Figure 2. Comparison of mean VO2max between Ugandan (study), Kenyan & Canadian cohorts by gender & age (Canadian & Ugandan data sourced from Leger et al. & Bustinduy et al., respectively).[2, 11]

Associations between Infection, Nutritional Status, & Aerobic Capacity

Unadjusted and multivariable-adjusted analyses examining VO2max as an outcome were performed using linear regression. Covariates studied included *S. mansoni* egg patent infection, fecal occult blood, malaria and stunting (based on validated charts). The analyses were stratified by gender due to the differences in aerobic capacity between males and females (S3 Table), and by altitude for the purposes of this study. Model selection was performed using a stepwise procedure, followed by Akaike's Information Criterion (AIC) as the model selection criterion. The model with the lowest AIC was selected. Tables 2 and 3 and S4 Table summarize these findings.

On unadjusted analysis, *S. mansoni* egg patent infection was a negative predictor of VO2max (Coeff -1.28, 95% CI -2.20 – 0.36, P = 0.007). Increasing *S. mansoni* intensity of infection correlated with decreasing VO2max (Coeff -0.496 95% CI -0.862 - -0.132, P < 0.05). No other covariates demonstrated significant associations with VO2max. The

correlation between *S. mansoni* egg patent infection and VO2max remained when adjusted for the presence of fecal occult blood, malaria, stunting (based on validated charts), and anemia (Coeff -4.91, 95% CI -6.31 – 2.07, P < 0.001, Table 2). Similarly, for girls, *S. mansoni* egg patent infection was associated with VO2max on unadjusted (Coeff -1.91, 95% CI -3.12 - -0.70, P = 0.002) and multivariable-adjusted (Coeff -5.04, 95% CI -8.80 - -1.28, P = 0.011) analyses (S4 Table). For boys, no significant correlations with VO2max were identified. For schools residing at low altitudes, *S. mansoni* egg patent infection negatively correlated with VO2max on both unadjusted (Coeff -1.30, 95% CI -2.39 - -0.21, P = 0.02) and multivariable-adjusted (Coeff -3.96, 95% CI -6.56 - -1.368, P = 0.004) analyses. For schools residing at high altitude, malaria infection positively correlated with VO2max on both unadjusted (Coeff 2.83, 95% CI 0.49 – 5.17, P = 0.019) and multivariable-adjusted (Coeff 5.52, 95% CI 0.08 – 10.96, P = 0.047) analyses (Table 3).

Associations between Infection, Anemia, Fecal Occult Blood, & Nutritional Status

Logistic regression was used to explore the association between fecal occult blood, anemia and stunting with infection status, with each covariate being recorded as dichotomous variables. *S. mansoni* egg patent infection positively correlated with fecal occult blood (OR 0.04, 95% CI 4.01 – 20.37, P < 0.05). *S. mansoni* egg patent infection was positively associated with anemia on unadjusted analysis (OR 1.85, 95% CI 1.08 – 3.15, P = 0.02), as was fecal occult blood (OR 1.51, 95% CI 1.11 – 2.07, P = 0.01). Multivariable-adjusted analysis revealed fecal occult blood to be the only positive predictor of anemia (OR 1.96, 95% CI 1.11 – 3.43, P = 0.02, S6 Figure).

Logistic regression was also used to analyse stunting (based on validated charts) as an outcome. *S. mansoni* egg patent infection positively correlated with stunting (OR 2.49, 95% CI 1.30 - 4.77, P = 0.01) on unadjusted analysis, however this association did not remain when adjusted for the presence of fecal occult blood, malaria, and anemia (OR 0.75, 95% CI 0.17 – 3.39, P = 0.71, S5 Table, S6 Figure).

TABLE 2: Linear Regression Models with VO2max as the Outcome.

	Unadjusted Analysis				Multivariable-adjusted Analysis			
	Coefficient	95% CI		P Value	Coefficient	95% CI		P Value
S. mansoni egg patent infection*	**-1.279**	**-2.199**	**-0.360**	**0.007**	**-4.191**	**-6.312**	**-2.070**	**< 0.001**
Fecal occult blood	-1.181	-0.767	0.404	0.542	0.404	-0.533	1.342	0.392
Malaria^	0.142	-1.057	1.341	0.815	-0.811	-2.824	1.203	0.424
Stunting~	-0.534	-1.650	0.583	0.348	-0.615	-2.934	1.704	0.598
Anemia#	-0.650	-1.663	0.363	0.208	0.364	-1.595	2.323	0.711

Statistically significant differences (P ≤ 0.05) indicated in **bold**. *As per dual Kato-Katz examination. ^As per malaria rapid diagnostic testing. ~According to validated stunting charts based on height-for-age Z-score ≤ 2 S.D. below mean.[36] #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5 - 11y), < 12.0 g dL^{-1} (12 - 14y). Hemoglobin adjusted for altitude.[40] For multivariable-adjusted analysis: n = 68. P Value = 0.009. R-squared = 0.2142. Adjusted R-squared = 0.1508. Akaike's Information Criterion = 373.447.

TABLE 3: Linear Regression Models with VO2max as the Outcome, Stratified by Altitude.

	Unadjusted Analysis				Multivariable-adjusted Analysis			
	Coefficient	95% CI		P Value	Coefficient	95% CI		P Value
S. mansoni egg patent infection*								
Low altitude	**-1.299**	**-2.389**	**-0.208**	**0.020**	**-3.962**	**-6.556**	**-1.368**	**0.004**
High altitude	-0.971	-2.712	0.770	0.271	0.452	-5.102	6.007	0.866
Fecal occult blood								
Low altitude	-0.610	-1.349	0.128	0.104	-0.226	-1.362	0.911	0.690
High altitude	0.592	-0.333	1.518	0.205	0.694	-1.094	2.482	0.424
Malaria^								
Low altitude	-0.938	-2.320	0.444	0.182	-2.121	-4.390	0.148	0.066
High altitude	**2.832**	**0.494**	**5.170**	**0.019**	**5.524**	**0.084**	**10.964**	**0.047**
Stunting~								
Low altitude	-0.448	-1.749	0.853	0.498	-0.126	-2.715	2.463	0.922
High altitude	-0.719	-2.875	1.438	0.510	-0.842	-6.230	4.547	0.746
Anemia#								
Low altitude	-0.924	-2.145	0.297	0.137	0.891	-1.418	3.201	0.440
High altitude	-0.326	-2.076	1.424	0.711	-1.834	-5.384	1.717	0.291

Statistically significant differences (P ≤ 0.05) indicated in **bold**. *As per dual Kato-Katz examination. ^As per malaria rapid diagnostic testing. ~According to validated stunting charts based on height-for-age Z-score ≤ 2 S.D. below mean.[36] #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5 - 11y), < 12.0 g dL^{-1} (12 - 14y). Hemoglobin adjusted for altitude.[40] For multivariable-adjusted analysis: **Low altitude: n = 45. P Value = 0.022.**

R-squared = 0.277. Adjusted R-squared = 0.184. AIC = 246.900. High altitude: n = 23. P Value = 0.202. R-squared = 0.326. Adjusted R-squared = 0.128. Akaike's Information Criterion = 125.111.

Comparison between Baseline & Follow-up

The prevalence of egg patent *S. mansoni* infection was similar at baseline and follow-up (20.8% vs 25.0%, P = 0.053). Median hemoglobin was significantly higher at follow-up (10.7 g dL^{-1} vs 10.2 g dL^{-1}, P < 0.001, Figure 2). Similarly, the prevalence of anemia was lower at follow-up (69.3% vs 72.9%, P = 0.001), particularly for those residing at low altitude. There was no difference in the prevalence of fecal occult blood between the two timepoints (22.9% vs 31%, P = 0.584, S2 Table).

In those residing at low altitude, median VO2max declined between baseline and follow-up (47.0 mL kg^{-1} min^{-1} vs 48.7mL kg^{-1} min^{-1}, P < 0.001), however remained similar between the two time-points in those residing at high altitude (46.3 mL kg^{-1} min^{-1} vs 46.3 mL kg^{-1} min^{-1}, P = 0.349, S2 Table). Median VO2max was higher in those who had been treated two weeks prior at baseline, compared with those who were newly recruited to the study (46.3 mL kg^{-1} min^{-1}, IQR 44.6 - 49.7 mL kg^{-1} min-1 vs 44 mL kg^{-1} min^{-1}, IQR 42.1 - 47.5 mL kg^{-1} min^{-1}, P < 0.001, Figure 3).

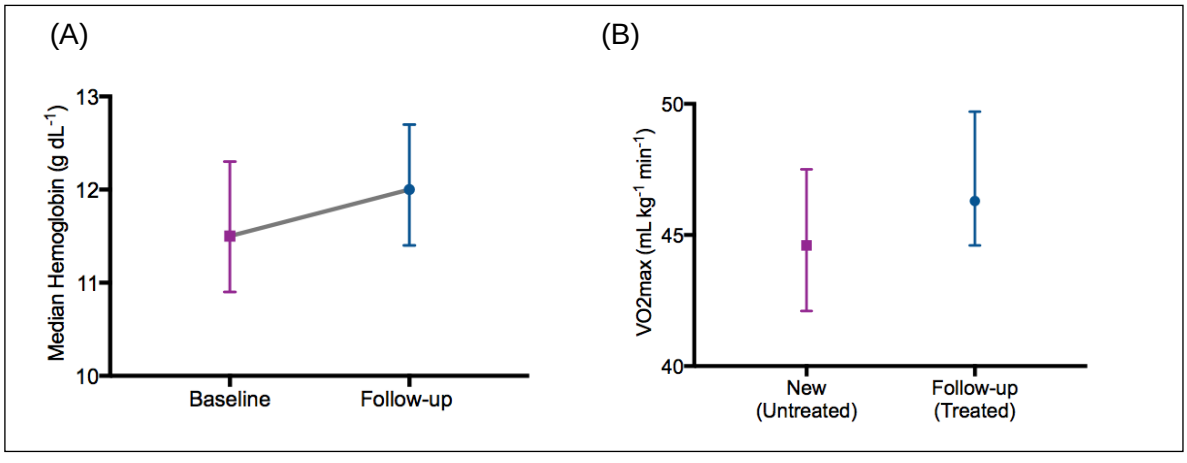


Figure 3: (A) Median hemoglobin at baseline & follow-up. (B) Scatter plot of VO2max for follow-up & new participants with median & interquartile range.

DISCUSSION

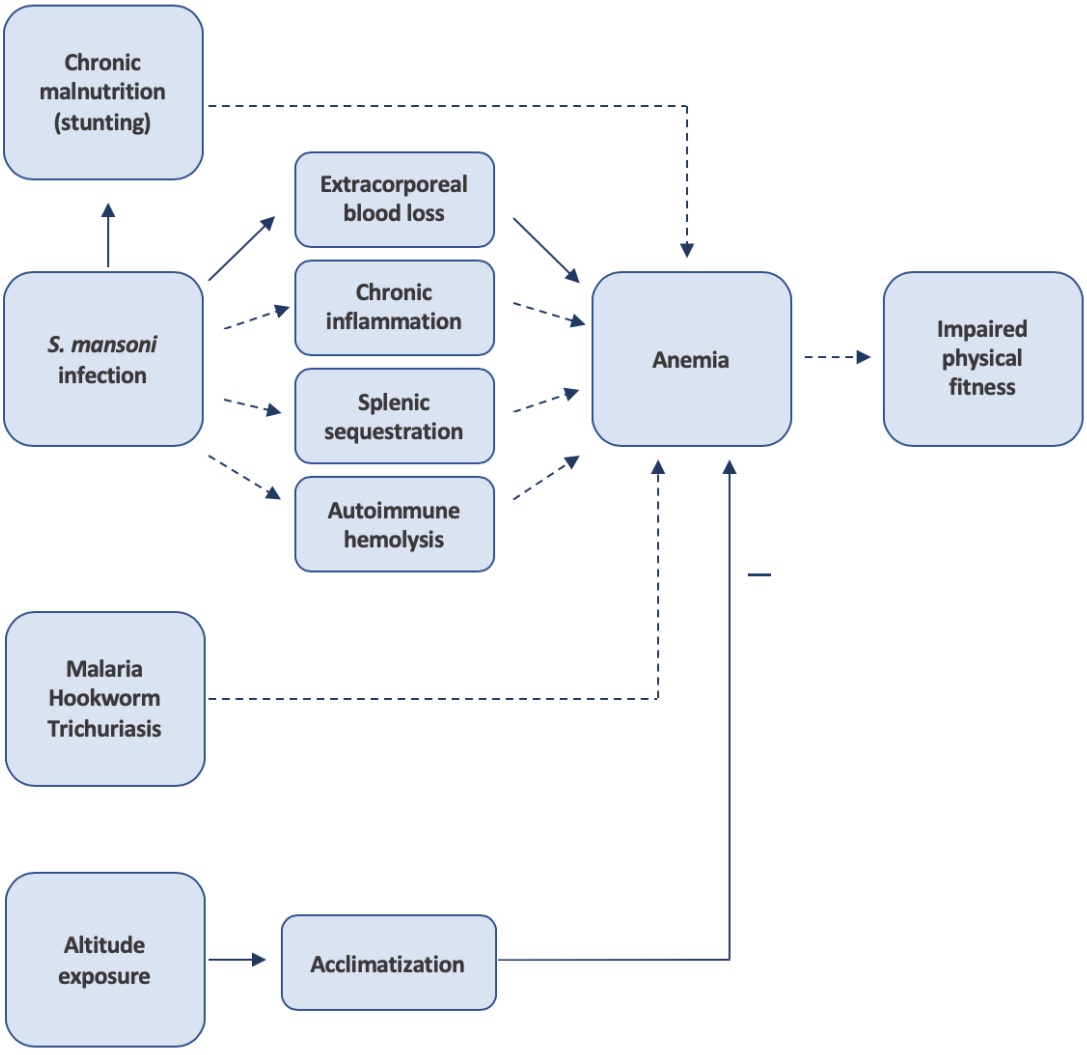
Chronic childhood morbidity secondary to *Schistosoma mansoni* infection has been previously overshadowed by a lack of feasible morbidity metrics adaptable to the pediatric population living within resource-poor settings. This study has shown that *S. mansoni* egg patent infection is associated with decreased aerobic capacity in Ugandan schoolchildren, with lower aerobic capacities seen in Ugandan compared with Canadian children. The 20mSRT proved to be a feasible and easily-implementable tool that may be harnessed for the identification of *S. mansoni*-related morbidity within the school setting.

Negative correlations between all *S. mansoni* infection intensities and VO2max were found in our study, highlighting the important contribution of light intensity infections to *S. mansoni*-related morbidity.[3, 4] These findings were based on the traditional Kato-Katz method which can miss up to 20-40% of active infections.[41] However, in the presence of infections of moderate-high intensity as was predominantly the case in this study, both urine-CCA and parasitological examination maintain high levels of accuracy.[42]

The pathway between *Schistosoma mansoni* infection and decreased aerobic capacity is multifactorial and complex. Anemia is a known downstream effector of *S. mansoni* infection and has been shown to be associated with decreased aerobic capacity.[2] Fecal occult blood is a proxy marker of intestinal inflammation and mechanism for anemia in *S. mansoni* infection.[27, 30, 43] *S. mansoni* egg patent infection and fecal occult blood both positively correlated with anemia in our study. Furthermore, *S. mansoni* egg patent infection was linked with stunting; another known pathway for anemia causation in *S. mansoni* infection.[2] Figure 4 integrates the findings of this study with current knowledge to suggest a potential, albeit simplified, pathophysiological basis for reduced physical fitness in children living in *S. mansoni*-endemic areas.

Previous studies have demonstrated a reduction in anemia, nutrition-related morbidity, fecal occult blood and increase in physical performance following praziquantel therapy.[18, 24, 30, 34, 44, 45] A reassuring decline in the prevalence of anemia was noted in the follow-up cohort after treatment for schistosomiasis at baseline. Furthermore, higher aerobic

409 capacities were seen in those who had been recently treated, compared with those who
410 were newly recruited to the study, emphasizing the reversibility of functional morbidities. It is
411 important to note however that disentangling chronic morbidity and the effects of
412 interventions in low resource settings is a challenging task. Chronic morbidity is confounded
413 by polyparasitic infections, nutritional deficiencies and numerous other factors, such as
414 socioeconomic status and food scarcity, which were unable to be accounted for within the
415 constraints of this study.[3, 4, 5, 46, 47]



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418 *Figure 4: Conceptual pathway for impaired physical fitness in* S. mansoni *infection in children.*

419 *Note: broken arrows represent relationships described elsewhere.*

420

Those children residing at high altitude exhibited higher aerobic capacities compared with those residing at low altitude. In the former, *S. mansoni* infection did not have a negative effect on aerobic capacity. With increasing altitude, barometric pressure and atmospheric partial pressure of oxygen decline, resulting in an increase in erythropoietin production. This occurs via the release of hypoxia inducible factor-alpha. Erythropoietin stimulates the bone marrow to increase iron turnover and production of nucleated red blood cells, thereby increasing red blood cell mass.[48, 49, 50] These adaptations may transpire at altitudes as low as ~1000m.[32] Such acclimatization may have dampened the deleterious effect of *S. mansoni* infection upon aerobic capacity in the children living at a higher altitude.

This study has several limitations. The small sample size achievable within the time frame has limited the strength of the inferences one can make from the findings, particularly with regard to baseline and follow-up cohorts. Nevertheless, the sample size calculation performed at the outset was achieved, and these findings provide a robust indication for further investigation into the pathway linking *S. mansoni* infection with physical fitness in children living in *S. mansoni*-endemic areas. In addition, testing resource availability was limited due to the unforeseen need of the local clinic to use the resources for medical indications. No specific method for ensuring the children reached their maximal aerobic capacity was employed. Such methods are usually time-consuming and cumbersome and were therefore purposely avoided as a means of maintaining the external validity of the 20mSRT as a school-based morbidity metric. The time period between baseline and follow-up testing was brief, limiting the speculations one could make with regard to outcomes following previous exposure to infection and treatment.

Areas requiring further investigation include: 1) the development of more rigorous diagnostic tests capable of detecting light infections and demonstrating antigenic cure, thereby illustrating treatment efficacy, 2) the innovation and application of feasible morbidity metrics with the ability to identify sequelae of *S. mansoni* infections of all intensities, 3) the degree of impact of various altitudes upon VO2max and interplay of these associations with parasitic infections and anemia, and 4) extended baseline-follow-up comparisons to

delineate the effects of treatment upon physical fitness within *S. mansoni*-endemic areas at different altitudes.

This is the first study to document a relationship between *S. mansoni* infection and decreased aerobic capacity at high and low altitudes. Altitude acclimatization may be partially protective of this effect. Whilst the cause of impaired physical performance is multifactorial, this study provides evidence to support the important contribution that *S. mansoni* infection has toward childhood morbidity. The lower aerobic capacities seen in the Ugandan children compared with Kenyan and Canadian children emphasize the inherent need for morbidity assessment in children residing within *S. mansoni*-endemic areas. Furthermore, a recent malacological survey identified schistosomiasis transmission in regions with an altitude beyond 1400m, indicating the need for the geographical expansion of morbidity assessment.[35, 51, 52] Widespread deployment of the 20mSRT throughout school settings represents a promising means by which schistosomiasis-related childhood morbidity may be rapidly detected and managed appropriately within these areas.

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DISCLOSURES REGARDING REAL OR PERCEIVED CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

S1 Table. Demographic, Hematologic, Immunochemical, Parasitological & 20m-Shuttle Run Test Findings in Villages of the Buliisa District at Low Altitude Compared with High Altitude.

Parameter	Total (n=304)	Low Altitude (n=210)	High Altitude (n=94)	P Value*
DEMOGRAPHY				
Median age in years (interquartile range)	11 (10-12)	11 (10-13)	11 (10-12)	0.876
% Female (n)	49.7 (151/304)	50.0 (105/210)	48.9 (46/94)	0.864
ANTHROPOMETRY				
Median height in centimeters (interquartile range)	134 (128.5-140.5)	135.5 (128.7-142.1)	133 (127.6-137.6)	**0.046**
% Stunted by HFA Z-score (n)**	36.7 (79/215)	39.5 (60/152)	30.2 (19/63)	0.197
% Stunted by validated charts (n)***	16.7 (49/293)	16.5 (33/200)	17.2 (16/93)	0.450
% Stunted (n)	15.7 (46/293)	15.0 (30/200)	17.2 (16/93)	
% Severely stunted (n)	1.0 (3/293)	1.5 (3/200)	0.0 (0/93)	
Median body mass index (interquartile range)	16.1 (14.8-17.3)	16.1 (14.8-17.3)	14.8 (13.2-16.3)	0.435
% Wasted (n)****	11.8 (8/68)	12.1 (8/66)	0.0 (2/2)	0.600
HAEMATOLOGY				
Median hemoglobin in g dL^{-1} (interquartile range)#	11.6 (10.7-12.4)	12.1 (11.4-12.7)	12.0 (11.4-12.7)	0.739
% Anemic (n)#	34.5 (86/249)	33.0 (57/173)	38.2 (29/76)	0.426
IMMUNOCHEMICAL				
% Fecal occult blood test positive (n)	41.2 (61/148)	42.6 (40/94)	38.9 (21/54)	0.654
PARASITOLOGY				
Schistosomiasis				
% *S. mansoni* infection by urine-CCA (n)~	80.5 (231/287)	80.3 (159/198)	80.9 (72/89)	0.906
% Egg patent *S. mansoni* infection (n)~~	44.3 (127/288)	47.0 (94/200)	37.5 (33/88)	0.135
Mean epg (95% confidence interval)	449.5 (330.1-568.9)	527.2 (366.4-687.9)	273.1 (137.5-408.8)	0.133

S. mansoni intensity~~				
% Negative (n)	55.9 (161/288)	53.0 (106/200)	62.5 (55/88)	
% Light (n)	10.1 (29/288)	12.0 (24/200)	5.7 (5/88)	
% Medium (n)	11.8 (34/288)	11.0 (22/200)	13.6 (12/88)	
% Heavy (n)	22.2 (64/288)	24.0 (48/200)	18.2 (16/88)	
Malaria				
% Malaria (n)^	65.2 (122/187)	60.9 (81/133)	75.9 (41/54)	0.051
% P. falciparum (n)	65.2 (122/187)	60.9 (81/133)	75.9 (41/54)	**0.015**
% Mixed (n)	11.2 (21/187)	10.5 (14/133)	13.0 (7/54)	0.768
Giardiasis				
% *Giardia duodenalis* infection (n)^^	21.4 (63/294)	22.2 (45/203)	19.8 (18/91)	0.128
20M-SHUTTLE RUN TEST				
Median VO2max in mL kg^{-1} min^{-1} (interquartile range)	45.4 (42.9-48.0)	44.8 (42.1-47.5)	46.3 (43.4-48.7)	**0.031**
Males	47.5 (43.9-49.0)	46.4 (43.8-49.0)	47.9 (46.0-49.6)	0.078
Females	43.9 (41.5-46.3)	43.9 (41.5-45.7)	44.3 (42.9-46.3)	0.258

*Indicates significance of differences among the villages by Kruskal-Wallis or Chi-squared analysis, paired T test or ANOVA. Statistically significant differences (P ≤ 0.05) indicated in **bold**. **As defined by height-for-age Z-scores ≤ 2 S.D. below mean.[37] ***According to validated stunting charts based on height-for-age Z-score: 'stunted' (≤ 2 - > 3 S.D. below mean), 'severely stunted' (≤ 3 S.D. below mean).[36] ****As defined by BMI-for-age Z-scores ≤2 S.D. below mean.[37] #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5 - 11y), < 12.0 g dL^{-1} (12 - 14y). Hemoglobin adjusted for altitude.[40] ~As per urine-cathodic circulating antigen testing. ~~As per dual Kato-Katz examination. Intensity defined by epg: 1 - 99 = light; 100 -399 = medium, ≥ 400 = heavy.[29] ^As per malaria rapid diagnostic testing. ^^As per Giardia/Cryptosporidium Quik Chek test.

720 S2 Table. Demographic, Hematologic, Anthropometric, Immunochemical, Parasitological &
721 20m-Shuttle Run Test Findings in Baseline & Follow-up Cohorts.
722

Parameter	Baseline (n=96)	Follow-up (n=96)	P Value*
DEMOGRAPHY			
Median age in years (interquartile range)	11 (9.5-12)	11 (9.5-12)	
Low altitude	11 (9-12)	11 (9-12)	
High altitude	11 (10-12)	11 (10-12)	
% Female (n)	51.0 (49/96)	51.0 (49/96)	
Low altitude	48.5 (16/33)	48.5 (16/33)	
High altitude	52.4 (33/63)	52.4 (33/63)	
HAEMATOLOGY			
Median hemoglobin in g dL^{-1}, adjusted (interquartile range)#	10.2 (9.6-11.7)	10.7 (9.7-12.1)	**<0.001**
Low altitude	11.8 (11.0-12.3)	12.5 (11.9-13.1)	**<0.001**
High altitude	11.5 (10.6-11.9)	112.0 (11.2-12.6)	**<0.001**
% Anemic, adjusted (n)#	47.9 (23/48)	69.3 (25/75)	**0.001**
Low altitude	42.9 (9/21)	20.8 (5/24)	**0.002**
High altitude	51.9 (14/27)	39.2 (20/51)	0.098
IMMUNOCHEMICAL			
% Fecal occult blood test positive	22.9 (11/48)	31.0 (18/58)	0.584
Low altitude	28.6 (6/21)	35.0 (7/20)	0.774
High altitude	18.5 (5/27)	29.0 (11/38)	0.137
PARASITOLOGY			
% *S. mansoni* infection by urine-CCA (n)~	62.5 (30/48)	76.1 (67/88)	**<0.001**
Low altitude	57.1 (12/21)	69.0 (20/29)	**0.005**
High altitude	66.7 (18/27)	79.7 (47/59)	**0.001**
% Egg patent *S. mansoni* Infection (n)~~	20.8 (10/48)	25.0 (22/88)	0.053
Low altitude	28.6 (6/21)	17.2 (5/29)	N/A
High altitude	14.8 (4/27)	28.8 (17/59)	**0.006**
Mean eggs per gram (95% confidence interval)~~	49.8 (-13.1-112.6)	251.5 (86.4-416.5)	0.375
Low altitude	75.4 (-64.6-215.5)	344.7 (-41.6-731.0)	0.277
High altitude	29.8 (-11.6-71.1)	205.6 (47.1-364.1)	0.663
S. mansoni intensity~~			
% Negative (n)	79.2 (38/48)	75.0 (66/88)	
% Light (n)	14.6 (7/48)	5.7 (5/88)	
% Medium (n)	2.1 (1/48)	6.8 (6/88)	
% Heavy (n)	4.2 (2/48)	12.5 (11/88)	
Low Altitude			
% Negative (n)	71.4 (15/21)	82.8 (24/29)	
% Light (n)	23.8 (5/21)	3.5 (1/29)	

% Medium (n)	0.0 (0/21)	0.0 (0/29)	
% Heavy (n)	4.8 (1/21)	13.8 (4/29)	
High Altitude			
% Negative (n)	85.2 (23/27)	71.2 (42/59)	
% Light (n)	7.4 (2/27)	6.8 (4/59)	
% Medium (n)	3.7 (1/27)	10.2 (6/59)	
% Heavy (n)	3.7 (1/27)	11.9 (7/59)	
% *Giardia duodenalis* infection (n)^^	20.8 (10/48)	14.9 (14/94)	1.000
Low altitude	13.3 (3/21)	12.5 (4/32)	0.732
High altitude	25.9 (7/27)	16.1 (10/62)	0.992
20M SHUTTLE RUN TEST			
Median VO2max in mL kg^{-1} min^{-1} (interquartile range)	47.45 (45.4-50.3)	46.3 (43.9-49.1)	**0.001**
Low altitude	48.7 (46.3-52.0)	47.0 (43.9-48.7)	**<0.001**
High altitude	46.3 (43.9-48.7)	46.3 (43.9-49.5)	0.349

Associations determined by linear regression. *Indicates significance of differences among the villages by Kruskal-Wallis or Chi-squared analysis, paired T test or ANOVA. Statistically significant differences ($P \leq 0.05$) indicated in **bold.** #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5 - 11y), < 12.0 g dL^{-1} (12 - 14y). Hemoglobin adjusted for altitude.[40] ~As per urine-cathodic circulating antigen testing. ~~As per dual Kato-Katz examination. Intensity defined by eggs per gram (epg): 1 - 99 = light; 100 - 399 = medium, ≥ 400 = heavy.[29] ^^As per Giardia/Cryptosporidium Quik Chek test.

S3 Table. Comparison of Mean VO2max between Study Participants & Reference Canadian Cohort.

Age	Gender	Canadian Cohort		Study Cohort		P Value
		n	Mean VO2max (S.D.)	n	Mean VO2max (S.D.)	
7	Male	297	51.23 (3.34)	2	46.30 (3.39)	**<0.001**
	Female	299	50.26 (2.63)	1	N/A	N/A
8	Male	303	51.67 (3.91)	9	44.66 (4.03)	**<0.001**
	Female	308	49.82 (3.44)	14	42.33 (3.05)	**<0.001**
9	Male	322	51.54 (4.39)	20	47.34 (4.28)	**<0.001**
	Female	322	49.20 (3.24)	22	44.42 (2.77)	**<0.001**
10	Male	404	51.64 (4.23)	30	47.32 (3.75)	**<0.001**
	Female	335	46.84 (2.76)	28	44.53 (4.10)	**0.006**
11	Male	386	51.13 (4.53)	23	46.47 (3.65)	**<0.001**
	Female	382	47.51 (4.04)	22	43.87 (4.18)	**<0.001**
12	Male	341	51.92 (5.16)	29	46.95 (3.78)	**<0.001**
	Female	292	46.65 (4.17)	29	44.72 (3.44)	**0.005**
13	Male	325	50.10 (5.21)	19	46.29 (3.51)	**<0.001**
	Female	298	44.42 (4.76)	19	43.05 (4.03)	0.1568
14	Male	289	50.11 (5.20)	20	45.92 (3.86)	**<0.001**
	Female	260	41.65 (4.72)	16	43.85 (3.57)	**0.026**
15	Male	333	50.20 (6.07)	1	48.80 (N/A)	**<0.001**
	Female	260	41.16 (5.07)	1	41.50 (N/A)	N/A

Canadian data obtained from Leger et al., 1988. Differences determined by one-way T test. Statistically significant differences (P ≤ 0.05) indicated in **bold.** S.D. = Standard Deviation.

S4 Table. Linear Regression Models with VO2max as the Outcome, Stratified by Gender.

	Unadjusted Analysis				Multivariable-adjusted Analysis			
	Coefficient	95% CI		P Value	Coefficient	95% CI		P Value
S. mansoni egg patent infection*								
Males	-0.842	-2.084	0.400	0.182	-2.407	-5.150	0.337	0.083
Females	**-1.912**	**-3.123**	**-0.700**	**0.002**	**-5.038**	**-8.794**	**-1.283**	**0.011**
Fecal Occult Blood								
Males	-0.077	-0.878	0.724	0.848	0.090	-1.233	1.412	0.891
Females	-0.442	-1.234	0.349	0.269	0.343	-0.988	1.673	0.601
Malaria^								
Males	-0.493	-2.039	1.054	0.529	-0.877	-3.524	1.770	0.504
Females	0.759	-0.942	2.460	0.378	-0.260	-3.592	3.071	0.874
Stunting~								
Males	-0.229	-1.966	1.508	0.795	-0.366	-3.541	2.809	0.815
Females	-0.345	-1.678	0.987	0.609	-1.251	-4.586	2.085	0.448
Anemia#								
Males	0.134	-1.261	1.529	0.849	1.731	1.000	4.462	0.205
Females	-1.264	-2.601	0.072	0.063	-0.311	-3.444	2.822	0.840

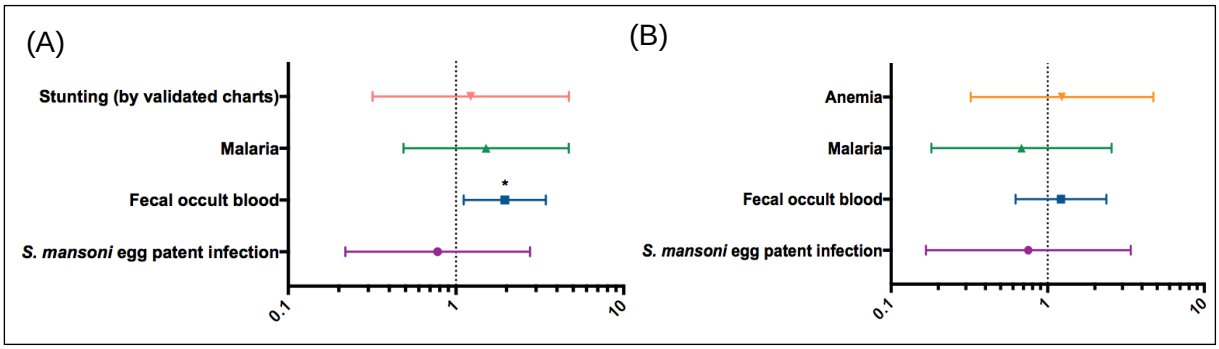
Statistically significant differences (P ≤ 0.05) indicated in **bold**. *As per dual Kato-Katz examination. ^As per malaria rapid diagnostic testing. ~According to validated stunting charts based on height-for-age Z-score ≤ 2 S.D. below mean.[36] #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5 - 11y), < 12.0 g dL^{-1} (12 - 14y). Hemoglobin adjusted for altitude.[40] For multivariable-adjusted analysis: Males: n = 36. P Value = 0.257. R-squared = 0.188. Adjusted R-squared = 0.053. AIC = 195.429. Females: n = 32. P Value = 0.052. R-squared = 0.330. Adjusted R-squared = 0.201. AIC = 176.918.

S5 Table. Linear Regression Models with Stunting (by validated charts) as the Outcome.

	Unadjusted Analysis				Multivariable-adjusted Analysis			
	Odds Ratio	95% CI		P Value	Odds Ratio	95% CI		P Value
S. mansoni egg patent infection*	**2.491**	**1.302**	**4.771**	**0.006**	0.752	0.167	3.390	0.711
Fecal occult blood	1.292	0.867	1.927	0.208	1.215	0.623	2.369	0.568
Malaria^	0.651	0.307	1.382	0.264	0.681	0.181	2.560	0.570
Anemia#	1.391	0.665	2.908	0.381	1.233	0.322	4.726	0.760

Statistically significant differences (P ≤ 0.05) indicated in **bold**. *As per dual Kato-Katz examination. ^As per malaria rapid diagnostic testing. #As per standardised hemoglobin cut-offs for age: < 11.5 g dL^{-1} (5-11y), < 12.0 g dL^{-1} (12-14y). Hemoglobin adjusted for altitude.[40] For multivariable-adjusted analysis: AIC = 73.21548. n = 70. P value = 0.92. Pseudo R-squared = 0.0144. likelihood ratio chi-squared test = 0.92.

S6 Figure. Adjusted Odds Ratios for Anemia (A) and Stunting (B; by validated charts).



The final models were controlled for (a) *S. mansoni* egg patent infection, fecal occult blood, malaria and stunting (by validated charts), & (b) *S. mansoni* egg patent infection, fecal occult blood, malaria and anemia. *OR 1.96; 95% CI 1.11 - 3.43, **P = 0.020.**