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

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

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

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

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

Keywords: Fibrosis, Liver, Madagascar, Schistosomiasis, Ultrasound.
Introduction

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

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

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

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

Performing ultrasound in many remote, endemic areas remains very challenging due to difficult access, lack of electrical power sources and lack of human resources (particularly
experienced ultrasound operators). Owing to these challenges, indirect indicators of morbidity are often used for monitoring. For example, the World Health Organisation (WHO) gives a morbidity control target of <5% prevalence of heavy-intensity infections (≥ 400 eggs per gram of stool).\textsuperscript{6}

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

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

\section*{Methods}

\subsection*{Study design and population}

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

Communities in the Marolambo District rely heavily on environmental water for drinking, cooking, washing and transport. Outside the main village of Marolambo, there is no
access to electrical power. Access to the villages upstream of Marolambo is via a single-track footpath which is impassable at certain times of the rainy season.

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

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

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

**Parasitological examination**

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

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

Two thick smears (containing 41.7mg of stool) were prepared from each stool sample using the Kato-Katz (KK; Vestergaard-Frandsen, Lausanne, Switzerland) method. Slides were prepared within six hours of receipt of the samples. Each slide was examined under light microscopy by one of four team members (with training and prior experience) to count, if
present, the number of *Schistosoma mansoni* eggs. The two slides from each sample were not interpreted by the same reader, and readers were blinded to the findings of others. Results of the two slides were used to calculate the mean number of eggs per slide. From this, the total number of eggs per gram (epg) was determined for each child.

Testing for *S. haematobium* was not performed as a cross-sectional study in these six villages in 2015 did not identify any *S. haematobium* infections in this area.\(^\text{12}\) Soil-transmitted helminths were not addressed in this study.

**Ultrasound examination**

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

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

**Ultrasound interpretation**
Interpretation of the ultrasound clips took place in the UK. A consultant radiologist (ECJ) and newly qualified doctor (HJR) interpreted the ultrasound clips of the first 15% of children together in order to train HJR. After this, HJR interpreted the remaining cases. Liver parenchyma were compared with standard image patterns included in an annex of the WHO protocol and assigned the letter it best aligned with. Image patterns A and B were considered normal, C-F corresponded to progressive degrees of periportal fibrosis, and Z was used for other abnormalities. Cases were only labelled with ‘image pattern C’ if there was clear thickening in the periphery of the parenchyma where portal branch walls should not normally be clearly visible with ultrasound. A random sample of the cases (10%) was second-read by the radiologist for quality assurance purposes. Interpreters were blinded to demographical and parasitological results.

**Health interventions and ethical considerations**

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

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

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

Results

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

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

Parasitological results

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

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

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

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

**Quality assurance of ultrasound interpretation**

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

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

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

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

Had it not been for the safety threat, we suspect that our research methods could have been applied in the district’s other villages. The kit was carried in rucksacks on foot, electrical equipment was charged by solar energy; the research methods can be reproduced in other
remote settings. However, the team recognise that, as in this district, there may be uncontrollable barriers preventing studies from taking place in some remote settings.

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

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

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

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

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

We present a method of performing ultrasound examination in this challenging setting: video clips recorded by relatively novice ultrasound operators using highly portable, solar-powered ultrasound devices and interpreted remotely. We returned to the UK with ultrasound images for interpretation and recognise that this is not a sustainable approach for ultrasound use on a larger scale to monitor morbidity associated with schistosomiasis. There are however possibilities to employ telemedicine to share images with central experts. In our study, the images were recorded by medical students who were able to record adequate clips for interpretation. Further work is needed to investigate the skill level required to obtain adequate
images, and also to even interpret images. If it is shown that minimal training is needed to assign image patterns accurately, perhaps ultrasound-based morbidity surveys could be carried out on a much larger scales by investigators requiring less training. This would lead to better mapping of morbidity in countries endemic for schistosomiasis and would guide national control programmes.

Limitations

There are a number of limitations to our study. The sample was selected from the school register meaning that non-school-attending children were excluded from the study. School attendance is affected by severe schistosomiasis and therefore our sample of school-attenders may be an underrepresentation of the true prevalence of periportal fibrosis in this area. The six villages involved in our study may not be representative of the whole district. There may also be observer bias; although interpreters were blinded to parasitological and demographical results, the high prevalence of schistosomiasis already known in the Marolambo District makes it difficult to avoid this bias. Unmeasured and unknown confounding factors may have influenced the results. Although sonographic evidence of periportal fibrosis is characteristic of *S. mansoni* infection, possible confounding factors such as concomitant infections and nutritional status of the children were not assessed during this study. The study was not adequately powered to assess for a relationship between schistosomiasis and periportal fibrosis, and the low number of study participants with fibrotic changes should be taken into consideration when interpreting statistical analyses. Interpretation of results is made harder as many of these children received praziquantel approximately 12 months prior to this study, but these specific individuals are not known. Population numbers and characteristics are not well recorded in this area and investigation of this was beyond the scope of our study. This however meant that the data could not be adjusted according to population density for each village and limited our ability to understand the effect of the sociodemographic characteristics of the participants’ families on the results. Finally, misclassification of children
with or without periportal fibrosis may have skewed the data in either direction, though the
93% accuracy in quality assurance is reassuring.

Conclusion

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

Authors’ contributions: HJR and ECJ conceived the study. HJR, JMStJP, CL, ECL, ALB,
JRS, DALR, EHA, LRM, EPR, AMR and SAS designed the study protocol. HJR, JMStJP and
CL carried out the ultrasound examination. HJR, JMStJP, CL, DALR, EHA, LRM, EPR, AMR
and SAS implemented the study. HJR and ECJ carried out interpretation of ultrasound
recordings. SAS carried out statistical analysis of the data. HJR and SAS drafted the paper.
HJR, JMStJP, CL, ACJ, ALB, JRS, DALR, EHA, LRM, EPR, AMR and SAS critically revised
the manuscript. All authors read and approved the final manuscript. HJR and SAS are
guarantors of the paper.

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References


Legends

Figure A: A still image captured from a video clip interpreted as ‘image pattern A’

Figure B: A still image captured from a video clip interpreted as ‘image pattern B’

Figure C: A still image captured from a video clip interpreted as ‘image pattern C’

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

Figure E: Association between age and sonographic evidence of periportal fibrosis; image patterns A and B are interpreted as ‘normal’ and image pattern C is interpreted as ‘periportal fibrosis’
Table 1: Ultrasound findings compared to age, CCA results and KK results using logistic regression analyses

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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masciama</td>
<td>45 (16.4)</td>
<td>32 (71.1)</td>
<td>0.0</td>
<td>45 (100.0)</td>
<td>0.0</td>
<td>9 (20.0)</td>
<td>0.43</td>
</tr>
<tr>
<td>Ampasimbola</td>
<td>38 (13.8)</td>
<td>29 (76.2)</td>
<td>0.0</td>
<td>38 (100.0)</td>
<td>0.0</td>
<td>4 (10.5)</td>
<td>0.24</td>
</tr>
<tr>
<td>Andohalo</td>
<td>45 (16.4)</td>
<td>33 (73.3)</td>
<td>0.0</td>
<td>42 (93.3)</td>
<td>0.0</td>
<td>5 (11.1)</td>
<td>0.25</td>
</tr>
<tr>
<td>Maroantsetra</td>
<td>48 (17.4)</td>
<td>31 (64.6)</td>
<td>0.0</td>
<td>47 (97.9)</td>
<td>0.0</td>
<td>4 (8.3)</td>
<td>0.12</td>
</tr>
<tr>
<td>Vohidanga</td>
<td>50 (18.2)</td>
<td>40 (80.0)</td>
<td>0.0</td>
<td>49 (98.0)</td>
<td>0.0</td>
<td>6 (12.6)</td>
<td>0.29</td>
</tr>
<tr>
<td>Betampona</td>
<td>49 (17.8)</td>
<td>38 (77.5)</td>
<td>0.0</td>
<td>48 (98.0)</td>
<td>0.0</td>
<td>3 (6.5)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Infection Intensity</th>
<th>Number of participants (%)</th>
<th>Perinatal fibrinolysis (%)</th>
<th>OR (95% CI) OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>72 (26.2)</td>
<td>6 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>86 (31.3)</td>
<td>7 (8.2)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>66 (24.0)</td>
<td>9 (13.6)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>51 (18.5)</td>
<td>9 (17.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Multiple logistic regression analyses controlling for the effect of age, village and gender
NS: not significant (p>0.05)