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A REVIEW OF HUMAN CARCINOGENS

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OPISTHORCHIS VIVERRINI AND CLONORCHIS SINENSIS

Opisthorchis viverrini and *Clonorchis sinensis* were considered by a previous IARC Working Group in 1994 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Taxonomy, structure and biology

1.1.1 Taxonomy

Opisthorchis viverrini (*O. viverrini*) and *Clonorchis sinensis* (*C. sinensis*) are pathologically important foodborne members of the genus *Opisthorchis*; family, Opisthorchiidae; order, Digenea; class, Trematoda; phylum, Platyhelminths; and kingdom, Animalia. They belong to the same genus (*Opisthorchis*) but to different species based on morphology; nonetheless, the genus *Clonorchis* is so well established in the medical literature that the term is retained here.

1.1.2 Structure

The adult of *O. viverrini* and *C. sinensis* are usually about 10–25 mm in length and 3–5 mm in width ([Liu & Chen, 1998](#); [Sripa et al., 2007](#)).

The yellowish-brown, ovoid eggs have a distinct operculum, which opens to release the miracidium – a fully formed larva. Eggs are on average 29 µm long by 17 µm wide for *C. sinensis* ([Liu & Chen, 1998](#)), and 27 µm by 15 µm for

O. viverrini ([Sadun, 1955](#)), and are difficult to differentiate between these two species ([Kaewkes et al., 1991](#)).

1.1.3 Structure of the genome

The genomic structures of *O. viverrini* and *C. sinensis* have not been reported.

O. viverrini is reported to have six pairs of chromosomes, i.e. $2n = 12$ ([Rim, 2005](#)), to have neither CpG nor A methylations, but to contain a highly repeated DNA element that is very specific to the organism ([Wongratanacheewin et al., 2003](#)). Intra- and inter-specific variations in the gene sequences of 18S, the second internally transcribed spacer region ITS2, 28S nuclear rDNA, and of the mitochondrial cytochrome C oxidase subunit I (mtCOI) DNA are low and nearly identical ([Ando et al., 2001](#)). A comparison of the ITS2 region sequences of *O. viverrini* versus *C. sinensis* show a 95% match; the sequences differ at 28 nucleotide positions ([Park, 2007](#)).

The chromosome number of *C. sinensis* is $2n = 56$, and the chromosomes can be divided into two groups based on their sizes, consisting of eight pairs of large and 20 pairs of small chromosomes. The mean total length of the diploid

complements of liver flukes collected in the People's Democratic Republic of China is slightly longer than that of those collected in the Republic of Korea ([Park et al., 2000](#)).

1.1.4 Host range

Three families of freshwater snails (Hydrobiidae, Bithyniidae, and Melaniidae) are first intermediate hosts ([Harinasuta & Harinasuta, 1984](#); [Liu & Chen, 1998](#)). Of these, *Parafossarulus striatulus*, *Alocinma longicornis* (Hydrobiidae), *Bithynia fuchsianus* (Bithyniidae) are currently considered to be of greatest importance in China in the life cycle of *C. sinensis* ([Lun et al., 2005](#)).

Over 130 species of fish (belonging to 16 families) are secondary intermediate hosts ([Komiya, 1966](#); [Vichasri et al., 1982](#); [Rim, 1986](#); [Joo, 1988](#); [Liu & Chen, 1998](#)). Fish in the family Cyprinidae are the major intermediate hosts ([Lun et al., 2005](#)).

In addition to human beings, other fish-eating mammals, for example dogs, cats, pigs, minks, weasels, civets, and house rats can be definite hosts, and some may act as reservoir hosts ([Wang, 1983](#); [Lun et al., 2005](#); [Rim, 2005](#)). There is also evidence that rabbits, guinea-pigs, hamsters, gerbils, mice, and rats are susceptible to the parasite in a laboratory setting ([Bhamarapravati et al., 1978](#); [Wang, 1983](#); [Boonmars et al., 2009](#)). Cats and dogs are considered to be the most important animal hosts in the endemic regions of China ([Lun et al., 2005](#)). In contrast with many other countries, most cats and dogs are not kept as pets in rural China but roam freely in villages, and thus have easy access to the remains of raw or undercooked fish in household waste ([Wang, 1983](#); [Jiang, 2001](#)).

1.1.5 Target organs

The adult liver flukes usually reside in the medium-sized or small intrahepatic bile ducts. In heavy infections, adult parasites may be found

in the gallbladder, the extrahepatic bile duct, and the pancreatic duct ([Pungpak et al., 1985](#); [Rim, 1986, 2005](#); [Lim, 1990](#); [Sripa, 2003](#)). Over 100 flukes were recovered from the gallbladder of one patient ([Evans et al., 1971](#)), and 5140 and 1348 flukes of *C. sinensis* were found, respectively, in the bile ducts and in pancreatic ducts of a child patient who died of clonorchiasis sinensis ([Chen et al., 1963](#)).

The pathophysiology and clinical manifestations for *O. viverrini* and *C. sinensis* and infection are very similar ([Lun et al., 2005](#); [Sripa, 2008](#)).

1.1.6 Life cycle

The eggs produced by the mature adult worms pass down the bile duct and are excreted in the faeces. If the eggs reach a freshwater body (small ponds, streams and rivers, flooded rice fields, and reservoirs), they are ingested by snails, which act as the primary intermediate hosts. Asexual reproduction in the snail results in daily release of thousands of cercariae, 1–2 months after infection of the snail. The free-swimming cercariae penetrate the tissue of freshwater fish, which act as the secondary intermediate host, and encyst to become fully infective metacercariae under the fish's skin or in muscle after 21 days.

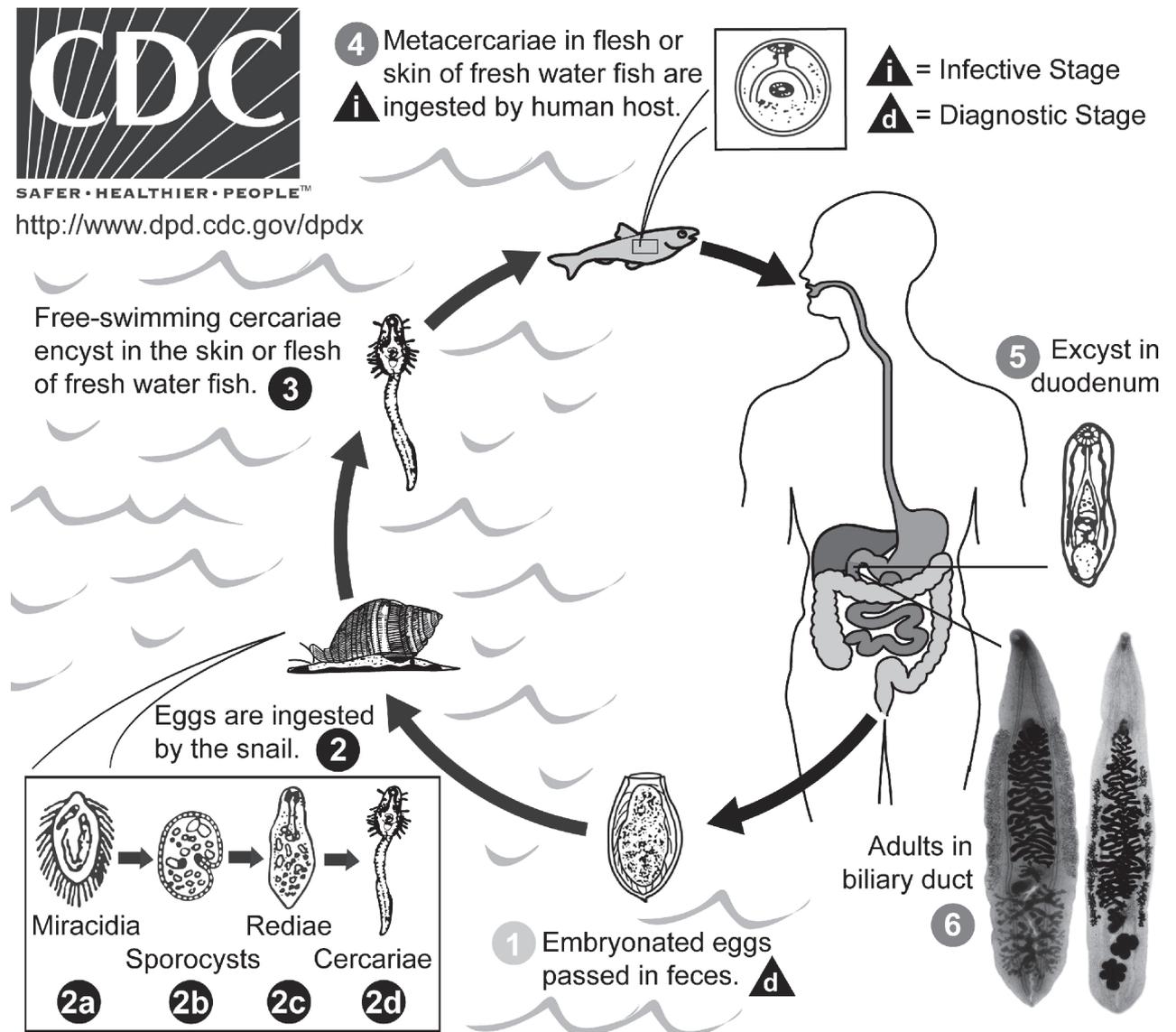
Humans or other fish-eating animals are infected through the ingestion of raw or undercooked (salted, pickled, or smoked) freshwater fish that contains metacercariae. After ingestion, the metacercaria excysts in the duodenum and ascends the biliary tract through the ampulla of Vater. Maturation to adulthood takes approximately 1 month.

The life cycle of the liver flukes is shown in Fig. 1.1 (for a review, see [Rim, 1986](#); [Sripa et al., 2007](#)).

1.1.7 Genes and gene products

[Laha et al. \(2007\)](#) constructed an *O. viverrini* cDNA library that covers ~14% of the entire transcriptome. About 20% of contigs were assigned

Figure 1.1 Life cycle of *Clonorchis sinensis* and *Opisthorchis viverrini*



Adapted from <http://www.dpd.cdc.gov/DPDx/HTML/Opisthorchiasis.htm>

Gene Ontology classifications. Frequently represented protein families included those involved in physiological functions that are essential to parasitism, such as anaerobic respiration, reproduction, detoxification, surface maintenance, and feeding. An assessment of evolutionary relationships showed that *O. viverrini* was similar to other parasitic flukes such as *C. sinensis* and *Schistosoma japonicum*. A total of 164 *O. viverrini* contigs contained open reading frames (ORFs) with signal sequences, many of which were platyhelminth-specific. Moreover, ORFs representing secreted proteins with known roles in tumorigenesis were identified such as granulin, kallikrein-like serine proteases, phospholipase A2 (PLA-2), saponin-like protein, and thioredoxin peroxidase. These proteins might play a role in the pathogenesis of *O. viverrini*-induced cholangiocarcinoma (Laha *et al.*, 2007). Gene expression profiling of adult *O. viverrini* was also constructed by the first 5' serial analysis of gene expression (5' SAGE) library, and vitelline B precursor protein and myoglobin were found to be the most abundant proteins (Chutiwitoonchai *et al.*, 2008).

By using the expressed sequence tag (EST) approach, Lee *et al.* (2003) constructed the *C. sinensis* adult cDNA library. A total of 220 genes were sorted into seven functional categories including: energy metabolism (38), gene expression/RNA metabolism (21), regulatory/signalling components (14), protein metabolism/sorting (98), the structure/cytoskeleton (29), membrane transporters (10), and antigenic proteins (10). The high frequency of cysteine protease expression (30/415 randomly selected clones) suggests an important role of this protein in the metabolism and/or pathogenesis of clonorchiasis. Also identified were Cu/Zn-superoxide dismutase and glutathione-S-transferase, which are believed to play a crucial role in protecting the parasite from the host immune effector mechanisms, and are being pursued as drug targets in other parasitic infections (Lee *et al.*, 2003). Cho *et al.* (2008)

reported gene expression profiles in *C. sinensis* metacercariae compared to those of adult worms. The genes expressed more abundantly in the metacercariae were a group of structural and cytoskeletal proteins, followed by transcription and translation machinery proteins, and a group of energy metabolism proteins. In contrast, adult *C. sinensis* has abundant mRNA clusters encoding for regulatory and signal proteins, other metabolic proteins and enzymes, and structural and cytoskeletal proteins, in decreasing order (Cho *et al.*, 2008). This may be explained by the fact that metacercariae in the muscles of freshwater fish are in a resting stage wherein they simply maintain a basal metabolic status, and adult *C. sinensis* have a high metabolic rate and produce a large numbers of eggs in mammalian hosts (Rim, 2005).

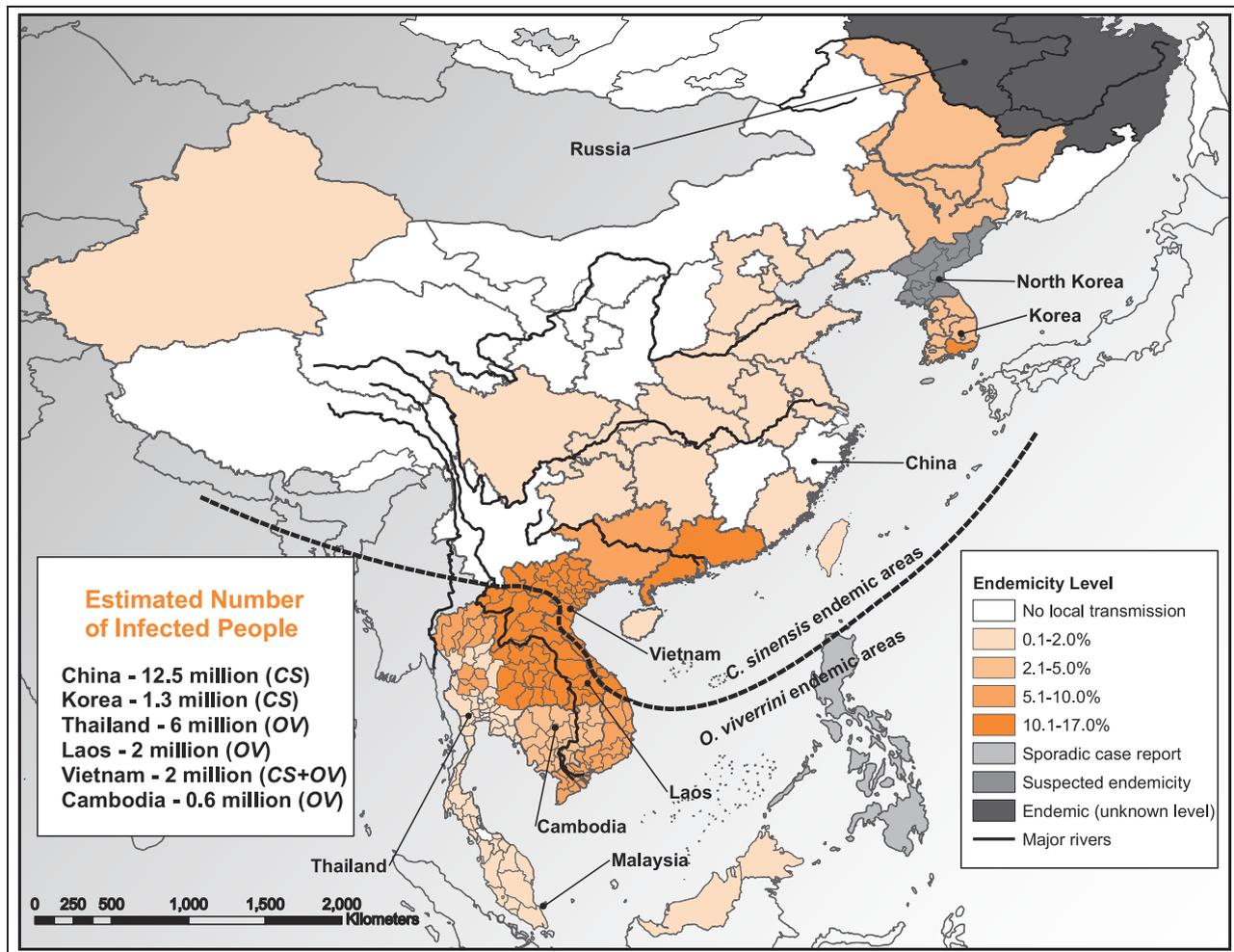
1.2 Epidemiology of infection

1.2.1 Prevalence, geographic distribution

Human liver fluke infection is endemic in China, Thailand, the Republic of Korea, the Democratic People's Republic of Korea, Viet Nam, Lao People's Democratic Republic, and Cambodia. Endemicity for *C. sinensis* is also suspected in the Russian Federation along the Amur River. Persons from Singapore and Malaysia with *C. sinensis* infection have been reported infrequently; many of them may be infected during travelling in other countries or through eating imported fish.

A very crude estimate of the global number of infected people is of the order of 45 million, comprising 35 million infected with *C. sinensis* (Korea Association of Health Promotion, 2004; Lun *et al.*, 2005; Fang *et al.*, 2008), and 10 million with *O. viverrini* (WHO, 1995; Jongsuksuntigul & Imsomboon, 2003). The geographic distribution of *O. viverrini* and *C. sinensis* is shown in Fig. 1.2.

Figure 1.2 Distribution of Liver fluke infection in Asia



C. sinensis is distributed in China, Republic of Korea, Democratic People's Republic of Korea, the Russian Federation, and northern parts of Viet Nam, and *O. viverrini* in Thailand, Laos, Cambodia, Malaysia, and southern part of Viet Nam. The data used for the map were derived from most recent national surveys and published literature.

Note that in the legend, "no local transmission" stands for "no reported local transmission".

A courtesy of Dr Song Liang, College of Public Health, The Ohio state University, USA, who did the art work based on data provided by the Working Group.

(a) *Opisthorchis viverrini*

Thailand is the most endemic country for opisthorchiasis due to *O. viverrini*. In Thailand and neighbouring countries, human opisthorchiasis is caused by *O. viverrini*. In 1980–81, the prevalence in the north, north-eastern, centre and the south of Thailand was 5.6%, 34.6%, 6.3%, and 0.01%, respectively, with an overall prevalence of 14% or 7 million people infected. As a

result of intensive and continuous control activities, the prevalence of infection in north-eastern Thailand declined to 15.7% in 2001, and the rates in other areas were as follows: the north (19.3%), the centre (3.8%) and the south (0%), with an average prevalence of 9.6% or 6 million people infected ([Jongsuksuntigul & Imsomboon, 2003](#)).

It was estimated that 1.7 million people were infected with *O. viverrini* in Laos in 1992 ([WHO,](#)

1995), mainly along the Mekong River, and as far as in the lowlands among people with close ethnic ties to the majority of the north-eastern Thai population. Based on a national survey of primary schoolchildren conducted in 2000–02 that included 17 provinces and the Vientiane Municipality, the prevalence of *O. viverrini* was 10.9% (29846 participants). Again, the regions along the Mekong River such as Khammuane, Saravane or Savannakhet Province showed a higher prevalence of *O. viverrini* (32.2%, 21.5%, 25.9%, respectively) (Rim *et al.*, 2003). More recently, a survey in the Saravane district revealed a high prevalence of *O. viverrini* infection (58.5%) among 814 persons from 13 villages (Sayasone *et al.*, 2007).

A few official reports or published data on *O. viverrini* infection in Cambodia are available. A small survey in primary schoolchildren from Kampongcham province showed a prevalence of *Opisthorchis* spp. of 4.0% from 251 fecal specimens in 2002 (Lee *et al.*, 2002).

Viet Nam has been reported to be endemic for *C. sinensis* in the northern part, and *O. viverrini* in the southern region (De *et al.*, 2003).

(b) *Clonorchis sinensis*

C. sinensis was first discovered in the bile ducts of a Chinese carpenter in Calcutta, India, in 1875. In 1994, archaeologists found a large number of *C. sinensis* eggs in the bowel content of a corpse buried at the middle stage of the Warring States Period (475–221 BC) in Hubei, China (Wu *et al.*, 1996), indicating that this parasite has been present in this province for more than 2300 years. In a nationwide sampling survey on the epidemiological status of parasitic diseases in China, a total of 356629 persons were investigated, and 2065 were found to be infected with *C. sinensis*, with an overall infection rate of 0.58% (Office of the National Survey on the Important Parasitic Diseases, 2005). In a recent survey in Chinese endemic areas, a total of 217829 persons were investigated, and 5230 were

found to be infected with *C. sinensis*, resulting in an infection rate of 2.4%. From this, an estimate of the number of infected persons in China was calculated to be 12.5 million (Fang *et al.*, 2008).

C. sinensis is currently the most prevalent human parasitic helminth in the Republic of Korea, as detected by faecal examination. There has been no decrease in the average national infection rate of *C. sinensis* for almost 30 years; the detection rate was 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 2.7% in 1986, 2.2% in 1992, 1.4% in 1997 and 2.9% in 2004, and about 1.3 million people in the Republic of Korea are estimated to be infected (Korea Association of Health Promotion, 2004; Rim, 2005). In endemic areas of the Republic of Korea, along the main rivers, prevalence values up to 40% have been reported (Rim, 1986, 2005).

Due to a lack of available data from their national survey, there is no accurate number for infected people in Viet Nam. A study of 1155 villagers in northern Viet Nam reported a prevalence of *C. sinensis* infection of 26% (Dang *et al.*, 2008).

A prevalence of *C. sinensis* infection is suspected in the south-eastern part of the Russian Federation, in the Amur River basin where, based on scarce reports, it was estimated at >20% in some villages (e.g. Nanay district) (Semenova *et al.*, 1995; Dyk *et al.*, 1997).

1.2.2 Transmission and risk factors for infection

The definitive host is infected by the liver fluke primarily through the ingestion of raw (dried, pickled or salted) or undercooked infected fish, which contain metacercariae – this is the infective stage in the life cycle of liver flukes (Sithithaworn & Haswell-Elkins, 2003). Many surveys show that people in Thailand (Kaewpitoon *et al.*, 2008), Viet Nam (Dang *et al.*, 2008), China (Fang *et al.*, 2008; Lun *et al.*, 2005), Laos (Hohmann *et al.*, 2001),

and the Republic of Korea ([Lim et al., 2006](#)) have these eating habits.

In southern China and among the Cantonese population in the Hong Kong Special Administrative Region, raw fish is traditionally eaten after being dipped in rice porridge. Alternatively, large fish are sliced and eaten with ginger and garlic known as “*yushen*.” This mode of transmission tends to increase with age. In contrast, many children in hilly areas of Guangdong and eastern China such as Jiangsu, Shandong, and Anhui provinces, often catch fish during play, and roast them incompletely before consumption. This mode of transmission tends to decline with age ([Fang et al., 2008](#)).

The population of the Republic of Korea eat raw fish soaked in vinegar, red-pepper mash or hot bean paste with rice wine at social gatherings. The fact that men do so more frequently than women has been given as a reason for the higher prevalence of infection among men; however, in heavily endemic areas, often no significant differences are seen between the genders. When fish is abundant, raw fish is eaten very regularly as opposed to being saved for special occasions ([Choi, 1984](#); [Rim, 1986](#)). Vietnamese people eat raw fish in salads ([Kiêu et al., 1990](#)).

In Thailand and the lowland region of Laos, three types of uncooked fish preparations are noted ([Sadun, 1955](#); [Sithithaworn & Haswell-Elkins, 2003](#)):

- *koi pla*, eaten soon after preparation;
- *pla som*, moderately fermented, and stored for a few days to weeks; and,
- *pla ra* and *jaewbhong*, extensively fermented, highly salted fish, stored for at least 2–3 months.

Koi pla is probably the most infective dish, followed by fish preserved for <7 days, then *pla ra* and *jaewbhong*, in which viable metacercariae are rare ([Sithithaworn & Haswell-Elkins, 2003](#)). Several factors can directly or indirectly lead to the transmission of the liver flukes to humans (for reviews, see [Sithithaworn &](#)

[Haswell-Elkins, 2003](#) for *O. viverrini*; [Lun et al., 2005](#) for *Clonorchis sinensis*): 1) poor educational level of local residents ([Jongsuksuntigul & Imsomboon, 2003](#)); 2) lack of sanitation: it is common in some endemic regions in China, particularly in the province of Guangdong and Guangxi, that “lavatories” are built adjacent to ponds, so that human excrement containing *C. sinensis* eggs enters the pond water ([Lun et al., 2005](#)). Also, in Laos, 95.5% of houses in some rural villages in Bolikhamxay Province do not have a latrine, and more than half of the village people use animal and/or human faeces as fertilizer ([Hohmann et al., 2001](#)); 3) habit to eat raw or undercooked freshwater fish; 4) freshwater aquaculture is developing rapidly, but adequate testing of fish products is lacking ([Fang et al., 2007](#)); 5) dinner-set contamination from infected fish ([Fang et al., 2007](#)); and 6) the absence of systematic control activities to limit transmission in many endemic areas ([Fang et al., 2007](#)).

1.2.3 Persistency and latency

It has been reported that *C. sinensis* may survive up to 26 years in a human host, as has been shown in a Chinese immigrant living in Australia ([Attwood & Chou, 1978](#)). The life expectancy of *O. viverrini* is approximately 10 years ([Sithithaworn & Haswell-Elkins, 2003](#)).

2. Cancer in Humans

2.1 Cholangiocarcinoma

2.1.1 *Opisthorchis viverrini*

The Working Group of the previous IARC *Monograph* on liver flukes ([IARC, 1994](#)) evaluated infection with *O. viverrini* based on a dozen of descriptive studies (case reports, cases series, and correlation studies), and three cross-sectional or case-control studies ([Kurathong](#)

Table 2.1 Descriptive study of *Opisthorchis viverrini* and liver cholangiocarcinoma

Reference Location	Area and period of study	Measure of exposure to Ov	Number of subjects	Egg positivity	Association	Comments
Sriamporn et al. (2004) Thailand	20 districts in Khon Kaen province 1990–2001	Stool microscopy	18393 total	Adjusted proportion of Ov-infected subjects, by age and sex (≥ 35 -yr-old)	Truncated incidence of CCA (age 35–69 yr)	The Pearson's correlation coefficient (r) for the overall districts was 0.009. Results reported for selected districts with more than 1000 tested for Ov
			1122	10.5	93.8 (Nam Phong)	
			1026	13.4	114.9 (Phon)	
			3884	21.5	288.6 (Mancha Khiri)	
			1003	25.7	135.7 (Muang)	
			4059	29.9	317.6 (Chonnabot)	

CCA, cholangiocarcinoma; Ov, *Opisthorchis viverrini*; yr, year or years.

[et al., 1985](#); [Parkin et al., 1991](#); [Haswell-Elkins et al., 1994](#)), which demonstrated a positive association between infection with *O. viverrini* and cholangiocarcinoma.

Currently, primary liver cancer is the leading cancer in Thailand in men (annual standardized ratio [ASR], 33.4/100000 population), and the third in women (ASR, 12.3/100000) ([Khuhaprema & Srivatanakul, 2007](#)), with cholangiocarcinoma being the predominant type. In addition, the highest incidence of liver cancer (ASR of up to 113.4/100000 in men) is found in the north-eastern regions where *O. viverrini* is endemic, and is 20 times higher than that in the south of Thailand where *O. viverrini* is rare ([Sripa & Pairojkul, 2008](#)). Furthermore, the proportion of histologically verified cases of cholangiocarcinoma in men diagnosed with liver cancer in the north-eastern regions has been reported to be as high as 85.5% compared to 10% in the south ([Khuhaprema & Srivatanakul, 2007](#)). A recent correlation study ([Sriamporn et al., 2004](#)) found a significant positive association between the incidence cases of cholangiocarcinoma from the cancer registry and *O. viverrini* infection in Khon Kaen, a province in north-east Thailand,

with the highest incidence of cholangiocarcinoma cancer in the world (see [Table 2.1](#)).

[Table 2.2](#) presents the results from all the available cross-sectional and case-control studies, all conducted in Thailand (descriptive studies are not presented). The odds ratios ranged from 1.3–27.1. The highest relative risk, reported by [Honjo et al. \(2005\)](#), was adjusted for sex, age, residence, alcohol consumption, and smoking. [Haswell-Elkins et al. \(1994\)](#) reported adjusted prevalence odds ratios (POR) of 1.7 in the light infection group, 3.2 in the moderate infection group, and 14.1 in the heavy infection group (based on 14 exposed cases stratified by intensity of infection).

2.1.2 *Clonorchis sinensis*

The Working Group of the previous IARC *Monograph* on liver flukes ([IARC, 1994](#)) evaluated infection with *C. sinensis* as probably carcinogenic to humans (Group 2A), based on nine case series and three case-control studies ([Gibson, 1971](#); [Kim, 1974](#); [Chung & Lee, 1976](#)). Since then, several studies have been published, and are summarized here.

Table 2.2 Cross-sectional and case-control studies on *Opisthorchis viverrini* infection and cholangiocarcinoma

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
Kurathong <i>et al.</i> (1985) Thailand 1981–83	13 cases clinically diagnosed and confirmed by ultrasound biopsy	479 in- and out-patients without hepatobiliary tract diseases	Stool specimens Stool, bile duct aspirate or liver biopsy	Eggs in stool Eggs in any tissue or fluid	9/13 13/13	[0.94 (0.26–4.22)] X ² test $p < 0.05$ [10.95 (1.10–108.48)] ^a		
Parkin <i>et al.</i> (1991) North-east Thailand 1987–88	103 consecutive patients from 3 hospitals	103 controls matched to cases by sex, age, residence, hospital, non-malignant diseases not related to tobacco or alcohol	Antibody titre by ELISA		NR	5.0 (2.3–11.0)	Consumption of 'sticky rice' and areca nut chewing	
Haswell-Elkins <i>et al.</i> (1994) Thailand, 1990–91	15 cases of suspected CCA among 1807 patients screened by ultrasound scanning		Stool microscopy	Ov eggs+ 0 EPG ≤1500 EPG 1500–6000 >6000 EPG	1 3 4 7	1.0 (ref) 1.7 (0.2–16.3) 3.2 (0.4–30) 14.1 (1.7–119)	Age, sex, district	
Honjo <i>et al.</i> (2005) Thailand, 2000	129 cases of CCA diagnosed by ultrasound, 9 with histology, serology and fetoprotein	129 population-based controls matched by age, sex, residence	Serology	Anti-Ov Ab+ ≤0.200	65	27.09 (6.30–116.57)	Smoking, alcohol, age, sex, residence	

^a Continuity correction was applied to calculate OR
CCA, cholangiocarcinoma; ELISA, enzyme-linked immunosorbent assay; EPG, egg per gram; NR, not reported; Ov, *Opisthorchis viverrini*

Table 2.3 Descriptive study of *Clonorchis sinensis* infection and cholangiocarcinoma

Reference, study location and period	Area	Number of subjects	Measure of exposure to Cs	Egg positivity (%)	Association	Comments
Lim et al. (2006)	Three areas by endemicity		Faecal egg		Incidence of cancer ^a per 100000 persons	In the survey, alcohol drinking and raw freshwater fish were significant risk factors for egg positivity (adjusted for age)
Korea 2000–04	Low (Chuncheon)	659		14 (2.1%)	0.3	
	Medium (Chungju)	568		44 (7.8%)	1.8	
	High (Haman)	1942		607 (31.3%)	5.5	

^a drawn from cancer registry in 1999–2001 (ICD-10, C22.1) Cs, *Clonorchis sinensis*

The incidence of primary liver cancer in the Republic of Korea is the highest in the world (ASR, 44.9 in men and 12.0 in women), with a proportion of microscopically verified cases of cholangiocarcinoma of 22.3% and 36.1% in men and women, respectively ([Curado et al., 2007](#)). According to the Korean Cancer Registry, the incidences of cholangiocarcinoma vary by geographic area, with up to 4-fold differences ([Shin et al., 2008](#)). The region with the highest incidence (7.2/100000 in men) was reported to be that with the highest prevalence of *C. sinensis* infection in a nationwide survey conducted 20 years ago ([Seo et al., 1981](#)).

A recent correlation study from the Republic of Korea showed a high correlation between the endemicity of *C. sinensis* infection with the incidence as well as mortality of cholangiocarcinoma ([Lim et al., 2006](#); [Table 2.3](#)).

Since the previous *IARC Monograph*, two case series from China have been published, both supporting a relationship between *C. sinensis* and cholangiocarcinoma ([Cheng et al., 2000](#); [Wang et al., 2003](#); [Table 2.4](#)). Furthermore, three case-control studies have been published from the Republic of Korea ([Table 2.5](#)). All three showed significant positive associations between *C. sinensis* infection and cholangiocarcinoma. The

study by [Choi et al. \(2006\)](#) reported an (unadjusted) odds ratio for any evidence of infection of 7.3 (95%CI: 3.9–13.3). [Shin et al. \(1996\)](#) reported an odds ratio of 2.7 (95%CI: 1.1–6.4), adjusted for alcohol consumption, smoking, hepatitis B and C, and [Lee et al. \(2008\)](#) found an odds ratio of 13.6 (95%CI: 6.1–30.3) after adjusting for hepatitis B, alcohol consumption, and liver cirrhosis.

In two of the studies ([Shin et al., 1996](#); [Choi et al., 2006](#)), higher odds ratios were reported for evidence of past *C. sinensis* infection (i.e. based on positive history, serology, skin test, radiology) compared to current infection (i.e. based on positive stool microscopy or pathology).

2.2 Hepatocellular carcinoma

2.2.1 *Opisthorchis viverrini*

A correlation analysis of the prevalence of *O. viverrini* infection and liver cancer incidence, conducted in five regions with different frequencies of cholangiocarcinoma and hepatocellular carcinoma (HCC), showed little geographic variation in the incidence of HCC, with a correlation of -0.37 ($P = 0.54$) for antibody titre $\geq 1:40$, and of 0.02 ($P = 0.96$) for faecal egg count ([Srivatanakul et al., 1991a](#)).

Table 2.4 Case series and case reports of cholangiocarcinoma associated with *Clonorchis sinensis*

Reference and study location	Case history	Clinical manifestations	Treatment	Pathological diagnosis
Liang (1995) Guangdong Province, People's Hospital, China	27 CCA cases with Cs 24 CCA cases without Cs	The same CT findings were observed in the cases with or without Cs	Operation	Development of CCA
Kim et al. (1999) Korea University Hospital, Seoul, Republic of Korea	69-yr-old man Eating raw freshwater fish, pulmonary tuberculosis	5-kg weight loss, moderate dilatation of left IHD and CBD, obstruction of proximal left HD, Cs eggs + by left HD cytology, CBD polyp	Hepaticojejunostomy, partial resection of left proximal HD Pzq, 75 mg/kg	Papillary hyperplasia
Cheng et al. (2000) Hospital, China	35 CCA cases (28 positive for Cs)	Cs egg+, abdominal pain, weight loss	Operation (14 cases)	CT finding pathology proven
Kim et al. (2000) Yonsei Medical Center, Seoul, Republic of Korea	64-year-old man	Abdominal pain, Cs worms were removed by percutaneous transbiliary drainage, CBD polyp	Pancreaticoduodenectomy	Composite small cell neuroendocrine carcinoma and adenocarcinoma
Wang et al. (2003) Guangzhou, Zhujiang Hospital, China	29 CCA cases	Clonorchiasis 100%	Operation	Average 20 years of liver fluke infection
Shim et al. (2004) Yonsei Medical Center, Seoul, Republic of Korea	69-year-old man Diabetes, cured tuberculosis	Abdominal pain, 8-cm-sized mass in right liver	Right hepatectomy, recurrent metastasis	Mucinous adenocarcinoma

CBD, common bile duct; CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; CT, computerized tomography; HD, hepatic duct; IHD, intrahepatic duct; Pzq, praziquantel

Table 2.5 Cross-sectional and case-control studies on *Clonorchis sinensis* infection and cholangiocarcinoma

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
Gibson (1971) Hong Kong SAR 1964–66	17 cases among 1484 autopsies, including 83 patients with HCC	1384 autopsies without CCA or HCC	Gross examination at autopsy		11/17	[3.1 (0.1–8.4)]		
Kim (1974) Low and high prevalence areas, Republic of Korea 1961–72	54 cases among 1843 records of autopsy and surgical specimens with liver diseases	1348 autopsies or surgery with non-cancerous liver lesions	Stool samples, liver tissue		NR	[6.5 (3.7–12)]		
Chung & Lee (1976) Pusan, Republic of Korea 1963–74	36 consecutive cases diagnosed in 2 hospitals	559 subjects admitted to hospital, with liver diseases	Stool specimen		NR	[6.0 (2.8–13)]		
Shin <i>et al.</i> (1996) Pusan Paik Hospital, Busan, Republic of Korea 1990–93	41 CCA cases	203 patients of other diseases (Control I), 203 healthy controls (Control II)	Stool microscopy	Cs eggs+ (current) Liver fluke history (past)	33.3 7.3	2.7 (1.1–6.4) 5.0 (1.2–21.3)	Age, sex, HBsAg, anti-HCV, drinking and smoking history, hepatitis history and SES	

Table 2.5 (continued)

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
Choi et al. (2006) Republic of Korea 2003–04	185 CCA cases identified from 1 hospital in Seoul: 51 intrahepatic CCA, 53 hilar CCA, and 81 extrahepatic CCA	185 patients with non-hepatobiliary diseases in the Department of Gastroenterology at same hospital	Stool microscopy, pathology, serology, radiology, history	Stool eggs + Pathology + Serology + Skin test + Radiology + History +	3 13 25 19 156 94	0.6 1.6 2.3 1.7 8.6 2.4	Age, sex, and area	
	51 cases of intrahepatic CCA among the 185 cases above	51 patients with non-hepatobiliary diseases		Any evidence + Stool eggs + Pathology + Serology + Skin test + Radiology + History +	167 0 0 7 5 36 22	7.3 (3.9–13.3) Not available Not available 1.75 0.8 5.0 1.5		
Lee et al. (2008) Seoul, Republic of Korea 2000–04	622 histologically confirmed intrahepatic CCA cases	2488 healthy controls admitted for routine examinations	Histology, stool, microscopy, serology, radiology, history	Any evidence + Stool eggs +	42 26	4.0 (1.5–10.7) 13.6 (6.1–30.3)	Age, sex, date of visit	

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; NR, not reported; SAR, Special Administrative Region

Table 2.6 Cross-sectional and case-control studies on infection with liver flukes and hepatocellular carcinoma

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method, fluke	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
<i>Opisthorchis viverrini</i>								
Kurathong et al. (1985) Thailand 1981–83	Cases among 72 patients with hepatobiliary tract diseases: 12 clinically diagnosed 5 biopsy proven	479 in- and out-patients without hepatobiliary diseases	Stool specimen	Eggs in stool	9/12	[1.21 (0.30–7.07)]	Crude ratio	
Srivatanakul et al. (1991b) North-east Thailand 1987–88	65 patients living and born in the area	65 patients with non-malignant diseases matched for sex, age, residence, hospital	ELISA for Ov antibody	Anti-OV titre $\geq 1/40$	4/5 NR	[1.62 (0.16–80.28)] 1.7 (0.8–3.7)		
<i>Clonorchis sinensis</i>								
Gibson (1971) Hong Kong SAR, China 1964–66	83 cases of HCC in a consecutive series of 1484 autopsies	1384 autopsies without HCC or CCA	Gross examination	Clonorchiasis	24	[0.73 (0.45–1.2)]	Age, sex	Expected proportion infected was 35%
Kim (1974) Seoul & Pusan, Republic of Korea 1961–72	386 and 109 cases in low and high prevalence areas, respectively; histologically proven cases among records of autopsies and surgical specimens	1061 and 287 subjects with liver diseases from low and high prevalence areas, respectively	Examination of liver tissue or stool samples	Cs infection	423	[1.2 (0.80–1.7)]		
Chung & Lee (1976) Pusan, Republic of Korea 1963–74	206 cases in consecutive series of 368 cases of primary liver carcinoma	559 subjects admitted to hospitals without liver disease	Stool specimens	Eggs in stool	170	1.1 (0.65–1.7)	None (crude odds ratio)	Overlap with study by Kim (1974) for cases from Pusan

CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; HCC, hepatocellular carcinoma; NR, not reported; Ov, *Opisthorchis viverrini*

One cross-sectional study ([Kurathong et al., 1985](#)) and one case-control ([Srivatanakul et al., 1991b](#)) study were carried out in north-east Thailand to evaluate the association between *O. viverrini* infection and the risk for HCC ([Table 2.6](#)). Neither study showed a significant association.

2.2.2 Clonorchis sinensis

A few studies have evaluated the association between *C. sinensis* infection and the risk for HCC ([Table 2.6](#)). One study was conducted in the Hong Kong Special Administrative Region ([Gibson, 1971](#)) and found no association.

Three studies were conducted in the Republic of Korea; one ([Kim, 1974](#)) in two separate regions, of low and high prevalence of *C. sinensis* infection, respectively; the other two studies were conducted in Pusan, one of the areas with the highest prevalence of *C. sinensis* infection ([Chung & Lee, 1976](#); [Shin et al., 1996](#)). In the two earlier studies, no increased risks for HCC were observed [from crude odd ratios]. In the most recent study ([Shin et al., 1996](#)), neither *C. sinensis* eggs in stool samples (OR, 2.7; 95%CI: 0.9–7.7) nor a history of liver fluke infection (OR, 2.6; 95%CI: 0.6–11.3) were significantly associated with HCC in a conditional logistic regression analysis adjusted for socioeconomic status ([Table 2.6](#)).

2.3 Cofactors

The intake of raw freshwater fish is traditionally combined with alcohol consumption in the Republic of Korea. In this country, one study reported a significantly increased risk of *C. sinensis* infection with alcohol consumption ([Lim et al., 2006](#)).

[Shin et al. \(1996\)](#) reported odds ratios of 4.6 (95%CI: 1.4–15.2) for heavy alcohol consumption, 5.0 (95%CI: 1.2–21.3) for a history of liver fluke infection, and 2.7 (95%CI: 1.1–6.3) for *C.*

sinensis in stool samples, all adjusted for the other factors. [Lee et al. \(2008\)](#) reported odds ratios of 6.6 (95%CI: 4.8–9.2) for heavy alcohol consumption and 13.6 (95%CI: 6.1–30.3) for *C. sinensis* in stool samples. [Honjo et al. \(2005\)](#) found odds ratios of 4.31 (1.12–16.57) for regular alcohol drinking and 27.09 (95%CI: 6.3–116.6) for presence of *O. viverrini* by antibody detection. No specific interactions between alcohol drinking and liver fluke infection were estimated in any of these studies.

3. Cancer in Experimental Animals

The association between *O. viverrini* and *C. sinensis* infections and cancers was extensively studied in experimental animal models in the 1970s and 1980s. All of these studies were reviewed in the previous *IARC Monograph* ([IARC, 1994](#)). Only one additional study has been published since ([Wang et al., 1994](#)).

[Thamavit et al. \(1978\)](#) first reported that hamsters given *O. viverrini* and *N*-nitrosodimethylamine in drinking-water could develop cholangiocarcinoma. The gross morphology and histology of the experimentally induced cholangiocarcinomas are similar to those found in humans, and are considered a suitable model for the study of cholangiocarcinoma. Following this experiment, many studies on the administration of *N*-nitroso compounds (*N*-nitrosodimethylamine or *N*-nitrosodihydroxydi-*n*-propylamine) in combination with *O. viverrini* infection were performed, and all resulted in increased incidences of cholangiocarcinoma. Intraperitoneal administration induced cholangiocarcinoma but also hepatic neoplastic nodules, and a few HCCs. All of these studies clearly established the role of *O. viverrini* in promoting cholangiocarcinoma in hamsters ([Flavell & Lucas, 1982, 1983](#); [Thamavit et al., 1987a, b, 1988a, b, 1993, 1994](#)).

Table 3.1 Studies in experimental animals exposed to liver flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<i>Opisthorchis viverrini</i>				
Hamster, Syrian golden (M) 23 wk Thamavit et al. (1978)	Ov 100 MC, NDMA 0.0025% at Week 4 in drinking-water for 10 wk Group 1: Untreated control (n=18) Group 2: NDMA alone (n=21) Group 3: Ov (n=18) Group 4: Ov+ NDMA (n=21)	CCA: Group 1: 0/18 Group 2: 0/21 Group 3: 0/18 Group 4: 15/15	[p <0.001] ^a	This is the first experiment of NDMA + liver fluke-induced CCA in the hamster
Hamster, Syrian golden (M) 490 d Flavel & Lucas (1982, 1983)	Ov 50 MC, NDMA 1.6 mg single oral dose Group 1: Ov+NDMA (41 days after infection) (n=50) Group 2: NDMA+Ov (96 h later) (n=46) Group 3: NDMA (n=30) Group 4: Ov (n=50)	CCA: Group 1: 5/50 (10%) Group 2: 9/46 (20%) Group 3: 0/30 (0%) Group 4: 0/50 (0%)	[NS] ^a [p <0.01]	High mortality in Ov+NDMA groups. Tumours found in right lobe. No significant difference between 2 combination groups for tumour latency
Hamster, Syrian golden (M) 40 wk Thamavit et al. (1987a)	Ov 12.5, 25, 50 or 100 MC NDMA 6 or 12.5 mg/L in drinking-water for 10 wk (2 wk later) Group 1: Untreated Groups 2: Ov 12, 25, 50 or 100 MC Groups 3: NDMA 3, 6 or 12 mg/L Groups 4: NDMA 6 or 12.5 mg/L + Ov 12, 25, 50 or 100 MC Total n=280	Group 1: No CCA Groups 2 and 3: No CCA in Groups 2 or 3 at doses of 3 or 6 mg/L Groups 3: CCA: 2/17 (12%) NDMA 12.5 mg/L Groups 4: CCA: 4/10, 7/10, 9/15, 13/19, 8/15, 10/17, 16/19, 14/15 in NDMA+Ov, respectively	p <0.01, all groups 4 (versus relevant group 3)	Cholangiofibrosis was also observed in Groups 3 and 4. Number of animals per group at start unspecified

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (F) 32 wk Thamavit et al. (1987b)	OV 60 MC, NDEA 10, 20 or 40 mg/L in drinking-water for 12 wk Group 1: Untreated control [n=20] Group 2: Ov only [n=20] Groups 3: Ov + NDEA (4 wk later) [n=20–30] Groups 4 NDEA only [n=20–25] [Total n= 180]	Group 1: 0/20 Group 2: 0/20 Groups 3: hepatocellular nodules, 12/19 with 2.5 nodules/animal (OV+NDEA20), 23/25 with 7.1 nodules/animal (Ov+NDEA40). Groups 4: 3/19 with 0.2 nodules/animal (NDEA20), 9/21 with 0.9 nodules/animal (NDEA40)	[p<0.01] ^b , [p<0.01]	Hamsters in group 3 showed high incidence of cholangiofibrosis. One CCA observed in Group 3 (OV+NDEA20)
Hamster, Syrian (M) 22 wk Thamavit et al. (1988a)	Ov 100 MC, NDHDPa 1000 mg/kg bw (two i.p. injections at 2 wk intervals) 2 wk later Group 1: Ov 100 MC + NDHDPa Group 2: DHPN Group 3: Ov 100 MC Group 4: Untreated control	Group 1: CCA, 6/19; liver neoplastic nodules, 9/19 Group 2: CCA, 0/20; liver neoplastic nodules, 0/20 Group 3: CCA, 0/14; liver neoplastic nodules, 0/14 Group 4: CCA, 0/14; liver neoplastic nodules, 0/14	p <0.05; p <0.01 (versus Group 2)	Initial number of animals not specified

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (M) 30 wk Thamavit et al. (1988b)	Ov 100 MC, 0.1% Sodium nitrite and 0.1% aminopyrine in the drinking-water for 8–12 wk Group 1: Untreated control Group 2 0.1% Sodium nitrite Group 3: 0.1% Aminopyrine Group 4: Sodium nitrite and Aminopyrine Group 5: Ov 100 MC Group 6: Ov 100 MC + sodium nitrite (4 wk later) Group 7: Ov 100 MC + aminopyrine (4 wk later) Group 8: Ov 100 MC + sodium nitrite and aminopyrine (4 wk later) Total <i>n</i> =150	Group 8 and 4: 8/18, 2/17 hepatocellular nodules and 14/18, 3/17 CCA, respectively; no tumours observed in group 1, 2, 3, 5, 6 and 7	P<0.05 (versus Group 4) and P<0.01 (versus Group 4)	Prior infection with Ov induced more inflammation and bile duct proliferation and is associated with a higher incidence of hepatocellular nodule, cholangiofibrosis and CCA
Hamster Syrian (M) 52 wk Moore et al. (1991)	Ov 80 MC, NDHDPa 500 mg/bw (3 i.p. injections at 1 wk interval) 16 wk later Group 1: Ov 80 MC + NDHDPa (<i>n</i> =40) Group 2: NDHDPa (<i>n</i> =30) Group 3: Ov 80 MC (<i>n</i> =20) Group 4: Untreated control (<i>n</i> =10)	Group 1: CCA, 8/16 Group 2: CCA, 0/16 Group 3: CCA, no tumours Group 4: CCA, no tumours	[<i>p</i> =0.001] ^a (versus Group 2)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (F) 38 wk Thamavit et al. (1993)	NDHDPA 1000 mg/kg bw (i.p.) at 2 wk intervals, Ov 60 MC, PZ 250 mg/kg bw suspended in corn oil at Weeks 4, 12 or 20	CCA: Group 1: 4/22 (18%) Group 2: 6/22 (28%) Group 3: 10/16 (63%) Group 4: 8/16 (50%) Group 5: 0/15 Group 6: 2/18 (11%) Group 7: 0/15 HCC: Group 1: 1/22 (5%) Group 2: 1/22 (5%) Group 3: 0/16 Group 4: 0/16 Group 5: 0/15 Group 6: 0/18 Group 7: 0/15	P<0.05 (between Group 1 and Group 4); [p=0.024 between Group 4 and 5] ^a	It was found that whereas praziquantel administration at the later two time points was ineffective at reducing cholangiocellular lesions. Significant reduction only being evident in hamsters treated 4 wk after parasite infestation. The findings thus indicate that promotion of DHPN-initiated bile duct carcinogenesis by opisthorchiasis is both rapid and to a large degree irreversible
Hamster, Syrian (M) 45 wk Thamavit et al. (1994)	Ov 80 MC, NDMA 20 mg/kg bw i.p. injection Group 1: NDMA + Ov (19 d later) (n=50) Group 2: NDMA (n=25) Group 3: Ov (n=15) Group 4: Untreated control (n=15)	Group 1: 19/43, CCA; 15/43, mucinous cystadenomas; 2/43, HCC. No such tumours in Group 2 (0/20), 3 (0/15) and 4 (0/15).	[p < 0.001] ^a , [p < 0.005], [NS]	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<i>Clonorchis sinensis</i>				
Rat, Fischer F334 (M) 40 wk Iang et al. (1990)	Cs 60 MC, NDMMA 25 mg/L in the drinking-water for 8 wk Group 1 Cs + NDMMA (4 wk later) (n=20) Group 2: Cs + NDMMA at the same time (n=20) Group 3: NDMMA + Cs 1 wk later (n=20) Group 4: NDMMA (n=19) Group 5: Cs (n=10) Group 6: Untreated control (n=12) [Total n=101]	0/101		No malignant tumours seen in the rat model. Animals infected before NDMMA administration had significantly ($p < 0.05$) increased numbers of glutathione S-transferase P-positive liver foci. No such effect was seen when animals were infected during or after exposure to NDMMA
Hamster, Syrian golden (F) 54 wk Iida (1985)	2-AAF 0.03% in the diet for 40 wk, Cs 40 MC Group 1: 2-AAF + Cs (n=60) Group 2: 2-AAF (n=50)	CCA: Group 1: 11/14 animals that lived beyond Week 25 Group 2: 6/17 animals that lived beyond Week 25	p<0.05	In group 1, of 11 animals with liver tumours, 5 had metastases. No metastases were observed in Group 2
Hamster, Syrian golden (NR) 11 wk Lee et al. (1993)	NDMA 15 mg/L in the drinking-water for 8 wk, Cs 10 MC. Group 1: NDMMA + Cs 10 MC (7 d later) (n=12) Group 2: NDMMA (n=12) Group 3: Cs (n=12) Group 4: Untreated control (n=12) Total n=48	Group 1: 6/8 CCA and 8/8 cholangiofibromas Group 2: 2/12 cholangiofibromas Group 3: 0/12 Group 4: 0/12	[p<0.001] ^a , CCA and cholangiofibromas	In the hamsters that received either DMN or <i>C. sinensis</i> alone, the livers showed only hyperplastic changes of the bile duct epithelial cells

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (NR) 13 wk Lee et al. (1994)	NDMA 15 mg/L in the drinking-water for 4 wk, Cs 15 MC, Praziquantel 200 mg/kg bw daily for 3 d Group 1: NDMA+ Cs (1 wk later) + praziquantel (5 wk later) Group 2: Cs (5 wk) + Praziquantel + NDMA (3 d later after treatment with praziquantel) Group 3: Cs + NDMA at the same time Group 4: NDMA alone Group 5: Cs alone Group 6: Untreated control Total n= 90, 15 animals/group	CCA: Group 1: 3/15 Group 2: 0/15 Group 3: 11/15 Group 4: 0/15 Group 5: 0/15 Group 6: 0/15	[p<0.001] ^a , versus all groups	Synergistic effect of <i>Clonorchis</i> infection and NDMA promoted the development of CCA
Hamster, Syrian golden (unspecified) 21 wk Wang et al. (1994)	Cs 20 MC, NDMA 25 mg/L in the drinking water for 17 wk (30 d later) Group A: Cs + NDMA Group B: NDMA Group C: Cs Group D: Untreated	HCC; CCA Group A: 4/11; 1/11 Group B: 3/15; 0/15 Group C: 0/12; 0/12 Group D: 0/15; 0/15	[NS] ^a	The authors concluded that <i>C. sinensis</i> “may” promote the formation of HCC though the comparison between Group A and B is not significant. Only one CCA in Group A.

^a Fisher Exact test

2-AAF, 2-Acetylaminofluorene; bw, body weight; CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; d, day or days; DHPN, 2,2'-dihydroxy-di-n-propylnitrosamine; DMN, dimethylnitrosamine; HCC, hepatocellular carcinoma; i.p., intraperitoneal; MC, Metacercariae; NDHPA, N-Nitrosodihydroxidi-n-propylamine; NDMA, N-Nitrosodimethylamine; NR, not reported; NS, not significant; Ov, *Opisthorchis viverrini*; PZ, Praziquantel; SAR, Special Administrative Region.; wk, week or weeks

Similar experiments were also performed following *C. sinensis* infection in combination with 2-acetylaminofluorene or *N*-nitroso compounds (*N*-nitrosodimethylamine or *N*-nitrosodihydroxydi-*n*-propylamine) in hamsters (Iida, 1985; Lee *et al.*, 1993, 1994; Wang *et al.*, 1994), and rats (Jang *et al.*, 1990). Three of these (Iida, 1985; Lee *et al.*, 1993, 1994) supported the role of *C. sinensis* in promoting cholangiocarcinoma in hamsters.

See [Table 3.1](#).

4. Other Relevant Data

4.1 Pathological changes in vivo

The main histopathological features of liver fluke infection both in man and the rodent models are inflammation, epithelial desquamation, epithelial and adenomatous hyperplasia, goblet cell metaplasia, periductal fibrosis, and granuloma formation. Liver fluke infection in humans may also result in cholangiocarcinoma, but not in rodents unless they are also given a chemical carcinogen (IARC, 1994; Sripa, 2003; Rim, 2005; Sripa *et al.*, 2007; see also Section 3).

Liver fluke antigens stimulate both inflammatory and hyperplastic changes in the bile ducts. The liver fluke excretes or secretes metabolic products from the tegument and excretory openings into the bile *in vivo* or culture medium *in vitro*, some of which are highly immunogenic (Wongratanacheewin *et al.*, 1988; Sripa & Kaewkes, 2000; Choi *et al.*, 2003). The metabolic products themselves, apart from inducing host immune responses, may be toxic to or interact with the biliary epithelium (Sripa, 2003). Sripa & Kaewkes (2000) demonstrated that *O. viverrini* excretory–secretory (ES) antigens can be detected in both the parasite and biliary epithelium. The presence of *O. viverrini* ES antigens in the biliary epithelium in association with severe inflammation has also been seen in the small

bile ducts, which the flukes cannot inhabit (Sripa & Kaewkes, 2000). Hong *et al.* (1993) observed strong stimulation of the proliferation of bile duct epithelial cells located at the base of the mucosal layer in Sprague-Dawley rats infected by *C. sinensis*. This finding was directly related to hyperplasia of the bile duct epithelium that may have been due to direct and local stimulation by *C. sinensis*.

4.2 Carcinogenicity of liver fluke infections

4.2.1 Cell proliferation in vitro

Adult *O. viverrini* worms were co-cultured with mouse NIH-3T3 fibroblasts. Even though worms and fibroblasts were separated by Transwell membrane, fibroblast proliferation was stimulated more than 4-fold. Moreover, *O. viverrini* ES products increased cell proliferation by stimulating the expression of phosphorylated retinoblastoma (pRB) and cyclin D1, the key proteins in driving cells through the G1/S transition point of the cell cycle. This led to the induction of cells going into the S-phase of the cell cycle (Thuwajit *et al.*, 2004). In similar experiments with *C. sinensis*, ES products, and the human embryonic kidney epithelial cell line HEK293, the ES products induce HEK293 cell proliferation, associated with the upregulation of cyclin E and the transcription factor E2F1 (Kim *et al.*, 2008a). Furthermore, *C. sinensis* ES products upregulate the phosphorylation of pRB and *N*-nitrosodimethylamine (NDMA) upregulates cyclin-dependent kinases, and both synergistically drive the cells to proliferate (Kim *et al.*, 2008b). An anti-apoptotic effect of *C. sinensis* ES products in human cholangiocarcinoma cells has been reported (Kim *et al.*, 2009).

Gene microarrays were used to explore transcriptional changes induced in NIH-3T3 murine fibroblasts co-cultured with *O. viverrini* ES products. mRNAs encoding certain

growth-promoting proteins such as transforming growth factor (TGF), PKC, EPS 8 and TGF- β II4, that are downstream of epidermal growth factor (EGF) or TGF- β -mediated signalling, were found to be overexpressed (Thuwajit *et al.*, 2006). Moreover, human cholangiocarcinoma cell line (KKU-100) underwent excessive proliferation upon stimulation with *O. viverrini* worms (Sripa, 2003). The promotion of proliferation *in vitro* is consistent with the histopathological findings of hyperplasia of biliary epithelial cells in opisthorchiasis and clonorchiasis (Bhamarapavati *et al.*, 1978; Sripa & Kaewkes, 2000; Rim, 2005).

4.2.2 Oval cell proliferation and differentiation *in vivo*

Oval cells are typically seen in response to certain liver injuries, and more than likely represent progenitor cells with the potential to differentiate along biliary or hepatocytic lineages, including into hepatic neoplasms (Sell & Leffert, 2008). Lee *et al.* (1997) reported the appearance of increased numbers of periductal oval cells in the portal and/or periportal areas of hamster liver infected with *C. sinensis* and administered NDMA.

4.2.3 DNA damage and adduct formation *in vivo*

Diffuse nitrosative and oxidative DNA damage (8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine [8-oxodG]) has been reported in the biliary epithelium of hamsters infected with *O. viverrini* (Pinlaor *et al.*, 2003). These DNA lesions still persisted for at least 180 days post-infection. Moreover, repeated infections with liver flukes result in enhanced biliary DNA damage (Pinlaor *et al.*, 2004a, b). This may be explained by the fact that repeated infection increased inducible nitric oxide synthase (iNOS) expression in the bile duct epithelium. The DNA damage in infected biliary cells is probably a result

of the inflammatory response caused by *O. viverrini* because 8-nitroguanine and 8-oxodG disappear after praziquantel treatment (Pinlaor *et al.*, 2006). However, in promoting parasite antigen dispersal, treatment with praziquantel may transiently increase inflammation, in association with increased NF- κ B and iNOS expression in the bile duct epithelium and inflammatory cells, and elevated levels of plasma nitrate, of end-products of nitric oxide, and of malondialdehyde in the treated hamsters (Pinlaor *et al.*, 2008).

Individuals infected with *O. viverrini* also show evidence of oxidative DNA damage. Urinary 8-oxodG levels were significantly higher in *O. viverrini*-infected patients ($4.45 \pm 0.25 \mu\text{g/g}$ creatinine) than in healthy subjects ($3.03 \pm 0.24 \mu\text{g/g}$ creatinine; $P < 0.01$). This level decreases significantly 2 months after praziquantel treatment, and is comparable with levels in healthy subjects 1 year after treatment. Urinary 8-oxodG levels were significantly correlated with leukocyte 8-oxodG levels (Thanan *et al.*, 2008).

The excretion of lipid peroxidation-derived etheno DNA adducts – 1, N^6 -etheno-2'-deoxyadenosine (ϵ dA) and 3, N^4 -etheno-2'-deoxycytidine (ϵ dC) – was measured in urine samples collected from healthy volunteers and *O. viverrini*-infected Thai subjects. Mean excreted ϵ dA and ϵ dC levels were 3–4 times higher in the urine of *O. viverrini*-infected patients and correlated with an increased level of urinary malondialdehyde, urinary nitrate/nitrite, and plasma alkaline phosphatase. After fluke elimination by treatment with praziquantel, the level of the two etheno adducts, malondialdehyde, nitrate/nitrite, and alkaline phosphatase was decreased. The promutagenic DNA etheno adducts that are thought to derive from bile duct epithelial cells may increase the risk of developing cholangiocarcinoma in *O. viverrini*-infected patients. This study highlights the biomarker potential of urinary ϵ dA and ϵ dC levels as non-invasive risk markers for developing

opisthorchiasis-related cholangiocarcinoma ([Dechakhamphu et al., 2008](#)).

[The Working Group noted that all the studies described above relate to *O. viverrini*; studies regarding DNA damage in response to *C. sinensis* infection were not available to the Working Group.]

4.3 Gene mutation, methylation, and altered expression in cholangiocarcinoma

4.3.1 *O. viverrini*-endemic areas

Differences in *Ki*-RAS mutational status have been described when comparing cholangiocarcinoma from Japanese patients (where fluke infections are very rare) with those from cholangiocarcinoma arising in patients living in areas of Thailand endemic for *O. viverrini* (the incidence of *Ki*-RAS mutation was higher in Thai patients) ([Kiba et al., 1993](#)). Hypermethylation of the promoter of the DNA mismatch repair enzyme hMLH1 has also been shown in another group of Thai patients ([Limpaiboon et al., 2005](#)). However, these studies did not specifically document liver fluke infection status in the two groups of patients.

Gene microarray transcriptional profiling of cholangiocarcinoma from Japanese versus Thai patients (again without certain knowledge of liver fluke status) led [Jinawath et al. \(2006\)](#) to propose a signature of *O. viverrini*-associated cholangiocarcinoma with an elevated expression of genes involved in xenobiotic metabolism (*UGT2B11*, *UGT1A10*, *CHST4*, *SULT1C1*) in cases from Thailand, but a lower expression of genes related to growth-factor signalling (*TGFBI*, *PGF*, *IGFBP1*, *IGFBP3*).

4.3.2 Studies in experimental animals

Few mutations of the *Ki*-RAS gene were observed in *O. viverrini*-NDMA-induced cholangiocarcinomas in hamsters ([Tangkawattana et al., 2008](#)), but *TP53* overexpression was reported in nearly all *O. viverrini*-induced hamster cholangiocarcinomas ([Tesana et al., 2000](#)).

[Loilome et al. \(2006\)](#) investigated the molecular mechanism of *O. viverrini*-NDMA-induced cholangiocarcinogenesis in hamsters by using fluorescence differential display-PCR, and found 23 upregulated and one downregulated transcripts among 149 differentially amplified bands. Among the upregulated genes in the liver was the signal transduction protein kinase A regulatory subunit 1 α (*Prkar1 α*), which was significantly higher in cholangiocarcinoma and its precursor lesion when compared with normal liver and normal gallbladder epithelia ($P < 0.05$). *Prkar1* expression tended to increase along with the progression of biliary transformation from hyperplasia and precancerous lesions to carcinoma.

4.4 Host immune system and genetic susceptibility

[Tesana et al. \(2000\)](#) explored the role of immunization in hamsters administered a subcarcinogenic dose of NDMA with *O. viverrini* infection. Pre-immunization with a crude somatic fluke antigen accelerated carcinogenesis at 30 weeks (71%) compared with the non-immunized group (20%), suggesting that an increased immune response to liver fluke antigens may increase the susceptibility of developing cholangiocarcinoma.

The relationship between immune responses to infection with *O. viverrini* and the synthesis of the carcinogen NDMA, nitric oxide (NO) and nitrosation of amines in humans has been described. The intake of exogenous nitrate and nitrite was minimized and assessments were carried out before and 4 months after elimination

of the infection with praziquantel treatment. No variation was observed in the amount of NDMA excreted in the urine between the control, moderate and heavy liver-fluke-infected groups ($n = 40-50$ subjects per group). However, during active infection, a strong negative association was observed between *in vitro* lymphoproliferative responses to some liver fluke antigens and NDMA excretion. This association was reduced after praziquantel treatment. Multivariate statistical models revealed a highly significant relationship between NDMA levels and urinary nitrate, stimulation indices for two T-cell responses to two parasite antigens (molecular weight, 37 kDa and 110 kDa), and gallbladder dimensions. NDMA levels after treatment were best described by the ratio between parasite-specific IgG2 and IgE, background levels of T-cell proliferation, a urinary marker of nitrosation (*N*-nitrosothioprolin), and a normal level of alcohol consumption. Thus, individual background immunological activity, parasite-specific responses and/or parasite products and NO synthesis may all be determinants of endogenous generation of nitrosamines in *O. viverrini*-infected humans (Satarug *et al.*, 1998).

In the only study of host genetic polymorphisms, a population-based case-control study in Thailand failed to show any association between glutathione *S*-transferase polymorphisms and cholangiocarcinoma risk (Honjo *et al.*, 2005).

4.5 Synthesis

Although liver fluke ES products may stimulate cell proliferation and anti-apoptosis directly, liver-fluke-induced cholangiocarcinoma is more likely the result of chronic inflammation (Holzinger *et al.*, 1999; Sirica, 2005; Kawanishi & Hiraku, 2006), involving the activation of oxidative stress pathways. Studies on *O. viverrini* provide most of the mechanistic data.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Opisthorchis viverrini*. Chronic infection with *Opisthorchis viverrini* causes cholangiocarcinoma.

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Clonorchis sinensis*. Chronic infection with *Clonorchis sinensis* causes cholangiocarcinoma.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Clonorchis sinensis*.

Chronic infection with *Opisthorchis viverrini* is *carcinogenic to humans* (Group 1).

Chronic infection with *Clonorchis sinensis* is *carcinogenic to humans* (Group 1).

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