

1 **A cross-sectional study of periportal fibrosis and**
2 ***Schistosoma mansoni* infection amongst school-**
3 **aged children in a hard-to-reach area of Madagascar**

4 **Hannah J. Russell ^{a,1*}, James M. StJ. Penney ^{a,2}, Cortland Linder ^{a,3}, Elizabeth**
5 **C. Joekes ^b, Amaya L. Bustinduy ^c, J. Russell Stothard ^b, Daniel A. L.**
6 **Rakotomampianina ^d, Emmanuel H. Andriamasy ^d, Lalarizo R. Mahary ^{d,4}, Elodie**
7 **P. Ranjanoro ^{d,5}, Alain M. Rahetilahy ^e, Stephen A. Spencer ^f**

8 ^aUniversity of Manchester Medical School, Manchester, England, M13 9PL; ^bLiverpool
9 School of Tropical Medicine, Liverpool, England, L3 5QA; ^cClinical Research Department,
10 London School of Tropical Medicine and Hygiene and Tropical Medicine, London, England,
11 WC1E 7HT; ^dFaculté de Médecine, Université d'Antananarivo, Antananarivo, Madagascar;
12 ^eMinistère de la Santé Publique, Antananarivo, Madagascar; ^fInfectious Diseases, North
13 Bristol NHS Trust, Bristol, England, BS10 5NB

14 ¹Present address: Red House, Guilsfield, Powys, Wales, SY21 9NH; ²Present address:
15 University Hospital Monklands, NHS Lanarkshire, Glasgow, Scotland, ML6 0JS; ³Present
16 address: Maidstone and Tunbridge Wells Hospital, Maidstone and Tunbridge Wells NHS
17 Trust, Kent, England, TN2 4QJ; ⁴Present address: Department of Immunology, Joseph
18 Ravoahangy University Hospital Centre, Antananarivo, Madagascar; ⁵Present address:
19 Compassion Madagascar University Hospital, Antananarivo, Madagascar

20 * Corresponding author: Telephone: +447581361324, Email: hannahrussell@doctors.org.uk

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23 **Background:** A cross-sectional survey was performed to estimate the prevalence of periportal
24 fibrosis in children based on ultrasound examination in the Marolambo District of the
25 Atsinanana Region of Madagascar. This is a remote area known to have a high prevalence
26 of intestinal schistosomiasis.

27

28 **Methods:** School-aged children (5-14 years) were selected from six villages for parasitological
29 and sonographic examination. Circulating cathodic antigen (CCA) tests and Kato Katz (KK)
30 stool microscopy were performed. Video clips of liver views were recorded with a SonoSite i-
31 Viz and interpreted in the UK by comparison with standardised images (WHO protocol).

32

33 **Results:** The prevalence of schistosomiasis according to CCA testing was 97.8% (269/275)
34 and 73.8% (203/275) by KK. Sonographic evidence of periportal fibrosis was observed in
35 11.3% (31/275). The youngest children with fibrosis were six years old. Fibrosis was more
36 common in older children ($p=0.03$) but was not associated with infection intensity category
37 ($p=0.07$) or gender ($p=0.67$).

38

39 **Conclusions:** Findings of periportal fibrosis amongst children in these hard-to-reach villages
40 suggests chronic *Schistosoma mansoni* infection from a very young age. This may reflect
41 other similarly remote schistosomiasis-endemic areas and reinforces the need to investigate
42 morbidity in neglected communities in order to understand the true extent of disease burden
43 in endemic countries.

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45 **Keywords:** Fibrosis, Liver, Madagascar, Schistosomiasis, Ultrasound.

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50 Introduction

51 Schistosomiasis is a parasitic disease associated with significant morbidity.¹ It is
52 estimated that at least 230 million people are infected with *Schistosoma* species globally, with
53 an associated loss of 3 – 70 million disability-adjusted life years.² Infection with *Schistosoma*
54 *mansoni* causes intestinal and hepatosplenic disease as the parasites' eggs lodge in tissue
55 causing inflammation and fibrosis. Symptoms include diarrhoea, abdominal discomfort and
56 blood in the stool. Hepatic periportal fibrosis can result in portal hypertension and gastro-
57 oesophageal varices which can be fatal upon variceal rupture.¹ Repeated chemotherapy with
58 praziquantel can lead to reversal of periportal fibrosis^{3,4} through action on existing egg
59 granulomas and by halting further egg deposition.⁵

60 The first step towards elimination of schistosomiasis is morbidity control by mass
61 treatment with praziquantel alongside complimentary public health interventions.⁶ Ultrasound
62 examination allows visualisation of hepato-splenic complications of *S. mansoni* infection and
63 is recommended as an indicator of schistosomiasis-related morbidity.⁷

64 Establishing the geographical distribution of schistosomiasis, associated morbidity and
65 the impact of treatment interventions remains priority.^{6,8} Ultrasound examination is
66 recommended as an important part of control programmes by regular examination of sentinel
67 groups to monitor morbidity and response to treatment.^{4,7}

68 The WHO protocol for sonographic examination of schistosomiasis-related morbidity⁷
69 appears to be the most widely used.⁹ It aims to provide a standardised protocol to facilitate
70 comparison of results between different surveys around the world.⁷ Although there are many
71 of causes of hepatomegaly and portal hypertension (complications of *S. mansoni* infection),
72 the distinctive pattern of periportal fibrosis seen with ultrasound is characteristic of *S. mansoni*
73 infection. Diagnosis of schistosomiasis-related hepatic disease can therefore be differentiated
74 from other hepatic pathology with ultrasound.¹⁰

75 Performing ultrasound in many remote, endemic areas remains very challenging due
76 to difficult access, lack of electrical power sources and lack of human resources (particularly

77 experienced ultrasound operators). Owing to these challenges, indirect indicators of morbidity
78 are often used for monitoring. For example, the World Health Organisation (WHO) gives a
79 morbidity control target of <5% prevalence of heavy-intensity infections (≥ 400 eggs per gram
80 of stool).⁶

81 In Madagascar, schistosomiasis is endemic in 107 of the 114 districts.¹¹ In the
82 Marolambo District in 2015, the prevalence of *S. mansoni* infection in school-aged children
83 (SAC) was 94%. A third of these children were found to have heavy-intensity infections. The
84 district is not endemic for *S. haematobium*.^{12,13} These parasitological findings in 2015
85 prompted annual mass treatment with praziquantel for SAC in the district, organised by the
86 Ministry of Health in Madagascar. However, no data were collected on hepatic morbidity.

87 The aim of this epidemiological survey was to determine the prevalence of periportal
88 fibrosis amongst a sample of SAC in this very remote area, hyperendemic for schistosomiasis.
89 This study was organised by Madagascar Medical Expeditions (MadEx), a voluntary research
90 organisation set up by students at The University of Manchester, UK.

91

92 **Methods**

93 **Study design and population**

94 This cross-sectional study took place in 2016 (May - June) in six villages lying along
95 the Nosivolo River in the Marolambo District of the Atsinanana Region in East Madagascar;
96 Marolambo, Ampasimbola, Ambohitelo, Marofatsy, Vohidamba and Betampona. These were
97 the only villages in the district that could be included due to warnings from local authorities
98 that the team's safety could not be guaranteed beyond these locations. Relationships had
99 already been formed with local community leaders and local organisations in these villages
100 during a MadEx study in 2015;¹² this ongoing support was essential to carry out research in a
101 setting such as this.

102 Communities in the Marolambo District rely heavily on environmental water for
103 drinking, cooking, washing and transport. Outside the main village of Marolambo, there is no

104 access to electrical power. Access to the villages upstream of Marolambo is via a single-track
105 footpath which is impassable at certain times of the rainy season.

106 There are very few known data recorded that give an indication of sociodemographic
107 characteristics of these communities. However, it is reported that around 95% of the
108 population are farmers.

109 The entire school register in each village was stratified by age and gender. All pupils
110 in the register were numbered and fifty children per village were selected by random with an
111 even spread across gender and age (5 – 14 years). After gaining parental consent, children
112 were invited to participate in the study. A total sample size of 300 SAC was chosen as the
113 limit that would be practicably possible to include (constrained by availability of time, funds
114 and human resources). Upon inclusion, each child was assigned a unique identifiable
115 number.

116 Praziquantel was administered to school-aged children in all six villages in 2015 but
117 prior to this there had been no mass treatment since 2008. It was not possible to accurately
118 know which study participants received treatment in 2015 as records were not available.

119

120 **Parasitological examination**

121 Each participant was given two sample containers (pre-labelled with unique identifiable
122 numbers) and asked to provide a urine and a stool sample.

123 A circulating cathodic antigen test (CCA; Rapid Medical Diagnostics Tests, Pretoria,
124 South Africa) was performed within six hours of receipt of the urine samples. Testing methods
125 were in line with the manufacturer's instructions and the results were read by two trained
126 technicians to ensure homogeneity. Results were recorded as either positive (presence of test
127 band) or negative (absence of test band).

128 Two thick smears (containing 41.7mg of stool) were prepared from each stool sample
129 using the Kato-Katz (KK; Vestergaard-Frandsen, Lausanne, Switzerland) method. Slides were
130 prepared within six hours of receipt of the samples. Each slide was examined under light
131 microscopy by one of four team members (with training and prior experience) to count, if

132 present, the number of *Schistosoma mansoni* eggs. The two slides from each sample were
133 not interpreted by the same reader, and readers were blinded to the findings of others. Results
134 of the two slides were used to calculate the mean number of eggs per slide. From this, the
135 total number of eggs per gram (epg) was determined for each child.

136 Testing for *S. haematobium* was not performed as a cross-sectional study in these six
137 villages in 2015 did not identify any *S. haematobium* infections in this area.¹² Soil-transmitted
138 helminths were not addressed in this study.

139

140 **Ultrasound examination**

141 An i-Viz (Fujifilm SonoSite, Inc.) portable ultrasound system with a phased array probe (5-
142 1MHz) was used. The operators were three senior medical students, trained by consultant
143 radiologists in the UK to capture a predefined set of video-clips. The students were trained to
144 obtain five-second video clips of the following views: longitudinal and transverse subcostal
145 views of the left lobe, transverse and oblique subcostal and oblique intercostal views of the
146 right lobe of the liver. These views were selected as they are listed in the WHO protocol.⁷
147 Views of the spleen were not included due to the endemicity of malaria in the district, in line
148 with the WHO protocol⁷. Once a good view was obtained at each of the sites, a video clip was
149 recorded as the operator fanned through the view (from one extreme to the other) with the
150 probe's footprint otherwise stationary. Video clips were stored under each child's unique
151 identifiable number and exported to a USB drive.

152 The children were asked to lie supine with their legs outstretched and examination took
153 place in the presence of a chaperone. The children in each village were scanned
154 opportunistically, in no particular order, by one of the three medical students (who each did an
155 equal share of the scanning).

156

157 **Ultrasound interpretation**

158 Interpretation of the ultrasound clips took place in the UK. A consultant radiologist
159 (ECJ) and newly qualified doctor (HJR) interpreted the ultrasound clips of the first 15% of
160 children together in order to train HJR. After this, HJR interpreted the remaining cases.

161 Liver parenchyma were compared with standard image patterns included in an annex
162 of the WHO protocol and assigned the letter it best aligned with. Image patterns A and B
163 were considered normal, C-F corresponded to progressive degrees of periportal fibrosis, and
164 Z was used for other abnormalities.⁷ Cases were only labelled with 'image pattern C' if there
165 was clear thickening in the periphery of the parenchyma where portal branch walls should
166 not normally be clearly visible with ultrasound.

167 A random sample of the cases (10%) was second-read by the radiologist for quality
168 assurance purposes. Interpreters were blinded to demographical and parasitological results.

169

170 **Health interventions and ethical considerations**

171 After the study, Malagasy members of the MadEx team delivered a schistosomiasis
172 education programme in the schools.

173 The study was timed to take place immediately before mass treatment of SAC in the
174 district, delivered as part of the national schistosomiasis control programme. A Ministry of
175 Health official travelled to the villages with the MadEx team to coordinate mass treatment
176 which took place in each village the day after testing finished. Throughout the study, the
177 participants and parents/guardians were repeatedly informed of the upcoming treatment
178 programme, the importance of which was reinforced by the education programme. At the end
179 of testing, the names of schistosomiasis-positive children were shared with the health centre
180 in their village (Centres de Santé de Base) to ensure that positive children had received
181 treatment.

182 The University of Manchester Research Ethics Committee (UREC3) approved
183 the project (#16153). Research permits were granted by the Ministry of Health,
184 Madagascar. Written consent for participation in the study was obtained from the child and
185 their parent / guardian. All data were anonymised.

186

187 **Data analysis**

188 Statistical analyses were performed using StataCorp 2017 (Stata Statistical Software
189 15. College Station, TX: StataCorp LLC). The chi-squared test was used to assess the
190 relationship between those diagnosed with schistosomiasis by either CCA or Kato Katz and
191 those with and periportal fibrosis. Ultrasound findings were compared to age, gender,
192 infection intensity and village by multiple logistic regression analyses.

193

194 **Results**

195 Twenty-four (8.0%) cases were excluded due to missing data: two (0.7%) children did
196 not attend on testing days at all, two (0.7%) children did not provide a stool sample, sixteen
197 (5.3%) children declined or did not attend for an ultrasound scan, and the ages of four (1.3%)
198 children were not recorded. One (0.3%) additional case was excluded as the recorded
199 ultrasound clips were not interpretable. There were therefore 25 (8.3%) children excluded from
200 the study.

201 Of the 275 children included in the results of this study, 141 (51.3%) were female and
202 134 (48.7%) were male. The number of children in the study by age and gender are listed in
203 Table 1.

204

205 **Parasitological results**

206 The prevalence of egg-patent *S. mansoni* infection according to CCA testing was
207 269/275 (97.8%) and 203/275 (73.8%) according to KK. The spread of low (1-99 epg), medium
208 (100-399 epg) and heavy (≥ 400 epg) infection intensities according to KK technique was
209 85/202 (42.1%), 66/202 (32.7%) and 51/202 (25.2%) respectively. For those with positive KK,
210 median egg count was 144 epg (range 24-5040 epg). Neither age nor gender were associated
211 with prevalence of *S. mansoni* infection by CCA ($p=0.26$, $p=0.95$, respectively) or KK ($p=0.61$,
212 $p=0.80$, respectively; Table 1).

213

214 **Ultrasound results**

215 Image patterns A, B and C were observed in 235/275 (85.5%), 8/275 (2.9%) and
216 31/275 (11.3%) of cases respectively (see Figures A, B and C). An example of the ultrasound
217 findings for a child without *S. mansoni* infection (negative CCA and KK testing) is shown for
218 comparison in Figure D. There were no cases of image patterns D, E or F. One case was
219 interpreted as Z. The youngest children with sonographic evidence of periportal fibrosis were
220 six years old. The six children with negative CCA results had a sonographically normal liver
221 parenchyma. Six KK-negative children were found to have sonographic evidence of fibrosis
222 (Table 2).

223 There was no evidence of an association between *S. mansoni*-positive cases detected
224 by either CCA or Kato Katz, and sonographic evidence of periportal fibrosis ($p=0.38$, $p=0.36$
225 respectively). The prevalence of periportal fibrosis amongst CCA-negative and CCA-positive
226 children was 0/6 (0.0%) and 31/269 (11.5%) respectively. The prevalence of periportal fibrosis
227 amongst Kato Katz-negative and Kato Katz-positive children was 6/72 (8.3%) and 25/203
228 (12.5%) respectively.

229 The prevalence of children with periportal fibrosis increased with age ($p_{adj}=0.03$, OR_{adj}
230 1.17, 95%CI 1.02-1.34; see Table 1, Figure E). Gender was not linked to periportal fibrosis: of
231 the 31 cases with pattern C, 17 (54.8%) were female and 14 (45.2%) were male ($p=0.67$;
232 Table 1). There was minimal evidence for an association between the prevalence of periportal
233 fibrosis and infection intensity when categorised as low, moderate or heavy ($p_{adj}=0.08$,
234 $OR_{adj}=1.38$, 95%CI 0.96-1.97; Table 2). Finally, there was no association between prevalence
235 of sonographic periportal fibrosis and village ($p_{adj}=0.19$).

236

237 **Quality assurance of ultrasound interpretation**

238 The random sample (10%) second read by the radiologist matched with the initial
239 interpretation by the junior doctor in 26/28 (93%) of cases.

240

241 **Discussion**

242 Periportal fibrosis, a well-recognised complication of chronic *S. mansoni* infection, was
243 detected in 11.3% of school-aged children in the Marolambo District, Madagascar. We
244 observed evidence of periportal fibrosis in children as young as six years old. This reflects
245 exposure to *S. mansoni* cercariae from a very young, preschool age in the absence of annual
246 treatment programmes. Consistent with other studies, presence of periportal fibrosis in our
247 study sample appears to be associated with chronicity of infection.¹⁴⁻¹⁹ Although no association
248 was found between *S. mansoni* infection and periportal fibrosis, there was some suggestion
249 that increasing infection intensities may be associated with periportal fibrosis.

250 The remoteness of the communities we studied posed a logistical challenge and
251 possibly explains why there had not been a schistosomiasis prevalence survey or morbidity
252 monitoring performed since 1961 prior to MadEx investigation in 2015.^{12,13} The lack of
253 contemporary prevalence data for the area has meant that the true need for treatment had not
254 been recognised and may explain the infrequency of mass treatment programmes in the
255 district.

256 Schistosomiasis is hyperendemic in the six villages included in this study. In addition
257 to the lack of regular mass treatment, there are many reasons which may explain the high
258 prevalence. Although not the particular focus of this study, many observations have been
259 made whilst working in this area. The communities are dependent on the Nosivolo River and
260 its surrounding streams for drinking water, bathing, washing clothes and plates, and
261 transportation. Many community members (including children) also pan for gold in the river.
262 The majority of the working population are farmers, and considerable time may be spent
263 tending to rice paddies.

264 Had it not been for the safety threat, we suspect that our research methods could have
265 been applied in the district's other villages. The kit was carried in rucksacks on foot, electrical
266 equipment was charged by solar energy; the research methods can be reproduced in other

267 remote settings. However, the team recognise that, as in this district, there may be
268 uncontrollable barriers preventing studies from taking place in some remote settings.

269 Periportal fibrosis has been demonstrated in preschool-aged children²⁰ and school
270 aged children in endemic countries.^{4,14-22} In a study in Western Zambia, ultrasound
271 examination was performed, and liver image patterns were assigned by a trained sonographer
272 in the field. Amongst 7-9-year olds and 10-14-year olds, the prevalence of liver fibrosis
273 (according to WHO protocol's image patterns C-F) was 14% (n=50) and 16.7% (n=96)
274 respectively.^{7,16} In Tanzania, a study of 354 children between 6-17 years old, identified
275 periportal fibrosis in 5.4% (n=354) of these children.²¹ In this study, the WHO protocol was
276 performed by experienced observers. In contrast, periportal fibrosis was not detected at all in
277 a population aged between 7 - 20 years in Kenya (88.5% of whom were excreting *S. mansoni*
278 eggs).²³ Ultrasound examination methods in this study again followed the WHO protocol and
279 fibrosis was classified as image pattern C-F.

280 Ultrasound has become relatively inexpensive and highly portable however,
281 interpretation can be subject to interobserver variance.²⁴ Measuring portal branch wall
282 thickness (PBWT) is time consuming, requires a high skill level and can be nonspecific. In a
283 review of WHO protocol usage, PBWT was measured in 19/41 studies, and only 2 of these
284 studies reported the results.⁹ Alternatively, assignment of an image pattern can be done
285 rapidly and apparently with a good degree of reproducibility.^{9,19} In our study, we elected to
286 focus solely on image patterns. We grouped image patterns A and B together as being 'normal'
287 scans however, some cases of pattern B may actually reflect early stages of fibrotic change.
288 Although the use of image patterns makes the assessment relatively simple, it can be
289 challenging to fit cases into distinct categories, particularly in the context of early-stage
290 fibrosis. Before unequivocal fibrosis develops, the morphology of the liver lies somewhere
291 between normal and abnormal.⁹ This is challenging stage to interpret with ultrasound, and the
292 stage that we expect many of our study participants may well have been in. To prevent
293 overdiagnosis, we therefore ensured that, cases were only defined as abnormal when findings
294 were unambiguous. This may have led to an underestimation of periportal fibrosis in our study.

295

296 **Recommendations**

297 Delivery of preventive chemotherapy to at-risk populations from an early age is key for
298 preventing development of hepato-splenic complications.² Our finding of fibrosis in children as
299 young as six reinforces the need for praziquantel administration to preschool-aged children in
300 order to halt (and hopefully reverse) periportal fibrosis.

301 Great variation in hepatosplenic disease between neighbouring villages in
302 Madagascar has been described suggesting that extrapolation of morbidity data to entire
303 regions may not be accurate.²⁵ This reinforces the need for high resolution morbidity mapping
304 to fully understand disease burden in a region. Regular monitoring of schistosomiasis-related
305 morbidity with ultrasound is recommended but there are many challenges to implementing
306 this.

307 There is a need for a quick, simple protocol that can be performed by relatively 'novice'
308 (non-expert) operators with highly portable, solar-powered ultrasound systems. This may be
309 achieved by assigning image patterns to clips from examinations which take less than two
310 minutes per case. Such a rapid assessment might increase the number of community surveys
311 that include ultrasound assessment alongside other morbidity tests, thus improving
312 understanding of the geographical distribution of schistosomiasis and its burden. However,
313 this abbreviated assessment should be tested against the full recommended WHO protocol to
314 ensure its accuracy and reliability.

315 We present a method of performing ultrasound examination in this challenging setting:
316 video clips recorded by relatively novice ultrasound operators using highly portable, solar-
317 powered ultrasound devices and interpreted remotely. We returned to the UK with ultrasound
318 images for interpretation and recognise that this is not a sustainable approach for ultrasound
319 use on a larger scale to monitor morbidity associated with schistosomiasis. There are however
320 possibilities to employ telemedicine to share images with central experts. In our study, the
321 images were recorded by medical students who were able to record adequate clips for
322 interpretation. Further work is needed to investigate the skill level required to obtain adequate

323 images, and also to even interpret images. If it is shown that minimal training is needed to
324 assign image patterns accurately, perhaps ultrasound-based morbidity surveys could be
325 carried out on a much larger scales by investigators requiring less training. This would lead
326 to better mapping of morbidity in countries endemic for schistosomiasis and would guide
327 national control programmes.

328

329 **Limitations**

330 There are a number of limitations to our study. The sample was selected from the
331 school register meaning that non-school-attending children were excluded from the study.
332 School attendance is affected by severe schistosomiasis and therefore our sample of school-
333 attenders may be an underrepresentation of the true prevalence of periportal fibrosis in this
334 area.²⁶ The six villages involved in our study may not be representative of the whole district.
335 There may also be observer bias; although interpreters were blinded to parasitological and
336 demographical results, the high prevalence of schistosomiasis already known in the
337 Marolambo District makes it difficult to avoid this bias. Unmeasured and unknown confounding
338 factors may have influenced the results. Although sonographic evidence of periportal fibrosis
339 is characteristic of *S. mansoni* infection,¹⁰ possible confounding factors such as concomitant
340 infections and nutritional status of the children were not assessed during this study.²⁷ The
341 study was not adequately powered to assess for a relationship between schistosomiasis and
342 periportal fibrosis, and the low number of study participants with fibrotic changes should be
343 taken into consideration when interpreting statistical analyses. Interpretation of results is made
344 harder as many of these children received praziquantel approximately 12 months prior to this
345 study, but these specific individuals are not known. Population numbers and characteristics
346 are not well recorded in this area and investigation of this was beyond the scope of our study.
347 This however meant that the data could not be adjusted according to population density for
348 each village and limited our ability to understand the effect of the sociodemographic
349 characteristics of the participants' families on the results. Finally, misclassification of children

350 with or without periportal fibrosis may have skewed the data in either direction, though the
351 93% accuracy in quality assurance is reassuring.

352

353 **Conclusion**

354 A high prevalence of periportal fibrosis has been detected amongst children as young
355 as six years old in the Marolambo District, an area with a 98% prevalence of *Schistosoma*
356 *mansoni* infection. This is of global relevance as it may reflect similarly remote areas of
357 endemic countries which have not yet been studied and reinforces the importance of including
358 hard-to-reach areas in surveys for a true understanding of disease burden. We present a
359 method of using ultrasound to examine children in a particularly remote area that we hope
360 could be reproduced in other regions in need of morbidity surveys and outreach work.

361

362 **Authors' contributions:** HJR and ECJ conceived the study. HJR, JMStJP, CL, ECL, ALB,
363 JRS, DALR, EHA, LRM, EPR, AMR and SAS designed the study protocol. HJR, JMStJP and
364 CL carried out the ultrasound examination. HJR, JMStJP, CL, DALR, EHA, LRM, EPR, AMR
365 and SAS implemented the study. HJR and ECJ carried out interpretation of ultrasound
366 recordings. SAS carried out statistical analysis of the data. HJR and SAS drafted the paper.
367 HJR, JMStJP, CL, ACJ, ALB, JRS, DALR, EHA, LRM, EPR, AMR and SAS critically revised
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376

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385 **References**

- 386 1. King CH and Dangerfield-Cha M. The unacknowledged impact of chronic
387 schistosomiasis. *Chronic Illn* 2008;4(1):65–79
- 388 2. Colley DG, Bustinduy AL, Secor WE and King CH. Human schistosomiasis. *Lancet*
389 2014;383(9936):2253-2264
- 390 3. Homeida MM, Eltoum IA, Ali MM et al. The effectiveness of annual versus biennial
391 mass chemotherapy in reducing morbidity due to schistosomiasis: a prospective study
392 in Gezira-Managil, Sudan. *Am J Trop Med Hyg* 1996;54(2):140-5
- 393 4. Sircar AD, Mwinzi PNM, Onkanga IO, Wiegand RE, Montgomery SP and Secor WE.
394 *Schistosoma mansoni* mass drug administration regimens and their effects on
395 morbidity among schoolchildren over a 5-year period – Kenya, 2010-2015. *Am J Trop*
396 *Med Hyg* 2018;99(2):362-9
- 397 5. Berhe N, Myrvang B and Gundersen SG. Reversibility of schistosomal periportal
398 thickening/fibrosis after praziquantel therapy: a twenty-six month follow-up study in
399 Ethiopia. *Am J Trop Med Hyg* 2008;78(2):228-34
- 400 6. World Health Organisation. Schistosomiasis: progress report 2001-2011 and strategic
401 plan 2012-2020. Geneva, Switzerland: WHO, 2013
- 402 7. Niamey Working Group. Ultrasound in schistosomiasis: a practical guide to the
403 standardized use of ultrasonography for the assessment of schistosomiasis related
404 morbidity. Geneva, Switzerland: WHO, 2000

- 405 8. Mutapi F, Maizels R, Fenwick A and Woolhouse M. Human schistosomiasis in the post
406 mass drug administration era. *Lancet Infect Dis* 2017;17(2):e42-8.
- 407 9. El Scheich T, Holtfreter MC, Ekamp H et al. The WHO ultrasonography protocol for
408 assessing hepatic morbidity due to *Schistosoma mansoni*. Acceptance and evolution
409 over 12 years. *Parasitol Res* 2014;113:3915-25
- 410 10. Abdel-Wahab MF, Esmat G, Milad M, Abdel-Razek S and Strickland GT. Characteristic
411 sonographic pattern of schistosomal hepatic fibrosis. *Am J Trop Med Hyg*
412 1989;40(1):72-6
- 413 11. Ministère de la Santé Publique de Madagascar. Cartographie des Maladies Tropicales
414 négligées à Chimiothérapie préventive Schistosomiasis-geo Helminthiases-Filariose
415 Lymphatique. Ministère de la Santé Publique de Madagascar: Antananarivo; 2016
- 416 12. Spencer SA, Penney JMStJ, Russell HJ et al. High burden of *Schistosoma mansoni*
417 infection in school-aged children in Marolambo District, Madagascar. *Parasit Vectors*
418 2017;10(307):1-8
- 419 13. Doumenge JP, Mott KE, Cheung C et al. Atlas de la répartition mondiale des
420 schistosomiasis / Atlas of the global distribution of schistosomiasis: Talence, CEGET-
421 CNRS, Geneva, OMS / WHO; 1987
- 422 14. Abebe N, Erka B, Medhin G and Berhe N. Clinico-epidemiological study of
423 *Schistosomiasis mansoni* in Waja-Timuga, District of Alamata, northern Ethiopia.
424 *Parasit Vectors* 2014;7:158
- 425 15. Booth M, Vennervald BJ, Kabatereine NB et al. Hepatosplenic morbidity in two
426 neighbouring communities in Uganda with high levels of *Schistosoma mansoni* but
427 very different durations of residence. *Trans R Soc Trop Med Hyg* 2004;98(2):125-36
- 428 16. Mutengo MM, Mwansa JC, Meduluza T et al. High *Schistosoma mansoni* disease
429 burden in a rural district of western Zambia. *Am J Trop Med Hyg* 2014;91(5):965-72
- 430 17. Kaatano GM, Min DY, Siza JE et al. *Schistosoma mansoni*-related hepatosplenic
431 morbidity in adult population on Kome Island, Sengerema District, Tanzania. *Korean J*
432 *Parasitol* 2015;53(5):545-51

- 433 18. Kardorff R, Gabone RM, Mugashe C et al. *Schistosoma mansoni*-related morbidity on
434 Ukerewe Island, Tanzania: clinical, ultrasonographical and biochemical parameters.
435 Trop Med Int Health 1997;2(3):230-9
- 436 19. King CH, Magak P, Salam EA et al. Measuring morbidity in schistosomiasis mansoni:
437 relationship between image pattern, portal vein diameter and portal branch thickness
438 in large-scale surveys using new WHO coding guidelines for ultrasound in
439 schistosomiasis. Trop Med Int Health 2003;8(2):109-117
- 440 20. Nalugwa A, Nuwaha F, Tukahebwa EM and Olsen A. *Schistosoma mansoni*-
441 associated morbidity among preschool-aged children along the shores of Lake Victoria
442 in Uganda. Trop Med Infect Dis 2017;2(4):58
- 443 21. El Scheich T, Hofer L, Kaatano G, et al. Hepatosplenic morbidity due to *Schistosoma*
444 *mansoni* in schoolchildren on Ukerewe Island, Tanzania. Parasitol Res
445 2012;110(6):2515–20
- 446 22. Shen Y, Wiegand RE, Olsen A et al. Five-year impact of different multi-year mass drug
447 administration strategies on childhood *Schistosoma mansoni*-associated morbidity: a
448 combined analysis from the Schistosomiasis Consortium for Operational Research
449 and Evaluation Cohort Studies in the Lake Victoria regions of Kenya and Tanzania.
450 Am J Trop Med Hyg 2019;101(6):1336-44
- 451 23. Vennervald BJ, Kenty L, Butterworth AE et al. Detailed clinical and ultrasound
452 examination of children and adolescents in a *Schistosoma mansoni* endemic area in
453 Kenya: Hepatosplenic disease in the absence of portal fibrosis. Trop Med Int Health
454 2004;9(4):461–70
- 455 24. Doehring-Schwerdtfeger E, Kaiser C, Franke D et al. Inter-observer variance in
456 ultrasonographical assessment of *Schistosoma mansoni*-related morbidity in young
457 schoolchildren. Acta Trop 1992;51(1):85-8
- 458 25. Boisier P, Ramarokoto CE, Ravoniarimbina P et al. Geographic differences in
459 hepatosplenic complications of schistosomiasis mansoni and explanatory factors of
460 morbidity. Trop Med Int Health 2001;6(9):699–706

- 461 26. World Health Organization. Working to overcome the global impact of neglected
462 tropical diseases: first WHO report on neglected tropical diseases. Geneva,
463 Switzerland: WHO, 2010
- 464 27. Silva PC, Leal TV and Domingues AL. Treatment and education reduce the severity
465 of schistosomiasis periportal fibrosis. Rev Soc Bras Med Trop 2013;46(4):472-7
466

467 Legends

468 Figure A: A still image captured from a video clip interpreted as 'image pattern A'



469

470 Figure B: A still image captured from a video clip interpreted as 'image pattern B'



471

472 Figure C: A still image captured from a video clip interpreted as 'image pattern C'



473

474 Figure D: A still image captured from a video clip interpreted as 'image pattern A';
475 recorded when examining a child without *S. mansoni* infection (negative CCA and KK
476 testing)



477

478 Figure E: Association between age and sonographic evidence of periportal fibrosis;
479 image patterns A and B are interpreted as 'normal' and image pattern C is interpreted as
480 'periportal fibrosis'

481

482

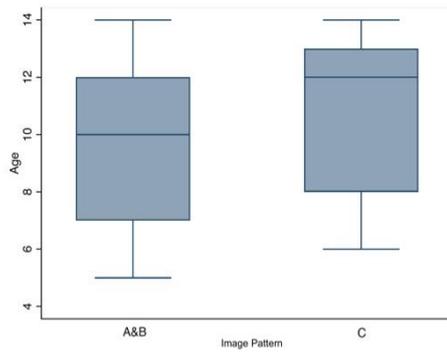


Table 1: Ultrasound findings compared to age, CCA results and KK results using logistic regression analyses

	Number of participants (%)	KK positive (%)	p	CCA positive (%)	p	Periportal fibrosis prevalence (%)	<i>P_{crude}</i>	OR (95%CI) _{crude}	<i>P_{adj}</i> *	OR (95%CI) _{adj} *
Overall	275	203 (73.8)		269 (97.8)		31(11.3)				
Age										
5	24 (8.7)	17 (70.8)		23 (95.8)		0 (0.0)				
6	27 (9.8)	20 (74.1)		25 (92.6)		3 (11.1)				
7	27 (9.8)	18 (66.7)		26 (96.3)		2 (7.4)				
8	27 (9.8)	19 (70.4)		27 (100.0)		4 (14.8)				
9	28 (10.2)	21 (75.0)		28 (100.0)		4 (14.3)				
10	29 (10.5)	20 (69.0)		29 (100.0)		1 (3.4)				
11	26 (9.5)	20 (76.9)		26 (100.0)		1 (3.8)				
12	28 (10.2)	23 (82.1)		27 (96.4)		5 (17.9)				
13	28 (10.2)	23 (82.1)		28 (100.0)		4 (14.3)				
14	31 (11.3)	21 (67.7)	NS	30 (96.8)	NS	7 (22.6)	0.03	1.16 (1.01 - 1.33)	0.03	1.17 (1.02 - 1.34)
Gender										
Female	141 (51.3)	105 (74.5)		138 (97.9)		17 (12.1)				
Male	134 (48.7)	98 (73.1)	NS	131 (97.8)	NS	14 (10.4)	NS	0.85 (0.40 - 1.80)	NS	0.87 (0.40 - 1.87)
Village			0.59		0.56		0.43		0.19	
<u>Marolambo</u>	45 (16.4)	32 (71.1)		45 (100.0)		9 (20.0)	ref	ref	ref	ref
<u>Ampasimbola</u>	38 (13.8)	29 (76.2)		38 (100.0)		4 (10.5)	0.24	0.47 (0.13 - 1.67)	0.29	0.50 (0.14 - 1.810)
<u>Ambohiteho</u>	45 (16.4)	33 (73.3)		42 (93.3)		5 (11.1)	0.25	0.50 (0.15 - 1.63)	0.16	0.43 (0.13 - 1.42)
<u>Marofatsy</u>	48 (17.4)	31 (64.6)		47 (97.9)		4 (8.3)	0.12	0.36 (0.10 - 1.28)	0.11	0.36 (0.10 - 1.27)
<u>Vohidamba</u>	50 (18.2)	40 (80.0)		49 (98.0)		6 (12.0)	0.29	0.55 (0.18 - 1.68)	0.29	0.54 (0.17 - 1.68)
<u>Betampona</u>	49 (17.8)	38 (77.5)		48 (98.0)		3 (6.1)	0.06	0.26 (0.07 - 1.03)	0.05	0.25 (0.06 - 1.01)

483

484

Table 2: Ultrasound findings compared to infection intensity category using logistic regression analyses

Infection Intensity	Number of participants (%)	Periportal fibrosis %	<i>P_{crude}</i>	OR (95%CI) _{crude}	<i>P_{adj}</i> *	OR (95%CI) _{adj} *
Negative	72 (26.2)	6 (8.3)				
Low	86 (31.3)	7 (8.2)				
Moderate	66 (24.0)	9 (13.6)				
High	51 (18.5)	9 (17.6)	NS	1.39 (0.97-1.98)	NS	1.38 (0.96-1.97)

*multiple logistic regression analyses controlling for the effect of age, village and gender
 NS: not significant ($p \geq 0.05$)

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