



Full length article

Improved latrines minimally impact *Schistosoma mekongi* transmission in Mekong islandsYouthanavanh Vonghachack^{a,b,c}, Peter Odermatt^{a,b}, Jürg Utzinger^{a,b}, Somphou Sayasone^{d,*}^a Swiss Tropical and Public Health Institute, Allschwil, Switzerland^b University of Basel, Basel, Switzerland^c Faculty of Basic Sciences, University of Health Sciences, Vientiane Capital, Lao People's Democratic Republic^d Lao Tropical and Public Health Institute, Ministry of Health, Vientiane Capital, Lao People's Democratic Republic

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ABSTRACT

Schistosoma mekongi and other intestinal helminth infections remain public health concerns in Lao People's Democratic Republic, especially in remote areas where access to sanitation is limited.

We performed an experimental study in four villages where latrine construction was coupled with two rounds of mass drug administration (MDA) with praziquantel and albendazole, and compared with two control villages that only received two rounds of MDA. The prevalence of helminth infections before (baseline) and after (follow-up) intervention were compared. Additionally, the prevalence in intervention and control villages were compared 12 months post-intervention. Kato-Katz, formalin-ethyl acetate concentration and Baermann techniques were employed to assess helminth infections.

We found infection prevalence of *S. mekongi* in the intervention and control villages was 28.6% and 1.8%, respectively. The prevalences of other helminth infections were as follows: *Opisthorchis viverrini*, 79.5% and 71.8%; hookworm, 48.8% and 65.6%; and *Strongyloides stercoralis*, 43.1% and 38.3%. Other helminth species were detected in less than 5% of the study participants. Latrine intervention coupled with two rounds of MDA in the intervention villages reduced the prevalence of *S. mekongi* infection by 6.0% (from 28.6% to 22.6%; $P < 0.001$), *O. viverrini* infection by 11.3% (from 79.5% to 68.2%; $P < 0.001$), hookworm infection by 22.6% (from 48.8% to 26.2%; $P < 0.001$), and *S. stercoralis* infection by 12.0% (from 43.1% to 31.1%; $P < 0.001$). The observed reductions were not significantly different when compared to the control villages, where only two rounds of MDA were implemented ($P > 0.05$). Study participants in both groups commonly engaged in behaviours such as open defecation, bathing in the Mekong River, consuming raw or undercooked fish dishes and walking barefoot. These practices and behaviours are associated with helminth infections. Concluding, this study showed only a marginal impact associated with latrine use in intervention communities. There is a need for longer term studies with integrated interventions, such as effective health education to foster behavioural changes related to open defecation, raw or undercooked food consumption, wearing protected footwear outdoors, and personal hygiene.

1. Background

Access to adequate sanitation facilities and their proper utilization play a crucial role in maintaining hygienic conditions within a community. Open defecation practices increase the risk of contracting numerous communicable diseases, including helminthiasis [1]. Helminth infections remain a considerable public health problem in areas with no or only limited sanitation, particularly in low- and middle-income countries

(LMIC) [2–4]. Helminth infections might impair physical and cognitive development of children and lead to chronic illness in childhood [5,6].

In Lao People's Democratic Republic (Lao PDR), sanitation coverage has improved in recent years. According to recent data from the Ministry of Health, the coverage of sanitation facilities and their proper use was recorded at 82% nationwide in 2022 [7,8]. This coverage, however, was slightly below the national target of 83.2% and there is considerable heterogeneity between provinces. While Vientiane Capital reported a

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complete 100% coverage, other provinces ranged between 87.2% (Champasack) and 47.8% (Phongsaly) [8]. At the onset of the current implementation study (2011), the overall coverage of latrine was 56.0% in Khong district and 40.6% in the communities where the current intervention was conducted [9,10]. This low coverage is a key factor linked to the high prevalence of helminth infections, including cases attributed to *Schistosoma mekongi*, within the Khong district [11,12].

This study aimed to assess the impact of improved sanitation practices on helminth infection prevalence in villages on islands in Khong district in the southern part of Lao PDR. Particular emphasis was placed on schistosomiasis, opisthorchiasis, soil-transmitted helminthiasis and strongyloidiasis. We used a rigorous parasitological diagnostic approach, including Kato–Katz, formalin-ethyl acetate concentration, and Baermann methods on multiple stool samples to assess infections in baseline and follow-up cross-sectional surveys. The helminth prevalence in a control village, where only mass drug administration (MDA) was employed, was compared with that in intervention villages that benefitted from both MDA and latrine construction.

2. Materials and methods

2.1. Study area and population

Khong district encompasses numerous Mekong islands, situated in the southernmost region of the Champasack province. This district is an endemic area for schistosomiasis caused by *Schistosoma mekongi* [11–13]. For decades, the Ministry of Health employed community-based chemotherapy with praziquantel to control schistosomiasis transmission [14]. However, *S. mekongi* remains endemic on some islands [15]. Furthermore, *Opisthorchis viverrini* is highly endemic within the communities of Khong district [11,12]. Chronic infections caused by *O. viverrini* lead to severe hepatobiliary diseases including, cholangiocarcinoma, a fatal bile duct cancer [16,17]. In addition, soil-transmitted helminths (mainly hookworm) and *Strongyloides stercoralis* are highly prevalent [9,18]. As open defecation practices are common in the endemic communities, the control and elimination of these helminths is difficult. This study was carried out from March 2011 to January 2013 on three islands; namely, Donlong, Donthan, and Donlieng. The islands are located in the Mekong River basin in Khong district, Champasack province, in the southern part of Lao PDR. Donlong island consists of four villages (Hualong, Longsong, Longkang, and Hanglong), where the intervention was implemented. The control villages (Donthan and Donlieng) are located on the other two islands (one village on each island). The study area and population have been described in detail elsewhere [9]. All study islands are endemic for *S. mekongi*. The selected villages had a very low coverage of households with latrines (40.6%) at the onset of this study [9]. Twenty to 30 households were randomly selected from each village, utilizing a random sampling approach based on a household registry at the village office. In each enrolled household, all members aged 2 years and above were invited to participate.

2.2. Intervention

We pursued an experimental pre- and post-intervention assessment, incorporating a single control group, to investigate the impact of latrine implementation on helminth infection, placing particular emphasis on *S. mekongi*, *O. viverrini*, hookworm, and *S. stercoralis*. We employed a household-based promotion of latrine construction approach to maximize the latrine coverage in the intervention villages. First, a cross-sectional baseline survey was carried out in March 2011 in intervention and control villages to assess helminth infections as well as people's knowledge, attitudes, and practices (KAP) related to latrine use, personal hygiene, and raw food consumption. After the baseline survey, all participants aged > 4 years in the intervention and control villages received praziquantel (single 40 mg/kg oral dose) and albendazole (single 400 mg oral dose) treatment, representing the first MDA [19]. Second, each

household in the intervention villages committed to completing its latrine construction within 9 months after the first MDA. The project subsidized the pit lining, septic tank, and slab installation, while household members provided labour for construction. After latrine construction, all individuals in the intervention and control villages received the second MDA, with praziquantel and albendazole. In this phase, those infected with *S. stercoralis* were treated with a single 200 µg/kg dose of ivermectin [20,21]. Finally, a follow-up survey was carried out 12 months after the second MDA, using the same methodology as in the baseline survey.

2.3. Field and laboratory procedures

For both baseline and follow-up surveys, the same parasitological methods were employed. Two stool samples were collected within a 5-day period from each study participant and examined using the Kato–Katz and Baermann techniques to detect helminth infections [21, 22]. The procedures of the Kato–Katz and Baermann preparations have been described in detail by Vonghachack et al. [9]. Laboratory technicians prepared a 41.7 mg Kato–Katz thick smear for each stool sample and examined it under a light microscope within 1 hour of preparation. Any helminth eggs observed during the microscopic examination were counted and recorded separately for each species. Furthermore, approximately 5 g of stool was divided from each sample for the Baermann test. This stool sample was placed on a gauze-lined mesh in a glass funnel equipped with a rubber tube and a clamp and covered with de-chlorinated tap-water. After 2 hours, the water (approximately 50 ml) was centrifuged for 5 min, and the sediment was examined under a light microscope for the presence of *S. stercoralis* larvae (Rhabditiform larvae).

2.4. Questionnaire survey

Two questionnaires were employed to collect information at the individual and household levels. An individual questionnaire was utilized to obtain data on demographics and KAP of latrine utilization and hygiene. Other behavioural risk factors related to helminth infections, such as raw or undercooked food consumption (i.e., fish, pork, beef and vegetables), walking barefoot and bathing in the Mekong River were also documented. The research team utilized a household questionnaire to interview the head of each study household about the house building materials (i.e., floor, wall and roof), owned assets (i.e., generator, battery, radio, television, refrigerator, motorcycle, truck and engine boat), owned agricultural land and owned livestock (i.e., cow, buffalo, goat and pig) for household socio-economic classification.

2.5. Data management and analysis

The collected data were double entered by two data clerks using the EpiData software, version 3.1 (Epidata Association; Odense, Denmark). Validation was performed to detect discrepancies and correct any entry errors. The validated data were transferred to STATA, version 14 (Stata Corporation; College Station, TX, USA) for analysis. Only study participants who submitted four stool samples (two samples at baseline and two at follow-up) and completed all study processes were included in the final analysis.

The study participants were categorised into six age groups: (i) ≤5 years, (ii) 6–11 years, (iii) 12–17 years, (iv) 18–35 years, (v) 36–59 years, and (vi) ≥60 years. The socioeconomic status of the households was assessed using a household asset-based approach proposed by the World Bank [23], as described in detail elsewhere [11,24]. In summary, principal component analysis was employed to construct the socioeconomic index based on the household's building materials, owned assets, owned agricultural land, and owned livestock. Following this, the study households were categorized into five socioeconomic quintiles, with the first quintile corresponding to the least wealthy households and the fifth quintile representing the wealthiest.

Frequency was used to describe the characteristics of the study participants and the prevalence of helminth infections in the baseline and follow-up assessments. The χ^2 test was used to compare the prevalence of helminth infections, KAP, and risky behaviours between participants in the intervention villages and those in the control villages. The McNemar test was used to compare the observations between the baseline and follow-up assessments. A two-independent sample *t*-test was used to compare each parasite's mean egg count per gram of stool (EPG) between the intervention and control groups, and a paired *t*-test was used to compare the mean EPG between baseline and follow-up. A *P*-value smaller than 0.05 was considered statistically significant.

3. Results

3.1. Demographic characteristics of participants

Complete parasitological and questionnaire data were obtained from 510 of 1,128 enrolled individuals from 247 households (Fig. 1). Of these, 283 and 227 individuals belonged to the intervention and control villages, respectively. Women constituted 52.9% of the study participants,

with 51.9% from the intervention villages and 54.2% from the control group. There was no significant difference in sex distribution between the intervention and control groups ($P = 0.614$). The age range of the participants was 2–95 years with a mean age of 29.9 years and 36.7 years for intervention and control villages, respectively. Farming and fishing were the main occupations for both the intervention (60.8%) and control (73.6%) groups. The socioeconomic analysis showed that 21.9% of households in the intervention group and 16.9% in the control group were classified in the lowest wealth quintiles ($P = 0.820$) (Table 1).

3.2. Effects of intervention

Parasitological analysis showed that *S. mekongi* was significantly more prevalent in the intervention villages than in the control villages at baseline (28.6% vs. 1.8%, $P < 0.001$) and follow-up (22.6% vs. 2.6%, $P < 0.001$). *O. viverrini* infection was also more prevalent in the intervention than in the control villages at baseline (79.5% vs. 71.8%, $P = 0.043$) and follow-up (68.2% vs. 59.9%, $P = 0.052$). In contrast, hookworm infection prevalence was significantly lower in the intervention villages than in the control villages at baseline (48.8% vs. 65.6%,

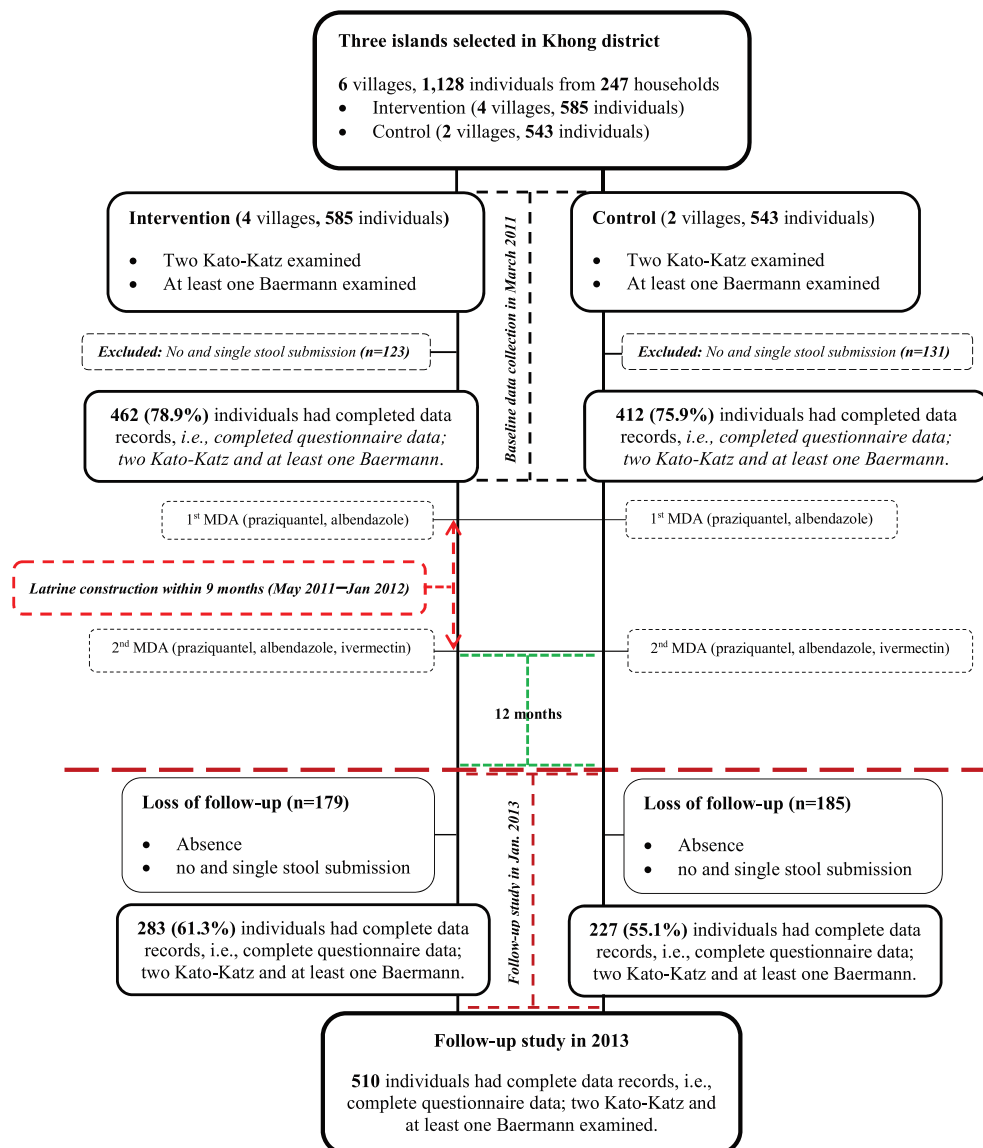


Fig. 1. Study diagram.

Table 1
Baseline characteristics of study participants in the intervention and control villages.

Characteristic	Overall, % (n)	Study areas		χ^2	P-value
		Intervention, % (n)	Control, % (n)		
n	510	283	227		
Sex					
Female	52.9 (270)	51.9 (147)	54.2 (123)	0.30	0.614
Male	47.1 (240)	48.1 (136)	45.8 (104)		
Age group (years)					
≤5	6.5 (33)	8.8 (25)	3.5 (8)	17.7	0.003
6–11	21.0 (107)	25.1 (71)	15.9 (36)		
12–17	8.6 (44)	8.8 (25)	8.5 (19)		
18–35	32.2 (164)	30.7 (87)	33.9 (77)		
36–59	19.8 (101)	17.7 (50)	22.5 (51)		
≥60	12.0 (61)	8.8 (25)	15.9 (36)		
Age (years)					
Mean (95% CI) *	32.9 (31.1–34.8)	29.9 (27.5–32.4)	36.7 (33.9–39.5)	–3.58	< 0.001
Educational level					
Pre-schooler	9.0 (46)	12.7 (36)	4.4 (10)	24.3	< 0.001
Illiterate	7.2 (37)	8.1 (23)	6.2 (14)		
Primary school	56.1 (286)	59.0 (167)	52.4 (119)		
High school and above	27.7 (141)	20.1 (57)	37.0 (84)		
Occupation					
Preschool child	9.0 (46)	12.7 (36)	4.4 (10)	20.3	< 0.001
Farmer/fisherman	66.5 (339)	60.8 (172)	73.6 (167)		
Primary school student	18.2 (93)	21.9 (62)	13.7 (31)		
High school student	6.3 (32)	4.6 (13)	8.4 (19)		
Socioeconomic status					
Poorest	20.3 (28)	19.2 (14)	21.5 (14)	1.5	0.820
Very poor	20.3 (28)	17.8 (13)	23.1 (15)		
Poor	19.6 (27)	21.9 (16)	16.9 (11)		
Less poor	20.3 (28)	19.2 (14)	21.5 (14)		
Least poor	19.6 (27)	21.9 (16)	16.9 (11)		

* P-value obtained from two independent sample t-test < 0.05, comparing intervention and control villages.

$P < 0.001$) and follow-up (26.2% vs. 38.3%, $P = 0.003$). *S. stercoralis* infection was similar in both groups at baseline (43.1% vs. 38.3%, $P = 0.275$) and follow-up (31.1% vs. 34.8%, $P = 0.375$). Other helminth species were found at prevalences of less than 5% in the intervention and control villages (Table 2).

3.3. Effects of latrine intervention

A comparison of the four helminthiasis of public health concern (i.e. *S. mekongi*, *O. viverrini*, hookworm, and *S. stercoralis*) between intervention and control villages after 1 year of the latrine intervention is shown in Table 3. Overall, *S. mekongi* infection decreased by 6.0% from 28.6% to 22.6% ($P = 0.046$) in the intervention villages, whereas it increased by 0.8% from 1.8% to 2.6% in the control villages. For *O. viverrini* infections, a similar prevalence was observed in both intervention and control villages. Hookworm infection was significantly reduced by 22.6% from 48.8% to 26.2% ($P < 0.001$), and *S. stercoralis* decreased by 12.0% from 43.1% to 31.1% ($P < 0.001$) when compared to the baseline for the intervention villages; however, these reductions were not statistically significant when compared with those of the control villages ($P > 0.05$).

Regarding the helminth infection intensities, the mean faecal egg counts for *S. mekongi* was significantly higher in the intervention than in the control villages at baseline (66.1 EPG vs. 0.7 EPG) and follow-up

Table 2
Prevalence of *Schistosoma mekongi*, *Opisthorchis viverrini*, soil-transmitted helminths, *Strongyloides stercoralis* and other helminth infections among study participants in the intervention (n = 283) and the control villages (n = 227) at baseline and follow-up studies.

Helminth parasites	Intervention, % (n)	Control, % (n)	χ^2	P-value
<i>Schistosoma mekongi</i>				
At follow-up	22.6 (64)	2.6 (6)	42.41	< 0.001
At baseline	28.6 (81)	1.8 (4)	65.44	< 0.001
<i>Opisthorchis viverrini</i>				
At follow-up	68.2 (193)	59.9 (136)	3.80	0.052
At baseline	79.5 (225)	71.8 (163)	4.10	0.043
Hookworm				
At follow-up	26.2 (74)	38.3 (87)	8.65	0.003
At baseline	48.8 (138)	65.6 (149)	14.83	< 0.001
<i>Strongyloides stercoralis</i>				
At follow-up	31.1 (88)	34.8 (79)	0.82	0.375
At baseline	43.1 (122)	38.3 (87)	1.20	0.275
<i>Trichuris trichiura</i>				
At follow-up	1.1 (3)	3.1 (7)	2.77	0.101
At baseline	3.5 (10)	1.8 (4)	1.53	0.224
<i>Ascaris lumbricoides</i>				
At follow-up	2.5 (7)	1.8 (4)	0.36	0.583
At baseline	0.3 (1)	0.4 (1)	0.02	0.876
<i>Enterobius vermicularis</i>				
At follow-up	0.4 (1)	1.3 (3)	1.50	0.218
At baseline	0	0	–	–
<i>Taenia</i> spp.				
At follow-up	0.4 (1)	0.4 (1)	0.02	0.876
At baseline	0	0.9 (2)	2.56	0.114

Table 3
Impact of latrine intervention on four helminthiasis with public health concerns, such as *Schistosoma mekongi*, *Opisthorchis viverrini*, hookworm, and *Strongyloides stercoralis* by study groups.

Helminth	Intervention, n = 283	Control, n = 227
<i>Schistosoma mekongi</i>		
At follow-up, %	22.6 (64) **	2.6 (6) **
At baseline, %	28.6 (81)	1.8 (4)
% of the difference (95% CI)	–6.0 (–11.7; –0.3)	+0.8 (–1.5; 3.2)
P-value*	0.046	0.754
<i>Opisthorchis viverrini</i>		
At follow-up, %	68.2 (193)	59.9 (136)
At baseline, %	79.5 (225)	71.8 (163)
% of the difference (95% CI)	–11.3 (–16.4; –6.2)	–11.9 (–15.3; –8.6)
P-value*	0.001	0.006
Hookworm		
At follow-up, %	26.2 (74) **	38.3 (87) **
At baseline, %	48.8 (138)	65.6 (149)
% of the difference (95% CI)	–22.6 (–28.7; –16.5)	–27.3 (–32.9; –21.7)
P-value*	< 0.001	< 0.001
<i>Strongyloides stercoralis</i>		
At follow-up, %	31.1 (88)	34.8 (79)
At baseline, %	43.1 (122)	38.3 (87)
% of the difference (95% CI)	–12.0 (–18.0; –6.0)	–3.5 (–9.8; 2.8)
P-value*	< 0.001	0.439

* P-value obtained from the McNamara test.

** P-value < 0.005, obtained from the Chi-square test.

(44.8 EPG vs. 0.6 EPG) ($P < 0.05$). For *O. viverrini* and hookworm, faecal egg count reductions were similar in both intervention and control villages (Table 3).

3.4. KAP for latrine, personal hygiene, and raw food consumption

At baseline, latrine availability was 34.3% in the intervention villages and 44.9% in the control villages ($P = 0.014$). Table 4 displays the availability of latrine, KAP of study participants toward latrine use, and behaviours including consumption of raw or undercooked food in the intervention and control villages one year after the completion of the intervention.

Table 4

Impact of latrine intervention on the intensity (EPG) of *Schistosoma mekongi*, *Opisthorchis viverrini* and hookworm infections among study participants in the intervention and control villages.

Helminth infections	Intervention, n = 283 Mean (95% CI)	Control, n = 277 Mean (95% CI)
<i>Schistosoma mekongi</i>		
At follow-up, EPG	44.8 (12.9; 76.6) *	0.6 (0.1; 1.2) *
At baseline, EPG	66.1 (39.2; 93.0) *	0.7 (-0.1; 1.5) *
Differences, EPG	-21.3 (-61.2; 18.5)	-0.1 (< -0.1; 1.5)
t	-1.05	-0.23
P-value **	0.293	0.819
<i>Opisthorchis viverrini</i>		
At follow-up, EPG	743.9 (477.5; 1010.3) *	260.0 (120.4; 399.6) *
At baseline, EPG	852.1 (627.5; 1076.8)	585.7 (386.5; 784.9)
Differences, EPG	-108.3 (-420.8; 204.3)	-325.7 (-476.4; -174.9)
t	-0.68	-4.26
P-value **	0.496	< 0.001
Hookworm		
At follow-up, EPG	113.5 (62.6; 164.4)	179.7 (83.6; 275.8)
At baseline, EPG	330.5 (196.5; 464.6)	521.3 (347.6; 694.9)
Differences, EPG	-217.1 (-334.9; -99.2)	-341.6 (-526.6; -156.6)
t	-3.63	-3.64
P-value **	< 0.001	< 0.001

* P-value obtained from two independent sample t-test < 0.05, comparing intervention and control villages.

** P-value obtained from paired t-test < 0.005, comparing follow-up and baseline study.

EPG: egg counts per gram of stool.

In the intervention villages, households without latrines constructed, achieving complete coverage at 100% (+65.7%). Meanwhile, latrine availability in the control villages remained unchanged. Study participants in both groups exhibited minimal awareness regarding the preventive benefits of latrine use. Only 9.9%, 3.2%, 13.4%, and 24.7% of the study participants in the intervention villages and 0.9%, 1.3%, 11.9%, and 24.7% in the control villages agreed that using latrines could protect them from *S. mekongi*, *O. viverrini*, soil-transmitted helminth, and diarrhoeal infections, respectively. Open defecation practices decreased by 46.3% in the intervention villages compared to only 4.8% in the control villages ($P < 0.001$). Approximately 15.9% of study participants in the intervention group and 3.1% in the control group perceived that the water availability in their villages posed a problem for latrine use ($P < 0.001$). Study participants widely practiced risky behaviours that could lead to helminth infections such as consuming raw or undercooked beef (5.7% and 4.0%), fish (36.6% and 39.2%) and vegetables (89.4% and 96.9%) within the last 7 days in the intervention and control villages, respectively. Daily bathing in the Mekong River was very common (93.3% and 99.1% of participants in the intervention and control villages, respectively). Walking barefoot was reported by 16.3% and 12.8% of the participants in the intervention and control villages, respectively (Table 5).

4. Discussion

Helminth infections pose a public health concern in communities residing on the islands of Khong district, Champasack province in the southern part of Lao PDR. In this study, we piloted a latrine intervention and determined its effect on the prevalence and intensity of helminth infection. Specifically, we compared the infection rates detected before and after the campaign to build and use latrines in the intervention villages, to those in the control group where no such campaign was held. Inhabitants of all six study villages (four intervention and two control villages) received MDA twice against *S. mekongi*, *O. viverrini* (using praziquantel), and soil-transmitted helminthiases (using albendazole). MDA coverage was more than 85% in the entire study population. For *S. stercoralis*, only the study participants whose stool analysis was positive for *S. stercoralis* larvae were treated with ivermectin.

Table 5

Changes in latrine availability, knowledge, attitude, and practices of study participants in the intervention and control villages.

Variables	Intervention, n = 283 (n)	Control, n = 227 % (n)	χ^2	P-value
Latrine availability				
At follow-up	100.0 (283)	44.9 (102)	206.43	<0.001
At baseline	34.3 (97)	44.9 (102)	6.01	0.014
% of changed	+65.7	0		
What diseases can be prevented by latrine use?				
<i>Schistosoma mekongi</i>				
At follow-up	9.9 (28)	0.9 (2)	18.5	<0.001
At baseline	13.1 (37)	6.2 (14)	6.68	0.010
% of changed	-3.2	-5.3		
<i>Opisthorchis viverrini</i>				
At follow-up	3.2 (9)	1.3 (3)	1.89	0.169
At baseline	2.8 (8)	7.9 (18)	6.78	0.009
% of changed	+0.4	-6.6		
<i>Soil-transmitted helminth</i>				
At follow-up	13.4 (38)	11.9 (27)	0.07	0.792
At baseline	11.3 (32)	10.6 (24)	0.27	0.606
% of changed	+2.1	+1.3		
<i>Diarrheal diseases</i>				
At follow-up	24.7 (70)	24.7 (56)	0.01	0.986
At baseline	23.7 (67)	33.9 (77)	6.53	0.011
% of changed	+1.0	-9.2		
Did you practice open defecation for the last time? YES				
At follow-up	19.4 (55)	50.7 (155)	55.27	<0.001
At baseline	65.7 (186)	55.5 (126)	5.54	0.019
% of changed	-46.3	-4.8		
Was the water a problem with your latrine use? YES				
At follow-up	15.9 (45)	3.1 (7)	5.77	0.017
At baseline	5.2 (12)	8.8 (20)	0.54	0.493
% of changed	+10.7	-5.7		
Washing hands with soap after your last defecation, YES				
At follow-up	20.9 (59)	18.1 (41)	0.62	0.431
At baseline	17.1 (48)	11.0 (25)	3.63	0.057
% of changed	+3.8	+7.1		
Washing hands with soap before your last meal, YES				
At follow-up	7.4 (21)	3.5 (8)	3.57	0.059
At baseline	7.7 (22)	4.0 (22)	3.20	0.074
% of changed	-0.3	-0.5		
Eating raw/undercooked beef dishes within the past seven days, YES				
At follow-up	5.7 (16)	4.0 (9)	0.77	0.380
At baseline	2.1 (6)	2.2 (5)	0.01	0.949
% of changed	+3.6	+2.2		
Eating raw/undercooked fish dishes within the past seven days, YES				
At follow-up	36.6 (103)	39.2 (89)	0.42	0.515
At baseline	29.0 (82)	37.9 (86)	4.53	0.033
% of changed	+7.6	+1.2		
Eating any raw vegetables within the past seven days, YES				
At follow-up	89.4 (253)	96.9 (220)	10.57	0.001
At baseline	91.2 (258)	97.4 (221)	8.46	0.004
% of changed	-1.8	-1.5		
Daily taking a bath in the Mekong River, YES				
At follow-up	93.3 (264)	99.1 (225)	10.85	0.001
At baseline	95.1 (269)	98.7 (224)	5.14	0.023
% of changed	-1.8	+0.4		
Observed walking barefoot currently, YES.				
At follow-up	16.3 (46)	12.8 (29)		
At baseline	19.1 (54)	14.1 (32)	3.67	0.055
% of changed	-2.8	-1.3		

In the baseline survey, the most common helminth species detected in the study participants were *S. mekongi*, *O. viverrini*, hookworm, and *S. stercoralis*; the respective prevalences were 28.6%, 79.5%, 48.8% and 43.1% in the intervention villages, and 1.8%, 71.8%, 65.6% and 38.3% in the control villages. These observed infection prevalences confirmed the high endemicity of helminth infections in these communities [9,11,12].

We conducted a follow-up 12 months after the completion of the intervention. Our findings showed a significant reduction in the prevalence of *S. mekongi*, *O. viverrini*, hookworm and *S. stercoralis* infection compared to the baseline. We attributed this reduction to the MDA campaign, which cleared the infections and reduced the prevalence and

intensity of infections in the initial stage of its implementation, particularly in the endemic areas with a high infection burden similar to the communities included in our study [25,26]. Surprisingly, at follow-up, we found high infection rates of *S. mekongi* (22.6%), *O. viverrini* (68.2%), hookworm (26.2%), and *S. stercoralis* (31.8%) in the intervention villages, which were counted as re-infections. These infection rates were not significantly different from that of the control group, except that for *S. mekongi*. This observation might indicate that the parasites were circulating extensively in the environment in this region and that latrine use in the communities could not immediately reduce transmissions. Additionally, we only tested the effect of promoting latrine use in controlling helminth infections, which were highly prevalent in the communities. Hence, the observed impact of the intervention was far from our expectations. Previous studies have proven that an integrated control package, including improving access to adequate sanitation, clean water, and hygienic conditions, is effective against helminth and intestinal protozoan infections [4,27–29]. Adding these activities to the current intervention might significantly increase the impact on controlling helminth infections in the study communities. Moreover, low awareness, inappropriate attitudes, and practices towards these infections are key risk factors for most cases of acquiring helminth infections [30].

The findings of our study revealed that less than one-sixth of the study participants believed that latrine use could prevent helminth transmission in their communities. Interestingly, about one-fifth of the study participants in the intervention villages continued open defecation practices, although latrine coverage reached 100% in their villages. This practice contributed to the increased risk of contracting hygienic-related infections [3]. The continuation of open defecation can be attributed to various factors. In our study villages, most residents are farmers or fishermen who depart their homes early in the morning for agricultural tasks in the rice fields or fishing in the Mekong River. This compels them to resort to open defecation outside their households. Nonetheless, the rate of open defecation witnessed a significant decline in the intervention group when the results of baseline and follow up assessment for the intervention and control groups were compared. Almost all the participants from both groups bathed in the Mekong River, which exposed them to *S. mekongi* infections [11]. Over one-third of the study participants in both groups consumed raw or undercooked fish dishes, making them susceptible to *O. viverrini* infections [31,32]. Every sixth study participant in both groups walked barefoot to the research station on the survey day, posing a risk of contracting hookworm and *S. stercoralis* infections [9,18]. These factors might contribute to the high re-infection rates observed in our current study in the intervention and control groups. Effective health education campaigns aiming at behavioural changes, increasing disease awareness, and improving proper practices might decrease a significant fraction of the re-infections in these endemic communities.

Our study has some methodological limitations. Firstly, this study did not apply a randomized, community-based controlled trial to test latrine utilization in controlling helminth infections. Hence, the similarity of internal and external variability between intervention and control groups could not be verified. Secondly, our study villages were deliberately selected from three islands within the endemic area, considering the historical prevalence of *S. mekongi*. However, this approach might not reflect the current status of the infections, potentially resulting in differences in prevalence between study groups. *S. mekongi* was highly prevalent in the intervention group compared to the control group. Third, our diagnostic approach consisting of Kato–Katz examination of multiple stool samples did not allow to distinguish between *O. viverrini* and minute intestinal fluke infections as the egg structure are very similar. Hence, some of the eggs reported as *O. viverrini* might be minute intestinal flukes. Finally, we only included participants who submitted four stool samples (two in the baseline and two in the follow-up study). This resulted in a considerable number of noncompliant participants, which might have affected the statistical significance of the data analysis.

5. Conclusions

The introduction of latrines has a limited short-term impact on controlling *S. mekongi*, *O. viverrini*, hookworm, and *S. stercoralis* infections within the intervention villages when compared with the control group. However, two rounds of MDA significantly reduced the prevalence and intensity of helminth infections compared to those in the baseline study. Study participants in both groups exposed themselves to considerable risks of helminth infections by practicing open defecation, consuming raw or uncooked fish dishes, walking barefoot when leaving home, and bathing in the Mekong River. Therefore, adding an effective health education campaign to the current intervention, aiming at behaviour changes, and increasing awareness of disease prevention might lead to significant reduction in helminth infections in these endemic areas.

Author contributions

Conceptualization, investigation, methodology, formal analysis, validation, investigation, writing – original draft: SS, and YV; methodology, writing – review & editing: PO and JU; funding acquisition: PO and JU.

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Institutional review board statement

Approval to conduct the study was granted by the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Lao PDR. A letter describing the study, its purpose, methods, potential risks, benefits of participation, and the protection of confidentiality was given to all eligible participants.

Informed consent statement

All study participants and parents or legal guardians of children below the age of 18 years consented and all children (above five years) assented to take part in the study. All infections diagnosed were treated with a single dose of albendazole (400 mg) and praziquantel (40 mg/kg) according to the Lao national treatment guidelines [19]. Those infected with *S. stercoralis* were treated with a single 200 µg/kg dose of ivermectin, free of charge [20,21].

Data availability statement

Data are available at the Lao TPHI and fully assessable to collaborators' institutions and all co-authors. Data will be shared with other institutions and researchers upon reasonable request.

Conflict of interest

Authors declare that there is no any conflict of interest.

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Abbreviations

CI	confidence interval
EPG	eggs per gram of stool
FECT	formalin-ethyl acetate concentration technique
Lao PDR	Lao People's Democratic Republic
MDA	mass drug administration

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