

Opinion

# Towards Evidence-based Control of *Opisthorchis viverrini*

Thomas Crellen <sup>1,\*</sup> Paiboon Sithithaworn <sup>2,3</sup> Opal Pitaksakulrat,<sup>2</sup> Narong Khuntikeo,<sup>3,4</sup> Graham F. Medley <sup>5</sup> and T. Déirdre Hollingsworth <sup>1</sup>

Transmission of the carcinogenic liver fluke *Opisthorchis viverrini* is ongoing across Southeast Asia. Endemic countries within the region are in different stages of achieving control. However, evidence on which interventions are the most effective for reducing parasite transmission, and the resulting liver cancer, is currently lacking. Quantitative modelling can be used to evaluate different control measures against *O. viverrini* and assist the design of clinical trials. In this article we evaluate the epidemiological parameters that underpin models of *O. viverrini* and the data necessary for their estimation, with the aim of developing evidence-based strategies for parasite control at a national or regional level.

## Assessing Interventions

Control initiatives against the liver fluke *O. viverrini* are driven by two related aims. Firstly, to reduce transmission of the parasite, leading to its **elimination** (see [Glossary](#)). Secondly, to halt the progression of *O. viverrini*-induced liver pathology, and thus prevent new cases of **cholangiocarcinoma** [1]. In endemic regions, definitive hosts become infected through consumption of raw or insufficiently cooked freshwater fish encysted with **metacercariae** and perpetuate the life cycle by defecating into water sources containing snails of the *Bithynia* genus. Programmes to control *O. viverrini* therefore have a number of interventions available to them, including health education to promote safe eating habits; case diagnosis and **anthelmintic treatment**; improvements to sanitation; and food safety controls [2]. Parasite-induced liver pathology can be tackled with ultrasound screening to detect periductal fibrosis, an early warning sign of cholangiocarcinoma; and operative surgery to improve survival rates [3]. With such a variety of tools on offer, policy makers, international health organisations, and affected communities are entitled to ask: what works? Which measures are most effective at interrupting transmission of the parasite and reducing mortality in a cost-effective, sustainable manner?

The traditional method for answering this question is through **randomised controlled trials**, which are the gold standard for assessing interventions [4]. Such trials, however, are costly, take many years, and require substantial manpower and expertise. It is also unethical to conduct trials in contexts where public health initiatives are ongoing and would have to be withdrawn for participants allocated into the control arm of a trial. An alternative approach is to use quantitative models to make predictions on the effectiveness of different control strategies. These models take myriad forms, can be parameterised from routinely collected field data, and provide considerable insight for a fraction of the cost of a trial. Different types of models that can capture **macroparasite** dynamics are summarised in [Box 1](#).

When the need to conduct a clinical trial is unavoidable, for example on novel therapeutics or diagnostics, **dynamic** simulations can provide powerful insights into trial design, power

## Highlights

Halting the transmission of *O. viverrini* is crucial to preventing cases of cholangiocarcinoma, yet there is no consensus on the best strategies for large-scale parasite control.

Quantitative modelling can provide insights and guide control programmes more rapidly and using fewer resources than clinical trials. To give reliable output, models depend on making accurate assumptions and using parameters inferred from relevant datasets.

Mathematical models of *O. viverrini* dynamics have recently been published and provide a valuable starting point. However, many key epidemiological processes remain poorly understood, including the links between parasite exposure, anthelmintic treatment, and carcinogenesis.

We highlight areas where new studies are required to fill knowledge gaps. Recent developments in statistical modelling and parasite genomics provide opportunities to make rapid gains.

<sup>1</sup>Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, Nuffield Department of Medicine, University of Oxford, Old Road Campus, Oxford, OX3 7LF, UK

<sup>2</sup>Department of Parasitology, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>3</sup>Cholangiocarcinoma Research Institute, Khon Kaen University, Khon Kaen 40002, Thailand

<sup>4</sup>Department of Surgery, Khon Kaen University, Khon Kaen 40002, Thailand



### Box 1. Modelling for Parasitologists

As parasitologists we aim to understand the processes underlying relationships between parasites and their hosts. In ecological systems, such as infections in human populations, we are usually restricted to collecting observational data. To make the leap from describing data, which inevitably suffers from biases and measurement errors, to inferring the underlying process and asking 'what if' questions requires a model.

Statistical modelling broadly seeks to learn from data and estimate biologically meaningful parameters, such as the distribution of parasites within a community, gene flow between populations, or the reinfection rate following treatment. The model may be mechanistic and use a function that explicitly captures the data-generating process, or use a less specific generalised linear model. Estimating (or inferring) parameters is an art in itself. The most common frameworks are maximum likelihood and **Bayesian** approaches. Bayesian methods require the incorporation of prior knowledge, and are increasingly favoured by statisticians as they permit parameter estimation for complex models and characterise uncertainty in an intuitive way [69]. An area where Bayesian statistical modelling has been successfully applied is the spatial distribution of parasitic diseases. These geostatistical models use survey data taken from discrete locations, and covariates from spatially continuous satellite data (such as temperature, rainfall, elevation) to infer the prevalence of a disease across regions or countries [70].

Mathematical modelling uses equations to describe a biological system. These typically take the form of dynamic compartmental models. Individual agents are not considered but rather populations that exist in discrete states (such as the number of parasites within intermediate or definitive hosts), and transitions between states are determined by rate parameters. The synthesis by Roy Anderson and Robert May on mathematical models remains a key text for macroparasite epidemiology [43].

Individual-based models consider disease processes affecting individuals, each with their own characteristics, such as sex, age, and disease susceptibility. Such models can consider detailed population processes such as movement between towns or children mixing in schools, in addition to tracking the infection history of individuals and their associated morbidity over time. Individual-based models have been developed for macroparasitic diseases, including onchocerciasis and lymphatic filariasis, which have been applied to clinical trial design for new anthelmintics [6].

calculations, and relevant outcomes by considering the transmission process and how it responds to perturbations [5]. For instance, Walker and colleagues simulated clinical trials to compare ivermectin, the existing treatment for onchocerciasis and lymphatic filariasis which kills microfilarial (juvenile) stages and temporarily reduces fecundity in *Onchocerca volvulus*, against hypothetical anthelmintic drugs that kill adult worms [6]. The study showed the importance of follow-up timeframes, as the benefits of anthelmintic drugs are most prominent 12–24 months after treatment, and calculated the required sample sizes under different scenarios. However, any model is only as useful as the underlying assumptions that drive it and the reliability of the data used for parameter inference. In this article we consider interventions to reduce transmission and morbidity, followed by a discussion of the knowns and unknowns in the epidemiology of *O. viverrini* which are most pertinent to developing evidence-based strategies for national or regional control.

### Interventions to Reduce Transmission

While there are several points in the *O. viverrini* life cycle where transmission could be interrupted, in practice implementing interventions against intermediate hosts can be challenging. The importance of cyprinid fish in the Lower Mekong Basin, as both a source of income and cheap protein, means that reducing fish stocks or using chemical molluscicides which disrupt freshwater ecosystems are unlikely to garner local support and be sustainable [7]. Furthermore, the informal nature of fish-distribution networks makes it challenging to impose food safety controls [7]. Controlling snail populations is also considered unfeasible as *Bithynia* snails inhabit many water sources, including rice paddies, and are resilient to cycles of drought and flooding [8,9]. Defecation into water sources occurs as fishermen and rice farmers work far from their homes for long periods of time [10]. The effectiveness of sanitation interventions, such as installing public latrines to reduce *O. viverrini* transmission, has not been quantified. However, greater latrine coverage does not necessarily result in increased usage [11].

<sup>5</sup>Faculty of Public Health and Policy, London School of Hygiene and Tropical Medicine, 15–17 Tavistock Place, London, WC1H 9SH, UK

\*Correspondence: [thomas.crellen@bdi.ox.ac.uk](mailto:thomas.crellen@bdi.ox.ac.uk) (T. Crellen).

Health education campaigns to discourage the consumption of raw fish have been a mainstay of control programmes for several decades [1]. As health education is often combined with treatment in 'integrated' programmes, it is difficult to measure its impact in isolation; particularly in Thailand, where there are ongoing government public health messages on television and radio [12]. Although there is some evidence that integrating health education with anthelmintic treatment prolongs the benefits of deworming for up to 1 year after treatment ends [13], this effect is not sustained over longer periods [14].

Anthelmintic treatment is the only intervention that impacts on both transmission and morbidity. Relatively few studies investigate the impact of multiple treatment rounds on *O. viverrini*. A trial in Northeast Thailand found that villages allocated to biannual or annual treatment of positive cases with praziquantel, in combination with health education, had sustained declines in *O. viverrini* prevalence and infection intensity over 3 years [15]. Villages allocated to treatment showed declines from 64% (biannual) and 57% (annual) prevalence at baseline; to 5% and 24%, respectively, at 12 months; and 4% and 5% after 3 years.

In Thailand, at the national level, *O. viverrini* prevalence has declined from 15% in 1980 to 5% in 2014, and in the Northeast region from 35% in 1980 to 10% in 2014 [16]. This fall could be attributed to (i) anthelmintic treatment during control programmes and the availability of praziquantel from pharmacies [17,18]; (ii) reduced raw fish consumption due to public health messaging; and/or (iii) improvements to sanitation resulting from socioeconomic development. Unpicking which factors were most influential in driving long-term trends would therefore be of value. In Laos and Cambodia, by contrast, the available evidence suggests that transmission of *O. viverrini* is either stable or increasing [16,19].

### Liver Fluke Infection Causes Cholangiocarcinoma

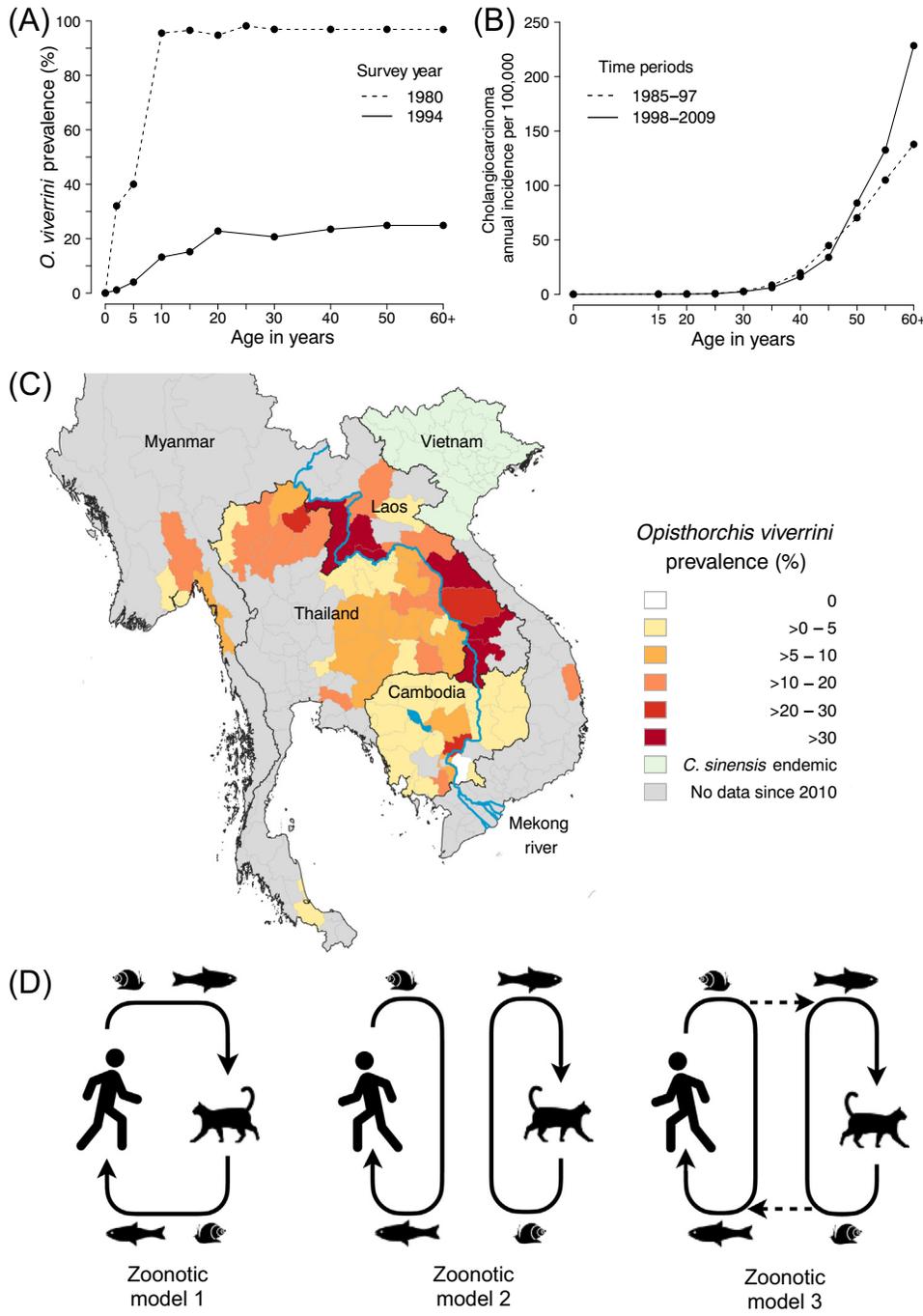
The International Agency for Research on Cancer has designated *O. viverrini* a class one carcinogen since 1994<sup>i</sup>, making it one of only ten infectious agents that are directly carcinogenic to humans<sup>ii</sup>. Evidence from epidemiological studies and animal models show that (i) heavier worm burdens increase the risk of developing cholangiocarcinoma [20]; (ii) proteins secreted by the parasite directly increase host liver damage [21]; and (iii) the pathology is more prevalent among older age groups, indicating a lag between infection in childhood and the onset of liver disease [22] (Figure 1A,B). This evidence suggests that chronic damage to the bile ducts accumulates primarily as a function of the length of parasite exposure and intensity of infection. Of those infected with *O. viverrini*, only around 5% will develop cholangiocarcinoma [23]. In addition to worm burden, cofactors associated with an increased risk of developing cholangiocarcinoma include consuming alcohol and food containing nitrosamines, while eating fruits and vegetables are associated with a reduced risk [23]. The host immune response to fluke infection can also cause liver damage, with proinflammatory cytokines implicated in the development of periductal fibrosis [24]. **Genome-wide association studies** have the potential to locate genetic variants that influence the progression of liver pathology [25], which would aid the identification of at-risk individuals.

### Deworming to Avert Liver Pathology

As infection with *O. viverrini* can lead to cholangiocarcinoma, removal of the parasite through deworming will halt or reduce parasite-induced chronic liver pathologies. This assertion is supported by three longitudinal studies conducted in hospital [26] and community settings [27,28] on the effects of anthelmintic treatment on *O. viverrini*-induced morbidity; all of these studies were conducted over 20 years ago. The largest of these studies, in Prachinburi Province, Thailand, reported hepatomegaly in 63% of patients at baseline which declined to 14% after 2 years following praziquantel treatment [28]. Symptoms in six patients with the most severe

### Glossary

- Anthelmintic treatment:** use of a deworming drug that kills adult worms within hosts.
- Basic reproduction number:** the expected number of adult parasites generated by a single adult worm in a generation.
- Bayesian:** a statistical framework which incorporates prior information on parameter values in addition to using a likelihood function to learn from the data, resulting in a posterior probability distribution for each parameter.
- Cholangiocarcinoma:** cancer of the bile duct.
- Density dependence:** a process that regulates population growth. In macroparasites the egg output per worm pair decreases as the total worm burden within a host increases.
- Dynamic:** relating to models. Variables within the model, such as the number of parasites in a population, change with respect to time.
- Elimination:** removal of an infectious pathogen from a specific area.
- Exome:** the protein-coding regions within a genome, excluding the noncoding intronic regions.
- Genome-wide association studies:** a case-control study in which participants have their genomes sequenced, and genetic variants are associated with a binary or quantitative phenotype.
- Latent class models:** a type of model used to infer a hidden state, in this case the 'true' prevalence of an infection in which diagnostic sensitivity and specificity are imperfect.
- Likelihood:** the joint probability of the observed outcomes, given a particular set of parameters. For a selected model, the optimal parameter values are those most consistent with the observed data or which 'maximise the likelihood'.
- Macroparasites:** infectious organisms, typically parasitic worms, that do not multiply within definitive hosts. Macroparasites have environmental stages in their transmission cycle and morbidity is usually chronic and governed by the worm burden (infection intensity).
- Mathematical model:** a model in which transitions between states (compartments) are governed by equations, either in continuous time (differential equations) or discrete time steps (difference equations).



**Mechanistic model:** a model which aims to simulate the underlying data-generating process.

**Metacercariae:** the infective stage for humans in the life cycle of *O. viverrini*: cysts in the muscle of freshwater fish; they encyst in the human stomach after consumption and transform into juvenile migrating worms.

**Microsatellites:** repetitive regions of the genome with reoccurring motifs that are assumed to evolve neutrally, and so provide insight into demographic processes rather than selection.

**Overdispersion:** where the variance for a set of count data exceeds the mean.

**Randomised controlled trials:** a method, for evaluating an intervention, in which participants are randomised into either a group with a novel intervention or into a control group with an existing treatment or placebo.

**Rate:** the expected frequency of an event occurring over a period of time.

**Statistical model:** a model which aims to estimate parameter values for a given model using data. Methods for estimating the parameters fall under frequentist, likelihood, or Bayesian frameworks.

**Variance:** a measure of the dispersion of values in a dataset, or by how much observations differ from the mean value.

**Figure 1. Epidemiology of *Opisthorchis viverrini*.** (A) Prevalence of *O. viverrini* (proportion egg positive) by age category. Data from surveys in Khon Kaen Province, Thailand, in 1980 (dashed line;  $n = 1651$ ) [78]; and a regional survey of Northeast Thailand in 1994 after 10 years of control efforts (solid line;  $n = 1912$ ) [17]. (B) Cholangiocarcinoma annual incidence in Khon Kaen Province, Thailand, per 100 000 population by age. Data from Kamsa-Ard *et al.* 2011 [79], assuming population sizes for the province of 1.66 million in the period 1985–1997 and 1.73 million in the period 1998–2009. (C) Prevalence of *O. viverrini* in Southeast Asia. Data shown from surveys conducted between 2010 and 2020. Grey areas indicate regions

(Figure legend continued at the bottom of the next page.)

liver pathology, intrahepatic cholangiocarcinoma, could not be reversed by deworming. Anthelmintic treatment of other parasitic flukes, namely schistosomes, reduces parasite-induced pathologies such as hepatomegaly, splenomegaly, and anaemia [29]. Several recent studies have controversially suggested that repeated deworming and reinfection with *O. viverrini* could accelerate the development of cholangiocarcinoma. The debate surrounding these claims is discussed in Box 2, where we argue that the weight of evidence supports a beneficial effect of deworming on morbidity.

### Building the Model

We now turn our attention to the areas of liver fluke epidemiology required for quantitative analysis to inform control strategies. Large-scale helminth-control programmes are increasingly reliant on models of transmission to predict the optimal timing, frequency, and spatial extent of interventions [30]. A major application for many macroparasitic diseases (schistosomiasis, soil-transmitted helminthiasis, lymphatic filariasis) is to assess the impact of mass drug administration (MDA), typically treating whole communities or schools with deworming drugs [30]. Forward projections from models are used to estimate the number of treatment rounds required to reach defined thresholds of endemicity or incidence, while conveying the uncertainty in their estimates [31]. The key components of a transmission model for *O. viverrini* are the parasite distribution at a given point in time; the rate of parasite acquisition; the contribution of zoonotic hosts; and the likely impact of interventions. Each of these components is discussed in more detail in the following text and summarised in Table 1. Relating transmission to the incidence of cholangiocarcinoma requires information on the relationship between parasite exposure and morbidity, and whether liver pathology resolves following treatment. Economic evaluations are an important consideration when assessing interventions but go beyond the scope of this article (reviewed in [32]).

The first **mathematical models** of *O. viverrini* transmission were recently published by Bürli and colleagues [33,34]. In a region of southern Laos the **basic reproduction number** was estimated as 1.1 and elimination was projected to be achievable within 20 years if annual MDA reached 44% of the population [34]. Formalising biological insight into a **mechanistic model** means that any assumptions are made explicit. In this case, the analysis incorporated multihost transmission from cats and dogs and assumed a sustained high efficacy of anthelmintic treatment. Mathematical models are useful for developing insights into a system; however, they struggle to incorporate heterogeneity which limits their ability to make detailed predictions over large spatial scales. A further challenge for models that consider the full parasite life cycle, plus transmission from nonhuman definitive hosts, is that they require many parameters to be estimated or assumed. This contrasts with simpler **statistical models** which infer fewer parameters and are more suited to exploring variation in transmission over space and time.

### Parasite Distribution and Aggregation

Capturing the spatial extent of infection is among the first priorities for any macroparasite control programme. Consumption of raw freshwater fish remains a substantial component of the diet across the Lower Mekong and has a cultural significance due to links with regional identity and male virility [12,35]. The current consensus is that *O. viverrini* is endemic across regions of

---

with no data since 2010. Estimates for North and Northeast Thailand taken from the Cholangiocarcinoma Screening and Care Program (CASCAP) survey data between 2013 and 2018, with an *O. viverrini* case defined as positive by either Kato-Katz, parasep, or formalin-ether concentration technique<sup>v</sup>. Estimates from Central and Southern Thailand, Cambodia, Vietnam, Myanmar, and Laos are taken from the literature (see Box S1 in the supplemental information online). (D) Three possible models of zoonotic *Opisthorchis* transmission in regions where humans and nonhuman definitive hosts (cats, dogs, possibly others) are infected. Zoonotic model 1: single transmission cycle where humans and other animals are alternate definitive hosts; Zoonotic model 2: separate life cycles for parasites which infect humans and other animals; or Zoonotic model 3: separate life cycles with limited cross infection and gene flow.

**Box 2. Is Repeated Deworming with Praziquantel Safe?**

The World Health Organization (WHO) advises that universal treatment with praziquantel should be adopted annually in regions where the prevalence of *O. viverrini* exceeds 20%, and biennially in regions where prevalence is less than 20%. Despite this recommendation, the only country which uses mass treatment as a control strategy against *O. viverrini* is Laos, and this is practiced exclusively in Champasak province which is coendemic for *Schistosoma mekongi* [80].

In a meeting convened by WHO in 2017, participants raised concerns about reliance on mass deworming due to high reinfection rates and a perceived link between repeated anthelmintic treatment and higher rates of cholangiocarcinoma<sup>4</sup>. These claims relate to two hospital-based cross-sectional studies which associated two or more previous praziquantel treatments (over a person's lifetime) with a higher odds of cholangiocarcinoma [71,72]. Neither study tested or controlled for infection with *O. viverrini*. Individuals in Thailand that regularly consume raw fish and have higher burdens of liver fluke are more likely to have been targeted by treatment programmes or self-medicate, as praziquantel can readily be acquired from private pharmacies [18]. Therefore the association between past treatments and cholangiocarcinoma is an example of statistical confounding which arises by failing to consider the main exposure, namely chronic infection with *O. viverrini* [73].

Previous work on animal models that used an indirect marker for liver cancer noted that the death of adult worms by praziquantel, and subsequent dispersion of worm antigens, led to host oxidative stress, which the authors suggested could exacerbate carcinogenesis [74]. Subsequent research, which examined cholangiocarcinoma directly in hamsters through magnetic resonance imaging, found instead that repeated cycles of reinfection with *O. viverrini* and treatment with praziquantel caused a slower progression to cholangiocarcinoma compared with untreated infections [75].

The combination of field studies examining the resolution of morbidity following anthelmintic treatment [26–28] and *in vitro* research [75] supports our assertion that praziquantel treatment against *O. viverrini* is safe and averts morbidity from cholangiocarcinoma<sup>4</sup>. However, the dearth of prospective cohort studies examining the progression of opisthorchiasis makes it challenging to quantify the benefits of deworming. The progression of chronic pathology resulting from macroparasite infection is generally nonlinear [76], and liver damage sustained early in life may trigger driver mutations for the cancer [77]. More data could therefore support mass treatment for younger individuals in endemic areas to prevent liver fluke burdens accumulating and triggering an irreversible cascade of cellular damage.

Thailand, Laos, Vietnam, and Cambodia infecting around 10 million people, although incomplete survey coverage and the low sensitivity of diagnostics suggest that these numbers underestimate the true burden [36]. Surveys in Myanmar uncovered endemic *O. viverrini* for the first time in 2017, finding a prevalence of 9.3% in a rural community of Lower Myanmar and later 0.9% in the capital Yangon [37,38]; however, the national prevalence is unknown (Figure 1C). As is commonly observed in macroparasite epidemiology, rates of transmission are heterogeneous across an endemic landscape and a number of 'hotspot' regions have persisted, including in Thailand<sup>4</sup>, despite the efforts of decades of control programmes (Figure 1C).

Adult *O. viverrini* worms can be obtained by (i) liver examination during autopsy, or (ii) worm expulsion following anthelmintic treatment. As the number of worms per host increases, the fecundity of each worm pair decreases due to space or nutritional constraints. The host egg count and number of adult worms therefore have a nonlinear relationship, which has implications for modelling transmission dynamics [39]. The last studies to quantify **density dependence** in *O. viverrini* are now 20 years old and the parameter estimates vary considerably [40–42]. Trials measuring egg counts and antigen concentrations as a function of worm burden are urgently needed across a range of prevalence settings to provide up-to-date estimates.

Within affected communities, the distribution of parasitic worms is aggregated such that the majority of worms are harboured by a small number of hosts while the majority of individuals have light or no infections [43]. Capturing the distribution of parasites within a community is crucial for control efforts as the more aggregated the distribution, the more important it becomes to attain high treatment coverage so that individuals with the largest worm burdens, who contribute disproportionately to transmission, receive treatment [39]. The negative binomial is a suitable model for parasite counts where the **variance** exceeds the mean. The distribution is governed

Table 1. Epidemiological Parameters of *Opisthorchis viverrini* Relevant to Control

Parameter	Method of estimation	Recent estimate
Mean worm burden ( $M$ )	Directly by worm expulsion or autopsy; indirectly from egg counts or worm antigen tests	21 worms/person in Savannakhet, Laos [44] (worm expulsion)
Parasite dispersion ( $k$ )	Fitting counts of worms or eggs to negative binomial distribution, or using Equation 1	0.10 from egg counts in Champasak, Laos [33] (Equation 1)
Relationship between <i>O. viverrini</i> exposure and cholangiocarcinoma	Cohort studies, or indirectly from age distributions of <i>O. viverrini</i> worm burden and cholangiocarcinoma incidence	5% lifetime risk of cholangiocarcinoma if infected with <i>O. viverrini</i> [23]
Anthelmintic efficacy	Comparing diagnostics, typically egg counts, before and post-treatment. Sample formula often used, though statistical model is preferable	Egg count reduction 94–100% with praziquantel 40 mg/kg dose [46,47,66] (sample formula)
Reinfection rate/force of infection	Expected number of new parasite infections per unit time. Can be estimated from longitudinal data or dynamic models	38 worms/person/year in Champasak, Laos [33] (dynamic model)
Feeding rate	Expected number, or weight, of cyprinid fish consumed per person per unit time	Not estimated
Distribution of metacercariae in fish	Sampling cyprinid fish, counting <i>O. viverrini</i> cysts, and fitting to negative binomial distribution	Mean of four cysts/fish in Mukdahan, Thailand and seven cysts/fish in Khammouane, Laos [67]
Probability that consumed metacercariae develop into adult worms	Experimental studies using animal models which consume infected fish. Difficult to observe directly in humans	Worm recovery in hamsters 52% from fish fermented 1 day, and 1% from fish fermented 4 days [68]
Diagnostic specificity	Probability that observed egg or worm is <i>O. viverrini</i> , rather than an intestinal fluke	Not estimated
Zoonotic contribution to <i>O. viverrini</i> transmission	Genetic relatedness of parasites from humans and animals in the same population	Not estimated

by two parameters: the mean  $M$ , and the dispersion parameter  $k$  which scales inversely with the variance. While population surveys of *O. viverrini* worm and egg counts consistently report **overdispersion** [22,44,45], it is surprising that there are no published accounts of the negative binomial distribution fit to data and subsequently no **likelihood**-based estimates of  $k$ . An alternative to model fitting has been to use the negative binomial relationship between mean worm burden or eggs counts ( $M$ ), prevalence ( $\rho$ ), and the dispersion parameter ( $k$ )

$$\rho = 1 - \left(1 + \frac{M}{k}\right)^{-k} \quad [1]$$

which can be rearranged to calculate  $k$  from the mean and prevalence.

### Anthelmintic Efficacy

Projecting the impact of interventions using deworming drugs requires an estimate of how effective anthelmintics are at clearing adult worms from infected hosts. The anthelmintic praziquantel remains the drug of choice for treatment of *O. viverrini* due to its excellent safety profile, short half-life, and high efficacy against the parasite. It is also effective against other trematodes, such as *Schistosoma mekongi*, which has an overlapping host range with *O. viverrini* [46]. The efficacy of an anthelmintic is measured by comparing diagnostics taken before and after treatment. These are typically parasitological diagnostics, though antigen tests are also informative [47]. The outcome can be defined as a binary cure rate; however, a quantitative measure of anthelmintic

efficacy, the egg reduction rate (ERR), is preferable as infection intensity is linked to morbidity and the rate of onward transmission [43].

Published estimates of praziquantel efficacy against *O. viverrini* suggest a high ERR (Table 1); however, the past exposure of the population to praziquantel is often unclear. Future efficacy studies would benefit from estimating ERR in longitudinal cohorts to understand if efficacy is maintained after repeated treatments. A simple 'sample formula' is generally used to calculate ERR; however, this is not recommended as there is no way to characterise uncertainty, incorporate covariates, or deal with repeated observations (reviewed in [48]). Likelihood-based methods, which have been applied successfully to detect waning anthelmintic efficacy against schistosomiasis [49], are preferable as they can account for the limitations of the sample ERR.

### Reinfection Following Treatment

Humans do not acquire protective immunity to *O. viverrini* and are susceptible to reinfection [50]. The **rate** of adult worm establishment in human populations over a period of time, also known as the force of infection, is informative about the strength of parasite transmission. An *O. viverrini* reinfection study from 2015 to 2017 found that the prevalence in Northeast Thailand was lower a year after treatment compared with baseline [47]. This contrasts with studies conducted in the 1980s where prevalence rapidly rebounded to equilibrium levels following treatment [51], and suggests that the force of infection has since declined in Northeast Thailand.

Estimating the reinfection rate from data requires parasite egg or worm counts from the same individuals over at least two time points. In the *O. viverrini* literature the term 'rate' is generally misused and instead, studies report the proportion of individuals cleared of parasites who are reinfected at a later time point. Bürli *et al.* used a dynamic model to estimate the average reinfection rate in a community in Laos as 38 worms per person per year, assuming a population of approximately 2000 infected fish, compared with 35 worms per year for cats and 3 for dogs [33]. A key question for control is whether the reinfection rate declines after consecutive rounds of mass treatment or is constant. A trial on the closely related liver fluke *Chlonorchis sinensis* in Heilongjiang Province, China, found that communities randomised to annual MDA with praziquantel showed a declining proportion of individuals reinfected over two timepoints [52].

The deterministic process governing reinfection is the product of (i) the host feeding rate of cyprinid fish, (ii) the expected number of *O. viverrini* metacercariae per fish, and (iii) the probability that a consumed metacercaria establishes as an adult worm (Table 1). Understanding which factors contribute to high reinfection rates can lead to more effective and targeted interventions for at-risk populations.

### Diagnostic Specificity

The low sensitivity of parasitological tests is a known problem for the diagnosis of macroparasites [53]. An additional complication for diagnosing *O. viverrini* is the presence of minute intestinal flukes (MIFs) – trematodes which are morphologically similar to *O. viverrini* in both the adult worm and egg stages and have overlapping host ranges, intermediate hosts, and modes of transmission [54]. Unlike *O. viverrini*, MIFs do not induce cholangiocarcinoma and are generally assumed to result in low or undetectable morbidity [55]. Due to the morphological similarities between the eggs of these trematodes, misdiagnosis is possible when using microscopy methods [56]. For the Kato–Katz thick smear, MIFs and *O. viverrini* are visually indistinguishable. In other parasitological assays, such as the formalin–ether concentration technique, experienced technicians may differentiate the eggs due to subtle differences in the parasite eggshell. Given the risks of misdiagnosis in areas where MIFs are coendemic, modifications should be made to estimates of prevalence and infection intensity to account for the reduced diagnostic specificity, for instance

using **latent class modelling** [57]. Recently developed antigen tests may also provide greater sensitivity and specificity for *O. viverrini* population surveys (reviewed in [58]).

### Zoonotic Transmission

It is a long-held assumption that *O. viverrini* infects nonhuman animals, which act as reservoirs and contribute to human transmission. Indeed, the presence of an animal reservoir was cited as the reason why liver flukes could not feasibly be considered for eradication<sup>iii</sup>. The basis for the assumption of zoonotic transmission relies on morphological similarity and geographic proximity [59]. We are not aware of any molecular studies which demonstrate that *O. viverrini* in humans and nonhuman animals represent the same population. Data from **microsatellite** studies on *O. viverrini* metacercariae from cyprinid fish and cercariae shed from snails has revealed structured parasite populations [60,61], leading to the suggestion that *O. viverrini* is a species complex. Diverse populations of liver flukes in intermediate hosts may represent a mixture of human and animal-adapted parasites. The extent to which zoonotic transmission cycles contribute to human infection is therefore unresolved.

The parasitological literature contains several cases of controversy over zoonotic transmission. The roundworms *Ascaris lumbricoides* in humans and *A. suum* in pigs were shown to be reproductively isolated species in population genetic studies using multiple nuclear markers [62], whereas studies using mitochondrial DNA or a single marker had previously reported cross transmission. Reports of hybridisation between the human blood fluke *Schistosoma haematobium* and livestock-infective species, which was thought to be new or ongoing, was limited to introgression in 3–8% of the *S. haematobium* genome 240 years ago according to a recent **exome** sequencing study [63].

There are several possible relationships between *O. viverrini*-like parasites in humans and other animals (Figure 1D) which can be quantified through an analysis of the molecular variance within and between populations, or by estimating gene flow. Such research is dependent on sequencing flukes from humans and animals in the same endemic foci, preferably making use of powerful molecular techniques to analyse larger sections of the genome [64].

### Concluding Remarks

Quantitative epidemiological analyses have made many contributions to the control of infectious diseases, of which the ongoing SARS-CoV-2 pandemic provides the most dramatic recent examples [65]. Technological advances in the areas of genomics and statistical modelling make this an exciting time to glean insights into neglected pathogens such as liver fluke. New, larger datasets are also forthcoming. An extensive programme was initiated by Khon Kaen University in 2015 to test and treat cases of *O. viverrini*, screen for liver fibrosis, and perform curative surgery on early-stage cholangiocarcinoma<sup>iv</sup>. As of January 2021, the programme has screened over 950 000 people for *O. viverrini*, examined 790 000 by ultrasound, and performed 3500 operations. This represents the first longitudinal cohort study on *O. viverrini* in over 20 years and promises substantial insights into the epidemiology of the parasite (see Outstanding Questions).

### Acknowledgments

T.C. is funded by a Sir Henry Wellcome Postdoctoral Fellowship from the Wellcome Trust (reference 215919/Z/19/Z). P.S. and N.K. are supported by the Cholangiocarcinoma Screening and Care Program (CASCAP) and Fluke-Free Thailand awarded by the National Research Council of Thailand, Invitation Research Fund to the Faculty of Medicine, Khon Kaen University. O.P. is supported by the Office of the Higher Education Commission and the Thailand Research Fund (grant number MRG6180091). G.F.M. and T.D.H. gratefully acknowledge funding of the NTD Modelling Consortium by the Bill & Melinda Gates Foundation (grant number OPP1184344). The authors thank Timothy M. Pollington and Anastasia Hernandez Koutoucheva for proofreading the manuscript.

### Declaration of Interests

No interests are declared.

### Outstanding Questions

Which interventions or changes caused the long-term decline in *O. viverrini* prevalence and infection intensity in Thailand since 1980?

Longitudinal studies are needed to characterise the relationship between exposure to liver fluke (intensity and duration of worm burdens) and the risk of developing cholangiocarcinoma. Are host genetic factors associated with the risk? To what extent does deworming with praziquantel reduce the risk?

How are *O. viverrini* worm burdens and egg counts currently distributed in communities? What estimates of the mean and dispersion parameter are obtained when fitting the negative binomial distribution to survey data?

What is the relationship between egg counts or antigen concentrations with worm burden across settings with differing transmission rates? How does a more sensitive diagnostic, such as worm antigen detection, affect estimates of the curative treatment and reinfection rates?

When minute intestinal flukes are coendemic with *O. viverrini*, how should estimates of prevalence and infection intensity be adjusted to account for reduced diagnostic specificity?

Are measures of anthelmintic efficacy, such as the ERR, consistently high across endemic populations? Is the efficacy maintained in regions where past exposure to praziquantel is high?

How do rates of reinfection vary over infected regions? Can the underlying drivers of reinfection (metacercariae burden in fish, host feeding rate) be quantified? Do reinfection rates fall after repeated mass deworming?

The contribution of zoonotic transmission cycles to human infection is currently unclear. Are *O. viverrini*-like parasites in cats, dogs, and other animals the same population as those in humans? What proportion of *O. viverrini* infections in humans result from adult worms in other animals?

Sufficient attention must also be given to the cultural processes that underlie

### Supplemental Information

Supplemental information associated with this article can be found online at <https://doi.org/10.1016/j.pt.2020.12.007>.

### Resources

- <sup>i</sup><https://monographs.iarc.fr/wp-content/uploads/2018/06/mono61.pdf>
- <sup>ii</sup><https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100B.pdf>
- <sup>iii</sup><https://www.cdc.gov/mmwr/PDF/rr/rr4216.pdf>
- <sup>iv</sup><https://cloud.casap.in.th/>
- <sup>v</sup><https://apps.who.int/iris/handle/10665/75209>
- <sup>vi</sup>[https://www.who.int/neglected\\_diseases/resources/RS-2017-GE-35-KOR/en/](https://www.who.int/neglected_diseases/resources/RS-2017-GE-35-KOR/en/)

### References

1. Sripa, B. *et al.* (2011) Opisthorchiasis and *Opisthorchis*-associated cholangiocarcinoma in Thailand and Laos. *Acta Trop.* 120, S158–S168
2. Khuntikeo, N. *et al.* (2018) Current perspectives on opisthorchiasis control and cholangiocarcinoma detection in Southeast Asia. *Front. Med.* 5, 117
3. Chamadol, N. *et al.* (2019) Association between periductal fibrosis and bile duct dilatation among a population at high risk of cholangiocarcinoma: a cross-sectional study of cholangiocarcinoma screening in Northeast Thailand. *BMJ Open* 9, e023217
4. Grossman, J. and Mackenzie, F.J. (2005) The randomized controlled trial: gold standard, or merely standard? *Perspec. Biol. Biol. Med.* 48, 516–534
5. Halloran, M.E. *et al.* (2017) Simulations for designing and interpreting intervention trials in infectious diseases. *BMC Med.* 15, 1–8
6. Walker, M. *et al.* (2020) Designing antifilarial drug trials using clinical trial simulators. *Nat. Commun.* 11, 1–11
7. Kim, C.S. *et al.* (2017) Role of socio-cultural and economic factors in cyprinid fish distribution networks and consumption in Lawa Lake region, Northeast Thailand: Novel perspectives on *Opisthorchis viverrini* transmission dynamics. *Acta Trop.* 170, 85–94
8. Brockelman, W.Y. *et al.* (1986) Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand: population changes of the snail intermediate host. *Int. J. Parasitol.* 16, 545–552
9. Kopolrat, K. *et al.* (2020) Influence of water irrigation schemes and seasonality on transmission dynamics of *Opisthorchis viverrini* in the snail intermediate host, *Bithynia siamensis goniomphalos* in rice paddy fields in Northeast Thailand. *Am. J. Trop. Med. Hyg.* 103, 276–286
10. Tangkawattana, S. and Sripa, B. (2018) Integrative EcoHealth/One Health approach for sustainable liver fluke control: the Lawa Model. *Adv. Parasitol.* 102, 115–139
11. Garn, J.V. *et al.* (2017) The impact of sanitation interventions on latrine coverage and latrine use: A systematic review and meta-analysis. *Int. J. Hyg. Environ. Health* 220, 329–340
12. Asavarut, P. *et al.* (2016) *Diet and Disease: Transgressing Boundaries between Science and Society – Understanding Neglected Diseases through the Lens of Cultural Studies and Anthropology*, Institute of Medical Ethics
13. Sormani, S. *et al.* (1984) Infection and re-infection rates of opisthorchiasis in the water resource development area of Nam Pong project, Khon Kaen Province, northeast Thailand. *Ann. Trop. Med. Parasitol.* 78, 649–656
14. Suwannatitorn, P. *et al.* (2013) A follow-up study of *Opisthorchis viverrini* infection after the implementation of control program in a rural community, central Thailand. *Parasit. Vectors* 6, 188
15. Saowakontha, S. *et al.* (1993) Field trials in the control of *Opisthorchis viverrini* with an integrated programme in endemic areas of northeast Thailand. *Parasitology* 106, 283–288
16. Suwannatrai, A. *et al.* (2018) Epidemiology of *Opisthorchis viverrini* infection. *Adv. Parasitol.* 101, 41–67
17. Jongsuksuntigul, P. and Imsomboon, T. (1997) The impact of a decade long opisthorchiasis control program in northeastern Thailand. *Southeast Asian J. Trop. Med. Pub. Health* 28, 551
18. Thinkhamrop, K. *et al.* (2019) Repeated praziquantel treatment and *Opisthorchis viverrini* infection: a population-based cross-sectional study in northeast Thailand. *Infect. Dis. Poverty* 8, 18
19. Khieu, V. *et al.* (2019) Is *Opisthorchis viverrini* emerging in Cambodia? *Adv. Parasitol.* 103, 31–73
20. Haswell-Elkins, M.R. *et al.* (1994) Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. *Int. J. Cancer* 59, 505–509
21. Arunsan, P. *et al.* (2019) Programmed knockout mutation of liver fluke granulin attenuates virulence of infection-induced hepatobiliary morbidity. *eLife* 8, e41463
22. Haswell-Elkins, M. *et al.* (1991) Distribution patterns of *Opisthorchis viverrini* within a human community. *Parasitology* 103, 97–101
23. Songserm, N. *et al.* (2012) Risk factors for cholangiocarcinoma in high-risk area of Thailand: role of lifestyle, diet and methylenetetrahydrofolate reductase polymorphisms. *Cancer Epidemiol.* 36, e89–e94
24. Sripa, B. *et al.* (2012) The tumorigenic liver fluke *Opisthorchis viverrini* – multiple pathways to cancer. *Trends Parasitol.* 28, 395–407
25. Tam, V. *et al.* (2019) Benefits and limitations of genome-wide association studies. *Nat. Rev. Genet.* 20, 467–484
26. Dhiansiri, T. *et al.* (1984) Roentgenographically controlled healing of gallbladder lesions in opisthorchiasis after praziquantel treatment. *Arzneimittel-Forschung* 34, 1175–1177
27. Mairiang, E. *et al.* (1993) Reversal of biliary tract abnormalities associated with *Opisthorchis viverrini* infection following praziquantel treatment. *Trans. R. Soc. Trop. Med. Hyg.* 87, 194–197
28. Pungpak, S. *et al.* (1997) *Opisthorchis viverrini* infection in Thailand: studies on the morbidity of the infection and resolution following praziquantel treatment. *Am. J. Trop. Med. Hyg.* 56, 311–314
29. Andrade, G. *et al.* (2017) Decline in infection-related morbidities following drug-mediated reductions in the intensity of *Schistosoma* infection: A systematic review and meta-analysis. *PLoS Negl. Trop. Dis.* 11, e0005372
30. Hollingsworth, T.D. *et al.* (2015) Quantitative analyses and modelling to support achievement of the 2020 goals for nine neglected tropical diseases. *Parasit. Vectors* 8, 630
31. Hollingsworth, T.D. (2018) Counting down the 2020 goals for 9 neglected tropical diseases: what have we learned from quantitative analysis and transmission modeling? *Clin. Infect. Dis.* 66, S237–S244
32. Turner, H.C. *et al.* (2014) Neglected tools for neglected diseases: mathematical models in economic evaluations. *Trends Parasitol.* 30, 562–570
33. Bürl, C. *et al.* (2018) Mathematical analysis of the transmission dynamics of the liver fluke, *Opisthorchis viverrini*. *J. Theoret. Biol.* 439, 181–194
34. Bürl, C. *et al.* (2018) Analysis of interventions against the liver fluke, *Opisthorchis viverrini*. *Mathemat. Biosci.* 303, 115–125

human behaviour. The persistence of liver fluke infection, despite decades of control efforts, suggests that understanding the societal drivers of infection will be essential for developing evidence-based interventions with sustainable outcomes.

35. Grundy-Warr, C. *et al.* (2012) Raw attitudes, wetland cultures, life-cycles: socio-cultural dynamics relating to *Opisthorchis viverrini* in the Mekong Basin. *Parasitol. Int.* 61, 65–70
36. Fürst, T. *et al.* (2012) Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect. Dis.* 12, 210–221
37. Aung, W.P.P. *et al.* (2017) First report and molecular identification of *Opisthorchis viverrini* infection in human communities from Lower Myanmar. *PLoS One* 12, e0177130
38. Sohn, W.-M. *et al.* (2019) Low-grade endemicity of opisthorchiasis, Yangon, Myanmar. *Emerg. Infect. Dis.* 25, 1435
39. Churcher, T. *et al.* (2005) Density dependence and overdispersion in the transmission of helminth parasites. *Parasitology* 131, 121–132
40. Ramsay, R.J. *et al.* (1989) Density-dependent fecundity of *Opisthorchis viverrini* in humans, based on faecal recovery of flukes. *Trans. R. Soc. Trop. Med. Hyg.* 83, 241–242
41. Sithithaworn, P. *et al.* (1991) Relationship between faecal egg count and worm burden of *Opisthorchis viverrini* in human autopsy cases. *Parasitology* 102, 277–281
42. Elkins, D. *et al.* (1991) *Opisthorchis viverrini*: relationships between egg counts, worms recovered and antibody levels within an endemic community in northeast Thailand. *Parasitology* 102, 283–288
43. Anderson, R.M. and May, R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*, Oxford University Press
44. Sato, M. *et al.* (2015) Patterns of trematode infections of *Opisthorchis viverrini* (Opisthorchiidae) and *Haplorchis taichui* (Heterophyidae) in human populations from two villages in Savannakhet Province, Lao PDR. *J. Helminthol.* 89, 439
45. Dao, T.T.H. *et al.* (2016) *Opisthorchis viverrini* infections and associated risk factors in a lowland area of Binh Dinh Province, Central Vietnam. *Acta Trop.* 157, 151–157
46. Lovis, L. *et al.* (2012) Efficacy of praziquantel against *Schistosoma mekongi* and *Opisthorchis viverrini*: a randomized, single-blinded dose-comparison trial. *PLoS Negl. Trop. Dis.* 6, e1726
47. Worasith, C. *et al.* (2020) Application of urine antigen assay to evaluate outcomes of praziquantel treatment and reinfection in opisthorchiasis in northeast Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 114, 751–761
48. Walker, M. *et al.* (2014) Models for measuring anthelmintic drug efficacy for parasitologists. *Trends Parasitol.* 30, 528–537
49. Crellen, T. *et al.* (2016) Reduced efficacy of praziquantel against *Schistosoma mansoni* is associated with multiple rounds of mass drug administration. *Clin. Infect. Dis.* 63, 1151–1159
50. Wongratanaheewin, S. *et al.* (2003) Immunology and molecular biology of *Opisthorchis viverrini* infection. *Acta Trop.* 88, 195–207
51. Upatham, E. *et al.* (1988) Rate of re-infection by *Opisthorchis viverrini* in an endemic northeast Thai community after chemotherapy. *Int. J. Parasitol.* 18, 643–649
52. Choi, M.-H. *et al.* (2010) Effect of control strategies on prevalence, incidence and re-infection of clonorchiasis in endemic areas of China. *PLoS Negl. Trop. Dis.* 4, e601
53. Nikolay, B. *et al.* (2014) Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. *Int. J. Parasitol.* 44, 765–774
54. Sithithaworn, P. *et al.* (2006) Epidemiology of food-borne trematodes and other parasite infections in a fishing community on the Nam Ngum reservoir, Lao PDR. *Southeast Asian J. Trop. Med. Pub. Health* 37, 1083
55. Carney, W.P. (1991) Echinostomiasis – a snail-borne intestinal trematode zoonosis. *Southeast Asian J. Trop. Med. Pub. Health* 22, 206–211
56. Johansen, M.V. *et al.* (2010) Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. *Adv. Parasitol.* 73, 171–195
57. Assefa, L.M. *et al.* (2014) Diagnostic accuracy and cost-effectiveness of alternative methods for detection of soil-transmitted helminths in a post-treatment setting in western Kenya. *PLoS Negl. Trop. Dis.* 8, e2843
58. Saijuntha, W. *et al.* (2018) Recent advances in the diagnosis and detection of *Opisthorchis viverrini sensu lato* in human and intermediate hosts for use in control and elimination programs. *Adv. Parasitol.* 101, 177–214
59. Tangkawattana, S. and Tangkawattana, P. (2018) Reservoir animals and their roles in transmission of *Opisthorchis viverrini*. *Adv. Parasitol.* 101, 69–95
60. Pitaksakurat, O. *et al.* (2017) Preliminary genetic evidence of two different populations of *Opisthorchis viverrini* in Lao PDR. *Parasitol. Res.* 116, 1247–1256
61. Namsanor, J. *et al.* (2020) Impact of geography and time on genetic clusters of *Opisthorchis viverrini* identified by micro-satellite and mitochondrial DNA analysis. *Int. J. Parasitol.* 50, 1133–1144
62. Betson, M. *et al.* (2014) Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of *Ascaris* in people and pigs. *J. Infect. Dis.* 210, 932–941
63. Platt, R.N. *et al.* (2019) Ancient hybridization and adaptive introgression of an invadysin gene in schistosome parasites. *Mol. Biol. Evol.* 36, 2127–2142
64. Doyle, S.R. and Cotton, J.A. (2019) Genome-wide approaches to investigate anthelmintic resistance. *Trends Parasitol.* 35, 289–301
65. Flaxman, S. *et al.* (2020) Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature* 584, 257–261
66. Sayasone, S. *et al.* (2017) Efficacy and safety of praziquantel against light infections of *Opisthorchis viverrini*: a randomized parallel single-blind dose-ranging trial. *Clin. Infect. Dis.* 64, 451–458
67. Namsanor, J. *et al.* (2020) Infection dynamics of *Opisthorchis viverrini* metacercariae in cyprinid fishes from two endemic areas in Thailand and Lao PDR. *Am. J. Trop. Med. Hyg.* 102, 110–116
68. Onsurathum, S. *et al.* (2016) Effects of fermentation time and low temperature during the production process of Thai pickled fish (pla-som) on the viability and infectivity of *Opisthorchis viverrini* metacercariae. *Int. J. Food Microbiol.* 218, 1–5
69. McElreath, R. (2016) *Statistical Rethinking: A Bayesian Course with Examples in R and Stan*, CRC Press
70. Forrer, A. *et al.* (2012) Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR. *PLoS Negl. Trop. Dis.* 6, e1481
71. Kamsa-Ard, S. *et al.* (2015) Association between praziquantel treatment and cholangiocarcinoma: a hospital-based matched case-control study. *BMC Cancer* 15, 776
72. Luvira, V. *et al.* (2018) Association between repeated praziquantel treatment and papillary, and intrahepatic cholangiocarcinoma. *Ann. Hepatol.* 17, 802–809
73. Pearl, J. (2009) *Causality*, Cambridge University Press
74. Pirlaor, S. *et al.* (2008) Oxidative and nitrate stress in *Opisthorchis viverrini*-infected hamsters: an indirect effect after praziquantel treatment. *Am. J. Trop. Med. Hyg.* 78, 564–573
75. Hanpanich, P. *et al.* (2017) Decreased risk of cholangiocarcinogenesis following repeated cycles of *Opisthorchis viverrini* infection-praziquantel treatment: Magnetic Resonance Imaging (MRI) and histopathological study in a hamster model. *Parasitol. Int.* 66, 464–470
76. Medley, G. and Bundy, D. (1996) Dynamic modeling of epidemiologic patterns of schistosomiasis morbidity. *Am. J. Trop. Med. Hyg.* 55, 149–158
77. Jusakul, A. *et al.* (2017) Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. *Cancer Discov.* 7, 1116–1135
78. Upatham, E. *et al.* (1984) Relationship between prevalence and intensity of *Opisthorchis viverrini* infection, and clinical symptoms and signs in a rural community in north-east Thailand. *Bull. WHO* 62, 451
79. Kamsa-Ard, S. *et al.* (2011) Trends in liver cancer incidence between 1985 and 2009, Khon Kaen, Thailand: cholangiocarcinoma. *Asian Pac. J. Cancer Prev.* 12, 2209–2213
80. Phongluxa, K. *et al.* (2015) Perceived illness drives participation in mass deworming campaigns in Laos. *Acta Trop.* 141, 281–288