Running head: Mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel against *Opisthorchis viverrini*

# A randomized, exploratory open-label trial on the efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel against *Opisthorchis viverrini*

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## Summary

**Background** A single drug, praziquantel, is available for the treatment of *Opisthorchis viverrini* infections. In vivo studies point to an activity of mefloquine, artesunate and tribendimidine against this liver fluke.

**Methods** In a randomized, exploratory open-label trial, the efficacy (cure rate [CR] and egg reduction rate [ERR]) and safety of mefloquine (25 mg/kg), artesunate (10 mg/kg as 3 split doses within 12 h)**,** mefloquine-artesunate (100 mg artesunate plus 250 mg mefloquine once daily for 3 consecutive days), and tribendimidine (200 or 400 mg single dose) compared to praziquantel (75 mg/kg in 2 divided doses) were studied against *O. viverrini* in 125 schoolchildren in Attapeu Province, Lao PDR.

**Results** Tribendimidine and praziquantel achieved CRs of 70.4% and 56.0%, respectively on the basis of intention to treat analysis. The corresponding ERRs were 99.3% and 98.4%. No or only very moderate effects were observed with mefloquine, artesunate and mefloquine-artesunate against *O. viverrini* (CRs: 0-4.2% and ERRs 30.2-41.3%). Children treated with tribendimidine experienced only mild and transient adverse events such as, headache, vertigo, nausea and fatigue. The most frequent adverse events, many of which were serious, were dizziness, nausea, vertigo, vomiting and headache were mainly experienced among those patients treated with mefloquine and mefloquine-artesunate.

**Interpretation** Tribendimidine is not only efficacious against various intestinal nematodes but also against *O. viverrini*. Large-scale clinical trials are warranted once additional preclinical studies for drug registration outside China will have been completed.



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## Introduction

Opisthorchiasis is a neglected tropical disease caused by the liver fluke *Opisthorchis viverrini*, which affects the poorest people in Northeastern parts of Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia and Vietnam.[1,2] An estimated 67.3 million people are at risk and 9 million are infected.[1] In Lao PDR, the highest prevalence rates (50% in school children and up to 90% in adults) of *O. viverrini* have been reported from villages adjacent to the Mekong River, particularly in the Southern and central provinces.[3,4] Though most human opisthorchiasis cases are asymptomatic, chronic infection with *O. viverrini* can cause obstructive jaundice, ascending cholangitis, cholecystitis, gallstones, hepatomegaly and an enhanced risk for cholangiocarcinoma.[5-7] Cholangiocarcinoma is a serious and fatal complication, incurable in the advanced stage, hence early diagnosis and treatment is imperative.[6,8-10]

Morbidity control through periodic treatment with praziquantel is the key control strategy for opisthorchiasis.[11,12] Praziquantel is the only available drug so that if resistance does develop to it, there will be no active drug left unless other treatments are developed. We have recently reported that the antimalarials artemether, artesunate (2 semisynthetic derivatives of artemisinin), mefloquine and the Chinese anthelminthic drug tribendimidine have interesting opisthorchicidal properties in rodents.[13,14] For example, administration of artesunate and artemether at a dose of 400 mg/kg to *O. viverrini*-infected hamsters resulted in worm burden reductions of 78.4% and 65.5%, respectively.[13,14] Similarly, high worm burden reductions were reported with a

single 300-mg/kg oral dose of mefloquine against juvenile and adult *O. viverrini* in vivo.[15] Finally, a 400 mg/kg oral dose of tribendimidine achieved a worm burden reduction of 95.7% in *O. viverrini* infected hamsters.[14]

The aim of the present study was to assess the efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine compared to the treatment of choice, praziquantel in patients with a parasitologically confirmed *O. viverrini* infection.

## Patients and methods

### Study site and population

The study was carried out in the Saysetha district, Attapeu province, Lao PDR, from February to April 2010. Attapeu Province has a total area of 10,320 $km^2$ and is the most south-easterly province of Lao PDR. A previous study showed *O. viverrini* prevalence rates of 21% among primary school children in Attapeu.[4] The province has approximately 93,000 inhabitants. The majority belong to the ethnic group of Lao Theung and is mainly engaged in subsistence rice cultivation.[16] The Saysetha upper and lower secondary school was selected for our study, where a total of 957 secondary schoolchildren were enrolled during the academic year 2008-2009. A preliminary survey showed that in this school the *O. viverrini* infection prevalence was higher than 50% (personal communication Mr. Thongsom, Provincial Hospital Attapeu).

### Study design

The study was designed as a randomized, exploratory open-label trial to assess the efficacy and safety of mefloquine, artesunate, mefloquine-artesunate and tribendimidine against *O. viverrini* infection among schoolchildren compared to the standard praziquantel treatment regimen. The sample size was based on a suggested sample size of 12 patients / group for proof-of-concept trials recommended by Julious.[17,18] To account for drop outs we aimed at 20-25 children per group.

On day 21-22 post-treatment we assessed the cure rate [CR], defined as the percentage of the children excreting eggs before treatment but in whom no eggs were found when reexamined and egg reduction rate [ERR], defined as the group's reduction of geometric mean [GM] egg output after treatment divided by the GM of the same patients pretreatment, multiplied by 100.

**Study procedures**

One week before the baseline screening survey, the National Institute of Public Health, Centre of Malaria, Parasitology and Entomology, Centre for Laboratory and Epidemiology, and the Provincial Department of Health and the Provincial Hospital of Attapeu, as well as the teachers were informed about the study objectives, procedures, benefits and potential risks. Overall, 214 schoolchildren aged between 10 to 15 years were invited to participate and the children and parents were asked to provide written informed consent. From each consenting and participating child at least 2 stool samples were collected within 5 consecutive days. Children with a parasitologically confirmed *O. viverrini* infection

(at least 2 of 4 slides positive), underwent a full clinical examination, including measurement of weight (using an electronic balance measuring to the nearest 0.1 kg, and axillary temperature using battery-powered thermometers to the nearest 0.01°C). In addition, a finger prick blood sample was taken from each child for a rapid malaria test (Paracheck Pf®) and a urine sample from all females for pregnancy testing (Quick-Check® hCG pregnancy test). Clinical malaria was defined as fever (axillary temperatures ≥ 37.5°c) and parasitaemia ≥ 100/microL.[19] Exclusion criteria included (i) presence of clinical malaria, (ii) pregnancy, (iii) presence of any abnormal medical condition (iv) history of any acute or severe chronic disease, (v) psychiatric and neurological disorders, (vi) use of artesunate, artemether, any artemisinin-based combination chemotherapy (ACT), mefloquine, or any anthelminthic treatment within the past month, and (vii) weight below 20 kg. Consenting children, who met all study criteria, were randomly assigned to one of the 5 different treatments using a computer-generated randomization code.

**Drugs and adverse events**

Mefloquine (Mephaquine® 250-mg/lactab) and mefloquine-artesunate (Artequin®) were the products of Mepha AG (Aesch, Switzerland). Artesunate (50-mg tablet) was kindly obtained from Dafra Pharma (Turnhout, Belgium). Tribendimidine (200-mg tablet) was the product of Shandong Xinhua Pharmaceutical Corporation. Tribendimidine is registered in China and the efficacy against soil-transmitted helminths and safety has been documented in

148 thousands of patients.[20,21] Praziquantel (600-mg tablet) was purchased from
149 Inresa (Bartenheim, France). Mefloquine and mefloquine-artesunate were
150 administered following the recommended malaria treatment schedules:
151 mefloquine: 25 mg/kg single dose (body weight < 30 kg) or a split dose spaced
152 by 6 hours at weights above 30 kg (e.g. at body weights 30-34 kg 2 lactabs were
153 administered followed by 1 lactab 6 hours later) and mefloquine-artesunate: 1
154 tablet of 100 mg artesunate and 1 lactab mefloquine 250 mg once daily for 3
155 consecutive days. Mefloquine and praziquantel were administered to the nearest
156 half tablet according to the calculate dose per kg of body weight. For artesunate
157 a previously used malaria treatment schedule (10 mg/kg as 3 split doses within
158 12 h) was used.[22] Tribendimidine was given following the manufacturer's
159 instruction for the treatment of soil-transmitted helminth infections: 200 mg (age
160 below 14 years) or 400 mg (age above 14 years) as a single dose. Finally,
161 praziquantel was administered according to Lao national policies: 75 mg/kg in 2
162 divided doses of 50 and 25 mg/kg spaced by 6 hours. All children received a
163 biscuit and water before drug administration to improve tolerability and increase
164 bioavailability.[23]

165 Children were supervised for at least 3 hours after treatment and were
166 asked to report any potential drug-related signs and symptoms at 24 h, 48 h, and
167 120 h after the first dosing using a standardized questionnaire. A full clinical
168 examination was performed by a study physician in case children reported
169 adverse events and an appropriate treatment was given. The intensity of adverse
170 events was graded as judged by study physicians (mild, moderate, severe,

serious or life-threatening). At the end of the study *O. viverrini* egg positive children enrolled in our study were treated with praziquantel (40 mg/kg). All schoolchildren received a single oral albendazole (400 mg) following Lao national scheme for mass drug administration in Lao PDR.[24,25]

**Laboratory procedures**

Filled stool containers were collected from children between 08:00 and 09:00 am and replaced with empty containers to obtain at least 2 stool samples from each child within a period of 5 days. Stool containers were then taken to the laboratory at the provincial hospital. From each stool sample 2 Kato-Katz (KK) thick smears using the standard 41.7 mg template were prepared and quantitatively examined under a light microscope at a x 100 magnification for helminth eggs. Each KK slide was read within 30 to 45 minutes after preparation. The number of *O. viverrini* eggs and soil-transmitted helminths eggs, i.e. *Ascaris lumbricoides*, hookworm, *Trichuris trichiura* and *Taenia* spp., were counted and recorded for each parasite species separately. 10% of the slides were reexamined for quality control by a senior microscopist. In addition, four samples (2 pre- plus 2 post-treatment) of stools were preserved in 10 ml sodium acetate-acetic acid-formalin (SAF) solution which contained exactly 500 mg of stool for examination with the formalin-ether concentration technique (FECT), respectively, which allows differentiating between *O. viverrini* and minute intestinal fluke infections (MIF).[26,27] Specimens of patients, for which pre- and post treatment samples could be preserved (per protocol analysis) were shipped

to a referral laboratory, at the Khon Kaen University, Thailand. For the FECT analysis the sample was centrifuged, and the sediment analyzed using a light microscope at 40 x and 100 x magnifications.[28]

**Ethical approval and consent**

The study was approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Swiss TPH, Basel, Switzerland) and the Ethics Committee of Basel (no 209/09). Ethical clearance was obtained from the National Ethics Committee (NEC), Ministry of Health (MOH) in Vientiane (no279/NECHR). The trial was registered with Current Controlled Trials, ISRCTN23425032. Permission for field work was provided by the MOH, the Provincial Health Department and the Provincial and District Education Office (DHO). Written informed consent was obtained from the parents or legal caretakers of each child. In addition, we also informed the participants and their parents that tribendimidine is currently registered only in China, and as such considered to be an investigational drug in Laos. We explained risk and benefits on the consent form in Lao language.

**Data management and statistical analysis**

All data were double entered using EpiData version 3.1 (Epidata Association; Odense, Denmark). Statistical analyses were performed with STATA statistical software version 10.1 (Stata Corp., College Station, TX, USA). Efficacy and safety were evaluated with intention to treat and per protocol analyses.

Intention to treat was defined as an analysis based on the initial treatment intent and per protocol analysis was defined as children who completed the entire clinical trial.

Descriptive statistics are presented as counts, percentages, means and standard deviations, as appropriate. Prevalence of *O. viverrini* was stratified according to the classification of infection intensities proposed by Maleewong *et al*.: light infections (1-999 eggs per gram of feces [epg]), moderate (1,000-9,999 epg) and severe (epg >10,000).[29] CR and ERR were assessed as efficacy outcomes. Logistic regression models were used to examine CRs of *O. viverrini* and hookworm among different treatment arms (comparison of odds of parasite clearance between treatment groups). Odds ratio of parasite clearance and 95% confidence intervals were reported. Negative binomial regression was applied to compare ERR between the numbers of *O. viverrini* eggs recovered in stool examination among mefloquine, artesunate, mefloquine-artesunate and tribendimidine compared to praziquantel. Egg reduction rate ratio (ERRR) and 95% confidence interval were reported.

Pearson's $x^2$ test was applied to compare the baseline binary characteristics and the proportion of the reported adverse events between the treatment arms. Statistical significance was estimated using a likelihood ratio test. Negative binominal models were fitted to compare the number of adverse events among the treatment groups. *P*-value below 5% was considered significant.

## Results

### Baseline characteristics

Of 214 schoolchildren screened with the Kato Katz method, 197 (92.1%) were *O. viverrini* positive (Figure 1). We excluded 72 children (36.5%) from the trial since they provided only a single stool sample (70 children), fever (1 child) and splenomegaly (1 child). 125 participants were randomly allocated to 5 treatment arms and included in the intention-to-treat analysis. The groups were not equal-sized (24 children in the artesunate and artesunate-mefloquine treatment groups versus 27 children in the tribendimidine group) since two patients were erroneously assigned to the tribendimidine group, instead of the artesunate and mefloquine-artesunate treatment groups. Of the 125 participants, 19 children (15.2%) were lost to follow-up at the end of study. Four stool samples (2 pre- plus 2 post-treatment) were available from 106 individuals (per protocol analyses).

Table 1 summarizes the demographic and laboratory baseline characteristics of the study participants. All baseline characteristics of the treatment groups were similar except for a slightly higher number of males in the mefloquine treatment group, which was however not statistical significant ($chi^2$, 3.97; $P$=0.41). Overall 63 males and 62 females, mean age 13.4±1.4 years, were included in the study. The intensity of *O. viverrini* infections was mostly mild to moderate. Overall, *O. viverrini* GM egg counts ranged from 609.1 to 3917.7 epg.

The overall prevalence of *A. lumbricoides, T. trichiura* and *Taenia* spp was below 16.0%, hence these parasites were not included in the efficacy evaluation. Hookworm infection rates ranged from 70.8% to 83.3%. Results of FECT confirmed the presence of an *O. viverrini* infection in all participants. The *O. viverrini* GM baseline egg counts obtained by FECT ranged from 82.5 to 639.0 epg (n=106) (data not shown). In 2 and 9 patients co-infections with MIF or intestinal protozoa were recorded.

**Efficacy evaluation**

As presented in Table 2 according to intention to treat analysis the highest CR was observed for tribendimidine (70.4%) followed by praziquantel (56.0%). No statistically significant difference was observed between the CRs of tribendimidine and praziquantel (Egg reduction rate ratio 1.87; *P*= .29). None of the children receiving mefloquine was cured and very low CRs were calculated for artesunate (4.2%), and mefloquine-artesunate (4.2%). Both tribendimidine and praziquantel resulted in almost complete egg elimination with ERRs of 99.3% and 98.4% (Table 3: Egg reduction rate ratio 1.0; *P*= .98), respectively. By contrast, mefloquine, artesunate, and mefloquine-artesunate had significantly lower ERRs of 30.2, 31.5 and 41.3%, respectively (Table 3: *P*< .01), except for a combination mefloquine-artesunate (Table 3: *P*= .08)

Results of the per protocol analysis (Kato Katz data) were similar to those of the intention-to-treat analysis. CRs of tribendimidine, praziquantel, mefloquine-artesunate, artesunate and mefloquine were 79.2, 63.6, 6.0, 4.2 and 0%, respectively (Table 2). Again both tribendimidine and praziquantel resulted in ERRs >98.0%, while ERRs in the mefloquine, artesunate and mefloquine-artesunate treatment groups were significantly lower (Table 3: $P< .01$), except for a combination mefloquine-artesunate (Table 3: $P= .06$). On the other hand, considering FECT analysis much higher CRs and ERRs were noted. CRs of tribendimidine, praziquantel, mefloquine-artesunate, artesunate and mefloquine were 95.8, 95.5, 47.1, 33.3 and 20.0%, respectively. The corresponding ERRs were 99.1, 99.0, 75.0, 60.0 and 71.0%. While no statistical significant difference was observed between CRs and ERRs of tribendimidine and praziquantel treatment (Table 3: $P= .25$ and .98, respectively) CRs and ERRs of the antimalarials were significantly lower (Table 3: $P< .01$), except for a combination mefloquine-artesunate (Table 3: $P= .14$)

Mefloquine, artesunate, mefloquine-artesunate and praziquantel had no effect against hookworms, whereas tribendimidine achieved CRs of 65.0% (both intention to treat and per protocol analysis) ($P= .004$).

**Safety evaluation**

Adverse events were assessed at 3, 24, 48 and 120 hours after the first dosing as summarized in Table 4. None of the symptoms were reported before

treatment. The majority of symptoms were reported to be mild 3 hours post-treatment, then increased in severity and subsided 48 hours post-treatment. In total, 92 (73.6%) mild, 47 (37.6%) moderate, 23 (18.4%) severe and 12 (9.6%) serious adverse events were reported (Table 5). 120 hours after treatment children were re-examined by the same physicians. None of them reported any adverse event and all children resumed their normal activities.

At least one adverse event was reported by 66.7, 74.0, 80.0, 88.0, and 96.0% of patients from the artesunate, tribendimidine, praziquantel, mefloquine and mefloquine-artesunate treatment groups, respectively (Table 5). No statistically significant difference was observed in the frequency of any adverse event among the tribendimidine, praziquantel and artesunate treatment groups. Most reported symptoms in the tribendimidine treatment group were mild including headache (44.4%), vertigo (33.4%), nausea (33.4%) and fatigue (18.5%) (Table 4). The most common symptoms reported, vertigo and nausea, were significantly more often observed in children treated with mefloquine ($P$= .02 and $P$= .007, respectively) than in any other treatment group. Additionally, dizziness was more common in patients who received mefloquine ($P$= .02), and mefloquine-artesunate ($P$= .001) than in patients who were treated with praziquantel. Twelve children treated with mefloquine or mefloquine-artesunate experienced serious adverse events including dizziness, nausea, vertigo, vomiting and were transferred to the provincial and local hospital. These children received a full clinical examination and proper medical mitigation measures including parentheral transfusion, antiemetic drugs, paracetamol or oral

rehydration. The children were closely monitored and after 48 hours of hospitalization all children had recovered and could be been discharged.

## Discussion

We evaluated the effectiveness and safety of the antimalarial drugs mefloquine, artesunate and mefloquine-artesunate and the Chinese broad spectrum anthelmintic drug tribendimidine in the treatment of *O. viverrini* patients. To our knowledge these drugs have not been studied to date against *O. viverrini* infections. It is interesting to note that another antimalarial drug, chloroquine was historically used for treating opistorchiasis; however, the CR and ERR were unsatisfactory.[30] Praziquantel served as reference, since it is the drug of choice for the treatment of *O. viverrini*.[25] Adverse events following praziquantel treatment are generally mild and transient, as confirmed in our study.[31] A single dose of 40 mg/kg praziquantel is widely used for community mass drug administration (MDA) in Southeast Asia. In Laos, MDA was initially introduced in the 1980s in high risk areas, under the close collaboration the Ministry of Health and WHO.[32] Since then the morbidity due to *O. viverrini* infections has declined considerable. In our study a split dose of 75 mg/kg praziquantel (75-mg/kg divided into two doses of 50 and 25-mg/kg) was used, which is recommended for individual treatment and is the most effective regimen.[24] We observed only moderate CRs following praziquantel treatment in our study which contrasts to previous studies which observed CRs between 96 and 100%.[33-35]

It is encouraging that a single 200 or 400 mg oral dose of tribendimidine achieved higher cure and egg reduction rates than a double dose of praziquantel, though this finding was not statistically significant since only a small number of children was included in our exploratory study. Tribendimidine is an amidantel derivative, first discovered and developed in China.[36] Preclinical and clinical studies have been launched to meet the international standard accepted by the FDA and European regulatory agencies, with the ultimate goal of gaining tribendimidine regulatory approvals for the treatment of soil-transmitted helminthiases outside of China and inclusion in the WHO's Essential Medicines List. Tribendimidine has a broad spectrum of activity against intestinal nematodes (e.g. *A. lumbricoides, Enterobius vermicularis* and the hookworms).[37] A recent study in China showed that single-dose oral tribendimidine was efficacious against *A. lumbricoides* and hookworm while showing promising activities against *Strongyloides stercoralis* and *Taenia* spp.[38] The good efficacy of tribendimidine against hookworm infections was confirmed in our study.

In contrast to recent laboratory findings, mefloquine, artesunate and a combination of mefloquine-artesunate showed no effect in the treatment of *O. viverrini* infections. In a recent proof-of-concept study against another trematode, *Schistosoma haematobium* in Côte d'Ivoire mefloquine and artesunate achieved similarly low CRs (21.0 and 25.0 %, respectively) however slightly higher ERRs (74.0 and 85.0%, respectively) were seen. Furthermore, promising results were observed with mefloquine-artesunate (CR: 61.0%, ERR >95.0%).[17]

We discriminated between *O. viverrini* infections and other common foodborne trematodes using FECT (Table 2). The results obtained with FECT confirmed the high efficacy of tribendimidine against *O. viverrini.* It is interesting to note that FECT yielded higher CRs of praziquantel and tribendimidine than the Kato Katz method, which might be explained by lower sensitivity of FECT technique compared to KK thick smears. Our findings are consistent with the study by Lovis and colleagues which demonstrated a lower sensitivity of FECT (49.4%) compared to one KK thick smear (62.3%).[39] Conversely, the lower sensitivity of FECT in this study contrasts with results obtained from a study conducted in the southern part of Lao PDR,[40] where FECT showed a sensitivity of 96.8% in the diagnosis of *O. viverrini* infections. It seems that the amount of stool used in FECT is not of primary importance. However, Sayasone and colleagues used the purging of patients as the reference "gold-standard" to calculate the validity of FECT.[40] Interestingly, two previous studies conducted in Lao PDR [39,40] using the same diagnostic tools to differentiate *O. viverrini* from *O. viverrini*-like parasites demonstrated that *O. viverrini* often coexists with other foodborne trematodes including MIF, including *Haplorchis taichui*. In our study very few coinfections with MIF were detected.

Children who were treated with praziquantel, artesunate and tribendimidine showed only mild adverse events, similar to previous studies.[38, 41] Moderate, severe and serious adverse events were observed in the mefloquine and mefloquine-artesunate treatment groups 24 hours after drug administration. Vertigo, dizziness and nausea were the most common adverse events reported.

All seriously affected patients were referred to the provincial hospital and provided appropriate medical care. Most of the patients recovered and were discharged from the hospital a day later. Surprisingly, patients in our current study were more likely to experience adverse events than observed in *S. haematobium* infected school-children in Côte d'Ivoire treated with mefloquine and mefloquine-artesunate.[17] Only mild and transient adverse events were observed in the latter study, with abdominal pain the most frequent adverse event reported.[17] We cannot explain at the moment why mefloquine and mefloquine-artesunate were not tolerated in our study population, but the *O. viverrini* infection and other host factors might play a role.

In conclusion, tribendimidine showed a promising activity against *O. viverrini* in our study. The nematocidal and opisthorchicidal properties of this drug are very intriguing as there is huge geographical overlap of these parasites and preventive chemotherapy is the mainstay of control. Once all preclinical studies have been completed to register the drug outside China large scale clinical studies should be conducted in *O. viverrini* endemic settings. Furthermore, it is interesting that in contrast to *in vivo* studies, antimalarial drugs are ineffective in the treatment of *O. viverrini* infections. Nonetheless, the deployment of antimalarials in areas of malaria-liver fluke coinfections might have marginal benefits as these drugs have been shown to slightly reduce *O. viverrini* egg counts. In addition, it might be of interest to study tribendimidine-praziquantel combinations in *O. viverrini* infected hamsters, as recently done in *Clonorchis*

413 *sinensis* infected rats,[42] since combination chemotherapy is a useful strategy to
414 delay the emergence of drug resistance.

**What this study adds:**

**Tribendimidine at recommended doses for the treatment of soil-transmitted helminths infections achieved high cure and egg reduction rates in secondary school children infected with *Opisthorchis viverrini***

**No or only very moderate effects were observed with mefloquine, artesunate and mefloquine-artesunate against *O. viverrini***

**Frequent adverse events, many of which were serious were observed following treatment with mefloquine, and mefloquine-artesunate. Only mild and transient adverse events were observed in secondary school children treated with tribendimidine**

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**Author Contributions** JK and PO conceived and designed the study; PS, JK, PO, SS, YV collected data; KA had the overall responsibility of data collection; PS, PO and PV analyzed data and interpreted results together with JK and CH; PS and JK wrote the manuscript; PO, KA and CH assisted with manuscript revisions; all authors read and approved the final submitted manuscript; PS and JK are guarantors of the paper.

**Conflict of interest**. None declared.

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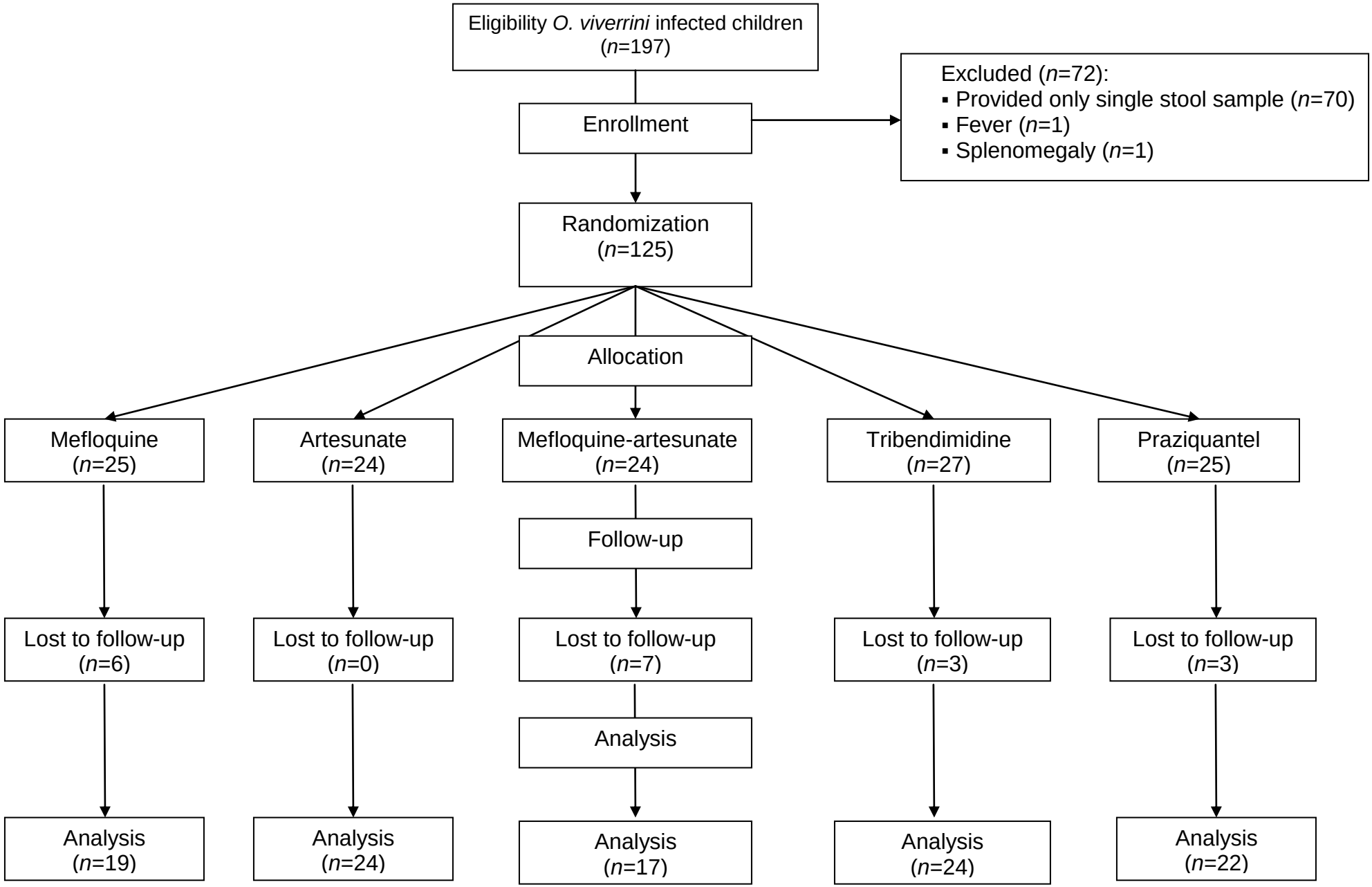
Eligibility O. viverrini infected children (n=197)
Enrollment
Excluded (n=72):
▪ Provided only single stool sample (n=70)
▪ Fever (n=1)
▪ Splenomegaly (n=1)
Randomization (n=125)
Allocation
Mefloquine (n=25)
Artesunate (n=24)
Mefloquine-artesunate (n=24)
Tribendimidine (n=27)
Praziquantel (n=25)
Follow-up
Lost to follow-up (n=6)
Lost to follow-up (n=0)
Lost to follow-up (n=7)
Lost to follow-up (n=3)
Lost to follow-up (n=3)
Analysis
Analysis (n=19)
Analysis (n=24)
Analysis (n=17)
Analysis (n=24)
Analysis (n=22)

Figure 1

***Table 1. Demographic and laboratory baseline characteristics of 125 schoolchildren infected with Opisthorchis viverrini at inclusion***

| Parameters | *Drugs* | | | | |
|---|---|---|---|---|---|
| | Mefloquine (*n* = 25) | Artesunate (*n* = 24) | Mefloquine-artesunate (*n* = 24) | Tribendimidine (*n* = 27) | *Praziquantel (n = 25)* |
| **Characteristics** | | | | | |
| Boys/girls | 16/9 | 13/11 | 12/12 | 10/17 | *12/13* |
| Mean (±SD) age, years | 13.4 (1.2) | 13.3 (1.6) | 13.4 (1.6) | 13.3 (1.3) | *13.6 (1.3)* |
| Mean (±SD) weight, kg | 39.5 (5.4) | 38.8 (6.8) | 38.0 (6.3) | 40.6 (7.0) | *39.4 (5.6)* |
| **Parasite infections** | | | | | |
| *Opisthorchis viverrini* infection [a] | | | | | |
| Overall GM epg | 1159.7 | 1368.0 | 1207.8 | 1968.1 | *1925.4* |
| GM epg (range) | (609.1- 2208.0) | (745.3- 2510.9) | (715.1-2040.0) | (988.7 -3917.7) | *(970.2-3821.2)* |
| No of light infection (1-999 epg) | 14 (56.0) | 11 (45.8) | 11 (45.8) | 9 (33.3) | *11(44.0)* |
| No of moderate infection (1000- 9999 epg) | 9 (36.0) | 12 (50.0) | 11 (45.8) | 14 (51.9) | *9 (36.0)* |
| No of heavy infection (>10 000 epg) | 2 (8.0) | 1 (4.2) | 2 ( 8.3) | 4 (14.8) | *5(20.0)* |
| Co-infection with soil-transmitted helminths | | | | | |
| Hookworm | 19 (76.0) | 20 (83.3) | 17 (70.8) | 20 (74.1) | *20 (80.0)* |
| *Ascaris lumbricoides* | 0 (0) | 0 (0) | 0 (0) | 3 (11.1) | *4 (16.0)* |
| *Trichuris trichiura* | 1 (4.0) | 1 (4.2) | 0 (0) | 1 (3.7) | *0 (0)* |
| *Taenia* spp | 2 (8.0) | 1 (4.2) | 1 (4.2) | 1 (3.7) | *3 (12.0)* |

[a] *According to guideline's classification put forward by WHO, based on Kato-Katz analysis; Data are no; (%) of subject, otherwise indicated (95% confident interval); GM, geometric mean; epg, eggs per gram of stool*

***Table 2. Prevalence and cure rate of mefloquine, artesunate, mefloquine-artesunate, tribendimidine and praziquantel schoolchildren infected with Opisthorchis viverrini at follow-up***

| | ***Intention-to-treat analysis*** | | | | |
|---|---|---|---|---|---|
| | Mefloquine (*n* = 25) | Artesunate (*n* = 24) | Mefloquine-artesunate (*n* = 24) | Tribendimidine (*n* = 27) | *Praziquantel (n = 25)* |
| **Kato-Katz thick smear technique** | | | | | |
| *Opisthorchis viverrini* | | | | | |
| No. of patients cured (%) | 0 (0) | 1 (4.2) | 1 (4.2) | 19 (70.4) | *14 (56.0)* |
| GM epg (range) | 1052.2 (537.8- 2058.4) | 1229.4 (625.1-2417.7) | 653.9 (323.9-1320.1) | 578.5 (47.7-7009.5) | *159.9 (38.1- 671.2)* |
| ERR (%) | 30.2 | 31.5 | 41.3 | 99.3 | *98.4* |
| Co-infection with hookworm | | | | | |
| No. of patients of sole hookworm infection (*n* = 86) | (*n* = 17) | (*n* = 20) | (*n* = 15) | (*n* = 17) | *(n = 17)* |
| No. of patients cured (%) | 3 (17.7) | 4 (20.0) | 3 (20.0) | 11 (64.7) | *2 (11.8)* |
| | ***Per-protocol analysis*** | | | | |
| | Mefloquine (*n* = 19) | Artesunate (*n* = 24) | Mefloquine-artesunate (*n* = 17) | Tribendimidine (*n* = 24) | *Praziquantel (n = 22)* |
| **Kato-Katz thick smear technique** | | | | | |
| *Opisthorchis viverrini* | | | | | |
| No. of patients cured (%) | 0 (0) | 1 (4.2) | 1 (6.0) | 19 (79.2) | *14 (63.6)* |
| GM epg (range) | 1114.1 (498.9-2488.1) | 1229.4 (625.1-2417.7) | 669.1 (320.8-1395.7) | 44.7 (11.6-171.7) | *43.1 (16.6-111.7)* |
| ERR (%) | 28.7 | 31.5 | 36.6 | 99.3 | *98.4* |
| Co-infection with hookworm | | | | | |
| No. patients of sole hookworm infection (*n* = 81) | (*n* = 15) | (*n* = 20) | (*n* = 12) | (*n* = 17) | *(n = 17)* |
| No. of patients cured (%) | 3 (20.0) | 4 (20.0) | 2 (16.7) | 11 (65.0) | *2 (13.0)* |
| **FECT technique** | | | | | |
| *Opisthorchis viverrini* | | | | | |
| No. of patients cured (%) | 4 (21.1) | 8 (33.3) | 8 (47.1) | 23 (95.8) | *21 (95.5)* |
| GM epg (range) | 182.3 (77.0-433.5) | 156.2 (82.2-297.0) | 114.0 (69.2-187.3) | na | *na* |
| ERR (%) | 71.0 | 60.0 | 75.0 | 99.1 | *99.0* |

***Note.*** *Data are no; (%) of subject, otherwise indicated (95% confident interval); GM, geometric mean; epg, eggs per gram of stool; ERR, egg reduction rate; ; na, not applicable*

***Table 3. Comparison of treatment outcome between groups***

| | | ***Intention-to-treat analysis*** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **MQ *vs* PZQ** | ***P*** | **AS *vs* PZQ** | ***P*** | **MQ-AS *vs* PZQ** | ***P*** | **TBD *vs* PZQ** | ***P*** |
| **Kato-Katz thick smear technique** | | | | | | | | | |
| *Opisthorchis viverrini* | | | | | | | | | |
| | OR | na | na | 0.03 (0.004-0.29) | 0.002 | 0.03 (0.004-0.29) | 0.002 | 1.87 (0.60-5.85) | *0.29* |
| | ERRR | 0.40 (0.21-0.72) | 0.003 | 0.43 (0.23 – 0.80) | 0.008 | 0.60 (0.31-1.10) | 0.08 | 1.00 (0.44-2.30) | *0.98* |
| Co-infection with hookworm | | | | | | | | | |
| | OR | 1.61 (0.23-11.09) | 0.63 | 1.88 (0.30-11.78) | 0.50 | 1.88 (0.27- 13.09) | 0.52 | 13.75 (2.32-81.49) | *0.004* |
| | | ***Per-protocol analysis*** | | | | | | | |
| | | **MQ *vs* PZQ** | ***P*** | **AS *vs* PZQ** | ***P*** | **MQ-AS *vs* PZQ** | ***P*** | **TBD *vs* PZQ** | ***P*** |
| **Kato-Katz thick smear technique** | | | | | | | | | |
| *Opisthorchis viverrini* | | | | | | | | | |
| | OR | na | na | 0.02 (0.003-0.22) | 0.001 | 0.04 (0.004-0.32) | 0.003 | 2.17 (0.58-8.08) | *0.25* |
| | ERRR | 0.36 (0.19-0.68) | 0.002 | 0.42 (0.22 -0.80) | 0.008 | 0.54 (0.28-1.03) | 0.06 | 1.00 (0.44-2.31) | *0.98* |
| Co-infection with hookworm | | | | | | | | | |
| | OR | 1.88 (0.27-13.09) | 0.53 | 1.88 (0.30-11.78) | 0.50 | 1.50 (0.18-12.46) | 0.70 | 13.75 (2.32-81.49) | *0.004* |
| **FECT technique** | | | | | | | | | |
| | OR | 0.01 (0.001-0.13) | <0.001 | 0.02 (0.003-0.21) | 0.001 | 0.04 (0.005-0.39) | 0.005 | 1.10 (0.06-18.64) | *0.95* |
| | ERRR | 0.54 (0.43-0.67) | <0.001 | 0.81 (0.70-0.94) | 0.009 | 0.87 (0.72-1.04) | 0.14 | 1.00 (0.86-1.16) | *0.99* |

***Note**. Data are odds ratios (OR, 95% confidence intervals) of parasite clearance; ERRR, egg reduction rate ratio; na, not applicable; MQ: mefloquine; AS: artesunate; MQ-AS Mefloquine-artesunate; TBD tribendimidine; PZQ Praziquantel*

***Table 4.1 Clinical symptoms reported 3-48 hour after drug administration among 125 schoolchildren, stratified by treatment group***

| | *No, (%) individuals with adverse event* | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mefloquine (n = 25) | | | | Artesunate (n = 24) | | | | Mefloquine-artesunate (n = 24) | | | | Tribendimidine (n = 27) | | | | *Praziquantel (n = 25)* | | | |
| **Adverse event / Grade** | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | At any Time point | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | *At any time point* |
| **Fatigue** | | | | | | | | | | | | | | | | | | | | |
| Mild | 3 | 4 | 2 | 7 (28.0) | 2 | 3 | 2 | 6 (25.0) | 4 | 5 | 7 | 12 (50.0) | 2 | 3 | 2 | 5 (18.5) | 5 | 8 | 2 | *11 (44.0)* |
| Moderate | 0 | 0 | 3 | 3 (12.0) | 0 | 1 | 1 | 2 (8.3) | 3 | 1 | 2 | 5 (20.8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 0 | 2 | 2 (8.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Asthenia** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 4 | 0 | 4 (16.0) | 0 | 2 | 0 | 2 (8.3) | 0 | 4 | 0 | 2 (8.3) | 0 | 1 | 1 | 1 (3.7) | 0 | 0 | 1 | *1 (4.0)* |
| Moderate | 0 | 6 | 0 | 6 (24.0) | 0 | 1 | 0 | 1 (4.2) | 0 | 3 | 0 | 3 (12.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 8 | 0 | 8 (32.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | *1 (4.0)* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Headache** | | | | | | | | | | | | | | | | | | | | |
| Mild | 4 | 3 | 2 | 7 (28.0) | 6 | 8 | 2 | 12 (50.0) | 6 | 9 | 6 | 14 (58.3) | 4 | 10 | 1 | 12 (44.4) | 12 | 7 | 2 | *16 (64.0)* |
| Moderate | 1 | 2 | 4 | 6 (24.0) | 1 | 2 | 1 | 4 (16.7) | 1 | 3 | 4 | 8 (33.3) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | *2 (8.0)* |
| Severe | 0 | 1 | 3 | 3 (12.0) | 0 | 1 | 0 | 1 (4.2) | 1 | 1 | 1 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | *2 (8.0)* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Vertigo** | | | | | | | | | | | | | | | | | | | | |
| Mild | 6 | 3 | 1 | 9 (36.0) [a] | 3 | 5 | 1 | 7 (29.2) | 3 | 8 | 6 | 12 (50.0) | 4 | 6 | 0 | 9 (33.4) | 11 | 8 | 2 | *16 (64.0)* |
| Moderate | 1 | 13 | 4 | 15 (60.0) | 1 | 0 | 0 | 1 (4.2) | 1 | 7 | 4 | 10 (41.7) | 0 | 1 | 1 | 1 (3.7) | 0 | 2 | 0 | *2 (8.0)* |
| Severe | 0 | 0 | 3 | 3 (12.0) | 0 | 1 | 0 | 1 (4.2) | 1 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 1 | 1 | 1 (4.0) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (4.2) | 0 | | 0 | 0 | 0 | 0 | 0 | *0* |
| **Vomiting** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 1 | 0 | 1 (4.0) | 0 | 1 | 0 | 1 (4.2) | 0 | 2 | 3 | 4 (16.7) | 0 | 1 | 1 | 1 (3.7) | 0 | 2 | 1 | *2 (8.0)* |
| Moderate | 0 | 7 | 6 | 10 (40.0) | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 3 (12.5) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | *1 (4.0)* |
| Severe | 0 | 6 | 1 | 6 (24.0) | 0 | 0 | 0 | 0 | 0 | 5 | 2 | 6 (25.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 5 | 0 | 5 (20.0) | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 4 (16.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Nausea** | | | | | | | | | | | | | | | | | | | | |
| Mild | 2 | 4 | 1 | 6 (24.0) | 2 | 3 | 2 | 5 (20.8) | 7 | 6 | 6 | 14 (58.3) | 3 | 7 | 0 | 9 (33.3) | 5 | 5 | 1 | *10 (40.0)* |
| Moderate | 1 | 8 | 5 | 11 (44.0) [b] | 0 | 0 | 1 | 1 (4.2) | 0 | 5 | 3 | 8 (33.3) | 1 | 0 | 1 | 2 (7.4) | 1 | 0 | 1 | *2 (8.0)* |
| Severe | 0 | 2 | 1 | 2 (8.0) | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 (12.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 2 | 1 | 2 (8.0) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 (8.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |

*[a] Significantly different from PQZ-treated children (p< .02); [b] Significantly different from PQZ-treated children (p< .007); [c] Significantly different from PQZ-treated children (p< .001)*

***Table 4.2 Clinical symptoms reported 3-48 hour after drug administration among 125 schoolchildren, stratified by treatment group***

| | *No, (%) individuals with adverse event* | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mefloquine (n = 25) | | | | Artesunate (n = 24) | | | | Mefloquine-artesunate (n = 24) | | | | Tribendimidine (n = 27) | | | | *Praziquantel (n = 25)* | | | |
| **Adverse event / Grade** | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | At any Time point | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | At any time point | 3 | 24 | 48 | *At any time point* |
| **Abdominal pain** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 (4.2) | 0 | 4 | 0 | 4 (16.7) | 0 | 1 | 0 | 1 (3.7) | 0 | 2 | 0 | *2 (8.0)* |
| Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Dizziness** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 (4.2) | 0 | 1 | 0 | 1 (4.2) | 0 | 1 | 1 | 1 (3.7) | 0 | 2 | 0 | *2 (8.0)* |
| Moderate | 0 | 12 | 0 | 12 (48.0) [a] | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 7 (29.2) [c] | 0 | 0 | 0 | 0 | 0 | 1 | 0 | *1 (4.0)* |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Somnolence** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 0 | 1 | 1 (4.0) | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 3 | 3 (12.5) | 0 | 1 | 1 | 1 (3.7) | 0 | 2 | 1 | *2 (8.0)* |
| Moderate | 0 | 0 | 1 | 1 (4.0) | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Anxiety** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 1 | 0 | 1 (4.0) | 0 | 2 | 1 | 2 (8.3) | 0 | 1 | 2 | 2 (8.3) | 0 | 1 | 1 | 1 (3.7) | 0 | 0 | 1 | *1 (4.0)* |
| Moderate | 0 | 1 | 0 | 1 (4.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| **Insomnia** | | | | | | | | | | | | | | | | | | | | |
| Mild | 0 | 2 | 0 | 2 (8.0) | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Moderate | 0 | 2 | 0 | 2 (8.0) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 (4.2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |
| Serious | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | *0* |

*[a] Significantly different from PQZ-treated children (p< .02); [b] Significantly different from PQZ-treated children (p< .007); [c] Significantly different from PQZ-treated children (p< .001)*

***Table 5. Summary of clinical symptoms recorded at 3-48 hour after drug administration, stratified by treatment group***

| **Adverse event arisen after treatment** | **Treatment group** | | | | | |
|---|---|---|---|---|---|---|
| | Mefloquine (*n* =25 ) | Artesunate (*n* =24 ) | Mefloquine-artesunate (*n* =24 ) | Tribendimidine (*n* =27 ) | *Praziquantel* (*n* =25 ) | *Total (n=125)* |
| At least 1 adverse event | 22 (88.0) | 16 (66.7) | 23 (95.8) | 20 (74.1) | 20 (80.0) | *101 (80.8)* |
| Mild | 18 (72.0) | 15 (62.5) | 20 (83.3) | 19 (70.4) | 20 (80.0) | *92 ( 73.6)* |
| Moderate | 19 (76.0) | 5 (20.8) | 16 (66.7) | 2 (7.4) | 5 (20.0) | *47 (37.6)* |
| Severe | 12 (48.0) | 2 (8.3) | 4 (16.7) | 1 (3.7) | 4 (16.0) | *23 (18.4)* |
| *Serious* | *4 (8.3)* | *0* | *8 (33.3)* | *0(0)* | *0(0)* | *12 (9.6)* |