EcoHealth 9, 328–341, 2012
DOI: 10.1007/s10393-012-0789-z

ECOHEALTH

Review

Examining Landscape Determinants of *Opisthorchis viverrini* Transmission

Yi-Chen Wang

Department of Geography, National University of Singapore, Block AS2, 1 Arts Link, Singapore 117570, Singapore

Abstract: Liver fluke (*Opisthorchis viverrini, O.v.*) infection, along with its associated cholangiocarcinoma, is a major public health problem in Southeast Asia. Despite the vast amount of epidemiological research, human *O.v.* prevalence remains high and varies greatly across the region. This paper examines the landscape determinants that influence *O.v.* transmission in relation to the three hosts of its life cycle and identifies areas that require further research so as to advance the understanding of the spatial variation in disease risk. A critical agent functionally connects all sequential life cycle stages of *O.v.* is water. Seasonality and water quality appear to affect the habitats and population dynamics of the two intermediate hosts, *Bithynia* snails and cyprinid fish. Land use practice through the construction of irrigation ditches increases the connections between the hosts, thereby functionally facilitating the disease transmission. Multi-season sampling data of host infections and habitat characteristics are needed for integration with analyses of landscape connectivity and human behavior to allow better understanding of the interactions among the landscape determinants on the spatial–temporal dynamics of disease transmission.

Keywords: connectivity, infectious disease, land use, *Opisthorchis viverrini*, spatial epidemiology, seasonality, water

Introduction

Environmental changes and ecological disturbances, both natural and human-induced, have exerted a marked influence on the emergence and proliferation of infectious diseases (Patz et al. 2000; Wilson 2009). Insights into the factors that affect the distributions of pathogens, hosts and vectors, and their likelihood of encountering, are essential for understanding disease dynamics (Ostfeld et al. 2005). A great deal of effort has evaluated the effects of biotic and abiotic conditions on disease dynamics, ranging from malaria (Obsomer et al. 2007), Lyme disease (Ostfeld and Keesing 2000), to schistosomiasis (Liang et al. 2007). Recognizing the role of the environment in disease ecology, an integrated landscape scale analysis has been highlighted for a better understanding of the interactions between land, people, disease vectors, and animal hosts (Lambin et al. 2010).

Little attention, however, has been given to the interactions between landscape determinants and spatial variations in disease risk of zoonotic parasitic diseases associated with food and water. Foodborne trematodiasis is an emerging public health problem worldwide (Keiser and Utzinger 2009). More than 680 million people are estimated to be at risk of infection caused by liver flukes of

Published online: August 14, 2012

Correspondence to: Yi-Chen Wang, e-mail: geowyc@nus.edu.sg

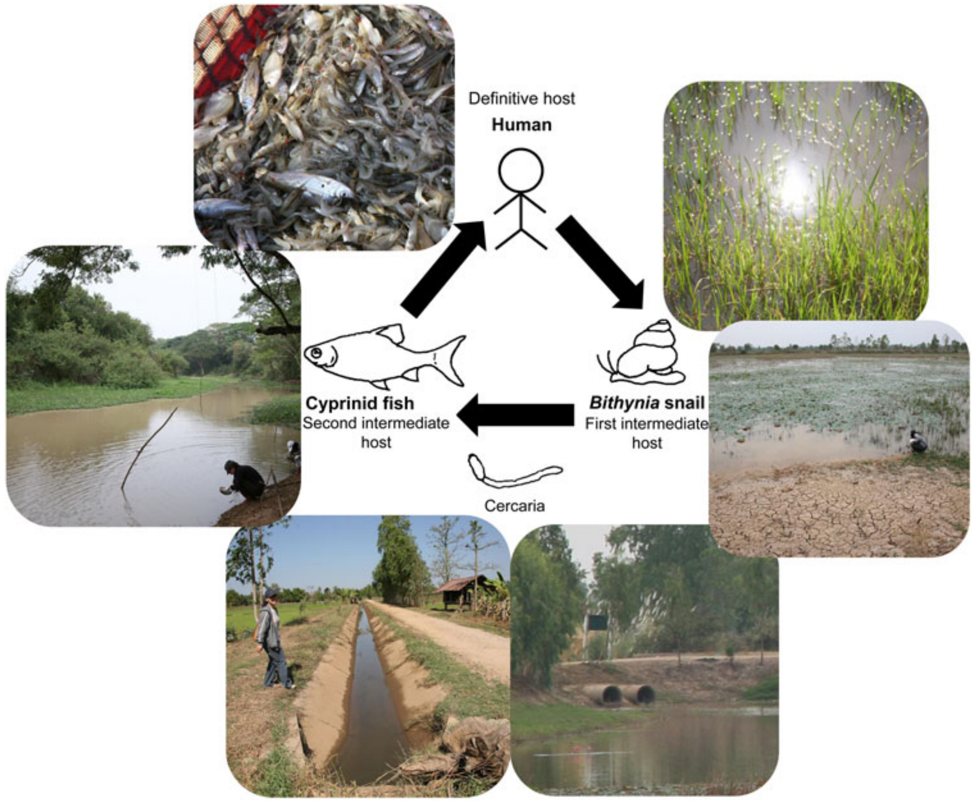

Figure 1. The life cycle of *O.v.* in a connected landscape. Snails of the genus *Bithynia* are the first intermediate host, fish of the family Cyprinidae as the second intermediate host, and humans as the definitive host. Fish-eating carnivores, such as cats and dogs, act as reservoir hosts. In its complex life cycle, eggs of *O.v.* are mainly shed by human hosts washing into freshwater habitats. The eggs of *O.v.* do not hatch directly in water; they have to be first ingested by the first intermediate host, *Bithynia* snails. These freshwater snails eat the eggs and become infected. Following multiplication in the snails, free-swimming cercariae emerge and are released into water. When a cercaria encounters cyprinid fish, it penetrates the scale of the fish, loses its tail, and becomes an oval cyst called metacercaria. Human become infected by consuming raw, semi-cooked, or fermented fish containing metacercariae. Graphics in the figure are not to scale.

Clonorchis sinensis and *Opisthorchis* spp. through the consumption of raw freshwater fish (Keiser and Utzinger 2005). Among the liver fluke species, *Opisthorchis viverrini* (*O.v.*) is endemic in Southeast Asia and has been classified as a Group1 carcinogen for cholangiocarcinoma (Shin et al. 2010). The bulk of research on *O.v.* has congregated in public health and medical fields (Sornmani et al. 1984; Haswell-Elkins et al. 1994; Sripa and Kaewkes 2000; Kiatsopit et al. 2011). Knowledge of the factors accounting for the distribution and transmission of the disease remains in its infancy. Human infection still varies greatly across geographic areas. To comprehend the spatial variations in disease risk, it would thus be desirable to scrutinize the landscape determinants of *O.v.* transmission.

The life cycle of *O.v.* echoes the need for an integrated landscape analysis for two reasons. First, *O.v.* transmission involves three hosts of different ecologies spanning terrestrial and freshwater environments (Fig. 1). The diversity of the hosts and their varying mobilities and habitat conditions emphasize the suitability of incorporating landscape determinants related to the characteristics and connectivity of the host habitats in scrutiny. Second, existing liver fluke mitigation measures have attempted to cut off the fish–human transmission by promoting safe eating habits and dispensing praziquantel, but only attained temporary success (Jongsuksuntigul and Imsomboon 1997; Sripa et al. 2011). Additional insights into the influences of landscape determinants can shed light on how to manage the human–snail and snail–fish transmissions. Hence, the paper aims at investigating how landscape determinants and their interactions contribute to *O.v.* transmission. By calling attention to landscape determinants, the paper contributes to advancing our understanding of the disease ecology of liver fluke.

Landscape Determinants Associated With the Infectivity of the *O.v.* Hosts

A set of principles for comprehending the influence of landscape determinants on disease risk has been formulated in Lambin et al. (2010), based on which the landscape determinants that largely affect the host habitats and transmission of *O.v.* are summarized in Fig. 2. Prior studies relevant to the association between the landscape determinants and the infectivity of the three *O.v.* hosts are first reviewed with future research avenues identified. The influence of habitat connectivity on disease transmission is then emphasized.

The First Intermediate Host–Snail

Landscape Attributes of Host Habitat

Landscape attributes affect the distribution, behavior, and population dynamics of non-human hosts, thereby influencing the level of disease transmission (Lambin et al. 2010). In the case of *O.v.*, the first intermediate host, *Bithynia* snail, prefers slow-running water habitats. Types of water bodies and water quality are therefore important landscape attributes that can influence snail distribution and infection.

The types of water bodies in which snails dwell are slow flowing, muddy rivers, ponds, lakes, reservoirs, wetlands, irrigation canals, and rice fields (Petney et al. 2012). *Bithynia* snails prefer shallow lentic environments along the edge of water bodies at depths of less than 30 cm (Brockelman et al. 1986). They can be found in deep water down to 3 m, albeit a much lower density (Suwannatrai et al. 2011). In Thailand, *Bithynia* snails are present in a variety of water bodies, but different taxa have their almost exclusive geographic distributions: *Bithynia (Digoniostoma) funiculata* occurs in the north region, *Bithynia siamensis siamensis* in the central, and *Bithynia siamensis goniomphalos* in the northeast (Sithithaworn et al. 2007). In Vientiane Province, Lao People's Democratic Republic (PDR), *B. s. siamensis* was found to predominate in rice fields, while *B. s. goniomphalos* occurred in reservoirs (Giboda et al. 1991). No regional separation of *Bithynia* snails has been reported in other parts of Southeast Asia, probably due to insufficient samplings.

For water quality, studies on the habitat attributes of *Bithynia* snails have examined temperature, pH, dissolved oxygen, turbidity, and conductivity (Table 1). *B. funiculata* was found in areas with higher turbidity and lower pH (Ngern-klun et al. 2006). Alternatively, salinity was the determinant for broad-scale distribution of *B. s. goniomphalos* (Suwannatrai et al. 2011). Information pertaining to water quality and other landscape attributes affecting *Bithynia* snail habitats is limited. Two research directions require special attention. First, at a broad scale, the physical environment is characterized by a range of geologic factors. It would be desirable to examine the influence of the geologic factors (e.g., bedrocks) on water quality (e.g., salinity), and their relationships with snail distribution and infection. Care, however, must be taken when examining the broad-scale relationship because recent analyses detect distinct genetic groups of *B. s. goniomphalos* snails and *O.v.* parasites in different major wetland systems in Thailand and Lao PDR, suggesting species complex within both the snail host and the parasite (Saijuntha et al. 2007). Second, the effect of temporal variation needs to be considered because water quality is dynamic, varying with meteorological conditions (Fig. 2). Multi-season sampling is needed to examine if certain water quality parameters exhibit seasonal differences, which may account for the variation in snail population and infection.

Seasonal Variability Influencing Water Availability

Seasonality has two other controls over snails and the transmission. First, the distribution and the population of snails are highly dependent on rainfall. During the rainy season, *B. s. goniomphalos* snails are abundant and distributed extensively in shallow water, but they disappear rapidly in the dry season (Brockelman et al. 1986). Similarly, *B. s. siamensis* prefers to attach to floating plants at the water surface during the rainy season (Upatham and Sukhapanth 1980). Second, snails have the ability to survive drought by burrowing in the mud for seasonal estivation (Brockelman et al. 1986). Over 90% of snails could die during the process of drying, but successfully dormant snails survive at a high rate for over a year. After the next flood, snails disperse to every available edge habitat and repopulate themselves. Because of the high dependence of snail survival on water availability, land use practices can impact snail population. Under natural circumstances, snails usually perish or enter estivation at dried-up localities during the dry season. However, in regions with double-cropping rice agriculture, irrigation takes place during the dry season, thereby altering the natural mortality cycle of snails and artificially boosting

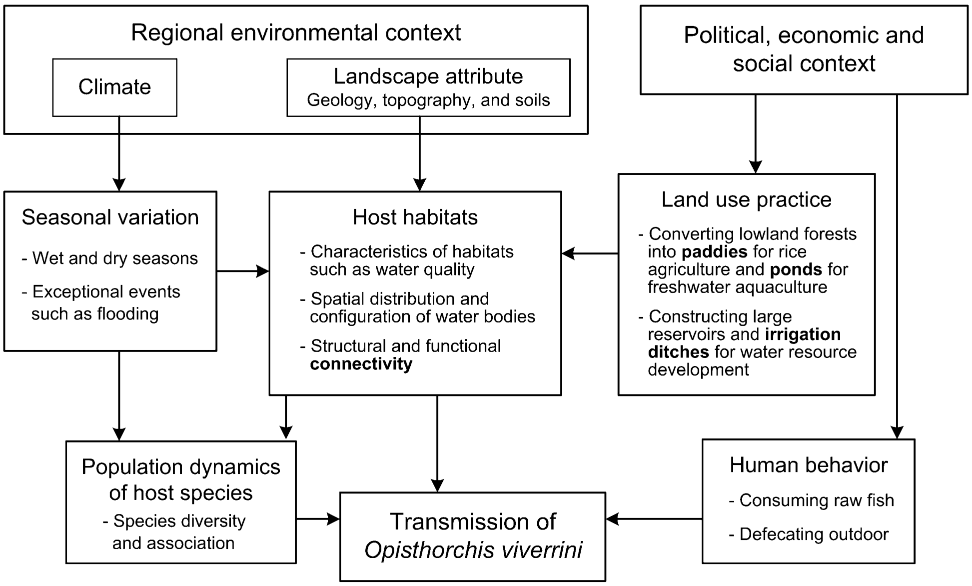

Figure 2. Landscape determinants and their relations for the transmission of *O.v.* The graphic representation is modified from Lambin et al. (2010). Specific landscape determinants in the context of *O.v.* disease ecology are incorporated.

their population in rice fields. Alternatively, land use practices can affect snail population through introduction of snail predators, such as ducks, to some water bodies. In areas where mixed duck and rice production system or free-range duck farming is practiced (e.g., Central Thailand and Vietnam), the presence of ducks is likely to alter snail population.

The lack of long-term multi-season sampling data has impaired the understanding of the influence of seasonality on *O.v.* infection in *Bithynia* snails. Although it is possible to have artificial control of *Bithynia* snails through molluscidng or habitat manipulation when the snails are confined to restricted habitats, the low prevalence of snail infection, typically less than 1% (Brockelman et al. 1986), makes such approaches unpromising. In addition, high water level during the rainy season facilitates the dispersal of snails, presenting a challenge to link the infected snails with the environments where they are found. Long-term studies across a range of snail habitats and their connectivity will be desirable.

The Second Intermediate Host–Fish

In contrast to the low infection rate in snails, the prevalence of infection in fish is much higher. Up to 90% of several cyprinid fish species have been reported to harbor *O.v.* metacercariae (Sithithaworn and Haswell-Elkins 2003). The intensity of fish infections differs greatly, and can be owing to variations in species, seasons, and water bodies.

Variation in Species

More than 80 cyprinid fish species are potential hosts for *O.v.* (WHO 1995). In Thailand, at least 15 species of native fish serve as sources of human infection (Sithithaworn et al. 2007). The most common cyprinids are in the genera of *Cyclocheilichthys*, *Puntius*, and *Hampala*, while other cyprinids have been reported to harbor *O.v.* metacercariae (Table 2). Studies investigating *O.v.* metacercariae have geographically concentrated in northeast Thailand (Table 2), probably due to the long history of public health research. Analyses have been conducted in Lao PDR (Ditrich et al. 1990; Manivong et al. 2009); recent effort has been devoted to Cambodia (Touch et al. 2009).

Infection in fish has been quantified using three measures: prevalence, computed as the proportion of fish found with *O.v.* metacercariae; intensity, calculated as the average number of metacercariae per infected fish; and density, derived as the average number of metacercariae per kilogram or gram of fish. Comparison of prior studies on *O.v.* infection in fish shows great variations across species and geographic areas (Table 3). The intensity of infection was generally higher in *Cyclocheilichthys* spp. than *Puntius* spp. in northeast Thailand (Vichasri et al. 1982; Komalamisra and Setasuban 1989); similar results were observed in southern Lao PDR (Rim et al. 2008) (Table 3). Research also compared its finding with other studies in the same province to suggest that implementing chemotherapy and proper latrine utilization had successfully decreased *O.v.*

Table 1. Comparison of the Water Quality Characteristics of *Bithynia* Snail Habitats

Species	Reference	Area	Sampling period	Variables					
				pH	Temperature (°C)	Salinity (ppt)	Conductivity (mS/cm)	Turbidity (NTU)	DO[a] (mg/L)
B. funiculata	Krueger et al. (2004)	Chiang Mai Province, north Thailand	Jul 2004	6.72–7.68	26.06–36.66	–	0.1368–0.3657	13–403	2.7–7.56
B. funiculata	Ngern-klun et al. (2006)	Chiang Mai Province, north Thailand	June to Oct 2004	6.58–7.56	24.48–31.78	–	0.000–0.2642	16–288	2.03–7.66
B. s. goniomphalos	Lohachit (2004–2005)[b]	Khon Kaen Province, northeast Thailand	June 1989 to Dec 1990	6.3–8.5	18–33	–	0.07–0.65	8–450	2.0–10.0
B. s. goniomphalos	Suwannatrai et al. (2011)	Khorat Basin, about 12 provinces in northeast Thailand	Oct 2006 to Aug 2009	6.02–8.07	21.9–38.6	0.05–32 (live snails 0.05–2.11)	0.12–63.4 (live snails 0.12–40.2)	3.2–420.3	0.01–6.47

[a]DO denotes dissolved oxygen and was recorded in ppm in Suwannatrai et al. (2011).
[b]Values for conductivity and turbidity were converted from micromhos/cm and FTU, respectively, to allow comparisons with other studies.

metacercariae in fish (Waikagul 1998). Nevertheless, at least three cautions are needed when making such comparison.

First, comparison should consider studies of similar sampling methodologies. Some researchers obtained fish from local markets (e.g., Waikagul 1998), while others sampled fish from water bodies (e.g., Sithithaworn et al. 1997). Although fish sold in the local markets are probably obtained from nearby water bodies, the exact fishing localities are often unknown, subsequently inhibits the comparison. Second, the intensity of infection is affected by other factors. For example, fish with larger body mass (e.g., *H. dispar*) may be easily targeted by cercariae (Sithithaworn et al. 1997). Hence, the diversity and composition of fish in the water bodies can play a role in the intensity of infection. An area of interest is to examine if the diversity of fish species plays a ''dilution effect'' on the metacercarial loads. Third, the transmission from humans to fish involves snails (Fig. 1). Attributing the decline of metacercariae in fish to the effective public health measures which reduced *O.v.* eggs being introduced into the environment would be inconclusive without additional data on human infection and snail–fish transmission.

Variation in Seasons

The intensity of infection in fish exhibits a seasonal variation. High infection often occurs from the late rainy season into the winter, approximately between September and February. High *O.v.* metacercariae were detected in fish in Khon Kaen Province, northeast Thailand (Vichasri et al. 1982; Sithithaworn et al. 1997) and in Kandal Province, southern Cambodia (Touch et al. 2009) during the rainy season. Conversely, *O.v.* metacercarial infection increased markedly during the dry season in Namdone and Napakane, central Lao PDR (Manivong et al. 2009). The infection in both snails and fish also exhibited a high prevalence in the dry season in Phnom Penh, southern Cambodia (Ngoen-klan et al. 2010).

The underlying processes responsible for the seasonal patterns of metacercarial abundance in cyprinids are not yet fully understood. It is possible that during the rainy season, a large number of *O.v.* eggs are washed into water bodies through heavy rainfall and dispersed through flooding, and this time period also coincides with the rapid increase in snail population. Attempt has been made to examine the impact of rainfall on the infectivity of fish (Wiwanikit 2005). However, with just five second-hand datasets of metacercarial infection and using average an-

Table 2. Cyprinid Fish Species Found to Harbor *O.v.* Metacercariae and Their Geographic Distribution

Species	Thailand				Lao PDR				Cambodia	
	CM	KK	MH	UT	SVH	VTM	KHA	NNR	KAT	PHP
Barbodes altus									√	
Barbonymus altus										√
Barbonymus gonionotus										√
Cirrhinus jullieni		√[b, d]								√
Cirrhinus microlepis										√
Cyclocheilichthys apogon		√[a, b]							√	
Cyclocheilichthys armatus		√[d]	√		√	√	√			
Cyclocheilichthys enoplos							√		√	
Cyclocheilichthys furcatus										√
Cyclocheilichthys repasson						√	√	√		
Cyclocheilichthys siaja				√						
Dangila lineate							√			
Esomus metallicus				√	√					
Hampala dispar		√[a, b]	√	√	√	√	√	√	√	√
Hampala macrolepidota	√							√	√	
Henicorhynchus spp.										√
Henicorhynchus lineatus							√			
Henicorhynchus siamensis									√	
Hypsibarbus lagleri						√				
Labiobarbus lineatus				√						
Labiobarbus burmanicus	√									
Mystacoleucus atridorsalis		√[d]								
Mystacoleucus margiinatus					√					
Onychostoma elongatum						√				
Osteochilus spp.				√						
Osteochilus hasseltii						√				√
Osteochilus melanopluerus										√
Osteochilus schlegelii										√
Osteochilus waandersii							√			
Puntioplites falcifer					√					
Puntioplites proctozysron							√		√	√
Puntius brevis					√	√			√	
Puntius gonionotus	√			√				√		

Table 2. continued

Species	Thailand				Lao PDR				Cambodia	
	CM	KK	MH	UT	SVH	VTM	KHA	NNR	KAT	PHP
Puntius leiacanthus		√[a–d]	√							
Puntius orphoides	√	√[b]		√						√
Puntius partipentazona		√[a, b]								
Puntius proctozysron				√						
Puntius rhombeus										√
Puntius viehoever				√						
Systomus orphoides									√	
Thynnichthys thynnoides	√								√	
Trichogaster microlepis										√

CM: Chiang Mai Province (Sukontason et al. 1999); KK: Khon Kaen Province, including [a] (Vichasri et al. 1982), [b] (Komalamisra and Setasuban 1989), [c] (Sithithaworn et al. 1997), and [d] (Srisawangwong et al. 1997); MH: Mahasarakham Province (Sithithaworn et al. 1997); UT: Udorn Thani Province (Wykoff et al. 1965); SVH: Savannakhet Province (Rim et al. 2008); VTM: Vientiane Municipality (Rim et al. 2008); KHA: Khammouane Province (Manivong et al. 2009); NNR: Nam Ngum Reservoir, Vientiane Province (Ditrich et al. 1990); KAT: on the border of Kandal and Takeo Provinces (Touch et al. 2009); PHP: Phnom Penh, Cambodia (Ngoen-klan et al. 2010).

nual rainfall data instead of monthly rainfall data that correspond to the timing of fish collection, the significance of the correlation could be in question. Previous experiment showed that water temperature, salinity, and pH affected the infectivity of *O.v.* metacercariae in hamsters (Kruatrachue et al. 1982). Although the environmental conditions for the infectivity in fish are likely to be different from those for hamsters, it underscores the importance to consider water quality parameters (Fig. 2). The prevalence of fish infection predicted for Thailand in Wiwanikit (2005) can therefore be improved by incorporating multiple environmental variables into modeling, rather than simply based on the relationship with rainfall.

The uneven seasonal distribution of *O.v.* metacercarial infection in fish affects the risk of human exposure to infection, such that exposure depends on the season and the fish species consumed (Haswell-Elkins et al. 1992). In northeast Thailand, high human infection has been reported at the end of the rainy season and at the beginning of the dry season when fish were easily caught and the intensity of infection was high (Sithithaworn et al. 1997). Since fish exhibited a high prevalence of infection in the dry season in Lao PDR (Manivong et al. 2009), it would be desirable to know if high human infection also follows the time period of high infection in fish in that area. Such information is useful for international health policy-makers to enforce the control programs at different time periods of a year. Large-scale community-based control programs can be undertaken when the intensity of metacercariae in fish is at its lowest level during which fish are less likely to transmit the disease, and the probability of human infection would therefore be the lowest.

Variation in Water Bodies

Studies on *O.v.* infection in fish have mainly focused on confined water bodies, such as reservoirs and large ponds (e.g., Ditrich et al. 1990; Sithithaworn et al. 1997). Comparison of metacercarial infection across various types of water bodies is scant. Research in southern Cambodian revealed that the prevalence of fish in the flood plain was fairly constant, while that in the lake varied. The number of samples was, however, considered insufficient to draw solid conclusions (Touch et al. 2009). In Vientiane Province, Lao PDR, fish caught in the irrigation ditches between the paddy fields and from the reservoir were found to carry *O.v.* metacercariae, while those caught directly from the flooded paddies were free of infection; the difference was attributed to various snail

Table 3. Comparison of Prevalence and Intensity of *O.v.* Metacercariae in the Most Common Cyprinid Fish Hosts, *Cyclocheilichthys* spp., *Puntius* spp., and *H. dispar*

Species	No. of fish examined	No. of fish infected	Prevalence (%)	Intensity	Geographic area (Province)	Reference
C. apogon	308	299	97.1	8.83–88.56[a]	NE Thailand (Khon Kaen)	Vichasri et al. (1982)
	250	172	68.8	28.43	NE Thailand (Khon Kaen)	Komalamisra and Setasuban (1989)
	217	66	30.4	4.06	S Cambodia (Kandal and Takeo)	Touch et al. (2009)
C. armatus	6	6	100.0	1989.8	S Lao PDR (Savannakhet)	Rim et al. (2008)
	72	31	43.1	13.55	Central Lao PDR (Khammouane)	Manivong et al. (2009)
C. enoplos	60	6	10.0	20.83	Central Lao PDR (Khammouane)	Manivong et al. (2009)
	143	3	2.1	2	S Cambodia (Kandal and Takeo)	Touch et al. (2009)
C. repasson	10	1	10.0	1	NW Lao PDR (Vientiane)	Ditrich et al. (1990)
	195	117	60.0	43.56	Central Lao PDR (Khammouane)	Manivong et al. (2009)
C. siaja	214	110	51.4	26	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
P. brevis	54	23	88.9	359.7	S Lao PDR (Savannakhet)	Rim et al. (2008)
	246	58	23.6	1.72	S Cambodia (Kandal and Takeo)	Touch et al. (2009)
P. gonionotus	34	1	2.9	3	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
	44	1	2.3	7	NW Lao PDR (Vientiane)	Ditrich et al. (1990)
P. leiacanthus	588	551	93.7	8.05–32.21[a]	NE Thailand (Khon Kaen)	Vichasri et al. (1982)
	408	285	69.9	19.35	NE Thailand (Khon Kaen)	Komalamisra and Setasuban (1989)
P. partipenta	47	23	48.9	17.41	NE Thailand (Khon Kaen)	Komalamisra and Setasuban (1989)
P. orphoides	426	276	64.8	79	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
	11	3	27.3	13.85	NE Thailand (Khon Kaen)	Komalamisra and Setasuban (1989)
P. proctozysron	112	9	8.0	1	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
P. viehoever	204	44	21.6	6	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
H. dispar	205	152	74.1	22	NE Thailand (Udorn Thani)	Wykoff et al. (1965)
	58	55	94.8	NA[b]	NE Thailand (Khon Kaen)	Vichasri et al. (1982)
	102	16	15.7	6	NW Lao PDR (Vientiane)	Ditrich et al. (1990)
	27	10	37.0	20.51	NE Thailand (Khon Kaen)	Komalamisra and Setasuban (1989)
	11	8	72.7	NA[c]	S Lao PDR (Savannakhet)	Rim et al. (2008)
	40	16	40.0	22.50	Central Lao PDR (Khammouane)	Manivong et al. (2009)
	27	9	33.3	15	S Cambodia (Kandal and Takeo)	Touch et al. (2009)

The prevalence rates, defined as percent of fish infected, differ slightly from some of the original studies because of different rounding. Intensity is defined as the mean number of metacercariae per infected fish.

[a]The values are shown in range because the results were presented by individual month rather than the overall intensity of infection.

[b]Not available as the result was provided for various parts of fish body.

[c]Not available because the result was shown as the number of metacercariae per gram of fish.

infections in different water bodies (Giboda et al. 1991). The probability of transmission increases when fish has direct contact with infected snails through overlapping habitats.

Research outside Southeast Asia shows that rice fields and irrigation ditches provide important habitats for cyprinid fish (Katano et al. 2003), and prolonged floods facilitate the survival of fish in the following season (Janáč et al. 2010). In Southeast Asia, water resource development activities, especially the construction of large reservoirs in northeast Thailand, have been related to the increase of cyprinids (Sornmani et al. 1981), thereby increasing the risk of *O.v.* infection in local population. To advance the understanding of the variation in fish infection across various water bodies, much effort is needed to investigate if various water bodies possess different environmental conditions that can cause differences in metacercarial infection (Fig. 2). Prior work has examined habitat characteristics of cyprinids in small rivers in central Thailand (Beamish et al. 2006); future endeavor is necessary to integrate the ecological studies of habitat characteristics with the parasitic analyses of fish infection. In addition, it is valuable to study whether the metacercariae intensity in fish exhibits distinct temporal variations across various types of water bodies. The finding, in conjunction with insights into the seasonal variation in fish infection, will provide beneficial information for setting up control strategies based on reduced environmental exposure to infection, which has currently received little attention.

Definitive Hosts

Fish-eating mammals, including humans, cats, and dogs, are the definitive hosts of *O.v.* Factors that contribute to human infection include human behavior of at-risk activities, global human movement and behavior change, and location and surrounding land use of human settlement.

Human Behavior of At-Risk Activities

At-risk activity of consuming raw and insufficiently cooked fish dishes in the Lower Mekong region is the main factor responsible for high human *O.v.* prevalence (Sayasone et al. 2007; Fig. 2). Although the consumption of raw fish dishes has now been confined to special social occasions in Thailand, other moderately preserved fish are still being consumed several times a week (Rangsin et al. 2009). In Lao PDR, the habit of eating undercooked fish remained high in rural areas, as reported in 75.1% of the villagers in Sayasone et al. (2007). Undercooked fish is also frequently consumed in Vietnam, ranging between 46 and 74% of the villagers across different provinces (Sithithaworn et al. 2012). In Cambodia, raw fish food consumption is common, particularly during the rainy season (Touch et al. 2009).

Prevalence of infection is reported in all age groups. The initial infection occurs between 5 and 9 years old. The intensity of infection in terms of fecal egg output (i.e., the number of eggs per mg feces examined) generally increases with age, with the highest intensity being reported for the middle and elderly age groups (Upatham et al. 1984; Maleewong et al. 1992). Some studies observed no notable gender differences (Sayasone et al. 2007); others, however, reported higher intensity of infection in males than in females (Changbumrung et al. 1989). The differences in the intensity of infection in age groups and gender are likely due to different eating behaviors. Male villagers, for example, commonly consume raw fish with alcohol, as such food practice is perceived as "a way of life since their ancestors" (Laithavewat et al. 2011). Indeed, food preparation habit and eating behavior are embedded cultural and dietary traditions in the region. How to better cope with human behavior presents one challenge in reducing the disease risk.

In some rural areas in Southeast Asia, sanitary facilities for defecation remain underdeveloped (WHO 2011). For example, in Lao PDR, sanitary facilities were present in only one of the 13 villages surveyed, and most of the study participants reported defecating outdoors (Sayasone et al. 2007). Better preventive measures in sanitary facilities and sewage treatment systems are critical to disrupt the life cycle of *O.v.*

Global Human Movement and Behavior Change

The unprecedented movements of migrant workers and the changing demographics and human behaviors globally have potentially expanded the distribution of parasitic infection. *O.v.* prevalence among the Thai workers in Taiwan was at 7% (Peng et al. 1993) and in Israel at 51.6% (Greenberg et al. 1994). It is unknown if high prevalence now occurs in other countries where the working force mainly consists of foreign workers from Southeast Asia. In addition, eating raw fish, such as sashimi, has become increasingly fashionable in many countries, leading to a rise in the incidence of fish-borne trematodiasis in previously uninfected ethnic groups. Since the life cycle of *O.v.* cannot be established in places where no intermediate hosts are available, the influence of these factors will have limited epidemiology relevance (Sithithaworn et al.

2007). However, a geographic focus on Southeast Asia is necessary because the similar wetland environments, livelihoods, and eating habits make the region a parasite paradise. One area for research attention is geographic circuits of movement, focusing on long established cross-border and international migration linkages within and between the hot spots of infection in Southeast Asia.

Location and Surrounding Land Use of Human Settlement

Location of human settlement can potentially contribute to the variation in disease risk. Residents of rural Thailand with agricultural occupations were reported to have higher *O.v.* prevalence than those of urban areas (Kurathong et al. 1987). High prevalence of over 90% was reported in lowland districts in Lao PDR, whereas low prevalence of 5.7% was recorded in a highland district (Sayasone et al. 2011). Conversely, in northeast Thailand, prevalence was almost twice higher in villages residing far from the rivers than those residing on the banks, despite the higher recording of raw fish consumption in the villages on the bank (Tesana et al. 1991). The differences in these findings suggest that proximity of villages to rivers alone does not fully explain the spatial variations in human infection. Quantitative approaches incorporating Geographic Information Systems and remote sensing to evaluate the surrounding land use compositions and landscape structure should be employed. Instead of visually determining the proximity of villages to rivers as was done previously, patchiness of water bodies and densities of streams and irrigation networks surrounding the villages should be quantified. Geospatial data of location and surrounding land use composition of human settlement also need to be integrated with data of human risk behavior and human mobility to better understand the geographic patterns of disease prevalence.

Potential Contribution of Canine and Feline Hosts

Canines and felines are recognized as the *O.v.* reservoir hosts. In Lao PDR, the prevalence of infection in felines ranged from 20 to 36% (Ditrich et al. 1990; Giboda et al. 1991; Scholtz et al. 2003). Recent surveys in northeast Thailand indicated a higher prevalence of infection in cats at approximately 36% than in dogs, for which the prevalence was 3.8% in Enes et al. (2010) and 0.37% in Aunpromma et al. (2012). The role of canines and felines as a reservoir in maintaining the presence of *O.v.* eggs in the environment has not been fully investigated because of the difficulty in monitoring their diet and movement and the relatively challenging process of sampling dogs and cats for fecal examination compared to the human stool tests. However, if similar prevalence patterns in reservoir hosts occur at a broader scale, the zoonotic role of dogs and cats in *O.v.* epidemiology should be considered in the development of improved control programs (Enes et al. 2010).

Connectivity Between the Hosts-Functional Roles of Irrigation Ditch

Liver fluke persistence is only possible when there are functional connections between the host habitats. In particular, the role of water in functionally connecting the two intermediate hosts needs scrutiny because it may shed light on the geographic variation in *O.v.* prevalence (Wang et al. 2011). A vital infrastructure which transports water in the lowland Southeast Asia is irrigation ditch (Fernando 1993; Fig. 2), and it has at least three functional significances in *O.v.* transmission.

First, irrigation ditches spatially act as corridors to provide structural connectivity between the habitat patches to facilitate the movement of snails, cercariae, and fish, thereby functionally facilitating the disease transmission. *Bithynia* snails have been found to clump together in canals as a result of water draining from rice fields into canals (Suwannatrai et al. 2011). Although little attention has probed the abundance of *O.v.* cercariae in irrigation ditches, these infrastructures have been found to carry infective cercariae of other parasites (Spear et al. 2004). As for fish, cyprinids have been found to use irrigation ditches to access rice fields for spawning and food in other lowland areas (Katano et al. 2003). The design of irrigation ditches can hence influence the degree of functional connectivity. Adding concrete lining in the construction of irrigation ditches increases the difference in water level between the ditches and the rice fields, potentially restricting the freedom of fish movement. Installing water pumps which allow water to pass from the ditch into the rice fields only through pumps can also restrict the passage of fish.

The second functional role of irrigation ditches is that the structure of irrigation ditches may influence water quality of the host habitats. It would thus be useful to investigate whether the physical shape and the network structure of ditches are associated with water quality and flow regime. Irrigation ditches with structural attributes that afford more conducive water quality for either one of the hosts may possess higher level of functional connectivity for disease transmission. Moreover, these anthropogenic water systems are potentially the dominant proponents of *O.v.* persistence during the dry season when natural hydrological input is minimal.

The third functional role of irrigation ditches is that they can change the extent of inundation during the rainy season. Increased flooding magnitude and duration can increase the contact between snails and fish through irrigation ditches. Furthermore, irrigation ditches functionally connect human and snail hosts through the transporting of feces from villages. In areas where sanitary facilities for defecation are underdeveloped, human feces with *O.v.* eggs can be flushed into water bodies directly or through irrigation ditches in heavy monsoon rain. Fecal bacteria densities are high in areas with high runoff (Gannon and Busse 1989), and the contamination is the greatest during the early part of the rainy season, which coincides with the rapid increase in snail population (Sithithaworn et al. 1997). Infected snails are also frequently found in water bodies nearby villages where high fecal contamination occurs (Kaewkes et al. 2012).

Two levels of assessments are needed for comprehending the functional connectivity of the landscape for *O.v.* transmission. At a fine scale, attention should be given to whether the corridor-patch (e.g., irrigation ditch and rice field) or patch–patch (e.g., between rice fields) interface allows movement of organisms such that contact between hosts is possible. An example for corridor–patch connectivity is how an outlet of irrigation ditches allows water and cercariae from rice fields to enter an irrigation ditch. At a broad scale, the influence of landscape configuration should be analyzed to address how efficiently the broad overall configuration of patches and corridors can facilitate the liver fluke to pass through the entire life cycle. For example, high resolution remote sensing imagery can be analyzed to delineate habitat patches and irrigation ditch corridors, and digital elevation models can be constructed to simulate water movement through different configurations of landscape units. In addition, concepts such as species–area relationships can be incorporated to consider freshwater habitats as islands of water surrounded by land, and to explore if the biophysical conditions, the spatial distribution and distance between these islands, and their structural connectivity affect the diversity and distribution of the intermediate hosts. An understanding of this can be useful for land planning and management to minimize liver fluke persistence.

Conclusions

This paper reviews the current understanding of the landscape determinants that influence the hosts and the transmission of *O.v.*, and identify areas that require future research. Seasonality and water quality appear to affect the habitats and population dynamics of the two intermediate hosts, *Bithynia* snails and cyprinid fish. Land use practices through the construction of irrigation ditches not only provide structural connections between the host habitats but also enable the movement of snails, cercariae, and fish, thereby functionally facilitating the disease transmission. Future endeavor is desired to integrate ecological studies of habitat characteristics, landscape studies of connectivity, and parasitic analyses of snail and fish infections so as to understand how environmental conditions affect differences in infection. Human behavior of eating raw or insufficiently cooked fish dishes is one of the key determinants responsible for high *O.v.* prevalence. Geospatial data of location and surrounding land use composition of human settlement thus need to be integrated with human behavior data and patterns of human mobility to comprehend the geographic variations in *O.v.* prevalence. In a research backdrop dominated by cross-sectional analysis, longitudinal surveys of infections in snails, fish, and human at the landscape scale are required for a better understanding of the interactions between the ecology of the intermediate hosts, seasonal variation in the environment, human behavior, and land use. Such insights are useful for planning of control strategies based on reduced environmental exposure to infection, which has been received little attention.

Acknowledgments

I wish to thank Paiboon Sithithaworn, Nonglak Laoprom, Chen-Chieh Feng, Xueyuan Ong, and Yikang Feng for useful discussion, two anonymous reviewers for valuable comments, and the National University of Singapore for funding support (Grant No. R-109-000-070-101).

References

Aunpromma S, Tangkawattana P, Papirom P, Kanjampa P Tesana S, Sripa B, et al. (2012) High prevalence of *Opisthorchis viverrini* infection in reservoir hosts in four districts of Khon Kaen Province, an opisthorchiasis endemic area of Thailand. *Parasitology International* 61:60–64

Beamish FWH, Sa-ardrit P, Tongnunui S (2006) Habitat characteristics of the Cyprinidae in small rivers in Central Thailand. *Environmental Biology of Fishes* 76:237–253

Brockelman WY, Upatham ES, Viyanant V, Ardsungnoen S, Cantanawat R (1986) Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in Northeast Thailand:

population changes of the snail intermediate host. *International Journal for Parasitology* 16:545–552

Changbumrung S, Tungtrongchitr R, Hongtong K, Supawan V, Kwanbunjan K, Prayurahong B, et al. (1989) Food patterns and habits of people in and endemic area for liver fluke infection. *Journal of Nutrition Association of Thailand* 23:133–146

Ditrich O, Scholz T, Giboda M (1990) Occurrence of some medically important flukes (Trematoda: Opisthorchiidae and Heterophyidae) in Nam Ngum water reservoir, Laos. *Southeast Asian Journal of Tropical Medicine and Public Health* 21:482–488

Enes JE, Wages AJ, Malone JB, Tesana S (2010) Prevalence of *Opisthorchis viverrini* Infection in the canine and feline hosts in three villages, Khon Kaen province, northeastern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 41:36–42

Fernando CH (1993) Rice field ecology and fish culture—an overview. *Hydrobiologia* 259:91–113

Gannon JJ, Busse MK (1989) *E. coli* and enterococci levels in urban stormwater, river water and chlorinated treatment plant effluent. *Water Research* 23:1167–1176

Giboda M, Ditrich O, Scholz T, Viengsay T, Bouaphanh S (1991) Human *Opisthorchis* and *Haplorchis* infections in Laos. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 85:538–540

Greenberg Z, Giladi L, Bashary A, Zahavi H (1994) Prevalence of intestinal parasites among Thais in Israel. *Harefuah* 126: 507–509, 563

Haswell-Elkins MR, Sithithaworn P, Elkins D (1992) *Opisthorchis viverrini* and cholangiocarcinoma in Northeast Thailand. *Parasitology Today* 8:86–89

Haswell-Elkins MR, Mairiang E, Mairiang P, Chaiyakum J, Chamadol N, Loapaiboon V, et al. (1994) Cross-sectional study of *Opisthorchis viverrini* infection and cholangiocarcinoma in communities within a high-risk area in northeast Thailand. *International Journal of Cancer* 59:505–509

Janáč M, Ondračková M, Jurajda P, Valová Z, Reichard M (2010) Flood duration determines the reproduction success of fish in artificial oxbows in a floodplain of a potamal river. *Ecology of Freshwater Fish* 19:644–655

Jongsuksuntigul P, Imsomboon T (1997) The impact of a decade long opistorchiasis control program in northeastern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 28:551–557

Kaewkes W, Kaewkes S, Tesana S, Laha T, Sripa B (2012) Fecal bacterial contamination in natural water reservoirs as an indicator of seasonal infection by *Opisthorchis viverrini* in snail intermediate hosts. *Parasitology International* 61:49–51

Katano O, Hosoya K, Iguchi K, Yamaguchi M, Aonuma Y, Kitano S (2003) Species diversity and abundance of freshwater fishes in irrigation ditches around rice fields. *Environmental Biology of Fishes* 66:107–121

Keiser J, Utzinger J (2005) Emerging foodborne trematodiasis. *Emerging Infectious Diseases* 11:1507–1514

Keiser J, Utzinger J (2009) Food-borne trematodiases. *Clinical Microbiology Reviews* 22(3):466–483

Kiatsopit N, Sithithaworn P, Boonmars T, Tesana S, Chanawong A, Saijuntha W, et al. (2011) Genetic markers for studies on the systematics and population genetics of snails, *Bithynia* spp., the first intermediate hosts of *Opisthorchis viverrini* in Thailand. *Acta Tropica* 118:136–141

Komalamisra C, Setasuban P (1989) Heterophyid flukes and *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from an endemic focus in northeast Thailand. *The Journal of Tropical Medicine and Parasitology* 12(1):22–28

Kruatrachue M, Chitramvong YP, Upatham ES, Vichasri S, Viyanant V (1982) Effects of physico-chemical factors on the infection of hamsters by metacercariae of *Opisthorchis viverrini*. *Southeast Asian Journal of Tropical Medicine and Public Health* 13:614–617

Krueger AM, Irvine KN, Prangkio C, Chaokasad K, Sukontason K, Sukontason KL, et al. (2004) Visualizing water quality trends in Chiang Mai rice paddies: possible links between environment and health risks. *Middle States Geographer* 37:1–8

Kurathong S, Lerdverasirikul P, Wongpaitoon V, Pramoolsinsap C, Upatham ES (1987) *Opisthorchis viverrini* infection in rural and urban communities in northeast Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81:411–414

Laithavewat L, Vaeteewootacharn K, Kaewpa C (2011) Liver fluke infection: situation analysis in upper northeastern part of Thailand, 2007. In: *The 96 Years of Opisthorchiasis: Past, Present and Future, International Congress of Liver Flukes*, Sripa B (editor), 7–8 March 2011, Khon Kaen: Klungnanavitha Press Limited Partnership, p 125.

Lambin EF, Tran A, Vanwambeke SO, Linard C, Soti V (2010) Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *International Journal of Health Geographics* 9:54

Liang S, Seto EYW, Remais JV, Zhong B, Yang C, Hubbard A, et al. (2007) Environmental effects on parasitic disease transmission exemplified by schistosomiasis in western China. *Proceedings of the National Academy of Sciences* 104:7110–7115

Lohachit C (2004–2005) Ecological studies of *Bithynia siamensis goniomphalos*, a snail intermediate host of *Opisthorchis viverrini*, in Khon Kaen Province, Northeast Thailand. *Malacological Review* 37/38:1–26.

Maleewong W, Intapan P, Wongwajana S, Sitthithaworn P, Pipitgool V, Wongkham C, et al. (1992) Prevalence and intensity of *Opisthorchis viverrini* in rural community near the Mekong River on the Thai-Laos border in northeast Thailand. *Journal of the Medical Association of Thailand* 75:231–235

Manivong K, Komalamisra C, Waikagul J, Radomyos P (2009) *Opisthorchis viverrini* metacercariae in cyprinoid fish from three rivers in Khammouane Province, Lao PDR. *The Journal of Tropical Medicine and Parasitology* 32:23–29

Ngern-klun R, Sukontason KL, Tesana S, Sripakdee D, Irvine KN, Sukontason K (2006) Field investigation of *Bithynia funiculata*, intermediate host of *Opisthorchis viverrini* in Northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 37:662–672

Ngoen-klan R, Piangjai S, Somwang P, Moophayak K, Sukontason K, Sukontason KL, et al. (2010) Emerging helminths infection in snails and cyprinoid fish in sewage treatment wetlands waters in Cambodia. *Asian Journal of Water, Environment and Pollution* 7(3):13–21

Obsomer V, Defourny P, Coosemans M (2007) The *Anopheles dirus* complex: spatial distribution and environmental drivers. *Malaria Journal* 6:26

Ostfeld RS, Glass GE, Keesing F (2005) Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends in Ecology and Evolution* 20:328–336

Ostfeld RS, Keesing F (2000) Biodiversity and disease risk: the case of Lyme disease. *Conservation Biology* 14:722–728

Patz JA, Graczyk TK, Geller N, Vittor AY (2000) Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30:1395–1405

Peng HW, Chao HL, Fan PC (1993) Imported *Opisthorchis viverrini* and parasite infections from Thai labourers in Taiwan. *Journal of Helminthology* 67:102–106

Petney T, Sithithaworn P, Andrews R, Kiatsopit N, Tesana S, Grundy-Warr C, et al. (2012) The ecology of the *Bithynia* first intermediate hosts of *Opisthorchis viverrini*. *Parasitology International* 61:38–45

Rangsin R, Mungthin M, Taamasri P, Mongklon S, Aimpun P, Naaglor T, et al. (2009) Incidence and risk factors of *Opisthorchis viverrini* infections in a rural community in Thailand. *The American Journal of Tropical Medicine and Hygiene* 81:152–155

Rim H-J, Sohn W-M, Yong T-S, Eom KS, Chai J-Y, Min D-Y, et al. (2008) Fishborne trematode metacercariae detected in freshwater fish from Vientiane Municipality and Savannakhet Province, Lao PDR. *The Korean Journal of Parasitology* 46:253–260

Saijuntha W, Sithithaworn P, Wongkham S, Laha T, Pipitgool V, Tesana S, et al. (2007) Evidence of a species complex within the food-borne trematode *Opisthorchis viverrini* and possible coevolution with their first intermediate hosts. *International Journal for Parasitology* 37:695–703

Sayasone S, Odermatt P, Phoumindr N, Vongsaravane X, Sensombath V, Phetsouvanh R, et al. (2007) Epidemiology of *Opisthorchis viverrini* in a rural district of southern Lao PDR. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101:40–47

Sayasone S, Mak TK, Vanmany M, Rasphone O, Vounatsou P, Utzinger J, et al. (2011) Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. *PLoS Neglected Tropical Diseases* 5(4):e1037

Scholtz T, Uhlírová M, Ditrich O (2003) Helminth parasites of cats from the Vientiane Province, Laos, as indicators of the occurrence of causative agents of human parasitosis. *Parasite* 10:343–350

Shin H-R, Oh J-K, Masuyer E, Curado M-P, Bouvard V, Fang Y–Y, et al. (2010) Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Science* 101:579–585

Sithithaworn P, Haswell-Elkins M (2003) Epidemiology of *Opisthorchis viverrini*. *Acta Tropica* 88:187–194

Sithithaworn P, Pipitgool V, Srisawangwong T, Elkins DB, Haswell-Elkins MR (1997) Seasonal variation of *Opisthorchis viverrini* infection in cyprinoid fish in north-east Thailand: implications for parasite control and food safety. *Bulletin of the World Health Organization* 75:125–131

Sithithaworn P, Yongvanit P, Tesana S, Pairojkul C (2007) Liver flukes. In: *Food-Borne Parasitic Zoonoses*, Darwin MK, Bernard F (editors), Berlin: Springer, pp 3–52

Sithithaworn P, Andrews RH, De NV, Wongsaroj T, Sinuon M, Odermatt P, et al. (2012) The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitology International* 61:10–16

Sornmani S, Schelp FP, Vivatanasesth P, Pongpaew P, Sritabutra P, Supawan V, et al. (1981) An investigation of the health and nutritional status of the population in the Nam Pong Water Resource Development Project, northeast Thailand. *Annals of Tropical Medicine and Parasitology* 75:335–346

Sornmani S, Vivatanasesth P, Impand P, Phatihatakorn W, Schelp FP (1984) Infection and re-infection rates of opisthorchiasis in the water resource development area of Nam Pong project, Khon Kaen province, northeast Thailand. *Annals of Tropical Medicine and Parasitology* 78:649–656

Spear RC, Zhong B, Mao Y, Hubbard A, Birkner M, Remais J, et al. (2004) Spatial and temporal variability in schistosome cercarial density detected by mouse bioassays in village irrigation ditches in Sichuan, China. *The American Journal of Tropical Medicine and Hygiene* 71:554–557

Sripa B, Kaewkes S (2000) Relationship between parasite specific antibody responses and intensity of *Opisthorchis viverrini* infection in hamsters. *Parasite Immunology* 22:139–145

Sripa B, Bethony JM, Sithithaworn P, Kaewkes S, Mairiang E, Loukas A, et al. (2011) Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Tropica* 120(Supplement 1):S158–S168

Srisawangwong T, Sithithaworn P, Tesana S (1997) Metacercariae isolated from cyprinoid fishes in Khon Kaen District by digestion technique. *Southeast Asian Journal of Tropical Medicine and Public Health* 28:224–226

Sukontason K, Piangjai S, Muangyimpong Y, Sukontason K, Methanitikorn R, Chaithong U (1999) Prevalence of trematode metacercariae in cyprinoid fish of Ban Pao District, Chiang Mai Province, northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 30:365–370

Suwannatrai A, Suwannatrai K, Haruay S, Piratae S, Thammasiri C, Khampoosa P, et al. (2011) Effect of soil surface salt on the density and distribution of the snail *Bithynia siamensis goniomphalos* in northeast Thailand. *Geospatial Health* 5: 183–190

Tesana S, Sithithaworn P, Prasongwatana J, Kaewkes S, Pipitgool V, Pientong C (1991) Influence of water current on the distribution of Opisthorchis viverrini infection in northeastern villages of Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 22:93–98

Touch S, Komalamisra C, Radomyos P, Waikagul J (2009) Discovery of *Opisthorchis viverrini* metacercariae in freshwater fish in southern Cambodia. *Acta Tropica* 111:108–113

Upatham ES, Sukhapanth N (1980) Field studies on the bionomics of *Bithynia siamensis siamensis* and the transmission of *Opisthorchis viverrini* in Bangna, Bangkok, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 11:355–358

Upatham ES, Viyanant V, Kurathong S, Rojborwonwitaya J, Brockelman WY, Ardsungnoen S, 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. *Bulletin of the World Health Organization* 62:451–461

Vichasri S, Viyanant V, Upatham ES (1982) *Opisthorchis viverrini*: intensity and rates of infection in cyprinoid fish from an endemic focus in northeast Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 13:138–141

Waikagul J (1998) *Opisthorchis viverrin*i metacercaria in Thai freshwater fish. *Southeast Asian Journal of Tropical Medicine and Public Health* 29:324–326

Wang Y-C, Feng C-C, Sithithaworn P, Feng Y, Petney TN (2011) How do snails meet fish? Landscape perspective needed to study parasite prevalence *EcoHealth* 8:258–260

World Health Organization (WHO) (1995) *Control of foodborne trematode infections*. Report of the WHO study group. WHO Technical Report Series, no. 849. Geneva: World Health Organization.

World Health Organization (WHO) (2011) *Report of the WHO expert consultation on foodborne trematode infections and taeniasis/cysticercosis*. www.who.int/neglected_diseases/preventive_chemotherapy/WHO_HTM_NTD_PCT_2011.3.pdf. Accessed 8 Feb 2012.

Wilson K (2009) Climate change and the spread of infectious ideas. *Ecology* 90:901–902

Wiwanikit V (2005) The correlation between rainfall and the prevalence of trematode metacercaria in freshwater fish in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Heath* 36:120–122

Wykoff DE, Harinasuta C, Juttijudata P, Winn MM (1965) *Opisthorchis viverrini* in Thailand: the life cycle and comparison with *O. felineus*. *The Journal of Parasitology* 51:207–214