Efficacy of drugs against clonorchiasis and opisthorchiasis: a systematic review and network meta-analysis

Men-Bao Qian, Chandni Patel*, Marta S Palmeirim, Xiaoming Wang, Christian Schindler, Jürg Utzinger, Xiao-Nong Zhou, Jennifer Keiser*

Summary

Background *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus* are the three most important human liver fluke species in the Opisthorchiidae family, infecting approximately 25 million people worldwide. Drug treatment is needed to control morbidity and is also useful in lowering transmission. Several drugs used in various regimens are available to treat these infections, but their comparative efficacy is uncertain. We aimed to compare the efficacy in terms of cure rate and egg reduction rate of currently registered drugs against human liver fluke infection.

Methods We conducted a systematic review using readily available electronic databases (MEDLINE, Embase, Cochrane Central Register of Controlled Trials, KoreaMed, China National Knowledge Infrastructure, and Wanfang Data) without language restrictions from inception until June 29, 2021. Clinical trials with pairwise comparison of drugs (praziquantel, albendazole, mebendazole, tribendimidine, or combinations of these drugs) against *C sinensis*, *O viverrini*, and *O felineus* were eligible, including trials comparing these drugs or their combinations with placebo. We compared efficacy in terms of cure rate by network meta-analysis. We conducted mixed binomial regression analyses for each species to derive predicted median cure rates for each drug regimen. The models included treatment and infection intensity as fixed factors, year of publication as covariate, and random effects of the different studies assumed to be normally distributed. We also assessed the quality of the included studies. This study was registered with PROSPERO (CRD42018109232).

Findings Overall, 26 trials from 25 studies were included, of which 18 involved *C sinensis*, seven studied *O viverrini*, and one focused on *O felineus*. These trials included a total of 3340 participants. The two long-term treatment courses against *C sinensis* infection using 400 mg of albendazole (400 mg twice a day for 5 days and 400 mg twice a day for 7 days) resulted in cure rates of 100%, while two other multiple-dose regimens of albendazole resulted in high predicted cure rates: 300 mg twice a day for 5 days (93·9% [95% CI 49·6–99·6]) and 400 mg twice a day for 3 days (91·0% [50·9–99·0]). The WHO-recommended praziquantel regimen (25 mg/kg three times a day for 2 days) also showed a high predicted cure rate (98·5% [85·4–99·9]) in *C sinensis* infection, and predicted cure rates were above 90% for several other multiple-dose praziquantel regimens, including 20 mg/kg three times a day for 3 days (97·6% [74·7–99·8]), 14 mg/kg three times a day for 5 days (93·9% [44·8–99·7]), and 20 mg/kg twice a day for 3 days (91·0% [50·9–99·0]). In *O viverrini* infection, the regimen of 50 mg/kg and 25 mg/kg of praziquantel given in a single day showed the highest predicted cure rate (93·8% [85·7–97·5]), while a single dose of 50 mg/kg praziquantel also resulted in a high predicted cure rate (92·1% [64·9–98·6]). The single dose of 400 mg tribendimidine showed a high predicted cure rate of 89·8% (77·5–95·8). A low quality of evidence was demonstrated in most studies, especially those published before 2000. Selection bias due to poor random sequence generation and allocation concealment was high, and performance and detection biases were frequently unreported.

Interpretation Praziquantel shows high efficacy against clonorchiasis and opisthorchiasis. Tribendimidine might serve as a treatment alternative and warrants further investigation. Although albendazole is efficacious when long treatment schedules (5 days or 7 days) are applied, limited size of studies and high risk of bias affect the interpretation of results. More high-quality studies are needed to promote the establishment of treatment guidelines for human liver fluke infection.

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Introduction

Clonorchis sinensis, *Opisthorchis viverrini*, and *Opisthorchis felineus* are important liver fluke species that parasitise humans.[1–3] Globally, an estimated 25 million people are infected with at least one of these three species.[3–5] An estimated 15 million cases of *C sinensis* infection occur in China, South Korea, northern Vietnam, and the far east part of Russia.[3–5] An estimated 8·6 million cases of *O viverrini* infection occur in Thailand, Laos, Cambodia, southern Vietnam, and Myanmar.[6–8] More than 1·5 million people are thought to be infected with *O felineus* in Russia, Kazakhstan, Ukraine, and some other European countries.[9]

Research in context

Evidence before this study

The three liver flukes of the Opisthorchiidae family—*Clonorchis sinensis*, *Opisthorchis viverrini*, and *Opisthorchis felineus*—affect millions of people and cause considerable morbidity and mortality, including cholangiocarcinoma. To control morbidity, anthelmintic treatment is needed. At present, only one drug (praziquantel) is recommended against clonorchiasis and opisthorchiasis by WHO and the US Food and Drug Administration. WHO recommends a dose of 25 mg/kg three times daily for 2 or 3 consecutive days or a single dose of 40 mg/kg. Additionally, albendazole (10 mg/kg once a day for 7 days) is recommended as an alternative drug by the US Centers for Disease Control and Prevention. Tribendimidine was more recently added to the anthelmintic armamentarium in China. However, the drug is not approved and there are no definitive treatment standards available for human liver fluke infections. To date, no systematic review and meta-analysis has been conducted to comparatively assess the efficacy of the available drugs and treatment regimens.

Added value of this study

To fill this identified gap, we conducted a systematic review and network meta-analysis assessing the efficacy of anthelmintic treatment against *C sinensis*, *O viverrini*, and *O felineus*. Several major English and Chinese electronic databases were consulted for studies published between inception of the databases and June 29, 2021. Our results confirm the high efficacy of multiple-dose praziquantel treatment schedules, as recommended by WHO, whereas a single 40 mg/kg oral dose applied in preventive chemotherapy programmes showed low efficacy against *C sinensis*. Long course and multiple-dose albendazole demonstrated high efficacy against *C sinensis*, but the evidence is based on studies with low numbers of participants and a high risk of bias. A single dose of tribendimidine or high dose of praziquantel showed similar efficacy against *O viverrini*.

Implications of all the available evidence

C sinensis, *O viverrini*, and *O felineus* infections can be treated effectively with the available drugs, with high doses and long treatment courses showing the best performance. Large-scale studies with tribendimidine against *C sinensis* and *O viverrini* are required and the drug should be investigated for the treatment of *O felineus* infections.

Persistent infection with these liver flukes leads to severe complications, mainly cholelithiasis, cholangitis, and cholecystitis.[10–14] Furthermore, both *C sinensis* and *O viverrini* can cause the fatal bile duct cancer cholangiocarcinoma.[15,16] To control morbidity, WHO recommends preventive chemotherapy as the main strategy.[17] At present, only praziquantel is recommended for clonorchiasis and opisthorchiasis by WHO and the US Food and Drug Administration. Additionally, albendazole is recommended as an alternative drug by the US Centers for Disease Control and Prevention.[18] Tribendimidine, an anthelmintic drug registered in China for soil-transmitted helminthiases, is also efficacious against these flukes.[19]

Efficacy, in terms of cure rate and egg reduction rate (ERR), of available drugs is dependent on infection intensity and drug dosage.[1] However, there are currently no definitive treatment standards available for human liver fluke infections, and treatment regimens are often arbitrarily selected.[20]

This systematic review aims to compare the efficacy in terms of cure rate and ERR of currently registered drugs against *C* sinensis, *O viverrini*, and *O felineus*, which should strengthen the evidence and guide control strategies.

Methods

Search strategy and selection criteria

This systematic review and meta-analysis adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for network meta-analysis (appendix pp 23–25). The review protocol is available in the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42018109232.

We conducted an electronic literature search using six databases, including MEDLINE, Embase, Cochrane Central Register of Controlled Trials, KoreaMed, China National Knowledge Infrastructure, and Wanfang Data, without language restrictions, for studies published between inception of these databases and June 29, 2021, using search terms relating to *C sinensis*, *O viverrini*, *O felineus*, and liver flukes (appendix pp 1–2). Bibliographies of papers that matched the eligibility criteria were hand-searched to identify any further relevant references.

All identified references were screened independently by two reviewers (M-BQ, CP, or MSP for English and M-BQ and XW for Chinese literature) using a two-stage approach. First, we reviewed the title and abstract. In the second step, papers deemed of relevance were subjected to full-text review. A senior scholar (JK) was consulted when inconsistency occurred. The following eligibility criteria were applied for efficacy studies. Clinical trials with pairwise comparisons of drugs (praziquantel, albendazole, mebendazole, tribendimidine, and combinations of these drugs) against any of the three human liver flukes (*C sinensis*, *O viverrini*, and *O felineus*) were eligible. Studies comparing one or more of the aforementioned drugs and drug combinations with placebo were also eligible. All

See Online for appendix

treatment doses were considered. Only studies which provided the number of individuals treated and cured for each treatment arm (or number of treated individuals and cure rate) were included. Reviews were regarded as irrelevant if they did not provide any additional original data that were not yet published elsewhere. In the case of articles with duplicate data, the one with more detailed data was included.

Compared with the published protocol, there were two revisions. First, safety data were not included in this review due to the low comparability among different trials, as the definition of symptoms is rather subjective and symptoms are difficult to distinguish from adverse events.[10] Second, the acceptable time for outcome assessment, which had not been specified in our original protocol, was set between 3 weeks and 6 weeks after the start of treatment.[1,21]

Data analysis

Data extraction from studies included year of publication, year and country of study, study population, diagnostic method, liver fluke species (*C sinensis*, *O viverrini*, or *O felineus*), drug(s) used (including doses and regimens), time of outcome assessment, number of all cases, number of cured individuals after treatment, cure rate (proportion of patients who turned from egg-positive to egg-negative), eggs per gram of faeces (EPG) before and after treatment, and ERR (percentage reduction of mean egg count). Study authors were contacted (when contact details were available) for clarification on their data if needed. The quality and risk of bias of eligible studies was assessed at the study level, using the Cochrane Risk of Bias tool.[22] In brief, risk of bias was evaluated across six domains. High, low, and unclear risks were attributed to each of the six domains. An attrition rate over 20% in any regimen of the study was judged as high attrition bias.

The applied unit for drugs involved two types, mg/kg or mg. We chose to use mg/kg for praziquantel, and mg for albendazole, mebendazole, and tribendimidine. When transforming between mg/kg and mg, the average weight of participants was assumed to be 40 kg for children, 60 kg for adults, and 50 kg for both.

Descriptive analysis was used to characterise the distribution of trials according to publication year, location, and risk of bias in different domains. Publication year was categorised into three periods: before 1990, between 1990 and 1999, and from 2000 onwards. The number of low biases in six domains was added by study and Student's *t* test was applied to compare the difference between different periods. Infection intensities were grouped into three EPG categories: light (1–999 EPG), moderate (1000–9999 EPG), and heavy (≥10 000 EPG).

For the network meta-analysis of cure rates, we used a method by Kessels and colleagues,[23] which consists of rebuilding the original datasets on the basis of published sample sizes and case numbers. All datasets from studies with one, two, or more treatment arms were pooled, and mixed binomial regression analyses were conducted within the pooled dataset, separately for *C sinensis* and *O viverrini*. The models included treatment and infection intensity as fixed factors, year of publication as covariate, and random effects of the different studies assumed to be normally distributed. Cure rates derived from these models with random effects being set to 0 will be referred to as "predicted median cure rates", as the median of the distribution of cure rates across studies is characterised by a random study effect of 0. To compare the different alternative treatments with the reference treatment, we also calculated the posterior probability after 100 000 simulations for the cure rate of the respective treatment to exceed that of the reference treatment; uninformative priors were used for both rates. In an additional mixed binomial regression analysis, cure rates of *C sinensis* and *O viverrini* were separately regressed on drug (albendazole, praziquantel, and tribendimidine), relative total dose, and relative total dose squared, while adjusting for year of publication and infection intensity. In this analysis, random effects of studies and of study arms within studies were considered. We calculated random effect variances and I^2 to describe heterogeneity. For *O viverrini*, interaction terms between the three drugs and the two variables of relative total dose further improved the model. Relative total dose was defined as ratio between the respective total dose and the mean total dose of the same drug. From these models, median cure rates were calculated for the three different drugs and for different levels of relative total dose, assuming mixed infection intensity and publication year of 2014 (*C sinensis*) and 2018 (*O viverrini*). These data were then plotted as estimated dose–response curves.

ERRs for *C sinensis* and *O viverrini* were first transformed to ensure that the resulting regression residuals were close to normally distributed, and the transformed rates were regressed against drug, relative total dose, and relative total dose squared, as well as interaction terms between these variables and drug, considering random effects of the different studies. Details on the transformation applied are provided in the appendix (p 3). Similarly as with the cure rates, median ERRs were derived from these models for the three different drugs and for different levels of relative total dose, again leading to plots of estimated dose–response curves. The studies reporting the efficacy by infection intensity were few in number. Thus, quantitative synthesis by infection intensity was not done.

All statistical analyses were done using Stata Statistical Software, Release 15.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

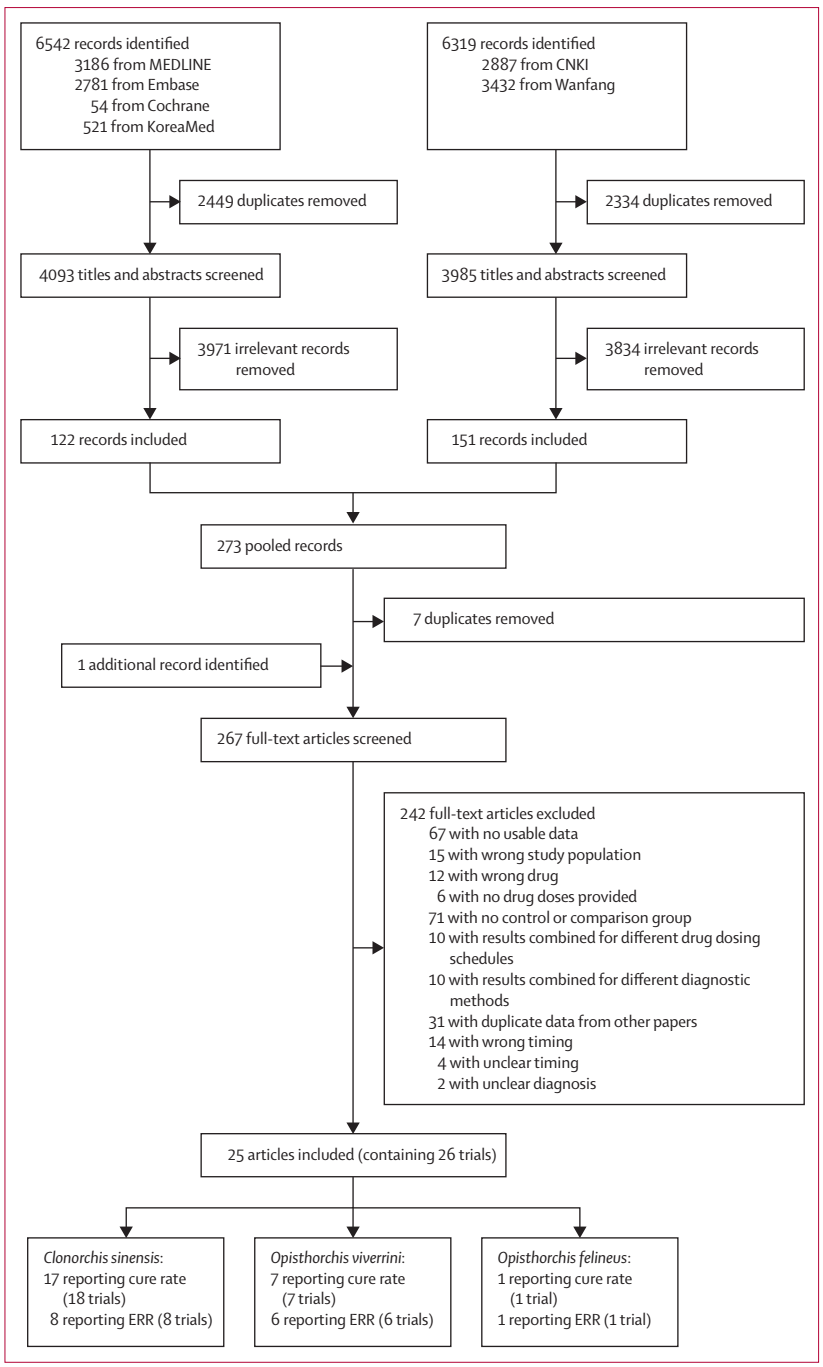

Figure 1: **Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram showing process of selection of studies for inclusion in the systematic review and meta-analysis**
ERR=egg reduction rate.

Results

More than 12 800 records were found across six databases with 8078 titles and abstracts screened after removal of duplicate records (figure 1). After title and abstract screening, 267 articles were included for full-text review. After reviewing the full texts, an additional 242 articles were excluded. Taken together, 25 studies were eligible, which included 26 trials. Among them, 18 trials in 17 studies assessed *C sinensis*,[24–40] seven trials *O viverrini*,[41–47] and one trial *O felineus*[48] (appendix pp 4–14). Among the 26 trials, 15 not only presented cure rates but also reported ERRs (eight on *C sinensis*, six on *O viverrini*, and one on *O felineus*; appendix pp 18–22). Across all trials there were a total of 3340 participants, comprising 2181 in *C sinensis* trials, 1128 in *O viverrini* trials, and 31 in the *O felineus* trial.

In *C sinensis* and *O viverrini* trials, praziquantel, albendazole, mebendazole, and tribendimidine were administered (appendix pp 26–27). For *C sinensis*, there were 35 different regimens of four drugs: albendazole (n=12), mebendazole (n=1), praziquantel (n=17), and tribendimidine (n=5). The most common regimen was praziquantel 25 mg/kg three times a day for 1 day (six trials), and four trials included a praziquantel regimen of 40 mg/kg single dose. For *O viverrini*, besides placebo, there were 12 different regimens tested: three different regimens of albendazole, one regimen of mebendazole, one regimen of tribendimidine, and seven different regimens of praziquantel. The most common regimens against *O viverrini* were praziquantel 50 mg/kg plus 25 mg/kg in a single day, and praziquantel 40 mg/kg single dose, which both were compared with other regimens in three trials. In *O felineus*, only two regimens of praziquantel (25 mg/kg once a day for 3 days and 40 mg/kg once a day for 3 days) were compared with each other (appendix p 28).

Several drugs and treatment courses showed high efficacy against *C sinensis*. The two long-term treatment courses against *C sinensis* using 400 mg of albendazole (400 mg twice a day for 5 days and 400 mg twice a day for 7 days) resulted in cure rates of 100% (table 1). However, the respective sample sizes were very low (<40 participants). Similarly, two other multiple-dose regimens of albendazole resulted in high predicted cure rates (300 mg twice a day for 5 days [93·9%] and 400 mg twice a day for 3 days [91·0%]), but they were drawn from a single study with a small sample size (<40 participants). The WHO-recommended praziquantel regimen (25 mg/kg three times a day for 2 days) assessed in two studies also showed a high predicted cure rate (98·5%). Additionally, predicted cure rates were above 90% for several other multiple-dose praziquantel regimens. The more frequently studied regimen, praziquantel 25 mg/kg three times a day for 1 day, showed a predicted cure rate of 79·8% (226 participants). Higher single doses (40–60 mg/kg) of praziquantel were tested in large sample sizes, which showed increasing cure rate by dose (ie, 32·4% for 40 mg/kg, 46·2% for 50 mg/kg, and 65·0% for 60 mg/kg). Of the five regimens of tribendimidine investigated in three studies, the highest predicted cure rates were demonstrated using 400 mg once a day for 3 days (75·4%) and 400 mg twice a day for 3 days (74·4%), whereas the lowest predicted cure rate (41·5%) resulted from the lowest dose tested (200 mg twice a day for

	Number of trials	Total participants followed up	Cured participants	Unadjusted cure rate, %	Predicted median cure rate, % (95% CI)	p value	Posterior exceedance probability
Albendazole 400 mg twice a day for 5 days	1[33]	39	39	100%	100%	1·0	0·62
Albendazole 400 mg twice a day for 7 days	1[32]	31	31	100%	100%	1·0	0·56
Praziquantel 25 mg/kg three times a day for 2 days	2[24,28]	63	62	98·4%	98·5% (85·4–99·9)	Reference	Reference
Praziquantel 20 mg/kg three times a day for 3 days	1[35]	66	65	98·5%	97·6% (74·7–99·8)	0·78	0·52
Albendazole 300 mg twice a day for 5 days	1[33]	24	23	95·8%	93·9% (49·6–99·6)	0·39	0·19
Praziquantel 14 mg/kg three times a day for 5 days	1[26]	34	33	97·1%	93·9% (44·8–99·7)	0·39	0·29
Albendazole 400 mg twice a day for 3 days	1[34]	34	32	94·1%	91·0% (50·9–99·0)	0·22	0·12
Praziquantel 20 mg/kg twice a day for 3 days	1[34]	34	32	94·1%	91·0% (50·9–99·0)	0·22	0·12
Praziquantel 12 mg/kg three times a day for 5 days	1[26]	33	31	93·9%	87·8% (35·0–99·0)	0·15	0·12
Praziquantel 25 mg/kg twice a day for 2 days	1[28]	41	33	80·5%	85·4% (54·4–96·7)	0·026	0·0009
Albendazole 560 mg twice a day for 3 days	1[34]	36	32	88·9%	83·4% (39·1–97·5)	0·073	0·023
Praziquantel 25 mg/kg three times a day for 1 day	6[24,25,28,29,37,39]	226	195	86·3%	79·8% (53·6–93·1)	0·0080	0·0021
Praziquantel 30 mg/kg twice a day for 1 day	1[27]	61	42	68·9%	77·6% (38·6–95·0)	0·013	<0·0001*
Albendazole 200 mg twice a day for 7 days	1[32]	32	27	84·4%	76·1% (28·2–96·3)	0·033	0·0061
Tribendimidine 400 mg once a day for 3 days	1[39]	24	14	58·3%	75·4% (44·5–92·1)	0·011	<0·0001*
Tribendimidine 400 mg twice a day for 3 days	1[38]	12	9	75·0%	74·4% (28·8–95·4)	0·047	0·0026
Tribendimidine 200 mg twice a day for 3 days	1[38]	19	13	68·4%	67·6% (26·3–92·4)	0·022	<0·0002
Praziquantel 10 mg/kg three times a day for 5 days	1[26]	33	27	81·8%	67·3% (16·6–95·5)	0·015	0·0024
Praziquantel 18·75 mg/kg twice a day for 2 days	1[40]	37	21	56·8%	65·2% (33·9–87·3)	0·0050	<0·0001*
Praziquantel 60 mg/kg single dose	1[31]	153	137	89·5%	65·0% (26·1–90·7)	0·0025	0·014
Albendazole 400 mg twice a day for 4 days	1[37]	293	264	90·1%	64·2% (30·2–88·2)	0·0029	0·014
Tribendimidine 400 mg single dose	2[39,40]	59	28	47·5%	60·6% (33·4–82·5)	0·0016	<0·0001*
Praziquantel 20 mg/kg three times a day for 1 day	1[29]	9	8	88·9%	58·2% (8·4–95·5)	0·017	0·048
Praziquantel 10 mg/kg three times a day for 3 days	2[36,38]	52	40	76·9%	56·6% (19·8–87·3)	0·0080	<0·0002
Albendazole 500 mg three times a day for 3 days	1[35]	62	38	61·3%	49·6% (13·8–85·8)	0·0019	<0·0001*
Praziquantel 50 mg/kg single dose	1[31]	149	119	79·9%	46·2% (14·4–81·3)	<0·0002	<0·0001
Praziquantel 16·7 mg/kg three times a day for 3 days	2[36]	32	30	93·8%	44·1% (3·5–94·6)	0·026	0·11
Tribendimidine 200 mg twice a day for 1 day	1[40]	33	11	33·3%	41·5% (15·7–72·9)	<0·0004	<0·0001*
Praziquantel 13·3 mg/kg three times a day for 3 days	1[36]	27	25	92·6%	40·0% (2·1–95·4)	0·031	0·084
Praziquantel 40 mg/kg single dose	4[25,27,29,31]	263	148	56·3%	32·4% (9·2–69·3)	<0·0001	<0·0001*
Albendazole 200 mg twice a day for 3 days	1[30]	71	25	35·2%	20·5% (3·0–68·0)	<0·0001	<0·0001*
Albendazole 400 mg once a day for 3 days	1[30]	33	2	6·1%	2·9% (0·2–27·0)	<0·0001	<0·0001*
Albendazole 400 mg single dose	1[30]	30	1	3·3%	1·6% (0·1–22·0)	<0·0001	<0·0001*
Mebendazole 400 mg single dose	1[40]	30	0	0%	0%	<0·0001	<0·0001*
Albendazole 200 mg twice a day for 1 day	1[30]	6	0	0%	0%	<0·0001	<0·0001*

Predictions of median cure rates were derived from a mixed binomial regression model with the factors treatment and infection intensity and the covariate year of publication, as well as random effects of the different studies. The unexplained variation of cure rates across studies is reflected in the variance of random study effects of 0·56 (~I^2=0·15). Predictions are adjusted to publication year 2014, mixed infection intensity, and a random study effect of 0. The p values relate to the difference between the respective cure rate and the cure rate of the reference treatment (praziquantel 25 mg/kg three times a day for 2 days). For cure rates different from 0% and 100%, p values were derived from the mixed logistic regression model; for cure rates of 0% and 100%, they were obtained by applying Fisher's exact test to the crude cure rates. Excess probability was defined as the posterior probability of the respective treatment's cure rate being higher than that of the reference treatment under the assumption of uninformative priors. The values displayed are estimates from 100 000 simulations. The WHO-recommended treatment schedule was defined as the reference regimen. *The estimate was 0 after 100 000 simulations.

Table 1: **Cure rates of different drug regimens against *Clonorchis sinensis***

1 day). The sole regimen of mebendazole (400 mg single dose) found no participants cured. Based on the ranking of predicted cure rates, the regimens with predicted cure rates above 87% were not significantly different from the WHO-recommended regimen (25 mg/kg three times a day for 2 days). Furthermore, based on the exceedance probability, only the long-term (5 days or 7 days) high-dose (400 mg twice daily) albendazole regimens, and the praziquantel regimen of 20 mg/kg three times a day for 3 days, had a posterior probability of more than 50% for their cure rate to be higher than that of the reference regimen (praziquantel 25 mg/kg three times a day for 2 days). The cure rate shows an increase with the dose of praziquantel, albendazole, and tribendimidine, up to a relative total dose of 1·5 (figure 2A). The ERR reveals an increase with the dose of albendazole up to a relative total dose of 1·5, while high ERRs were observed for all relative doses tested for praziquantel and tribendimidine (figure 2B). Three trials reported the cure rates stratifying by light, moderate, and heavy infection intensities,[27,31,39]

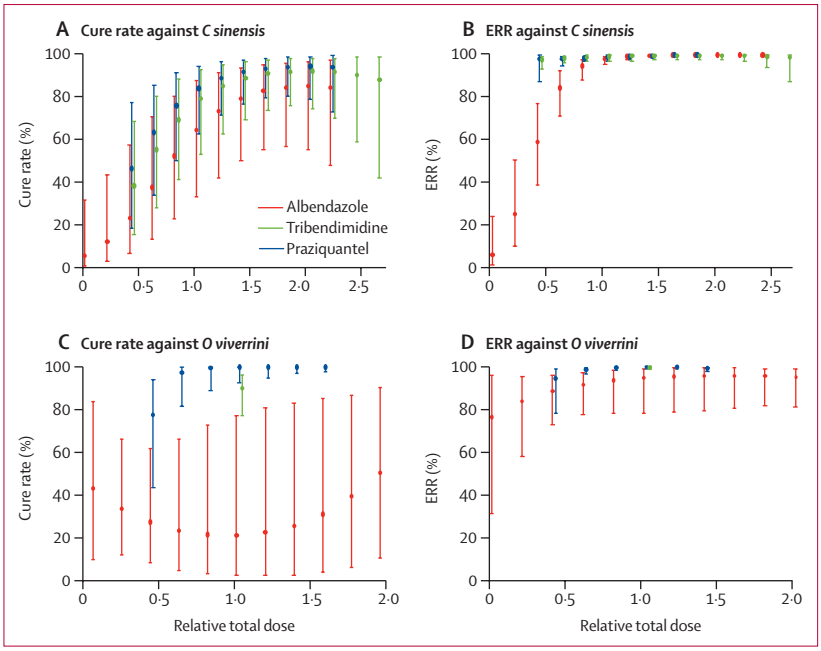

Figure 2: **Predicted median cure rate and ERR of different drugs as a function of relative total dose**
(A) Cure rate against *Clonorchis sinensis*, (B) ERR against *C sinensis*, (C) cure rate against *Opisthorchis viverrini*, and (D) ERR against *O viverrini*. Error bars show 95% CIs. Relative total dose was defined as the ratio between the total dose of a treatment and the mean total dose of all treatments with the respective drug. Median cure rates were predicted from mixed binomial regression models including the variables type of drug, relative total dose, and relative total dose squared (and in the case of *O viverrini* also interaction terms between type of drug and the variables of relative total dose), while adjusting for infection intensity (reference=mixed intensity) and year of publication (reference=2014 in *C sinensis* and 2018 in *O viverrini*). The models included random effects of studies and of arms within studies, whose variances were 0·62 and 0·46 in the case of C sinensis, and 0·24 and 0·19 in the case of O viverrini. Models of ERRs are described in the appendix (p 3). ERR=egg reduction rate.

whereas moderate and heavy intensities were not differentiated in another two trials (appendix pp 15–16).[35,37]

Treating *O viverrini* infection, the regimen of 50 mg/kg plus 25 mg/kg of praziquantel in a single day (reference treatment in Laos) showed the highest predicted cure rate (93·8%) based on three studies with a total of 352 participants (table 2). A single dose of praziquantel 50 mg/kg also resulted in a high predicted cure rate (92·1%). The praziquantel regimens of 25 mg/kg twice a day for 1 day, 25 mg/kg three times a day for 1 day, 30 mg/kg single dose, and 40 mg/kg single dose also showed predicted cure rates of over 80%. The single dose of tribendimidine (400 mg single dose) showed a high predicted cure rate of 89·8% based on two studies. Albendazole and mebendazole showed low predicted cure rates (<33%) against this infection. Higher dose and longer regimens of praziquantel, and the single dose of 400 mg tribendimidine were not found to be significantly different in predicted cure rates than the reference regimen of praziquantel. An increase of cure rate and ERR can be seen for praziquantel in the lower range of relative total dose, and the curve flattens off at higher doses (figure 2C, D). However, no dose–response relation can be observed for albendazole. Stratifying cure rates according to infection intensities was possible only for a single study (appendix p 17).[44] The efficacy was presented by age in one study, comparing the single dose of tribendimidine (400 mg, or 200 mg in children younger than 14 years) with a praziquantel regimen of 50 mg/kg plus 25 mg/kg.[47] In the tribendimidine group, the cure rate was 100% (38 of 38) in children, and 92·6% (238 of 257) in adults, while it

	Number of trials	Total participants followed up	Cured participants	Unadjusted cure rate, %	Predicted median cure rate, % (95% CI)	p value	Posterior exceedance probability
Praziquantel 50 mg/kg and 25 mg/kg given in a single day	3[43,44,47]	352	335	95·2%	93·8% (85·7–97·5)	Reference	Reference
Praziquantel 50 mg/kg single dose	1[46]	44	42	95·5%	92·1% (64·9–98·6)	0·79	0·40
Tribendimidine 400 mg single dose	2[43,47]	319	295	92·5%	89·8% (77·5–95·8)	0·11	0·074
Praziquantel 25 mg/kg twice a day for 1 day	1[41]	26	24	92·3%	89·6% (51·3–98·6)	0·60	0·16
Praziquantel 25 mg/kg three times a day for 1 day	1[46]	41	38	92·7%	87·5% (57·1–97·3)	0·40	0·17
Praziquantel 30 mg/kg single dose	1[46]	41	38	92·7%	87·5% (57·1–97·3)	0·40	0·17
Praziquantel 40 mg/kg single dose	3[41,44,46]	124	106	85·5%	82·9% (60·9–93·8)	0·069	<0·0004
Albendazole 400 mg single dose	1[45]	45	15	33·3%	32·7% (10·7–66·3)	<0·0001	<0·0001*
Praziquantel 25 mg/kg single dose	1[41]	10	4	40·0%	31·4% (5·9–77·1)	<0·0008	<0·0001*
Albendazole 400 mg twice a day for 7 days	1[42]	27	9	33·3%	30·6% (4·2–81·8)	0·0026	<0·0001*
Mebendazole 500 mg single dose	1[45]	33	8	24·2%	23·6% (6·6–57·7)	<0·0001	<0·0001*
Placebo	1[46]	41	12	29·3%	18·2% (5·4–46·2)	<0·0001	<0·0001*
Albendazole 400 mg twice a day for 3 days	1[42]	25	3	12·0%	10·6% (1·0–58·8)	<0·0002	<0·0001*

Predictions of median cure rates were derived from a mixed binomial regression model with the factors treatment and infection intensity and the covariate year of publication as well as random effects of the different studies. The unexplained variation of cure rates across studies is reflected in the variance of random study effects of 0·38 (~I^2=0·10). Predictions are adjusted to publication year 2018, mixed infection intensity, and a random study effect of 0. The p values relate to the difference between the respective cure rate and the cure rate of the reference treatment (praziquantel 50 mg/kg and 25 mg/kg given in a single day). Excess probability was defined as the posterior probability of the respective treatment's cure rate being higher than the cure rate of the reference treatment under the assumption of uninformative priors. The values displayed are estimates from 100 000 simulations. The treatment schedule applied in Laos was defined as the reference regimen. *The estimate was 0 after 100 000 simulations.

Table 2: **Cure rates of different drug regimens against *Opisthorchis viverrini***

was 100% (40 of 40) and 96·9% (253 of 261), respectively, in the praziquantel group.

Only one study assessed two different regimens of praziquantel against *O felineus* infection.[48] Cure rates were 59·1% (13 of 22) for the group receiving 25 mg/kg once a day for 3 days and 44·4% (four of nine) for the group receiving 40 mg/kg once a day for 3 days, and the corresponding geometric mean ERRs were 95·8% and 99·7%, respectively (appendix pp 14, 22).

Among 18 studies for *C sinensis*, only five had an adequate method of randomisation and only two properly concealed allocation of group assignment (figure 3). In the majority of trials (n=16) the risks associated with potential imperfect blinding were unclear (performance and detection biases), while the two remaining trials had a high risk of performance bias and a low risk of detection bias. Of the studies assessing *C sinensis*, 12 had a low risk of attrition bias. All studies had a low risk of reporting bias. Five studies pertaining to *O viverrini* had a low risk of selection bias for both random sequence generation and allocation concealment. Two studies had sufficient blinding of participants and personnel, and the risk of detection bias was low in five studies. Six studies had a low risk of attrition bias. All seven studies had a low risk of reporting bias. The one study pertaining to *O felineus* infection showed a low risk only in the domains of attrition and reporting biases. Overall, across all domains, the average number of domains showing low bias in studies published before 1990 was 2·1, with a statistically significant increase to 3·7 in studies published from 2000 onwards (p=0·027). Study heterogeneity of *C sinensis* and *O viverrini* trials is described in the legends of table 1, table 2, and figure 2.

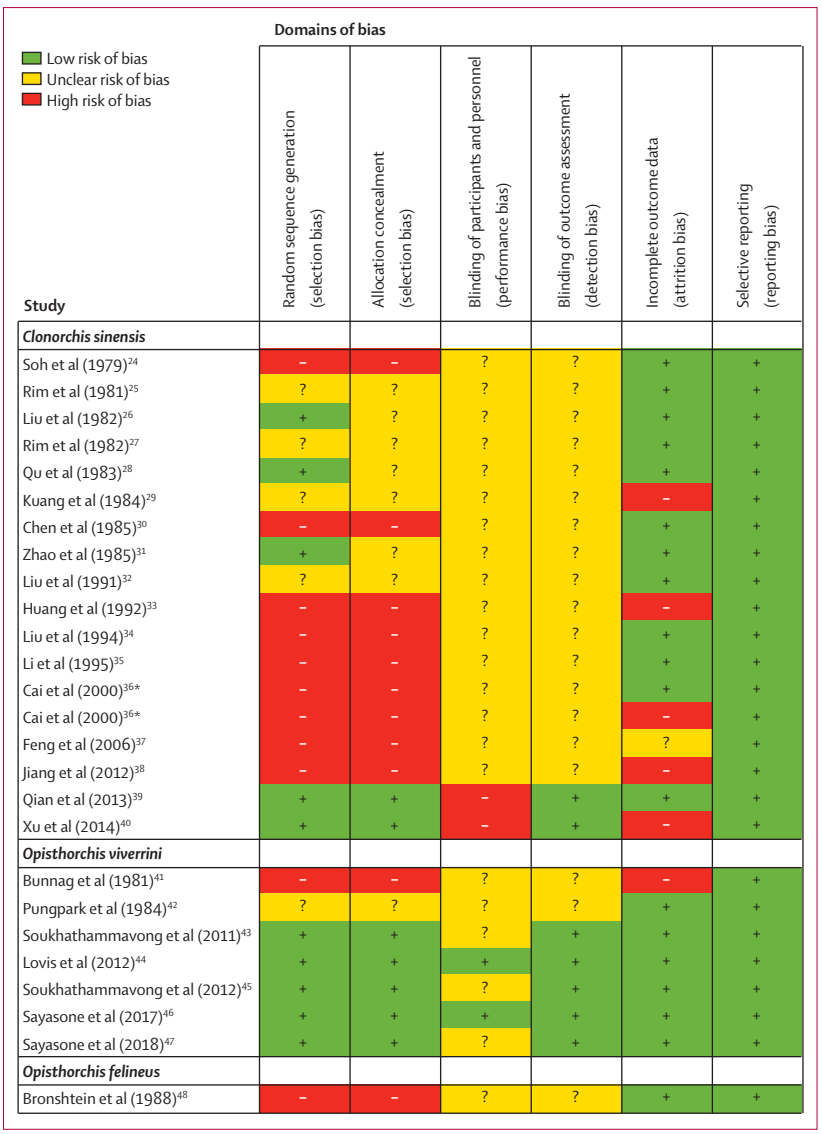

***Figure 3*: Risk of bias assessment of included trials**
*Considered two separate trials, although results limited to one publication.

Discussion

This is the first systematic review and network meta-analysis examining the efficacy of different drug regimens of albendazole, mebendazole, praziquantel, and tribendimidine in the treatment of the three species of human liver flukes belonging to the Opisthorchiidae family: *C sinensis*, *O viverrini*, and *O felineus*. The inclusion of many studies in endemic countries with clonorchiasis and opisthorchiasis makes this a comprehensive review of the literature.

The studies mainly fell into two periods, before 1990 and from 2000 onwards. Praziquantel was developed in the late 1970s.[49,50] At that time, several other drugs were used for the treatment of human liver flukes, of which the efficacy was low and adverse events were considerable.[1] Thus, many trials were conducted to evaluate praziquantel in treatment of human liver fluke infection in major endemic countries. In the 1980s, albendazole was approved for human use and was then also assessed in terms of efficacy and safety against human liver fluke infection.[1] In the new millennium, tribendimidine, which showed promising efficacy against *C sinensis* and *O viverrini* in vitro and in vivo,[19] was followed up in clinical trials in China and Laos. Although high heterogeneity was present in the eligible studies, as evidenced by the large variances of random study effects, and many different regimens were used, an increase in total dosage usually resulted in higher cure rates against both *C sinensis* and *O viverrini*. The regimens of praziquantel 25 mg/kg three times a day for 2 days or 3 days are recommended by WHO for individual treatment against these three liver fluke infections, and a single dose of 40 mg/kg is recommended for preventive chemotherapy in public health measures.[17] However, in *C sinensis* studies, the most frequently tested regimen was 25 mg/kg three times a day for 1 day, followed by 40 mg/kg single dose, and the eligible regimens in this systematic review did not include 25 mg/kg three times a

day for 3 days. The regimen of 25 mg/kg three times a day for 2 days was superior to the other two regimens. Although inferior to the 2 days' treatment, the regimen of 25 mg/kg three times a day for 1 day still showed a high cure rate. However, the regimen of 40 mg/kg single dose resulted in a lower cure rate compared with the other two regimens.

In *O viverrini* studies, a slightly different dose of praziquantel, namely 50 mg/kg plus 25 mg/kg on a single day, was frequently used according to national guidelines in Laos. This regimen resulted in a high cure rate, which was significantly higher than the cure rate of a single dose of 40 mg/kg. However, a single dose of 40 mg/kg also exhibited a high cure rate. Notably, cure rates were higher against *O viverrini* than *C sinensis* when the same drug regimen was used. We speculate that this finding is attributable to higher reported infection intensities among the studies on *C sinensis* compared with *O viverrini*.

Several studies have demonstrated the efficacy of albendazole against *C sinensis*. High efficacy was documented in long treatment courses (5 days or even 7 days).[32,33] However, results on a strong effect of prolonged treatment should be interpreted with caution due to small sample size. Furthermore, cure rates might be overestimated because cases with low infection intensities remained undetected due to imperfect diagnostic assays. With regard to mebendazole, due to the small number of studies, we could only draw the conclusion that short treatment courses of mebendazole provide low efficacy against liver fluke infections.

For tribendimidine, a single dose of 400 mg was frequently applied against both *C sinensis*[39,40] and *O viverrini*.[43,47] A single dose of 400 mg of tribendimidine showed similar efficacy to praziquantel (50 mg/kg plus 25 mg/kg) against *O viverrini*, which both resulted in high cure rates. However, it was inferior to praziquantel (25 mg/kg three times a day for 1 day) in *C sinensis*.

In some studies, the efficacy on co-infection with other helminth species was also recorded. Good efficacy was demonstrated by albendazole against soil-transmitted helminths, especially *Ascaris lumbricoides* and hookworm,[34,35,42,45] tribendimidine against hookworm,[40,43,47] and praziquantel against *Schistosoma mekongi*.[44]

Our review has several limitations. First, diverse regimens hinder a synthesised analysis. For example, only a single study could be included for *O felineus*, and therefore no conclusions can be drawn for this liver fluke species. Second, the differences in infection intensity, which was often not taken into account in the calculation of cure rates, compromise the comparability across studies. Third, although a clear positive effect of dose on the cure rate of *C sinensis* can be inferred from our data, the heterogeneity of cure rates across studies and across study arms within studies limits the comparability of the effects of the different drugs at the same relative total doses. Fourth, overall, a high risk of bias was present in many studies, which indicates the lack of high-quality evidence. Finally, adverse events, which could impact compliance in preventive chemotherapy campaigns, were not included in the formal analysis of this systematic review.

Taken together, our multiple treatment comparisons demonstrated that several treatment courses achieve high efficacy against clonorchiasis and opisthorchiasis. We observed that longer treatment courses and higher doses show better efficacy against the three liver fluke infections studied here. Conversely, the single 40 mg/kg dose of praziquantel recommended by WHO showed only moderate efficacy against *C sinensis*. Infection intensity is highly relevant to the performance of these drugs, with lower cure rate in high infection intensity. High-quality studies are needed to verify the efficacy of albendazole with long treatment courses using praziquantel as comparator. Larger trials are warranted to consolidate the efficacy of tribendimidine against *C sinensis* and *O viverrini* infections. Recent in-vitro and in-vivo studies have also demonstrated efficacy of tribendimidine in *O felineus*,[51] and hence, clinical trials are warranted.

Contributors

M-BQ, JU, X-NZ, and JK conceived of the study. M-BQ, CP, and MSP did the literature search. M-BQ, CP, MSP, and XW abstracted and curated all data. CS did the data analysis. M-BQ and CP wrote the first draft of the manuscript. All authors reviewed, edited, and approved the final version of the manuscript. All authors had full access to all the data. M-BQ and CP verified all data reported in the manuscript. JK had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The data supporting this meta-analysis are from previously reported studies and datasets, which have been cited. The processed data are all presented in the appendix.

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