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Invited Review

The zoonotic, fish-borne liver flukes *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini*

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ABSTRACT

Clonorchis sinensis, *Opisthorchis felineus* and *Opisthorchis viverrini* are the three most important liver flukes involved in human health, infecting more than 45 million people worldwide. Both *C. sinensis* and *O. viverrini*, and possibly *O. felineus*, can induce human cholangiocarcinoma as well as inducing other hepatobiliary pathology. Although the life cycles of all three species are similar, only that of *O. felineus* in Europe remains predominantly zoonotic, while *O. felineus* in Asia and *C. sinensis* have a stronger mixture of zoonotic and anthroponotic components in their life cycles. *Opisthorchis viverrini* from the Mekong area of southeastern Asia is predominantly anthroponotic. Here we discuss the comparative epidemiology of these three taxa comparing in detail the use of first, second and final animal hosts, and consider the potential role of humans in spreading these pathogens. In addition we discuss the genetic structure of all three species in relation to potentially cryptic species complexes.

1. Introduction

Many important pathogens causing human zoonotic disease are transmissible to human beings from animals by eating raw or partially cooked meat or fish (Keiser and Utzinger, 2009; Grundy-Warr et al., 2012). These include a number of fish-borne zoonotic liver flukes, with currently more than 45 million people estimated to be infected (Keiser and Utzinger, 2009). Of these fish-borne trematodes, *Clonorchis sinensis*, *Opisthorchis felineus* and *Opisthorchis viverrini* (family Opisthorchiidae, subfamily Opisthorchiinae) are not only the most important but provide an interesting zoonotic perspective. *Opisthorchis felineus* is a parasite predominantly of carnivores throughout much of its range, only occasionally causing human disease (Pozio et al., 2013) except in eastern Russia where prevalences in humans can be high and poor sanitary conditions make the spread of eggs via human faeces possible (Mordvinov et al., 2012). *Clonorchis sinensis* holds an intermediate position, with a number of significant native and domestic reservoir animal hosts but also with high levels of human faecal contamination of the environment playing a significant role in the transmission cycle (Lun et al., 2005). *Opisthorchis viverrini* provides a quite different perspective. This designation represents a number of taxa (Andrews et al., 2008; Sithithaworn et al., 2012b), all of which are predominantly parasites of humans with some input into the life cycle likely to come from domestic carnivores, particularly cats (Sithithaworn et al., 2007a; Aunpromma et al., 2012). There is almost no information on endemic wildlife involvement in the life cycle of *O. viverrini* sensu lato except for a single record from a fishing cat (*Prionailurus viverrinus*), the worms from which were used to describe the species late in the 19th century (Poirier, 1886). Thus, only *O. felineus* can provide us with an idea of the natural zoonotic dynamics of infection, *C. sinensis* provides an intermediate perspective, while the zoonotic cycle has largely disappeared for *O. viverrini*, being replaced with a predominantly anthropogenic cycle.

The combined range of these liver flukes covers much of Europe across to east and southeastern Asia (Sithithaworn et al., 2007a). All three are very important trematodes with a high impact on human health in many areas in which they are found (Sithithaworn et al., 2007a; Keiser and Utzinger, 2009). This is especially true

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for *C. sinensis* and *O. viverrini* which are major causative agents of bile duct cancer (cholangiocarcinoma; CCA) within their areas of distribution (Shin et al., 2010; Sithithaworn et al., 2012a). They are both classified as class I carcinogens (Bouvard et al., 2009), and data are accumulating which suggest that *O. felineus* also fits this pattern (Parkin, 2006; Mordvinov et al., 2012).

Approximately 700 million people are at risk of infection by these liver flukes through the consumption of raw or partially cooked freshwater (mainly) cyprinid fish (Keiser and Utzinger, 2005, 2009). The disease caused is endemic in China, the Republic of Korea, eastern Russia, Japan, Taiwan, Hong Kong and northern and central Vietnam (*C. sinensis*), the eastern states of the Russian Federation including Siberia (*O. felineus*), and the north and northeast of Thailand, Lao PDR, Cambodia and southern Vietnam (*O. viverrini*). For *C. sinensis*, 35 million people are estimated to be infected with 600 million people at risk of infection (Lun et al., 2005; Keiser and Utzinger, 2009; IARC, 2012), while in China, where clonorchiasis is considered to be one of the fastest growing food-borne parasitic diseases, *C. sinensis* occurs in 27 of the 34 provinces/autonomous regions/municipalities (Li et al., 2010). Opisthorchiasis due to *O. felineus* has a population of 12.5 million at risk, at least in Kazakhstan, the Russian Federation, Siberia and the Ukraine. An estimated 1.6 million people are infected (Keiser and Utzinger, 2009). In southeastern Asia, more than 90 million people are at risk of infection, and at least 10 million people are estimated to be infected by *O. viverrini* (Keiser and Utzinger, 2005; Andrews et al., 2008). In fact, in Thailand alone, at least 8 million people are known to be infected, most of whom live in the northern and northeastern regions of the country (Sripa et al., 2011). Reliable data from Cambodia, Lao PDR and southern Vietnam are lacking, although endemicity can be high locally in all of these countries (Sithithaworn et al., 2012a).

2. Animals involved in the life cycles of fish-borne liver flukes

All three trematodes have a three host life cycle with the first intermediate hosts being freshwater snails, the second fish, mostly often belonging to the family Cyprinidae, while mammals, in most cases carnivores and humans, act as final hosts (Fig. 1; Sithithaworn, 2012a).

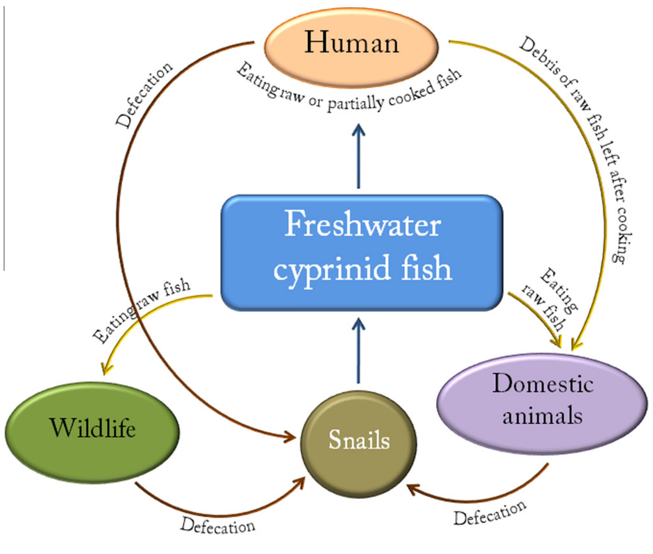

Fig. 1. A generalized life cycle for zoonotic opithorchiid liver flukes.

2.1. First intermediate hosts

Unlike the two *Opisthorchis* spp. whose first intermediate hosts belong to a single genus of snails (*Bithynia*), *C. sinensis* has eight major freshwater intermediate snail host species belonging to five families (Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, Thiaridae; Lun et al., 2005). Levels of prevalence of infection in these snails vary locally in China and are usually low, although they can reach up to 27% for *Alocinma longicornis* (Hydrobiidae) in parts of Guangdong, China and 8% for *Bithynia fuchsianus* (Bithyniidae) in parts of Guangxi, China (Lun et al., 2005).

In the case of *O. felineus* and *O. viverrini*, there are currently only a limited number of first intermediate hosts which are recognized, all of which belong to the genus *Bithynia* (family Bithyniidae) (Sithithaworn and Haswell-Elkins, 2003; Mordvinov et al., 2012). The taxonomic status of various members of this genus is currently being investigated in several areas. Research has shown that taxa previously classified as individual species can frequently be divided into species groups based on their isolation in different freshwater bodies (Glöer and Yildirim, 2006; Glöer and Pesic, 2007; Glöer et al., 2007; Lazutkina et al., 2009). Although considerably more work is required to determine the exact taxonomic status of the intermediate hosts of both *O. felineus* and *O. viverrini*, the number of known hosts of *O. felineus* has recently increased from one to three morphologically similar species, *Bithynia inflata*, *Bithynia leachi* and *Bithynia troscheli* (Lazutkina et al., 2009; Mordvinov et al., 2012). Evidence suggests that the number of snail intermediate hosts of *O. viverrini* has also been underestimated (Saijuntha et al., 2007; Kiatsopit et al., 2013).

Relatively few data are available on the prevalence of *O. felineus* in its intermediate hosts, although Erhardt et al. (1962) indicated that 73% of the *B. leachi* tested experimentally were susceptible to infection. De Liberato et al. (2011), using a pooling technique, found a minimum prevalence of 0.6% in *Bithynia* spp. in Lake Bolsena and 0.04% in Lake Bracciano in central Italy. Hering-Hagenbeck and Schuster (1996) found a prevalence of 1.2% in *B. leachi* from Brandenburg in Germany.

Based on our current knowledge, *O. viverrini* is known to infect *Bithynia funiculata*, *Bithynia siamensis gomiomphalos* and *Bithynia siamensis siamensis* (Petney and Taraschewski, 2011). *Bithynia funiculata* is distributed in the northern provinces of Thailand and is found in association with *B. s. siamensis* in Chiang Mai, Mae Sot and Lampun (Brandt, 1974). *Bithynia s. siamensis* occurs throughout the whole of the Malaccan peninsula including central, western, northern and southeastern Thailand, Cambodia and southern Vietnam, and into Myanmar from Mandalay southwards (Brandt, 1974). *Bithynia s. goniomphalos* is distributed from the northeast of Thailand to Tonkin and probably to southern China (Brandt, 1974). The distributional limits of *B. s. gomiomphalos* are unclear but Brandt (1974) indicated that all specimens from the Tonle Sap, Cambodia and from central Vietnam belong to this species while *B. s. siamensis* is found in southern Cambodia and southern Vietnam. No regional separation of *Bithynia* snails has been reported in other parts of southeastern Asia, probably due to insufficient surveys (Kiatsopit et al., 2013). The main areas of human infection in Thailand lie within the range of *B. s. gomiomphalos* (Sithithawon et al., 2012a).

The prevalence of *O. viverrini* infection in *Bithynia* snails is usually low, with many uninfected individuals being found. Prevalence as determined by cercarial release is commonly less than 2% but Kiatsopit et al. (2012) reported several collections with between 6% and 9% prevalence of infection. One of the major unanswered questions involves the dynamics of snail infection. Given that many locations appear to be infection-free and where infection does occur, prevalences are usually low, where and how do snails come into contact with infected faecal material? Kaewkes et al.