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Liver morbidity due to *Schistosoma mekongi* in Cambodia after seven rounds of mass drug administration

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Summary Severe liver disease due to *Schistosoma mekongi* was frequent in northern Cambodia. Between 1995 and 2002, seven rounds of mass chemotherapy (praziquantel) reduced infection from 50% to below 3%. In 2002, we assessed hepatosplenic morbidity by historical, clinical and ultrasonographic investigations in adults (older than 14 years) from endemic ($n = 342$) and non-endemic ($n = 103$) areas (Kratie province). Clinical hepatomegaly (25 vs. 0%), splenomegaly (55 vs. 0%), reported blood in stool (41 vs. 20%) and abdominal pain (78 vs. 57%) were significantly higher in the endemic area. In this area, significantly more subjects reported a family history of death due to schistosomiasis (12 vs. 0%); 63% (vs. 0%) reported having at least three treatments of praziquantel in previous years; and only 11% (vs. 99%) had normal liver ultrasonographic examination. Periportal fibrosis with portal hypertension was diagnosed in 46% (vs. 0%) of people in this area; 18% (vs. 0%) and 5% (vs. 0%) of portal hypertension was classified as moderate and severe, respectively. People aged between 24 and 35 years were mostly affected. There was no gender difference. The pathology in the endemic district is most probably residual morbidity of *S. mekongi* infections. Contributions of co-infections (hepatitis) cannot be excluded. Careful monitoring of the affected communities is required.

1. Introduction

Schistosoma mekongi infection may lead to severe intestinal, hepatosplenic and cerebral morbidity. Mortality due to this parasite was shown to be dramatic in non-treated transmission areas (Biays et al., 1999; Urbani et al., 2002), occurring mostly through oesophageal bleeding. Recently, a

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case of cerebral infection has been described (Houston et al., 2004).

In 1968, the first cases of *S. mekongi* originating from Kratie, Cambodia, were diagnosed (Audebaud et al., 1968; Tournier-Lasserve et al., 1970). In the subsequent period of war and political instability, follow-up field investigations in the province of Kratie were not possible. Ongoing transmission led to a high degree of morbidity and mortality in the districts of Sambo and Kratie, situated in the northern part of Kratie province.

In 1994, approximately 10 patients with severe schistosomiasis were hospitalized each month in the district hospital of Sambo and more than 150 affected patients were treated in the outpatient clinic (Biays et al., 1999). The clinical picture of the severe cases included advanced hepatosplenomegaly, ascites and other signs of portal hypertension. Death due to spleen rupture or oesophageal bleeding was frequently observed in Sambo District Hospital and Kratie Provincial Hospital and was often reported from the communities.

In 1995, the entire population of three villages of Sambo district (Samerong, Chatnaol and Achen) was examined. Forty-nine per cent were clinically diagnosed with hepatomegaly, reaching as high as 90% in children aged 10—14 years and 60% in young adults aged 15—19 years (Stich et al., 1999). In addition, in a sample from 20 primary schools in the districts of Sambo and Kratie, the prevalence of infection and hepatomegaly in school children reached 70 and 90%, respectively (Stich et al., 1999).

In the same year, a control programme based on annual chemotherapy was started. The intervention targeted the schistosomiasis-endemic districts of Sambo and Kratie populated by approximately 60 000 people. Up to 2002, seven annual mass drug administration campaigns were carried out in which all individuals (except children <2 years and pregnant women) were treated with praziquantel (40 mg/kg body weight) in combination with mebendazole (500 mg). The annual coverage of treatment was between 62 and 74% (Sinuon et al., 2007).

Over the control programme period, between 1995 and 2002, monitoring surveys showed that liver and intestinal morbidity was reduced. Clinical manifestations, such as hepatomegaly and splenomegaly, and/or reports of blood and mucus in stool have been drastically reduced in schoolchildren in four sentinel villages, between 1995 and 2002, as have the numbers of schistosomiasis patients diagnosed in the health facilities in the endemic area (Urbani et al., 2002). The infection rates were monitored annually by stool analysis of schoolchildren in four sentinel villages. The data showed a marked decline in infection from more than 60% to less than 3% (Ohmae et al., 2004). Health promotion activities and the provision of safe water sources led to general improvements in the target communities. Overall, the intervention was very successful with regard to infection and severe morbidity control (Sinuon et al., 2007).

Ultrasonography is used to assess liver morbidity due to *Schistosoma* infection (Richter et al., 2003). Guidelines for standardised ultrasonographic assessment are available (WHO, 2000) and allow the evaluation of large-scale control programmes (Hatz, 2001). Periportal fibrosis and portal hypertension are the primary indicators for the disease. Thus ultrasonography has been used to assess *S. japonicum* foci (Hatz et al., 1992), but only very few data are available for *S. mekongi*-endemic areas.

The objective of the present study conducted in 2002 was to measure the prevalence of liver morbidity attributable to *S. mekongi* in the endemic area of Sambo district following seven rounds of annual mass drug administration, by comparing it with signs, symptoms and measures of pathology in the non-endemic untreated area of Chhlong district. The study was conducted using family histories, clinical examinations and ultrasonographies.

2. Materials and methods

The study was carried out in the province of Kratie in May and June 2002. A cross-sectional sample survey was conducted in four schistosomiasis-endemic villages of Ampil Teuk, Kampong Krabei, Tongsaong Thleak and Sambo (Table 1) in the district of Sambo (district population: 39 402 habitants in 2002), in which in 1994, during a baseline survey prior to control activities, a high prevalence of liver morbidity was found (Stich et al., 1999). Households were identified by random selection from the list of households provided by the village chief. All subjects older than 14 years and present on the survey day were enrolled.

Chryothmar village (Table 1), located on the shore of the Mekong and capital of the Chhlong district (district population in 2002: 47 919 habitants), was chosen as a comparison. Enrolment procedures were the same as in the Sambo district.

Table 1 Population and study sample

District	Village	Population	Sample			
			n	% female	Age range (years)	Mean age (years)
Sambo	Tongsaong Thleak	1634	72	68.1	15—69	34.8
	Kampong Krabei	662	94	62.8	14—55	31.2
	Ampil Teuk	1063	96	58.3	15—53	29.1
	Sambo	2245	80	60.0	15—55	29.3
Subtotal		5604	342	62.0	14—69	30.9
Chhlong	Chryothmar	3614	103	56.3	16—63	37.0
Total		9218	445	60.7	14—69	32.3

Chhlong district in the southern part of Kratie province is only marginally affected by *S. mekongi* transmission. It is considered a non-endemic area. The river ecology is profoundly different. Sandy shores prevail and are unfavourable for the development of the intermediate snail host *Neotricula aperta*, which requires rocky underground material (Attwood et al., 2004; Ohmae et al., 2004). *Schistosoma mekongi* infections have rarely been reported in this district and a sero-epidemiological survey, using *S. japonicum* antigens, showed that fewer than 20% of the schoolchildren tested positive (Ohmae et al., 2004). In contrast, the districts of Sambo and Kratie had a seroprevalence of more than 95%. Consequently, no treatment campaigns were performed in Chhlong district.

All enrolled people were interviewed for a history of haematemesis, bloody stool, abdominal pain and diarrhoea in the past 2 weeks, family history of deaths due to schistosomiasis and the number of treatments with praziquantel. Subsequently, each subject underwent a directed clinical examination for the presence of hepatomegaly, splenomegaly and shunt circulation, ascites and jaundice. Hepatomegaly was scored by palpating the left liver lobe along the xiphoid-umbilicus line in supine position. Hepatomegaly was defined as absent when the left liver lobe was not palpable and as present when the left liver lobe was palpable under the xiphoid. The presence of ascites was assessed by percussion and fluid thrill. Spleen enlargement was registered using the Hackett score (Hackett, 1944).

Liver pathology was assessed with an ultrasonographic examination following the Niamey protocol (WHO, 2000) by using a Fukuda Denshi UF-4000 (scan sectorial FUT-C111A 3,5 MHz 60R convex probe) portable ultrasonography machine. We assessed the score of image patterns of the liver parenchyma (IP score), the height-standardised score for periportal thickening (PT score) and periportal hypertension (PH score). PH score consisted of the sum of the height-standardised portal vein diameter (portal vein score: 0, 4 or 6), the presence of collateral veins (collateral score: 0 or 4) and the presence of ascites (ascites score: 0 or 3). The final score consisted of the IP score, PT score and the PH score. Liver surface, shape of the caudal liver edge and height-standardised lengths of the right and left liver lobes were assessed but reported independently of the scoring results.

We aimed to enrol 82 adults per village, which allowed for estimating a factor of unknown prevalence ($P = 0.5$) with a precision of 0.15 (power 80%, alpha 0.05) at the village level and 0.08 for all the four villages in the *S. mekongi*-endemic area ($n = 328$). All data were entered into EpiInfo version 6.04 (CDC, Atlanta, GA, USA). Data were analysed with STATA version 8.2 (Stata Corp., College Station, TX, USA). Excess risk was calculated for the *S. mekongi*-endemic area by subtracting prevalence of absolute risks from the endemic area minus the non-endemic area and exact 95% confidence intervals were calculated (95% CI ER). For data on the portal hypertension score and the final score of the endemic district, an ordered logistic regression was used to test whether age groups and gender independently contribute to the observed morbidity pattern. The data were grouped in four age groups: 14—24 years, 25—34 years, 35—44 years and 45 years and older.

The study followed the ethical conduct defined in the guidelines of the Declaration of Helsinki (1964). All patients gave their informed oral consent before being included in the study.

3. Results

A total of 445 people was enrolled in the study (Sambo district 342, Chhlong district 103). Details of the sample are given in Table 1. In Sambo district 34.2, 33.3, 18.7, 13.7 and 1.5% fell into the 14—24, 25—34, 35—44 and 45 years and older age group, respectively, while in Chhlong district 18.5, 23.3, 29.1 and 29.1% of the subjects were in the respective age groups. The median age in Sambo district was 30 years, significantly younger than in Chhlong district (37 years, $P < 0.001$). There was no difference in mean age between the sexes, or sex ratio between the two districts ($P = 0.3$).

The socio-economic status of the examined population of Sambo was significantly lower than Chhlong district. A significantly smaller proportion of the study participants in Sambo district attended secondary school (14.6 vs. 33.0%, $P < 0.001$, controlled for age group). Most of the studied Sambo population were rice farmers, whereas this activity was rare in the studied village of Chhlong district (79.8 vs. 9.7%).

Clinical examination revealed schistosomiasis-related morbidity in Sambo district (Table 2). Hepatomegaly (24.3%), splenomegaly (54.7%) and clinical signs of portal hypertension such as ascites (1.2%) and collateral veins (2.0%) were seen only in people in the schistosomiasis-endemic district. Jaundice was diagnosed in three patients (1.2%). Blood in stool and abdominal pain was reported significantly more often in Sambo than in Chhlong district ($P < 0.001$), while no difference was recorded for diarrhoea.

Twelve per cent of people in the district of Sambo reported to have lost at least one family member due to schistosomiasis in recent years compared with no reports in the non-endemic district of Chhlong.

In Sambo district, at least three treatments with praziquantel during mass drug administration were reported by 62.5% of the people and 21.9% reported to have been treated six or seven times. No treatment reports were recorded in Chhlong district.

Ultrasonographic investigations revealed major liver pathology in the schistosomiasis-endemic district of Sambo, while very little pathology was found in Chhlong district (Table 3). In almost half the examined subjects (45.5%), we diagnosed advanced periportal fibrosis with portal hypertension. In Sambo district, more than one-fifth of the subjects (23.1%) had moderate or severe portal hypertension (PH score) and periportal vein thickening was prevalent. In Sambo, 40% of subjects showed highly echogenic ruff areas around the portal bifurcation and the main stems of the liver parenchyma. In addition, we frequently diagnosed severe fibrotic liver parenchyma, consisting of high echogenic patches extending from the main portal vein and branches into the parenchyma (23% of subjects). No reticular patterns of liver morbidity were identified.

Analysis of the liver morbidity scores in Sambo district revealed a similar pattern in all villages and in both

Table 2 Results of the history and clinical examination of 445 study participants

	Sambo ($n = 342$) %	Chhlong ($n = 103$) %	Excess risk in endemic area	
			%	95% CI
History				
Haematemesis	5.9	1.0	4.8	1.8—8.0
Blood in stool (past 2 weeks)[a]	40.6	20.4	20.2	10.9—29.6
Abdominal pain (past 2 weeks)[a]	77.5	57.3	20.2	9.7—30.7
Diarrhoea (past 2 weeks)[b]	44.4	35.9	8.5	2.1—19.2
Family death due to schistosomiasis				
None	88.0	100	—	—
1 person	7.6	0	7.6	4.8—10.4
2 or 3 people	4.4	0	4	2.2—6.6
Praziquantel treatment				
0	7.0	100	—	—
1—2 times	30.4	0	30.4	25.5—35.3
3—5 times	40.6	0	40.6	35.4—45.8
6—7 times	21.9	0	21.9	17.5—26.3
Clinical examination				
Hepatomegaly (present)	24.3	0	—	19.7—28.8
Splenomegaly (Hackett score)[c]				
0	45.3	100	—	—
1	12.6	0	12.6	9.1—16.1
2—3	32.1	0	32.1	27.2—37.1
4—5	9.9	0	9.9	6.8—13.1
Ascites (present)	1.2	0	1.2	0.03—2.3
Collateral veins (present)	2.0	0	2.0	0.5—3.5
Jaundice (present)	1.2	0	1.2	0.03—2.3

[a] Significant difference between districts at $P < 0.001$, controlled for age.
[b] Significant difference between districts at $P < 0.05$, controlled for age.
[c] See Hackett (1944).

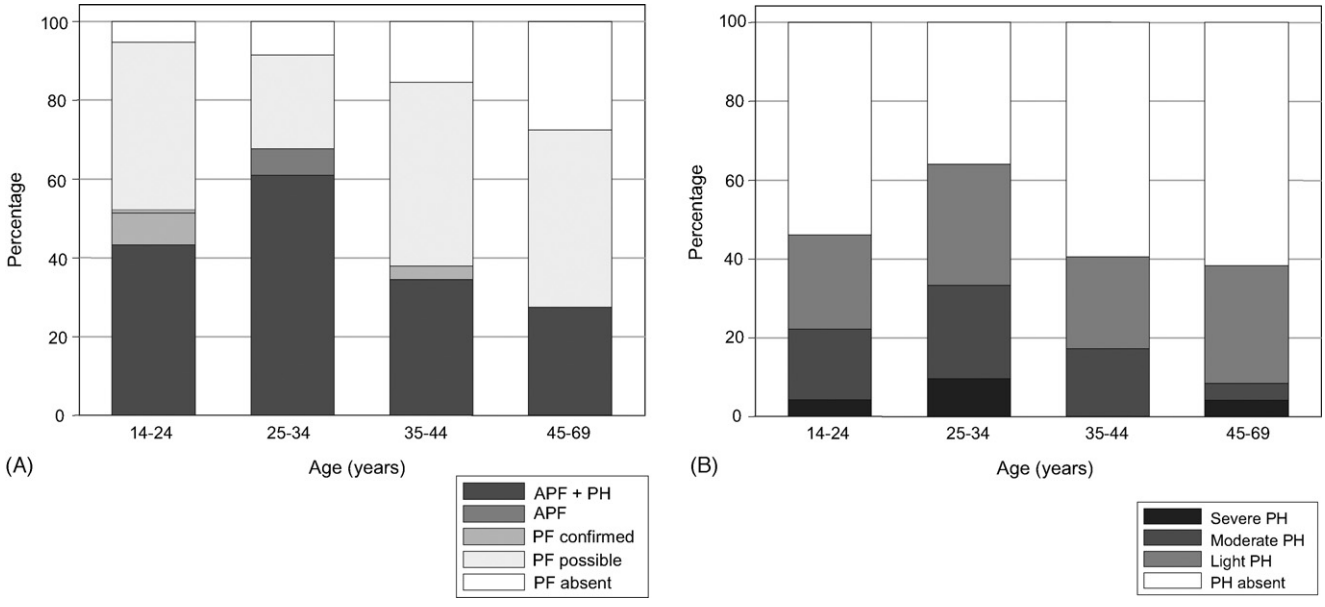

Figure 1 (A) Final score of ultrasonography assessment by age in Sambo district ($n = 342$; PF: periportal fibrosis; APF: advanced PF; PH: portal hypertension). (B) Portal hypertension (PH) score by age in schistosomiasis-endemic district of Sambo ($n = 342$).

Table 3 Results of ultrasonographic examinations in Sambo and Chhlong districts

	Sambo ($n = 342$) %	Chhlong ($n = 103$) %	Excess risk in endemic area	
			%	95% CI
Final score[a]				
No PF	11.2	99.0	–	
PF possible	37.3	1.0	36.2	30.5–40.9
PF confirmed	1.0	0	1.0	0.1–2.0
APF	5.1	0	5.1	2.7–7.5
APF + portal hypertension	45.5	0	45.5	40.0–50.0
PH score (grouped)				
0 normal	50.0	95.1	–	–
4 light	26.9	4.9	22.0	15.8–28.3
6–8 moderate	17.8	0	17.8	13.8–21.9
10–13 severe	5.3	0	5.3	2.9–7.6
PT score				
0 normal	0.9	1.9	–	–
1 slightly increased	86.6	98.1	–	–
4 increased	10.2	0	10.2	7.0–13.4
8 much increased	2.3	0	2.3	0.7–3.9
IP score[b]				
0	0.9	1.9	–	–
1	10.8	97.1	–	–
2	24.6	1.0	23.6	18.7–28.5
4	39.5	0	39.5	34.3–44.7
6	22.5	0	22.5	18.1–26.9
8	1.8	0	1.8	0.4–3.1
Liver surface (frontal)				
Regular	6.4	98.1	–	–
Lightly nodular	74.0	1.9	72.0	66.7–77.4
Nodular	19.6	0	19.6	15.4–23.8

PF: periportal fibrosis; APF: advanced PF; PH: portal hypertension; PT: periportal vein thickening; IP: image pattern.
[a] $n = 412$ (92.6% of total); for 33 patients final score could not be established.
[b] Short description of IP score: 0, normal; 1, diffuse foci; 2, ring echoes; 4, ruff zones around portal bifurcation and main stem; 6, patches extending from main portal vein and branches to parenchyma; 8, bands and streaks extending from main portal vein and its bifurcation to liver surface.

sexes. However, substantial age differences were observed. The population between 25 and 34 years of age was mostly affected. Of them, 61.0 and 64.4% suffered from advanced periportal fibrosis with portal hypertension and portal hypertension, respectively, while in other age groups corresponding rates were much lower (Figure 1).

Ordered multiple regression analysis showed that age group rather than gender contributes to the advanced periportal fibrosis and portal hypertension. Subjects aged 25 to 34 had a 1.75-fold risk of an increased final score compared with the youngest age group, controlled for sex and age group (95% CI 1.04–2.94, $P = 0.033$). Similar results were obtained for the portal hypertension score. Subjects aged 25 to 34 years had double the risk for portal hypertension compared with the youngest age group, controlled for sex and age group (OR = 2.02, 95% CI 1.24–3.29, $P = 0.005$). The risks for portal hypertension for the older age groups were not significantly different from those of the 14–24 year olds (OR = 0.76, 95% CI 0.42–1.38, $P = 0.368$ and OR = 0.64, 95% CI 0.33–1.24, $P = 0.187$ for subjects aged 35–44 and 45–69 years, respectively).

4. Discussion

Our study reveals a high degree of sub-clinical liver morbidity, reaching a prevalence of more than 60% of advanced periportal fibrosis and portal hypertension in young adults (aged 25–34 years) in the district of Sambo, Kratié Province, Cambodia. Younger and older age groups were also affected, but substantially less so. Furthermore, very little liver pathology could be detected in the district of Chhlong, suggesting that the observed morbidity in Sambo district was an excess morbidity attributable to *S. mekongi* infections.

In 1995, in Sambo district, left liver lobe enlargement was clinically observed in high frequencies in the communities (Biays et al., 1999). In the hospitals of this province these observations were confirmed. Furthermore, the consequences of severe portal hypertension, such as ascites and oesophageal bleeding, were frequent and led to a great number of casualties. In 1998, 106 chronic patients with portal hypertension were identified. Eleven were eligible for surgical decompression procedures in order to decrease portal hypertension (Dumurgier et al., 2006).

Histological liver examinations of six of these patients revealed a surprisingly high degree of fibrosis (Monchy et al., 2006). No similar observations were made in the Chhlong district.

Sub-clinical liver morbidity could not be assessed in 1995. Therefore, no data are available for a direct comparison with our study. However, an ulrasonographic assessment was performed in 1997 in a *S. mekongi*-endemic village in the Stung Treng province (north of Kratie province). Of 223 subjects, 73% were found positive for *S. mekongi*, and periportal thickening and/or portal vein enlargement was diagnosed in 84% (Urbani et al., 2002). Therefore, it is reasonable to assume that sub-clinical morbidity was present in comparable frequencies for 1995 in the Kratie province.

Undoubtedly, between 1994 and 2002, the situation has much improved in terms of infection and morbidity (Sinuon et al., 2007). The prevalence of clinically observed hepatomegaly has dropped from over 60% to an undetectable level in annually examined sentinel primary schools (Ohmae et al., 2004). Our study diagnosed hepatomegaly in 25% of our subjects. In 1994, in comparable age groups, more than 80% had the same finding.

Based on the high number of mass drug administrations we assumed that most *S. mekongi* infections were cured. Consequently, the question arises whether the observed subclinical liver morbidity is indeed a residual part of the *S. mekongi* infection-induced pathology in the population, or provoked by other factors such as hepatitis B (HBV) and C (HCV) infections. Hepatitis infections may lead to hepatic reticular lesions of similar ultrasonographic appearance (Kardorff et al., 1999). As a community survey in 1991 indicated that 8.0% of the Cambodian population was hepatitis B surface antigen positive and 6.5% was anti-HCV positive (Thuring et al., 1993), these infections could have potentially contributed to our morbidity findings. Furthermore, Cambodia has one of the world's highest overall injection usages (Vong et al., 2005), suggesting a high rate of overuse and unsafe injection practices, which may lead to these infections. Therefore, a contribution of hepatitis viruses to the observed liver morbidity cannot be excluded. However, we believe that a major part of this liver pathology is attributable to *S. mekongi* infection, for the following reasons.

Firstly, we observed liver pathology in a high prevalence of 46%. In 1998, in an attempt to select chronic schistosomiasis patients for surgical treatment, 106 severe schistosomiasis cases were examined, 30% of whom were found positive for HBV or HCV antigen (19% HBV, 9% HCV, 2% both; Dr Muth Sinuon, National Malaria Centre, Phnom Penh, personal communication). In a sample of 421 individuals in Kratie province, Chigusa and colleagues found 20.2% positive HBV (Chigusa et al., 2006). Such a prevalence could be expected in our study population. But most probably it was lower because subjects in our study were not sick and therefore had less frequent contact with a health facility compared with the chronic severe cases of schistosomiasis. Hence, our study population was at lower risk for hepatitis infection.

Secondly, hepatitis infections provoke hepatic reticular lesions, mainly at the late stage of the disease. Therefore, one would expect to find liver pathology in older individuals. However, in our study we found pathology predominantly in young adults (aged 25—34 years) and adolescents (aged 14—24 years).

Thirdly, a 2-year cohort study of liver morbidity due to *S. japonicum* showed that after a single treatment with praziquantel, sub-clinical liver morbidity was significantly reduced (Li et al., 2000), but even after 5 years follow-up the resolution was not complete (Li et al., 2002). It was concluded that pathological changes around the portal tree might regress to a large degree but that 'network pattern' abnormalities do not normally resolve (Richter, 2000). A comparable finding was obtained in a 2-year cohort study in Stung Treng province in Cambodia for *S. mekongi* between 1997 and 1999 (Hatz, 2001). While at the start of the cohort only 18.1% of subjects had a normal liver ultrasonographic assessment (pattern A or B of Niamey protocol), after 2 years 81.6% did not reveal pathological liver lesions (Urbani et al., 2002). But, in all patients, the resolution of liver morbidity was incomplete. Based on these findings, liver pathology is to be expected in the *S. mekongi*-endemic areas.

Finally, very little pathological finding was found in Chhlong district, where *S. mekongi* is rare. We would expect that with a high rate of injection drug use, and a resulting high risk for hepatitis, at least some hepatic lesions should have been found. This, however, was not the case.

A surprising finding was that a substantial number of subjects in Chhlong district reported blood in stool. Serological studies showed that some infections with *S. mekongi* were present in this district, which explains these reports to some extent (Ohmae et al., 2004). In addition, other infections, such as by *Strongyloides* spp., might have contributed to the observation. Furthermore, mis-reporting cannot be excluded.

Our study followed the recommendations on the morbidity assessment procedures for Asian schistosomiasis established by WHO expert committee in Phnom Penh (Cambodia, 2002) and Yueyang (Hunan Province, China, 2005) (WHO, 2007). The study suffers from epidemiological weaknesses. The sampling procedures resulted in a female-dominated sample population, as many men were away in the rice fields. The sample was not proportional to the population size, and no information could be obtained from non-participating individuals. Furthermore, the districts could not be matched for other prognostic variables. These factors limit the strength of the conclusions that can be drawn about the general population. In addition, due to lack of resources we were not able to perform laboratory investigations such as stool examinations for *S. mekongi* and serological analysis for hepatitis virus infection.

Nonetheless, our study demonstrates that a substantial amount of sub-clinical morbidity is present in a schistosomiasis-endemic district, which was probably to a large extent residual morbidity due to *S. mekongi*. The strength of our study is that we included a control group from a non-endemic district and demonstrated the amount of sub-clinical morbidity in a community where little active *S. mekongi* infection is present. It furthermore showed that gross clinical morbidity was rare, providing further evidence for a successful intervention. Careful follow-up and monitoring are required, and necessary action needs to be taken to maintain and consolidate gains from preceding control efforts.

Authors' contributions: PO, HK, SOB and CH designed the study protocol; HK carried out assessments under the supervision of PO and SC; HK, PO and AD analysed and interpreted the data; HK wrote the draft manuscript. All authors contributed to and approved the final version. PO, CH and AD are guarantors of the paper.

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Ethical approval: Ministry of Health and the provincial health department of Kratie province, Cambodia.

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