HUMAN LIVER FLUKES

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Search strategy and selection criteria

References for this review were conducted using PubMed and EMBASE. Relevant articles, websites, and book sections were considered. Non-English articles have been included in some cases. Search terms included "Clonorchis sinensis", "clonorchiasis", "Opisthorchis viverrini", "Opisthorchis felineus", "Opisthorchiasis", "Liver fluke", "Food-borne trematodes", "Food-borne trematodiasis", "Cholangiocarcinoma". The final reference list was generated on the basis of relevance to the broad scope of the review.

SUMMARY

Human liver fluke infections are encountered worldwide. In some areas of the developing world a combination of ecological, agricultural and culinary factors leads to a very high prevalence of infection, but in the developed world they are uncommon. Infection is associated with considerable morbidity and several are recognised as biological carcinogens. In this article, we review the epidemiology, clinical significance, and diagnostic and treatment strategies of human infection with these pathogens.

INTRODUCTION

Flukes are large, multicellular, leaf-like organisms of the phylum Platyhelminthes, class trematoda. More than 6000 species have been described and at least 100 of these are capable of infecting humans. In order to use vertebrates as a definitive host, the adult fluke must migrate to a tissue that provides a portal of exit for its ova. The common names given to these pathogens reflect tissue tropism of the adult fluke in humans. Adult members of the genera *Fasciola, Clonorchis* and *Opisthorchis* (the common liver flukes) reside in the

biliary tract. *Paragonimus spp*. (lung flukes) migrate to pulmonary tissue, *Echinostoma* and *Fasciolopsis* (intestinal flukes) remain in the small bowel and species of *Schistosoma* are sometimes referred to as blood flukes as the adult worm resides in blood vessels.

Together the liver, lung, and intestinal flukes are referred to as the food-borne trematodiases. Collectively, these have been designated as one of 17 Neglected Tropical Diseases (NTDs). In 2012, the World Health Organization (WHO) released a roadmap detailing strategy and specifying targets for the control of NTDs[1]. Food-borne trematodiases remain amongst the most neglected of NTDs[2]. Estimates of prevalence are hard to calculate as most trematodiases are geographically and ecologically circumscribed, with areas of hyperinfestation interspersed with areas which are free of infection. Within endemic communities, parasites are also highly aggregated, with a few individuals harbouring the majority of parasites[3]. Overall, the WHO estimates that 56 million individuals are infected with one of the four main genera of food-borne trematodes, a figure that is very likely to be an underestimate [4].

Flukes known to parasitise the human liver include the Opisthorchidae *(Clonorchis sinensis* [the Chinese liver fluke]*, Opisthorchis felineus, Opisthorchis viverrini, Metorchis conjunctus* [the North American liver fluke], *Metorchis bilis, Amphimerua noverca, Opisthorchis guayaquilensis, Pseudamphistomum truncatum*, and possibly *Metorchis orientalis);* the Fasciolidae *(Fasciola hepatica* and *Fasciola gigantica)* and the Dicrocoeliidae *(Dicrocoelium dendriticum* [the Lanceolate liver fluke] , *Dicrocoelium hospes,* and *Eurytretna pancreaticum)*[5]*.* The most important of these are *C. sinensis,* causing human clonorchiasis, *O. viverrini* and *O. felineus* (the Far Eastern Liver Flukes), causing human opisthorchiasis and *F. hepatica* and *F. gigantica,* which cause human fascioliasis. These will be addressed in most detail in this review.

Disease associated with liver fluke infection tends to be insidious although acute disease associated with parasite migration through tissue can occur. Chronic infection with *Clonorchis sinensis* or *Opisthorchis viverrini* is strongly associated

with the development of cholangiocarcinoma and both have been recognised as class 1 biological carcinogens[6].

PARASITE LIFE CYCLES

The life cycles of *Clonorchis* and *Opisthorchis* can be considered together. Eggs are excreted in the faeces of infected definitive hosts, with each egg containing a single miracidium. When an egg is ingested by a suitable intermediate freshwater snail host, a miracidium is hatched and develops into a sporocyst then rediae. Within the snail, asexual reproduction occurs, resulting in the production of thousands of cercariae which are periodically released into the environment. Free swimming cercariae penetrate and embed themselves in the flesh of cyprinoid freshwater fish (second intermediate host) and encyst as metacercariae. Fish eating mammals (ie: cats, dogs, pigs) are common definite hosts. Humans are often incidental hosts, but can be definite hosts in *O. viverrini* infection. Local culinary practices which fail to kill encysted metacercariae (salting, pickling, smoking, or eating raw food) may result in human infection. Metacercariae excyst in the duodenum of the definitive host, migrate through the ampulla of Vater and ascend the biliary tree to small and medium-sized biliary ducts, where they mature into adult flukes over 30-45 days and sexually reproduce. *C. sinensis* adult flukes grow to between 10 and 25mm and can produce approximately 4000 eggs per fluke per day. Adult *O. viverrini and O. felineus* flukes grow to between 8 and 12mm and can shed approximately 200 eggs per fluke per day[5].

The lifecycle of the Fasciolids, *Fasciola hepatica* and *Fasciola gigantica,* is similar to *Clonorchis* and *Opisthorchis*, with the distinction that neither requires a vertebrate second intermediate host. The definite hosts of both species are domestic livestock, predominantly sheep and cattle. *F. hepatica* is also known to infect goats, buffalo, horses, donkeys, mules, camels, hogs, rats, deer and rabbits as definite hosts. Eggs are released into the biliary ducts and then faeces of the host and become embryonated in water where they hatch, releasing free-swimming miracidia. Miracidia penetrate the freshwater snails intermediate host and, like *Clonorchis* and *Opisthorchis,* develop into sporocysts then rediae,

reproduce asexually, and are subsequently released as cercariae from the snail [5]. *Fasciola* cercariae encyst (as metacercariae) on aquatic vegetation such as the watercress, water lotus, water caltrop, water chestnut, or water lily[7] and infection of the definitive or incidental (human) host is acquired from eating contaminated plants. Dandelion leaves, lettuce, spearmint, algae, corncob, alfalfa, spinach, broccoli, morning glory have also been described as vectors in human infection[5, 7]. Metecercariae are also found on water surfaces, and use of contaminated water for drinking, or washing food or kitchen utensils may result in human infection[7]. Outbreaks have also been seen in chewers of Khat, a narcotic leaf which may become contaminated by water used to keep it fresh during transport[8].

Once ingested, *Fasciola* metacercariae excyst in the duodenum. In contrast to the *Opisthorchidae,* which ascend the biliary tree, *Fasciola* larvae bore directly through the intestinal wall, peritoneal cavity and liver parenchyma, reaching the large biliary ducts where they mature into adults. Aberrant migration is well described with ectopic fascioliasis recorded in many tissue types. Adult *F. hepatica* flukes can grow up to 2.9cm and produce up to 25,000 eggs per fluke per day[5]. *F. gigantica,* as the name suggests, are much larger (growing up to 5.2cm) and can produce 8000-10,000 eggs per fluke per day[5].

AFFECTED POPULATIONS

Human trematodiases are geographically limited to areas where the necessary intermediate hosts coexist and local culinary habits fail to kill metacercariae. In general, warm and wet rural areas with poor sanitation have the highest prevalence of liver fluke infection. Human migration and environmental, behavioural and agricultural changes mean that the geographical distribution of the individual species is in flux. Liver fluke infection may be diagnosed in those with a travel history to endemic areas. Primary infection may also occur in areas where these trematodes do not live if metacercariae in fish or vegetable products are imported[8]. There are also reports of liver fluke transmission via infected

liver transplants[9].

Diagnostic difficulty and species misidentification mean that there may be considerable unrecognized overlap in epidemiology of individual species, with co-endemicity potentially under reported.

Clonorchis sinensis infection is endemic in China, Korea, Taiwan, Northern Vietnam, and far eastern Russia(10). Local prevalence is as high as 85% in some regions of China[11]. Worldwide, an estimated 601 million people are at risk, with an estimated 35 million infected, 15 million of whom are in China[12-14]. *C. sinensis* was previously described in Japan, but with improved sanitation and disease control there have been no reported locally transmitted cases since 1991[11]. The incidence of clonorchiasis in China is rising, potentially due to an increase in commercial freshwater fish farming[12].

Opisthorchis viverrini is endemic in Thailand, Laos, Cambodia and central and south Vietnam. An estimated 10 million people are infected worldwide, 80% of whom reside in Thailand[7]. A study in Thailand showed *O. viverrini* infection rates ranging between 2 and 71% in different districts[15].

Opisthorchis felineus is found in the former USSR including Belarus and Ukraine and has been found in animals in Germany, Italy, Poland, Portugal, and Spain over the last 50 years[5, 16]. Locally transmitted human infection has been reported in Germany and Italy, with a recent outbreak reported from the region around Lake Bolsena[17]. An estimated 1.2 million people are affected worldwide, predominantly in Russia[5].

Fasciola hepatica is a far more widespread parasite than the Chinese and oriental liver flukes. It has a worldwide distribution and is a common zoonosis throughout Eurasia and parts of South America and Africa[5]. Previous estimates suggest between 2·4 and 17.0 million people are infected worldwide[7]. Cattle and sheep herders are at higher risk and, in endemic areas, infection is more common in children[5]. Prevalence in the Northern Bolivian Altiplano approaches

100%[18] and notably high prevalence has also been reported in the Mantaro valleys (34·2%) and Puno region (15·64%) of Peru, Corozal in Puerta Rico (10·9%), and the inner Porto area of Portugal (3·2%)[19]. The wide range of *F. hepatica* may be related to the ability of the metacercariae to encyst on aquatic plants and water surfaces without the requirement and associated selective constraint of a specific second intermediate host.

Fasciola gigantica is known to be endemic in Africa and Asia. As these areas harbour both *F. hepatica* and *F. gigantica,* and the ova of which cannot be reliably distinguished on stool microscopy, the exact distributions of each species remain unclear. The distribution of *F. gigantica* is currently understood to extend from the Nile Delta in Egypt through Africa and Asia as far as Japan. The distribution of *F. gigantica* is limited by the distribution of its intermediate host, the *radix* group of Freshwater snail[20]. Reports of human infection were rare before 1989, when an outbreak of fascioliasis caused by *F. gigantica* was reported in Iran[21]. Recently, an outbreak was reported in China[22], where infection had not previously been documented.

It should be noted that the estimates of prevalence of the human liver flukes are in most cases decades old and likely inaccurate. Likewise knowing their true distribution is limited by the sparsity of up to date information, the high incidence of asymptomatic human infection, and the nature of the liver flukes as zoonoses.

CLINICAL MANIFESTATIONS

CLONORCHIASIS AND OPSITHORCHIASIS

Acute and chronic infection with *Clonorchis sinensis* or *Opisthorchis viverrini/felineus* is usually asymptomatic, with symptoms only reported in 5-10% of cases[23]. Studies in Korea and Thailand demonstrate that intensity of infection, inferred by stool egg counts, is positively associated with incidence of symptoms[24]. Acute symptoms of clonorchiasis and opisthorchiasis include malaise, weakness, anorexia, flatulence, nausea, vomiting, abdominal pain and

diarrhoea. Obstructive jaundice may occur in heavy infections[23]. Hepatomegaly, often more pronounced in the left hepatic lobe[25], is common and can be greater than 7cm below the costal margin in heavy infections. Splenomegaly is a rare finding[23]. Acute symptoms seem to occur more frequently in *O. felineus* infections than in *C. sinensis* and *O. viverrini*, and fever is sometimes seen [7]. Acute symptoms usually resolve within a few weeks [7].

Clonorchis and *Opisthorchis* infections are long lasting. *C. sinensis* parasites have been reported to survive for up to 26 years and *O. viverrini* up to 10 years in humans[26]. Chronic infections are often benign but various complications can occur late in the clinical course. Chronic symptoms may mirror acute symptoms, and may be due to chronic irritation of bile ducts or physical obstruction by liver flukes. Cholangitis, cholecystitis, obstructive jaundice, periportal fibrosis, cholelithiasis, and cholangiocarcinoma are all recognised consequences of clonorchiasis and opisthorchiasis[27]. Pancreatitis, as a result of pancreatic duct obstruction, has been described but is rare and usually mild[5]. Recurrent pyogenic cholangitis (RPC) is the single most common complication[28].

Cholangiocarcinoma

The most important complication of chronic liver fluke infection is cholangiocarcinoma , a rare cancer with a high mortality which is in part due to the absence of early symptoms and consequent late diagnosis [29]. *Clonorchis sinensis* and *Opisthorchis viverrini* are both listed as Class 1 (carcinogenic to humans) biological carcinogens by the International Agency for Research on Cancer (IARC)[6].

The association between cholangiocarcinoma and liver fluke infection was first demonstrated from studies of *Opisthorchis viverrini* infection in Thailand. Thailand has a high incidence of cholangiocarcinoma, and regions within Thailand with the highest prevalence of *O. viverrini* infection also have the highest incidence of cholangiocarcinoma[30]. In the Khon Kaen region of North-east Thailand, where *O. viverrini* prevalence has historically approached 100%, the age-standardised incidence rates (ASR) of cholangiocarcinoma was

113·4/100,000 population/year in men (compared to 0·3-1·5/100,000 population/year worldwide)[30].

The association between *Clonorchis sinensis* infection and cholangiocarcinoma has been shown in Korean studies. Korea also shows the same pattern of higher incidence of cholangiocarcinoma in districts with the highest prevalence of *C. sinensis* infection[31].

There is a paucity of studies on a potential link between *Opisthorchis felineus* and cholangiocarcinoma. There are some epidemiological data from Russia suggesting a higher incidence of cholangiocarcinoma in areas endemic for *O. felineus*[16] and a high prevalence of *O. felineus* infection in autopsy studies of patients with primary liver malignancies (fluke infection seen in 42 of 44 liver malignancy patients in one study)[32]. Additionally there are some animal data suggesting cholangiocarcinoma as a sequalae of *O. felineus* infection[33]. Lower prevalence and a lower intensity of research into *O. felineus* compared to the other Far Eastern liver flukes may explain the difficulty in proving a link between *O. felineus* infection and cholangiocarcinoma, although it does seem likely that such a link exists.

The mechanism of carcinogenesis is incompletely understood and is the subject of ongoing research[34, 35]. Much of the data relate to *Opisthorchis viverrini* induced cholangiocarcinoma. As *Clonorchis* or *Opisthorchis* feed on biliary epithelium, mechanical damage, as well as release of fluke excretory-secretory products, lead to inflammation and ulceration of biliary epithelium, resulting in epithelial metaplasia and eventually periductal fibrosis and dysplasia[36].

Chronic periportal inflammation caused by *Opisthorchis viverrini*, results in liver cell necrosis and deposition of fibrotic tissue, with periportal fibrosis similar to that seen in primary sclerosing cholangitis (a strong risk factor for cholangiocarcinoma[37]). Fibrosis replaces the periportal inflammation over time[38]. Re-infection, which is common and often continual in endemic areas, results in more severe inflammation, and accelerated fibrosis[39]. Induction of

fibrosis after inflammation is an important part of the pathway of pathological changes that lead to cholangiocarcinoma[40]. These abnormalities do not occur in all patients, with a subset producing a stronger inflammatory response and then faster and more advanced periportal fibrosis deposition, predisposing them to cholangiocarcinoma[36]**.** Genetic or environmental factors, such as alcohol, smoking, and levels of fruit and vegetable intake, are likely involved here[41]. The presence of the fermented foods present in a North-Eastern Thai diet may enhance the development of fibrosis in *O. viverrini* infected individuals[42]. Melatonin, known to protect against liver injury in multiple cancer types, has been shown to reduce tumour burden, and increase survival in hamsters with *O. viverrini*-related cholangiocarcinoma[43].

Excretory-secretory products of liver flukes likely have additional effects promoting tumourigenesis. Secreted carcinogenic oxysterols, which can induce production of other carcinogens, have been found in liver tissue of *Opisthorchis viverrini* infected hamsters, and human cholangiocarcinoma tissue[37, 44].
A granulin-like growth factor secreted by *O. viverrini* has been shown to promote tumour cell growth[45]. Excretory-secretory products of *Clonorchis sinensis* may induce hyperplasia and metaplasia of biliary cells with subsequent transformation to cholangiocarcinoma[46], and have been shown to suppress apoptosis of cholangiocarcinoma[47], and hepatocellular carcinoma[48] cells *in vitro*. *C. sinensis* excretory-secretory products may also induce cancer-related microRNA expression[49]. Excretory-secretory products of *O. viverrini* induce expression of Interleukin-6 (IL-6)[50]. Extracellular vesicles containing excretory-secretory products, are taken up by cholangiocytes, and induce release of IL-6, as well as changes in protein expression associated with cancer[51]. IL-6 drives release of human pro-granulin, which is associated with development of many cancers, including cholangiocarcinoma[52], and may represent a biomarker for development of advanced periportal fibrosis and cholangiocarcinoma[36, 50].
Other cytokines, especially Interferon-γ (IFN-γ), Interleukin-10 (IL-10), and Lymphotoxin-α (LT-α) are significantly raised in *O. viverrini* related cholangiocarcinoma, and may indicate dysregulation of the immune response[53]. Similar cytokine overexpression and dysregulation is seen in *C. sinensis*

infection[34].

Upregulation of inducible nitric oxide synthase (iNOS) and subsequent oxidative/nitritative DNA damage has been demonstrated in *Opisthorchis viverrini*-related cholangiocarcinoma in humans[54], and in *Clonorchis sinensis* infected mice[49, 55]. Oxidative/nitritative damage to proteins via reactive oxygen species may prolong the oxidative stress[37]. iNOS is a target for nuclear factor κB (NFκB), and other targets of NFκB have been shown to be upregulated in *O. viverrini* infection[56]. NFκB-mediated inflammation is also seen in *C. sinensis* infection[46, 55]. Likewise inhibition of NFκB with cepharanthine has been shown to have anti-tumour activity against cholangiocarcinoma in mice[57], and may represent a potential future treatment. Use of ciclosporin to suppress the raised levels of cyclophilin A seen in *O. viverrini* has been shown reduce cholangiocarcinoma cell proliferation and tumour growth in a mouse model[43]. Long-term (three months) curcumin treatment has been shown to reduce periductal fibrosis in *O. viverrini* infected hamsters[58].

Opisthorchis viverrini induced cholangiocarcinomas have been found to have different gene expression profiles to non-liver fluke related cholangiocarcinoma, with high expression of *UGT2B11*, *UGT1A10*, *CHST4*, *SULT1C1* genes related to xenobiotic metabolism, whereas non-liver fluke related cholangiocarcinomas have upregulation of genes related to growth factor signalling[59]. The lesser *K-ras* gene mutation, is seen in 56% of *O. viverrini*-related cholangiocarcinoma (versus 0.0-16.7% of cholangiocarcinoma from other causes), and *p16* DNA methylation is seen in 100% of *O. viverrini*-related cholangiocarcinoma (versus 28.3% of cholangiocarcinoma from other causes)[59-62]. Additionally novel cholangiocarcinoma-related genes such as *BAP1, ARID1A, MLL3, IDH1/2, RNF43, PEG3, GNAS*, and *ROBO2* have been identified in *O. viverrini*-related cholangiocarcinoma[63]. NFκB, a pivotal regulator of gene expression, is known to be specifically involved in the expression of genes of the UGT2B family[64]. There are a number of other molecular targets that may be useful in recognition or treatment of liver-fluke cholangiocarcinoma in the future[56].

There has been a great deal of research into the involvement of *Helicobacter spp.* in the development of cholangiocarcinoma in *Opisthorchis viverrini* infection. *Helicobacter pylori* can be found in 90% of *O. viverrini* infected hamsters (versus 43.3% of non-*O. viverrini* infected hamsters), and *Helicobacter bilis* can be found in 40% of *O. viverrini* infected hamsters (versus 26.7% of non-*O. viverrini* infected hamsters). Prevalence of *H. pylori* and *H. bilis* was lower in *O. viverrini* infected hamsters treated with praziquantel and antibiotics, rather than antibiotics alone, indicating *O. viverrini* is a potential reservoir for *H. pylori* and *H. bilis*[65]. *O. viverrini* infection may also cause changes in bile acid composition that reduce alkalinity which may allow for easier colonisation of *H. pylori* [66, 67], and may even directly contribute to biliary inflammation[68]. Chronic cholestasis may also contribute to a low pH environment favourable to *H. pylori*[69]. *H. bilis* causes similar pre-malignant pathological changes to those seen in liver fluke infection in the biliary tree in an animal model (chronic hepatitis, hepatic dysplasia, fibrosis, and biliary hyperplasia)[70]. Cell inflammation and proliferation of biliary and gallbladder epithelial cells has been shown to be significantly higher in *O. viverrini*-related cholangiocarcinoma with *H. pylori* DNA detected in bile samples, compared to those without detectable biliary *H. pylori* infection[71]. The increased inflammatory and proliferative process in these patients was more pronounced in biliary infections with *H. pylori* harbouring the *cagA* gene[71], an important virulence factor known to induce the severe inflammation associated with gastric adenocarcinoma[72]. *H. bilis* and *H. pylori* infection is associated with cholangiocarcinoma in patients in both areas endemic for human liver fluke infection and in areas where human liver fluke infection is rarely seen[73-75].

There is no difference in treatment for *Opisthorchis viverrini*-related cholangiocarcinoma compared to non *O. viverrini*-related cholangiocarcinoma, and prevention through improved public health measures, education, and mass *O. viverrini* treatment may help reduce the burden of disease. There are a number of molecular targets for potential future therapies, and further research into the role of anti-*Helicobacter* antibiotics, melatonin, ciclosporin and curcumin his needed.

In 2009, the IARC found no evidence of an association between liver fluke infection and hepatocellular carcinoma[6], however a recent meta-analysis of seven studies did demonstrate a positive association[27]. Severin, an excretory/secretory product of *Clonorchis sinensis* inhibits apoptosis of HCC cell lines *in vitro*[48]. Co-infection with *C. sinensis* in chronic hepatitis B infection, has been shown to be associated with higher hepatitis B virus (HBV) DNA titres[76]. More research is needed in this area.

FASCIOLIASIS

Infections with *Fasciola hepatica* and *F. gigantica* are clinically indistinguishable and can be split into acute, latent (when parasites mature into adults), and chronic phases. Acute symptoms are caused by the tissue destruction and acute inflammation associated with larval migration through intestine, peritoneum and liver parenchyma, as well as a generalised allergic reactions to parasite antigens[5]. Fever and abdominal pain (which may be excruciating), are the most frequently reported symptoms[5, 77]. Anorexia, flatulence, nausea, diarrhoea, urticaria and cough are also common. Hepatomegaly, splenomegaly, jaundice, and ascites may be seen. Acute symptoms may last 2-4 months, but in endemic areas re-infection may cause episodic symptoms and overlapping of acute and chronic symptoms[5].

Human fascioliasis is a chronic disease, with adult flukes living up to 13·5 years.[78] The flukes cause inflammation and hyperplasia of biliary epithelium, portal triad and duct dilatation, periductal fibrosis, necrotising arterial vasculitis and portal venous thrombosis[5]. The large size of the flukes mean that obstruction of the large biliary ducts occurs commonly. Cholangitis (which may be recurrent), cholecystitis, cholelithiasis, liver abscesses, subcapsular haemorrhages, cirrhosis, and pancreatitis are known complications of human fascioliasis[5]. There is no known association between *Fasciola* infection and cholangiocarcinoma.

DIAGNOSIS OF LIVER FLUKE INFECTIONS

HAEMATOLOGICAL AND BIOCHEMICAL ABNORMALITIES

Eosinophilia is often the most apparent haematological abnormality in clonorchiasis and opisthorchiasis however, even in heavy infections, eosinophilia may not always be present [5, 24].

Eosinophilia is reported in up to 100% of cases of fascioliasis in the acute phase, and is often associated with hypergammaglobulinaemia[5]. Anaemia is common in fascioliasis and is related to chronic blood loss from damaged biliary epithelium[5]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) and bilirubin are often raised during larval migration [79], and during the chronic phase.

PARASITOLOGICAL DIAGNOSIS

Parasitological diagnosis can be obtained by microscopic identification of eggs in faeces, duodenal aspirates, bile specimens or surgical samples. Identification of eggs in stool by microscopy is the most common method of diagnosis. Wet mount preparations are widely used, but may miss light infections[80]. Concentration techniques using formalin-ether or centrifugation are therefore used in hospital laboratories to detect light infections[81]. However, difficulty in using egg morphology to differentiate species of liver fluke, and even confusion with intestinal flukes or non-pathogenic trematodes, reduces the specificity and sensitivity of this method[81, 82]. Therefore eggs of light, or early infections may not be detected[24, 83]. Eggs do not appear in stool until 3-4 months post ingestion in fascioliasis, and 3-4 weeks in clonorchiasis and opisthorchiasis[5, 82], when the flukes have finished migration and reached maturity. If flukes cause obstruction of the biliary tree, eggs may not be passed to stool, and so endoscopic or percutaneous biliary aspirates may be more useful for diagnosis[5]. Adult flukes may be seen in stool examination after expulsion from the biliary tree post-treatment. The sensitivity of microscopic diagnosis may be increased by

repeating samples[84], but there is a need for improved point-of-care diagnostic methods both for the eggs, as a proxy to adult worm burden and measure of environmental contamination, as well as for direct detection of the adult worms or adult worm antigens.

IMMUNODIAGNOSIS AND MOLECULAR DIAGNOSTICS

Crude adult somatic extracts for enzyme-linked immunosorbent assay (ELISA) , Excretory/secretory (ES) antigens, and several other antigens from *Clonorchis sinensis* and *Opisthorchis viverrini* have been used with higher sensitivity than stool egg microscopic detection, and may therefore be of use assessing treatment success[85]. Stool antigen tests for *Clonorchis* and *Opisthorchis* have also been developed, and may be of use in detection of light infections[85]. Stool PCR assays of *O. viverrini* DNA have also been developed with 100% sensitivity and 98% specificity in heavy infections, and although less sensitive for light infections (68%)[86], may enable detection of light or early infection when stool microscopy is negative[85]. Access to serological, antigen and PCR diagnostics for *Clonorchis* and *Opisthorchis* varies, and validation of these tests for widespread use is still required.

Immunological tests for *Fasciola hepatica* are better validated than those for *Clonorchis* and *Opisthorchis*, and allow confirmation of early acute disease 2-4 weeks after infection[82]. The CDC currently recommend enzyme immunoassays (EIA) with excretory-secretory (ES) antigens combined with confirmation of positives by immunoblot.

IMAGING

Ultrasound may be of use in diagnosis of liver fluke infections. The findings of hepatomegaly, gallstones, sludge, intrahepatic duct stones, poorly-functioning gall bladder, increased portal vein diameter, intrahepatic bile duct dilatation, increased splenic thickness, or high hepatic pulsation index are non-specific but supportive[87, 88]. The most specific finding for clonorchiasis and opisthorchiasis are visualisation of aggregates of flukes as floating echogenic foci[89] and periductal fibrosis. Degree of periductal fibrosis measured by echogenicity is

associated with burden of disease.[88]. Computed Tomography (CT) provides similar information, and is more sensitive in detecting dilated and thickened small bile ducts associated with recurrent cholangitis[90]. Magnetic Resonance Imaging (MRI) may reveal flukes as filling defects in the biliary system[91] and may identify bile duct dilatation, enhancement, and thickening. Magnetic Resonance Cholangiopancreatography (MRCP) is the most sensitive modality for the detection of cholangiocarcinoma[92].

In acute fascioliasis, multiple, small (2-3cm) liver lesions, representing migratory tracks, have been reported to be present in up to 90% of confirmed fascioliasis cases presenting to a hospital in Turkey[93]. These are usually hypoechoic on US, but may be hyper or anechoic. If not visible on US they may still be seen on CT scans. Larger lesions are often necrotic. Perihepatic or subcapsular collections may be seen in acute fascioliasis[93]. The migration tracks of *Fasciola* in the parenchymal phase, may be visible as subcapsular enhancing hypo- or hyperintense lines on T1W and T2W images on MRI[94].

Adult *Fasciola* flukes may also be visualised directly as mobile structures in the common bile duct on US or CT during the latent or chronic phase[93]. Enlarged porta hepatis nodes are visible in 51% on US or CT[93]. Biliary abnormalities are present in 45% of cases and may include visible parasites in the gallbladder or bile ducts, or biliary duct dilatation, thickening, oedema or abnormal enhancement[93, 95]. After eight weeks ductal ectasia may be seen with bile duct thickening, dilatation and tortuosity occurring after 12 weeks[95]. Other findings include mild splenomegaly, ectopic inflammation in the abdominal wall or bowel[93].

ENDOSCOPIC DIAGNOSIS

Endoscopic Retrograde Cholangiopancreatography (ERCP) is an effective diagnostic tool with endoscopic aspiration of bile demonstrating *Clonorchis sinensis* ova in 100% and live worms in 50% of patients in a study of 18 patients presenting to a hospital in Taiwan[96]. Bile duct aspiration may allow for parasitological diagnosis even when stool sample are negative[97]. *Clonorchis*

flukes may also be visualised directly as filamentous or elliptical filling defects, which are pathognomonic clonorchiasis on ERCP[96]. This finding has been reported as occurring in 83% of patients at ERCP, but may be obscured by contrast medium[96]. It has been suggested that careful observation of contrast medium and use of diluted contrast may increase sensitivity[96]. Other findings include small, irregular filling defects, diffuse tapering of intrahepatic ducts with intra- and extra-hepatic bile duct dilatation and hazy appearance of the intrahepatic ducts, solitary or multiple intrahepatic cystic dilatations producing a mulberry-like appearance, and biliary duct ectasia[96].

ERCP is considered the gold standard for bile duct imaging in fascioliasis. Typical findings include dilated large bile ducts, with a jagged appearance distally, containing small linear filling defects and cresenteric shadows representing adult *Fasciola* flukes which can be retrieved endoscopically[98]. Fascioliasis may also be diagnosed by direct visualisation of fluke as a floating linear filling structure, or spiral shaped mobile membrane, with common bile duct dilatation on endoscopic ultrasound[99].

SURGICAL DIAGNOSIS

Laparoscopy commonly demonstrates hepatomegaly and multiple raised vermilliform nodules on the surface of the liver and peritoneum in fascioliasis [100]. Adult liver flukes of all species are large enough to be visible macroscopically, and so may be seen during cholecystectomy, or explorative laparotomy[5].

HISTOLOGICAL DIAGNOSIS

In clonorchiasis and opisthorchiasis common histological findings include biliary epithelial adenomatous proliferation, and goblet cell metaplasia, with periductal fibrosis and bile duct thickening in long standing infection[5]. Fluke eggs may embed in damaged epithelium and cause granulomatous inflammation with eosinophilic, mononuclear and neutrophilic infiltration[5]. Necrotising arterial vasculitis and portal venous thrombosis have been described[5]. Mucinous and

squamous metaplasia of the pancreatic duct is seen in pancreatitis caused by fluke infection[5].

In fascioliasis, histology of liver lesions most commonly show central necrosis, cellular debris with Charcot-Leyden crystals surrounded by an eosinophilic and inflammatory cell infiltrate and multiple calcific foci. Eggs or adult flukes are occasionally seen on liver biopsy[101]. Egg granulomas and eosinophilic infiltrate may be seen in all liver fluke infections[102].

MANAGEMENT

MEDICAL MANAGEMENT

For *Clonorchis sinensis, Opisthorchis viverrini a*nd *Opisthorchis felineus* infection, WHO recommends praziquantel treatment with either 25mg of drug/kg body weight (mg/kg) 3 times daily for 2-3 consecutive days, or a single dose of 40mg/kg. The Centers for Disease Control and Prevention (CDC) recommend a higher dose of 75mg/kg/day for 2 days for treatment of *Opisthorchis* infections, with this dose shown to be safe, and effective[103]. Recommendations for use are extended to pregnant and lactating women although it remains a category B drug as formal randomised trials are lacking. WHO also recommend use in children, although data are lacking in the under 4 years of age group. Praziquantel treatment yields cure rates of 85-100% in *Clonorchis* infections[104, 105], and 100% cure rates with praziquantel have been reported in *O. viverrini* and *O. felineus* infections with 25mg/kg/day over 3 days[106],. Cure rates of 90-95% are reported with the 40mg/kg single dose in *Opisthorchis* infections[107], although cure rates are much lower (22.7-33.3%) in *Clonorchis* infections[104].

The CDC recommends albendazole 10mg/kg/day for 7 days as second line treatment, this is a category C drug in pregnancy, but is approved for use in the second and third trimester. Albendazole should be used with caution in lactating women, and effects in children under 6 years of age are thought to be minimal, but are not fully investigated. Albendazole has however been used in WHO supported prevention programmes in children as young as 1 year[108]. 7 days of

albendazole is effective in treating *Clonorchis* in animal models, with cure rates of 90-92·6% at 6 months [109]. Cure rates are only 63% after 7 days of albendazole in human Opisthorchiasis[110]. 89-94% cure rates have been reported after 20-30 days of mebendazole in human opisthorchiasis[111], and may represent an alternative to albendazole.GH

Resistance to praziquantel has not been described in *Clonorchis* or *Opisthorchis*. There is a single report of failure of praziquantel treatment in Vietnam, with cure rates of only 29% in 21 patients, although this may have been due to inadequate dosing rather than drug resistance[112]. A new drug, tribendimidine, appears as effective as praziquantel for clonorchiasis with fewer side-effects[113]. A recent phase two clinical trial demonstrated cure rates up to 91·5% in adults, and up to 98·5% in children in *O. viverrini* infections treated with 400mg and 100mg of tribendimidine, respectively[114].

Fascioliasis is the only trematode infection which does not respond to praziquantel, and triclabendazole is the only drug recommended for this infection by WHO[115]. Triclabendazole can be used in both acute and chronic fascioliasis as it is active against both immature and adult flukes. It is given as a single dose of 10mg/kg[116]. Double-dose therapy (10mg/kg dose given, and repeated at 12-24 hours) is recommended in heavy infections, or if there is no response to single-dose therapy[116]. Cure rates of 92·2-100% are described in adults with a two-dose regimen[117, 118], with 79·2% cure rates described with the single-dose regimen[119]. Cure rates of 95% using the 10mg/kg dose have recently been described in children, with 100% cure rates using two doses of 7.5mg/kg[120]. Reports of resistance to triclabendazole in livestock are increasingly common, and more recently likely resistance in human fascioliasis has been detected in the Netherlands and Peru[121, 122]. Evidence from animal studies suggests triclabendazole resistance may be overcome by co-administration with the enzyme inhibitor ketonazole[123]. The artemisinins artesunate, artethemer, and OZ78 are active against triclabendazole-resistant *Fasciola hepatica*[124], and combination therapy may provide a solution to triclabendazole-resistant flukes, although further work is required[125].

Nitazoxanide 500mg/day twice daily, for 7 days can be used as an alternative therapy for fascioliasis and has been shown to be efficacious in treating human *Fasciola gigantica* infections with cure rates of 78%[126]. Nitazoxanide is considered second line treatment for human fascioliasis by the CDC[116]. Cure rates of 60% in adults and 40% of children with *F. hepatica* infection have been reported[127]. A study of Mexican children with *F. hepatica* infection, produced cure rates of 94% with nitazoxanide 7.5mg/kg twice daily, for 7 days, rising to 100% with a second treatment course [128].

WHO recommend mass treatment with praziquantel 40mg/kg at 12 or 24 month intervals in populations endemic for clonorchiasis or opisthorchiasis, and mass treatment with triclabendazole 10mg/kg as a single dose of school age children, or the entire community, in sub-districts, villages or communities where cases of fascioliasis appear to be clustered[129].

ENDOSCOPIC MANAGEMENT

ERCP has a well-described role in the treatment of biliary obstruction, and may be of use in any liver fluke infection causing biliary obstruction cholangitis, or cholecystitis from flukes or secondary stones or bacterial infection[130-132]. Sphincterotomy and direct retrieval of flukes may be of value in diagnosis and treatment, but needs to be supported by medical therapy. There are cases reports of successful endoscopic management of fascioliasis in refractory cases using ERCP and flushing of the biliary system with povidone iodine[133]. Further work is needed to establish the validity of this treatment.

RARE LIVER FLUKES

Infection with *Dicrocoelium dendriticum*, which may be acquired through the ingestion of infected ants, has been reported sporadically and can manifest in a similar way to clonorchiasis and opisthorchiasis[134]. Successful treatment with praziquantel and triclabendazole have been reported[135]. *Metorchis conjunctus* has been described as causing asymptomatic carriage in Sioux populations of Ontario, Canada[136]. An outbreak of 19 patients related to consumption of white

sucker, prepared as sashimi has been reported [137]. *Metorchis bilis* is an important cause of human liver fluke infection in the Ob river basin of western Siberia, and has a similar presentation to *Opisthorchis felineus*[138]. *Metorchis orientalis* eggs have been found in the stool of 4·2% of the human population of Ping Yuan County of Guangdong Province, China and, although not identified in the biliary system of humans directly, has been identified in the biliary systems of local ducks, cats and dogs[139]. Human infection with *Amphimerua noverca*, *Opisthorchis guayaquilensis*, *Pseudamphistomum truncatum*, *Dicrocoelium hospes*, and *Eurytretna pancreaticum* have been described but are limited to a handful of case reports[5].

CONCLUSION

Liver fluke infection is rare in developed countries and, with the exception of *Fasciola hepatica*, is usually limited to travellers from endemic areas. More work is needed on the epidemiology of individual species, which can be difficult to distinguish from one another. Diagnosis to the level of genus is usually made by examination of stool for ova, although this lacks both sensitivity and specificity. The association of some of these parasites with cholangiocarcinoma makes identification and treatment of liver fluke infections important. Treatment with praziquantel for clonorchiasis and opisthorchiais, or triclabendazole for fascioliasis is highly effective, and mass treatment in endemic areas is advised.

Conflicts of interest

We declare that we have no conflicts of interest

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