THE LIVER FLUKES: CLONORCHIS SINENSIS, OPISTHORCHIS SPP, AND METORCHIS SPP.
Summary

The liver and intestinal fish-borne zoonotic trematodes (flukes) are important parasites of humans and animals and are estimated to infect more than 18 million people, especially in Asia. The diseases caused by fish-borne liver flukes, clonorchiasis, opisthorchiasis and metorchiasis, can be severe. Infection with high worm burdens has high impact on health status in endemic areas; a recent estimation of the effect of liver flukes on morbidity yielded DALY value of 275,370. Because fish are a major source of protein and an important export commodity in western Siberia and South East Asia these diseases are of both economic and public health concern.

Clonorchis sinensis is endemic in southern China, Korea and northern Vietnam, whereas O. viverrini is endemic in the Lower Mekong Basin, including Thailand, Lao People's Democratic Republic), Cambodia and south and central Vietnam. Opisthorchis felineus has been documented in at least 12 countries of the European Union, Belarus, Ukraine, and in Western Siberia (Russia). Methorchis species are wide spread, and reported from North America, Eurasia, and East Asia; however, information on human infections is very limited. The infective stage for humans, as well for animals, is the larval metacercaria stage present in fish that matures to the adult stage in the hepatobiliary system of humans and other fish-eating mammals. A significant feature of the epidemiology of these parasites is their wide definitive host range, which includes not only domestic animals but also sylvatic mammals such as rodents and carnivores. The adult flukes can survive for up to ten years in the host, producing around 200 eggs per day. This results in considerable contamination of the environment. Water becomes contaminated with fluke eggs from indiscriminate deposition of infected human and animal excreta. These zoonotic helminths are of public health concern because of the serious pathology they can induce in the liver and bile ducts (Sithithaworn et al., 2007a; Pakharukova and Mordvinov, 2016). According to the Food and Agriculture Organization and the World Health Organization (FAO/WHO, 2014), they rank 8th overall in global health importance among 24 food-borne parasites. Because their life cycles require intermediate hosts that are aquatic (snails and fish) infected due to excretion of the eggs of this parasite from feces of infected humans and other mammals, they may, especially when associated with aquaculture systems, be a consideration in the design of sanitation systems for human and animal excreta.

1.1 Global Burden of Disease

1.1.1 Global distribution

Figure 1 shows the distribution area of Opisthorchis felineus in western Siberia and Europe and Figure 2 shows the distribution areas of Clonorchis sinensis and Opistorchis viverrini in Cambodia, China, Laos, Thailand and Vietnam.

Figure 1. Distribution area of Opisthorchis felineus. Information on the distribution of O. felineus in western Siberia orginated from Pakharukova and Mordvinov, 2016. Information on the distribution of O. felineus in Europe is from Pozio and Gomez Morales, 2014. (permission obtained from Dr. Edoardo Pozio, Istituto Superiore Di Sanita’, Department of Infectious, Parasitic and Immune-Mediated Diseases)

The Liver Flukes: Clonorchis sinensis, Opisthorchis spp, and Metorchis spp.

1.0 Epidemiology of the Disease and Pathogens

Trematode parasites of the genera Clonorchis, Opisthorchis and Metorchis, commonly referred to as liver flukes, are transmitted to humans and other mammals by the ingestion of fish infected with their larval stages which ultimately come from snails infected due to excreta and polluted waters (Chai et al., 2005; Mordvinov et al., 2012; Petney et al., 2013). These zoonotic helminths are of public health concern because of the serious pathology they can induce in the liver and bile ducts (Sithithaworn et al., 2007a; Pakharukova and Mordvinov, 2016). According to the Food and Agriculture Organization and the World Health Organization (FAO/WHO, 2014), they rank 8th overall in global health importance among 24 food-borne parasites. Because their life cycles require intermediate hosts that are aquatic (snails and fish) infected due to excretion of the eggs of this parasite from feces of infected humans and other mammals, they may, especially when associated with aquaculture systems, be a consideration in the design of sanitation systems for human and animal excreta.

Further, the snail intermediate host species are diverse and abundant in water bodies. These features make control of these zoonotic parasites difficult and focuses prevention on human food behaviors, and mass drug treatment of communities. Procedures to limit contamination of ponds, lakes, and rivers, with human and animal feces containing liver fluke eggs are limited, but methods focusing on the education of consumers, farmers, and fishermen will be discussed.
Rough distribution area of *O. viverrini* in Cambodia, Laos and Thailand (striped red). (permission obtained from Dr. Edoardo Pozio, Istituto Superiore Di Sanita’, Department of Infectious, Parasitic and Immune-Mediated Diseases)

1.1.1.1 *Clonorchis sinensis*

Infection with *C. sinensis* and the disease it causes, clonorchiasis, occurs primarily in East Asia, where it is widely distributed; it is currently endemic in South Korea, China, Taiwan, northern Vietnam, and eastern Russia (De et al., 2003; Chai et al., 2005; Lun et al., 2005; Sithithaworn et al., 2007a; Sithithaworn et al., 2012). The number of people infected in this region is estimated to be 7-15 million (WHO, 1995, 1999; Fürst et al., 2011) and prevalence varies widely, from <1.0% in Guang Xi, China to >40% in North Vietnam, to >70% in Guangdong Pr., China. Importantly, Fürst et al. (2011) calculated that 1.1 million of infected people had heavy infections (> 1000 eggs/gram feces).

1.1.1.2 *Opisthorchis viverrini*

Infection with *O. viverrini* and the disease it causes, opisthorchiasis, occurs in Cambodia, Lao PDR (mainly southern areas), Thailand (mainly northeastern areas), and southern Vietnam (De et al., 2003; Andrews et al., 2008; Sohn et al., 2011, 2012; Yong et al., 2012; Doanh and Nawa, 2016). The number of people infected in these countries is estimated to be eight million in Thailand and two million in Laos (Sithithaworn and Haswell-Elkins, 2003); little prevalence data are available in Vietnam even if the presence of *O. viverrini* infection in humans has been documented (De et al.; 2003; Sithithaworn et al., 2006; Sayasone et al., 2007; Doanh and Nawa, 2016).

1.1.1.3 *Opisthorchis felineus*

Infection with *O. felineus* and the disease it causes, opisthorchiasis, occurs in Byelorussia, Kazakhstan, Russia, Ukraine and Siberia, and in scattered foci of the European Union (Germany, Greece, Italy, Poland, Portugal and Spain) (Pozio et al., 2013). In Russia, Ukraine, and Kazakhstan, 12.5 million people have been considered to be at risk for *O. felineus* (Keiser and Utzinger, 2005). In these foci, both humans and domestic animals (cats and dogs) play the role as final hosts (Mordvinov et al., 2012). In the Tomsk region of Siberia, the prevalence of opisthorchiasis in humans increased from 495 cases per 100,000 inhabitants to 649 cases per 100,000 inhabitants between 1997 and 2006 (Mordvinov et al., 2012). Other endemic foci of *O. felineus* in Siberia are the Ob river and the Irtysh river basins.

1.1.1.4 Trade impact

The liver flukes are ranked 6th among 24 food-borne parasites for impact on trade in endemic countries; their impact on overall socioeconomic wellbeing of affected communities is ranked 5th (FAO/WHO, 2014). An important factor affecting the evaluation of trade impact of liver flukes is that their primary source are wild-caught freshwater fish rather than fish produced in aquaculture (see Section 1.3.2). Further, non-intensive aquaculture farms generally produce for local domestic markets rather than for international trade. However, in Italy, wild tenches fished from central Italy lakes, where *O. felineus* is highly endemic, are exported to several fish markets outside the country and have caused opisthorchiasis outbreaks (Traverso et al., 2012).

1.1.2 Symptomology

In general, all the liver fluke infections induce chronic inflammatory diseases of the hepatobiliary system and in chronic high worm burden infections this may lead to bile duct cancer termed cholangiocarcinoma (CCA) (Sithithaworn et al., 2007a; Pakharukova and Mordvinov, 2016; Qian et al., 2016). Most of these manifestations are mild and asymptomatic. However, once advanced CCA develops, clinical manifestations such as jaundice occurs in approximately half of the cases, while the other half may have no specific symptoms (Chai et al., 2005).

Infections with less than 100 worms may be asymptomatic (Armignacco et al., 2008, 2013; Pakharukova and Mordvinov, 2016; Qian et al., 2016). Infection with one-hundred to thousands of worms, however, may cause jaundice, indigestion, epigastric discomfort, anorexia, general malaise, diarrhea, and mild fever (Chai et al., 2005). Over time, without treatment, infection may lead to liver enlargement, allergic lesions, congestion of the spleen, bile stone development, cholecystitis, and liver cirrhosis. The most serious possible outcome, however, is the development of CCA. Benign hepatobiliary diseases are characterized by cholangitis, obstructive jaundice hepatomegaly, periductal fibrosis, cholecystitis, and cholelithiasis (Chai et al., 2005; Sithithaworn et al., 2007a).

1.1.2.1 Morbidity and mortality

Because of the potentially severe consequences of all liver fluke infections (e.g., hepatic lesions, cholangitis, and, most seriously, CCA), chronic infections with high worm burdens have a high impact on health status in endemic areas; a recent estimation of the effect of clonorchiasis on morbidity yielded DALY value of 275,370 (Fürst et al., 2011), a relatively high impact for a helminthic disease. In
highly endemic foci of *O. felineus* in Western Siberia, CCA was detected in 77% of patients with opisthorchiasis, versus 34.2% of patients without opisthorchiasis (Pakharukova and Mordvinov, 2016).

### 1.2 Taxonomic Classification of the Agents

The fishborne liver flukes of public health importance belong to the trematode family Opisthorchiidae (Scholtz, 2008). The most prevalent and important species are *Clonorchis sinensis*, *Opisthorchis viverrini*, and *O. felineus*, members of the subfamily Opisthorchiinae. These species are similar in morphology, life cycles, and modes of transmission, which often causes difficulties in specific diagnosis. Their geographic distributions are basically allopatric, however. Other species of the *Opisthorchis* genus have been reported from humans only rarely and will not be considered further in this chapter.

#### 1.2.1 Physical description of the agents

*Clonorchis sinensis*: The adult worms are flat, elongated, leaf or lanceolate shaped, generally 8-15 mm in length, and 1.5-4.0 mm wide (Chai et al., 2005; Rim, 1990). As shown in Figure 3, *C. sinensis* is morphologically similar to *Opisthorchis viverrini* and *O. felineus*, but differs particularly in having highly branched testis (Scholtz, 2008). The larval stage transmitted through fish to humans and other mammals is termed a metacercaria, which is encysted in various tissues of the fish host. The metacercaria is round to oval, measuring 0.13-0.14 X 0.09-0.10 mm (Figure 4) (Chai et al., 2005).

**Figure 3.** Hematoxylin and eosin stained adult worms of the most important liver flukes. A, *Opishorchis felineus*, scale bar 2 mm; B, *Opithorchis vivverini*, scale bar 1 mm; and C, *Clonorchis sinensis* scale bar 2 mm. There is no proportion between worm size. (permission obtained from Dr. Edoardo Pozio, Istituto Superiore Di Sanita’, Department of Infectious, Parasitic and Immune-Mediated Diseases)
**Figure 4.** First intermediate host of liver flukes, and infecting stages for fish and mammals. 1, *Bythinia* sp., major snail host of liver flukes, scale bar 8 mm; 2 *Opisthorchis felineus* cercaria, the swimming larval stage of liver flukes infecting fish, scale bar 100 µm; 3, metacercaria of *Clonorchis sinensis* scale bar 100 µm; 4, metacercariae of *Opisthorchis viverrini*, scale bar 150 µm; and 5, metacercariae of *Opisthorchis felineus*, scale bar 200 µm. (permission obtained from Dr. Edoardo Pozio, Istituto Superiore Di Sanita’, Department of Infectious, Parasitic and Immune-Mediated Diseases)

*Opisthorchis viverrini*: The adult worms are flat, elongated, leaf or lanceolate shaped, generally 5.5-10 mm in length, and 0.8-1.6 mm wide (Figure 3) (Pozio and Gomez Morales, 2014). The metacercaria is round to oval, measuring 0.19-0.25 x 0.15-0.22 mm in size (Figure 4).

*Opisthorchis felineus*: The adult worms are flat, elongated, leaf or lanceolate shaped, generally 7-12 mm in length, and 1.5-2.5 mm wide (Figure 3) (Pozio et al., 2013). The metacercaria is oval, measuring 0.25-0.30 x 0.19-0.23 mm in size (Figure 4).

Variation in the size of adults depends on the intensity of infection and the diameters of the bile ducts they inhabit.

*Metorchis* spp: This genus belongs to a separate opisthorchid subfamily, the Metorchiinae, and is readily differentiated morphologically from *C. sinensis* and *Opisthorchis* spp. (Scholtz, 2008); their life cycle features, however, are similar the other liver flukes (Mas-Coma and Bargues, 1997). The species reported from humans are *M. conjunctus, M. bilis, M. orientalis*, and *M. taiwanensis*. Because their overall prevalence and geographic distributions are limited compared to that of *C. sinensis* and *Opisthorchis* spp., there is comparatively little information on their epidemiology, health burden, and control (Mas-Coma and Baruges, 1997; Chai et al., 2005; Furst et al., 2011; Mordvinov et al., 2012). For these reasons, species of the *Metorchis* genus will not be discussed further in this chapter.

Although the metacercariae of the liver flukes species are very similar, they can be differentiated morphologically and by molecular methods (Figure 4).

### 1.3 Transmission

#### 1.3.1 Life cycle and routes of transmission
The basic life cycle of the fish-borne liver flukes is shown in Figure 5. Liver flukes utilize as their first intermediate host freshwater snails belonging to several genera. The egg contains a mature miracidia that emerges from the egg if it reaches freshwater and is ingested by an appropriate snail species. In the snail, the miracidial stage then develops to a sporocyst, which then undergoes asexual multiplication producing rediae, which mature in the snail hepatopancreas within about 17 days. Each redia, in turn, produces 4-50 cercariae, which emerge from the snail into the surrounding water. The time between trematode egg ingestion by the snail and the emergence of the cercariae is influenced by the water temperature; in tropical regions, this takes about 14-17 days after ingestion of the egg (Rim et al., 1982). An infected snail may produce and release into the water 500 to 5,000 pleurolophocercus cercariae (Figure 4) per day, depending on the infection level (Rim, 1990). The cercariae are phototactic and geotropic and are able to survive in the water up to 24 hours at temperatures ranging from 12°C to 27°C. The second intermediate hosts are freshwater fish of the family Cyprinidae; however, metacercariae of Clonorchis sinensis in fish of other families have been reported (WHO, 1995). Fish movements attract cercariae, which, on contact with the fish, penetrate under the scales, lose their tails and encyst, mainly in the muscles, subcutaneous tissues, and to a lesser degree in the fins and gills (Rim et al., 1982). In the fish, the metacercaria reaches maturity in about 5-6 weeks, and may remain infective for the definitive host for at least 30 days, probably much longer although this has not been well characterized (Rim et al., 1982). When a metacercaria is ingested by the definitive mammalian host (e.g., humans), it excysts in the duodenum and migrates to the common bile duct and then to the biliary ducts within 4-7 hours. The hermaphroditic adult worm reaches sexual maturity and, in four-six weeks, begins producing eggs that are expelled with the host’s feces. The longevity of adult liver flukes has been estimated to be 15-26 years (Lai et al., 2016). In humans, adult worms may shed 1000 to 4000 eggs per day, depending on the worm burden, which is density-dependent (Mas-Coma and Bargues, 1997).

The risk for human infection is closely related to social and cultural traits that determine food behaviors, such as a fondness for raw or inadequately prepared fish (i.e., cooked, frozen, or pickled). The consumption of raw or undercooked fish is widely practiced, particularly in localities near lakes, reservoirs, streams and ponds where fresh fish are readily available (WHO, 1995). For example, in China, raw fish is commonly served after dipping briefly
in boiling soup and immediately eaten, or in hot rice congee. In Thailand, a major source of infection with *O. viverrini* is consumption of raw or inadequately cooked, frozen, salted, or smoked fish in a dish called *Koi-pla*. In Italy, large *O. felineus* human outbreaks occurred from 2007 to 2011 from the consumption of marinated tench fillets at restaurants or during social events (Pozio et al., 2013). A strong risk factor for *C. sinensis* infection, especially for males, is the consumption of raw fish at social gatherings where alcoholic drinks are served (Chai et al., 2005). Studies have shown that liver fluke metacercariae in fish tissue are moderately tolerant to low levels of heat, freezing, and pickling (Table 1).

![Table 1. Reports on the preservation and treatment parameters necessary to inactivate liver fluke metacercariae in fish](image)

The data presented are the reported treatment conditions that yielded complete inactivation of metacercaria in the bioassays employed; the details of test protocols and results can be found in the appropriate reference; 

1.3.2 Epidemiological role of the intermediate and reservoir hosts

There is an age and gender bias in human infections with *C. sinensis* and *O. felineus*. Infection rates of *O. felineus* are generally higher in men than in women, and higher in adults than in children (Pozio et al., 2013). Analysis of clonorchiasis cases have revealed that men 25-55 years old and women over 45 years are the most highly affected groups in Southern China, Korea, and North Vietnam (De et al., 2003; Chai et al., 2005). Similar infection patterns are reported for *O. viverrini* outbreaks (Sithithaworn et al., 2007a). This probably reflects behavioral patterns of men and alcohol, as mentioned above. The age influence is evident from clinical studies that indicate that initial infections are acquired at an early age, and repeated exposure results, in the absence of immunity and worm expulsion, in increasing worm burdens and disease (Sithithaworn et al, 2007a). It is important to distinguish human infections acquired in endemic foci, where people frequently consume raw infected fish, from human infections acquired sporadically in endemic foci where the consumption of raw fish is infrequent, as in Italy.
The liver fluke *C. sinensis* utilizes, as first intermediate host, snail species including *Alocinna longicornis*, *Bithynia* spp., *Melanoides tuberculatus*, *Parafossarulus* spp., and *Thiara* (WHO, 1995; Sithithaworn et al., 2007a; Hung et al., 2013). The major snail hosts for *O. viverrini* and *O. felineus* belong to the genus *Bithynia*. The prevalence of larval stages in snails is always quite low and does not often exceed 1% in endemic foci (De Liberato et al., 2011).

The second intermediate hosts are mainly fish of the family Cyprinidae. Metacercariae of *C. sinensis* have been recovered from fish belonging to the genera *Acanthogobius*, *Abbottina*, *Carassius*, *Cirrhinus*, *Crassiodes*, *Cultrichthys*, *Cyprinus*, *Ctenopharyngodon*, *Erythrocultor*, *Gnathophogon*, *Hemibarbus*, *Hemiculter*, *Hypomesus*, *Hypophthalmichthys*, *Ischikauia*, *Osparsichthys*, *Oreochromis*, *Parabramis*, *Pseudogobio*, *Pseudorabora*, *Pungtungia*, *Rhodeus*, *Sarcocheilichthys*, *Toxobramus*, *Xenocypris*, and *Zacco* (WHO, 1995; Hung et al., 2013). Metacercariae of *O. viverrini* were detected in fish of the genera *Carassius*, *Channa*, *Cyclocheilichthys*, *Hampala*, *Esomus*, *Osteochilus*, *Puntiploites*, and *Puntius* (WHO, 1995). Metacercariae of *O. felineus* were detected in fish of the genera *Alburnus*, *Abramis*, *Aspius*, *Blicca*, *Carassius*, *Chondrostoma*, *Cobitis*, *Cyprinus*, *Gobio*, *Leucaspis*, *Leuciscus*, *Phoxinus*, *Rutilus*, *Scardinius*, and *Tinca* (Erhardt et al., 1962; Pozio et al., 2013; Pakharukova and Mordvinov, 2016). There are reports of shrimp found infected with metacercariae that morphologically were identified as *C. sinensis* (Chen et al., 2010), but follow up studies to verify their identity do not appear to have been conducted. Because shrimp are intermediate hosts for other trematode species, this report must be provisional.

The large number of fish species reported infected with liver fluke metacercariae (see above) implies that these parasites have low host specificity (WHO, 1995; Chai et al., 2005). It is therefore important to be aware that in locations where these parasites are found, infections may occur in several fish species, and the relative infection rates may fluctuate independently, an important consideration for epidemiological studies. Importantly, wild fish from clean fresh water sources, such as rivers and reservoirs, are usually preferred for preparing raw fish dishes.

All fish eating mammals, including humans, are potential final hosts of liver flukes. Hosts for *C. sinensis* and *O. viverrini*, besides humans, include feral and domestic cats and dogs, and pigs and rats (*Rattus*); these host species play the crucial role of reservoir host (Mas-Coma and Bargues, 1997; Lun et al., 2005; Lan-Anh et al., 2009; Petney et al., 2013). However, *C. sinensis* has also been reported from sylvatic animals such as martins, civet cats, badgers, monkeys, weasels, muskrats, foxes and rice rats (Mas-Coma and Bargues, 1997; Hung et al., 2013; Petney et al., 2013). The role of sylvatic reservoir hosts is very important in the epidemiology of *O. felineus*, which has an even wider spectrum of final hosts; it has been reported from domestic (e.g., cats, dogs, pigs), synanthropic (e.g., muskrats, rats) and 28 wild animals (e.g., otters, polecats, polar and red foxes, sable, seals, wild boar, wolverines) (Mordvinov et al., 2012; Pozio et al., 2013; Chai et al., 2005).

The role of a sylvatic cycle in the epidemiology of *C. sinensis* and *O. viverrini* has not been well studied. A role for wild animal reservoirs is suggested from surveys of fish infections in endemic areas, which frequently demonstrate that the prevalence of liver fluke metacercariae is often higher in wild fish from reservoirs, canals, streams and rice fields than in fish from farm ponds (Mas-Coma and Bargues, 1997; Chai et al., 2005; Phan et al., 2010; Li et al., 2013).

Cats, dogs and pigs are considered to be the most important reservoir hosts in the domestic habitat, because of their wide distribution and large populations (WHO, 1995). Although it is a common assumption that farm households, including humans, dogs, pigs, and cats, play essential roles in liver fluke epidemiology, the greatest infection risk factor for domestic cats and dogs is the common practice of allowing such animals to roam and scavenge freely in the communities (Lan-Anh et al., 2009; Aunpromma et al., 2012; Petney et al., 2013). Epidemiological studies on the role of cultured and wild-caught fish in liver fluke transmission have demonstrated that sylvatic hosts, both fish and mammals, can sustain the life cycle and risk for humans in the absence of a domestic cycle (Li et al., 2013; Clausen et al., 2015).

The probable explanation for the higher infection rates of *C. sinensis* and *O. viverrini* in wild caught fish maybe related to both biotic and abiotic factors associated with the different aquatic habitats of vector snails, especially *Bithynia* spp. Recent research in Thailand and Vietnam on the ecology and distribution in various aquatic habitats of a major snail host in the genus *Bithynia*, revealed a greater abundance in rice fields, streams, and small canals than in lakes and in farm ponds (Broekelman et al., 1986; Ngernklun et al., 2006; Petney et al., 2012; Doanh and Nawa, 2016). Further, investigations on the abiotic factors affecting the abundance of *Bithynia siamensis gonoiocephalus*, the major snail host for *O. viverrini*, revealed the importance of water depth and temperature, level of dissolved oxygen, pH, and salinity (Nithithai et al., 2002). These conditions may not be met in farm ponds that are generally stagnant, warm, and with low oxygen levels.

### 1.4 Population and Individual Control Measures

#### 1.4.1 Treatment options

Prevention and control of human liver fluke infections must begin with an effective education effort directed at enabling consumers to understand the risks associated with eating raw or undercooked fish, regardless of source. Currently, the major strategies for community prevention and control encompasses fecal examination and treatment of individual cases with praziquantel (25 mg/kg three times daily for 2-3 days), and environmental sanitation by building and use of household latrines (Chai et al., 2005; Sithithaworn et al., 2007a). Mass chemotherapy with praziquantel (40 mg in a single dose) is recommended by WHO (1995), but the sustainability of control by this approach is uncertain (Chai et al., 2005; Clausen et al., 2015). *O. felineus* infection in humans can be treated with praziquantel (25 mg/kg orally 3×/day for 1-2 days) or
albendazole (10 mg/kg/day orally in 2 doses for 7–14 days). The treatment with praziquantel is, as a rule, effective, whereas treatment with albendazole can fail and the individual has to be treated again with another drug (Armignacco et al., 2008, 2013). After the second treatment, eggs are generally not detected in fecal samples; however, in very few cases, eggs have been detected after a period from 5 to 6 months up to 2 years despite treatment of the patients with albendazole (Pozio et al., 2013). Although the effectiveness of treatment can be determined based on the search for eggs in stool samples, very few eggs are produced in cases of unsuccessful treatment; for this reason, ELISA could be used, although the antibody levels decrease very slowly to the cut-off value (Pozio et al., 2013). Vaccines to prevent liver fluke infection in humans have not been developed.

1.4.2 Hygiene measures

Emphasis on hygienic measures applicable to households and restaurants may be the most fruitful approach to control in endemic areas, at least for the immediate future. Treatment of fish to inactivate the metacercariae by heating to temperatures of 70 °C or above are effective (WHO, 1995). Table 1 lists parameters for inactivation by salting and freezing, pickling, and irradiation that can be effective (WHO, 1995). Other food preparation methods for household use that may inactivate metacercariae, such as microwaving, smoking, fermenting, and marinating, need further investigation before guidance on their use can be formulated. *O. felineus* metacercariae, for example, can survive in smoked fish causing human infections (Yossepowitch et al., 2004), nor does marinating does not kill *O. felineus* metacercariae present in tench muscles (Armignacco et al., 2008, 2013; Traverso et al., 2012).

Education of food handlers on the risk from fish in endemic areas and on the need to keep preparation counters and utensils clean between individual fish preparations is highly recommended. It should be noted that many home freezers can reach temperatures of -6°C or -12°C, but domestic freezers that can operate at a temperature of -18°C or below are more effective; infected fish need to be frozen in all parts of the products for longer than 24 hours to ensure that all parasites are inactivated.

Investigations in Thailand have demonstrated that low dose irradiation of freshwater fish can prevent infectivity of *O. viverrini* metacercariae when such fish are prepared in local dishes made from raw or semi processed fish (Bhaiobulaya, 1985). Experimental studies should be extended in order to evaluate the usefulness of this control measure in food processing (EFSA, 2010).

2.0 **Environmental Occurrence and Persistence of Larval Liver Fluke Stages**

2.1. Detection Methods

Detection is made most often by microscopy however trained parasitologists are needed to make the identification. Eggs containing the miracidia of liver flukes can be detected in the feces by using standard fecal exam methods (e.g., Kato-Katz technic). Fecal deposits picked up from the ground adjacent to fish ponds, rice paddies, reservoirs and streams, and from latrines and pig pens, can be collected and tested. This approach has been successful in identifying reservoir hosts with aquaculture activities and assessing their role in the epidemiology of fishborne flukes (Lan-Anh et al., 2009).

Possible ongoing transmission may be detected by examining snails collected from fishponds and local waterbodies and examining them for the presence of opisthorchid sporocysts and/or rediae, and for cercariae. This can be done either by crushing the snail and viewing the remnants under a stereomicroscope or by allowing the snails to shed cercariae directly into water-filled containers. However, because snails may be infected with other trematodes with pleurolophocercous type cercaria (Figure 4), especially heterophyid intestinal flukes, a specific identification of liver fluke cercariae may not be possible.

Examination of local fish can also provide an indication of liver fluke transmission in the area. Fish tissue can be examined directly by microscope for metacercariae (Figure 4) or, preferably, after pepsin digestion to free any metacercariae present before examination (WHO, 1995; Thu et al., 2007; De Liberato et al., 2011).

Use of molecular methods such as PCR, can make detection more reliable (Jeon et al., 2012). Distinguishing liver fluke eggs from the intestinal heterophyid fluke eggs can also be difficult (Ditrich et al., 1992), and molecular methods applicable to egg identification have also been developed (Sato et al., 2009; Sanpool et al., 2012; Armignacco et al., 2008).

2.2 **Environmental Contamination with Eggs**

There are no data on the occurrence of liver fluke eggs in sewage or various types of polluted waters. The number of eggs per gram of human feces can be used to roughly calculate potential egg concentration in sewage. In a study carried out in a focus of clonorchiasis of South Korea, the mean concentration of eggs was reported to be 2.8 x 10³ per gram of human feces, with a range of 12 to 6.6x10³ per gram (Kim et al., 2011). On average, humans excrete approximately 100 grams of feces per person per day and in highly endemic foci of clonorchiasis the prevalence of infection can reach 70%. Eggs could sediment out into the solids, such as sludge or carried along in wet sewage, and if these waste materials are emptied into water bodies (rice fields, ponds, streams, canals, or rivers) that contain suitable snail hosts and fish, the liver fluke life cycle could be sustained.

2.3 **Survival (Persistence) of Eggs in the Environment**

Because of a lack of a desiccation-resistant protective coating, trematode eggs and cercariae are extremely fragile. Limited studies under laboratory conditions have yielded some information on their survival. Faust and Khaw (1927) and Komiya (1966) reported that *C. sinensis* eggs
survived in isotonic solution at 2°C - 4°C for up to 3 months, and at 26°C for up to 1 month. In fresh night soil, the survival time was 2 days at 25°C; survival decreased with age of the night soil. Drozdov (1962) observed survival of O. felineus eggs for 160 days in river water held at 0°C - 5°C. Field research on egg persistence in soil and water under natural environmental conditions is needed. Similarly, studies on the survival of eggs in sewage, sludge, surface water, wastewater and irrigation water are insufficient to draw any conclusions.

3.2 Control of Snails

There are many methods employed or proposed to control snails, including chemical control (molluscicides), physical control (Hoffman, 1970; Khamboonraung et al., 1997), ecological control (Wang et al., 2007), and biological control (Hung et al., 2014). Although these methods are applicable to aquaculture ponds, control of snails in marshes, rivers, reservoirs and rice paddies is impractical and may be environmentally harmful. Many molluscicides, such as copper sulfate (Reddy et al., 2004), endosulfan (Othulid et al., 2004), and bayluscide (Dai et al., 2010), have been used but they have toxic effects on fish, water plants, and small organisms, and in modern aquaculture systems they are either prohibited or discouraged. Biobased molluscicides, including plant components, may have less toxic effects on humans, aquatic animals, and plants, and research on them should be encouraged, especially their cost-effectiveness. Studies on biological control with predatory fish (on snails) such as black carp have recently shown promise, but this approach is still in the early phase of research and development (Hung et al., 2013).

3.3 Sanitation Technologies

There are no data available on removal of the eggs by wastewater treatment. Their small size (average 27 X 15 μm) may account for their ability to escape removal from water by standard filtration systems or sedimentation. Also there are very few recommendations on standard sanitation measures for the control of human liver flukes, the lack of which may be attributed to the unique epidemiology of human infections and the ecology of the liver flukes. As discussed in Section 1.3.2, the liver flukes have a wide range of both domestic and sylvatic reservoir hosts, and surveys of snails and fish for liver fluke metacercariae consistently reveal a major cycling of liver flukes through wild, non-cultured fish residing in lakes, reservoirs, streams, rivers, marshes and rice fields (Sithithaworn et al., 2007b; Thu et al., 2007; Li et al., 2013). In contrast, surveys of fish produced in aquaculture ponds generally reveal low prevalence of C. sinensis, and O. viverrini (Chi et al., 2008; Thien et al., 2007; Li et al., 2013; Chen et al., 2010; Pitaksakulrat et al., 2013), even when the human prevalence of C. sinensis, for example, in a community is high (Dung et al., 2007). Further, the human prevalence of liver flukes in aquaculture systems may be overestimated because of diagnostic confusion between their eggs with those of fishborne intestinal flukes (Heterophyidae) (Ditrich et al., 1992; De et al., 2003; Chi et al., 2008; Thien et al., 2007).

The major snail vectors of liver flukes (e.g., Bythinia spp., Parafossarulus spp.), however, are not common in fish ponds, preferring instead moving water associated with lakes, reservoirs, streams and canals (Petney et al., 2012).

In contrast, the major snail host for the heterophyids, Melanoidies tuberculata, is very common in aquaculture ponds (Dung et al. 2010; Madsen and Hung 2014). Because
most people in endemic areas consume both wild caught and cultured fish, it is difficult to determine the actual source of the infected fish. Importantly, wild fish are readily available in local markets and they are often preferred for raw fish dishes because they are obtained from water that is cleaner and less polluted than that normally found in farm ponds. The importance of wild caught fish in the transmission of liver flukes underscores the difficulty of applying standard sanitation approaches for prevention and control of liver flukes.

3.4 Role for Sanitation Interventions in Aquaculture

When there is evidence that significant transmission from cultured fish is an important source of human infections, there are interventions that can be implemented in aquaculture systems to control fish-borne liver and intestinal flukes. These interventions, developed in extensive field trials (Khambooraung et al., 1997; Clausen et al., 2015), are designed to control fish infections by eliminating egg contamination of the ponds and reducing the snail populations. They require a strong program of farmer education and improvements in management practices and pond infrastructure, as follows:

1. Education:
   - Before initiating the program’s changes to pond infrastructure and management, the farmers should receive training on the basics of the biology and epidemiology of fishborne zoonotic trematodes and the health benefits to themselves and their families for the prescriptive prevention and control interventions.
   - Household members must be encouraged to avoid eating raw fish and to prevent consumption of raw or dead fish by farm animals, including dogs, cats and pigs.

2. Interventions to prevent egg and host fecal contamination of the pond environment require:
   - Modification of pond embankments to prevent surface water run-off from entering the pond by installing a cement barrier at least 10-15 cm above the bank top.
   - Installation of fencing to exclude reservoir hosts, especially cats and dogs, from the immediate pond environment.
   - Prevent discharge into the pond of all waste from latrines and livestock pens.

3. Interventions to prevent and control snails in the fish pond require that:
   - Before restocking the ponds with juvenile fish between the harvests, the pond should be drained and dried completely for at least 5 days. The top 3-5 cm of bottom mud should be removed to a site not adjacent to the pond.
   - All vegetation in ponds should be removed, and a liner (plastic or cement) applied to the banks of the ponds.
   - Aquatic vegetation must be removed at least 3 m from the water intake portal (the inlet for pond water replenishment) and all in-coming water filtered through a 5 mm mesh screen before entering the pond.

4. Additional public health actions for endemic communities. Educate households:
   - on the risk from inadequately prepared fish food;
   - to avoid contaminating water bodies with human and animal waste to the extent possible;
   - on the signs and symptoms of liver fluke infection, and to seek medical treatment when infection is suspected.
The Liver Flukes: Clonorchis sinensis, Opisthorchis spp, and Metorchis spp.

References


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