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Invited review

Fascioliasis and other plant-borne trematode zoonoses

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Abstract

Fascioliasis and other food-borne trematodiases are included in the list of important helminthiases with a great impact on human development. Six plant-borne trematode species have been found to affect humans: *Fasciola hepatica*, *Fasciola gigantica* and *Fasciolopsis buski* (Fasciolidae), *Gastrodiscoides hominis* (Gastrodiscidae), *Watsonius watsoni* and *Fischoederius elongatus* (Paramphistomidae). Whereas *F. hepatica* and *F. gigantica* are hepatic, the other four species are intestinal parasites. The fasciolids and the gastrodiscid cause important zoonoses distributed throughout many countries, while *W. watsoni* and *F. elongatus* have been only accidentally detected in humans. Present climate and global changes appear to increasingly affect snail-borne helminthiases, which are strongly dependent on environmental factors. Fascioliasis is a good example of an emerging/re-emerging parasitic disease in many countries as a consequence of many phenomena related to environmental changes as well as man-made modifications. The ability of *F. hepatica* to spread is related to its capacity to colonise and adapt to new hosts and environments, even at the extreme inhospitality of very high altitude. Moreover, the spread of *F. hepatica* from its original European range to other continents is related to the geographic expansion of its original European lymnaeid intermediate host species *Galba truncatula*, the American species *Pseudosuccinea columella*, and its adaptation to other lymnaeid species authochthonous in the newly colonised areas. Although fasciolopsiasis and gastrodiscoidiasis can be controlled along with other food-borne parasitoses, fasciolopsiasis still remains a public health problem in many endemic areas despite sustained WHO control programmes. Fasciolopsiasis has become a re-emerging infection in recent years and gastrodiscoidiasis, initially supposed to be restricted to Asian countries, is now being reported in African countries.

Keywords: Plant-borne trematode zoonoses; Fascioliasis; Fasciolopsiasis; Gastrodiscoidiasis

1. Introduction

The importance of food-borne trematode infections and the need to implement control measures against them have been emphasised by the World Health Organization (WHO) (1995b). Plant-borne trematodes have recently been included in the list considered by the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition (Orlandi et al., 2002). More recently fascioliasis and other food-borne trematodiases were added to the list of important helminthiases with a great impact on human development, at the Third Global Meeting of the Partners for Parasite Control held in WHO Headquarters Geneva in November 2004 (Anonymous, 2004). Moreover, present climate and global changes appear to increasingly affect those snail-borne helminthiases, which are heavily dependent on the environment for dissemination. Fascioliasis is a good example of an emerging/re-emerging parasitic disease in many countries as a consequence of many phenomena related to both environmental change and man-made modifications.

Six plant-borne trematode species have been found to affect humans: *Fasciola hepatica, Fasciola gigantica, Fasciolopsis buski, Gastrodiscoides hominis, Watsonius watsoni* and *Fischoederius elongatus*. Whereas *F. hepatica* and *F. gigantica* are hepatic parasites, the four other species are intestinal parasites. The fasciolids and the gastrodiscid cause important zoonoses distributed throughout many countries, while *W. watsoni* and *F. elongatus* have only been accidentally detected in humans.

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Watsonius watsoni has been reported only twice in humans. The first finding took place during the autopsy of an emaciated West African who had died of severe diarrhoea in Liberia in 1904. Many worms were recovered from the intestine, some attached to the duodenal and jejunal wall, others free in the lumen of the colon. The second case, also affected by diarrhoea, was detected in Nigeria and shed numerous specimens too. Various species of primates in eastern Asia and Africa are natural hosts of this parasite. The complete history of those two cases and a study on the parasite and its symptomatology and pathology in primates were described by Pick (1964, 1967). The morphology of the parasite was described by Stiles and Goldberger (1910). Its life cycle is unknown, but infection is probably acquired by ingesting vegetation on which the metacercariae have encysted (Yu and Mott, 1994).

Fischoederius elongatus is a parasite of ruminants infected by ingesting aquatic plants or snails (*Lymnaea acuminata*, *Lymnaea succinea*, *Gyraulus euphraticus*) carrying attached, encysted metacercariae. It is transmitted by lymnaeid snails, such as *Lymnaea luteola*, in Asia (Yamaguti, 1975). The first human infection was reported from Guangdong, China (Li, 1991). A 35-year-old woman complained of epigastric pain for months and vomited a worm of this species one morning while brushing her teeth, after which the symptoms disappeared. It is unknown how the patient became infected.

2. Fascioliasis

Fascioliasis is an important disease caused by *F. hepatica* and *F. gigantica*. Whereas in Europe, the Americas and Oceania only *F. hepatica* is concerned, the distributions of both species overlap in many areas of Africa and Asia. Two hosts are needed for these species to complete their life cycle. The definitive host range is very broad and includes many herbivorous mammals, including humans. Intermediate hosts are freshwater snail species of the family Lymnaeidae (Gastropoda: Basommatophora). The literature concerning the disease in animals (see Dalton, 1999) and humans (Boray, 1982; Chen and Mott, 1990; Mas-Coma and Bargues, 1997; Mas-Coma et al., 1999b) is considerable. The following summarises recent information on this disease.

2.1. Etiology

2.1.1. Fasciolid genotyping

Different molecular techniques and DNA markers have been applied to fasciolid flukes. These molecular techniques appear to be very useful for the development of both diagnostic and epidemiological tools as well as for studies of intraspecific variability of the causal agents.

Most studies on fasciolid proteins have concentrated on isoenzymes. Unfortunately, only very few studies have 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 differed according to host (Blair, 1993). Isoelectric focusing of fluke soluble proteins was used to confirm the presence of both *F. hepatica* and *F. gigantica* in Egypt (Lotfy et al., 2002), although profiles of whole-body proteins and excretory/secretory (E/S) products obtained with this technique differ among worms from different hosts such as sheep and calves (Lee et al., 1992). Random amplified polymorphic DNA (RAPD) markers were used to quantify the genetic diversity in *F. hepatica*. These studies showed that the majority of variance occurred within, rather than between, hosts and was also greater within than between populations. Individual cows were found to be infected by numerous genetically different liver flukes, suggesting the influence of migrations and transportation of definitive hosts (Semyenova et al., 2003).

Parts of the mitochondrial DNA of *F. hepatica* showed length heterogeneity, suggesting differences among individual mitochondrial genomes (Zurita et al., 1988). The whole mitochondrial genome of *F. hepatica* has recently been sequenced and will be suitable for studies of variation (Le et al., 2001).

Nicotinamide adenine dinucleotide dehydrogenase subunit I (NDI) and cytochrome c oxidase subunit I (COI) sequencing results (Itagaki et al., 1998) showed that Japanese *Fasciola* forms were more closely related to *F. gigantica* than to *F. hepatica*, contrary to previous predictions obtained from the similar band pattern detected between fasciolids via the PCR-single-strand conformational polymorphism (SSCP) method (Itagaki et al., 1995). Moreover, a high intraspecific variation (8.3%) in a 145-bp-fragment of the NDI sequence of *F. gigantica* and a low one (0.2%) in a 474-bp-fragment of the COI sequence of *F. hepatica* and the Japanese form of *Fasciola* were found (Itagaki et al., 1998).

Restriction fragment length polymorphism (RFLP) patterns were analysed for the whole mitochondrial DNA of *F. hepatica* from Australia, *F. gigantica* from Malaysia and *Fasciola* sp. from Japan after digestion with three four-base-cutting endonucleases: *Hinf* I, *Msp* I and *Rsa* I (Hashimoto et al., 1997). The mtDNA digestion patterns differed markedly between the three fasciolids. For each enzyme, there were some bands specific for each geographical isolate, the Japanese *Fasciola* sp. sharing more bands with *F. gigantica* than with *F. hepatica*. However, given the variation observed within the short region of the COI gene sequenced, there are likely to be considerable differences in RFLP patterns even between quite closely related forms. Additionally, 25–28 nucleotide differences were detected in a 395-bp-long COI fragment between *F. hepatica* and *Fasciola* sp./*F. gigantica*, and 4–5 between *Fasciola* sp. and *F. gigantica*. Moreover, intraspecific variation was found at one nucleotide site between

different specimens from the same *F. gigantica* population from Malaysia (Hashimoto et al., 1997).

Restriction endonuclease maps of the rRNA genes were distinct for *F. hepatica* and *F. gigantica*, Japanese *Fasciola* sp. being identical in its restriction map to *F. gigantica*. No intraspecific variations in the maps of *F. hepatica* (11 isolates from six countries) or of *F. gigantica* (two isolates from different countries) were detected, but length heterogeneity was noted in the intergenic spacer, even within individual worms (Blair and McManus, 1989).

In a fragment of less than 200 bp of the D1 domain of the 28S rRNA gene, a total of six differences were detected between *F. hepatica* found in sheep from Ipswich and *F. gigantica* in cattle from Malaysia (Barker et al., 1993), but, unfortunately, no intraspecific variation studies were performed.

The second internal transcribed spacer (ITS-2) of the rDNA has been used several times for fasciolid classification. One nucleotide difference in a 263-bp-long ITS-2 fragment between the *F. hepatica* population from Mexico and those from Australia, Hungary and New Zealand, and no difference in the 213-bp-long ITS-2 fragment between *F. gigantica* from Indonesia and Malaysia were found. *Fasciola hepatica* and *F. gigantica* differed at six nucleotide sites among the 213 nucleotides compared, and *Fasciola* sp. from Japan differed at seven nucleotide positions from *F. hepatica* and in one from *F. gigantica* (Adlard et al., 1993). Later, similar results were found in the near-complete, 362-bp-long ITS-2 sequence, although no difference was found between Japanese *Fasciola* sp. and *F. gigantica* (Hashimoto et al., 1997).

The ITS-2 sequences of the seven Japanese triploid fasciolids were divided into two distinct types: F.sp.I, almost identical to that of *F. hepatica* from Uruguay, and F.sp.II, similar to *F. gigantica* from Indonesia and Japan from previous reports. No intraspecific variation was detected between two *F. hepatica* specimens from Uruguay. However, two different sequences were obtained from individuals of *F. gigantica* from Zambia and a third sequence from *F. gigantica* from Indonesia, which was identical to that from Malaysia. It was concluded that the Japanese triploid form of *Fasciola* might be a hybrid between *F. hepatica* and *F. gigantica* because the NDI and COI sequences of F.sp.I were almost identical to those of *F. gigantica* from Zambia but not to *F. hepatica* from Uruguay (Itagaki and Tsutsumi, 1998). ITS-2 sequences were also used to characterise the liver flukes from mainland China. *Fasciola hepatica*, *F. gigantica* and an intermediate genotype, including polymorphism among ITS-2 copies within the same fluke individual, were found (Huang et al., 2004).

The complete ITS-2 sequence of *F. hepatica* from Spain and Bolivia proved to be identical and differ from other geographic origins at least in one position. Similarly, the complete, 433-bp-long ITS-1 showed no nucleotide difference between Spain and Bolivia. These results suggest that the Bolivian fluke derives from Iberian ones, due to their recent introduction, which most probably took place at or after the time of Spanish colonisation (Mas-Coma et al., 2001). According to molecular clock estimations based on ITS sequences, very few ITS nucleotide differences between different regions of the world suggest a very recent geographical diffusion of *F. hepatica* from Europe into the other continents (Mas-Coma et al., 2003).

Five of six microsatellite markers were shown to be polymorphic in *F. hepatica* from Bolivia. No genetic differences between sampling sites or between definitive host species (sheep, cattle, pig) were found. However, the significant variability that was detected suggests a preferential outcrossing mode of reproduction for this hermaphroditic parasite (Hurtrez-Bousses et al., 2004), a process which may be influenced by present day livestock transport.

Several interesting attempts have been undertaken to develop a nucleic acid probe capable of sensitive and specific detection of *F. hepatica* in lymnaeid-transmitting snails. The value of several *F. hepatica* 18S rRNA sequences was already tested by Shubkin et al. (1992) and Ronglie et al. (1994), achieving different results. An assay using the reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify a region of *F. hepatica* 18S rRNA, followed by hybridisation to a *F. hepatica*-specific probe, was able to detect individual infected snails immediately after miracidial exposure and throughout the parasite's development period. However, the assay was not species-specific because of cross-reaction with another fasciolid, *Fascioloides magna*. A DNA probe for *F. hepatica* was described that can be used to detect the fluke larvae in the intermediate host by the squash-blot method (Heussler et al., 1993; 1998). The high sensitivity of a DNA probe for the detection of *F. hepatica*-infected snails which does not cross-hybridise with DNA of *F. magna* and other trematodes (Kaplan et al., 1995) was further improved by using chemiluminescent detection (Kaplan et al., 1997). Similar results were also obtained in the detection of *F. hepatica* in European *Galba truncatula* by applying a specific repeated, non-coding and very abundant DNA sequence of 124 base pairs (Kozak-Cieszczyk et al., 2002 in XXXII Annual Meeting, European Society for New Methods in Agricultural Research, Warsaw).

2.1.2. Adult stage phenotyping

Exhaustive morphometric comparisons of *F. hepatica* adults and eggs from sheep, cattle, pigs and donkeys of the Bolivian Altiplano, as well as *F. hepatica* adults and eggs experimentally obtained in Wistar rats infected with Altiplanic sheep, cattle and pig isolates, were made following measurement standardisation and allometric growth studies with computer image analysis system (CIAS) (Valero et al., 1996; 1999). Statistical analyses of the allometries showed that fluke adult populations from sheep, cattle and pigs significantly differ in the functions of: (i) body length versus body width; and (ii) body length

versus distance between posterior end of body and ventral sucker. Statistical analysis of *F. hepatica* egg size showed characteristic morphometric traits in each definitive host species. In experimentally infected rats, fluke adult allometry and egg morphometry did not vary in parallel with the Altiplanic definitive host species isolate. This study revealed that the definitive host species influences the size of *F. hepatica* adults and eggs, and that this influence does not persist in a heterologous host (Valero et al., 2001b). Eggs shed by both naturally and experimentally infected murid rodents (wild *Mus musculus* and *Rattus rattus* from Corsica island and *Rattus norvegicus* Wistar laboratory strain) were smaller in size than those shed by naturally infected cattle from the same region (Valero et al., 2002).

Applying the same method, Valero et al. (1999) were able to detect slight but significant differences between sheep liver flukes from the Northern Bolivian Altiplano and Spain. The results obtained did not show differences in egg size, and in adults, only a smaller size was found in the majority of the parameters in the Bolivian material. The minor differences can be accounted for by geographical variability related to effects of altitude or genetic isolation and suggest that the Bolivian population is a recent isolation from Iberian populations. Interestingly, Bolivian sheep and cattle liver fluke populations proved to have a uterine size smaller than that of European populations. Although this may be attributable to intraspecific variability, these uterus differences between populations of highlands and lowlands were thought to be related to high altitude influences (Valero et al., 2001a). High altitude hypoxia conditions could be the origin of a reduced egg production by the flukes. Moreover, although the uterus in digeneans has traditionally not been considered a storage organ but mainly an organ adapted to the developmental time of the eggs (in fasciolids, eggs are layed unembryonated, the miracidium beginning its development in eggs once in freshwater), in the Northern Bolivian Altiplano, climatic conditions, freshwater body characteristics and lymnaeid ecology enable fascioliasis transmission to take place throughout the year (Mas-Coma et al., 1999c; Fuentes et al., 1999). Thus, egg storage a priori is not needed as in the northern hemisphere latitudes where fascioliasis transmission is typically seasonal (Valero et al., 2001a).

The overlapping distribution of *F. hepatica* and *F. gigantica* has become the basis of a long running controversy on the taxonomic identity of the *Fasciola* species in countries of the Far East, especially Japan, Taiwan, the Philippines and Korea, in which a wide range of morphological types has been detected. At the extremes of this morphological range, some resemble *F. hepatica*, whereas others resemble *F. gigantica*, with intermediate forms also occurring and involving phenomena such as abnormal gametogenesis, diploidy, triploidy and mixoploidy, parthenogenesis, and hybridisation events between different genotypes (see review in Mas-Coma and Bargues, 1997). The existence of hybrids was fairly recently confirmed when it was shown that given Japanese triploid forms presented rDNA ITS-2 sequences almost identical to those of *F. hepatica* and mtDNA NDI and COI sequences almost identical to those of *F. gigantica* (Itagaki and Tsutsumi, 1998). The recent finding of an aspermic triploid, asexually reproducing, liver fluke isolate in the UK suggests that facultative gynogenesis may be widespread in this parasite (Fletcher et al., 2004).

2.2. Transmission and spread

2.2.1. Larval development

The ability of *Fasciola* to spread is related to the large capacities of fasciolids to colonise and adapt to new environments, even to the extreme inhospitality of very high altitude. The development of *F. hepatica*/*G. truncatula* from the Northern Bolivian Altiplano high altitude endemic area (3800–4100 m) was compared with that of the European homologue. At altitude particular aspects appear modified in the way that transmission is favoured, such as the longer cercarial shedding period and the higher cercarial production. Both aspects are related to the greater survival capacity of the infected lymnaeid snails. Moreover, a discrepancy was found in the distribution of cercariae throughout the shedding period, as in the Bolivian model the highest daily numbers of cercariae are shed in the first weeks, whereas in the European model acrophases appear delayed (Mas-Coma et al., 2001).

Lymnaeid survival in the experiments carried out with *F. hepatica* and *G. truncatula* from the Northern Bolivian Altiplano are worth mentioning. The longevity of the experimentally parasitised Bolivian molluscs after the moment of the miracidial infection was longer than in the European species *G. truncatula*, and also longer than in other American lymnaeids such as *Lymnaea viatrix* and *Lymnaea bulimoides*. The capacity of Altiplanic lymnaeids to survive for more than 4 months after the end of the shedding period is surprising, as in Europe snails die during the shedding period, immediately after the end of shedding or shortly after it. Moreover, the absence of survival differences between parasitised and non-parasitised molluscs suggests a better parasite–host adaptation in Bolivia (Mas-Coma et al., 2001).

The capacity of *F. hepatica* to produce more larval stages when infecting specimens of the same lymnaeid species from another place may also be related to its fast geographic spreading capacity. This was detected in Europe when experimentally infecting *G. truncatula* from different geographic origins with the same liver fluke isolate (Gasnier et al., 2000).

2.2.2. Adaptation to new definitive hosts

Fasciola hepatica has succeeded in expanding from its European origin due to the exportation of European livestock to colonise five continents, where it has adapted to other autochthonous mammal species such as camelids in

Africa, aukenids in South America and marsupials in Australia (Mas-Coma et al., 2003).

Recent studies on isoenzymes, protein sequences and mitochondrial DNA sequences suggest that fasciolids are able to develop a capacity for definitive host species selection (Miller et al., 1993; Panaccio and Trudgett, 1999; Spithill et al., 1999). This finding poses the question of liver fluke circulation within an endemic area (Valero et al., 2001b). In Belorussia, for example, it has been found in seven wild animal species: the elk *Alces alces* (5.6% prevalence), the red deer *Cervus elaphus* (31.3%), the reo deer *Capreolus capreolus* (6.3%), the wild boar *Sus scrofa* (7.1%), the beaver *Castor fiber* (10.0%), the otter *Lutra lutra* (4.0%) and the hare *Lepus europaeus* (9.1%) (Shimalov and Shimalov, 2000). The fast capacity of *F. hepatica* to quickly adapt to new definitive host species is illustrated by the examples of the black rat in Corsica island (Valero et al., 1998, 2002; Mas-Coma et al., 2003), the nutria in France (Menard et al., 2001) and the pig in Andean countries (Mas-Coma et al., 1997; Valero and Mas-Coma, 2000; Valero et al., 2001a, b). In all these cases, the newly acquired hosts now play an important role as reservoir hosts in fascioliasis transmission, contributing to the expansion of the disease, and must, consequently, be taken into account when applying control measures.

2.3. Lymneid intermediate hosts

2.3.1. Fasciolid/lymnaeid spread and adaptation

The expansion of *F. hepatica* from its original European range to other continents is also related to: (i) the geographic expansion of its original European lymnaeid intermediate host species *G. truncatula*; (ii) secondarily spread of the American species *Pseudosuccinea columella*; and (iii) its adaptation to other lymnaeid species autochthonous of the newly colonised areas. The smaller geographic distribution of *F. gigantica* seems to be related to the weaker diffusion capacity of its intermediate snail hosts, the African *Radix natalensis* and the Eurasian *Radix auricularia*.

The European *G. truncatula* most probably spread to other continents together with the commercial exportation of livestock (i.e. in mud attached to the feet of sheep and cattle). The spreading capacity of *G. truncatula* is also related to its capacity for ecological niche widening, as observed in the low human hypoendemic, Mediterranean island of Corsica (Gil-Benito et al., 1991). This large island is very mountainous and its climatic conditions (rainfall, temperature) make it difficult to understand why fascioliasis is endemic throughout the island. Studies showed that *G. truncatula* is distributed throughout the insular periphery (coastal zones) as well as in the inner regions of the island, at an altitude of up to 1500 m. Its habitats in Corsica can be classified into two different types: reservoir habitats (permanent presence and renewal of water) and invasion habitats (only seasonal presence of water). From an ecological point of view, numerous different biotopes can be distinguished: from large to small rivers, from natural (rivers; water-filled fields; flooding zones; pasture plains) to man-made habitats (large water reservoirs; irrigation canals; fountains; animal drinking-troughs of different types; road ditches) (Oviedo et al., 1992 in European Multicolloquium of Parasitology VI, The Hague, p. 218.). Occupying several atypical habitats may be regarded as ecological niche widening that in turn is related to the wide distribution of the disease on the island.

Another lymnaeid species related to the spread of fascioliasis is *P. columella*. This is a rapidly colonising, aquatic, heat-tolerant species, which is thought to have originated from Central America, the Caribbean and the southern part of North America, but today is present in South America, Europe, Africa, Australia, New Zealand and even Tahiti. In Brazil, for instance, *P. columella* appears to be the only lymnaeid present in many fascioliasis areas. Interestingly, a strain of *P. columella* resistant to liver fluke infection was recently detected in Cuba, where fascioliasis is transmitted by both *Fossaria cubensis* and *P. columella* (Gutierrez et al., 2003a, b; Fernandez Calienes et al., 2004). This finding opens research opportunities to look for the genes responsible for resistance and future application in control strategies.

Fasciola hepatica utilised many different lymnaeid species as intermediate hosts to spread in the Americas, Asia, Hawaii, Papua New Guinea, Philippines, Japan, Australia, and New Zealand (see recent reviews in Mas-Coma and Bargues, 1997; Bargues et al., 2001). The reports of other lymnaeid (*Lymnaea palustris*, *Lymnaea turricula*, *Omphiscola glabra*, *Catascopia occulta*, *Radix ovata*) and planorbid (*Planorbis leucostoma*) species as alternative or facultative natural host species for transmitting *F. hepatica* in Europe are extremely rare, as such a chance only exists when the infection of the lymnaeid individual occurs during the first few days of the snail's life (Dreyfuss et al., 1994, 2002; Bargues et al., 2001). In the laboratory, *O. glabra*, *L. palustris* and *Lymnaea fuscus*, and even *Lymnaea stagnalis*, *Radix peregra* and *Myxas glutinosa* can be infected only if the miracidium penetrates very young snails, although a high mortality occurs (Oviedo et al., 1996; Dreyfuss et al., 2000, 2002). In Egypt, the finding of the planorbid *Biomphalaria alexandrina* naturally infected by *F. gigantica* (Farag and El Sayad, 1995) still requires confirmation.

2.3.2. New tools for lymnaeid classification and genotyping

Present knowledge on lymnaeid genetics and lymnaeid–fasciolid interrelationships is far from complete: (i) this molluscan family is subject to a systematic–taxonomic confusion; (ii) numerous lymnaeids show an interspecific morphological and anatomic uniformity, which often creates serious difficulties in specimen classification at species level, sometimes impeding it; and (iii) intraspecific variation of shell shape is particularly well marked within

lymnaeids (Bargues et al., 2001). There is a need for tools to enable species distinction and population characterisation.

Isoenzyme electrophoresis, DNA microsatellites and RAPD analysis may be useful at the population level, but specific DNA sequence markers have proved to be the best tool. The large subunit (16S) mitochondrial ribosomal DNA makes it possible to distinguish between several lymnaeid species and to analyse their phylogenetic relationships, but has not been used to advance lymnaeid systematics and taxonomy (see review in Bargues and Mas-Coma, in press).

The entire 18S nuclear ribosomal RNA gene could be used to differentiate between species belonging to different genera and subgenera, and sometimes between species of the same genus. It was, however, not useful when comparing populations of the same species (Bargues and Mas-Coma, 1997; Bargues et al., 1997).

Most of the valuable information used to clarify systematic and taxonomic aspects and population genetic characterisation of lymnaeids has been furnished by nuclear rDNA ITS sequences. ITS-2 has been the molecular marker most frequently used and appears to be a useful tool for resolving supraspecific, specific and population relationships in Lymnaeidae and also seems to be an excellent marker for systematic and taxonomic purposes (Bargues et al., 2001, 2003; Mas-Coma et al., 2001). The rDNA ITS-1 has been used to a lesser extent and may offer further information at population level than ITS-2 (Bargues et al., 2005).

Very recently, nucleotide mutations in both ITS-2 and ITS-1 have been shown to be useful to distinguish between populations of the species *P. columella* susceptible and resistant to *F. hepatica* miracidial infection in Cuba (Gutierrez et al., 2003b).

2.4. Epidemiology

2.4.1. Palaeoparasitological surveys

There are many studies on human coprolites in which *Fasciola* eggs have been found, suggesting that fascioliasis was common in prehistoric humans (Bouchet, 1991, 1994, 1995a, b, 1997; Bouchet et al., 1998; Dommelier et al., 1998; Aspöck et al., 1999; Lavazec et al., 2000; Dittmar and Teegen, 2003). *Fasciola* has even been found in prehistoric human populations of the Stone Age, living at the end of the Mesolithic period, 5000–5100 years ago and the Neolithic, a period marked by the domestication of animals and the development of agriculture. *Fasciola hepatica* has also been found in the Bronze Age, as well as in ancient Europeans of the Gallo-Roman period and the Middle Ages (Bouchet et al., 2003).

Interestingly, liver fluke eggs have been found in many palaeoparasitological studies performed in Europe (France, the Netherlands, Denmark, Germany, Austria, Poland, Switzerland,) but never in coprolites from the New World (Carvalho Gonzalves et al., 2002), which clearly indicates that fascioliasis in the Americas is the consequence of a relatively recent introduction. Taking these data into account, the present situation may be catalogued as re-emerging or emerging depending on the geographical area in question.

2.4.2. Classification of human fascioliasis

Studies carried out in recent years have shown human fascioliasis to be an important public health problem (Chen and Mott, 1990; WHO, 1995b; Mas-Coma et al., 1999a, b). The incidence of human cases has been increasing in 51 countries of the five continents (Esteban et al., 1998; Mas-Coma et al., 1999a, b). An exhaustive review compiled 7071 human reported cases from 51 countries across several continents in the last 25-year period: Europe, 2951; America, 3267; Asia, 354; Africa, 487; Oceania, 12 (Esteban et al., 1998). Different diagnostic limitations and the fact that human fascioliasis is not a disease of obligatory declaration suggest that the number of human cases is much greater than that published. Recent papers estimate human infection up to 2.4 million (Rim et al., 1994), up to 17 million people (Hopkins, 1992), or even higher depending on the hitherto unknown situations in many countries, mainly of Asia and Africa (Mas-Coma, 2004a, b).

An epidemiological classification of human infection situations has recently been proposed (Mas-Coma et al., 1999a). It includes: (i) autochthonous, isolated, non-constant cases; (ii) imported cases; (iii) hypoendemic; (iv) mesoendemic; (v) hyperendemic; (vi) epidemics in non-human endemic but animal endemic areas; and (vii) epidemics in human endemic areas.

2.4.3. Main human endemic areas

Major health problems are encountered in Andean countries (Bolivia, Peru, Chile, Ecuador), the Caribbean area (Cuba), northern Africa (Egypt), western Europe (Portugal, France and Spain) and the Caspian area (Iran and neighbouring countries) (Mas-Coma, 2004a). In all these regions, the existence of true human endemic areas is of utmost importance. Interestingly, high prevalences in humans do not seem to be related to high prevalences in livestock, so that the expected correlation between animal and human fascioliasis is not a consistent finding.

In Andean countries, well-known human hyperendemic areas are the Northern Altiplano in Bolivia (see reviews in Mas-Coma et al., 1995, 1999c), the Puno Altiplano (Esteban et al., 2002), the Cajamarca valley (Knobloch, 1985; Knobloch et al., 1985) and the Mantaro valley, all in Peru (Bendezu, 1969; Stork et al., 1973; Marcos Raymundo et al., 2004). In the Caribbean, in Cuba, in Pinar del Rio Province more than 10,000 people were infected between 1947 and 1948 (Mitterpak, 1968), in Villa Clara Province an outbreak involved more than 1000 subjects in 1983 (Gonzalez et al., 1985, 1987; Diaz et al., 1990), a new outbreak involving 81 subjects took place in 1995 (Perez et al., 1997 in XIII Federation of Latin American Parasitologists Congress,

La Habana, pp. 122–123), and patients are continuously diagnosed (Millan et al., 2000).

In Europe, France is considered an important endemic area (Anonymous, 1988). The first large modern epidemic of human fascioliasis occurred in 1956 (Coudert and Triozon, 1958). Between 1950 and 1983, 3297 cases were catalogued from published reports (Gaillet et al., 1983). Most cases were reported from the areas of Lyon, Bretagne Nord—Pas de Calais and Sud-Ouest. Recent reports on Sud-Ouest France refer to more than 300 cases (Giap, 1987; Ripert et al., 1987). At any rate, cases compiled in the present review only refer to published reports; the paper by Danis et al. (1985), which reports on 5863 human cases recorded at merely nine hospitals between 1970 and 1982, demonstrates that published data largely underestimate the real situation. The French Mediterranean island of Corsica maintains at low hypoendemicity (Gitard et al., 1965; Gil-Benito et al., 1991).

The disease is also important in Portugal, the northern part of the country being an endemic area. Rombert and Gracio (1984) reported on 77 cases and Sampaio Silva (in Chen and Mott, 1990) refered to 561 cases in only three communities in northern Portugal. Sampaio-Silva et al. (1996) refered to 1011 cases diagnosed in the laboratory of Porto between 1970 and 1992.

In Spain, according to the review by Sorribes et al. (1990 in Meeting of the International Zoonoses Association, Valencia, p. 186), human fascioliasis appears to be underestimated and mainly distributed in northern Spain (autonomous communities of the Pais Vasco, Castilla-León, Cantabria, Navarra and Rioja). More recently, imported cases have been added to authochthonous ones (Turrientes et al., 2004).

In Africa, the biggest problems have been encountered in Egypt, where numerous human cases have been detected in many governorates (Curtale et al., 2000; 2003a, b; Haseeb et al., 2002; Esteban et al., 2003). Initial estimates of 830,000 subjects affected in the Nile Delta region (WHO, 1995b) probably underestimate the real situation, considering the high prevalences of up to 18–19% in total population recently found in concrete villages (Esteban et al., 2003).

Among Asian countries, Iran is worth mentioning. Although human cases have been reported throughout the country for a long time (Sahba et al., 1972), the greatest problems appear to be concentrated in the Gilan province, on the Caspian Sea. In this northwestern area of Iran, high fascioliasis prevalences in livestock and human infections have been known for a long time (Sabokbar, 1960). Moreover, at the end of the 1980 s and during the 1990 s several large epidemics, including thousands of human cases, were reported (Massoud, 1990; Talaie et al., 2004; Ashrafi et al., 2004). In Mazandaran province, fascioliasis has very recently shown to be a major human health problem too (Moghaddam et al., 2004a, b). The recent detection of a 1.8% human prevalence in a village in eastern Turkey (Yilmaz and Gödekmerdan, 2004) suggests that the endemic area around the Caspian Sea may be widespread.

In eastern Asia, cases in Japan and Korea appear to be sporadic, but the situation may be different in the south-eastern peninsula (Mas-Coma, 2004b). In Thailand only a few cases were diagnosed from the northern part of the country (Tesana et al., 1989), but recent information on Vietnam causes concern. Sporadic cases of human fascioliasis were reported in Vietnam until the 1990 s, but over 500 human cases have been diagnosed between 1997 and 2000 (De et al., 2003). The majority of the infected subjects were from central and southern provinces, especially from the coastal and central highland areas (Hien et al., 2001b; Cong et al., 2001; Xuan et al., 2001). The emergence of fascioliasis in Vietnam is an enigma (De et al., 2003). Hien et al. (2001a) consider the sudden emergence of this large number of human cases as puzzling and raise many multidisciplinary questions.

2.4.4. Prevalences and intensities in human hyperendemic areas

The highest prevalences and intensities have been found in the Northern Bolivian Altiplano. In this area, prevalences detected in some communities were up to 72 and 100% in coprological and serological surveys, respectively (Hillyer et al., 1992, 1995c; Mas-Coma et al., 1999; Bjorland et al., 1995; Esteban et al., 1997a, b, 1999; O'Neill et al., 1998), and intensities reached up to more than 5000 eggs per gram (epg) in children (Esteban et al., 1997a, b, 1999). The results of the surveys proved that although more prevalent and intense in children (with a peak in the 9–11-year-old age group), adults of the 21–40 and $>$ 40-year-old age groups were also infected, with prevalences exceding 40% in both groups and arithmetic mean intensities of up to 752 and 616 epg, respectively, in given communities (Esteban et al., 1997a, b, 1999). Although of a lower level, prevalence and intensity found in other Andean and African countries (Peru, Egypt) were similar (Esteban et al., 2002, 2003).

Prevalences and/or intensities in human hyperendemic areas are significantly higher in females. Females shed significantly more eggs than males in Andean countries (Esteban et al., 1999, 2002), and in Egypt the prevalence in females appears to be statistically significantly higher than in males (Esteban et al., 2003). This result contrasts with Andean countries, where prevalences do not differ between the sexes (Esteban et al., 1999, 2002). The gender role in Egypt may be related to cultural, hygienic and behavioural factors, females being occupied in washing household items in large canals where transmitting lymnaeids are present, and agricultural tasks in irrigated plantations such as rice fields. They are also central to meal preparation in houses including management of freshwater plants potentially carrying attached metacercariae. In Egypt, many species of vegetables and weeds are eaten raw in salads. At childhood ages, girls may be more in contact with

transmission foci, according to data collected showing that girls are away from schools more than boys (Esteban et al., 2003).

2.4.5. Environment variation in human endemic areas

Despite liver fluke development being very dependent on environmental characteristcs, fascioliasis has become the vector-borne parasitic disease with the widest latitudinal, longitudinal and altitudinal distribution known (Mas-Coma, 2004b). Fascioliasis is unique in being capable of giving rise to human endemic areas from below sea level (on the shores of the Caspian Sea) up to very high altitude (as in Bolivia, Peru, Ecuador and Venezuela) (Mas-Coma et al., 2003).

When comparing different human endemic areas, a large diversity of situations and environments appears, 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 up to 4200 m, hot and cold weather, seasonal and all-year round constant temperatures, scarce to pronounced annual rainfall, low and high mean annual potential evapotranspiration, and a lack of dry period to a lack of wet period through different dryness/humidity rates. Moreover, from the landscape point of view, these areas include altiplanos to valleys, islands to mainlands, natural to artificial irrigation, lakes to lagoons, large rivers to small streams, and permanent as well as temporary water bodies (Mas-Coma et al., 2003).

2.4.6. Epidemiological patterns

In the different continents, fascioliasis in human hypo- to hyperendemic areas present a very wide spectrum of transmission and epidemiological patterns related to the very wide diversity of environments, including mainly:

(i) a very high altitude pattern related to *F. hepatica* only, 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 (Mas-Coma et al., 1999c); e.g. the Northern Bolivian Altiplano and the Puno Altiplano;
(b) the valley pattern, with seasonality (Claxton et al., 1997) and prevalences and intensities related to altitude; e.g. the valleys of Cajamarca and Mantaro;
(ii) a Caribbean insular pattern, including reduced but repeated outbreaks in a human hypoendemic area and lymnaeid species other than the main vector species involved in the transmission elsewhere (Gonzalez et al., 1985, 1987; Diaz et al., 1990; Perez et al., 1997 in XIII Federation of Latin American Parasitologists Congress, La Habana, pp. 122-123; Millan et al., 2000); e.g. Pinar del Rio Province in Cuba;
(iii) 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 (Esteban et al., 2003); e.g. the Behera Governorate in the Nile Delta region in Egypt;
(iv) a pattern related to Caspian surrounding areas, including human hypoendemic areas in which large epidemics occur, sometimes involving up to 10,000 people and with *F. hepatica* and *F. gigantica* overlapping and several *Galba–Fossaria*, *Radix* and stagnicoline lymnaeids (Ashrafi et al., 2004); e.g. is the area of Rasht and Bandar-e Anzali in Gilan province in Iran.

The parasite distribution appears irregular within a human endemic area. 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).

2.4.7. Climatics and human fascioliasis forecast indices

Climatic factors are decisive in the transmission of *F. hepatica*. The annual incidence of infection of definitive hosts is related to air temperature, rainfall and/or potential evapotranspiration. Climatic fascioliasis forecast indices are calculated with different equations, which take into account variations in these climatic factors. Several of these indices have been successfully applied to animal fascioliasis in different areas of Europe, Africa and the USA (see review in Fuentes et al., 1999). Until the study of Fuentes et al. (1999), climatic fascioliasis forecast indices had never been applied to human fascioliasis or to the extreme environmental conditions of the very high altitude endemic areas. Climatic factors taken into account by these indices vary markedly with both altitude and latitude. The analyses show that the very high altitude climatic characteristics of the Northern Bolivian Altiplano human endemic region also differ from those of fascioliasis endemic lowland areas.

Climadiagrams furnished results on the duration of wet and dry seasons, which did not fit the real conditions of the endemic area. Consequently, modifications for high altitude and low latitude were proposed by Fuentes et al. (1999) in the two most useful indices: the Mt index and the Water budget-based system index (Wb-bs index). Values of both modified Mt index and Wb-bs index reflected the possibility of optimum transmission during the December–March period. The results were statistically significant for the Wb-bs index when a meteorological station in which no lymnaeids were found was excluded. The modified Mt index was not accurate enough. The modified Wb-bs index values made it possible to classify the degree of transmission of

human and animal fascioliasis in the Altiplanic zones studied into low, moderate and high-risk areas.

2.4.8. Remote sensing and geographic information systems

For application to infectious diseases, a complex suite of detectable environmental factors are important, many of which can be observed to a greater or lesser degree from space-borne platforms (Huh and Malone, 2001). Concerning fascioliasis, the following may be useful: (i) temperature, air, soil and surface water (diurnal temperature maximum and minimum, diurnal temperature difference, sea/water/land surface temperature); (ii) water, including soil moisture, standing water and atmospheric water vapor; (iii) condition of vegetation canopy above the ground; (iv) structure and dynamics of the lower atmosphere plus composition and dimensions of air-borne particulates (aerosols) contained; and (v) topography and mineralogy, i.e. terrain relief and bedrock/soil types.

Remote Sensing (RS) and Geographic Information Systems (GIS) have been used for animal fascioliasis (Malone and Yilma, 1999). In GIS for animal fascioliasis, surface hydrology, vegetation indices and temperature data based on previous knowledge have proved to be very useful. This is why the development of GIS for human fascioliasis was encouraged by several specialists (WHO, 1995b; Hillyer and Apt, 1997). The first attempt to apply these technologies to a human fascioliasis endemic area was that of Fuentes and Malone (1999) in Chile. Annual normalised difference vegetation index (NDVI) values were calculated for each region, using specialised computer software to extract values from advanced very high-resolution radiometer (AVHRR) 10-day (dekade) composite satellite images. Based on different NDVI classes, a risk map of fascioliasis transmission was created for each of the administrative zones, and a model was proposed for forecasting fascioliasis transmission in Chile.

More recently, studies were undertaken to analyse whether a GIS prediction model would be viable and useful in the Northern Bolivian Altiplano human endemic zone (Mas-Coma et al., 1999c). The prediction capacity of the remote sensing map based on NDVI data extracted from 10-day (dekade) composite images from the 1-km AVHRR, acquired by the National Oceanic and Atmospheric Administration's Television Infrared Observation Satellite (Fuentes et al., 2001), appeared to be greater than that of forecast indices merely based on climatic data (Fuentes et al., 1999). A complete overlap between real ranges of human fascioliasis prevalence and predicted ranges of fascioliasis prevalence (transmission risk through NDVI) was obtained. NDVI data maps represent a further step towards creating a GIS-based prediction of epidemiological and transmission situations of human fascioliasis in high altitude endemic areas in Andean countries (Fuentes, 2004) and a GIS forecast model to achieve this was recently proposed (Fuentes et al., 2005). This model allows us, through the classification of the transmission degree into low-, moderate- and high-risk areas, to identify those areas requiring the implementation of control activities. Studies performed in many human endemic areas of Bolivia, Peru and Ecuador demonstrate the validity and approximation of this forecast model.

2.4.9. Climate and landscape change and human fascioliasis

Current evidence suggests that inter-annual and inter-decadal climate variability have a direct influence on the epidemiology of vector-borne diseases. Moreover, climatic anomalies such as those associated with the El Niño-Southern Oscillation (ENSO) phenomenon and resulting in drought and floods are expected to increase in frequency and intensity (Githeko et al., 2001). Links between ENSO events and outbreaks of human fascioliasis in the western coast countries of South America, mainly Peru and Ecuador, are to be expected.

The colonisation of man-made irrigation systems by *F. hepatica* giving rise to human disease was recently described in the Nile Delta, Egypt and the Puno Altiplano, Peru. In the Egyptian Behera Governorate, human fascioliasis is unexpectedly emerging after many years of successful control measures against schistosomiasis, including human treatments with praziquantel and molluscicide applications against freshwater snails (Curtale et al., 2000). In Peru, the Asillo zone of the Northern Altiplano is a man-made irrigation area built in the 1956–1974 period, to which both liver fluke and lymnaeid snails quickly adapted. This Peruvian area is isolated from the Northern Bolivian Altiplano natural endemic area. This shows the high health risk, which such water resource constructions pose and raises questions about the wisdom of building several irrigation projects proposed for the Peruvian and Bolivian Northern Altiplanos (Esteban et al., 2002).

2.5. Diagnosis

2.5.1. Techniques

Direct parasitological techniques, indirect immunological tests and other non-invasive diagnostic techniques are presently used for human fascioliasis diagnosis. New biological data emphasise the importance of quantitative coprological data analyses in epidemiological surveys, as well as in postreatment (and future postvaccination) monitoring. 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 the liver and/or other organ biopsy materials. Serological, intradermal and stool antigen detection tests have been developed and employed. Immunological techniques provide the advantages of being applicable during all stages of the disease, but especially during the invasive or acute phase, and in other situations in which coprological techniques are

inappropriate. On the other hand, immunological techniques present other problems related to poor sensitivity and specificity. Various serological tests have been used for human diagnosis. Almost all of these techniques concern the detection of circulating antibodies and only a few are designed to detect circulating antigens and immune complexes. Several serological techniques have also recently proved to be useful for monitoring post-treatment evolution. Non-invasive diagnostic techniques, which can be used for human diagnosis, are radiology, radioisotope scanning, ultrasound, computer tomography and magnetic resonance (see reviews in Esteban et al., 1998; Hillyer, 1999).

2.5.2. Fasciola hepatica/F. gigantica *differential diagnosis*

The differential diagnosis between *F. hepatica* and *F. gigantica* in humans cannot be achieved by clinical, pathological, coprological or immunological methods. This is a problem in those areas of overlapping range since differential diagnosis is very important due to the different pathological, transmission and epidemiological characteristics of the two fasciolids. To distinguish between *F. hepatica* and *F. gigantica*, a simple and rapid PCR-restriction fragment length polymorphism (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, provides unambigous 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*92 II and *Rca* I has recently been applied to differentiate between Chinese liver flukes (Huang et al., 2004).

2.5.3. *Increasing sensitivity and specificity in serological tests*

Specialists are concentrating efforts on obtaining purified E/S antigens and/or recombinant molecules to improve serological tests, because parasitological diagnosis has problems caused by the delay in their usefulness in the acute phase (coprological examination positive only after 3–4 months p.i.), 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).

Cysteine proteinases are probably the most abundant proteins among E/S products from fasciolids and have shown to be a very valuable source of antigens for diagnosis. These enzymes are secreted by the adult and juvenile forms (Dalton et al., 2003; Law et al., 2003) and are highly antigenic in animals (Cornelissen et al., 1999, 2001; Neyra et al., 2002) and humans (Cordova et al., 1997). 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) as well as for *F. gigantica* infection (Maleewong et al., 1996, 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, providing results similar to native antigens.

2.6. Pathology

Fascioliasis is a highly pathogenic disease. In humans, pathogenesis depends on the number of infecting flukes, and appears to be similar to that reported in animals (Chen and Mott, 1990; Mas-Coma and Bargues, 1997; Mas-Coma et al., 1999a, 2000).

Despite the existence of a decrease of the prevalence from children and young subjects to adult subjects, results demonstrate that in high endemic zones 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 that the life span of the adult fluke in man is between 9 and 13.5 years (Chatterjee, 1975; Dan et al., 1981). Such a picture suggests that in those areas, the majority of adult subjects should be in the chronic phase, acute lesions by repetitive infections being superimposed on chronic disease with relative frequency. Thus, the acute phase may be prolonged and overlap with both the latent and the obstructive phase.

A useful approach for pathological research in the advanced chronic period appears to be the laboratory rat model (Valero et al., 2000, 2003). In Wistar rats experimentally infected with *F. hepatica*, pigment stone (PS) presence increased with infection time, and the lithogenic induction by *F. hepatica* infection became manifest in situations of advanced chronicity state over 100 days p.i. The relative risk of gallstone disease rose when the number of flukes per rat increased. The presence of gallstones was strongly associated with the number of flukes located in the bile duct and with serum high-density lipoproteins and triglyceride levels. The risk of PS 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 repetitive reinfections, usually in human endemic areas of developing countries, imply a higher lithiasis risk. 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).

The clinical 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 (Brady et al., 1999). Interestingly, 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 pathogenic 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 be at the base of the high morbidity and mortality rates of Aymara children inhabiting the Northern Bolivian Altiplano (Mas-Coma et al., 1995).

2.7. Treatment

Many drugs have been used to treat fascioliasis in human patients. Dehydroemetine was considered the therapy of choice a few decades ago, but its toxicity allowed bithionol to become the drug of choice for years, despite its long treatment course. The lack of consensus about the therapy of choice for human fascioliasis came to an end when it was proved that at appropriate dose rates triclabendazole, a benzimidazole derivative [6-chloro-5-(2,3-dichlorophenoxy)-2-methylthiobenzimidazole], was highly efficient in humans (Esteban et al., 1998; Mas-Coma et al., 1999b). Triclabendazole for human use (Egaten®) is at present the drug of choice for human fascioliasis caused by both *F. hepatica* and *F. gigantica* (Savioli et al., 1999).

Triclabendazole is more effective if administered after meals (Lecaillon et al., 1998) and 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 by Apt et al. (1995), and 79.4% and 93.9%, respectively, by El-Morshedy et al. (1999). Although recent studies show that triclabendazole appears to keep its efficacy in human endemic areas over many years (Talaie et al., 2004), Millan et al. (2000) found six of 77 (8%) patients still shedding eggs in faeces on day 60 post-therapy with two doses, although all these cases responded to a third triclabendazole dose.

Although the possibility of reinfection after treatment due to living in an endemic area cannot be ignored, the risk of appearance of resistance to triclabendazole cannot be ignored, taking into account: (i) the long-term veterinary use of triclabendazole (Fasinex®) for livestock treatment in endemic areas; (ii) the tradition of human self-treatments with Fasinex® owing to the very general availability of this drug; and (iii) the recent appearance of triclabendazole resistance in the Old World. In animals, triclabendazole resistance was first described in Australia (Overend and Bowen, 1995), later in Ireland (Lane, 1998; O'Brien, 1998) and Scotland (Mitchell et al., 1998) and more recently in the Netherlands (Moll et al., 2000; Gaasenbeek et al., 2001). The strategies to minimise the development of resistance include the use of synergistic drug combinations (Fairweather and Boray, 1999), although this approach carries the risk of building up multiple drug resistance (Gaasenbeek et al., 2001). Moreover, it must be considered that there are no drug alternatives for human treatment at present, drugs such as bithionol and others being no longer commercially available (Millan et al., 2000). Additionally, nitazoxanide, very recently marketed in Mexico and reported to be effective against fascioliasis in human patients in treatments of 7-day duration (Rossignol et al., 1998), and myrrh (Mirazid®), registered in Egypt (Massoud et al., 2001), still require more studies on efficacy and tolerability.

2.8. Prevention and control

Human infection was always related to animal endemicity so that prevention and control measures recommended were the same as those applied for veterinary fascioliasis (see reviews by Roberts and Suhardono, 1996; Torgerson and Claxton, 1999; Spithill et al., 1999). However, recent studies in human endemic areas have shown that well-known epidemiological patterns of fascioliasis may not always explain the transmission characteristics in a given area, so that control measures must consider the results of the ecoepidemiological studies undertaken in the zone concerned, as adaptation to new environmental conditions may change transmission characteristics (Mas-Coma et al., 1999b). Accordingly, communities in endemic areas should be appropriately informed about the disease, its transmission and its danger.

The prevention of human fascioliasis may be achieved by strict control of watercress and other metacercariae-carrying aquatic plants for human consumption, especially in endemic zones. Among vegetables incriminated, freshwater plant species differ according to geographic zones and human dietary habits (Mas-Coma et al., 1995, 1999c; Mas-Coma, 2004a). The amphibious characteristics of vector species, such as *G. truncatula*, are linked to the transmission foci in plantations of non-aquatic vegetables requiring frequent irrigation (El Sayed et al., 1997; Motawea et al., 2001). Moreover, recent results suggest that humans consuming raw liver dishes prepared from fresh liver infected with immature flukes may also become infected (Taira et al., 1997; Cho et al., 1999).

Metacercarial infectivity is dependent upon storage time, being lower when metacercariae are older (maximum longevity of 48 weeks) and independent from the animal reservoir source, demonstrating that flukes from secondary reservoirs, such as pigs and donkeys, involve the same potential risk as those from main reservoirs, such as sheep

and cattle (Valero and Mas-Coma, 2000). The long survival capacity and resistance to desiccation of metacercariae explain human contamination by consumption of terrestrial plants collected in dry habitats but which were submerged in water a few weeks or months before collection, as for example in Iran (Mas-Coma, 2004a). Due to transport, plants carrying metacercariae can be sold in non-controlled city markets giving rise to urban infection (Mas-Coma, 2004a). Local beverages have also been incriminated, as, for example, in Iran and Cape Verde (WHO, 1995b; Mas-Coma, 2004a).

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). Water containing metacercariae may also contaminate food, kitchen utensils or other objects and thus become the source of fortuitous infection (Mas-Coma, 2004a). 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 protozoans and helminths, such as *Giardia intestinalis* in Andean countries (Esteban et al., 1997a, 2002) or *Entamoeba coli*, *Chilomastix mesnili*, and *Schistosoma mansoni* in Egypt (Esteban et al., 2003). 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 in the Peruvian Altiplano, inhabitants do not consume freshwater plants (Esteban et al., 2002). In the Nile Delta region, people living in houses where piped water is present showed to have a higher infection risk (Curtale et al., 2003). In the Egyptian locality of Tiba, where a 18.0% prevalence was initially found, human infection has markedly decreased after the construction and utilisation of the so-called 'washing units', in which water is appropriately filtered (Mas-Coma, 2004a).

3. Fasciolopsiasis

3.1. Etiology, location and definitive hosts

Fasciolopsis buski is one of the largest digeneans infecting humans, with a body of 2–10/0.8–3 cm and eggs of 130–140/80–85 μm (Kumar, 1980). It inhabits the duodenum and jejunum, and can also be found in much of the intestinal tract, including the stomach, in moderate and heavy infections (Graczyk et al., 2001).

Pigs are the only important reservoir, although they harbour few flukes (usually only three to 12) (Rim, 1982), and producing fewer eggs/adult than in humans. Animal species showed susceptibility differences in experimental tests: mice, rats, monkeys and dogs were completely refractory, and guinea-pigs only partially susceptible, but rabbits were susceptible (Malviya, 1985), similar to squirrel monkeys, *Saimiri sciureus petrinus* (Kuntz and Lo, 1967). Rarely reported in monkeys, such as rhesus monkeys *Macaca mulatta* (Hartman, 1961), there is no report incriminating monkeys in endemic areas. Studies showed no infection in cows, buffaloes, dogs or horses in areas where pigs were harbouring the parasite (Manning and Ratanarat, 1970). There is still disagreement as to whether there is only one or more strains of *F. buski*, more or less adapted to either humans or animals (Roy and Tandon, 1993).

3.2. Geographical distribution

Fasciolopsiasis is present in many Asian countries (Cross, 1969; WHO, 1995b).

In China, infections have been reported from 10 provinces including both humans and pigs, including prevalences up to 85% in Chekiang and Kiangsi Provinces. In other areas of China, it varies from less than 1 to 5% (Weng et al., 1989; Gai et al., 1995; Lo and Lee, 1996). Recent nationwide surveys suggest a fasciolopsiasis decrease (Yu et al., 1994; Xu et al., 1995).

In Taiwan, the most severely affected endemic area is southern Taiwan Hsieh (Lee, 1986; Lee et al., 1989; Lo and Lee, 1996), where infection rates reached 48% (Hsieh, 1960) and even 61% in the village of Pa-Weng (Lee, 1972). A prevalence of 25% was found in schoolchildren (Shyu et al., 1984). In Liuying area, the intensity per person was estimated to be about 10 worms (Hsieh, 1960). It was also detected in immigrant Thai labourers (Lo and Lee, 1996; Cheng and Shieh, 2000).

In Bangladesh, surveys showed 39.2 and 8.6% infection in two villages (Muttalib and Islam, 1975). Prevalences in schoolchildren reached 50% in one endemic focus (Gilman et al., 1982). Other studies in humans were those of Idris et al. (1980) and Rahman et al. (1981).

In India, 60% of people were found infected and harbouring 1–57 adult worms in Assam (Buckley, 1939). Infections in pigs and humans were reported in Calcutta, and Bombay, where Shah et al. (1966) found 29% in the city emphasising *F. buski* absence in pigs, similar to Assam. New human foci were detected in Maharastra (Manjarumkar and Shah, 1972). A more recent study on pigs is that of Roy and Tandon (1992).

In Vietnam, old studies reported infections in both Asians and Europeans (Fournier, 1954; Harinasuta et al., 1984), and both humans and pigs were found to be positive (Yoshihara et al., 1999). A curious case of a boy who vomitted eight live *F. buski* adults was described by Le et al. (2004).

In Thailand, the central part of the country is the main endemic area, with an estimated 100,000 individuals infected among 500,000 (Manning and Ratanarat, 1970; Manning et al., 1971). A total of 13% out of 1500 people from three provinces were infected (Sadun and Maiphoom, 1953). In areas such as Pak Hai, 100% of the indigenous population is likely to be infected. A lower prevalence of 10% was found by Bunnag et al. (1983). A prevalence of 7.1% has been found in northeastern Thailand, although

immigrants from other parts of the country seem to be involved (Wiwanitkit et al., 2002). Infection rates in pigs closely parallel those found in humans.

Infections were also reported from Laos (Waikagul, 1991; Giboda et al., 1991). In Cambodia, prevalences of 0.04% in humans and 5% in pigs were detected in Phnom-Penh (Waikagul, 1991). It was also reported from Borneo and Sumatra, Indonesia (Rim, 1982; Hadidjaja et al., 1982; Margono, 2003), where 27.0% prevalence was detected in residents of Kalimantan, and 20.3% outside that area, including the highest prevalence in the 5–14-year-old group (56.8%) but decreasing with age, and a male/female ratio of 1.4:1 (Handoyo et al., 1986). The parasite was also detected in Kampuchea, the Philippines, Singapore (Waikagul, 1991), Burma (Rim, 1982), and Malaysia (Shekhar, 1991).

Reports on Korean and Japanese subjects showed that they did not seem to have been infected in their respective countries (Rim, 1982). Reports from the USA, Venezuela, Australia, Guatemala, Israel and Cuba may be due to immigrants from the Far East (i.e. Basnuevo and Seuc-Chiu, 1950; Greenberg et al., 1994) or to misidentification of faecal eggs (Schubert and Granz, 1981).

3.3. Life cycle and intermediate host

Fasciolopsis buski produces a great number of eggs in humans (13,000–26,000, mean 16,000/worm per day) (Hsieh, 1960). Eggs are unembryonated when shed with faeces and must reach freshwater to continue the cycle. The miracidium development period is 16–77 days, with a mean of 22 days, the optimum being a water temperature of 27–30 °C and a pH range of 6.5–7.2 (Barlow, 1925).

Snail hosts are limited to small planorbids of the genera *Segmentina, Hippeutis* and *Gyraulus*, as *Segmentina hemisphaerula (syn. Segmentina coenosus, Segmentina nitidella, Segmentina calathus, Segmentina largeillierti), Hippeutis cantori (syn. Hippeutis smackeri), Hippeutis umbilicalis* and *Gyraulus convexiusculus* in China, Vietnam and Taiwan (Hsu, 1964; Wang et al., 1977; Nguen Tkhi Le, 1978). In India (Assam), the snail hosts are *Segmentina trochoideus, S. hemisphaerula* and probably also *H. umbilicalis* (Tripathi et al., 1973). *Segmentina hemisphaerula, S. trochoideus* and *Gyraulus chinensis* are involved in Thailand (Manning and Ratanarat, 1970; Nguen Tkhi Le, 1978), *S. hemisphaerula* in Taiwan (Hsieh, 1960), and *H. umbilicalis* in Laos (Ditrich et al., 1992), *S. trochoideus* and *H. umbilicalis* in Bangladesh (Gilman et al., 1982; Graczyk et al., 2000), and *Indoplanorbis exustus* in India (Preet and Prakash, 2001).

A total of 17% *S. hemisphaerula* and 50% *H. cantori* were found naturally infected in Shaohsing, China (Barlow, 1925), 0.66% *S. hemisphaerula* in Canton, and 1.92% *S. hemisphaerula* in Lin-Ying, Taiwan (Hsieh, 1960). The prevalence of *H. umbilicalis* infection was 1% in Bangladesh (Gilman et al., 1982) and 1.9% in Laos (Ditrich et al., 1992). In India, *I. exustus*, prevalence of 5–20% was inversely proportional to the snail number per month (Preet and Prakash, 2001).

Sporocyst, mother and daughter rediae and cercariae develop in the snail. Sporocysts are elliptical, of 131–169/34–83 mm. Mother rediae develop rapidly inside the sporocyst, emerging within 9–10 days after miracidium penetration. Mother rediae migrate to the ovotestis and are 701/159 μm in size. Daughter rediae may be up to 2.8 mm when mature and harbour up to 45 cercariae. *Gymnocephalous cercariae* include a body of 195/145 μm and a tail of 498/57 μm. Cercariae leave rediae after 25–30 days but emerge from the snail after an average of 49 days, since they require a maturation period in snail tissues (Barlow, 1925). Other incubation periods recorded are 46–59 days at 22–24 °C, 85–100 days at 18–22 °C (Lo, 1967), and 31 days (Buckley, 1939). A very short prepatent period of only 21 days has been found in *S. trochoideus* and *H. umbilicalis*, in which *F. buski* causes 100% snail mortality due to mechanical damage to the ovotestis (Graczyk et al., 2000).

The emergence of cercariae is dependent on light, with great variation in daily emergence patterns (Preet and Prakash, 2001). Cercariae swim in water until encystment on a substrate, mainly aquatic plants and debris. Its survival in water varies from 64 to 72 days (Gilman et al., 1982; Nguyen Van Tho, 2002b). Important are various consumption water plants, such as water caltrop (*Trapa natans* in China, *Trapa bispinosa* in Taiwan and *Trapa bicornis* in Bangladesh and Thailand), water chestnut (*Eliocharis tuberosa*), water hyacinth (*Eichhornia* sp.), water bamboo (*Zizania* sp.), water lotus (*Nymphaea lotus*), water lily (*Nymphaea* sp.), watercress, gankola (*Otelia* sp.), and water morning glory (*Ipomoea aquatica*). Metacercarial cysts on plants are visible to the naked eye (average 3.9/2.1 mm). Up to 200 cysts may be found on the skin of one water caltrop, but the usual number is about 15–20.

Cercariae may also encyst on water surface. The number of floating metacercariae is 3.6% of the total. It was found that 10.3–12.8% of the patients and 35.1–40% of the infested pigs had possibly been infected by drinking water (Weng et al., 1989).

Definitive host contamination takes place by ingestion of plants or water carrying metacercariae. Metacercariae excyst in the duodenum and attach to the intestine wall to grow to mature flukes within 3 months.

3.4. Epidemiology

In endemic areas, the disease is underreported and most prevalent in remote rural places and semi-urban areas (Lee, 1972; Shah et al., 1973; Idris et al., 1980; Rahman et al., 1981; Gilman et al., 1982; Bunnag et al., 1983; Harinasuta et al., 1984; Gai et al., 1995), mainly in schoolchildren in whom the number of worms per child can exceed 800 (Gilman et al., 1982; Weng et al., 1989).

A higher prevalence among females (16%) than males (11%) was detected in coprological surveys in Thailand

(Sadun and Maiphoom, 1953). The prevalence varied from 8% among adults 15 years and over to 15% in children 5–14 years old, with an average of 13% for all age groups. The severity of infection increased up to 10–14 years of age and decreased in older groups, prevalence and intensity differences being related to activities. Similar gender and age relationships were found in Thailand (Manning and Ratanarat, 1970), Bangladesh (Rahman et al., 1981) and Taiwan (Hsieh, 1960). Prevalences detected in children were 57% in mainland China (Lee, 1972; Weng et al., 1989), 25% in Taiwan (Shyu et al., 1984), 60% in India (Muttalib and Islam, 1975), 50% in Bangladesh (Rahman et al., 1981), and 10% in Thailand (Bunnag et al., 1983).

Fasciolopsiasis occurs focally, is widespread, linked to freshwater habitats with stagnant or slow moving waters, and is associated with common social and agricultural practices and promiscuous defaecation (Kuntz and Lo, 1967; Gilman et al., 1982; Cross, 1984; Weng et al., 1989). In pigs, it is seasonal (Roy and Tandon, 1992).

Humans and pigs contract the infection by eating metacercarial cysts through consumption of raw or undercooked aquatic plants, drinking or using untreated water, and handling or processing water-derived plants (Kuntz and Lo, 1967; Gilman et al., 1982; Weng et al., 1989). Human infection takes place when peeling off the hull or skin of infected plants between the teeth. Cultivation of aquatic, metacercariae-carrying plants for consumption on a large scale and the pollution of the areas in which they are grown with human and animal (mainly pig) excreta are important spreading factors (Kuntz and Lo, 1967; Manning and Ratanarat, 1970; Cross, 1984). Prevalences among people living near water caltrop plantations were much higher than in villages far away from plantations (Sadun and Maiphoom, 1953; Hsieh, 1960). However, the common habit of dipping water caltrops in water to retain their freshness might favour the spread to areas where these plants are not grown.

In several countries, fasciolopsiasis is aggravated by social and economic factors, such as poverty, malnutrition, and a uncontrolled food market associated with lack of food inspection, poor sanitation, other helminthiasis, and declining economic conditions (Muttalib and Islam, 1975; Gilman et al., 1982; Cross, 1984; Weng et al., 1989; Yu et al., 1994; WHO, 1995a). The differences of incidence in the same area are due to factors such as economic status, educational background, standard of health and way of life (Jaroonvesama et al., 1980).

The pig is the main reservoir (Kuntz and Lo, 1967; Weng et al., 1989; Roy and Tandon, 1992). Fresh aquatic green fodder and untreated water used to raise pigs are the main infection sources in farm animals (Weng et al., 1989; D'Souza et al., 2001). Different pig infection rates were reported: 30% in India (Tripathi et al., 1973); 10% in China (Hsu, 1964); 52% in Taiwan (Hsieh, 1960).

3.5. Pathology, symptomatology and clinical manifestations

Morbidity in endemic areas is high, and the disease can be fatal (Lee, 1972; Idris et al., 1980; Gilman et al., 1982; Bunnag et al., 1983; Lee, 1986; Weng et al., 1989). Worms cause extensive intestinal and duodenal erosions, ulceration, haemorrahage, abscesses and catarrhal inflammation. Absorption of toxic and allergic worm metabolites causes ascites and both general and facial edema, e.g. cheek and orbital edema (Jaroonvesama et al., 1986). Pathological changes may be traumatic, obstructive, and toxic, especially in heavy infections, in which worms disturb secretion of intestinal juices, cause excess mucus secretion and obstruct the passage of food. At autopsy of a fatal case, the mucosa of stomach and small and large intestine was hyperemic (Viranuvatti et al., 1953). Faeces are profuse, yellow-brown, and contain pieces of undigested food due to malabsorption (Jaroonvesama et al., 1986).

Clinical symptoms are related to parasite load. Light infections are usually asymptomatic except for diarrhoea, alternating with periods of constipation and abdominal pain. Light infections include anemia with eosinophilia, headache, dizziness, stomach ache, gastric pain, loose stools (Gilman et al., 1982), asthenia, pallor, malnutrition, protuberant abdomen and abdominal distention (Chandra, 1976). Moderate and heavy infections are associated with malnutrition, severe epigastric and abdominal pain, diarrhea or bowel obstruction, poor appetite, mild abdominal colic, vomiting, fever, nausea (occurring especially in the morning and resolving after the first meal), acute ileus, anasarca, marked eosinophilia and leucocytosis, and a significant lowering in serum vitamin B_{12} content (Arekuul et al., 1979; Rahman et al., 1981; Gilman et al., 1982; Handoyo et al., 1986; Jaroonvesama et al., 1986).

In heavy infections, there are generalised toxic and allergic symptoms, usually in the form of edema, particularly of the face, abdominal wall and lower extremities. Generalised abdominal pain and ascites are common, in addition to poor appetite, bitemporal headache, giddiness, a low-grade fever, non-palpable liver and spleen, nausea and vomitting (Viranuvatti et al., 1953). The clinical disease becomes evident only in massive infections. Patients purged of the worms usually recover completely, although advanced, heavy infections can be fatal. Mortality has been reported in heavily infected children (Sadun and Maiphoom, 1953; Viranuvatti et al., 1953; Yu and Mott, 1994). However, little, if any, evidence has been found to suggest that this parasite is harmful to man, if present in less than massive numbers (Plaut et al., 1969; Jaroonvesama et al., 1986).

3.6. Diagnosis and treatment

Although highly suggestive in endemic areas, the clinical picture is usually not distinctive. Hence, diagnosis is carried out with coprological techniques by examining for eggs,

or occasionally by examination of expelled adult worms vomitted or passed in faeces (Rim, 1982; Gilman et al., 1982; Weng et al., 1989; Le et al., 2004).

Two sequences corresponding to the 18S rRNA gene of *F. buski* are deposited in the GenBank. The sequence from a fluke vomitted by a Vietnamese child was recently used to verify the diagnosis, as only two nucleotide substitutions had been found when comparing the alleles (Le et al., 2004).

Many drugs have been used. Tetrachloroethylene proved to be effective (Shah et al., 1966), and was suggested as the drug of choice (Plaut et al., 1969; Chandra, 1976). Niclosamide was less effective than tetrachloroethylene (Suntharasamai et al., 1974; Idris et al., 1980). Thiabendazole, mebendazole, levamisole and pyrantel pamoate were ineffective (Rabbani et al., 1985). Later, praziquantel showed its efficacy even in severe fasciolopsiasis, so that a single dose of 15 mg/kg was proposed as the treatment of choice (Bunnag et al., 1983; Harinasuta et al., 1984; Taraschewski et al., 1986; Lee, 1986; Handoyo et al., 1986), although this drug could not save the life of a heavily infected 20-year-old woman (Gupta et al., 1999). More recently, the efficacy of triclabendazole, oxyclozanide and rafoxanide has been evaluated in pigs. The high efficacy (97.12%) of triclabendazole is worth mentioning, followed by oxyclozanide (93.27%) and rafoxanide (83.17%), none exhibiting side effects (Datta et al., 2004).

3.7. Prevention and control

Control can be achieved by treatment of people and livestock, preventing reinfection and instituting modern pig farming (Kuntz and Lo, 1967; Muttalib and Islam, 1975; Cross, 1984; Gai et al., 1995). Individual prevention is very simple by avoiding eating raw, water-derived food. However, it is extremely difficult to enforce this in the face of century-old traditions (Rim, 1982; Graczyk et al., 2001). Infections follow a familial trend, as food preparation and eating habits are passed from one generation to the next (Gai et al., 1995). In addition, water plants are a common food source because they are cheap and readily available (Gilman et al., 1982; Weng et al., 1989; Gai et al., 1995).

Control includes prevention of pollution of ponds where aquatic plants are cultivated, i.e. avoiding human excreta as fertilizers, promiscuous defaecation, and washing of pig excreta into neighbouring water bodies (Nguyen Van Tho, 2002a). Dried aquatic plants are not harmless because desiccation and direct solar radiation kill metacercariae with time (Lo, 1967; Rim, 1982; Weng et al., 1989). The prevention relies on consistent educational programmes stressing the importance of thoroughly cooking aquatic plants, immersing plants and fruit in boiling water for a few minutes, and boiling water where treated water is not available (Cross, 1984; Weng et al., 1989; Gai et al., 1995). Moreover, metacercariae are killed in 1.0% HCl in 18 days, 2% acetic acid in 9 days, 3.0% acetic acid in 6 days, 5% salt solution in 3 h, through soybean sauce in 30 min, and 10% cane sugar in 3 days (Komiya, 1964).

In Taiwan, the number of human cases drastically decreased in the 1980 s, thanks to aggressive education programs (Lee, 1986; Lee et al., 1989). Although reduction of fasciolopsiasis was recognised in endemic regions, this tendency was not stable, because many cultures still enjoy eating raw food (Lee et al., 1989; Weng et al., 1989).

Fasciolopsiasis can be controlled along with other food-borne parasitoses (Mott et al., 1995). Unfortunately, despite control programs, it still remains a public health problem in endemic areas (WHO, 1995a, b) and where it was thought to be controlled, as in Uttar Pradesh, where no case was detected in the 1990 s, there have been reports of a re-emerging infection (Bhatti et al., 2000; Muralidhar et al., 2000).

4. Gastrodiscoidiasis

4.1. Etiology, location and definitive hosts

Gastrodiscoides hominis is the only common amphistome of man. Adults are thick, fleshy and pyramidal in shape and bright pink in colour, when alive. Their size is 8.0–14.0/5.5–7.5 mm (Shrivastav and Shah, 1970; Kumar, 1980). The body is divided into two parts: the anterior portion is short and conical-cylindrical, whilst the posterior portion is large, discoidal, of up to 8.0 mm wide and ventrally excavated (Ahluwalia, 1960; Kumar, 1980, 1999). Eggs are ovoid and have a pale greenish grey colour in fresh faecal specimens and measure 127–160/62–75 μm (mean 146/66 μm), with the abopercular end generally thickened and rarely provided with a spine-like elongation (Dutt and Srivastava, 1972).

This amphistome inhabits the caecum and colon of pigs and humans (Ahuwalia, 1960; Kumar, 1980, 1999). The pig is the normal host species, in which it may reach very high numbers of up to 1886 in an individual pig and intensity averages of up to 227 in pig populations (Dutt and Srivastava, 1972). Porcine forms differ from human forms in their smaller size, characters of their genital papilla/cone, and shape and arrangements of testes, justifying a separate variety *G. hominis* var. *suis* (Varma, 1954), although those differences are not constant (Shrivastav and Shah, 1970).

Besides the domestic pig, a wild pig was also detected infected in the Thekaddy forest area in Kerala, India (Easwaran et al., 2003). It was also found in the Napu mouse deer (*Tragulus napu*) from Malaysia, the field rat (*Rattus brevicaudatus*) from Java, and rhesus monkeys (*M. mulatta*) in India (Buckley, 1964; Fox and Hall, 1970). There are also records concerning other monkeys (*Macaca fascicularis*, *Macaca irus*, *Macaca philippinensis*, *Macaca cynomolgus*) (Herman, 1967) and primates (orangutan, *Pongo pygmaeus*) (Pester and Keymer, 1968). *Gastrodiscoides hominis* was recently found in the American muskrat (*Ondatra zibethica*)

introduced in the Volga Delta, Russia where it locally infects wild boars (Ivanov and Semenova, 2000).

4.2. Geographical distribution

It has a large distribution in the Old World, which covers India (Assam, Bengal, Bihar, Uttar Pradesh, Madhya Pradesh and Orissa) (Shrivastav and Shah, 1970; Murty and Reddy, 1980), Pakistan, Burma, Thailand, Vietnam, the Philippines, China, Kazakstan and the Volga Delta in Russia. It has even been found in Indian inmigrants in Guyana (Ahuwalia, 1960; Buckley, 1964; Kumar, 1980; Harinasuta et al., 1987; Yu and Mott, 1994; Ivanov and Semenova, 2000; Fried et al., 2004).

In Africa (Goldsmid, 1975), it is found in countries such as Zambia (Hira, 1983), and it was also reported in a 7-year-old girl in Nigeria diagnosed by egg finding in stools (Dada-Adegbola et al., 2004).

4.3. Life cyle and intermediate host

Gastrodiscoides hominis follows a diheteroxenous life cycle. Eggs when laid include an embryo in a very early stage of segmentation. The miracidium appears fully mature on day 9 at a temperature of 24–33 °C (Zablotski, 1964; Dutt and Srivastava, 1972). Hatching takes place between days 9 and 14 depending on temperature. Longer periods of up to 3 months have also been observed (Dutt and Srivastava, 1972). The tiny aquatic snail species *Helicorbis coenosus* (= *S. coenosus*) is the only intermediate host known. Experiments to infect other species (*I. exustus*, *G. convexiusculus*) failed (Dutt and Srivastava, 1966; Dutt and Srivastava, 1972). Mother and daughter rediae are found in the digestive gland. These are sausage shaped, 148–747/45–140 μm in size, and lack collar and locomotor organs. The birthpore is salient and located near the level of the saccular intestine (Dutt and Srivastava, 1972). The prepatent period ranges from 28 to 152 days, depending on the seasonal variations of temperature. This period is shortest in summer and longest in winter. Snail longevity is 10–147 days after the first shedding day, shedding cercariae intermittently for 6–40 days.

The cercaria has an ovoid to pyriform body of 403–865/192–310 μm, with a narrower anterior end, and is provided with a pair of dark pigmented eye-spots. The tail is slightly longer than the body, of 468–923/56–94 μm. The daily number of cercariae shed is one to 34 (mean 6.8) per snail, with a total number of cercariae shed of seven to 238 (mean 74.7) per snail (Dutt and Srivastava, 1972). Cercarial emergence takes place in morning hours. The free swimming life of cercariae lasts from 1 to more than 24 h, at the end of which they encyst. Encystation generally takes place on the bottom. A few of them encyst on the shell of the snail host or any other substrate available as a stalk of *Nymphoea*. The metacercarial cyst, formed in about 2–4 min, is hemispherical and brownish, 201–227 μm (mean 216 μm) in diameter (Dutt and Srivastava, 1972).

When experimentally infecting pigs, all (100%) became infected and the mean percentage of metacercariae established was 37% (Dutt and Srivastava, 1972). Juvenile flukes, released from metacercariae in the small intestine, descend to reach the caecum and colon, where they mature and live as adults in the lumen by attaching to the intestinal mucosa (Kumar, 1999).

Reports in Africa pose the question of the intermediate snail host in that continent. A closely related species, *Gastrodiscus aegypticus*, which mainly parasitises equines (horse, donkey, mule) in various parts of Africa and was also found in pigs and wild boars, uses planorbid species of the *Bulinus (Bulinus) forskalii* group (Malek, 1971).

4.4. Epidemiology

High prevalences have been detected in humans, i.e. 41% mainly in children in Kamrup District, Assam, northern India. High intensities were also found in individual cases when removed by treatment. Since pigs were scarce in Kamrup, it was concluded that human infection was maintained without pig participation (Buckley, 1939).

However, the pig is the main reservoir, and, hence the prevalence in pigs is of epidemiological importance. The mollusc, *H. coenosus*, abounds in the water reservoirs around pigsties, where this trematode infection is endemic (Kumar, 1980).

The fact that *G. hominis* and *F. buski* use the same snail intermediate host in India makes it possible to understand much of the epidemiological data. Among 233 slaughter-pigs in Bareilly, India, 27% were infected with this amphistome, and in 50% of those cases the infection was concomitant with *F. buski* (Dutt and Srivastava, 1972). Surveys showing 59.7 and 41% infection rates by *F. buski* and *G. hominis*, rerspectively, in the same population of 221 human subjects analysed may be explained in a similar manner (Buckley, 1939).

In surveys of pigs in the tribal populations of Shillong (Mehhalaya), a hilly city of north-east India, where rearing of pigs is a common household practice, *G. hominis* showed a seasonal occurrence similar to that of *F. buski*. During the months from June to September, their prevalence rose to a peak, declining thereafter to a low level during winter and early spring (November–March). *Gastrodiscoides hominis* infection was not present during the first 3 months of the year (Roy and Tandon, 1992).

As metacercariae are able to attach to different substrates, it has been suggested that human contamination may occur when encysted metacercariae are swallowed with tainted vegetation (aquatic plants) or with animal products, such as raw or undercooked crustaceans (crayfish), squid, molluscs, or amphibians (frogs, tadpoles), as is the case in other species of the same family Gastrodiscidae (Yu and Mott, 1994; Fried et al., 2004).

4.5. Pathology, symptomatology and clinical manifestations

Pathology and symptomatology of *G. hominis* infection are uncertain. In humans, the parasite causes inflammation of the mucosa of the caecum and ascending colon with attendant symptoms of diarrhoea. It causes ill health in a large number of individuals, and deaths among untreated patients, especially children, have been attributed to this infection (Kumar, 1980). The specimens can be collected from the caecum, especially near the ileocaecal valva (Yu and Mott, 1994).

In pigs, gross lesions have a characteristic appearance. The acetabulum is found to drag the mucosa like a plug, which stands like a papilla and occupies an eccentric position in the sharply defined circular area which develops by continued impact of the discoidal region on the caecum mucosa (Ahluwalia, 1960). Caeca may be infected to such an extent that healthy tissue can hardly be seen (Shrivastav and Shah, 1970).

Sections of the lesions showed mucosal desquamation, infiltration with eosinophils, lymphocytes and plasma cells. The submucosa was also infiltrated, displayed oedema and tended to thicken, resulting in a subacute inflammation of the caecum and mucoid diarrhoea (Ahluwalia, 1960; Yu and Mott, 1994). Histopathological examination shows marked desquamation of epithelial lining, hypersecretion of mucus and necrosis of the mucuous glands. The lamina propria shows massive infiltration of eosinophils in between the crypts of Lieberkuhn. The muscularis mucosa becomes thickened, due to infiltration of eosinophils, lymphocytes, plasma cells and macrophages. Blood vessels appear hyperaemic and some show thickening of tunica intima and infiltration by eosinophils and lymphocytes (Shrivastav and Shah, 1970). A similar picture might be expected in human infection.

4.6. Diagnosis, treatment, prevention and control

Human infection by *G. hominis* is easily recognisable by finding its characteristic eggs in faeces. They are easily distinguishable from the eggs of *F. buski*, since the eggs of the latter are oval and yellowish-brown. The adult amphistome worm is unmistakable when passed spontaneously or expelled following treatment with soap water enemas or anthelmintics (Buckley, 1964).

A specific treatment for *G. hominis* infection is not known (Kumar, 1980), although it appears to respond to antihelmintics usually used against trematodiases (thymol, carbon tetrachloride, tetrachloroethylene). Although assays are lacking, Praziquantel appears to be the drug of choice. Even a single dose of 500 mg mebendazole was efficient in clearing a girl who was shedding thousands of *G. hominis* eggs in stools (Dada-Adegbola et al., 2004).

Owing to the similarities in intermediate and reservoir hosts, as well as in transmission and contamination characteristics, measures useful against *F. buski* may also be applied to *G. hominis*.

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