Chapter 5
Clonorchiasis and Opisthorchiasis

Edoardo Pozio and Maria Angeles Gomez-Morales

Abstract Clonorchiasis and opisthorchiasis are helminthic diseases caused by the liver flukes *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*, respectively. Humans acquire these trematode infections by consuming raw or partially cooked freshwater fish infected with the larval stage metacercariae. More than 45 million people have been estimated to be infected. These infections are prevalent in developing countries and are closely linked to poverty, pollution, and population growth, as well as to cultural food habits and tradition. However, people living in industrialized countries are not exempted to acquire these pathogens due to an increasing consumption of raw fish. Near one third of infected persons are asymptomatic. Besides being the etiological agents of helminthic diseases, *C. sinensis* and *O. viverrini* have been classified as class I carcinogens, since they are the causative agents of cholangiocarcinoma in chronically infected people. The drug of choice is praziquantel. Health education and implementation of food safety measures can prevent infections and morbidity.

5.1 History

An early documentation of clonorchiasis in the human beings dates back some 2,000 years to the Ming and Western Han dynasties. In 1956, *C. sinensis* eggs were detected in desiccated fecal remains from a mummy of the Ming dynasty (about XV century) in the Guangdong province of China. Again in 1975, *C. sinensis* eggs were detected in fecal remains from a corpse buried during the West Han dynasty (206 BC–23 AD) in the Hubei province (Lun et al. 2005). *Clonorchis sinensis* was first discovered in the bile ducts of a Chinese man in India in 1875, and the first autochthonous case was documented in China in 1908 (Lun et al. 2005). The first

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and second intermediate hosts of *C. sinensis* were discovered by two Japanese researchers, Masatomo Muto and Harujiro Kobayashi, in 1918 and in 1912, respectively (Yoshida 2012).

Opisthorchis felineus was described for the first time in cats and dogs in Pisa (Italy) and was referred to as *Distoma felineum* (Rivolta 1884). In 1891, this species was included in the new genus *Opisthorchis* (Blanchard 1895). The first human infections were described as caused by *Distomum sibericum* in the liver of eight persons from Siberia (Winogradoff 1892). The complete life cycle of *O. felineus* was described in Germany in 1934 (Vogel 1934; Schuster 2010).

The third species, *O. viverrini*, collected from the liver of a fishing cat (*Felis viverrinus*, now *Prionailurus viverrinus*), was described at the end of the nineteenth century as *Distoma viverrini* (Poirier 1886), but the life cycle was fully described only in 1965 (Anonymous 2012). The first human case of *O. viverrini* infection was described in 1915 (Leiper 1915).

5.2 The Life Cycle

The natural life cycle is similar among the three liver fluke species. The adult hermaphrodite worms (*C. sinensis*: 10–25 × 3–5 mm; *O. felineus*: 7–12 × 1.5–2.5 mm; *O. viverrini*: 5.5–10 × 0.8–1.6 mm) are dorsoventrally flattened with an anterior oral sucker, a centrally located ventral sucker, and a uterine pore. These worms parasitize mainly the intrahepatic bile ducts and gallbladder and less frequently the extrahepatic and pancreatic ducts of humans and other fish-eating mammals (Fig. 5.1). About 1–2 months after infection, operculate eggs (22–35 × 10–22 μm) containing the larval stage, or miracidium, are shed with feces (Fig. 5.1). An adult worm can produce from 1,000–4,000 eggs per day for at least 6 months, depending on the mammalian host species and worm burden. When the eggs reach a body of freshwater and are ingested by snails of the genus *Bithynia* for *O. felineus* and *O. viverrini* (Pozio et al. 2013; Kiatsopit et al. 2013) or by snails belonging to five families for *C. sinensis* (Lun et al. 2005), they hatch in the gastrointestinal tract of the snail, and the miracidium develops into a sporocyst in the intestinal wall or in other organs to undergo asexual reproduction. The sporocyst produces rediae which mature in the hepatopancreas within about 17 days. The cercariae, about 5–50 per rediae, leave the snail during the day, when it is warm and sunny, approximately 1–2 months after the snail is infected; however, the duration of development in the snail body is strongly influenced by the water temperature. The free-swimming cercariae, which are characterized by a positive photo- and geotropism, have a long tail with a long dorsal and some shorter ventral fins, a finely spined tegument, penetration and cystogenous glands, and a pair of eyespots (Fig. 5.1). They shed their tail, penetrate fish tissue between the scales (mainly near the fins), and encyst as metacercariae under the skin or in the musculature approximately 3 weeks later. In doing so, the carcariae lose their eyespots and develop a saclike excretory bladder filled with coarse, refractile granules. The metacercarial stage is usually

ovoid (140 × 120 μm) with a thin wall (Fig. 5.1). Freshwater fish of the family Cyprinidae act as second intermediate hosts for all the three liver flukes; however, metacercariae of *C. sinensis* have been also detected in other fish families and in crustaceans even if their epidemiological importance in the natural cycle seems to be lower than that of the Cyprinidae fish (Lun et al. 2005; Chen et al. 2010). When infected fish are ingested by mammals, including humans, the metacercariae excyst in the duodenum and the juvenile flukes migrate (within about 30 min) up through the ampulla of Vater and the common bile duct into the intrahepatic bile ducts where they attach to the bile duct epithelium using their suckers. Then, flukes develop into adults after at least 1 month. Adult flukes have been also detected in the duodenum and stomach. In humans, flukes can survive for 20–25 years (Kaewpitoon et al. 2008).

5.3 Taxonomy and Genetic Variability

According to the current taxonomy, *C. sinensis*, *O. felineus*, and *O. viverrini* belong to the phylum Platyhelminthes, class Trematoda, subclass Digenea, order Plagiorchiida, and family Opisthorchiidae (Mordvinov and Furman 2010). The genetic variability of the three liver flukes has been investigated at the inter- and intraspecies level with differences according to the target sequences (CO1, CO3, mDNA, ITS1, and ITS2) (Kang et al. 2008; Saijuntha et al. 2008; Liu et al. 2012; Brusentsov et al. 2013). Different populations were identified in each fluke species according to the region of origin, but the genetic differences were in general low. The study of microsatellite markers of *O. viverrini* allowed to identify different populations in Lao People' s Democratic Republic (PDR) and Thailand (Laoprom et al. 2010, 2012).

5.4 Geographical Distribution

Large foci of *O. felineus* are present in the European Russia, Kazakhstan, and West Siberia (Mordvinov et al. 2012). In central, southern, and western Europe, *O. felineus* has been detected as isolated foci in Belarus, Ukraine, and in 13 countries of the European Union (EU) (Pozio et al. 2013) (Fig. 5.2, panel A).

C. sinensis is circulating in almost all of the Chinese provinces (excluding eight northern and western provinces or autonomous regions), North and South Korea, Taiwan, northern Vietnam (Lun et al. 2005), the Amur basin in East Russia (Hong and Fang 2012), and Central Thailand (Traub et al. 2009) (Fig. 5.2, panel B).

O. viverrini is endemic in Cambodia, Lao PDR (mainly southern areas), Thailand (mainly northeast areas), and southern Vietnam (Andrews et al. 2008; Sohn et al. 2011, 2012; Yong et al. 2012) (Fig. 5.2, panel B).

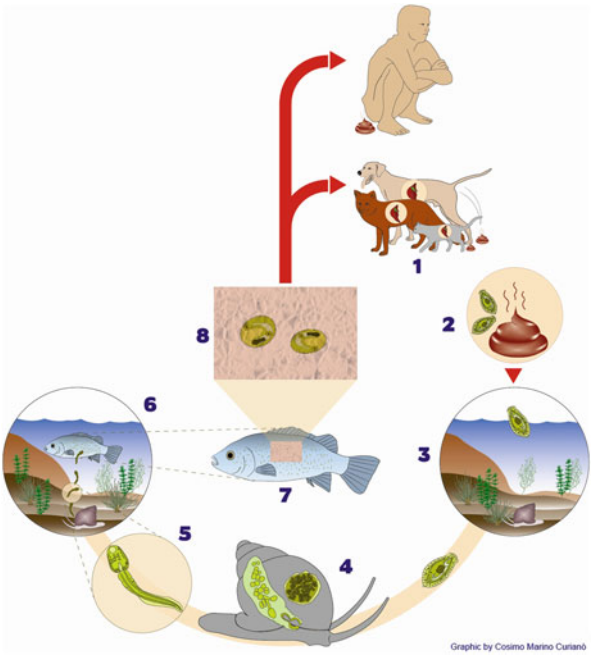

Fig. 5.1 The natural life cycle of *Clonorchis sinensis*, *Opisthorchis felineus*, and *O. viverrini*

5.5 Intermediate Hosts

The prevalence of infection of gastropoda molluscus with the larval stages of liver flukes (sporocyst and rediae) is, in general, low (<0.1 %), even in highly endemic areas; however, a prevalence of up to 27 % has been documented in some Chinese foci of *C. sinensis* (Lun et al. 2005). An increasing number of reports suggest that the genetic diversity of the gastropoda molluscus that act as intermediate hosts of liver flukes is higher than expected (Lazuthina et al. 2009; Mordvinov et al. 2012). The snail populations show strong seasonality due to the temperature and rainfall variations (Brockelman et al. 1986).

A broad spectrum of mollusc species belonging to five families (Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, and Thiaridae) acts as first intermediate host for *C. sinensis* (Lun et al. 2005). Three mollusc species of the genus *Bithynia* (*B. inflata*, *B. leachi*, and *B. troscheli*) play the role of first intermediate hosts for *O. felineus* (Erhardt et al. 1962; Hering-Hagenbeck and Schuster 1996; Lazuthina et al. 2009; Mordvinov et al. 2012). Larval stages of *O. viverrini* have been detected in *Bithynia funiculata* and in two subspecies of *B. siamensis* (*B. siamensis siamensis* and *B. siamensis goniomphalos*) (Petney et al. 2012; Sithithaworn et al. 2012).

The number of fish species as well as the prevalence and intensity of infection is much higher than that of the first intermediate hosts. Fish species acting as second intermediate host of *C. sinensis*, *O. felineus*, and *O. viverrini* belong prevalently to

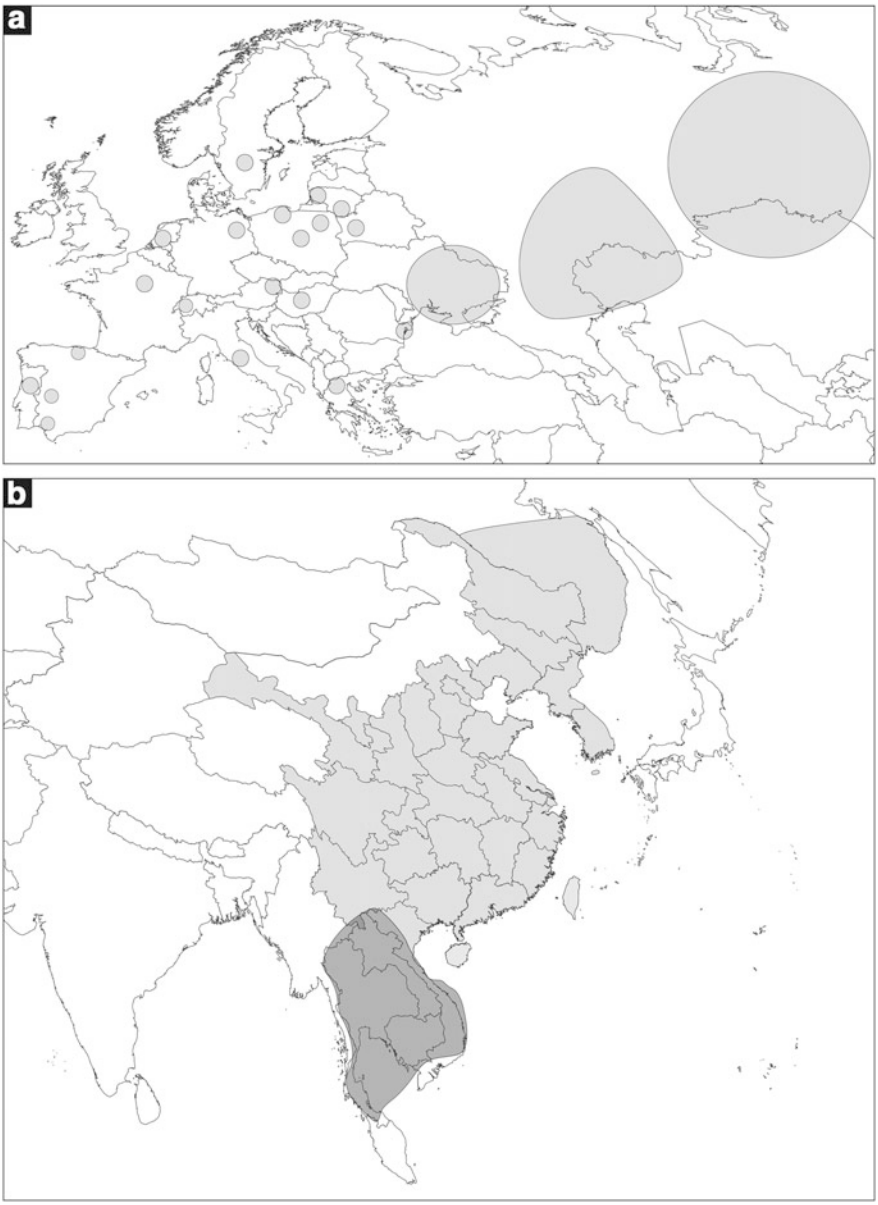

Fig. 5.2 Distribution areas of *Clonorchis sinensis*, *Opisthorchis felineus*, and *Opisthorchis viverrini*. Panel A, *O. felineus* distribution area. Panel B, *C. sinensis* distribution area (*light gray*); *O. viverrini* distribution area (*dark gray*)

the family Cyprinidae. According to Lun et al. (2005), some additional 60 fish species not belonging to the family Cyprinidae can host *C. sinensis* metacercariae. A prevalence of *C. sinensis* infection of 80 % and 95 % has been detected in *Parabramis pekinensis* and in *Abbottina sinensis*, respectively (Lun et al. 2005). In the Mekong area, *O. viverrini* metacercariae have been isolated from 40 fish species of 18 genera of the family Cyprinidae (Sithithaworn et al. 2007). In Europe, *O. felineus* metacercariae have been detected in *Alburnus alburnus*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Idus idus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, and *Tinca tinca* with prevalences up to 95 % (De Liberato et al. 2011; Pozio et al. 2013). The most important second intermediate hosts of *O. viverrini* are cyprinoid fish of the genera *Cyclocheilichthys*, *Hampala*, and *Puntius* (Wykoff et al. 1965).

The number of metacercariae in fish varies by season, species, and physical and biological parameters of the water bodies (Sithithaworn et al. 2007). The metacercarial burden of *C. sinensis*, *O. felineus*, and *O. viverrini* peaks in spring and summer, summer and autumn, and winter, respectively (Sithithaworn et al. 2007; De Liberato et al. 2011). The number of metacercariae per fish ranges from one to hundreds, but over 30,000 parasites have been detected per fish with more than 6,000 metacercariae/g depending on species and biological, ecological, and epidemiological circumstances (Chen et al. 1994). However, a fish can harbor metacercariae of several zoonotic and non-zoonotic fluke species (both liver and intestinal flukes); therefore, metacercariae should be carefully identified by morphology or molecular methods.

5.6 Final Hosts

All fish-eating mammals, including humans, can act as final hosts of *C. sinensis*, *O. felineus*, and *O. viverrini* (Table 5.1), but their role as reservoir hosts is strongly influenced by biological, ecological, and epidemiological factors, including the human impact on fishing. Humans are the main final hosts for *C. sinensis*, *O. viverrini*, and *O. felineus* in Siberian foci (Keiser and Utzinger 2009; Mordvinov and Furman 2010; Mordvinov et al. 2012), whereas domestic and wild carnivore animals are the main final hosts of *O. felineus* in European foci (Pozio et al. 2013). However, in *C. sinensis*, *O. viverrini*, and *O. felineus* foci where humans play the most important role of reservoir, the role of animals should be considered. In fact, if fecal contamination from humans is stopped by mass treatment and proper sanitation, animals may maintain the natural cycle at a hypo-endemic level.

Table 5.1 Epidemiological pattern of the etiological agents of opisthorchiasis and clonorchiasis

Etiological agent	Distribution	Main hosts species	References
Clonorchis sinensis	China, Korea, Taiwan, Vietnam, Thailand	I intermediate hosts Gasteropods of the families: Assimineidae, Bithyniidae, Hydrobiidae, Melaniidae, Thiaridae	Lun et al. (2005) Traub et al. (2009)
		II intermediate hosts 132 species	Lun et al. (2005)
		Final hosts Human, dog, cat, pig, rat	Lun et al. (2005)
Opisthorchis felineus	Belarus, Russia, West Siberia, Ukraine, Austria, France, Germany, Greece, Hungary, Italy, Lithuania, the Netherlands, Poland, Portugal, Romania, Spain, Scandinavia	I intermediate hosts *Bithynia inflata*, *B. leachi*, *B. troscheli*	Erhardt et al. (1962) Hering-Hagenbeck and Schuster (1996)
		II intermediate hosts *Alburnus alburnus*, *Abramis brama*, *A. ballerus*, *Blicca bjoerkna*, *Idus idus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*	Lazuthina et al. (2009) Mordvinov and Furman (2010)
		Final hosts Human, dog, cat, fox (3 species), seal (3 species), wolf, mustelids (11 species), raccoon dog, raccoon, wild boar, rodent (4 species)	Mordvinov et al. (2012) Pozio et al. (2013)
Opisthorchis viverrini	Cambodia, Lao PDR, Thailand, Vietnam	I intermediate hosts *Bithynia funiculate*, *B. siamensis siamensis*, *B. siamensis goniomphalos*	Andrews et al. (2008), Petney et al. (2012)
		II intermediate hosts *Cyclocheilichthys*, *Hampala*, *Puntius*	Sithithaworn et al. (2012)
		Final hosts Human, dog, cat	Wykoff et al. (1965)

5.7 Epidemiology

Over 10 % of world inhabitants, i.e., 750 million people, was estimated to be at risk of foodborne trematodiases (WHO 1995). According to Sithithaworn et al. (2007), 35 million people are infected with *C. sinensis*, and of them, 15 million are Chinese people (Lun et al. 2005). In Southeast Asia, 67 million people were estimated to be at risk of *O. viverrini* infection (Keiser and Utzinger 2005). In 2003, Sithithaworn and Haswell-Elkins estimated a prevalence of *O. viverrini* infection in the Mekong region of eight million in Thailand and two million in Laos. No prevalence data are

available in Vietnam even if the presence of *O. viverrini* infection in humans has been documented (Sithithaworn et al. 2006; Sayasone et al. 2007). In Cambodia, a high endemicity of *O. viverrini* infections in humans has been documented. In a southern area of the country, the prevalence was assessed as high as almost 50 % among the surveyed people (Yong et al. 2012). The number of *O. felineus* infections in humans has been estimated to be 1.2 million (WHO 1995).

The transmission patterns of the three liver flukes are substantially different. In most of *C. sinensis* foci, both humans and domestic and wild fish-eating mammals play the role of final hosts, i.e., a zoonotic and an anthroponotic cycle occur concurrently. In China, the prevalence of human clonorchiasis reaches 16.4 % and 9.8 % in the Guangdong and Guangxi provinces, respectively (Hong and Fang 2012). *C. sinensis* has been detected in cats, dogs, and pigs with a prevalence of up to 100 %, 100 %, and 25 %, respectively (Lun et al. 2005). There are also some reports on *C. sinensis* in cattle and rats (up to 14 %) (Lun et al. 2005). This liver fluke was present in three localities of southern Taiwan (Rim 2005); however, no recent information is available on its prevalence in the human population of Taiwan. In Korea, a prevalence rate of 2.9 % of *C. sinensis* in the general population was recorded, with an estimated prevalence of 1.3 million people with clonorchiasis (Kim et al. 2009). The Amur river basin in Eastern Russia is also an endemic area for *C. sinensis* where 1 million people are estimated to be infected (Figurnov et al. 2002). In North Vietnam, 1 million people were estimated to be infected (Dang et al. 2008). Globally, 1.5–2 million people infected with *C. sinensis* are symptomatic and 10 % of them are heavily infected with complications (Hong and Fang 2012).

In *O. viverrini* foci, humans play an important role as final host and the biomass of parasites present in fish-eating mammals was considered to be low when compared to that in humans living in the same areas. However, a recent investigation shows that cats are the most important animal reservoir of human opisthorchiasis in Northeast Thailand with a prevalence of infection of 35.51 % (Aunpromma et al. 2012). In this region, the prevalence of human opisthorchiasis (16.6 %) is the highest in the world, and it is coincident with the highest incidence of cholangiocarcinoma (CCA) (Sithithaworn et al. 2012; Sripa et al. 2012).

Opisthorchis felineus shows two well-distinct transmission patterns in the EU, Eastern Europe (Byelorussia, Russia, and Ukraine), and Siberia. In the EU, the cycle is typically zoonotic with domestic (cats and dogs) and wild fish-eating mammals (red foxes) playing the most important role of definitive hosts. Human infections are sporadic (five cases in Germany and two cases in Greece in the last 50 years) with few exceptions related to a change in food behavior as observed in Italy in the last 10 years, where more than 200 cases were documented (Pozio et al. 2013). Some of the people who acquired opisthorchiasis in Italy were tourists who developed the disease when they returned home in Austria and the Netherlands (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.

5.8 Pathology and Pathogenesis

The magnitude of the pathology caused by these parasites depends on their number, duration of the infection, and susceptibility of the host (Sripa 2003). Pathological features of liver fluke infections have been reported both in humans and in experimental animal models.

5.8.1 Clonorchis sinensis

In light *C. sinensis* human infections, the liver appears normal, whereas in massive infections, a localized dilation of the thickened peripheral bile ducts can be seen on the surface beneath the Glisson's capsule (Rim 2005). The intraductal flukes cause a mechanical injury by their feeding and migrating activities, contributing to the biliary damage (Fig. 5.3, panel A). Both oral and ventral suckers of the fluke hook up the biliary epithelium, resulting in tissue damage, which starts by the formation of an edema followed by tissue desquamation mainly in the areas close to the flukes. Periductal infiltrates of mononuclear cells are frequently found; however, inflammation of the bile duct walls is slight in uncomplicated cases. On the contrary, in chronic infections, epithelial cells proliferate with metaplasias of the biliary epithelial cells into mucin-producing cells. Goblet cells can proliferate to produce many small gland-like structures in the mucosa (adenomatous hyperplasia), leading to an excessively high mucus content in the bile which, in combination with the presence of eggs and worm fragments, causes cholestasis and serves as support for bacterial superinfection and intrahepatic stone formation (cholelithiasis) (Lim 2011). Secondary bacterial infections, mainly of enteric origin, may occur, and *Escherichia coli* is the pathogen most frequently identified. These alterations may progress to a pyogenic cholangitis, liver abscess, and hepatitis (Rim 2005). Persistent infections result in varying degrees of periductal fibrosis. These changes are distinctive features of clonorchiasis; therefore, when they are observed in patients in an area of endemic infection, they are suggestive of infection (Sithithaworn et al. 2007). As the fibrosis proceeds, the epithelia proliferation appears milder and fecal egg production drops markedly. The cholelithiasis is one of the most serious complications that can lead to the biliary obstruction (Sithithaworn et al. 2007).

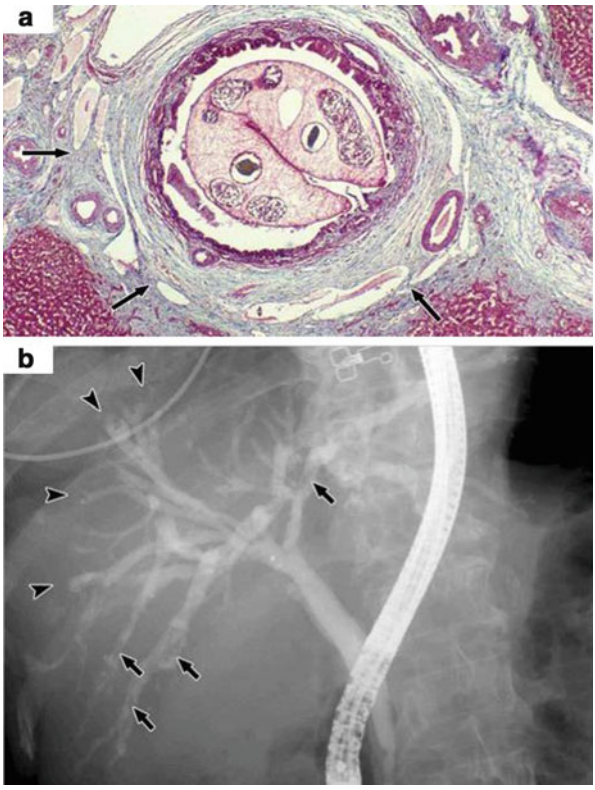

Fig. 5.3 (**a**) Photomicrograph of a pathologic specimen shows an adult fluke of *Clonorchis sinensis* in an intrahepatic bile duct. Note adenomatous hyperplasia of mucosa and severe fibrous thickening of bile duct wall (*arrows*). Masson's trichrome stain X 40. (**b**) Endoscopic retrograde cholangiogram shows innumerable elongated or elliptic small filling defects, indicating adult *C. sinensis* (*arrows*) in peripheral small branches of bile ducts. Many peripheral bile ducts are occluded by flukes (*arrowheads*) (From Lim et al. 2007, with permission)

5.8.2 Opisthorchis viverrini

In the early stage of light infections caused by *O. viverrini*, no detectable changes in biliary epithelium and periductal areas of the liver can be found. However, in community-based studies in Northeast Thailand, using ultrasonography and cholecystography, an increase in the frequency and severity of the gallbladder disease has been demonstrated, specifically wall irregularity, enlargement, and bile sludge, among apparently healthy individuals with a moderate *O. viverrini* infection (Elkins et al. 1996; Mairiang et al. 1992). In heavy *O. viverrini* infections, the liver may be enlarged and its weight may be more than double the normal (3,000–3,500 g or more). In the liver, the predominant changes are desquamation of the biliary epithelium, epithelial hyperplasia, bile duct hyperplasia, and periductal fibrosis (Riganti et al. 1989; Sripa et al. 2012). The adult flukes may be seen in the gallbladder, common bile duct, and pancreatic duct. In the large and medium-size bile ducts, the flukes can cause chronic cholecystitis. In case of a superimposed bacterial infection, empyema of the gallbladder may exist. Cholelithiasis is not particularly frequent in *O. viverrini* infections; however, biliary sludge is often seen in heavy infections. The enlargement of the gallbladder is commonly found either

in autopsy (Riganti et al. 1989) or ultrasonographic studies (Mairiang et al. 1992, 2012). Following antihelmintic treatment, many of the gallbladder abnormalities can be eliminated, as indicated by the reduction of the length and regained contractility (Mairiang et al. 1993). Granulomatous inflammation around the parasite eggs is occasionally seen in the gallbladder wall during *O. viverrini* infections (Riganti et al. 1989).

5.8.3 Opisthorchis felineus

In *O. felineus* human infections, pathological changes seem to be similar to those induced by the others liver flukes; however, the information is scarce. In patients with heavy or prolonged infections or superinvasion, 84 % develop duodenal hypertension, 94 % gastric hypertension, and 75 % duodenogastric reflux with formation of chronic gastritis (Suvorov et al. 2004). Reflux of gastric contents into the esophagus causes chronic esophagitis. Moreover, regurgitation of intestinal contents into the pancreatic duct is a cause of chronic indurative pancreatitis of the head of the gland. In cases of duodenal hypertension, the rates of pancreatic *O. felineus* invasion are as high as 93.7 % (Suvorov et al. 2004). Sonographic studies carried out in *O. felineus*-infected people have evidenced disturbances in the gallbladder ranging from dyskinesia to cholestatic syndrome; the most profound abnormalities in the indices were seen in the early phase of the disease (Bronshteĭn et al. 1989). Unspecific findings by an abdominal ultrasonography scan and multiple hypodense nodules with hyperenhancement in the arterial phase by a computed tomography have been recently reported in some patients. In a patient who underwent liver biopsy, acute inflammatory signs with dilatation of portal spaces and eosinophilic infiltration with lymphocytes and monocytes were found (Traverso et al. 2012).

5.8.4 Associated Pathologies

Renal alterations have been described in human infected with *O. viverrini* (Boonpucknavig and Soontornniyomkij 2003) and *O. felineus* (Lapteva 1990). From a series of 113 cases of nephropathy that coincided with chronic opisthorchiasis caused by *O. felineus*, Lapteva (1990) detected signs of renal lesions: nephritis, pyelonephritis, dyskinesia of the urinary system, a tendency to right-sided lesion, involvement of interstitium, and chronic renal failure. An acute renal failure in obstructive jaundice due to CCA, which is associated with opisthorchiasis caused by *O. viverrini* in Thailand, was observed in nearly all patients (Mairiang et al. 1992).

The lesions induced by *C. sinensis* and *O. viverrini* enhance the susceptibility of DNA to carcinogens. The association between CCA and liver flukes has been

observed since approximately 60 years (Viranuvatti et al. 1955). The International Agency for Research on Cancer (IARC) has considered *O. viverrini* and *C. sinensis* as group 1 carcinogen agents and *O. felineus* as group III (IARC 1994; Bouvard et al. 2009). Chronic *O. viverrini* infection and CCA have been considered to be the strongest association between a parasitic infection and cancer based on the epidemiological data collected from South Asia (Aunpromma et al. 2012; Sripa et al 2012).

5.8.5 Carcinogenesis Induced by Liver Flukes

Infection in hamsters with *O. viverrini* closely mimics the carcinogenic processes in humans. This process starts by phase 1 which is characterized by edema and desquamation of the bile duct epithelium, followed by epithelial hyperplasia, pseudostratification of the biliary epithelium, and mucin-secreting cell metaplasia. During phase 2, metaplastic squamous cells appear in conjunction with glandular proliferation, periductal infiltrates composed of plasma cells, lymphocytes, and other mononuclear cell types, producing high levels of pro-inflammatory cytokines. In phase 3, the final phase (>12 weeks), the now chronically inflamed biliary tree shows advancing fibrosis along its length. Periductal fibrosis is considered the precursor event to CCA and, similarly to human opisthorchiasis, progression of infection to CCA is accelerated by the inclusion of dietary nitrosamines (Sripa et al. 2007, 2012).

Epidemiological studies revealed that current or past *C. sinensis* infections are the major risk factor of intrahepatic CCA (Honjo et al. 2005; Choi et al. 2006). Moreover, the highest risk factor is the elevated serological positivity associated to the host genetic polymorphism of glutathione S-transferase mu 1 (GSTM1) gene. Additional risk factors were the area of residence, alcohol consumption, age (older than 60), sex (male), smoking, and consumption of fermented raw fish (Fried et al. 2011).

There are two premalignant lesions in cholangiocarcinogenesis: biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the bile duct (Zen et al. 2006). Even if an understanding of the mechanisms leading from liver fluke infection to CCA is not complete, the general mechanisms proposed to contribute to CCA through chronic infection are mainly mechanical damages to the biliary epithelia (see Sect. 5.9), toxic effects of parasite excretory/secretory (ES) products, and the immunopathology due to infection-related inflammation (Sripa et al. 2012).

5.8.6 Toxic Effects of Parasite Excretory/Secretory Products

The fluke secretes or excretes several metabolic products from the tegument and excretory opening into the bile (or in a culture medium), some of which are highly immunogenic (Sripa and Kaewkes 2000; Wongratanacheewin et al. 2003). Apart from inducing host immune response, the metabolic ES products may be toxic to or may interact with the biliary epithelium. Experimental studies clearly indicate that ES products of *O. viverrini* can induce cell proliferation which corresponds to hyperplasia of biliary epithelial cells in opisthorchiasis (Sripa and Kaewkes 2000). To understand the cellular response to *O. viverrini* ES products, gene expression analysis of NIH-3 T3 non-contact co-cultured fibroblasts with *O. viverrini* adults was compared with that without *O. viverrini* ES product treatment. Among all genes, 885 genes showed upregulation of twofold or more after stimulation by *O. viverrini* ES products. Among these genes, 239 had cell proliferation-related functions. The TGF-β and EGF signal transduction pathways have been indicated as the possible pathways of *O. viverrini*-driven cell proliferation (Thuwajit et al. 2006). Recently, the expression of a protein kinase implicated in coordination of membrane cytoskeleton events which can control the reattachment, migration, and invasion of the CCA cells has been demonstrated (Techasen et al. 2012).

O. viverrini ES products comprise a complex mixture of proteins, some of which have homologs in the human host that are associated with cancer, including proteases, protease inhibitors, orthologs of mammalian growth factors, and anti-apoptotic proteins. A protein (Ov-GRN-1) with a sequence similar to that of the mammalian growth factor, granulin, has been identified in ES products from *O. viverrini*. It is the only helminth-derived growth factor reported to date that causes proliferation of mammalian cells (Smout et al. 2009, 2011) and can bind to biliary epithelial cells of *O. viverrini*-infected hamsters (Sripa et al. 2012). The human pro-GRN (PGRN) is overexpressed in many human tumors and stimulates angiogenesis, suppresses apoptosis, and promotes tumor invasion and anchorage independence, all of which support tumor expansion in an unfavorable interstitial environment (Monami et al. 2006; Frampton et al. 2012). ES products from *C. sinensis*, as well as from *O. viverrini*, induce the proliferation of human embryonic kidney cells via regulation of the transcription factor E2F1 (Kim et al. 2008).

5.8.7 Immunopathology Due to Infection-Related Inflammation

During liver fluke infections, macrophages, mast cells, eosinophils, epithelial cells, and neutrophils that infiltrate the sites of inflammation activated by the parasite-specific T cells and cytokines synthesize nitric oxide (NO) (Haswell-Elkins et al. 1994). Further, NO reacts with superoxide anion (O_2^-) to form peroxynitrite,

a highly reactive species that causes nitrative and oxidative DNA damage to the cells. In fact, an increased level of urinary nitrates and salivary nitrites in *O. viverrini*-infected subjects in Northeast Thailand has been reported; the concentration of these substances decreased following treatment with praziquantel (Haswell-Elkins et al. 1994). Moreover, a tenfold greater potential for endogenous nitrosation among people living in endemic areas with positive antibody titers for *O. viverrini* as compared to uninfected controls has been demonstrated (Pinlaor et al. 2005, 2006). Nitrosamines play a very important role in inflammation-associated carcinogenesis, especially if they are generated in situ and their production is both chronic and located in close proximity to cells containing P450 enzymes which can metabolize the nitrosamine to DNA methylating agents. The nearby biliary epithelium may be highly susceptible to malignant transformation due to chronic proliferation, which is another pathologic response to infection. This combination of events could explain the very high risk of CCA associated with liver fluke infection (Satarung et al. 1998).

Increased levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-alpha and oxidative stress response enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), encoded by genes activated by the transcription factor NF-kappaB (NF-κB), are also involved in the inflammatory processes (Hussain et al. 2003; Karin and Greten 2005).

Mediators produced from the inflammatory cells, such as reactive oxygen species, are also toxic to the fluke. Despite this potent oxidative assault, *O. viverrini* can survive in infected hosts for many years. Recently, a thioredoxin peroxidase (Ov-TPx-1), an antioxidant enzyme, has been identified and characterized from *O. viverrini* and suggested to be the main enzyme that protects the parasite from reactive oxygen species produced by host effectors' cells (Suttiprapa et al. 2008, 2012).

Even if no association has been reported for *O. felineus* and CCA, epidemiological and clinical data in humans and animals suggest that *O. felineus* can be the cause of neoplasia (Pozio et al. 2013). In Russia, the highest incidence of bile duct cancer in humans was documented in the same area (i.e., Tyumen Oblast) with the highest prevalence of *O. felineus* infection in humans (Mordvinov et al. 2012). In Italy, precancerous or cancerous lesions have been found during autopsy in *O. felineus* naturally infected animals, in particular cats and dogs (Pozio et al 2013).

5.9 Immune Response

5.9.1 Opisthorchis viverrini

O. viverrini induces a strong antibody response to somatic and ES antigens in the human host, mainly constituted by specific IgG, IgA, and IgE, with IgG being the predominant subclass followed by IgA and IgE . The specific immune response is

detectable in serum and bile and it comes along with a marked increase of total IgE in serum (Wongratanacheewin et al. 1988). There is a significant positive correlation of the IgG antibody titer and the severity of disease (Haswell-Elkins et al. 1991; Pinlaor et al. 2012). In infected individuals, the level of serum antibodies decreased slowly but remained elevated for several months after the praziquantel treatment. This can be due to a long-lasting immunological memory or to new antigenic stimulations by reinfections with the same or other parasites (cross-reacting) (Ruangkunaporn et al. 1994).

The fact that many individuals from endemic areas can harbor many parasites (Haswell-Elkins et al. 1994) suggests than reinfection occurs, in spite of the strong cellular and humoral immune response elicited by the parasites during the primary infection, as it has been demonstrated in hamsters (Wongratanacheewin et al. 1991). The role of T cells and cytokines in immunity and pathogenesis of opisthorchiasis is not well known. The involvement of T cells in pathogenesis is supported by the fact that T-cell-deprived *O. viverrini*-infected hamsters show less severe damage of the bile duct tract (Flavell and Flavell 1986). In experimentally infected hamsters, the parasite stimulates the expression of the Th1-inducing cytokine, IL-12, in the early stage of infection (2 weeks post infection), whereas the expression of the Th2-inducing cytokine, IL-4, and the regulatory cytokines, TGF-β and IL-10, are significantly increased in chronic and/or heavy infections (Jittimanee et al. 2007). It has been hypothesized that the high level of expression of these regulatory cytokines following *O. viverrini* infection may play an important role in the disease process by inhibiting T-cell proliferation that in turn leads to prolonged worm survival (Maizels and Yazdanbakhsh 2003).

The antigenic recognition molecules of T and B cells have been found to be different (Wongratanacheewin et al. 2003). Reinfection with metacercariae elicits high levels of IgG.

Resistance to *O. viverrini* challenge in hamsters can be induced only in animals harboring a low number of worms (Flavell 1982). The lack of a protective immune response can be related to the suppression caused by the parasite, which can be abolished by antihelmintic treatment (Wongratanacheewin et al. 1987).

5.9.2 Clonorchis sinensis

Humans are susceptible to infection, reinfection, and superinfection by *C. sinensis* (Hong and Fang 2012). However, rats, mice, and rabbits resist against reinfection and superinfection of *C. sinensis* (Sohn et al. 2006). Only a few worms survive as small and immature forms in the bile duct of reinfected or superinfected rats; however, the rat resistance was not observed in immune-suppressed or nude rats (Zhang et al. 2008a, b). Specific antibodies to *C. sinensis* are produced in serum and bile, mainly IgE in serum and IgA in bile, and their levels correlated with resistance in rats (Zhang et al. 2008a, b). The immune response to *C. sinensis* is of the Th2 type as confirmed by the significant production of IgG1, IL-4, and IgE in rats

(Wang et al. 2009). It has been demonstrated that *C. sinensis*-derived total proteins can suppress the development of allergen-specific asthma by induction of an increase in the number of CD4 + CD25 + Foxp3+ Treg cells, which maintain immune response homeostasis and interfere with the priming of naïve T cells by airway dendritic cells (Jeong et al. 2011).

5.9.3 Opisthorchis felineus

As other liver flukes, *O. felineus* elicits a humoral immune response already detectable from the third week after infection (Armignacco et al 2008, 2013; Traverso et al. 2012). The main antigens recognized by human antibodies have been associated to the tegument, muscles, uterus, gonads, intestine, and eggs of the liver fluke, as showed by immune electron microscopy. These findings have led to the conclusion that the surface structures of liver flukes stimulate a low B-cell immune response, whereas the structures linked to the ES system of the parasite and their products contain main antigens able to induce B-immune response in man (Kotelkin et al. 2001).

5.10 Clinical Manifestations

The types of clinical diseases caused by liver fluke infections seem to vary. Most of the reported *O. viverrini* and *C. sinensis* infections are dormant and the infected people are asymptomatic, except for the patients with very heavy infections and for those presenting complications. On the contrary, there are many reports detailing specific signs and symptoms accompanying well-defined clinical stages of the *O. felineus* infection, in which acute infections are frequently reported (Bronshteĭn et al. 1989; Lim 2011).

5.10.1 Opisthorchis viverrini

In chronic infections, an increased frequency of hepatomegaly, as revealed by community studies based on physical examination, can be found (Mairiang et al. 2012). Hematological and liver function tests are generally unremarkable, regardless of infection intensity; instead, ultrasound examinations have shown high frequencies of left lobe liver and gallbladder enlargement, sludge and stones in the gallbladder, and poor hepatobiliary function. In patients with a severe infection, the clinical signs and symptoms include lassitude, hepatomegaly, and nonspecific abdominal complaints such as anorexia, nausea, vomiting, abdominal discomfort, diarrhea, indigestion, weight loss, ascites, and edema (Furst et al. 2012). Jaundice is

due to the mechanical obstruction caused by large number of flukes in the bile ducts in patients with a heavy infection, or it is due to bile duct obstruction caused by stone, cholangitis, or CCA as a late complication of chronic infection (Lim 2011).

There is no report of acute *O. viverrini* infections. Most subjects with opisthorchiasis have nonspecific symptoms or no symptoms at all. Mild hepatomegaly occurs in 14 % of the heavily infected persons (egg counts > 10,000/g). Enlargement of the gallbladder is only detected by ultrasonography and is reversed after elimination of flukes by praziquantel (Mairiang et al. 1993). Intrahepatic duct stones and recurrent suppurative cholangitis is not a common manifestation of opisthorchiasis caused by *O. viverrini* but can be present. Since jaundice is the main clinical manifestation of the CCA, whenever jaundice and ascending cholangitis are detected in endemic areas, the fluke-related CCA is suggested (Uttaravichien et al. 1999). Obstructive jaundice can be presented alone, with fever or with acute abdominal complications, such as cholangitis, acalculous cholecystitis, and generalized bile peritonitis (Uttaravichien et al. 1999). Non-jaundiced patients may present dyspeptic pain, anorexia, weight loss, and right upper abdominal mass (Chunlertrith et al. 1992).

5.10.2 Clonorchis sinensis

In chronic infections, an increased frequency of hepatomegaly, as revealed by community studies based on physical examination, can be found (Choi et al. 2005). The clinical manifestations of clonorchiasis tend to reflect the worm burden. Most patients with mild infections, i.e., with fewer than 100 flukes, have few symptoms. Early symptoms may include general malaise, abdominal discomfort, and diarrhea. In 10–40 % of patients, peripheral eosinophilia accompanies a fluctuating jaundice that is usually obstructive. Moderate infection (generally fewer than 1,000 flukes) presents with fever and chills, as well as fatigue, anorexia, diarrhea, weight loss, discomfort, and abdominal distension. Up to 20,000 flukes may be present in patients with severe disease, who present with acute right upper quadrant pain, often superimposed on the signs and symptoms seen in moderate infections. In the late stage of severe cases, jaundice, diarrhea, portal hypertension, hepatosplenomegaly, ascites, and edema can occur. Pyogenic cholangitis, cholelithiasis, chronic cholecystitis, pancreatitis, and CCA have been described as potential long-term complications of clonorchiasis. Many hepatic and biliary diseases can mimic clonorchiasis in their clinical presentation. Differential diagnoses of clonorchiasis include acute or chronic hepatitis, cancer along the bile ducts, hepatocholedocholithiasis with recurrent pyogenic cholangitis, sclerosing cholangitis, Caroli's disease, and *Fasciola hepatica* infection (Choi et al. 2006; Keiser and Utzinger 2009).

5.10.3 Opisthorchis felineus

The clinical manifestations caused by *O. felineus* during the acute stages of the infection in humans are characterized by fever, abdominal pain, headache, asthenia, arthralgia, lymphadenopathy, skin rash, diarrhea, nausea, hepatitis-like symptoms, eosinophilia, and increased liver enzymes (Mairiang and Mairiang 2003; Mordvinov and Furman 2010; Traverso et al. 2012; Pozio et al. 2013). Acute opisthorchiasis occurs early in infection and may be associated with primary exposure to a large dose of metacercariae (Furst et al. 2012). These clinical features may lead to misdiagnosis as acute viral hepatitis (Belova et al. 1981) and rheumatic disease (Gordon et al. 1984). In endemic regions such as Ukraine, Russia, and Siberia, where people frequently consume raw fish, the number of worms in the bile ducts can be very high, inducing chronic infection, which is characterized by anorexia, dyspepsia, dryness and bitter taste in the mouth, fatigue, intolerability to greasy foods, nausea, and pain in the right hypochondrium (Mordvinov and Furman 2010). Other frequently reported symptoms include cholecystitis, duodenitis, and pancreatitis. In persons with a high-worm burden, chronic infection can become severe, being characterized by acute pancreatitis, bile peritonitis, hepatic abscesses, obstruction of bile ducts with jaundice, and recurrent cholangitis (Mordvinov and Furman 2010). In non-endemic areas, such as EU countries, most infected persons show pauci-symptomatic or asymptomatic forms, and in some cases, clinical disease and the seroconversion can develop up to 2 months after infection. In Italy, about 1/3 of infections was asymptomatic (Pozio et al. 2013). In symptomatic persons, during the acute stage, the more frequently observed signs and symptoms were asthenia, headache, abdominal pain, and fever, which started about 2–3 weeks after the infection (Armignacco et al. 2008; Traverso et al. 2012; Pozio et al. 2013). Jaundice was not observed. The main laboratory findings were leukocytosis, eosinophilia, and increased transaminases, e.g., aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase (Armignacco et al. 2008; Traverso et al. 2012). In an outbreak in Italy, in persons not diagnosed during the acute phase and then not treated, there was a spontaneous remission of the clinical symptoms within 2–3 months of infection, although the liver flukes were still present and produced eggs (Armignacco et al. 2013).

5.11 Diagnosis

In non-endemic areas, liver fluke infections are very difficult to diagnose because of the lack of pathognomonic signs and symptoms and the decreasing number of professionals able to identify opisthorchiid eggs in stool samples (Yossepowitch et al. 2004; Pozio et al. 2013). Moreover, in some cases the clinical disease and the seroconversion can develop up to 2 months after infection (Pozio et al. 2013). In endemic areas, the presence of signs revealing injury of the bile ducts by ultrasound

or other imaging techniques is suggestive of infection (Sithithaworn et al. 2007) (Fig. 5.3, panel B). However, in any case, the clinical diagnosis of liver flukes infections should be confirmed by the detection of eggs in stools.

5.11.1 Parasitological Diagnosis

Fecal examinations by the Kato-Katz (KK), the formalin–ether concentration technique (FECT), and Stoll's dilution egg count methods have been frequently used for diagnosis of liver fluke infections. However, FECT seems more sensitive than the other methods, especially for the diagnosis of extremely low burden infections and for the follow-up examination after treatment (Sithithaworn et al. 2007). The KK and FECT methods are commonly used for mass screening in endemic areas for *O. viverrini* and *C. sinensis*, and both are considered to have a comparable sensitivity and reliability (Hong and Fang 2012; Sithithaworn et al 2007). Stoll's dilution egg count method presents a detection rate slightly inferior to KK but is believed to be suitable for the measurement of the intensity of *O. viverrini* infection (Viyanant et al. 1983). The sensitivity of stool examination for *O. viverrini* eggs by formalin–ether concentration and Stoll's dilution methods has been assessed in autopsied subjects grouped according to the number of worm recovered from their liver. At a worm burden >20, the rate of egg detection by both methods was comparable with the worm recovery (sensitivity 100 %), but at a worm burden of 10–19 worms, false negative samples are detected (Sithithaworn et al. 1991). The strength of these methods is the possibility of determining the infection intensity, as expressed by the number of parasite eggs per gram of feces, which allows quantifying treatment outcomes both in terms of cure rate and egg reduction rate (Wood et al. 1995). However, the sensitivity of these direct diagnostic tests, in particular for low-intensity infections, is frequently insufficient. Hence, multiple stool sampling or the combination of different diagnostic tests should be considered to enhance diagnostic accuracy (Bergquist et al. 2009; Johansen et al. 2010). Promising results have been obtained with a new multivalent flotation method (FLOTAC), which allows considerably larger amounts of feces to be examined and showed a considerably higher sensitivity than the classical methods (Cringoli et al. 2010). It is necessary to take into account that there are several species of foodborne trematodes which have similar egg morphology (as Opisthorchiidae, Heterophyidae, and Lecithodendriidae families); consequently, recognition of the egg morphology is essential for a correct diagnosis (Chai and Lee 2002).

5.11.2 Detection of Parasite Antigens in Stools

ELISAs for antigen detection in stools from infected persons have been developed using monoclonal antibodies (MAb) to different antigenic proteins of *O. viverrini* (Chaicumpa et al. 1992; Sirisinha et al. 1995). A highly sensitive (limit of detection 20 ng/mL) polyclonal antibody capture ELISA for the detection of *C. sinensis* coproantigens in rat feces has been developed (Rahman Mazidur et al. 2012), but further evaluation of the method on a human large population from endemic areas has to be still carried out.

5.11.3 Detection of Parasite DNA in Stools

The detection of parasite DNA by PCR and sequencing or by real-time PCR constitutes an alternative to parasitological diagnosis and is a very sensitive and specific way to identify cryptic infections. Since there are species-specific PCR tests to identify *O. viverrini* (Ando et al. 2001; Wongratanacheewin et al. 2002), *C. sinensis* (Le et al. 2006; Traub et al. 2009; Sato et al. 2009; Kim et al. 2009; Cai et al. 2010, 2012; Huang et al. 2012; Arimatsu et al. 2012), and *O. felineus* (Pauly et al. 2003; Müller et al. 2007) from various parasite stages (eggs, metacercariae, and adult worms), these methods have been largely applied for the diagnosis. The sensitivity can vary and it reaches 100 % in moderate to severe infections (eggs per gram >1,000), whereas in light infections (eggs per gram <200), the sensitivity drops to 68.2 % (Wongratanacheewin et al. 2002). The presence of PCR inhibitors in human fecal specimens can strongly reduce PCR sensitivity (Wongratanacheewin et al. 2003; Sithithaworn et al. 2007).

5.11.4 Serological Diagnosis

The detection of anti-liver fluke antibodies is widely used, since the sensitivity and specificity of the serological tests have greatly improved. Moreover, studies in humans have shown a close relationship between parasite-specific IgG (in serum and saliva), salivary parasite-specific IgA, and intensity of *O. viverrini* infection (Elkins et al. 1991; Haswell-Elkins et al. 1991; Sawangsoda et al. 2012). Furthermore, in *O. viverrini* infections, the level of parasite-specific IgG is correlated to the severity of the clinical disease rather than to the egg count in stools (Haswell-Elkins et al. 1991; Tesana et al. 2007). Since the time between infection and the detection of antibodies in serum ranges from 3 to 8 weeks for *O. felineus* infections (Armignacco et al. 2008, 2013, Traverso et al. 2012; Pozio et al. 2013), and it is around 2 weeks for *O. viverrini* (Sripa and Kaewkes 2000), the detection of specific antibodies, mainly IgG, has been considered as a complementary tool to establish a

definitive diagnosis of the infection (Sripa and Kaewkes 2000; Upatham and Viyanant 2003).

The serodiagnosis of liver fluke infections caused by *O. viverrini* and *C. sinensis* has been attempted using crude adult extracts, metabolic products, and egg antigens together with different immunodiagnostic methods, producing results of varying degrees of sensitivity and specificity (Wongratanacheewin et al. 1988; Haswell-Elkins et al. 1991; Sawangsoda et al. 2012; Sakolvaree et al. 1997; Pinlaor et al. 2012). A main problem in the serological diagnosis of parasitic infections, and especially for those caused by helminths, is the cross-reactivity, in particular when parasite crude extracts (CE) are used. In fact, using CE from adults, metacercariae and eggs, and ES products from adults, some authors have reported that the specificity in the detection of circulating antibodies to *O. viverrini* is limited by the cross-reactive nature of the antigens (Wongratanacheewin et al. 1988; Sirisinha et al. 1995; Wongsaroj et al. 2001; Sawangsoda et al. 2012). Serum titers of anti-*O. viverrini* antibodies have been found to be higher in cases of CCA than in patients with cholangitis caused by the liver fluke. In infected patients, the detection of IgG and IgG4 levels in serum yielded good sensitivity (99.2 % and 93 %, respectively) but poor specificity (23.1 % and 29.6 %, respectively), whereas the detection of IgG and IgG4 levels in urine had much lower sensitivity (43 % and 45.9 %, respectively) but better specificity (64.5 % and 67.2 %, respectively) (Tesana et al. 2007).

ELISA is widely used in Korea for *C. sinensis* infections (Choi et al. 2003; Lee et al. 2010; Kim et al. 2010) and has a 93.1 % sensitivity when ES are used as antigen and 87.8 % when CE is used (Choi et al. 2003). Several recombinant proteins from *C. sinensis* have been produced and identified (Kim et al. 2010; Shen et al. 2009; Ju et al. 2009; Chen et al. 2011; Nagano et al. 2004; Na et al. 2008) and shown to be sensitive and specific for serodiagnosis of clonorchiasis, but not enough to replace CE (Hong and Fang 2012).

The indirect hemagglutination test, intradermal test, and ELISA have been developed using *O. felineus* CE from adult worms as antigens (Wongratanacheewin et al. 2003). According to Meniavtseva et al. (1996), ELISA shows the best performance among all the serological tests. Recently, ELISA based on ES products has been validated for *O. felineus* infection in humans from low endemic areas (Gómez-Morales et al. 2013).

5.12 Human Treatment

Currently, the drug of choice to treat people with clonorchiasis or opisthorchiasis is praziquantel (2-(cyclohexylcarbonyl)-1,2,3,6,7,*11b*-hexahydro-4H-pyrazine-[2,1-a]-isoquinoline-4-one). The commercial preparation contained a racemic mixture of equal portion of levo- R(-) and dextro- S(-) isomers, but only the R(-) enantiomer has antihelmintic activity (Mordvinov and Furman 2010). Since 2004, the commercial preparation contains only the R(-) enantiomer. This drug is safe for

pregnant and lactating women (Olds 2003). It is recommended to use praziquantel only for children older than 4 years of age. Praziquantel induces a muscle contraction and a vacuolization of the tegumental syncytium of the flukes. This drug is absorbed rapidly and peak serum levels occur 1–3 h after administration; then, it is excreted with bile and urine within 24 h. It follows that the praziquantel treatment has little or no effect on subsequent exposure to infection. According to WHO (1995), the recommended dose for mass treatment is 40 mg/kg body weight. Praziquantel should be administered at a daily dose of 75 mg/kg body weight divided into three subdoses of 25 mg/kg body weight at 4–5 h intervals. This treatment gives 100 % and 80–85 % cure rate for *O. viverrini* and *C. sinensis* infection, respectively (WHO 1995). However, in endemic areas, the praziquantel treatment alone is not sufficient when people get reinfected by continuously consuming raw fish (Hong et al. 2001). Quite frequently (up to 90 % of treated people), praziquantel can cause side-effect reactions such as abdominal pain, nausea, headache, dizziness, and drowsiness.

The second drugs of choice are the benzimidazole derivates mebendazole and albendazole. However, these two drugs are effective only when given over a long period or at high doses (Jaroonvesama et al. 1981; Pungpak et al. 1984; Armignacco et al. 2008). The treatment of *O. felineus* infections with albendazole (10 mg/kg body weight daily in two doses for 7 days) failed to eradicate all the flukes from one patient involved in an outbreak in Italy (Armignacco et al. 2013). Indeed, 2 years later, the patient still shed *O. felineus* eggs in her feces in the absence of reinfection (Armignacco et al. 2013). In this patient, the albendazole treatment probably induced the flukes to stop the egg production, suggesting a false recovery.

5.13 Prevention and Control

Prevention and control measures should be proportionate to the epidemiological situation. The snail control by molluscicides was approached to reduce the first intermediate host populations but failed to reduce the prevalence of the infection in addition to cause considerable ecological damages (Mordvinov and Furman 2010; Tesana and Thapsripair 2012). In high endemic areas, morbidity can be prevented or controlled by treatment, health education, improved sanitary conditions, and implementation of food safety measures (WHO 1995; Sithithaworn et al. 2007). The ultimate aim is to change human behavior, because the consumption of raw or undercooked freshwater fish is the key risk factor for acquiring clonorchiasis and opisthorchiasis. Vaccines are not available yet for the prevention of these zoonoses. The use of chemotherapy with or without health education failed to eradicate or control these infections in humans. Successful results were obtained only when a mass praziquantel treatment was combined to an extensive improvement of the healthcare system and to socioeconomic development (Jongsuksuntigul and Imsomboon 2003).

To prevent clonorchiasis and opisthorchiasis, freshwater fish should be cooked until the core reaches 65 °C for at least 1 min (EFSA 2010). *O. felineus* metacercariae in fish fillets can be killed by freezing at −28 °C for 20 h, at −35 °C for 8 h, and at −40 °C for 2 h (Fattakhov 1989). According to Lloyd and Soulsby (1998), metacercariae may be killed by freezing at –10 °C for 5–70 days, depending on the size of the fish. Metacercariae of *O. felineus* present in tench muscles were devitalized at −18 °C for 96 h (Pozio et al. 2013); however, only freezers marked with three or four stars reach a temperature of −18 °C. Metacercariae of *O. felineus* can survive in smoked fish causing human infections (Yossepowitch et al. 2004). Marinating does not kill *O. felineus* metacercariae present in tench muscles (Armignacco et al. 2008, 2013; Traverso et al. 2012). Metacercariae of *O. viverrini* are killed in fish at 13.6 % NaCl after 24 h (Kruatrachue et al. 1982).

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