Chapter 4
Fascioliasis

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Abstract   Human fascioliasis is an important public health problem in many regions and livestock infection is a veterinary problem worldwide. This disease is caused by the liver fluke species *Fasciola hepatica* of worldwide distribution and *F. gigantica* restricted to regions of Africa and Asia. Their two-host life cycle is similar, including specific freshwater lymnaeid snails as vectors. The major human health problems are known in Andean countries, the Caribbean, Northern Africa, Near East, Southeast Asia and Western Europe. In human hyperendemic areas, children and females are the most affected. Human fascioliasis shows a marked heterogeneity of epidemiological situations and transmission patterns. Variation of climatic factors and anthropogenic environmental modifications give rise to different fascioliasis seasonality and long-term disease risk trends. There are many human infection sources, local diet and cultural traditions being important. The rapid and potent ability of fasciolids to suppress the immune response explains why hosts do not develop resistance and the frequency of pathogen coinfections. The disease is chiefly confined to the liver, including hepatic lesions, fibrosis and chronic inflammation. Juvenile flukes may cause ectopic fascioliasis. Clinical manifestations are evident in both invasive and biliary periods. Diagnosis is mainly made by coprological and serological techniques. Among the useful drugs, triclabendazole is of choice at present. Prognosis depends on treatment promptness. Serious complications, sequelae and death causes should be highlighted. New knowledge has allowed improvement of individual infection prevention measures and community control. Challenges appear in vaccinology, indicating that a human vaccine is still far from affordable.

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4.1   Introduction

Fascioliasis is a well-known veterinary problem worldwide. Moreover, in the last two decades, many surveys have shown it to be an important public health problem as well (Chen and Mott 1990; Mas-Coma et al. 1999a, 2009a), including estimations of 2.4 million up to 17 million people or even higher depending on the hitherto unknown situations in mainly Asia and Africa (Mas-Coma 2004).

The number of human case reports is increasing in many countries of the five continents, many human endemic areas have already been assessed, and recent results of studies on pathogenicity and immunity underlie the decision to consider fascioliasis an important human parasitic disease henceforth (Mas-Coma et al. 1999b) and include it as a food-borne trematode disease priority within the agenda of the World Health Organization (WHO 2013).

4.2   The Agent

This parasitic disease is caused by two digenean trematodes, *Fasciola hepatica* of worldwide distribution and *F. gigantica* restricted to given regions of Africa and Asia. Their adult stage has a leaf-shaped body and is anatomically characterised by the branching of their caeca, testes and ovary; the very numerous small vitellaria extending bilaterally up to the hind body; and a short uterus located between the ovary and the caecal bifurcation (Fig. 4.1). The eggs are operculated, ovoid, yellow and non-embryonated when laid.

Fasciola gigantica is more elongated and narrower, with lateral walls tending to be parallel and with non-existent or less marked shoulders of the cephalic cone (Fig. 4.1). The adult stage of *F. hepatica* has a maximum length/width of 29.0/ 14.1 mm, whereas *F. gigantica* is bigger, with a maximum size reaching 52.3/ 11.8 mm. Hybrid specimens may give rise to intermediate forms in those endemic areas where the two species overlap (Mas-Coma et al. 2009a).

Adult worms parasitise the large biliary passages and the gall bladder of ruminants, mainly sheep, goats, cattle and many other herbivorous domestic and wild animals, including horses, donkeys, mules and also Old and New World camelids. Buffalo, deer, wild sheep, wild pig, various marsupials, rabbit, hare and nutria are also susceptible hosts. Grazing domestic pigs may also be infected, but this host usually shows a higher natural resistance against the liver fluke (Mas-Coma and Bargues 1997).

Sheep and cattle are the livestock species most infected by *F. hepatica*, whereas the buffalo appears to be the most important for *F. gigantica*. Among wild definitive hosts in Europe, *F. hepatica* seems to be less adapted to the roe deer (*Capreolus capreolus*) when compared to other deer (red and fallow deer), and the introduced nutria (*Myocastor coypus*) has become an important reservoir in France and may also be so in areas of South America where it originated.

Fig. 4.1 Adult stages of pure *Fasciola hepatica* and pure *F. gigantica*. Note different size and shape; hybrid forms usually show an intermediate form. The two photographs are at the same scale (Orig. S. Mas-Coma)
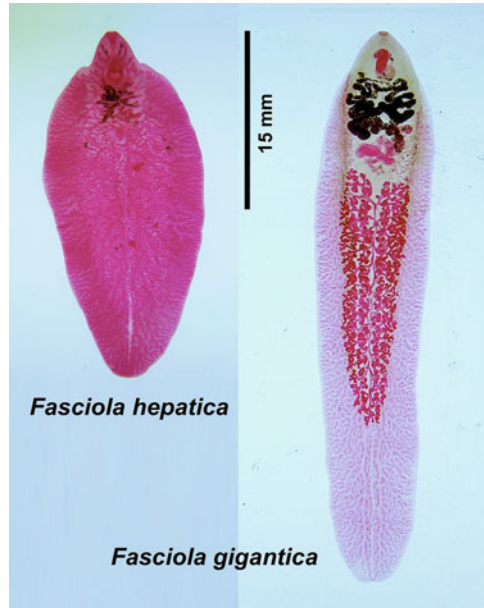
Humans are susceptible hosts to the infection by both *Fasciola* species (Mas-Coma et al. 2009a).

The two-host life cycles of both fasciolids are similar and take about 14–23 weeks. These comprise four phases (Mas-Coma and Bargues 1997):

(a) The definitive host harbours fluke adults producing eggs which reach the external milieu by way of bile and intestine; the definitive host is infected by ingestion of metacercariae; in humans, the flukes attain sexual maturity in 3–4 months and their life span is between 9 and 13.5 years.

(b) The transit between definitive mammal host and intermediate snail host includes the long resistance phase of the egg and the short active phase of miracidium; eggs shed with the mammal faeces will continue their development in freshwater of appropriate physico-chemical characteristics (mainly temperature of 15–25 °C).

(c) The development at snail level includes miracidium penetration, sporocyst, redial generations, production of cercariae and shedding of the latter into water; the prepatent period (38–86 days) is dependent on temperature, and higher temperatures reduce the period.

(d) The transit between snail and mammal host includes the short swimming phase of cercaria and the long resistance phase of metacercaria; the shedding process takes place between 9° and 26 °C, independent of light or darkness; cercariae swim for a short time until contacting a solid support, mostly leaves of water plants above or below the water line, to attach and encyst (Fig. 4.2); metacercarial cysts become infective within 24 h. Liver fluke development is very

Fig. 4.2 Metacercarial body of *Fasciola hepatica* shortly after detaching from its swimming tail and already starting encystment process (Orig. S. Mas-Coma)
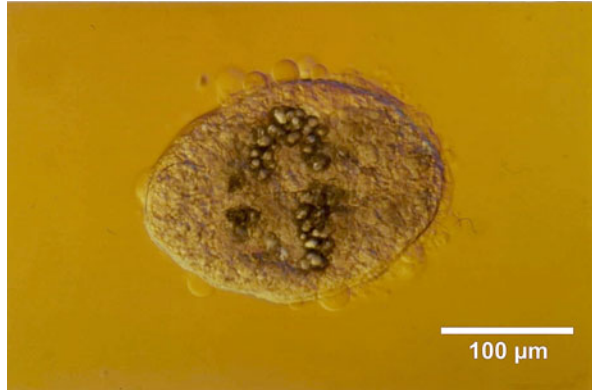
dependent on the environmental characteristics according to phases B, C and D and markedly influenceable by human activities at phase A.

Vectors of *Fasciola* are freshwater gastropod snails of the family Lymnaeidae. Different lymnaeid species transmit the two fasciolids, which show a marked and different specificity. There are species of Lymnaeidae which cannot transmit fasciolids, other lymnaeid species which transmit *F. hepatica*, other species which transmit *F. gigantica* and a very few which are able to transmit the two fasciolid species.

Fasciola hepatica is mainly transmitted by species of small size belonging to the so-called *Galba/Fossaria* group (Bargues et al. 2007, 2011a), including *Galba truncatula* as the main vector and the only one in Europe, but also present in Africa, Asia and South America. Other species of this group act as vectors in the Americas. *Lymnaea tomentosa* is the transmitter in Australia. *Fasciola gigantica* is transmitted by species of the genus *Radix*, mainly *R. natalensis* in Africa and varieties of *R. auricularia* and *R. viridis* in Asia. In Europe, however, *Radix* species do not show any epidemiological importance, given that only *F. hepatica* is present (Bargues et al. 2001). *Pseudosuccinea* is a monospecific genus including the species *P. columella* which has colonised all continents and appears to be able to transmit both *Fasciola* species (Bargues et al. 2011c).

The presence of lymnaeid vectors not only defines the distribution of fascioliasis but may also explain the distribution of human infection within a country, as has been recently observed in Venezuela (Bargues et al. 2011b) and Chile (Artigas et al. 2011), and within an endemic area, as well as its seasonality or permanent transmission (Mas-Coma et al. 1999c). Similar to other waterborne parasitic diseases, the transmission foci are patchily distributed within a human endemic area and linked to the presence of appropriate water collections, and human prevalences in schoolchildren appear to be related to the distance to waterbodies presenting lymnaeids (Mas-Coma et al. 1999c).

4.3 Epidemiology of Infection

A global analysis of the geographical distribution of human cases shows that the expected correlation between animal and human fascioliasis only appears at a basic level. High prevalences in humans are not necessarily related to areas where fascioliasis is a great veterinary problem. The major human health problems are known in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean (Cuba), Northern Africa (Egypt), Near East (Iran and neighbouring countries), Southeast Asia (Vietnam) and Western Europe (Portugal, France and Spain) (Esteban et al. 1998; Mas-Coma et al. 2005, 2009a).

In the human hyperendemic areas, though more prevalent and intense in children (with a peak in the 9- to 11-year age group), adult subjects are also infected. Adult subjects either maintain the parasites acquired when young or can be newly infected because of the high infection risk. The gender effect in fascioliasis is worth mentioning. Prevalences and/or intensities in human hyperendemic areas appear to be significantly higher in females. In Andean countries, females shed pronouncedly and significantly more eggs than males (Esteban et al. 1999, 2002), whereas in Egypt the prevalence in females appeared to be significantly higher than in males (Esteban et al. 2003).

4.3.1 Epidemiological Heterogeneity of Human Fascioliasis

After many years of studies on different areas presenting human infection by fasciolid liver flukes throughout the world, the classification of epidemiological situations proposed by Mas-Coma et al. (1999a) still appears to be fully valid and useful. This classification includes the following situations: (1) autochthonous, isolated, non-constant cases; (2) imported cases; endemic situations including (3) hypoendemic, (4) mesoendemic and (5) hyperendemic; and also epidemic situations comprising (6) epidemics in non-human endemic but animal endemic areas and (7) epidemics in human endemic areas.

Fascioliasis presents a very wide spectrum of transmission and epidemiological patterns in human hypo- to hyperendemic areas. These are related to the large diversity of environments, including different human endemic/epidemic situations; different human demographies, races, diets, habits, traditions and religions; different domestic and wild mammal reservoir species; different lymnaeid transmitting species; zones in both the Northern and Southern Hemispheres; altitudes from –27 m up to 4,200 m; hot and cold weathers; seasonal and yearly constant temperatures; scarce to pronounced annual rainfall; low and high mean annual potential evapotranspiration; and seasons from lack of dry period to lack of wet period through different dryness/humidity rates. From the landscape point of view, these areas include from altiplanos to valleys, from islands to mainlands, from

natural to artificial irrigations, from lakes to lagoons, from large rivers to small streams and from permanent to temporal waterbodies (Mas-Coma et al. 2003).

A classification of transmission patterns has been proposed (Mas-Coma 2005) and is progressively updated to offer a baseline for future research (Mas-Coma et al. 2009a). Up to the present, the following patterns have been distinguished: (1) a very high altitude pattern in Andean countries including the altiplanic pattern and the valley pattern (Valero et al. 2012c), (2) a Caribbean insular pattern, (3) a pattern related to Afro-Mediterranean lowlands, (4) a pattern related to Caspian surrounding areas and (5) a pattern related to lowland areas in Southeast Asia.

Thus, well-known situations and patterns of fascioliasis may not always explain the disease characteristics in a given area. Only once epidemiology and transmission characteristics of the new area are sufficiently assessed may appropriate control measures be designed for the endemic area in question.

4.3.2   Seasonality and Long-Term Impacts of Climate and Global Changes

Climatic factors are decisive in the transmission of fascioliasis, mainly temperature, rainfall and/or potential evapotranspiration (Mas-Coma et al. 2009b).

Variation of mainly rainfall and temperature gives rise to different fascioliasis seasonality. In Europe, the transmission of the disease is typically bi-seasonal, due to the activity periods of the lymnaeid vectors in spring and autumn. In the Bolivian Altiplano, however, the transmission takes place throughout the year, lymnaeid vector populations being always present because of inhabiting permanent waterbodies instead of temporary ones due to the high evapotranspiration rates at the very high altitude (Mas-Coma et al. 1999c). In other areas, the transmission appears mono-seasonal, due to the existence of only one intra-annual period with water availability.

Climate change overlaps other anthropogenic and environmental modifications which are included in the broad term of "global change" (Mas-Coma et al. 2009b). Thus, artificial field irrigation appears to be sufficient by its own to allow for fascioliasis transmission in the Peruvian Altiplano (Esteban et al. 2002). In the province of Punjab, in Pakistan, transmission includes bi-seasonality with a peak related to natural rainfall and another peak related to man-made irrigation (Afshan et al. 2014). Punjab is the first endemic area where the emergence of human infection has been correlated with a significant increase of fascioliasis transmission risk due to an impact of climate change throughout a 20-year period (Afshan et al. 2014).

4.3.3 Sources of Human Infection

Metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older. The maximum longevity was 48 weeks. Moreover, metacercarial viability and infectivity did not show differences between isolates from different reservoir species (Valero and Mas-Coma 2000).

The ingestion of infective metacercariae by humans may occur by different ways. Several infection sources have been distinguished (Mas-Coma 2004):

– Ingestion of freshwater wild plants: important in animal endemic areas
– Ingestion of freshwater cultivated plants, mainly watercress
– Ingestion of terrestrial wild plants: collected in dry habitats but which were submerged in water a few weeks or months before
– Ingestion of terrestrial cultivated plants needing frequent irrigation
– Drinking of contaminated water
– Ingestion of dishes and soups made with contaminated water
– Washing of kitchen utensils or other objects with contaminated water
– Ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration

Cultural traditions prove to be important in given endemic areas. Experimental studies showed the role that plant-made foods may play in human infection in Gilan Province, Iran (Ashrafi et al. 2006).

In Mexican children, an association between fascioliasis and the habit of eating raw vegetables was identified. The link of fascioliasis risk with consumption of raw vegetables other than watercress should be highlighted, as it suggests contamination when washing terrestrial vegetables with untreated water and/or in plant cultures using natural water for irrigation (Zumaquero-Ríos et al. 2013).

4.4 The Host Response to the Parasite

Immunologically, cell- and/or antibody-mediated response varies from host to host and in the same host according to the phase of the infection. Similarly, immunity to reinfection differs greatly from host to host.

In humans, studies on immunity are limited. It is generally believed that humans are not a suitable host, most migrating flukes becoming trapped in the liver parenchyma and dying without reaching the bile ducts. Considerable tissue reaction and calcification of the bile passages due to the flukes have been recorded (Acosta-Ferreira et al. 1979).

4.4.1 Immunological Processes

Fasciolid trematodes promote their own survival through several strategies to downregulate the host's immune response during the early phase of infection (Brady et al. 1999). Another study proved that immune response modulation occurs in advanced chronic fascioliasis too. The results indicated that during early chronic infection there was a predominance of a Th2 response, which decreased in the advanced chronic infection characterised by a persistent immune suppression (Girones et al. 2007). Fascioliasis is a potent inducer of Th2 responses which impair the ability to mount any effective Th1 responses against bacteria and other pathogens (Brady et al. 1999; O'Neill et al. 2000; Jaffar et al. 2004).

The rapid and potent ability of fasciolids to suppress the immune response explains why infected hosts do not develop resistance. Within 24 h after oral infection, peritoneal macrophages express markers for the Th2-associated phenotype and display a reduced ability to respond to Th1 stimulants. This implies that by the time the newly excysted juveniles have penetrated the intestinal wall and entered the peritoneum, they have already initiated the immune events that will dominate throughout infection. So, these early-stage parasites secrete immunomodulatory molecules that influence the function of innate cells (dendritic cells, macrophages, neutrophils, mast cells, etc.) in the intestinal wall and peritoneal cavity. A systemic antigen-specific Th2 response is firmly established already at 7 days post infection and is characterised by the secretion of IL-4, IL-5 and IL-13 from splenocytes. As the infection develops (3 weeks), regulatory macrophages (TGF-ß and IL-10 producing) and dendritic cells (IL-10 producing) are recruited to the peritoneum, and dendritic cell maturation is inhibited. Mast cells recruited to the site of infection exhibit impaired Th1-promoting abilities. Most CD4* T cells in the peritoneum secrete IL-10 but not IL-4 or IFN-γ. IL-10-secreting Tregs are induced which exert a suppression of both Th1 and Th2 cells that become non-responsive to parasite-specific antigens, and mesenteric lymph nodes produce IL-10 and IL-5, but not IFN-γ and IL-17, in response to stimulation by parasite antigens (Dalton et al. 2013).

The chronic disease is also typified by Th2 responses and suppressed by Th1 responses. Serologically, this polarity of immune response is strikingly displayed in the isotype of circulating antibodies. Fluke-infected animals secrete high titres of IgG1 antibodies and virtually no IgG2. Furthermore, blood macrophages are non-responsive to stimulation with endotoxin and exhibit elevated levels of arginase indicative of a phenotype that metabolises L-arginine and are important in promoting Th2 responses and facilitating tissue repair and fibrosis (Dalton et al. 2013).

4.4.2 Associations with Other Parasites

A consequence of liver fluke infection is the suppression of immune responses directed against concurrent pathogenic infections. The synergistic capacity of fasciolids in co-infection with other pathogenic agents is well known, immunological responses to pathogen antigens being markedly suppressed and concomitant infection being exacerbated following fascioliasis infection. The parasitological spectrum of protozoan and helminthic species found in the inhabitants of the human endemic areas, the multiparasitisms and the associations between liver fluke infection and infection by other pathogenous parasites all appear to be similar in the different human endemic zones (Esteban et al. 1997a, b, 1999, 2002, 2003; Gonzalez et al. 2011). These synergistic associations of fascioliasis with other pathogens are believed to underlie the high morbidity and mortality rates of Aymara children inhabiting the Northern Altiplano (Mas-Coma 2004).

In animals, the clinic synergistic capacity of *F. hepatica* in concomitant infection with other pathogenic agents is well known. In humans, the association of *F. hepatica* with other parasites has been assessed in recent large surveys performed in human fascioliasis endemic areas. In the Bolivian Altiplano endemic zone, among the samples positive to *F. hepatica*, the number of other parasite species found varied from one to eight. In 35.5 % of the cases with *F. hepatica* eggs, the liver fluke showed to be the sole parasite species present in which a pathogenic capacity is well known. *Fasciola hepatica* infections associated with one (34.9 %), two (23.7 %), three (4.6 %) and four (1.3 %) of the other generally recognised pathogenic parasites were recorded. A significant positive association of great health importance in children was found with *Giardia intestinalis*, in both Bolivia and Peru (Esteban et al. 1997a, b, 1999, 2002).

4.5 Pathology

Pathogenesis depends on the number of flukes. The penetration of the duodenum or jejunum wall by metacercariae may cause focal haemorrhages and inflammation, although lesions may not be clinically evident. The fluke migration through the liver parenchyma for 4–6 weeks or longer induces the major pathological changes. Parasites digest hepatic tissue and cause extensive parenchymal destruction with intensive haemorrhagic lesions and immunological and inflammatory reactions. Migration tracks are observed in histological sections. Migratory flukes sometimes die leaving cavities filled with necrotic debris, and considerable liver areas may subsequently be replaced by scar tissue (Mas-Coma et al. 1999b, 2000).

A small proportion of the flukes may reach the bile ducts where they may live for even years. Less pathogenic effects may be caused in the bile ducts, although inflammation resulting in fibrosis, thickness and expansion is common (Fig. 4.3) (Chen and Mott 1990). The ultrastructural picture revealed bile ductular

Fig. 4.3 Histopathological section showing a specimen of *Fasciola hepatica* in a rat biliary duct. See section of large sucker inside and prominent spines in the tegumental external surface of the worm (Orig. S. Mas-Coma)
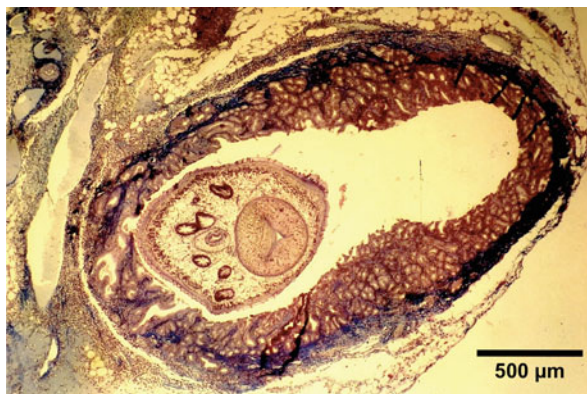
hyperplasia, fibrosis of portal tracts, widening of the interhepatic spaces by many microvilli and dilated Disse space with collagen fibres. Bile ductular hyperplasia may be the initial factor to fibrinogenesis, which subsequently enhances the development of the microvilli on the surface of the hepatocytes.

Anaemia is one of the most characteristic symptoms, especially in heavier infections. Blood loss into the bile seems most probably to be an important factor contributing to severe anaemia. An association between anaemia and fluke burden (the most important), epg, fluke body area, presence of blood in faeces, IgG1 and eosinophil levels and % of splenic weight was verified in a multivariate analysis. These results lead to the assumption that a high risk of anaemia in subjects with a heavy parasitic burden in human hyperendemic areas is to be expected (Valero et al. 2008). These results are crucial, because although there were several reports listing anaemia in patients from endemic areas, results could only be considered with great caution because coinfections were never excluded in those papers and in fact it becomes very difficult, not to say almost impossible, to find subjects from endemic areas only infected by fascioliasis. And among those parasites coinfecting fascioliasis-affected subjects, many are also known to cause anaemia.

The process may be summarised as follows: (a) the fluke is a blood feeder but may also feed on tissue; (b) haemorrhages may occur from the erosion of the biliary epithelium due to the infection; (c) reticulocytes are increased in the peripheral blood; (d) generalised haemolysis is absent; (e) there is no evidence of plasma iron or vitamin B_{12} deficiencies, although a significant reduction in serum iron has recently been detected in patients with chronic fascioliasis.

The most important pathogenic sequelae are hepatic lesions and fibrosis, and chronic inflammation of the bile ducts, given that the disease is chiefly confined to the liver. Unlike clonorchiasis or opisthorchiasis, no association with biliary carcinoma has been reported (Mas-Coma and Bargues 1997).

4.5.1 Liver and Biliary Tract

The liver appears usually enlarged with a smooth or uneven surface. The most common macroscopic lesions are multiple soft, yellowish or grey-white nodules ranging from 2 to 30 mm in diameter, which correspond to eosinophilic abscesses. Nodules are also observed in the parietal peritoneum proximal to the liver and on the round liver ligament. Haemorrhagic stippling appears at the margin of the nodules. White or yellow striae, surrounded by telangiectasia, are observed on the liver capsule. Close to the nodules, ribbed or vermiform formations with similar colour and consistency as nodules are also observed under laparoscopy. Hepatic capsular thickening of varying degree appears, and in a few cases the entire hepatic capsule appears thickened. Subcapsular lymphatic vessels are dilated. The lymph nodes near the porta hepatis may be markedly enlarged. Multiple subcapsular cavities filled with necrotic material were observed below the liver capsule in one patient who died. Several reddish-purple tracks radiated from the nodules, whereas others were greyish white and fibrous. The tracks extended from the liver capsules and ended in subcapsular cavities. Most of the lesions are less than 20 mm below the capsules (Acosta-Ferreira et al. 1979). In cases with marked involvement of the peritoneal wall and the liver surfaces, yellow and opalescent ascites was present. Apart from mild splenomegaly in two patients, no significant portal hypertension was found in laparoscopic examinations of several patients.

The common bile ducts are usually large (Fig. 4.3) and dilated, and the wall is thickened upon palpation. The gall bladder wall is greatly thickened and oedematous. Multiple greyish-white subserous nodules are present, and adhesions of the gall bladder to adjacent structures are common. The mucosal folds of the gall bladder appear prominent. The wall of the gall bladder appears thickened owing to muscular hypertrophy and perimuscular fibrosis. There is glandular epithelial hyperplasia. All layers of the wall contain patchy infiltrates with lymphocytes, plasma cells and eosinophils (Acosta-Ferreira et al. 1979).

Lithiasis, often multiple, in the common bile ducts and gall bladders is very frequent (Arjona et al. 1995), whereas cirrhosis does not appear to be so (Marcos et al. 2009). The presence of gallstones was experimentally proved to increase with infection time. Therefore, the lithogenic induction by infection becomes manifest in situations of advanced chronicity. Gallstone presence was strongly associated with the number of flukes located in the bile duct. The risk of pigment stones appears to depend mainly on factors that favour bile duct obstruction (cholangitis, fluke body development versus time, intensity of infection). Situations of undiagnosed cases, as in subjects presenting undistinguishable symptoms or in those keeping their infection for a long time because of non-treatment or of repetitive reinfections, usually in human endemic areas of developing countries, imply a higher lithiasis risk. Thus, a high gallstone risk may be expected in subjects inhabiting human hyperendemic areas where very high egg outputs detected in humans suggest that liver fluke burdens may also be very high (Valero et al. 2003).

Generally, the migration tracks can be found in the liver and other organs. The walls of the tracks in the liver often contain Charcot–Leyden crystals and eosinophils. The cavities of the tracks are filled with necrotic cellular debris, including hepatocytes, fibrin and red cells. A considerable eosinophilic infiltrate surrounds the tracks. Longer tracks can cross several hepatic lobules. In older lesions, macrophages, lymphocytes, eosinophils and fibrous tissue are observed. Focal calcification is sometimes seen in the margin of the necrotic debris. Calcifications may form the outline of a dead fluke (Acosta-Ferreira et al. 1979).

Egg granulomas have been reported. Multinuclear giant cells surround a single egg with subsequent layers of epithelioid cells and fibrous tissue, plasma cells, lymphocytes and eosinophils. The portal triads are dilated and oedematous with infiltrates of lymphocytes and eosinophils. Bile duct proliferation, periductal fibrosis, necrotising arterial vasculitis and portal venous thrombosis are frequent (Acosta-Ferreira et al. 1979; Chen and Mott 1990).

4.5.2   Other Locations and Ectopic Fascioliasis

Juvenile flukes may deviate during migration, enter other organs and cause ectopic fascioliasis. In humans, the most frequent ectopic lesions are those of the gastrointestinal tract (Acosta-Ferreira et al. 1979). Other ectopic locations reported are the subcutaneous tissue, heart, blood vessels, lung and pleural cavity, abdominal wall, appendix, pancreas, spleen, inguinal nodes, cervical node, skeletal muscle and epididymis. Such ectopic flukes almost never achieve maturity (Mas-Coma et al. 2014). The usual pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis. Parasites may be calcified or become incorporated in a granuloma (Arjona et al. 1995; Mas-Coma and Bargues 1997).

Very recently, a wide analysis has shown that neurofascioliasis or intracranial infection by *Fasciola* and ophthalmofascioliasis or direct affection of the eye by migrating flukes may be rare, although not sporadic as previously believed. However, manifestations including a very wide range of neurological symptoms, signs and syndromes, together with meningeal, psychiatric or neuropsychic manifestations, and ocular disorders caused at distance by flukes infecting the liver may be frequent but underestimated due to misdiagnosis, mainly in low-income regions. The impressive clinical pictures should be highlighted. They include from hemiplegia and paraplegia to disturbances and difficulties of walking capacity, speech disorders, convulsions, epilepsy and coma, amnesia or visual hallucinations and permanent blindness, only to mention a few, plus the clinical complexity of the puzzling polymorphisms, the disconcerting multifocality of the manifestations and their changes along the evolution of the disease in a same patient, as well as differences between the clinical pictures shown by different patients. Moreover, these studies emphasise post-treatment sequelae and mortality in neurological

patients and the need to consider neurological fascioliasis when estimating the global burden of this disease (Mas-Coma et al. 2013, 2014).

4.6 Clinical Manifestations

The following clinical periods can be distinguished: incubation period (from the ingestion of metacercariae to the appearance of the first symptoms) and infection periods including the invasive or acute phase (fluke migration up to the bile ducts), the latent phase (maturation of the parasites and starting of oviposition) and the biliary, chronic or obstructive phase. Of these four periods, the invasive or acute phase and the biliary or chronic phase are the most important and in which most of the patients are detected.

The incubation period varies considerably depending on the number of metacercariae ingested and the host's response. The period of incubation in humans has not yet been accurately determined: only "a few" days, 6 weeks, 2–3 months or even more (Mas-Coma et al. 1999b).

The latent phase can last for months or years. The proportion of asymptomatic subjects in this phase is unknown. They are often discovered during family screening after a patient is diagnosed, confirmed after clinical suspicion or in epidemiological surveys by finding the eggs in the duodenal fluid and/or in the stool (Arjona et al. 1995). An unexplained, prominent eosinophilia may already be suggestive of infection (Gil-Benito et al. 1991). These persons may have gastrointestinal complaints or one or more relapses of the acute symptoms during this phase (Mas-Coma and Bargues 1997).

4.6.1 Invasive or Acute Phase

In this phase, the symptomatology is due mainly to the mechanical destruction of the liver tissue and of the abdominal peritoneum by the migrating larvae causing localised or generalised toxic and allergic reactions lasting 2–4 months. However, in endemic areas, *F. hepatica* infection is usually repetitive and the acute lesions are superimposed on chronic disease. Thus, the acute phase may be prolonged and overlap on to a latent or an obstructive phase.

The major symptoms of this period include (Mas-Coma et al. 1999b):

– *Fever*: it is usually the first symptom, usually low or moderate but may reach 40 °C and in heavily infected cases as high as 42 °C; it may be remittent, intermittent or irregular with higher temperature in the evening; in some cases, a low, recurrent fever lasted for a long time (4–18 months).

– *Abdominal pain*: from mild to excruciating, sometimes vague, it may be gener-
   alised at the outset but is usually localised in the right hypochondrium or below
   the xiphoid.
– *Gastrointestinal disturbances*: loss of appetite, abdominal flatulence, nausea and
   diarrhoea are common, whereas vomiting and constipation are infrequent.
– *Urticaria*: it is, with dermatographia, a distinctive feature in the early stage of
   the fluke invasion and may be accompanied with bouts of bronchial asthma.
– *Respiratory symptoms*: cough, dyspnoea, haemoptysis and chest pain occur
   occasionally but in some cases are the first manifestation of infection.

The following signs may appear in the invasive phase on physical examination
(Mas-Coma et al. 1999b):

– *Hepatomegaly and splenomegaly*: the liver is usually enlarged and tender,
   sometimes reaching down to the right iliac fossa, but it is never hard; the degree
   of hepatomegaly seems to increase during the course of the disease, and hepatic
   abscesses are detected; splenomegaly is not common but has been many times
   reported.
– *Ascites*: this sign has been reported several times; it is yellow with a high
   leucocyte count, eosinophils predominating; the pathogenesis is considered to
   be an inflammatory response to a large number of juvenile flukes penetrating the
   intestinal walls, irritation of the peritoneum and penetration through the liver
   capsule during their migration rather than hepatic failure per se.
– *Anaemia*: mild to moderate anaemia can be seen; pallor of the skin and mucosa is
   commonly associated with lassitude, dizziness, palpitation and weakness.
– *Chest signs*: on auscultation, dry or moist rales can occasionally be elicited upon
   coughing at the base of the right lung probably due to migration of the juvenile
   flukes; pleural rub with effusion and even spontaneous pneumothorax have been
   reported; parenchymal infiltrates resembling the Loeffler syndrome and pleural
   effusion are the most common radiologic manifestations; pyopneumothorax has
   been also reported.
– *Jaundice*: it is infrequent and when it appears, it is milder than that seen in the
   chronic phase.

In human endemic zones, there is usually a decrease of the prevalence from
children and young subjects to adult subjects. Despite this, results demonstrate that
adult subjects either maintain the parasites acquired when young or can be newly
infected as the consequence of inhabiting a zone of high infection risk (Esteban
et al. 1999). It must be considered here that the life span of the adult fluke in humans
is between 9 and 13.5 years (Mas-Coma and Bargues 1997). Such a picture suggests
that, in those areas, the majority of adult subjects should be in the biliary period,
acute lesions by repetitive infections being superimposed on chronic disease with
relative frequency. Thus, the acute period may be prolonged and overlap with both
latent and biliary periods.

4.6.2 Biliary or Chronic Phase

This phase may develop after months to years of infection. Adult flukes in the bile ducts cause inflammation and hyperplasia of the epithelium. There is thickening and dilatation of the ducts and the gall bladder walls ensue. The resulting cholangitis and cholecystitis, combined with the large body of the flukes, are sufficient to cause mechanical obstruction of the biliary duct which is comparatively small in diameter. The proportion of those whose infection develops into the obstructive phase or their prognosis has not been defined.

In this phase, biliary colic, epigastric pain, fatty food intolerance, nausea, jaundice, pruritus, right upper-quadrant abdominal tenderness, etc., are clinical manifestations indistinguishable from cholangitis, cholecystitis and cholelithiasis of origins other than *Fasciola* infection. Hepatic enlargement may be associated with an enlarged spleen or ascites (Acosta-Ferreira et al. 1979).

The common bile ducts are usually seen as distended and thickened. The diameters are 1.5–3.0 times the normal size. The most frequent site of obstruction is the common bile duct. A diverticulum in this duct has been observed, and the head of the pancreas was enlarged and firm.

In case of obstruction, the gall bladder is usually enlarged and oedematous with thickening of the wall. The gall bladder may measure $12 \times 7 \times 7$ cm and the lower edge reaches the umbilicus. Fibrous adhesions of the gall bladder to adjacent organs are common. Lithiasis of the bile duct or the gall bladder is frequent, and the stones are usually small and multiple (Chen and Mott 1990; Arjona et al. 1995). The bile duct and the gall bladder may contain blood mixed with bile (haemobilia), blood clots and fibrinous plugs.

Symptomatology in children from human endemic areas of Peru includes abdominal pain localised in the epigastrium, the Murphy symptom and jaundice as the most frequent clinical biliary characteristics, the rest of the symptoms being non-specific (Marcos Raymundo et al. 2002).

The duration and intensity of fasciolid infection and liver damage have been experimentally verified to be associated with bacterobilia by *Escherichia coli* (45 % of cases), *Enterococcus faecalis* (45 %) and *Klebsiella pneumoniae* (10 %). This supports that the obstruction caused by advanced chronic fascioliasis may be related to biliary sepsis. These results lead to the reconsideration of treatment features in human disease, i.e. therapeutic strategies should also consider the possibility of bacterial co-infection (Valero et al. 2006b).

4.6.3 Clinical Laboratory Analyses

The outstanding abnormal laboratory findings concern leucocytosis, eosinophilia, anaemia, erythrocyte sedimentation rate, hepatic functions and serum immunoglobulin levels (Chen and Mott 1990; Mas-Coma et al. 1999b, 2000):

– *Leucocytosis and eosinophilia*: in the acute phase, the leucocyte counts are usually over 10,000/mm^3 up to 43,000/mm^3. The eosinophil count is nearly always greater than 5 % of the total leucocytes and may be as high as 83 %. In a developed country, blood eosinophilia and the ingestion of watercress or any other suggestive freshwater plant in anamnesis are extremely useful in guiding towards a fascioliasis diagnosis. Unfortunately, these two aspects are usually not helpful in human endemic areas of developing countries, where eosinophilia may be also caused by other helminth infections and local food traditions including the ingestion of many uncooked plants may mask liver fluke infection sources (Mas-Coma et al. 2014).

– *Anaemia*: anaemia is common, but usually not very severe and mostly between 7.0 and 13.5 g dl^{-1} haemoglobin levels. Levels as low as 2.8 and 4.0 g dl^{-1} have been reported.

– *Erythrocyte sedimentation rate*: the erythrocyte sedimentation rate may be high in the acute phase, reaching 165 mm in an hour, normal in the latent phase and normal or only moderately high in the obstructive phase.

– *Hepatic functions*: abnormal results in liver function tests may be found in both the invasive and biliary periods.

In the invasive period, data are inconsistent. Abnormal results may be obtained in hepatic function tests. In the acute phase, results sometimes include a rise of the two aminotransferases (formerly transaminases) most frequently utilised, namely, alanine aminotransferase (ALT, formerly serum glutamic pyruvate transaminase—SGPT) and aspartate aminotransferase (AST, formerly serum glutamate oxaloacetic transaminase—SGOT), as well as elevated thymol turbidity, zinc sulphate turbidity, serum globulin and serum bilirubin. In other cases, tests give normal results, with the exception of alkaline phosphatase (AKP or ALP). Serum electrophoresis may show an increase of α_2- and γ-globulins. Serum triglycerides and very-low-density lipoproteins have been seen to increase, while total serum cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol exhibited a significant decrease. These changes were due to the degenerative necrotic damage of the hepatocytes. Other reported findings include abnormally high levels of β-glucuronidase.

In the biliary period, jaundice is a prominent feature. Serum bilirubin levels between 2.0 and 8.6 have been reported. Biliary colic is usually followed by a higher level of serum bilirubin as well as dark urine positive for bilirubin. Serum bilirubin may be normal in this phase and between attacks of biliary colic. AKP, GPT, GOT and serum globulin (mainly γ-globulin) are often elevated in this phase, while albumin is decreased.

– *Immunoglobulins*: levels for IgG, IgM and IgE are usually elevated. Specific IgE antibodies were detected in 48 % of the patients. Total and specific IgE levels have been shown to be positively correlated with the egg burden, age, clinical features and degree of eosinophilia. IgA levels are usually normal but may be sometimes elevated.

4.7 Diagnosis

Several suggestive clinical presentation aspects may be useful, mainly in human endemic areas where physicians are aware about liver fluke infection risk in humans. However, verification needs the use of at least one among the direct parasitological techniques or indirect immunological tests. Other non-invasive diagnostic techniques presently available may be additionally helpful. Non-invasive diagnostic techniques which can be used for human diagnosis are radiology, radioisotope scanning, ultrasound, computed tomography and magnetic resonance (see reviews in Esteban et al. 1998 and Hillyer 1999).

For the differential diagnosis of *F. hepatica* and *F. gigantica*, clinical, pathological, coprological or immunological methods are useless. This is a problem in overlapping areas because this differential diagnosis is very important owing to the different pathological, transmission and epidemiological characteristics of the two fasciolids, as well as due to intermediate forms in which egg measurements may overlap. Despite the recent development of many molecular tests, DNA marker sequencing still remains as the only appropriate method for both the haplotyping of the two pure fasciolid species and the detection of hybridisation in intermediate forms. For such a purpose, the complete sequences of the two rDNA spacers ITS-2 and ITS-1 together with those of the complete mtDNA genes *cox*1 and *nad*1 have so far proved to be the markers of choice, and an exhaustive baseline and nomenclature for these four markers have already been provided (Mas-Coma et al. 2009a).

4.7.1 Direct Techniques

Detection and identification of fasciolid eggs in stool sample, duodenal contents or bile continue to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognised lower sensitivity of egg detection in faecal samples and its uselessness for the diagnosis of patients in the acute period, as well as the lack of an accurate relationship between the egg counts per g of faeces and the fluke burden (Valero et al. 2006a, 2009). Identifying fluke adults obtained during an endoscopy after surgical intervention by either microscopic morphometry (Periago et al. 2006) or molecular tools (Mas-Coma et al. 2009a) may also be performed nowadays, although such occasions are evidently not frequent at all. Moreover, the infrastructure for endoscopy or surgery is in general not available in rural endemic areas.

Techniques ranging from a simple direct smear to different concentration methods may be used. Egg concentration has been achieved by flotation and sedimentation techniques. The sedimentation techniques appear to be more accurate and sensitive than flotation techniques (Esteban et al. 1998; Mas-Coma et al. 1999a).

The size of the fluke eggs has always been used for human diagnosis. Basing on studies in livestock, the borderlines allowing differentiation between the two species were traditionally considered to be 150 μm in length and 90 μm in width, lower values representing *F. hepatica* and higher values *F. gigantica*. A study on morphometric traits of fasciolid eggs from different continents revealed that eggs shed by humans show traits different from eggs shed by animals. In humans, *F. hepatica* eggs are bigger and *F. gigantica* eggs are smaller than reported to date from livestock, and their measurements overlap when compared. Measurements of *F. hepatica* and *F. gigantica* eggs originating from humans and animals from sympatric areas overlap, and, therefore, they do not allow differential diagnosis when within this overlapping range (Valero et al. 2009; Mas-Coma et al. 2014). These new results should aid clinicians since the application of the classic egg size range in human samples may lead to erroneous conclusions. Consequently, fasciolid egg size in human stool samples ought to be corrected in books and monographs as well as in guides of medical parasitology and tropical medicine.

Quantitative coprological analyses become important in epidemiological surveys as well as post-treatment monitoring. Egg burden is also crucial in the moment of deciding the appropriate treatment dose. The 400-epg threshold has been proposed for identifying high-intensity infections. To avoid risk of colic, a repeated, timely spaced mid-dose is recommended in patients shedding more than 400 eggs (WHO 2007; Valero et al. 2012a). The second half of the regimen is administered 24 h later, once the absence of secondary effects verified. The Kato–Katz technique appears to be appropriate, because of its simplicity, very low cost and reproducibility (Mas-Coma et al. 1999b). Its low sensitivity may be solved by repeated application.

Besides eggs in coprological analyses, adults and eggs may be also found elsewhere by means of other invasive techniques: obtaining duodenal fluid and duodenal and biliary aspirates, surgery (laparotomy, cholecystectomy, sphincterotomy), histological examination of liver and/or other organ biopsy materials (Mas-Coma et al. 1999b).

4.7.2   Indirect Techniques

Numerous serological, intradermal and stool antigen detection tests have been developed. Immunological techniques present the advantages of being applicable during all periods of the disease but fundamentally during the invasive or acute period, as well as to other situations in which coprological techniques may present problems. However, immunological techniques offer other types of problems related mainly to sensibility and specificity and are not able to differentiate between infection by *F. hepatica* and that by *F. gigantica*.

Efforts have been concentrated in obtaining purified excretory/secretory antigens and/or recombinant molecules to improve serological tests, owing to the

problems of the parasitological diagnosis because of the delay in its usefulness in the acute period (coprological examination positive only after 3–4 months post infection), intermittent egg output dynamics, very low or even absence of egg shedding in cases of only one or a few adult flukes and old, chronic infections, ectopic infections and "false" fascioliasis related to eggs in transit after ingestion of infected liver from domestic animals or flukes unable to attain maturity in human subjects in non-human endemic areas (Esteban et al. 1998; Mas-Coma et al. 1999b).

Cysteine proteinases offer highly sensitive and specific markers for human fascioliasis serodiagnosis for *F. hepatica* (O'Neill et al. 1999; Strauss et al. 1999; Espinoza et al. 2007; Mezo et al. 2004) as well as for *F. gigantica* infection (Maleewong et al. 1999; Intapan et al. 1998; Tantrawatpan et al. 2005). *Fasciola hepatica* recombinant cysteine proteinases produced in yeast (O'Neill et al. 1999) or in *Escherichia coli* (Carnevale et al. 2001) have been used in ELISA methods for human infection diagnosis.

Studies in Bolivia and Peru have shown that the MM3 coproantigen-detection test allows for high sensitivity and specificity, fast large mass screening capacity, detection in the chronic period, early detection of treatment failure or reinfection in post-treated subjects and usefulness for surveillance programmes. However, this technique falls short when evaluating the fluke burden on its own (Valero et al. 2012a). The use of a new preservative/diluent CoproGuard[TM], developed for preservation of *Fasciola* coproantigens, proved to enhance coproantigen extraction and the antigenicity throughout the complete observation period (Ubeira et al. 2009).

The commercialised DRG *Fasciola hepatica* IgG (human) ELISA proved to be highly sensitive and specific, with a high negative predictive value but a low positive predictive value. No correlation with egg output was observed. This test may be used both as an individual serodiagnostic test when backed up by a compatible clinical history together with a second diagnostic technique for other cross-reactive helminth infections and in future large-scale epidemiological studies (Valero et al. 2012b).

A new lateral flow test (SeroFluke) for human diagnosis appears to be a useful step forward (Martinez-Sernandez et al. 2011). In comparison with an ELISA test (MM3-SERO), the SeroFluke test showed maximal specificity and sensitivity and the advantage of being applicable to both serum and whole blood samples. Its simplicity allows it to be used in major hospitals as well as in endemic/hyperendemic regions.

4.8 Treatment

Emetine and the better tolerated dehydroemetine were used widely and still continue to be in use today, given intramuscularly or subcutaneously at doses of 1–10 mg/kg a day for 10 days. However, the use of emetine was progressively

abandoned due to their toxic side effects involving heart, liver and digestive tract (Mas-Coma et al. 2014).

Chloroquine improved the symptoms when applied in the acute phase. Bithionol was proposed as the drug of choice for fascioliasis treatment during the last three decades of the last century. It was usually applied at a dose of 30–50 mg/kg daily, divided into three oral doses on alternate days for 20–30 days. Occasionally, the patients required a second course to obtain a complete cure. The side effects were usually mild (Chen and Mott 1990; Esteban et al. 1998).

Regarding praziquantel, it is generally accepted today that *Fasciola* may be the only trematode genus that has practically no response to praziquantel. Metronidazole and albendazole and sporadically also mebendazole have been also applied for human fascioliasis treatment with more or less success.

Triclabendazole (Egaten®) has become the drug of choice for human fascioliasis caused by both *F. hepatica* and *F. gigantica* at present (Savioli et al. 1999). This drug is better adsorbed if administered after meals (Lecaillon et al. 1998). The recommended dosage is two separate regimens of 10 mg/kg. Cure rates of 79.2 % when first used and 100 % after a second round of therapy were found in Chile (Apt et al. 1995) and 79.4 % and 93.9 %, respectively, in Egypt (El-Morshedy et al. 1999). Triclabendazole appears to keep its efficiency at standard regimes in human endemic areas after years (Talaie et al. 2004), although the need for a third dose has been reported in Cuba (Millan et al. 2000).

Unfortunately, the risk of appearance of resistance to triclabendazole cannot be forgotten. Triclabendazole resistance was first described in Australia, later in European countries such as Ireland, Scotland, the Netherlands and Spain (see review in Mas-Coma et al. 2007). Very recently it has also been found in Southern Brazil (Oliveira et al. 2008) and Argentina (Olaechea et al. 2011). Up to that moment, triclabendazole resistance only concerned livestock in animal endemic areas, but unfortunately it has very recently been also described (Ortiz et al. 2013) in a human highly endemic area such as Cajamarca, Peru (Gonzalez et al. 2011).

Nitazoxanide is a good alternative to triclabendazole, at least for the chronic stage of fascioliasis, mainly in those countries where Egaten® is still not registered but nitazoxanide is for several years. Nitazoxanide had demonstrated its efficacy against human fascioliasis in a few trials, in Egypt (Rossignol et al. 1998; Kabil et al. 2000) and Peru (Favennec et al. 2003). Its long 7-day treatment course may nevertheless become a problem. However, its usefulness for the treatment of human cases not responding to triclabendazole (Gargala et al. 2005) is of important additional value. A good nitazoxanide efficacy has recently been reported when applied to liver fluke-infected children in Mexico (Zumaquero-Ríos et al. 2013). However, differences in fasciolid susceptibility to nitazoxanide may exist depending on geographical strains. Thus, no response to nitazoxanide treatment was reported in 24 cases of liver fluke infection in Esmeralda, Camagüey, Cuba (Del Risco Barrios et al. 2001), and a triclabendazole-resistant *F. hepatica*-infected patient not responding to nitazoxanide treatment has recently been reported in the Netherlands (Winkelhagen et al. 2012).

4.9 Prognosis, Complications, Sequelae and Death Causes

The prognosis of fascioliasis depends on the promptness of the treatment. At the phase of hepatic invasion (acute phase), the treatment most usually gives rise to cure without sequelae. However, when treated late, the prognosis becomes subordinated to the importance of the affection of the biliary ducts.

With regard to cure criteria, from the clinical point of view, the improvement of the general condition of the patient with fever disappearance and weight recovery indicates a good prognosis, although one should be cautious as relapses are possible. The normalisation of blood eosinophilia as best criterion in all cases, the disappearance of eggs in stools in cases of patients diagnosed in the biliary phase and the progressive disappearance of hepatic function test abnormalities are helpful indicators of treatment effectivity (Mas-Coma et al. 2014).

One complication may be bleeding. Subcapsular haematoma and severe haemobilia have been reported. Haematemesis and melaena were associated with obstructive jaundice, epigastric pain and severe anaemia. The sources of the bleeding were all identified from laparotomy, and other causes of upper gastrointestinal bleeding have been carefully ruled out. In one case, bleeding occurred due to an ulcer in the common bile duct, and in other cases no single bleeding point was detected in the common bile ducts and/or in the gall bladders.

Biliary cirrhosis is another complication. During the course of the infection, inflammation, hyperplasia and hypertrophy of the bile duct epithelia may induce periductal fibrosis. Prolonged heavy infection rarely leads to biliary cirrhosis. The outstanding findings on physical examinations were firm untender hepatomegaly with or without jaundice and ascites. Splenomegaly was not prominent. Sclerosing cholangitis with biliary cirrhosis was detected by endoscopic retrograde cholangiopancreatography.

Another serious complication is the multiple extrahepatic venous thromboses. In the post-mortem examination of one patient who died suddenly, multiple thromboses of the ovarian, suprahepatic, mesenteric and myocardial veins, along with massive pulmonary embolism, were disclosed. During the invasion period, another patient developed a complete thrombosis of the superior vena cava (Mas-Coma et al. 1999b, 2000).

The seriousness of fascioliasis was emphasised in many cases, with regard to the progressive general deterioration of the patients with slimming, anorexia and physical and neuropsychic asthenia, as well as the fact that cure does not mean total recovery but that fascioliasis left them as handicapped and frail subjects. In several patients presenting neurological and ocular manifestations, given sequelae were noted to persist despite treatment. These sequelae were sometimes important, even making difficult or impeding the professional activity of the patient (Mas-Coma et al. 2014).

Several deaths related to fascioliasis have been reported in the recent literature. Post-mortem examination of a patient who died from an acute liver condition showed *F. hepatica* to be responsible for blockage of the bile duct. More than

40 flukes were detected from the liver of a patient at autopsy. Between 1 and 14 flukes were found in each liver at post-mortem in 81 inhabitants of the Samarkand region in 1968–1986, although deaths were not presumed to be due to fascioliasis. However, in human hyperendemic zones with depauperate socio-economic status, unhygienic conditions and high child morbidity and mortality, studies are still needed to ascertain whether fascioliasis may be related to death, above all in very young children (Mas-Coma and Bargues 1997; Mas-Coma et al. 2000). Many fatal cases have been reported among patients suffering from neurological disorders, whether due to direct or indirect affection of the central nervous system (Mas-Coma et al. 2013, 2014).

4.10 Prevention and Control

Studies on human endemic areas performed in the last two decades have shown that traditional epidemiological patterns of animal fascioliasis may not always explain the characteristics of human infection in a given area. Therefore, control measures for human fascioliasis should consider the results of the ecoepidemiological studies previously undertaken in the area concerned (Mas-Coma et al. 2009a). This is the reason why the World Health Organization (WHO) launched a worldwide initiative against this disease including different control strategies depending on the human endemic areas and countries.

4.10.1 Prevention of Human Infection

The prevention of human infection may be achieved by strict control of the human infection sources, mainly with regard to watercress and other aquatic plants for human consumption, especially in endemic zones. Unfortunately, potassium permanganate, which had been suggested to be the most effective preventive tool for killing metacercariae attached to leaves and vegetables used in salads, has been shown to have no effectivity on metacercarial viability, even at very high doses (Ashrafi et al. 2006).

Moreover, it should be considered that infection risks shall not be restricted to only ingestion of freshwater vegetables, as always mentioned. The different human infection sources may be taken into account, mainly in human endemic areas. Drinking of natural freshwater should be avoided in human endemic areas. In the Nile Delta region, persons living in houses where piped water is present showed to have a higher infection risk (Curtale et al. 2003).

The possibility of human infection in urban areas should not be neglected. Thanks to transport of vegetables (both aquatic and terrestrial) from rural endemic zones to cities, plants carrying metacercariae can be sold in non-controlled city markets giving rise to urban infection (Fig. 4.4) (Mas-Coma 2004).

Fig. 4.4 Uncontrolled sale of vegetables involved in the transmission of human fascioliasis in a city market in Quy Nhon, Vietnam (Orig. S. Mas-Coma)

   Education should always be included within general control measures to be applied in human endemic areas, mainly to let inhabitants know about the human infection sources. The community should be appropriately informed about the disease, its pathogenicity, its transmission and where to go for diagnosis if suggestive symptoms appear.

4.10.2 Control Measures at Community Level

The availability of triclabendazole prompted the WHO to launch a decisive step forwards within its worldwide initiative against human fascioliasis (WHO 2007, 2008) in recent years. This initiative includes action in human fascioliasis endemic areas presenting different epidemiological situations and transmission patterns (Mas-Coma 2005; Mas-Coma et al. 2009a). Pilot schemes were designed to assess the best control strategies according to the different epidemiological situations and transmission patterns in the way to decrease morbidity, mainly in children. Selective patient treatment after passive detection in hospitals was the strategy applied in Vietnam, and infected subject treatment after active detection in surveys the one applied in Egypt. The Northern Altiplano in Bolivia was chosen as an example of the altiplanic pattern, while Cajamarca, Peru, was chosen as an example of the valley pattern. The pilot interventions in these two Andean areas demonstrated the absence of serious side effects in triclabendazole treatments of schoolchildren (Villegas et al. 2012), which subsequently allowed for the launching of mass treatments. Many other countries are nowadays receiving yearly triclabendazole donations through WHO for the treatment of their patients, in an expansion of the aforementioned WHO initiative.

   In countries where watercress is included in food traditions, such as France, commercial growing of watercress should be carried out under completely

controlled conditions, without access for ruminants and snail vectors to the water-cress cultures.

In Egypt, the construction and utilisation of the so-called washing units, in which the water was appropriately filtered, gave rise to a marked decrease of human infection in a locality of the Nile Delta region where a high prevalence in humans was initially found (Mas-Coma 2004).

4.10.3 Progress in Vaccinology

Initial research focused on the identification of molecules of fasciolid parasites that played critical functions at the host–parasite interface, by way of isolating and characterising the molecules that were secreted by the flukes. ES products from adult worms showed two fractions: a >200-kDa fraction including several proteins and other molecules and a 40-kDa fraction consisting of cathepsin L cysteine peptidases subsequently fractioned into two subfractions presenting distinct enzymatic activities—cathepsin L1 (FhCL1) and cathepsin L2 (FhCL2) (Dalton et al. 1996). Cattle vaccination with FhCL1 induced protection levels of up to 69.5 % and combination vaccines reached a 72.4 %. These vaccines also exhibited significant anti-fecundity effects of reduced egg output and lower egg viability in up to 98 %. Thus, vaccines were showing for the first time that they could potentially block the transmission of the disease. Assays were further developed by using different adjuvants.

The development of high-level protective vaccines that can also impact on disease transmission may require combinations of various parasite molecules. Therefore, two new important vaccine candidates have been identified, peroxidase (FhPrx) and helminth defence molecule (FhHDM), that also perform potent immunomodulatory functions (Robinson et al. 2013). Several other *Fasciola* molecules hold promise as components of combination vaccines, including glutathione S-transferase (FhGST), cathepsin B (FhCB1–10), fatty acid-binding protein (FhFABP) and leucine aminopeptidase (FhLAP) (Spithill et al. 2012).

In sheep, FhLAP was shown to induce protective responses both alone and in combination with FhCL1 and FhCL2 in native form (Piacenza et al. 1999). Moreover, impressive levels of protection have been observed in sheep (up to 87 %) vaccinated with a recombinant FhLAP formulated in adjuvants that induce high-titre IgG1 and IgG2 (Maggioli et al. 2011).

Unfortunately, two challenges appear far from being solved at present. The first is the rapid and potent ability of *F. hepatica* to suppress the protective arm of the immune response, which explains why infected hosts do not develop immune resistance and provides a reasonable explanation for why efforts have been largely unsuccessful in developing efficacious vaccines against *Fasciola* in animals. Indeed, fluke-induced immunomodulation/immunosuppression is induced rapidly upon *Fasciola* invasion and maintained through the chronic infection. It is mediated by fluke molecules that alter the function of innate immune cells (dendritic cells,

macrophages, mast cells) and the quality and magnitude of adaptive immune cell (T and B cells) responses (Dalton et al. 2013). The second challenge refers to the differences in cell- and/or antibody-mediated responses depending on host species, which means that even being successful in obtaining an effective vaccine against animal infections, this will not allow for a direct extrapolation to a vaccine for humans. However, the wide knowledge obtained on the immunological processes and molecules involved will undoubtedly facilitate the development of a vaccine for human use in the future.

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