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Opisthorchis viverrini: an underestimated parasite in world health

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Abstract

Infection with *Opisthorchis viverrini* and its associated cholangiocarcinoma (CCA) is an underestimated problem in the Mekong region of Southeast Asia, despite the widespread use of praziquantel and health education measures for parasite control. Although data from Cambodia, Laos and Vietnam are rare, data from Thailand often show wide-ranging variability in epidemiological parameters, including human morbidity and the prevalence and incidence of CCA. The recent discovery of high levels of population genetic variability in *O. viverrini* in different wetlands in Thailand and Laos, which indicates the presence of sibling species, suggests that we have underestimated the complexity of this epidemiological situation. Future research should determine the relationship between the genetic variability of *O. viverrini* and patterns of opisthorchiasis-related disease.

Opisthorchis viverrini *in Southeast Asia*

The World Health Organization (WHO) estimates that approximately five million people with HIV live in the Asia-Pacific Region [1]. This can be compared with a conservative estimate of ten million people infected with the liver fluke *Opisthorchis viverrini* in Thailand and Laos alone [2]. People infected with *Opisthorchis* parasites are at risk of developing cholangiocarcinoma (CCA), and those who have advanced CCA will almost certainly die. As with HIV, infection with *O. viverrini* and the likelihood of developing CCA is related to culturally dependent behavioural patterns, which, if modified, can prevent infection [3]. Here, we briefly review epidemiological aspects of opisthorchiasis and CCA, and discuss these in relation to the recently published evidence that *O. viverrini* is a species complex.

O. viverrini: an underestimated parasite in world health

O. viverrini is an underestimated parasite in world health [4] because it is mistakenly thought to represent a limited, local problem in Southeast Asia, particularly in Thailand [5]. This is predominantly because of the fact that reasonably accurate spatial and temporal morbidity data and CCA incidence are available from Thailand but not from elsewhere within the Mekong region where *O. viverrini* occurs [6-9]. Although *O. viverrini* infection in humans is often asymptomatic, this parasite can be pathogenic in its own right and cause hepatobiliary diseases [10]. Its greatest impact, however, is as a direct risk factor for CCA of

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the bile duct [5,7], and *O. viverrini* has been recognized as a type-1 carcinogen since 1994 [11,12]. Thus, the infection is not immediately life threatening; cancer develops 30-40 years after infection, with death occurring within 3-6 months of diagnosis [5]. No pharmaceutical treatment is available, and surgery and supportive treatment are complicated [13] and often not accessible for victims in developing countries.

Economically, opisthorchiasis and CCA are estimated to cost US$120 million annually in medical care and lost wages in Thailand alone [14]. The cost to families and communities is enormous. CCA causes social, economic, community and family problems because it is more prevalent in males, the principal financial earners. Infection also occurs in young children, who are then at considerable risk of developing liver cancer when they are 30-40 years old, during their most productive years.

O. viverrini has a three-host life cycle (Figure 1). The first intermediate hosts are prosobranch snails, *Bithynia* spp. of the family Bithyiniidae [15]. At least 18 species of cyprinid fish act as second intermediate hosts, and carnivores (e.g. cats and dogs), but more specifically humans, are definitive hosts [6]. Humans become infected following the consumption of raw or insufficiently cooked fish containing metacercariae.

The prevalence of *O. viverrini* in snails is generally low (<1%), whereas in cyprinid fish it is extremely high (90-95%) [2,16,17]. The parasite burden in fish is highly aggregated and the majority of infected fish harbour one to two metacercariae, so a repeated, low infectious dose is likely (P. Sithithaworn *et al.*, unpublished).

Prevalence in humans

The most recent estimate of the number of people infected with *O. viverrini* in the Mekong region is eight million in Thailand and two million in Laos [2]. This is a substantial underestimate of its prevalence because no data are available for Cambodia or Vietnam, although opisthorchiasis in known to be common in parts of these countries [18,19]. In Thailand, an average of 9.6% of the population is infected [20], with the liver fluke being distributed mainly in the North (19.3% prevalence) and Northeast (15.7% prevalence) [2,21]. There has been a substantial decline in the prevalence of infection in Thailand from 34% (in 1992) to 10% (in 2002) [21], which has been attributed to intensive and continuous control activities. Disturbingly, substantial variation in prevalence ranging from 4% to 33% still remains in affected provinces [20]. Within Khon Kaen province (northeast Thailand), *O. viverrini* infection ranges from 2% to 71% between different districts [22]. Although data on the prevalence of infection are available, data on incidence are scant [23,24].

Incidence of CCA

CCA has caused millions of deaths in southeast Asia [25]. The most important risk factor for CCA is *O. viverrini* infection, with risk being dependent on intensity [26], previous or current exposure to infection and host genetic polymorphism [27]. A recent study detected residual *O. viverrini* DNA in both cancerous and non-cancerous tissue in CCA patients in Thailand [28]. By contrast, the risk factors for CCA in areas where liver flukes are not endemic include primary sclerosing cholangitis, hepatolithiasis and choledochal cysts [29]. Its incidence is 98 per 100 000 people in the Thai province of Khon Kaen, which is at least an order of magnitude higher than in areas outside of the Mekong region, even though CCA is becoming increasingly frequent in the Western world [30,31].

Because cancer registration is available in Thailand but not other countries in the Mekong region [32], a systematic investigation to estimate the socioeconomic impact of CCA is currently impossible [33]. Nevertheless, although <10% of people infected with *O. viverrini* develop CCA, a high proportion of the population are infected in many areas [8,22].

Importantly, the prevalence, intensity and medical significance of *O. viverrini* infection in humans varies geographically within the Mekong region, as do clinical symptoms and prevalence of CCA. Variation in the incidence of CCA in different communities in Thailand, however, is not correlated with the prevalence of *O. viverrini* [22].

Genetic and molecular characterization of *O. viverrini*

The study of the molecular genetics of O. viverrini has received considerable impetus from the recent publication that provides the first catalogue of expressed genes (~14% of the entire transcriptome) for this species [34]. This advance will enhance our ability to conduct comprehensive molecular analyses of the systematics and population genetics of *O. viverrini* in a similar manner to that proven useful for other parasites, as has been the case for the parasitic protozoan *Giardia intestinalis* utilizing the genes encoding allozyme markers [35]. In addition, the possible role of genetic variation among *O. viverrini* populations in the local and regional variability in human disease, including CCA, has also begun to receive attention recently.

Analyses using random amplification of polymorphic DNA have detected genetic polymorphisms among *O. viverrini* isolates from Thailand and Laos [36]. This finding was supported by allozyme electrophoresis analyses at 32 enzyme loci [37-39]. Allozyme analyses have shown that *O. viverrini* is a species complex that consists of at least two, but possibly six, genetically distinct but morphologically similar, hence, ‘cryptic’ sibling species. The species are associated with different major wetland systems, including the Chi, Mun, Songkram and Wang Rivers in Thailand and the Nam Ngum River in Laos [39] (Figure 2).

The separation of *O. viverrini* into at least two sibling species is supported by preliminary evidence from other molecular and biological data (W. Saijuntha *et al.*, unpublished) and coincides with the division of the intermediate host *Bithynia siamensis goniomphalos* into two major genetic groups [39]. The role that specific wetlands have between species and populations of *Opisthorchis* parasites in limiting gene flow requires investigation.

Current and future threats

The movement of people is a major component of globalization in Asia [40]. *O. viverrini* is regularly carried to regions outside of its normal distribution by tourists and Thai labourers [2,41]. Because infection is often asymptomatic and of a long duration, infected migrants are likely to remain undiagnosed and shed eggs for long time periods. However, the introduction of *O. viverrini* to human populations outside of the Mekong region is more likely to come from fish imports. Asia is the world’s major producer (90%) of aquaculture products and there has been an enormous recent expansion in aquaculture in *O. viverrini* endemic countries [25,42]. The increasing use of fish aquaculture, with high usage of cyprinid species [43], provides ample opportunities for species-switching by parasites, including those with complex life cycles [44]. The export and import of fish and fish products worldwide provides a transfer route for parasites [45], which in the case of *O. viverrini* are favoured by the fact that the metacercariae are resilient and can remain viable in fish muscle even if the fish is pickled or fermented [46].

The possible effects of global climate change on *O. viverrini* distribution are debatable. Regional projections for Southeast Asia indicate higher temperatures approaching the mean global increase with potentially higher rainfall depending on the pattern of tropical cyclones

[47]. Increased temperature is likely to reduce the developmental time of immature stages and might potentially reduce cercarial host-searching time [48,49]. An increase in rainfall could lead to the extension of wetlands and, therefore, suitable new habitat, with an increased likelihood of parasitic gene flow between them through the expansion of snail and fish host distributions.

Prevention

The availability of praziquantel provides effective chemotherapy for *Opisthorchis* parasite infection [46]. There is, however, evidence that repeated infection and treatment could increase the risk of CCA [50,51]. Thus, a more basic approach involving prevention by reducing the consumption of infected fish is likely to have better long-term chances of success. It is difficult to achieve this goal, however, because it involves changing traditional patterns of eating behaviour. A control program for *O. viverrini* in Thailand operates through the primary care system and employs a selective treatment approach [21]; however, high transmission variation between communities is common [22]. Control activity should be targeted to communities with high *O. viverrini* intensities and areas with high risk of CCA [8]. Inflammatory biomarkers recently reported in opisthorchiasis and CCA are potentially useful tools for surveillance and control [52,53]. For adults, the campaign should emphasize a reduction in reinfection, and emphasis should be placed on health education for children. By building local knowledge of parasitic diseases and CCA into curricula, information could be systematically disseminated to schoolchildren.

A minimum requirement should be that an adequate health system be set up to enable appropriate surveillance work. This is particularly pertinent for Cambodia, Laos and Vietnam, which lag behind Thailand in the development of such systems.

The success of control programs is dependent on several epidemiological factors. First, humans seem to be the main, definitive hosts of the parasite, which is rarely found in animal reservoir hosts. Thus, by breaking the cycle at the human host, only a limited sylvatic input is likely. Second, population genetic data indicate that gene flow between areas is limited (W. Saijuntha, unpublished) and that once control measures have taken effect, the reintroduction of *O. viverrini* is likely to be slow if human movement is limited from areas where prevalence is still high. Here, it is important to understand more about the ecology of *O. viverrini* and whether disease characteristics are likely to change (i.e. towards higher virulence in a susceptible population) if new introductions occur.

Concluding remarks and future directions

Although our knowledge of the biology of *O. viverrini* is steadily increasing, fundamental studies on epidemiology and ecology, in addition to genomics and proteomics, are still needed. In addition, investigation of the molecular basis of fluke-induced CCA is likely to be of great importance in the development of biomarkers for the diagnosis of this devastating condition.

The scientific challenge of providing a comprehensive epidemiological understanding of the *O. viverrini* species complex remains immense. Finding substantial genetic variation among *O. viverrini* from different wetlands and among *B. s. goniomphalos* from different geographical areas provides a potential basis from which to investigate the epidemiological variability in opisthorchiasis and CCA. Furthermore, how abiotic and biotic characteristics of different wetlands influence the ecology and genetic variation within *O. viverrini* and its intermediate hosts needs to be explored, particularly in relation to future climate change. Understanding such relationships will have major implications for the surveillance, control and treatment of opisthorchiasis.

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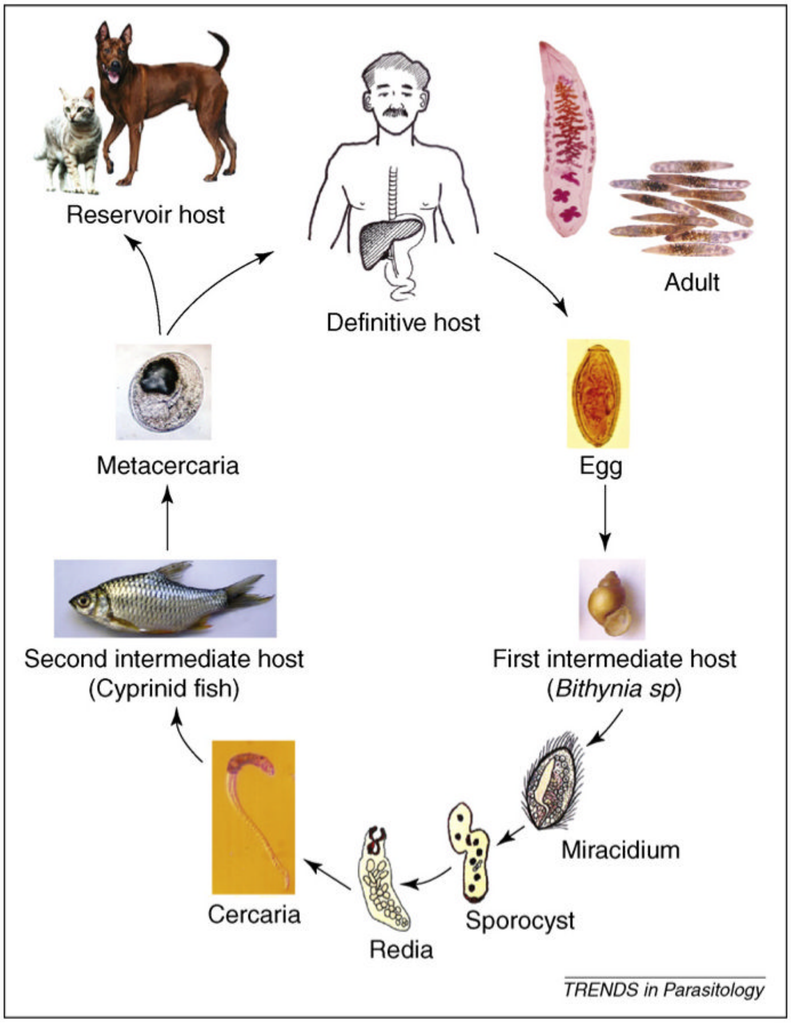

Figure 1.

The life cycle of *O. viverrini*. Humans become infected by ingesting metacercariae in uncooked fish. The ingested metacercariae excyst in the duodenum and enter the bile duct, where they develop into sexually mature adult worms. Eggs are produced and discharged with bile fluid into the intestine and faeces. When eggs reach a body of freshwater and are ingested by an appropriate snail, miracidium hatch and develop into sporocysts and rediae. Different species of *Bithynia* snails serve as first intermediate hosts. The rediae gave rise to cercariae and, when exposed to appropriate cyprinid species of fish (the second intermediate hosts), the free-swimming cercariae penetrate into the tissues or skin of freshwater fish and become fully infective metacercariae, which completes the life cycle. Humans act as definite hosts, along with fish-eating carnivores (e.g. cats and dogs), which act as reservoir hosts.

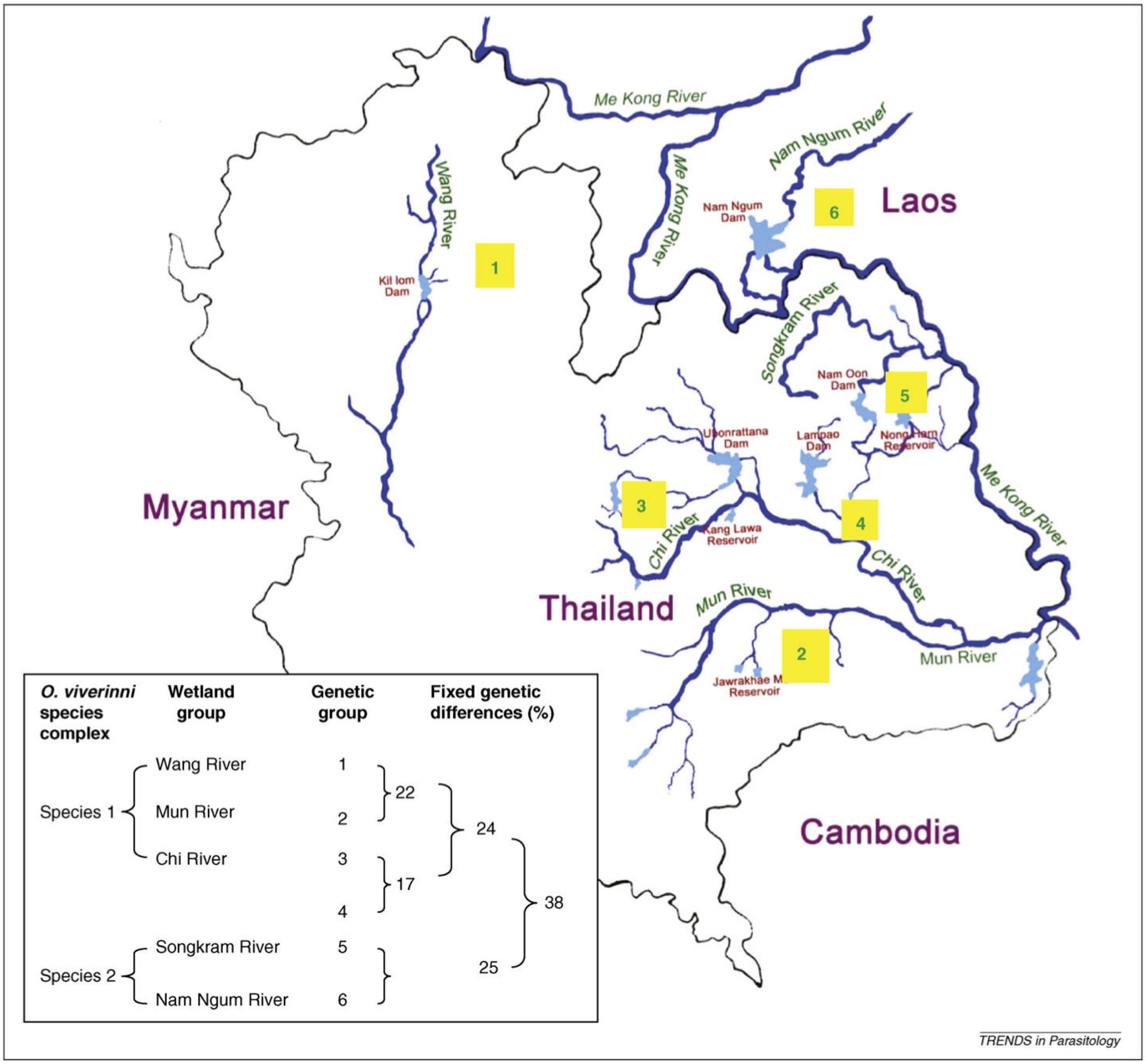

Figure 2.

A summary of the systematics of the *O. viverrini* species complex. Depicted are the numbers of genetic groups (in yellow boxes) detected within each of the currently detected sibling species; the magnitude of fixed genetic differences between genetic groups; the levels of fixed genetic differences that define each species, and correlations of species and genetic groups with distinct wetlands after the application of the molecular genetic technique of allozyme electrophoresis. Fixed genetic differences occur when samples being compared do not have any alleles in common at the enzyme locus being examined. In the case of *O. viverrini*, 33 independent enzyme loci were examined [36]. The geographical distribution of *O. viverrini* includes Thailand, Laos, Cambodia, Vietnam and, possibly, Myanmar (no data are currently available).