Fascioliasis

S. Mas-Coma, M.A. Valero, and M.D. Bargues

Contents

Abstract Fascioliasis in Latin America is caused by *Fasciola hepatica*. Animal fascioliasis is distributed throughout. Human infection occurs in many countries, mainly Cuba, Mexico, and all Andean countries. Peru is the country where more people are affected, mainly in altitude areas. The Northern Altiplano, in Bolivia and Peru, is the area with higher prevalence and intensities in humans. Children and females are the most affected. Cattle and sheep are the main reservoirs throughout. Pigs and donkeys play an additional reservoir role in human endemic areas. Snail vectors belong to the *Galba/Fossaria* group of lymnaeids, excepting *Pseudosuccinea columella*. Human fascioliasis in Latin America includes hypoendemic, mesoendemic, and hyperendemic situations, human epidemics in both human and nonhuman endemic areas, and other areas with authochthonous

S. Mas-Coma (✉) • M.A. Valero • M.D. Bargues
Departamento de Parasitologia, Universidad de Valencia, Av. Vicent Andres Estelles s/n, 46100 Burjassot, Valencia, Spain
e-mail: S.Mas.Coma@uv.es

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isolated cases and imported cases. The altiplanic and valley patterns at high altitude and the Caribbean insular pattern are the most important transmission patterns. Different freshwater and terrestrial vegetables, local traditional beverages, and water drinking have been reported as human infection sources. Pathology and clinical manifestations in Latin America do not differ from the main pictures known elsewhere, including from no symptoms up to death. Diagnosis mainly relies on stool and serological techniques. Triclabendazole is of choice for human treatment, although resistance to this drug has already been reported in South America, including even a human endemic area. The large WHO control initiative is based on the costless availability of triclabendazole. Successful pilot action in Bolivia and Peru furnished the base for the present mass treatments of children.

Keywords Human fascioliasis • *Fasciola hepatica* • Latin America • Livestock • Animal reservoirs • Lymnaeid snail vectors • Geographical distribution • Transmission patterns • Epidemiological situations • Disease burden • Human infection sources • Pathology and major manifestations • Diagnosis • Treatment • Control

1 Introduction

This food-borne trematodiasis is a great veterinary problem of worldwide distribution. In the last two decades, many surveys have also shown it to be an important public health problem as well (Chen and Mott 1990; Mas-Coma et al. 1999a, 2009a), including estimations of 2,4 million, up to 17 million people, or even higher depending on the hitherto unknown situations in mainly Asia and Africa (Mas-Coma 2004). Many human fascioliasis endemic areas have already been described and the number of human case reports is increasing in many countries of the five continents, comprising mainly developing countries (Mas-Coma 2005; Mas-Coma et al. 2005, 2009a, 2014a), but also developed countries (Arjona et al. 1995).

Moreover, studies on pathogenicity have demonstrated that this disease may be highly pathogenic in humans throughout its long biliary chronic phase, and not only in its invasive acute phase as hitherto considered (Valero et al. 2003, 2006a, 2008), including impressive clinical pictures, high pathogenicity, many sequelae, and a higher mortality rate than the one usually noted (Mas-Coma et al. 2014b). Research on immunity aspects has also shown that fasciolid flukes downregulate the host's immune response during both the early phase of infection (Brady et al. 1999) and the chronic phase (Girones et al. 2007). A consequence of liver fluke infection is the suppression of immune responses directed against concurrent 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 (Esteban et al. 1999, 2002, 2003; Gonzalez et al. 2011; Zumaquero-Ríos et al. 2013).

Additionally, results of recent research showing that human fascioliasis is pronouncedly influenced by climate change and global change add concern on the present situation of this disease in many endemic areas (Mas-Coma et al. 2009b; Afshan et al. 2014).

Such a worrying global scenario underlies the decision to consider fascioliasis an important human parasitic disease henceforth (Mas-Coma et al. 1999b) and include it as a food-borne trematode disease priority within the agenda of the World Health Organization (WHO 2013).

2 The Causal Agent: Systematics, Phenotype, and Genotype

This parasitic disease is caused by two digenean trematodes, *Fasciola hepatica* of worldwide distribution and *F. gigantica* restricted to given regions of Africa and Asia. In the Americas, only *F. hepatica* is present. Two other species of Fasciola were described in the Americas some time ago, namely *F. californica* from the lymnaeid *L. bulimoides* in California and adult experimentally obtained in the rabbit, and *F. halli* found in the liver of cattle and sheep in Texas and Louisiana, transmitted by the lymnaeid *L. bulimoid*es and experimental adults obtained in sheep, although both fasciolids were later synonymized with *F. hepatica* (Mas-Coma et al. 2009a).

Adult worms have a leaf-shaped body, with a broadly pointed posterior end. The two suckers are relatively small and located close to 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 opening 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 adult stage of *F. hepatica* has a maximum length of 29.0 mm and a maximum width of 14.1 mm (Fig. 1) (Periago et al. 2006), although its morphometric characteristics vary according to the different definitive host species (Valero et al. 2001). Two phenotypic patterns could be distinguished in *F. hepatica* adult size in Andean endemic areas: the valley pattern (Cajamarca and Mantaro, Peru) and the altiplanic pattern (Northern Altiplano, Bolivia). Results showed that the Andean valley population presented a phenotypic homogeneity with European standard populations. The Altiplano population showed a large size range with a pronouncedly lower minimum size indicating that uterus gravidity is reached at a

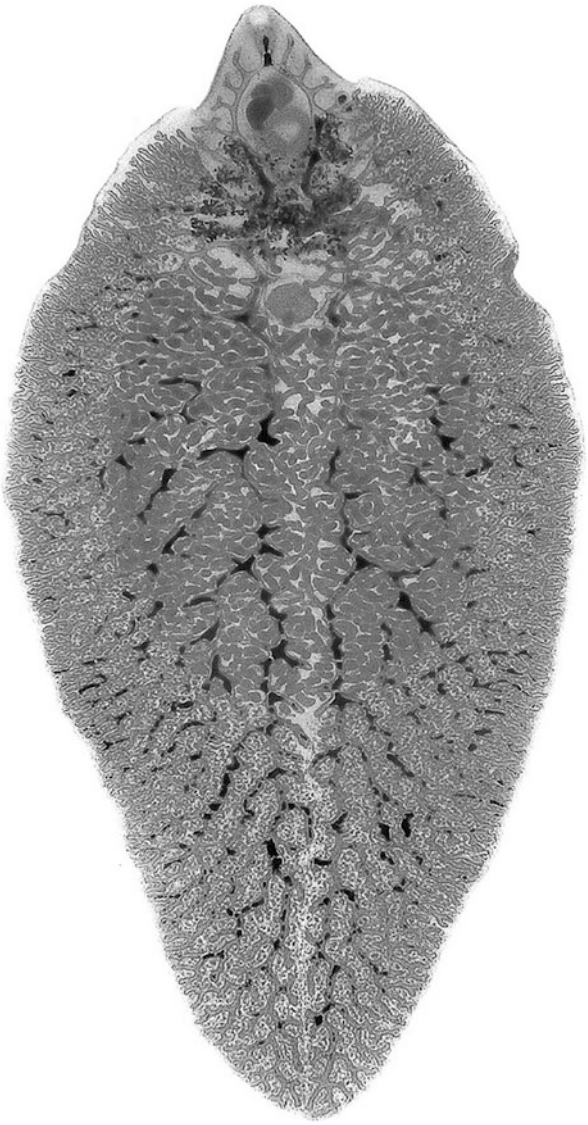

Fig. 1 Adult stage of *Fasciola hepatica* found in a biliary canal of cattle. Note marked shoulders and leaf-shaped body (original S. Mas-Coma)

size lower than in valley populations. The results of this study demonstrated that there is no apparent relationship between the shape of fasciolid adults with regard to the difference in altitude or geographical origin and that allometry-free shape appears as a more stable trait than size in fasciolid species (Valero et al. 2012a).

The eggs of *F. hepatica* are operculated, ovoid, yellow, and nonembryonated when laid (Fig. 2). Their measurements also vary depending on the definitive host species. They are 100.6–162.2/65.9–104.6 μm in humans and 73.8–156.8/58.1–98.1 μm in animals (Valero et al. 2009).

Fig. 2 Egg of *Fasciola hepatica* found in stools of a human patient. Note that fasciolid eggs are still unembryonated when laid and shed with feces (original S. Mas-Coma)

In a wide multicountry genotyping study in different continents, within the nuclear ribosomal DNA (rDNA) operon of *F. hepatica*, the 432-bp-long ITS-1 appeared to be fully uniform (only one haplotype FhITS1-A distributed everywhere), whereas up to four haplotypes of the 364-bp-long sequence of the ITS-2 could be distinguished (FhITS2-1 to 4), of which FhITS2-1 and FhITS2-2 have been found in Latin America: FhITS2-H1 in Peru, Argentina, Chile, Bolivia, Venezuela, Ecuador, México, and Uruguay and FhITS2-H2 in Peru, Argentina, Bolivia, Mexico, and Uruguay (Mas-Coma et al. 2009a).

In the mitochondrial DNA (mtDNA) of *F. hepatica*, a total of 69 different haplotypes of the 1533-bp-long *cox*1-coding gene (Fh*cox*1-1 to 69) and 23 different haplotypes in the corresponding 510-aa-long aminoacid COX1 protein (FhCOX1-1 to 23) were found in this worldwide study. Of the 23 COX1 protein haplotypes, one is the most abundant and present in all countries studied, whereas several countries such as Argentina, Bolivia, Peru, and Mexico present exclusive haplotypes not detected in any of the other countries studied. In the same mitochondrial genome, a total of 51 different haplotypes of the 903-bp-long *nad*1 coding gene (Fh*nad*1-1 to

51) and 15 different haplotypes in the corresponding 300-aa-long aminoacid NAD1 protein (FhNAD1-1 to 15) were found in the same study. Of the 15 NAD1 protein haplotypes, five were exclusive for Argentina, two for Bolivia, two for Peru, one for Mexico, and another for Europe (Spain and Poland). The other haplotypes were shared by different countries (Mas-Coma et al. 2009a).

3 Parasites Life Cycle and Disease Transmission

The two-host life cycle of *F. hepatica* follows a transmission pattern which takes about 14–23 weeks and comprises four phases (Mas-Coma and Bargues 1997; Mas-Coma et al. 2003):

(a) The definitive host harbors the fluke adult stage in the large biliary passages and gallbladder, eggs reaching the external milieu by way of bile and intestine; the definitive host is infected by the ingestion of metacercariae; 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 reach the liver within 6 days after excystment; in the liver, they migrate for 5–6 weeks, preferentially feeding directly on liver tissues; they eventually 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 feces) 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. 2006b); in man, a period of at least 3–4 months is necessary for the flukes to attain sexual maturity; several studies show that the life span of the parasite in sheep can be as long as 11 years and 9–12 months in cattle; different estimations suggest a life span of the adult fluke in man of between 9 and 13.5 years

(b) The transit between definitive mammal host and intermediate snail host includes the long resistance phase of the egg and the short active phase of miracidium; eggs shed with the mammal feces will only continue their development if they reach freshwater of appropriate physicochemical characteristics; if the climatic conditions are suitable (15-25 ° C), the miracidia develop and hatch in about 9–21 days; if conditions are unfavorable, 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

(c) The development at intermediate host level includes miracidium penetration into the snail, sporocyst, redial generations, production of cercariae, and shedding of the latter into water; up to four redial generations have been found, although 3 generations are usually produced after a monomiracidial infection; the redial generations follow the same developmental pattern in different lymnaeid species; the complex development of redial generations has

been recently described (Rondelaud et al. 2009); cercariae develop within 6–7 weeks at 20–25 ° C; at lower temperatures the development is delayed; the prepatent period is dependent on temperature, with higher temperatures reducing the period (15 ° C: 56–86 days; 20 ° C: 48–51 days; 25 ° C: 38 days)

(d) The transit between intermediate snail host and definitive mammal host includes the short swimming phase of cercaria and the long resistance phase of metacercaria until its ingestion by the definitive host; the shedding process takes place between 9° and 26 ° C, independent 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 and quickly encyst, changing into metacercariae; metacercarial cysts become infective within 24 h after encystment

The development of this trematode is thus very dependent on the environmental characteristics according to the nature of phases B and D, which take place fully in the external freshwater milieu, and phase C, which develops completely within a 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). Additionally, it is markedly influenceable by human activities at phase A, which explains its relationships with human behavior (e.g., eating traditions, livestock management) and also anthropogenic modifications of the environment as a component of global change (e.g., artificial irrigation for agriculture, animal export/import) (Afshan et al. 2014). Despite these restrictions, *F. hepatica* has succeeded in expanding from the Near East original geographical area up to actually colonizing the five continents, including the Americas where it was introduced by the Spanish conquistadores 500 years ago (Mas-Coma et al. 2001, 2009a). At present, fascioliasis by *F. hepatica* is the vector-borne parasitic disease presenting the widest latitudinal, longitudinal, and altitudinal distribution known (Mas-Coma et al. 2003).

4 The Animal Reservoirs

Adult fasciolid worms parasitize ruminants, mainly sheep, goats, and cattle, and many other herbivorous domestic and wild mammals, including horses, donkeys, mules, and also Old and New World camelids. Buffalo, deer, wild sheep, domestic and wild pig, various marsupials, rabbit, hare, and nutria are also susceptible hosts (Mas-Coma and Bargues 1997; Mas-Coma et al. 2009a).

In Latin America, cattle and sheep are the main reservoir hosts in the human endemic areas (Mas-Coma et al. 1999c), followed by donkeys and pigs (Mas-Coma et al. 1997; Valero et al. 2001). The capacity of donkeys and pigs to contribute to the transmission and spread of the disease in these areas has proven to be similar to that of cattle and sheep (Mas-Coma et al. 1997; Valero and Mas-Coma 2000).

The Andean camelids, llama, alpaca, guanaco, and vicuña, are very susceptible to *F. hepatica*. Liver fluke infection induces a high pathogenicity in these autochthonous mammals, giving rise to serious economic losses in their husbandry (Timoteo et al. 2005).

5 The Snail Vectors

Freshwater snails of the family Lymnaeidae (Gastropoda) act as intermediate hosts or vectors of fasciolid flukes. Although livestock species play an important reservoir role, transmission studies have shown that the metacercarial infective stage from different origins, such as sheep, cattle, pig, and donkey, represents similar infectivity sources (Valero and Mas-Coma 2000; Valero et al. 2001). On the contrary, the specificity of fasciolid species regarding concrete lymnaeid species (Bargues et al. 2001) represents a crucial factor in establishing not only the geographical distribution of the disease in both animals and humans but also prevalences and intensities due to more or less appropriate ecological characteristics (population dynamics, anthropophylic characteristics, type of water bodies, etc.) of the different lymnaeid intermediate host or vector species. That is why different lymnaeid species appear linked to different transmission patterns and epidemiological scenarios of this very heterogeneous disease in humans (Mas-Coma 2005; Mas-Coma et al. 2009a). The continental differences in lymnaeid faunas also explain that in the Americas fascioliasis is only caused by *F. hepatica*, due to the absence of lymnaeids of the genus *Radix* which act as transmitters of *F. gigantica* (Bargues et al. 2001). Similarly, as in other vector-borne diseases, this relationship supports the use of lymnaeids as biomarkers of the disease at both local and large scales and can thus be useful for the validation of mathematical modeling and remote sensing—geographical information system (RS-GIS) tools for the control of the disease (Fuentes et al. 1999, 2001).

At lymnaeid species level, the problems are found mainly because of the interspecific morphological and anatomic uniformity numerous species show, usually giving serious difficulties in specimen classification, sometimes even impeding it (Bargues et al. 2007a, 2011a; Bargues and Mas-Coma 2005). Moreover, intraspecific variation of shell shape is particularly well marked within lymnaeids depending on environmental conditions. Thus, there are many specimen classification problems, mainly related to species of the "stagnicoline" group, the "radix" group, and the "fossarine" or "*Galba/Fossaria*" group (Bargues et al. 2011a). Fortunately, there are sequence markers in both the nuclear rDNA and the mtDNA which allow today for the appropriate specimen classification (Bargues et al. 2001, 2011a; Bargues and Mas-Coma 2005).

In the Americas, except the only species of the genus *Pseudosuccinea*, *P. columella*, all lymnaeid species involved in the transmission of *F. hepatica* belong to the *Galba/Fossaria* group, a fact that makes specimen classification by simple malacological tools pronouncedly difficult. In northern and central America, as well

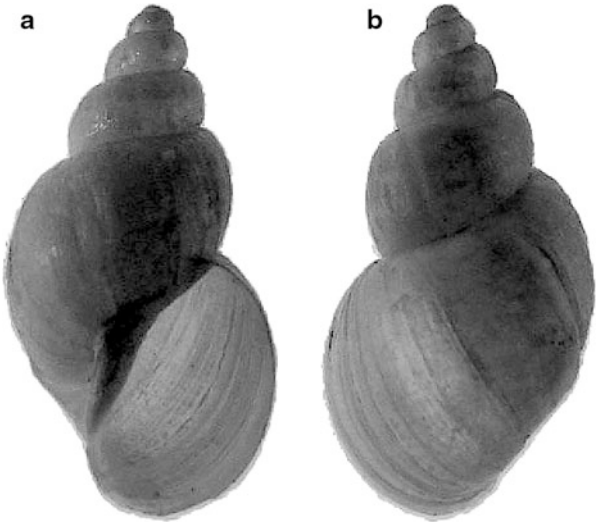

Fig. 3 Specimen of *Galba truncatula*, the main lymnaeid snail vector responsible for human infection in endemic areas of the Andean countries: (**a**) ventral view; (**b**) dorsal view (original S. Mas-Coma)

as in the Caribbean islands and lowlands of northern Venezuela, the lymnaeid vector species are *Lymnaea cubensis* and *P. columella*, and secondarily *L. humilis* and *L. bulimoides* in northern mainland territories of the USA and Mexico (Bargues et al. 1997, 2011a, b).

In South America, the main species related to endemic areas of altitude in the Andean region are *Galba truncatula*, *L. neotropica*, *L. cubensis*, and *L. cousini* in northern countries such as Venezuela, Colombia, Ecuador, and Peru (Bargues et al. 2007a, 2011a, b, c, 2012a). In the human endemic high altitude area of the Northern Altiplano of both Bolivia and Peru, only *G. truncatula* is involved (Fig. 3) (Mas-Coma et al. 2001). In Brazil, the transmission is mainly assured by *P. columella*, and in the Southern Cone countries of Uruguay, Chile, and Argentina, the latter species appears accompanied by the species *G. truncatula*, *L. neotropica*, *L. viator* (= *L. viatrix*), and *L. diaphana* as the main vectors (Bargues et al. 2007a, b, 2012b; Mera y Sierra et al. 2009; Artigas et al. 2011).

6 Epidemiology

Traditionally, the epidemiological characteristics of animal fascioliasis were extrapolated to human fascioliasis. However, in the last two decades, numerous field studies have demonstrated that this was a misunderstading and that human fascioliasis only shows common features with animal fascioliasis at a basic level (Mas-Coma 2005; Mas-Coma et al. 2009a).

6.1 Geographical Distribution

Whereas animal fascioliasis shows an almost worldwide distribution, human infection appears more geographically focused, including human endemic areas in the Near East, Southeast Asia, Western Europe, Northeastern Africa, and Latin America. This does not mean, however, that patients infected by *Fasciola* have been diagnosed in many other countries outside of these hotspot areas (Mas-Coma et al. 2005, 2009a, 2014a).

In Latin America, human infection appears mainly in altitude areas of the Andean region. In the Bolivian Altiplano, human prevalences were of 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, both higher in females than in males (Fig. 4) (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). 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).

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 1,840 m. Fascioliasis prevalences indicate this area to be mesoendemic, with isolated hyperendemic foci, a situation which adds concern

Fig. 4 Recreational and washing activities of the people in a fascioliasis transmission focus of the Northern Bolivian Altiplano, the endemic area with higher prevalences and intensities known in humans (original S. Mas-Coma)

about possible human fascioliasis underestimation in other areas of Mexico (Zumaquero-Ríos et al. 2013).

In the Caribbean region, human fascioliasis mainly poses problems in Cuba, where the first human case was already diagnosed in the first half of the last century (Kouri et al. 1938), 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). Unfortunately, appropriate field surveys are still lacking (Rojas et al. 2009) and 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 some time ago (Clay and Straight 1961).

6.2 Transmission Patterns

Human infection by the liver fluke 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 above-noted known 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.

In Latin America, the following human fascioliasis transmission patterns have been highlighted among the different ones which have been described throughout (Mas-Coma 2005; 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 from seasonal to permanent; at very high altitude, experimental studies have demonstrated that fascioliasis transmission becomes enhanced mainly due to:

   (a) A longer cercarial shedding period
   (b) A higher metacercarial production per snail, and
   (c) A longer survival of the lymnaeid vectors (Mas-Coma et al. 2001).

   Within this category, two subpatterns may be distinguished according to physiographic and seasonal characteristics:

   (i) The altiplanic pattern, with transmission throughout the whole year due to high evapotranspiration rates and the consequent restriction of lymnaeids to

permanent water bodies, e.g., in the Northern Bolivian Altiplano (Fig. 4) and the Puno Altiplano (Mas-Coma et al. 1999c; Esteban et al. 2002)

(ii) The valley pattern, with seasonality and prevalences and intensities related to altitude, higher human infection rates correlating an altitude increase (Gonzalez et al. 2011); examples of such human endemic areas are the valleys of Cajamarca and Mantaro (Valero et al. 2012a); other *Galba*/*Fossaria* vector species appear accompanying *G. truncatula* in these areas (Bargues et al. 2012a)

2. A Caribbean insular pattern, with reduced but repeated outbreaks in human hypoendemic areas and lymnaeid species (*L. cubensis*, *P. columella*) other than the main vector species being involved in the transmission, e.g., the Pinar del Rio Province and other areas in Cuba

Studies are at present under way to assess whether other human fascioliasis transmission patterns may be defined according to more recent descriptions of additional human endemic areas.

6.3 Epidemiological Situations and Disease Burdens

This trematodiasis presents a very wide spectrum of transmission and epidemiological patterns In human hypo- to hyperendemic areas. These are related to the large diversity of environments, including different human endemic/epidemic situations, different human demographies, races, diets, habits, traditions, and religions, different domestic and wild mammal reservoir species, different lymnaeid transmitting species, zones in both the Northern and Southern hemispheres, altitudes from −27 m up to 4,200 m, hot and cold weathers, seasonal and yearly constant temperatures, scarce to pronounced annual rainfall, low and high mean annual potential evapotranspiration, and 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).

The only classification of epidemiological situations hitherto proposed (Mas-Coma et al. 1999a) still appears to be fully valid and useful.

In Latin America, human fascoliasis shows all of these epidemiological situations depending on countries and areas. Authochthonous, isolated, nonconstant cases are typical in Colombia, Brazil, Uruguay, and most of Argentina (e.g., Bargues et al. 2011c; Mera y Sierra et al. 2011), and imported cases from one country to another have been reported several times. Examples of hyperendemic situations are those in the Northern Bolivian Altiplano (Hillyer et al. 1992; Bjorland et al. 1995; Esteban et al. 1999; Mas-Coma et al. 1999c), rural altitude areas throughout the Andean chain of Peru (Esteban et al. 2002; Raymundo et al. 2004; Gonzalez et al. 2011), and an area in central Chile (Apt et al. 1993; Artigas et al.

2011). A mesoendemic situation has recently been described in Mexico, even including a short number of hyperendemic foci (Zumaquero-Ríos et al. 2013). Areas described in Ecuador and Venezuela fit a hypoendemic situation's characteristics (Trueba et al. 2000; Bargues et al. 2011b).

Epidemics in nonhuman endemic but animal endemic areas, including familiar outbreaks involving only a few subjects, have been reported several times from Argentina (Mera y Sierra et al. 2011). A situation of relatively large epidemics involving many subjects in human endemic areas is the one typically reported from Cuba (Esteban et al. 1998).

6.4 Human Infection Sources

The infectivity of metacercariae is dependent upon storage time. In experimental assays, it proved to be lower when metacercariae are older: the maximum longevity was 31 and 48 weeks using doses of 20 and 150 metacercariae per host, 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).

The ingestion of infective metacercariae by humans may occur by different ways. Several infection sources have been distinguished in studies performed in the last two decades (Mas-Coma 2004): (1) ingestion of freshwater wild plants (important in animal endemic areas); (2) ingestion of freshwater cultivated plants, mainly watercress; (3) ingestion of terrestrial wild plants, including even those collected in dry habitats but which were submerged in water a few weeks or months before; (4) ingestion of terrestrial cultivated plants needing frequent irrigation; (5) drinking of contaminated water; (6) ingestion of dishes and soups made with contaminated water; (7) washing of kitchen utensils or other objects with contaminated water; and (8) ingestion of raw liver infected with migrating metacercariae which may keep the capacity to restart migration.

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 (Ashrafi et al. 2006).

In the Northern Bolivian Altiplano, a study was performed on the presence of metacercariae in semi-aquatic plants collected from a swamp in an endemic locality. According to the fluke number obtained in guinea pigs experimentally infected with 100 g of plant, the plants could be classified into seven risk levels: Compositae: 56,3; *Eleocharis* sp.: 50,9; *Senicio* sp.: 12,0; *Vallisneria* sp.: 10,3; *Scirpus* sp.: 3,3; Ranunculaceae: 2,6; and Liliaceae: 0. In this high human endemic area, reports suggest that human infection is related to traditional consumption of uncooked aquatic plants, including:

– matara: *Juncus andicola* (Juncaceae);
– totorilla = "kosko-oskosko": *Juncus ebracteatus* (Juncaceae);
– watercress = berros = "okororo": *Mimulus glabratus* and *Nasturtium officinale* (Scrophulariaceae);
– brown algae = cochaguyo = "llayta": *Nostoc* sp. (Cianofitas);
– and others still undetermined (Mas-Coma et al. 1995).

The typical plant of the Lake Titicaca, *Schoenoplectus californicus* ssp. *tatora*, known as totora or "chullu" (Cyperaceae), had been also involved in the transmission, but subsequent analyses showing that the roots of this plant produce molluscicidal secretions enabled to rule out its participation (Mas-Coma et al. 1999c).

In Mexican children, an association between fascioliasis and the habit of eating raw vegetables was identified, including from more to less risk as follows: watercress and radish with pronouncedly higher relative risk than lettuce, corncob, spinach, alfalfa juice, and broccoli. The link of fascioliasis risk with the 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). In Peru, patients mentioned having eaten watercress (45.6 %), lettuce (31.6 %), alfalfa (10.5 %), or spinach (5.3 %), drinking natural water from small streams (10.5 %), or emollients (warm beverages made from various plants, chiefly alfalfa and watercress, and supposed to be good for liver diseases) (5.3 %), among others (Blancas et al. 2004).

Water is often cited as a human infection source. In the Bolivian Altiplano, 13 % of the metacercariae of all isolates are floating (Bargues et al. 1996). This becomes very important owing to the very high number of cercariae-shedding lymnaeids which may be found: 31.6 % prevalence in lymnaeids from the endemic locality of Tambillo, where up to 7 metacercariae were found in only half a liter of water from the small river crossing this locality (Mas-Coma 2004). The importance of fascioliasis transmission through water is supported by many indirect results. There are significant positive associations between liver fluke infection and infection by other waterborne parasites, such as *Giardia intestinalis* in both Bolivia and Peru (Esteban et al. 1997a, 2002). Moreover, in many human hyperendemic areas of the Americas, people do not have a history of eating watercress (Hillyer and Apt 1997), and in zones as the Asillo irrigation area of the Peruvian Altiplano, inhabitants do not consume freshwater plants (Esteban et al. 2002).

7 Pathology and Major Manifestations

In human fascioliasis, four clinical periods may be distinguished (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 the other of these two periods almost always when diagnosed.

In the invasive or acute period, symptoms are 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 diarrhea, respiratory symptoms such as cough, dyspnea, hemoptysis, and chest pain, and also urticaria.

In the biliary or chronic period, adult flukes cause inflammation, hyperplasia of the epithelium, and thickening and dilatation of the bile duct and gallbladder 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 gallbladder is frequent, whereas cirrhosis does not appear to be so (Marcos et al. 2009). The bile duct and the gallbladder may contain blood mixed with bile (hemobilia), 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 nonspecific (Marcos et al. 2002).

The very long life span of fasciolid flukes in humans, of up to 13.5 years (Mas-Coma & Bargues 1997), underlies many problems regarding complications and sequelae in long-term chronicity. The most common clinical complications listed in the literature are biliary obstruction, cholecystitis, recurrent cholangitis, liver abscesses, and subcapsular hemorrhages (Arjona et al. 1995). Bleeding, multiple extrahepatic venous thrombosis, pancreatitis, and biliary colics have also been reported. Anemia, lithiasis, and bacteriobilia have recently proven to be additional important complications in the chronic period (Valero et al. 2003, 2006a, 2008). In human endemic areas of developing countries, coinfections with other protozoan and helminth parasites may add more complications to the patients, mainly due to the immunesuppression induced by the liver fluke infection.

Hepatic lesions may appear persistent many years after a successful treatment which allowed for short-term normalization of symptoms and laboratory values (Rondelaud et al. 2006). Clinical recovery is much faster than radiological clearance (Kabaalioglu et al. 2007).

In human hyperendemic zones with depauperated socioeconomic status, unhygienic conditions, and high child morbidity and mortality as the Bolivian Altiplano (Mas-Coma et al. 1995), studies are needed to ascertain whether fasciolosis may be related to death, above all in very young children and on the new light about immunesupression, coinfections, and complications.

Ectopic fascioliasis comprises clinical pictures caused by fasciolids in locations of the human body different from the liver. 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. 2014b). 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; Mas-Coma et al. 2014b). Pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis.

A recent wide analysis has shown that neurofascioliasis or intracranial infection by *Fasciola* and ophthalmofascioliasis or direct affection of the eye by migrating flukes may be rare, although not sporadic as previously believed. However, manifestations including a very wide range of neurological symptoms, signs, and syndromes, together with meningeal, psychiatric, or neuropsychic manifestations, and ocular disorders caused at distance by flukes infecting the liver may be frequent but underestimated due to misdiagnosis, mainly in low-income regions. The impressive clinical pictures should be highlighted. They include from hemiplegia and paraplegia to disturbances and difficulties of walking capacity, speech disorders, convulsions, epilepsia and coma, amnesia, or visual hallucinations and permanent blindness, only to mention a few, plus the clinical complexity of the puzzling poymorphisms, 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 postreatment 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. 2014b).

8 Diagnosis

In a developed country, blood eosinophilia and the ingestion of watercress or any other suggestive freshwater plant in anamnesis are extremely useful in guiding towards a fascioliasis diagnosis. Unfortunately, these two aspects are usually not helpful in human endemic areas of developing countries, where eosinophilia may be also caused by other helminth infections and local food traditions including the ingestion of many uncooked plants may mask liver fluke infection (Mas-Coma et al. 2014b).

Abnormal laboratory findings concern leucocytosis, eosinophilia, anemia, erythrocyte sedimentation rate, hepatic functions, and serum immnunoglobulin levels (Chen and Mott 1990; Mas-Coma et al. 1999b, 2000). Several suggestive clinical presentation aspects may be useful, mainly in human endemic areas where physicians are aware about liver fluke infection risk in humans. However, verification needs the use of at least one among the direct parasitological techniques or indirect immunological tests (Mas-Coma et al. 2014a). Other noninvasive diagnostic techniques presently available may be additionally helpful. Noninvasive diagnostic techniques which can be used for human diagnosis are radiology, radioisotope scanning, ultrasound, computed tomography, and magnetic resonance (Esteban et al. 1998; Hillyer 1999).

Finding and identification of fasciolid eggs in fecal samples, duodenal contents, or bile continue to be the most appropriate diagnostic strategy for both detection of infection and estimation of intensity. This is even in spite of the recognized lower sensitivity of egg detection in fecal 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 g of feces and the fluke burden (Valero et al. 2006b, 2009). 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. 2014a).

Egg counting is crucial in the moment of deciding the appropriate treatment dose. The 400-epg threshold has been proposed for identifying high intensity infections. To avoid the risk of colic, a repeated, timely spaciated middose is recommended to patients shedding more than 400 eggs (WHO 2007; Valero et al. 2012b). The second half of the regimen is administered 24 h later, once the absence of secondary effects is 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. Quantitative coprological analyses also become important in epidemiological surveys as well as postreatment monitoring (Mas-Coma et al. 2014a).

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

Many 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 other situations in which coprological techniques may present problems. However, immunological techniques offer other types of problems related mainly to sensibility and specificity. 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 (1) the delay in its usefulness in the acute period (coprological examination positive only after 3–4 months postinfection), (2) intermitent egg output

dynamics, (3) very low or even the absence of egg shedding in cases of only one or a few fluke adults and old, chronic infections, (4) ectopic infections, (5) "false" fascioliasis related to eggs in transit after ingestion of infected liver from domestic animals, or (6) flukes unable to attain maturity in human subjects in nonhuman endemic areas (Mas-Coma et al. 2014a).

Cysteine proteinases offer highly sensitive and specific markers for human fascioliasis serodiagnosis for *F. hepatica* (O'Neill et al. 1999; Strauss et al. 1999; Mezo et al. 2004; Espinoza et al. 2007). *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.

In Bolivia and Peru, the MM3 coproantigen-detection test allowed 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 programs. However, this technique falls short when evaluating the fluke burden on its own (Valero et al. 2012b). The use of a new preservative/diluent CoproGuard™, developed for the preservation of *Fasciola* coproantigens, proved to enhance coproantigen extraction and the antigenicity throughout the complete observation period (Ubeira et al. 2009).

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

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

9 Treatment

Drugs such as emetine and the better tolerated dehydroemetine were used widely in the past 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 (Mas-Coma et al. 2014b). Chloroquine improved the symptoms when applied in the acute phase. Bithionol was proposed as the drug of choice for fascioliasis treatment during the last three decades of the last century. It was usually applied at a dose of 30–50 mg/kg daily, divided into 3 oral doses on alternate days for 20–30 days. Occasionally, the patients required a second course to obtain a

complete cure. The side effects were usually mild (Chen and Mott 1990; Esteban et al. 1998).

With regard to praziquantel, fasciolids are the only trematodes that have practically no response to this drug. Metronidazole and albendazole and sporadically also mebendazole have been also applied for human fascioliasis treatment with more or less success.

Triclabendazole (Egaten®) has become the drug of choice for human fascioliasis 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).

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

Nitazoxanide may be an alternative to triclabendazole, at least for the chronic stage of fascioliasis and for patients with low burdens, 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, a triclabendazole-resistant *F. hepatica*-infected patient not responding to nitazoxanide treatment has recently been reported in the Netherlands (Winkelhagen et al. 2012), and no response to nitazoxanide treatment was reported in 24 cases of liver fluke infection in Esmeralda, Camagüey, Cuba (Del Risco et al. 2001).

10 Control and Elimination

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. Commercial growing of watercress should be carried out under completely controlled conditions, without access for snails and ruminants. 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 unfortunately shown to have no effect on metacercarial viability, even at very high doses of 300 mg/l, 600 mg/l, and 1,200 mg/l (Ashrafi et al. 2006).

Human infection risk shall not be restricted to only ingestion of freshwater vegetables, as always mentioned. The aforementioned different human infection sources may be considered, mainly in high human endemic areas. Drinking of natural freshwater should be avoided in human endemic areas. Moreover, the possibility of human infection in urban areas should also be taken into account. Thanks to transport of vegetables (both aquatic and terrestrial) from rural endemic zones to cities, plants carrying metacercariae can be sold in noncontrolled city markets giving rise to urban infection (Mas-Coma 2004).

Education should always be included within general control measures to be applied in human fascioliasis endemic areas, 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 transmission, and its danger.

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). 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 different epidemiological situations and transmission patterns in the way to decrease morbidity, mainly in children. Bolivia and Peru were the 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 mass treatments of mainly children in these two Andean countries. Many other countries are nowadays receiving yearly triclabendazole donations through WHO for the treatment of their patients.

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 (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; and (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) (Mas-Coma and Bargues 1997).

Lymnaeid snail vector control has unfortunately not received the attention from public health officials 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, and requires labor and equipment, and regular yearly strategic molluscicide applications. Moreover, the application of molluscicides in the fight against vector species of the *Galba/Fossaria* group becomes very difficult and sometimes unaffordable when the snails inhabit small temporary waterbodies from rainfall. The ecological requirements of *P. columella* may, however, be more appropriate for molluscicidal application.

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