# Fascioliasis

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Santiago Mas-Coma, Mª Adela Valero, and Mª Dolores Bargues

## 4.1 Introduction

Considered a well-known veterinary problem of worldwide distribution, fascioliasis is the vector-borne parasitic disease presenting the widest latitudinal, longitudinal, and altitudinal distribution known at present (Mas-Coma et al. 2003, 2005). In the last two decades, many surveys have shown it to be an important public health problem as well (Chen and Mott 1990; WHO 1995; 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 mainly in several regions of Asia and Africa (Mas-Coma 2004a).

The increasing number of human case reports in many countries of the five continents and the results of studies on pathogenicity and immunity, mainly regarding the chronic period of the disease, are the reasons why it has been decided to no longer consider fascioliasis merely a secondary zoonotic disease, but an important human parasitic disease (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).

S. Mas-Coma (✉) · M. A. Valero · M. D. Bargues
Departamento de Farmacia y Tecnología Farmacéutica y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain
e-mail: S.Mas.Coma@uv.es; Madela.Valero@uv.es; M.D.Bargues@uv.es

## 4.2 Systematics and Morphology of Causal Agents

Fascioliasis is caused by two species which belong to the subfamily Fasciolinae: *Fasciola hepatica* and *F. gigantica*. This subfamily includes digeneans which infect the liver and more rarely duodenum and lungs of their mammal hosts, are morphologically characterized by branched caeca and dendritic testes, and are transmitted by snails of the family Lymnaeidae (Mas-Coma et al. 2009a).

The adult stage of both fasciolid species has a leaf-shaped body, with a broadly pointed posterior end. The two suckers are relatively small and located close one another in a cone-like anterior extension of the body. The pharynx is well visible. The intestinal caeca are long, reaching the posterior end of the body and presenting a large number of lateral branches. The two branched testes are located in a longitudinal tandem, within the second and third fourth of the body. The cirrus pouch, containing a protrusible spined cirrus, is prominent, preacetabular and opens in a postbifurcal genital pore. The branched ovary is pretesticular and dextral. The vitellaria extend bilaterally up to the hindbody. The short uterus is located between the ovary and the caecal bifurcation. The eggs are operculated, ovoid, yellow, and non-embryonated when laid (Fig. 4.1).

The two species differ in size. The adult stage of *F. hepatica* has a maximum length of 29.0 mm



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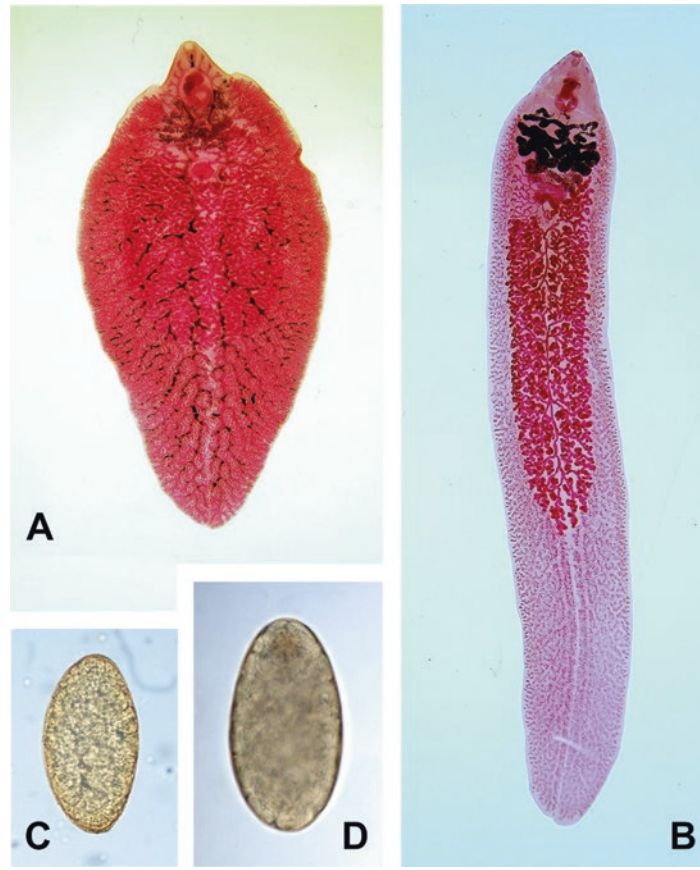


**Fig. 4.1** Adults and eggs of fasciolid species: (**a**) adult stage of *Fasciola hepatica* from Bolivia; (**b**) adult stage of *F. gigantica* from Burkina Faso; (**c**) egg of *F. hepatica* found in stools of a human patient from the Bolivian Altiplano endemic area; (**d**) egg of *F. gigantica* found in a faecal sample of a bovine from Bobo Dioulasso, in Burkina Faso. Note almost absence of shoulders and parallel lateral body borders in the adult of *F. gigantica* (**b**). (**a**, **b**) at the same scale; (**c**, **d**) at the same scale. For measurements of adult stages and eggs of both fasciolids see text and Table 4.1 (Orig. S. Mas-Coma)

**Table 4.1** Measurements of eggs of *Fasciola hepatica* and *F. gigantica* in different world regions according to the absence or existence of overlap of the two fasciolid species (intermediate hybrid forms have egg size ranges different from pure species)

| Endemic areas | Geographical distribution | *Fasciola hepatica* | | *Fasciola gigantica* | |
|---|---|---|---|---|---|
| | | In humans | In animals | In humans | In animals |
| Areas where *F. gigantica* is absent | The Americas and Europe | 100.6–162.2/ 65.9–104.6 | 73.8–156.8/ 58.1–98.1 | – | – |
| Areas where both fasciolid species are present | Parts of Africa and Asia | 106.5–171.5/ 63.9–95.4 | 120.6–163.9/ 69.2–93.8 | 150.9–182.2/ 85.1–106.2 | 130.3–182.8/ 74.0–123.6 |
| Areas where *F. hepatica* is absent | Parts of Africa | – | – | 137.2–191.1/ 73.5–120.0 | 129.6–204.5/ 61.6–112.5 |

Size given in length/width. All measures in μm
Data from Valero et al. (2009) and Mas-Coma et al. (2014a, b)

and a maximum width of 14.1 mm (Fig. 4.1a), whereas in *F. gigantica* it shows a maximum size reaching 52.3 mm and 11.8 mm (Fig. 4.1b), respectively. Thus, *F. gigantica* is more elongate and narrower, with lateral walls tending to be parallel, and with non-existent or less marked shoulders of the cephalic cone. Moreover, in *F. gigantica* caeca are more branched, mainly those towards the midline of the body, and the branches of the ovary are more numerous and longer. Morphometrically, all the measurements overlap in specimens of "pure" *F. hepatica* and "pure" *F. gigantica*, except the maximum body length, maximum body width, body length–body width ratio, body roundness, and the distance between the ventral sucker and the posterior end

of the body (Periago et al. 2006). These features allow for the phenotypical differentiation between the two species.

However, hybrid specimens may give rise to intermediate forms in those endemic areas where the two species overlap (Mas-Coma et al. 2009a). The presence of such phenotypically intermediate adult and egg forms has been proved in Egypt (Periago et al. 2008), Iran (Ashrafi et al. 2006b), Pakistan (Afshan et al. 2014a), and Bangladesh (Ahasan et al. 2016). Additionally, comparisons of adults and eggs of liver fluke populations from different host species, and adults and eggs experimentally obtained in laboratory rats infected with isolates from different natural hosts revealed that the definitive host species decisively influences the size of adult worms and eggs, and that this influence does not persist in a heterologous host (Valero et al. 2001). Thus, morphometric comparisons of fasciolid populations should always be made inside the same definitive host species.

## 4.3 Life Cycle

The adult stage of *F. hepatica* and *F. gigantica* parasitizes the large biliary passages and the gallbladder of ruminants, mainly sheep, goats, and cattle, and many other herbivorous domestic and wild animals, including horses, donkeys, mules, and also Old and New World camelids. Buffalo, deer, wild boar, 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). Several African wild animals and many rodent species have been found naturally infected, and other species are usually used for experimental purposes (Losos 1986; Mas-Coma et al. 1987, 1988). Humans are susceptible hosts for the infection by both *Fasciola* species (Mas-Coma et al. 2009a).

The life cycle of the two fasciolids takes around 14–23 weeks and follows a similar pattern (Mas-Coma and Bargues 1997; Mas-Coma et al. 2003).

Fasciolid adults produce eggs inside the mammal host. These eggs reach the external milieu by way of bile and intestine. The transit between the definitive mammal host and the 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 only continue their development if they reach freshwater of appropriate physico-chemical characteristics. If the climatic conditions are suitable (15–25 °C), the miracidia develop and hatch in about 9–21 days. However, when conditions are unfavourable, they may not mature but may remain viable for several months.

The miracidium hatches under light stimulation and swims rapidly until it contacts an appropriate aquatic or amphibious snail host. The development takes place inside the intermediate snail host and includes miracidium penetration into the snail, sporocyst, redial generations, production of cercariae, and shedding of the latter into water. A maximum of four redial generations have been found although 3 generations are usually produced after monomiracidial infection. The redial generations follow the same developmental pattern in different lymnaeid species. Redial generations follow a complex development (Rondelaud et al. 2009). The stage of cercaria develops within 6–7 weeks at 20–25 °C, its development being delayed at lower temperatures. Thus, the prepatent period is dependent on temperature, higher temperatures reducing it (15 °C: 56–86 days; 25 °C: 38 days).

A short swimming phase of cercaria and a long resistance phase of metacercaria allow for the transit between snail host and mammal host. The shedding process takes place between 9 and 26 °C, independently of light or darkness. Cercariae swim for a short time (1 h) until contacting a solid support, mostly leaves of water plants above or below the water line. They then lose their tails, quickly encyst and become infective within 24 h.

The definitive host is infected by ingestion of metacercariae (Fig. 4.2). Metacercariae excyst in the small intestine within an hour after ingestion, penetrate the host's intestine wall, and appear in the abdominal cavity by about 2 h after ingestion. Most migrating juveniles reach the liver within 6 days after excystment. In the liver they migrate for 5–6 weeks, preferentially feeding directly on

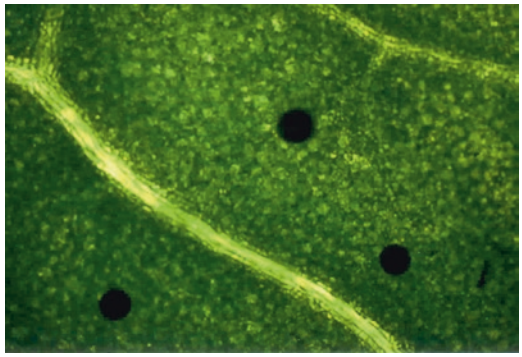

**Fig. 4.2** Encysted metacercariae of *Fasciola hepatica* attached to a leaf of a freshwater plant (Orig. S. Mas-Coma)


**Fig. 4.3** Main species of lymnaeid vectors of fascioliasis in dorsal view: (**a**) specimen of *Galba truncatula* from Europe; (**b**) specimen of *Radix natalensis* from Africa. Note larger size of the latter (photographs at the same scale) (Orig. S. Mas-Coma)

liver tissue. They finally penetrate into the bile ducts where they become sexually mature.

The prepatent period (from the ingestion of metacercariae to the first appearance of the first eggs in the faeces) is about 2 months (6–13 weeks) in sheep and cattle, varies according to the host, and also depends on the number of the adult flukes in the liver (Valero et al. 2006a). In humans, a period of at least 3–4 months is necessary for the flukes to attain sexual maturity. The lifespan of the parasite in sheep can be as long as 11 years and 9–12 months in cattle. In humans, estimations from several long-term case reports suggest a lifespan of the adult fluke between 9 and 13.5 years.

## 4.4 Lymnaeid Snail Vectors

The development of fasciolid larval stages is very dependent on the environmental characteristics according to the nature of the free living phases which take place in the external freshwater milieu, and the parasite phase which develops inside the freshwater snail, in its turn also very dependent on the environment. That is why this disease is pronouncedly influenced by climate change (Mas-Coma et al. 2009b). The similarity in the relationships between snails and climate/environment resembles the one known in arthropods participating in the transmission of many infectious diseases and underlies the recent trend of using the term vector also for the intermediate lymnaeid snail hosts transmitting fascioliasis.

Vectors of *Fasciola* are freshwater gastropod snails of the family Lymnaeidae (Fig. 4.3). 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 lymnaeid species which transmit *F. gigantica* and a very few which are able to transmit the two fasciolid species. However, recent molecular studies on lymnaeids have shown that lymnaeid species misclassifications have been usual (Bargues et al. 2001) and additionally hybridization phenomena between the two *Fasciola* species were unknown in the past, so that results of many of the old fasciolid–lymnaeid specificity experiments should be re-assessed (Mas-Coma et al. 2009a).

Molecular studies indicate that *F. 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 (Fig. 4.3a); *Lymnaea humilis*, *L. bulimoides*, and *L. cubensis* in North America, *L. cubensis* in the Caribbean; *L. neotropica*, *L. cousini*, and *L. viator* in South America; and *L. tomentosa* in Australia. The recent discovery of *L. schirazensis*, another species of the same *Galba*/*Fossaria* group which appears to have

been always confused with *G. truncatula* and other similar vector species, in Asia, Europe, Africa, the Caribbean, North America, and South America, has highlighted potential specimen classification problems distorting fasciolid–snail specificity/susceptibility and fascioliasis geographical distribution data. This unexpected finding now recommends the need to review a large body of literature on *G. truncatula* (Bargues et al. 2011a).

The species *Fasciola gigantica* is transmitted by species of the genus *Radix*, mainly *R. natalensis* in Africa (Fig. 4.3b) and varieties of *R. auricularia* and *R. viridis* in Asia (Bargues et al. 2001). *Pseudosuccinea* is a monospecific genus including the species *P. columella* which has colonized all continents and appears to be able to transmit both *Fasciola* species (Bargues et al. 2011c).

A few species among the lymnaeid group of the stagnicolines have proved their capacity to transmit *F. hepatica* under exceptional or local natural conditions in a few areas, such as *L.* (*Stagnicola*) *palustris* and *L.* (*S.*) *fuscus*, and closely related species such as *Omphiscola glabra* (Bargues et al. 2003).

Lymnaeid vectors, with their geographical distribution, define not only 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, its seasonality or permanent transmission (Mas-Coma et al. 1999c).

## 4.5 Epidemiology

Despite the restrictions imposed by the necessary climate/environment thresholds, *F. hepatica* has succeeded in expanding from the Near East original geographical area up to actually colonize the five continents. In its turn, *F. gigantica* appears restricted to areas of Africa and Asia where *Radix* vectors allow for their transmission (Mas-Coma et al. 2009a). It should be emphasized, however, that a global analysis of the geographical distribution of human infection shows that the expected correlation between animal and human fascioliasis only appears at a basic level. High prevalences in humans do not seem to be necessarily related to high prevalences in livestock.

Similarly to other water-borne parasitic diseases such as schistosomiasis, within a human endemic area, it has been seen that human and animal infection appears irregularly distributed. The transmission foci are patchily distributed and linked to the presence of appropriate water collections, and human prevalences in school children appear to be related to the distance to water bodies presenting lymnaeids (Mas-Coma et al. 1999c).

### 4.5.1 Distribution of Human Fascioliasis

In Europe, France is the endemic area where a higher number of human cases have been reported (Anonymous 1988). The first large modern epidemic of human fascioliasis occurred in that country in 1956 (Coudert and Triozon 1958). Between 1950 and 1983, a total of 3297 cases from published reports were catalogued (Gaillet et al. 1983). Most cases were reported from the areas of Lyon, Bretagne Nord—Pas de Calais and Sud-Ouest. More recent reports on Sud-Ouest France refer to more than 300 cases (Giap 1987; Ripert et al. 1987). Reports on 5863 human cases were recorded from only nine hospitals between 1970 and 1982 (Danis et al. 1985), demonstrating that published data were largely underestimating the real situation. The disease is also important in Portugal, with the northern part of the country as a marked endemic area, including 1011 cases diagnosed in Porto between 1970 and 1992 (Sampaio-Silva et al. 1996). In Spain, human fascioliasis appears to be underestimated and mainly distributed in the northern part (Sorribes et al. 1990), with imported cases recently added to autochthonous ones (Turrientes et al. 2004). In other parts of Europe, human infection appears to be sporadic although reported from almost all countries (Esteban et al. 1998).

In Asia, the Near East appears as an important focus of human infection, concerning mainly Iran and Turkey. In Iran, human cases appear above

all concentrated in the province of Gilan, at the Caspian Sea, where several large epidemics, including thousands of human cases, were reported from the end of the 1980s and during the 1990s (Massoud 1990, 1993; Ashrafi et al. 2004). In Mazandaran, fascioliasis has recently shown to be a human health problem too (Moghaddam et al. 2004), and many reports have very recently been published on human cases diagnosed in other provinces. In Turkey, human infection does not seem to be rare. The detection of a 1.8% human prevalence in a village in Eastern Turkey (Yilmaz and Gödekmerdan 2004) suggests that this endemic area may be largely widespread throughout the eastern part of the country.

In southern Asia, whereas only sporadic isolated cases have been diagnosed in India and Afghanistan, a wide human endemic area in the Punjab province and child infection in another area have been recently reported from Pakistan (Qureshi et al. 2016, 2019), and a worrying scenario has been described in Nepal (Sah et al. 2018).

In the Far East, cases in Japan and Korea are sporadic, but recent information on Vietnam becomes bothering (Mas-Coma 2004b). Only occasional cases of human fascioliasis were reported in Vietnam until the 1990s, but over 500 human cases have been diagnosed between 1997 and 2000 (De et al. 2003) and with non-stop increasing numbers thereafter (De et al. 2006; Hung and Dung 2011). A recent report of a 13.8% human prevalence in a village of Laos (Quang et al. 2008) may be interpreted as an epidemiological situation with a broader spread throughout southeastern Asia. Additionally, a human infection focus has been reported from southern China (Chen et al. 2013).

In Africa, numerous human cases have been detected in many governorates of Egypt, mainly children (Fig. 4.4) (Curtale et al. 2000, 2003a, b; Haseeb et al. 2002; Esteban et al. 2003). Initial estimations of 830,000 subjects affected in the Nile Delta region (WHO 1995) probably underestimate the real situation if the high prevalences reaching 18–19% in total population in concrete villages (Esteban et al. 2003) are considered. More recently, human endemic areas have been reported from Ethiopia (Fentie et al. 2013) and Tanzania (Lukambagire et al. 2015), and sporadic human infection has been reported from the Maghreb countries, sub-Saharan countries and even up to South Africa, where infection proved sometimes to be fatal (Black et al. 2013).

**Fig. 4.4** Transmission focus of human fascioliasis in the Nile Delta region, in Egypt, with *Galba truncatula*, *Radix natalensis caillaudi* and *Pseudosuccinea columella* as vector species transmitting both *Fasciola hepatica* and *F. gigantica* infecting children (Orig. S. Mas-Coma)

In Latin America, human infection appears mainly in altitude areas of the Andean region. In the Bolivian Altiplano, human prevalences were up to 72% and 100% in coprological and serological surveys, respectively (Hillyer et al. 1992; Bjorland et al. 1995; Esteban et al. 1997a, b, 1999; Mas-Coma et al. 1999c), and intensities reached up to more than 8000 eggs per gram (epg) in children (Mas-Coma et al. 2009a). Similar situations, although with lower intensities, have been described in other altitude areas of Peru, such as in Puno (Esteban et al. 2002), Mantaro valley (Raymundo et al. 2004), and Cajamarca (Gonzalez et al. 2011). Human infection has also been described in altitude areas of Ecuador (Trueba et al. 2000), Colombia (see review in Bargues et al. 2011c), Venezuela (see review in Bargues et al. 2011b), and recently also in Argentina (Carnevale et al. 2013; Bargues et al. 2016). A few human endemic areas have also been described in lowland areas in countries of the Southern Cone, such as Argentina (Mera y Sierra et al. 2011) and Chile (Apt et al. 1993; Artigas et al. 2011), whereas in Uruguay only a relatively low number of cases have been reported (Bargues et al. 2017).

Very recently, a human fascioliasis endemic area has been described for the first time in North America. Children proved to be infected in the state of Puebla, at a mean altitude of 1840 m. Fascioliasis prevalences indicate this area to be mesoendemic with isolated hyperendemic foci, a situation which adds concern about possible human fascioliasis underestimation in other areas of Mexico (Zumaquero-Rios et al., 2013).

In the Caribbean region, human fascioliasis mainly poses problems in Cuba, where the first human cases were already diagnosed in the first half of last century (Kouri and Arenas 1932; Vasquez 1943), many outbreaks have been reported (Esteban et al. 1998) since the first one (Arenas et al. 1948), losses in livestock husbandry due to fascioliasis are very high (Brito Alberto et al. 2010), and patients are continuously diagnosed (Millan et al. 2000; Diaz Fernandez et al. 2011), even in high numbers (Gonzales-Santana et al. 2013). In that island, the disease transmission is assured by two lymnaeid vectors, *L. cubensis* and *Pseudosuccinea columella* (Fig. 4.5). Unfortunately, appropriate field surveys are still lacking (Rojas et al. 2009) and

**Fig. 4.5** Typical focus of transmission of animal fascioliasis in Cuba, with *Lymnaea cubensis* as vector species (Orig. S. Mas-Coma)

hence the real situation in the different parts of the island remains unknown. Puerto Rico may still be considered a human infection risky area after the epidemiological situation in the past (Hillyer 1981), and Haiti has recently proved to be also affected by this disease at human level nowadays (Agnamey et al. 2012) although human infection was already detected in Haiti time ago (Clay and Straight 1961).

### 4.5.2 The Present Epidemiological Baseline

The present baseline on human fascioliasis pronouncedly differs from the knowledge available on human infection two decades ago. Many new concepts have been reached on human fascioliasis from the 1990s up to the present. A list of key aspects may be enumerated (Mas-Coma et al. 2009a):

1. In many areas, there are true human fascioliasis endemic situations, from hypo- to hyperendemics, which is very different of the old concept of humans only becoming infected sporadically in animal endemic areas.
2. In those endemic areas, high prevalences in humans (up to more than 70% by coprology and even reaching 100% by serology) do not appear to be necessarily related to high prevalences in domestic animals.
3. In human endemic areas, fascioliasis mainly affects children and females, with flukes infecting even at very precocious age (1–2-year-old children), usually showing a peak around 9–11 years and declining thereafter, although it may keep high prevalences in adults too (up to 40% in given communities).
4. Worldwide estimations raised from the 2500 reports of 1990 to 2.4 million, 17 million people and may even be higher at present if the almost total lack of knowledge about the situation of this disease in humans in many African and Asian countries is taken into account.
5. Human infection has been reported in 51 different countries from the five continents, showing how geographically expanded the problem might be.
6. The analysis of the distribution of the disease has shown that fascioliasis is the vector-borne parasitic disease showing the widest latitudinal, longitudinal, and altitudinal distribution known.
7. Such a broad distribution including from under sea level (as in the Caspian area) up to the very high altitude (4200 m at the Paso del Condor in Venezuela) is the consequence of the great capacity of both liver flukes and lymnaeid vectors to colonize new areas and their great capacity for adaptation to very different environments, habitats, and climates, even of extreme conditions as the very high altitude regions in Andean areas, where mathematical models well-known for fascioliasis in lowlands of the Northern Hemisphere indicated that the disease could not exist.
8. In human endemic areas, intensities, estimated from amounts of epg of faeces, may reach up to more than 8000 and amounts higher than 400 epg may be frequent in given communities, which markedly differs from the very low burdens (usually from less than 1 to 1–2 epg) reported before the 1990s.
9. Domestic animal species other than the usual sheep and cattle may also play an important role as reservoirs for humans in many different endemic areas, as mainly pigs, donkeys, and buffaloes, depending on the regions.
10. The snail family of lymnaeids shows a systematic-taxonomic chaos which even impedes correct classification of snail specimens by malacology experts, as demonstrated by DNA sequencing methods; classification errors underlie a concept of fasciolid–lymnaeid specificity which must be revisited.
11. Lymnaeid species linked to the disease transmission in many human endemic areas were erroneously classified as local lymnaeid spe-

cies, whereas in fact lymnaeid vector species imported from other continents were involved. This, together with importation/exportation of fasciolid-infected livestock, has given an international dimension to the public health problem in many areas where the disease was previously given local repercussion only.

The above-mentioned issues have given rise to a new platform for the analysis and interpretation of the human disease which is very different from a simple extrapolation from the traditional knowledge of fascioliasis in livestock. Unfortunately, sometimes not sufficient importance is given to this new base or it is not considered at all and consequently incorrect interpretations and erroneous conclusions are increasingly appearing in the recent literature.

### 4.5.3 Epidemiological Heterogeneity of Human Fascioliasis

After many years of studies on different areas presenting human infection by fasciolid liver flukes in South and Central America, Europe, Africa, and Asia, 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 human infection situations:

- *Autochthonous, isolated, non-constant cases*: humans acquire the infection in an area where they live and where animal fascioliasis is also present; these human cases appear sporadically, without any constancy.
- *Imported cases*: human cases diagnosed in a zone lacking the parasite, even in animals, who were infected in an area where transmission occurs.
- *Endemic*: three types of endemic situations can be distinguished according to human prevalences in the total population obtained by coprological diagnosis (data from serological tests may be somewhat higher).
  - *Hypoendemic*: prevalence less than 1%; arithmetic mean intensity less than 50 epg; high epg numbers only in sporadic cases; human participation in transmission through egg shedding may be neglected; hygiene–sanitation characteristics usually including latrines and waste or sewage disposal facilities; outdoor defaecation is not commonly practised.
  - *Mesoendemic*: prevalence between 1 and 10%; 5–15-year-old children may present higher prevalences (holoendemic); arithmetic mean intensity in human communities usually between 50 and 300 epg; individual high epg numbers can be found although intensities over 1000 epg are rare; human subjects may participate in transmission through egg shedding; hygiene–sanitation characteristics may or may not include latrines and waste or sewage disposal facilities; outdoor defaecation may be practised.
  - *Hyperendemic*: prevalence more than 10%; 5–15-year-old children usually present higher prevalences (holoendemic); arithmetic mean intensity in human communities usually more than 300 epg; individual very high epg numbers are encountered, intensities over 1000 epg being relatively frequent; human subjects significantly participate in transmission through egg shedding; hygiene–sanitation characteristics not including the use of latrines; no proper waste or sewage disposal facilities; indiscriminate defaecation is commonly practised.
- *Epidemic*: there are different types of outbreaks according to the endemic/non-endemic situation of the zone.
  - *Epidemics in non-human endemic but animal endemic areas*: outbreaks appearing in zones where previous human reports have always been isolated and sporadic; such outbreaks usually concern a very few subjects infected from the same contamination source (family or small group reports; contaminated wild, home-grown or com-

mercially grown watercress or other metacercariae-carrying vegetables).
- *Epidemics in human endemic areas*: outbreaks appearing in zones presenting human endemics; a more important number of subjects may be concerned; usually related to previous climatic conditions having favoured both the parasite and the snail life cycles; epidemics can take place in hypoendemic, mesoendemic, and hyperendemic areas.

Fascioliasis presents a very wide spectrum of transmission and epidemiological patterns in human hypoendemic 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 4200 m, hot and cold weathers, seasonal and yearly constant temperatures, scarce to pronounced annual rainfall, low and high mean annual potential evapotranspiration, and 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 water bodies (Mas-Coma et al. 2003).

### 4.5.4 Transmission Patterns in Human Fascioliasis Areas

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):

1. A very high altitude pattern related to only *F. hepatica* transmitted by imported *G. truncatula* in Andean countries following transmission throughout the year; within this category, two subpatterns may be distinguished according to physiographic and seasonal characteristics.
   (a) The altiplanic pattern, with transmission throughout the whole year, e.g. in the Northern Bolivian Altiplano and the Puno Altiplano.
   (b) The valley pattern, with seasonality and prevalences and intensities related to altitude, e.g. in the valleys of Cajamarca and Mantaro (Valero et al. 2012c);
2. A Caribbean insular pattern, with reduced but repeated outbreaks in human hypoendemic areas and lymnaeid species other than the main vector species being involved in the transmission, e.g. the Pinar del Rio Province in Cuba.
3. A pattern related to Afro-Mediterranean lowlands, including overlapping *F. hepatica* and *F. gigantica* and several *Galba*/*Fossaria* and *Radix* lymnaeids together with secondary transmitting *Pseudosuccinea*, and where seasonality is typical, e.g. the Behera Governorate in Nile Delta region in Egypt.
4. A pattern related to Caspian surrounding areas, including human hypoendemic areas in which large epidemics occur, occasionally involving up to 10,000 people and with overlapping of *F. hepatica* and *F. gigantica* and several *Galba*/*Fossaria*, *Radix*, and stagnicoline lymnaeids, e.g. the area of Rasht and Bandar-e Anzali in the Gilan province in Iran.
5. A pattern related to lowland areas in Vietnam, which may perhaps be extrapolated to other neighbouring South East Asian countries; this pattern is able to give rise to large human epidemics and is related to only/mainly *F. gigantica* and consequently *Radix* lymnaeids.
6. Another pattern has recently been described in Argentina (Bargues et al. 2016); this pattern is very different from the typical fascioliasis transmission foci because of the desertic-arid/semiarid conditions surrounding the transmission foci in which lymnaeids are confined to lateral river side floodings and small man-made irrigation systems, water availability only depends on the rivers flowing from neighbouring mountains, and all disease transmission

factors are concentrated in small areas where humans and animals go for water supply, vegetable cultures and livestock farming, remembering the schistosomiasis transmission foi in African oases of the Sahara desert.

Human fascioliasis shows a marked heterogeneity of different epidemiological situations and transmission patterns throughout the world. Thus, well-known situations and patterns of fascioliasis may not always explain the disease characteristics in a given area. In other terms, when dealing with an endemic zone not previously studied, the aforementioned situations and patterns of human infection must always be taken into account merely as the starting base. 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.

The lymnaeid vector species show a relationship with the transmission pattern. Lymnaeids present pronouncedly different ecological and ethological characteristics depending on the species. Factors such as type of water collection habitats, population dynamics, temperature thresholds, seasonality, or susceptibility regarding liver fluke infection, are crucial for fascioliasis. As in other well-known vector-borne parasitic diseases, lymnaeids constitute excellent markers of the disease characteristics useful for the differentiation between different human fascioliasis situations and patterns, and consequently their assessment is necessary before the appropriate control strategies may be designed.

### 4.5.5 Seasonality and Long-Term Impacts of Climate and Global Changes

Climatic factors are decisive in the transmission of fascioliasis. The yearly definitive host infection incidence of fascioliasis has been related to air temperature, rainfall, and/or potential evapotranspiration. These factors affect the intermediate snail host population dynamics and the parasite population at the level of both the free living larval stages of egg and metacercaria and the intramolluscan parasitic larval stages of sporocyst, rediae, and cercariae.

Seasonal variation of mainly rainfall and temperature gives rise to different fascioliasis seasonality depending on the areas. 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 water bodies 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 1-year period with water availability and another period of dryness covering the rest of the year.

Man-made modifications of the environment may also modify the seasonality of fascioliasis in a given endemic area. Thus, artificial field irrigation appears to be sufficient by its own to allow for fascioliasis transmission in Cambodia (Tum et al. 2004, 2007). In the province of Punjab, in Pakistan, a complex transmission model has recently been described, including bi-seasonality with a peak related to rainfall and another peak related to man-made irrigation (Afshan et al. 2014b).

Unfortunately, climate change overlaps other anthropogenic and environmental modifications which are included in the broad term of "global change". Global change refers to many man-made environmental changes such as hydrological changes, e.g. construction of dams, irrigation canals, water reservoirs that establish suitable new environments for the snail vectors that transmit the parasites (Mas-Coma et al. 2009b). Hence, global change factors are able to pronouncedly influence parasitic diseases by their own, so that establishing the causality of disease emergence by climate change is usually not an easy task. However, the aforementioned Pakistani province of Punjab is the first endemic area where the emergence of human infection has been correlated with an increase of fascioliasis transmission risk due to an impact of climate

change throughout a 20-year period by means of an analysis of forecast indices and remote sensing data (Afshan et al. 2014b).

### 4.5.6 Sources of Human Infection

The ingestion of infective metacercariae by humans may occur by different ways. A very recent worldwide, detailed analysis has clarified all human fascioliasis infection sources, their diversity in the different areas and countries, the related incidence factors, and the methods for the study and analysis of these sources. The high diversity of infection sources and their heterogeneity in different countries underlie the large epidemiological heterogeneity of human fascioliasis throughout (Mas-Coma et al. 2018). The following infection sources have been distinguished:

- Ingestion of freshwater wild plants: main aspects to be considered are the plant markers of transmission foci, watercress, other freshwater wild plants, and wild plants sold in urban markets.
- Ingestion of freshwater cultivated plants, mainly watercress.
- Ingestion of terrestrial cultivated plants needing frequent irrigation.
- Ingestion of terrestrial wild plants: collected in dry habitats but which were submerged in water a few weeks or months before.
- Ingestion of traditional local dishes made with contaminated sylvatic plants.
- Ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration.
- Drinking of contaminated water.
- Drinking of beverages and juices made from local plants.
- Ingestion of dishes and soups made with contaminated water.
- Washing of vegetables, fruits, tubercles, kitchen utensils, or other objects with contaminated water.

Among the incidence factors, disease transmission seasonality, the infectivity of metacercariae under field conditions, as well as several community, familial and social factors in infection risk appear in the forefront. Among the methods to assess human infection sources, the following should be considered: (1) detection of metacercariae attached to plants or floating in freshwater; (2) anamnesis in individual patients, and (3) questionnaire surveys in human endemic areas (Mas-Coma et al. 2018).

Cultural traditions prove to be highly important in given endemic areas. Experimental studies performed with plant-made foods showed the role they may play in human infection in the province of Gilan, Iran (Fig. 4.6) (Ashrafi et al. 2006a).

**Fig. 4.6** Freshwater plant usually included in human diet in a focus of fascioliasis transmitted by *Galba truncatula* in Talesh Mountains, province of Gilan, in Iran (Orig. S. Mas-Coma)

In Mexican children, an association between fascioliasis and the habit of eating raw vegetables was identified, including watercress and radish with pronouncedly higher relative risk than lettuce, corncob, spinach, alfalfa juice, and broccoli. 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).

It shall be considered that metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older: the maximum longevity was 31 and 48 weeks using doses of 20 and 150 metacercariae per rat, respectively, although in the latter case only a very low percentage was viable. Moreover, metacercarial viability and infectivity did not show differences between isolates from different reservoir species, demonstrating that flukes from secondary reservoirs as pigs and donkeys involve the same potential risk as those from the main ones sheep and cattle (Valero and Mas-Coma 2000).

## 4.6 Pathology, Symptomatology, and Clinical Manifestations

Four clinical periods may be distinguished in fascioliasis (Chen and Mott 1990; Mas-Coma and Bargues 1997; Mas-Coma et al. 1999b, 2000). The incubation period includes from the ingestion of metacercariae to the appearance of the first symptoms. In man, this period has not been accurately determined (only "a few" days, 6 weeks, 2–3 months, or even more). The invasive or acute period comprises fluke migration up to the bile ducts. The latent period includes maturation of the parasites and starting of oviposition. This period can last for months or years and the proportion of asymptomatic subjects in this phase is unknown, being often discovered during family screening after a patient is diagnosed (Arjona et al. 1995). Patients may have prominent eosinophilia suggestive of infection, gastrointestinal complaints, or one or more relapses of the acute symptoms. Finally, the biliary, chronic, or obstructive period may develop after months to years of infection. Of these four periods, the second and fourth are the most important because patients are in one or another of these two periods almost always when diagnosed.

### 4.6.1 Invasive or Acute Period

The symptomatology which appears during this period is due mainly to mechanical destruction of liver tissue and abdominal peritoneum by the migrating larvae causing localized or generalized toxic and allergic reactions lasting 2–4 months. The major symptoms of this phase include fever, abdominal pain usually in the right hypochondrium or below the xyphoid, gastrointestinal disturbances such as loss of appetite, abdominal flatulence, nausea and diarrhoea, respiratory symptoms such as cough, dyspnoea, hemoptysis and chest pain, and also urticaria.

### 4.6.2 Biliary, Chronic, or Obstructive Period

Once in the bile ducts, adult flukes cause inflammation, hyperplasia of the epithelium, and thickening and dilatation of duct and gall bladder walls. The resulting cholangitis and cholecystitis, combined with the large body of the flukes, are sufficient to cause obstruction. This phase includes biliary colic, epigastric pain, fatty food intolerance, nausea, jaundice, pruritus, and right upper-quadrant abdominal tenderness, among others. Lithiasis of the bile duct or the gall bladder is frequent, whereas cirrhosis does not appear to be so (Marcos et al. 2009). 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 localized 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).

### 4.6.3 Clinical Highlights

Little was known about pathogenicity of *F. gigantica* in comparison with *F. hepatica*, mainly due to difficulties in assessing the moment of a patient's infection, the differential diagnosis with *F. hepatica*, and the lack of an animal model similarly susceptible to both fascioliods. However, a long-term, 24-week, experimental study comparing *F. hepatica* and *F. gigantica* has recently been successfully made for the first time in the same animal model host, Guirra sheep (Valero et al. 2016). Serum biochemical parameters of liver damage, serum electrolytes, protein metabolism, plasma proteins, carbohydrate metabolism, hepatic lipid metabolism and inflammation were analysed on a biweekly basis as morbidity indicators. Serum anti-*Fasciola* IgG, coproantigen, and egg shedding were simultaneously followed up. rDNA and mtDNA sequencing and the morphometric study by CIAS showed that fasciolids used fitted standard species characteristics. Results demonstrated that *F. gigantica* is more pathogenic, given its bigger size and biomass. *Fasciola gigantica* proved to follow a delayed development of 1–2 weeks regarding both the biliary phase and the beginning of egg shedding, with respective consequences for biochemical modifications in the acute and chronic periods. The higher *F. gigantica* pathogenicity contrasts with previous studies which only reflected the faster development of *F. hepatica* observed in short-term experiments (Valero et al. 2016).

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 also be caused by other helminth infections and local food traditions including the ingestion of many uncooked plants may mask liver fluke infection (Mas-Coma et al. 2014a, b).

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 lifespan of the adult fluke in man 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 (Valero et al. 2017). Thus, the acute period may be prolonged and overlap with both latent and biliary periods.

An association between anaemia and fluke burden (the most important), epg, fluke body area, presence of blood in faeces, IgG1 and eosinophil levels, and percentage 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 duration of fasciolid infection, 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 a reconsideration of treatment features in human disease, i.e. therapeutic strategies should also consider the possibility of bacterial coinfection (Valero et al. 2006b).

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).

Clinical pictures caused by fasciolids in locations of the human body different from the liver are known as ectopic fascioliasis. Flukes may deviate during migration, enter other organs and cause ectopic fascioliasis. In almost all patients, the causal agent is an immature juvenile, but a reduced number of ectopic cases caused by mature flukes shedding eggs have also been reported (Mas-Coma et al. 2014a, b). In humans, the most frequent ectopic lesions are in the gastrointestinal tract. Other such lesions are in abdominal wall, pancreas, spleen, subcutaneous tissue, heart, blood vessels, the lung and pleural cavity, skeletal muscle, appendix and epididymis (Mas-Coma and Bargues 1997). Pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis.

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 (Mas-Coma et al. 2013, 2014a). The impressive clinical pictures should be highlighted. They include from hemiplegia and paraplegia to disturbances and difficulties of walking capacity, speech disorders, convulsions, epilepsia 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 emphasize 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, 2014a).

Very recently, interactions between diverse *Fasciola* infection situations and non-imbalancing fibrinolysis system alterations have been for the first time proposed to explain the complexity, heterogeneity, and timely variations of neurological disorders (Gonzalez-Miguel et al. 2019). Proteomic and mass spectrometry analyses of the *Fasciola hepatica* excretome/secretome identified numerous, several new, plasminogen-binding proteins enhancing plasmin generation. This may underlie blood–brain barrier leakage whether by many simultaneously migrating, small-sized juvenile flukes in the acute phase, or by breakage of encapsulating formations triggered by single worm tracks in the chronic phase. Blood–brain barrier leakages may subsequently occur due to a fibrinolytic system-dependent mechanism involving plasmin-dependent generation of the proinflammatory peptide bradykinin and activation of bradykinin B2 receptors, after different plasminogen-binding protein agglomeration waves. Additionally, inflammation and dilation of blood vessels may be due to contact system-dependent generation bradykinin. This baseline allows for search of indicators to detect neurological risk in fascioliasis patients and experimental work on antifibrinolytic treatments or B2 receptor antagonists for preventing blood–brain barrier leakage (Gonzalez-Miguel et al. 2019).

## 4.7 Immunobiology and Coinfections

Fasciolid trematodes promote its 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 characterized 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 protective arm of the immune response explains why infected hosts do not develop immune 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 postinfection and is characterized 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-gamma. 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-gamma 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 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 metabolize L-arginine and are important in promoting Th2 responses and facilitating tissue repair and fibrosis (Dalton et al. 2013).

A consequence of liver fluke infection is the suppression of immune responses directed against concurrent or secondary pathogenic infections. The synergistic capacity of fasciolids in coinfection 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 fascioliasis 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). These synergistic associations of fascioliasis with other pathogens are believed to underlie the high morbidity and mortality rates of Aymara children inhabiting the Northern Bolivian Altiplano (Mas-Coma 2004a).

## 4.8 Genomics and Proteomics

Many different tools known to be useful for intraspecific variability analyses have been applied to fasciolids. Most studies on fasciolid proteins have concentrated on isoenzymes. Only a very few studies considered individual or population-level variation. The same isoenzymes of *F. hepatica*

were detected regardless of the host species (cattle, sheep, goats), but densities of some isoenzyme bands did differ according to host (Blair 1993). Profiles of whole-body proteins and excretory/secretory products obtained with isoelectric focusing differ among worms from different hosts (Lee et al. 1992), and isoelectric focusing is therefore not a good technique. Random amplified polymorphic DNA (RAPD) markers applied to *F. hepatica* showed that the majority of genetic diversity occurred within, rather than between hosts and was also greater within than between populations. Individual cows were infected by numerous genetically different liver flukes, suggesting the influence of mainly migrations and transportation of definitive hosts (Semyenova et al. 2003). Five among six microsatellite markers proved to be polymorphic in *F. hepatica* from Bolivia. No genetic differentiation between sampling sites or between definitive host species (sheep, cattle, pig) was found when applying these microsatellites (Hurtrez-Bousses et al. 2004).

Similarly, the restriction fragment length polymorphism (PCR-RFLP) technique has been applied repeatedly to fasciolids (Marcilla et al. 2002; Huang et al. 2004; Rokni et al. 2010), but unfortunately these assays are only useful for the differentiation of pure species, but not for hybrid forms (Mas-Coma et al. 2009a). Indeed, this was already initially detected three decades ago. Restriction endonuclease maps of rRNA genes were distinct for *F. hepatica* and *F. gigantica*, Japanese *Fasciola* sp. being identical to *F. gigantica*. No intraspecific variations in the maps of *F. hepatica* or of *F. gigantica* were detected, but length heterogeneity was noted in the intergenic spacer, even within individual worms (Blair and McManus 1989).

The aforementioned problems posed by isoenzymes, RAPD, microsatellites, and RFLP techniques explain why these genetic tools have been abandoned or only sporadically used in *Fasciola*. That is why genetic studies on fasciolids mainly rely on DNA sequencing techniques at present.

In an initial approach, a total of six differences were detected between *F. hepatica* from Ipswich and *F. gigantica* from Malaysia in a 28S rRNA gene D1 domain fragment (Barker et al. 1993). However, within the nuclear ribosomal DNA (rDNA) operon. Studies in invertebrates in general have shown that the ITS spacers are the most adequate markers for species differentiation (Mas-Coma and Bargues 2009). The complete sequence of ITS spacers of fasciolids was obtained for the first time at the beginning of this century (Mas-Coma et al. 2001). Nowadays, a large amount of literature is already available about the variability of ITS-1 and ITS-2 in *F. hepatica*, *F. gigantica*, and also hybrid or intermediate forms from throughout. This large information has recently been reviewed, corrected, and a useful standardized nomenclature for DNA markers in fasciolids proposed (Mas-Coma et al. 2009a).

In a large analysis of samples of "pure" *F. hepatica* from numerous countries and continents, only two haplotypes of the ITS-2 differing in only one mutation were found: the most spread FhITS2-H1 and the apparently more geographically restricted FhITS2-H2. On the contrary, the sequence of the other spacer ITS-1 always proved to be identical in "pure" *F. hepatica*: FhITS1-HA. In "pure" *F. gigantica*, only one haplotype in ITS-2 (FgITS2–1) and similarly one in ITS-1 (FgITS1-A) were found. When comparing ITS-2 sequences, the two haplotypes of *F. hepatica* (FhITS2-H1 and H2) with the only one of *F. gigantica* (FgITS2-H1), five polymorphic sites enable the two species to be distinguished. When comparing ITS-1 sequences, the only haplotype of *F. hepatica* (FhITS1-HA) differs from the only haplotype of *F. gigantica* (FgITS1-HA) also in five polymorphic sites (Mas-Coma et al. 2009a).

A comparative study with sequences obtained in countries not included in that countrywide study allowed to reach the conclusion that up to four haplotypes of ITS-2 could be distinguished in *F. hepatica* (FhITS2–1 to 4) and up to five in *F. gigantica* (FgITS2–1 to 5). On the contrary, the ITS-1 appeared to be fully uniform in both *F. hepatica* and *F. gigantica* everywhere (FhITS1-A and FgITS1-A) (Mas-Coma et al. 2009a).

With regard to mitochondrial DNA (mtDNA), the complete genome of *F. hepatica* has been already sequenced, which will be suitable for

studies of variation (Le et al. 2001). Unfortunately, only small fragments of the mtDNA codifying genes *cox*1 and *nad*1 have been used in the numerous local studies, and this becomes a problem due to the biased information gene fragments furnish (Mas-Coma and Bargues 2009).

Only in the aforementioned wide multicountry analysis of samples of "pure" *F. hepatica* and "pure" *F. gigantica* from different continents were these mitochondrial genes analysed in its complete length (Mas-Coma et al. 2009a). "Pure" *F. hepatica* showed a mtDNA *cox*1 codifying gene providing a total of 69 different haplotypes (Fh cox1–1 to 69), including a total of 78 polymorphic sites. A total of 23 different haplotypes of the COX1 protein were found (FhCOX1–1 to 23). In its turn, the mtDNA *nad*1 codifying gene provided a total of 51 different haplotypes (Fh nad1–1 to 51). A total of 15 different haplotypes of the NAD1 protein were found (FhNAD1–1 to 15). "Pure" *F. gigantica* showed a *cox*1 gene providing a total of 11 different haplotypes (Fg cox1–1 to 11). A total of 5 different haplotypes of the COX1 protein were found (FgCOX1–1 to 5). In its turn, the *nad*1 gene provided a total of 15 different haplotypes (Fg nad1–1 to 15). A total of 10 different haplotypes of the NAD1 protein were found (FgNAD1–1 to 10) (Mas-Coma et al. 2009a).

Proteomic tools have provided wide information about the profiles of soluble proteins secreted by fasciolids, mainly on cathepsin L and cathepsin B family of peptidases and their temporal expression as the parasites progresses from tissue to tissue (McVeigh et al. 2012), as well as about the expression and function of several antioxidant molecules, glutathione S-transferases, fatty acid binding proteins and peroxiredoxin which, besides protecting the parasite from damaging reaction, may also have immunoprotective functions (Spithill et al. 2012).

Given the intimate contact between the fluke and host tissues through its migration, antigens associated with the tegument of *Fasciola* also modulate host immune cell function. The tegument of *Fasciola* is a unique syncytial layer that plays the interface between host and parasite. In recent proteomic studies on the adult stage, extracted fractions of *F. hepatica* showed to contain 172–229 proteins, providing valuable insights into the complex protein composition within the tegumental layer as a whole (Dalton et al. 2013). Regarding *Fasciola* juveniles, the enzymatic shaving of peptides from the surface of liver flukes and their subsequent identification, has allowed at least some of the tegumental surface proteome to be identified (De La Torre et al. 2011).

The genome of *F. hepatica* has proved to be among the largest known pathogen genomes at 1.3 Gb, a size which cannot be explained by genome duplication or expansion of a single repeat element. The substantial levels of polymorphism found have tentatively been linked to the evolutionary potential for rapid adaptation to changes in host availability, climate change or to drug or vaccine interventions (Cwiklinski et al. 2015). Intriguingly, the genome of *F. hepatica* isolated from sheep of North America showed a markedly higher repeat content (55.29%) than the aforementioned genome of *F. hepatica* isolated from cattle of United Kingdom (32.0%) (McNulty et al. 2017).

## 4.9 Diagnosis

Although some suggestive clinical presentation aspects may be useful, mainly in human endemic areas where physicians are aware about liver fluke infection risk in humans, 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).

Stool and blood techniques, the main tools for diagnosis in humans, have been improved in the last two decades. Present availabilities for human diagnosis have recently been reviewed exhaustively, focusing on advantages and weaknesses, sample management, egg differentiation,

qualitative and quantitative diagnosis, antibody and antigen detection, post-treatment monitoring, and post-control surveillance (Mas-Coma et al. 2014b). Main conclusions refer to the pronounced difficulties of diagnosing fascioliasis in humans given the different infection phases and parasite migration capacities, clinical heterogeneity, immunological complexity, different epidemiological situations and transmission patterns, the lack of a diagnostic technique covering all needs and situations, and the advisability for a combined use of different techniques, at least including a stool technique and a blood technique (Mas-Coma et al. 2014b).

### 4.9.1 Coprological and Other Direct Diagnostic Techniques

Analysis for the detection and identification of fasciolid eggs found in stool sample (Fig. 4.3), duodenal contents or bile continues to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognized 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 egg counts per gram of faeces and the fluke burden (Valero et al. 2006a, 2009). Identifying fluke adults obtained during an endoscopy of after surgical intervention either by microscopic morphometry (Periago et al. 2006) or molecular tools (Marcilla et al. 2002; 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. Based 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*. However, large variations were first observed in the size of *F. hepatica* eggs in livestock from different geographical locations (Tinar 1984). Furthermore, it has been experimentally shown that the final host species (sheep, cattle, pig, and donkey) decisively influences the size of the *F. hepatica* eggs even within the same endemic area (Valero et al. 2001). Additionally, the existence of intermediate forms between the two fasciolid species and genetic hybrids of both in overlapping areas increases the problem. The existence of these intermediate forms posed a question mark on whether egg characteristics are suitable as a tool for the differential diagnosis of fascioliasis caused by either species (Valero et al. 2009). A concrete example of this problem was already emphasized in the diagnosis of fascioliasis in humans (Inoue et al. 2007). All in all, the aforementioned problems plus the use of serological tests, all of which unable to differentiate between the two fasciolids, or the lack of a calibrated microscope for measurements with an ocular micrometer explain why subjects diagnosed from areas where both species co-exist, such as in areas of Africa and Asia, are currently referred to as infected by *Fasciola* sp. or simply *Fasciola* (Mas-Coma et al. 2005).

A study of fasciolid eggs from different continents, using a computer image analysis system (CIAS), revealed that eggs shed by humans show morphological 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 (Table 4.1) (Valero et al. 2009; Mas-Coma et al. 2014a, b). These new results should aid clinicians since the application of the classic egg size range in human samples may lead to erroneous conclu-

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

### 4.9.2 Serological and Other Indirect Diagnostic 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 the other situations in which coprological techniques may present problems. However, immunological techniques offer other types of problems related mainly to sensitivity and specificity. Different serological tests have been used for human diagnosis. Almost all these techniques concern the detection of circulating antibodies and only a very few are designed to detect circulating antigens and immune complexes.

In recent years, 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 postinfection), intermittent egg output dynamics, very low or even absence of egg shedding in cases of only one or a few fluke adults and old, chronic infections, ectopic infections, "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).

Several cysteine proteinases offer highly sensitive and specific markers for human fascioliasis serodiagnosis for *F. hepatica* (Sampaio-Silva et al. 1996; Cordova et al. 1997, 1999; O'Neill et al. 1998, 1999; Strauss et al. 1999; Rokni et al. 2002; Espinoza et al. 2007; Mezo et al. 2003, 2004) as well as for *F. gigantica* infection (Maleewong et al. 1999; Ikeda 1998; Intapan et al. 1998, 2004; 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.

Very recent studies in two human hyperendemic areas of 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™, developed for preservation of *Fasciola* coproantigens, proved to enhance coproantigen extraction without affecting the detection limit of the assay, and the antigenicity of *Fasciola* coproantigens in faecal samples stored at 37 °C was retained throughout the entire observation period (Ubeira et al. 2009). Thus, MM3-COPRO ELISA combined with the use of CoproGuard™ may be a very useful tool for the diagnosis of human fascioliasis.

Another study demonstrated that the commercialized DRG *Fasciola hepatica* IgG (human) ELISA is highly sensitive and specific, has a high negative predictive value but has a low positive predictive value. No correlation between egg output and the *F. hepatica* IgG ELISA test values was observed. It was concluded that this test could be used both as an individual serodiagnostic test for human fascioliasis 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 of human fascioliasis worldwide (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). This test was constructed with a recombinant cathepsin L1 from *F. hepatica*, and uses protein A and mAb MM3 as detector reagents in the test and control lines, respectively. 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 where point-of-care testing is required.

### 4.9.3 Fasciolid Species Differentiation by Molecular Tools

Infection by *F. hepatica* and *F. gigantica* cannot be differentiated by clinical, pathological, coprological, or immunological methods. 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.

To distinguish between *F. hepatica* and *F. gigantica*, a simple and rapid PCR-RFLP assay, using the common restriction enzymes *Ava*II and *Dra*II, has recently been described. It is based on a 618-bp-long sequence of the 28S rRNA gene recently obtained from populations of South America, Europe, and Africa. This sequence showed no intraspecific variations within each species and a few nucleotide differences between both fasciolids. This assay provides unambiguous results and may be useful for both individual subject diagnosis and epidemiological surveys of humans and animals in endemic regions of sympatry in Africa and Asia (Marcilla et al. 2002). A similar PCR-RFLP assay using restriction endonucleases *Hsp*92II and *Rca*I has been recently applied to differentiate between Chinese liver flukes (Huang et al. 2004). Another such PCR-RFLP method was later developed (Rokni et al. 2010).

Unfortunately, these three aforementioned PCR-RFLP assays are only useful for the differentiation of pure species, but not for hybrid forms (Mas-Coma et al. 2009a). A similar comment may be applied to the recently dev eloped single step duplex PCR for simultaneous detection of both fasciolid species (Le et al. 2012), as well as to the TaqMan real-time PCR-based assay (Alasaad et al. 2011), and other specific PCR-based assays (Ai et al. 2010). None of these methods proves to be able to detect the wide introgression capacity the two fasciolid species have (Mas-Coma et al. 2009a).

Therefore, DNA marker sequencing still remains as the only appropriate method for both haplotyping of the two pure fasciolid species, as well as for the detection of hybridization 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 mtDNA genes *cox*1 and *nad*1 have so far proved to be the markers of choice, and a complete baseline and nomenclature for these four markers have already been provided (Mas-Coma et al. 2009a).

## 4.10 Treatment

Many drugs have been used to treat human fascioliasis. Emetine derivatives, the classic drugs, were used widely and still continue to be used 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. The same occurred with dehydroemetine despite its better tolerability. Dehydroemetine, at a usual dose of 1 mg/kg daily for 10–14 days, was even considered the therapy of choice a few decades ago (Mas-Coma et al. 2014a, b).

Chloroquine was also used to treat *F. hepatica* infection. Although no cidal effects on the flukes were shown, treatment by this aminoquinoline derivative improved the symptoms dramatically when applied in the acute phase. Among xylol derivatives, hexachloro-para-xylol was effectively used, mainly in the old Soviet Union and China. Bithionol, a halogenated phenol derivative, was proposed as the drug of choice for the treatment of *F. hepatica* infection during the last three decades of last century. It was usually applied at a dose of 30–50 mg/kg daily, divided into 3 oral doses on alternate days for 20–30 days. In cases of fascioliasis resistant to emetine and praziquantel treatment, bithionol achieved cure in dosages of 50 mg/kg daily for 10 alternate days or 40 mg/kg daily for 14–15 alternate days. Occasionally, the patients required a second course to obtain a complete cure. The side effects, including diarrhoea, anorexia, nausea, vomiting, pruritus, urticaria and abdominal pain, were usually mild (Chen and Mott 1990; Esteban et al. 1998). Another halogenated phenol derivative such as niclofolan was also assayed for liver fluke treatment in humans but rapidly abandoned due to its wide side effects.

Praziquantel is an isoquinoline-pyrazine derivative which was widely applied for the treatment of human fascioliasis during the 1980s and 1990s, based on the fact that it is the drug of choice for human trematode infections. However, controversial results were found already from the beginning of its application to fascioliasis patients, including many reported praziquantel failures even at high doses. Today, it is generally accepted that *Fasciola* may be the only trematode genus that has practically no response to praziquantel.

Metronidazole and albendazole and sporadically also mebendazole are imidazole derivatives which have been also applied for human fascioliasis treatment with more or less success. But another imidazole derivative as 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. A cure rate of 79.2% when first used and 100% after a second round of therapy was 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).

The risk of appearance of resistance to triclabendazole can neither be forgotten taking into account the veterinary use of triclabendazole (Fasinex®) for livestock treatment in endemic areas since long ago, the tradition of human self-treatments with Fasinex® owing to the very general availability of this drug, and the appearance of triclabendazole resistance in animals in different countries. 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), and thus already in the New World. 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 the Andean valley of Cajamarca, Peru (Gonzalez et al. 2011). The strategies to minimize the development of resistance include the use of synergistic drug combinations (Fairweather and Boray 1999) although this approach has the risk of building up multiple drug resistance (Gaasenbeek et al. 2001). Additionally, studies suggest that our understanding of the mechanism of resistance to triclabendazole remains far from complete (Fairweather 2005, 2009; Brennan et al. 2007), so that the spreading capacity of triclabendazole resistance remains unknown.

Nitazoxanide is a pyruvate ferredoxin oxidoreductase inhibitor with reported efficacy on a broad parasitological spectrum, such as intestinal protozoans and helminths. It may be considered 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 since 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).

Mirazid® is a drug prepared commercially from myrrh (Arabian or Somali) which is an oleo-gum resin obtained from the stem of thorny trees *Commiphora molmol* and other species of the family Burseraceae. Introduced to the local Egyptian market, it has been highlighted by its efficacy against human fascioliasis in many reports although a recent evaluation proved that it showed only an insignificant activity against the liver fluke (Botros et al. 2009).

Artemisinin derivatives initially showed a high fasciolicidal activity in sheep infection, which was encouraging. Artesunate and artemether, given by the intramuscular route, yielded high egg and worm burden reductions. A study in Vietnam showed that the complete response rate at 3 months was lower than in triclabendazole although those treated with artesunate were significantly more likely to be free of abdominal pain (Hien et al. 2008). Unfortunately, a last study in Egypt demonstrated that artemether, administered at malaria treatment regimens, shows no or only little effect against fascioliasis, and hence does not represent an alternative (Keiser et al. 2011).

## 4.11 Control

Prevention and control measures recommended for human fascioliasis were traditionally the same to be applied for veterinary fascioliasis, at the levels of domestic animals, snails, and field (Roberts and Suhardono 1996; Torgerson and Claxton 1999; Spithill et al. 1999). However, 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 eco-epidemiological studies previously undertaken in the area concerned (Mas-Coma et al. 2009a). This is the reason why the WHO launched a worldwide initiative against this disease including different control strategies depending on the human endemic areas and countries.

The large heterogeneity of the human fascioliasis infection sources underlies a considerable control complexity linked to the many different ways the parasite may follow for successfully accessing the human host. This complexity of the highly heterogenic scenario globally conformed by fascioliasis, that both individual and general prevention measures should face, has deeply been very recently analysed for the first time, including many new concepts, strategies, and even newly proposed specific legislation initiatives (Mas-Coma et al. 2018). Health responsibles, physicians, governmental officers involved, authorities and any other person working on this disease should refer to this freely online available article of the WHO initiative including exhaustive details about measures for the control and fight against human fascioliasis.

In the following, only the traditional frame of control is summarized owing to space restric-

**Fig. 4.7** City market showing uncontrolled sale of vegetables involved in the transmission of human fascioliasis in Quy Nhon, Vietnam (Orig. S. Mas-Coma)

tions. With regard to individual measures, the prevention of human infection may be achieved by strict control of the human infection sources in each place, mainly with regard to watercress and other metacercariae-carrying 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 the very high doses (Ashrafi et al. 2006a).

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 many human hyperendemic areas of the Americas, people do not have a history of eating watercress or other freshwater plants (Esteban et al. 2002). In the Nile Delta region, persons living in houses where piped water is present showed to have a higher infection risk (Curtale et al. 2003b).

The problem does not only concern rural areas, as usually believed. 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. 4.7) (Mas-Coma 2004a).

Within general control measures to be applied in human fascioliasis endemic areas, education should always be included, mainly with regard to the need to let know inhabitants 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.

The availability of a very effective drug against fascioliasis as triclabendazole prompted the WHO to launch a decisive step forward 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 treatments after passive detection in hospitals was the strategy applied in Vietnam, and infected subject treatment after

active detection in surveys in the Nile Delta high human endemic region the one applied in Egypt. Bolivia and Peru were the other two countries selected for priority intervention due to the very large public health problem posed by this disease. The Northern Bolivian Altiplano was chosen as an example of the Altiplanic pattern, while the Cajamarca valley was chosen as an example of the valley pattern. The respective pilot interventions in the two Andean human endemic areas demonstrated the absence of serious side effects in triclabendazole treatments of schoolchildren (Villegas et al. 2012), which subsequently allowed for the launching of annual campaigns of preventive chemotherapy by mass treatments in these two Andean countries. 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.

In Egypt, the construction and utilization 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 2004a).

Regarding veterinary control, previous epidemiological studies may provide for general recommendations on the appropriate time for treatment with effective drugs to achieve economic control, and better information from the livestock farming community. Forecasts of outbreaks may be made based on climatological data and epidemiological models. Recommendations for control measures should be made on a preventive rather than a curative basis, and all measures have to be considered from the point of view of the economy and assessment of local topographical and meteorological conditions. The efficiency of fascioliasis control depends on the correct and integrated application of several measures (Mas-Coma and Bargues 1997): (1) reduction of the parasite load of the animal hosts and pasture contamination by regular strategic use of drugs (preventive treatment in appropriate year periods according to different regions); (2) reduction of the number of snails by physical, chemical, and biological means; (3) reduction of the risks of infection through correct farm management practices (rotational system through fluke-infected and fluke-free paddocks, combined with effective treatment).

Owing to the similarity of the life cycles of the two fasciolid species, prevention and control measures follow the same patterns for both *F. hepatica* and *F. gigantica*. However, the peculiarities of *F. gigantica* should be considered. Thus, in enzootic areas of *F. gigantica*, contraction of the infection by the animals and their contamination of the area with eggs shed with faeces take place when the animals go to drink, rather than when they are grazing in the pasture as is the case in *F. hepatica*. Accordingly, avoiding the watering of the animals from swampy banks of rivers and from bodies of water rich in vegetation would considerably reduce infection chances (Mas-Coma and Bargues 1997).

Lymnaeid vector control has unfortunately not received, by public health officers, the sufficient attention required to definitively eliminate transmission (Chen and Mott 1990). Intensive agricultural methods must be applied to reduce suitable snail habitats. Besides physical methods, there are available control strategies which consist of the use of chemical molluscicides, natural molluscicides of plant origin, biological control (including predators, competitors, the decoy effect and related phenomena, parasitic castration, interspecific trematode antagonism, and pathogens), genetic manipulation, and engineering control. However, the practical application of chemical methods in the control of snails is of doubtful value, requires labour and equipment, and regular yearly strategic molluscicide applications. Moreover, the application of molluscicides in the case of the small *Galba–Fossaria* vector species showing marked amphibious behaviour becomes almost impossible, due to the small size of the water bodies these vectors inhabit.

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