

Review

Dynamics of host–parasite interactions: the example of population biology of the liver fluke (*Fasciola hepatica*)

Sylvie Hurtrez-Boussès*, Cécile Meunier, Patrick Durand, François Renaud

Centre d'études sur le polymorphisme des microorganismes (CEPM), UMR CNRS-IRD 9926, Equipe 'Evolution des Systèmes Symbiotiques',
IRD, 911 avenue Agropolis, BP 5045, 34032 Montpellier cedex 1, France

ABSTRACT – Knowledge of the population dynamics of parasites and their hosts is essential to build veterinary and health programs. The example chosen is that of *Fasciola hepatica*, a food-borne trematode responsible for severe human and animal infections on the five continents. In this paper, we review the relationships between the liver fluke and its intermediate (mollusc) and definitive (vertebrate) hosts. © 2001 Éditions scientifiques et médicales Elsevier SAS

host–parasite interactions / population genetics / liver fluke / intermediate and definitive host

1. Introduction

At the beginning of this new millennium, the knowledge of evolutionary processes is one of the major challenges of life sciences. Although we currently cannot formalize, or define all of the mechanisms involved, several studies have revealed that parasites and pathogens (symbiotic organisms which live at the expense of other organisms (i.e. hosts); see [1] for a definition of these terms) constitute a major evolutionary component. As suggested by Barbault [2], three principal reasons explain why such symbiotic systems are so important in evolutionary dynamics.

1. All living species can be involved in parasitism, either as parasites or as hosts [3]; human societies do not escape this biological constraint, as evidenced by statistics supplied by different health organizations (WHO, CDC, etc). Indeed, AIDS, malaria, yellow fever, Lyme disease, bilharziosis, cholera, trypanosomiasis or fascioliasis (to cite only a few examples) are all human diseases caused by a symbiotic organism (in the sense defined by [1]). Moreover, we cannot ignore the emergence or re-emergence of many of these diseases around the world.

2. Parasitism is a major factor limiting natural populations and must therefore play an important role in the equilibrium of ecosystems.

3. Parasitism, which is probably the most specialized type of interspecific relationship, implies strong genetic interactions between the parasite and its host, which leads to coadaptation between the two partners.

In this context, host–parasite interaction studies have to address the following questions.

- How is the genetic information transmitted in different environments? Which reproductive strategies are selected in host and parasite populations?
- How does gene flow spread within and between parasite populations on one hand, and host populations on the other hand? Are host and parasite genotypes randomly associated or not?
- What are the levels of adaptation between parasites and their hosts? Which roles do the biological and ecological parameters of host (i.e. 'habitat-resource' system of the parasite) play in mechanisms of specialization of parasites?
- What are the consequences of parasitic pressures on host life history traits (i.e. survival, fecundity, behavior, sexual selection, spatial and temporal distribution)? Do hosts and parasites co-evolve (evolution of resistance and virulence)?

Although most of these points belong to ecological and evolutionary topics, investigations on human, animal and plant diseases concern all branches of biology (i.e. medical, pharmacological, veterinary, genetics, biochemistry, ecology and evolutionary biology). Therefore, the development of prophylactic tools requires good fundamental knowledge, and information acquired from ecological and evolutionary studies is essential for public and veterinary health programs (epidemiology, pathology, control and therapy). Thus, it is necessary to develop strong interactions between different approaches in order to enhance our efforts against current or future biotic aggressors.

*Correspondence and reprints.
E-mail address: hurtrez@mlp.ird.fr (S. Hurtrez-Boussès).

The biological model we have chosen is the liver fluke *Fasciola hepatica*, a food-borne trematode which constitutes a severe public-health problem across the world [4]. The purpose of this paper is to analyse the ecological and evolutionary relationships between the liver fluke and its intermediate and definitive hosts, and to suggest and discuss future investigations for the control of this disease.

2. Current knowledge of fasciolosis around the world

An estimated 40 million people are infected by at least one of the different trematode species and several infections caused by these parasites are re-emerging [5]. Among them, fasciolosis is caused by *F. hepatica* or, to a lesser extent, by *F. gigantica*. These liver flukes, which are among the largest parasites infecting the human host (each adult measures 25–30 mm in length and about 10–15 mm in width) are digenean parasites (see [6, 7] for details concerning the life cycle). *Figure 1* shows the three steps of the *F. hepatica* life cycle. The definitive host is a vertebrate, most often a mammal (human, cow, sheep, rabbit, etc.), but infection has also been reported in birds [8]. The intermediate host is a hermaphroditic lymnaeid snail (mollusc gastropod) inhabiting intermediate ponds. Major sources of infection for definitive hosts are plants associated with water (e.g., watercress, mint) or spring water [9]. When swallowed by a vertebrate host, the encysted infective stage (metacercaria) excysts in the lumen of the intestine, and migrates toward the liver, where it feeds upon the parenchymal cells, causing extensive haemorrhage. *F. hepatica* grows slowly, achieving sexual maturity after 2 months in the bile ducts. The fluke is hermaphroditic, and self-mating may occur. Eggs are produced by each worm after approximately one more week of development. Eggs are laid unembryonated and are passed from

the common bile ducts into the duodenum and subsequently into feces. The egg must be deposited in fresh water to complete its development and hatch. Hatching usually takes place within 10–15 days, but at low temperatures, development can be delayed for several months. Immediately after hatching, the ciliated miracidium swims actively and seeks its appropriate snail host. Upon encountering it, the miracidium penetrates the snail and develops within its tissue, where it multiplies asexually. Morphogenesis within the snail proceeds sequentially from the miracidium to the sporocyst, and then to the redia stage. Each new stage signals an increase in the number of individual immature flukes. Each redia gives rise to many cercariae, which penetrate out of the snail and into the water. The cercariae encyst upon aquatic plants or, in a lower proportion, in water. The encysted cercariae, now termed metacercariae, are resistant to mild changes in temperature and other environmental parameters.

Fasciolosis, which is probably the most common helminth infection of cattle (prevalences 30–90%, mainly in tropical areas [10]) has long been recognized as a true veterinary problem. Indeed, infection of livestock induces productivity losses (e.g., meat, milk, wool, see review in [10]), with important economic consequences [4, 11]. Fasciolosis is now also recognized as an important re-emerging human disease [12]: 17 million humans are considered to be infected with liver fluke [13], and 180 million are at risk of infection [14]. In some regions, prevalence of infection with *Fasciola* is extremely high, particularly in South America (Peru and Bolivia), where fasciolosis is regarded as a serious health problem. The most striking description of a human hyperendemic area [15] is the Bolivian Altiplano, in which prevalences between 72 and 100% have been recorded in different surveys [16, 17]. Hillyer and Apt [11] estimated that over 350 000 humans are infected in the Bolivian altiplano alone.

Figure 1. Life cycle of the liver fluke *F. hepatica* L. 1758 (modified from [6] and [7]).

a. Intra-mollusc stages: asexual reproduction of larval stages of *F. hepatica* (sporocysts, redia and cercaria) occurs in the intermediate host. In this part, the major research aims on population biology should be:

A. Mollusc:

1. Genetic characterization and allelic cartography of *L. truncatula* in the different geographic areas of its distribution; 2. Genetic structure and reproduction mode of snail populations under different parasitic pressures; 3. Spatial and temporal analyses of bottleneck and demography of snail populations; 4. Evolution of life history traits (survival, fecundity, resistance to infection, etc.) in different endemic areas.

B. Liver fluke:

1. Comparative analyses of population genetic structures of larvae vs adults of *F. hepatica*; 2. Evolution of life history traits (infestation rates, intra-host survival, cercarial production, etc.) of *F. hepatica* depending on the definitive host species and the genetic variability of the snails.

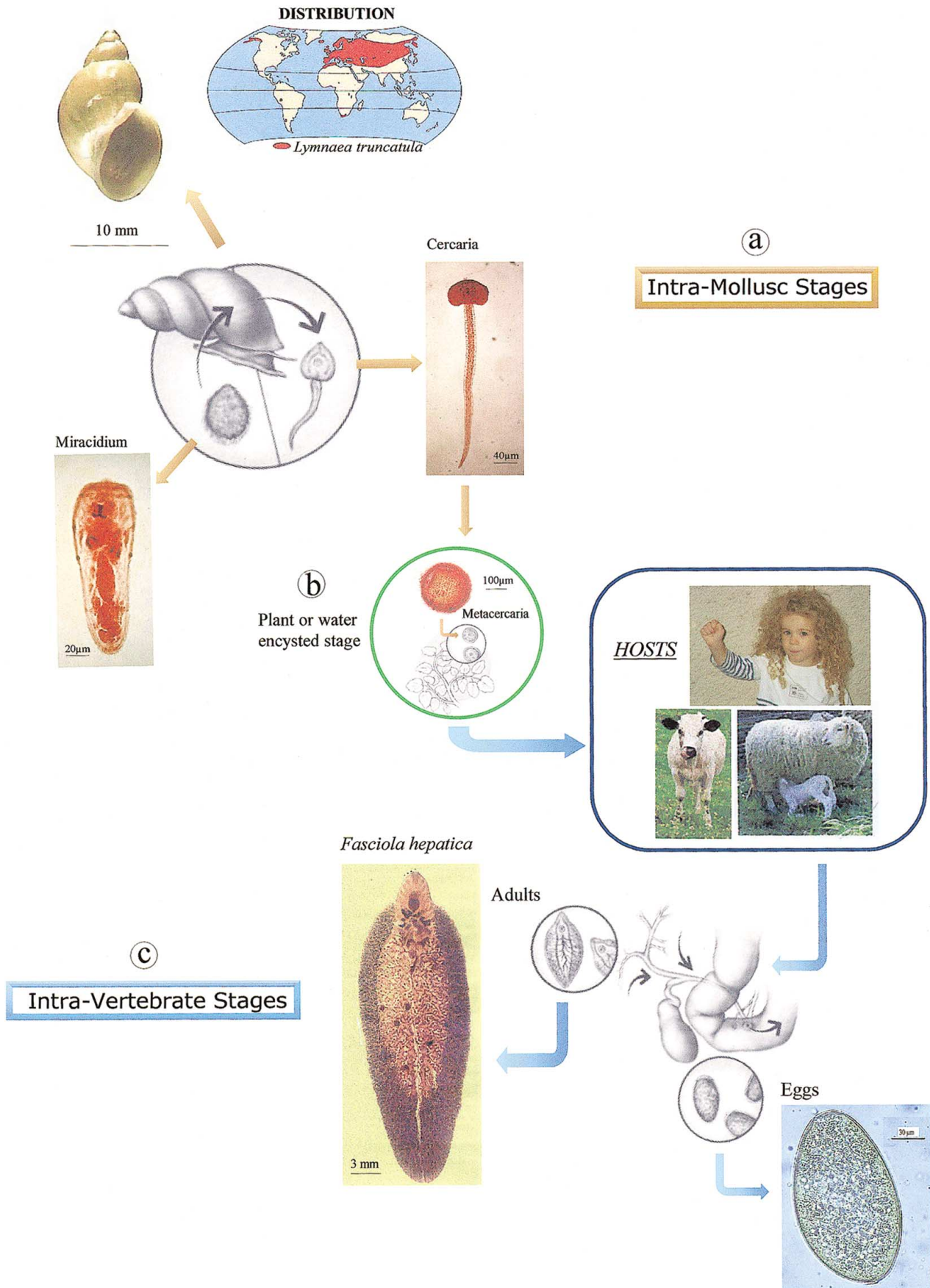
C. Biosystem: 'Mollusc-fluke':

1. Genetic co-structures of *Lymnaea* and *Fasciola* populations in different endemic areas: 'Who transmits who, where and when?'; 2. Compatibility polymorphisms between snails and flukes depending on their geographic origins.

b. Plant or water encysted stage (metacercaria), which is the infective stage for the vertebrate host.

c. Intra-vertebrate stages. Sexual reproduction (selfing or outcrossing) occurs for the hermaphroditic adult of *F. hepatica* in the definitive host. In this part, different research aims on the population biology of the liver fluke should be addressed:

1. Genetic characterization and allelic cartography of *F. hepatica* in different geographic areas of its distribution; 2. Genomic specificity of *F. hepatica* among definitive host taxa; 3. Evolution of life history traits (survival, fecundity, egg hatching rates) of *F. hepatica* depending on host species and geographic origins; 4. Population genetic structures of *F. hepatica* and reproduction mode among endemic areas. Does only one genetic entity of *F. hepatica* exist?



Fasciolosis is cosmopolitan in distribution, with human and animal cases being reported on the five continents (reviews in [10, 18, 19]). Only *F. hepatica* has a worldwide distribution, whereas *F. gigantica* is restrained to Africa and South America [5]. *F. hepatica* originated in Europe and was successfully introduced into America, Australia, New Zealand and Africa [20]. When introduced, *F. hepatica* has adapted to new definitive (e.g., llamas and alpacas in South America [15]) and intermediate hosts (e.g., *Lymnaea tomentosa* in Australia [20]). However, in the Bolivian altiplano, a population-genetics study conducted by Jabbour-Zahab et al. [21] has revealed that the European intermediate host *Lymnaea truncatula* had also been introduced from Europe, and is responsible for the transmission of liver fluke (see figure 2; see also [22, 23], for morphological and molecular arguments).

3. Relationships between liver fluke and molluscs (intermediate hosts)

Although different lymnaeid snails can be infected by *F. hepatica*, *L. truncatula* is the predominant intermediate host [20, 24]. The liver fluke has proved to have several detrimental effects on its intermediate host, including castration or decrease in fecundity, increased mortality, destruction of the digestive gland, metabolic changes (reallocation of energy from reproduction to growth, inducing gigantism), increased sensitivity to environmental stress [24, 25]. Since the liver fluke affects both survival and fecundity of its intermediate host, it most probably exerts strong selective pressures on snail populations. According to the Red Queen hypothesis (see section 3.1), it would be expected that co-selection between host and parasite favours the maintenance of sex in host and parasite populations [26], resulting in the maintenance of polymorphism on loci acting in host–parasite interactions. However, Meunier et al. [27] have shown that selfing is the predominant mode of reproduction and that, in the Bolivian altiplano, *L. truncatula* are monomorphic at six microsatellite loci (see figure 3). This suggests that another effect (i.e. bottleneck event due to introduction) would impair the Red Queen hypothesis in the Bolivian liver fluke–lymnaeid snail system. However, surprisingly, liver flukes infecting snails in the Bolivian altiplano present microsatellite polymorphism (Hurtrez-Boussès et al., unpublished data).

Natural prevalence of *F. hepatica* in lymnaeid snails is high [28], but highly variable among sites. For instance, in the region of Limoges (France), Rondelaud [29] showed that natural prevalence ranged from 0% in sites without potential definitive hosts (river banks) to 12.3% in sites usually occupied by livestock (meadows). These differences were also observed at very small scale (ca. 400 m), between drainage areas (prevalence: 3.8%) and brook banks (0%) in a same hydrographic network [30]. In six cattle ranches in Florida, Kaplan et al. [31] found that the prevalence of *F. hepatica* in its local intermediate host, *Fossaria cubensis*, ranged from 0.1 to 3.1%. In the Bolivian altiplano, Meunier et al. [27] reported a mean prevalence of 11.6% (± 23.3 , $n = 245$ snails dissected), ranging from 0 to 88.3% ($n = 13$ sites).

Experimental infestations have revealed that the liver fluke induces higher mortality in snails originating from populations with low natural prevalences than in those originating from populations with high prevalences [29, 32, 33] (but see [34] for contradictory results), indicating that there would be co-adaptation between host and parasite (see section 3.1). Moreover, Boray [20] has experimentally shown that Australian strains of *F. hepatica* are more infective than European strains for the local intermediate host (*L. tomentosa*), suggesting a local adaptation of the introduced parasite to its new host.

As pointed out by van der Knaap and Loker [35], the variations in compatibility between trematode and snail might result from different traits (ecological, behavioural, physiological, immunological), and their study will be useful in our knowledge of the dynamics of host–parasite systems (figure 1). Such information would be particularly relevant in the elaboration of control programs based on the management of snail populations.

3.1. The Red Queen principal

In Lewis Carroll's book *Through the looking glass and what Alice found there*, published in 1872, the Red Queen runs, dragging Alice away (see figure 4). She explains to Alice that she needs to run continuously in order to stay in the same place, because the background is always moving. Van Valen [36] has transposed this principle to evolutionary ecology: since a given species interacts with other species (competitors, predators, parasites) which are continuously evolving ('the moving landscape'), the 'Red Queen species' has to keep adapting ('to run') to avoid extinction ('to stay at the same place'). The 'arms race' developed by hosts and parasites, named coevolution, is an illustration of this principle: parasites continuously evolve to evade strategies developed by the hosts against them and, in turn, hosts must adapt to the parasite changes. According to the Red Queen principle, the evolutionary success of parasites on common host genotypes leads to: 1) selection for sexual reproduction (Hamilton et al. [37]) and 2) local adaptation between parasites and hosts (Lively and Dybdahl [38]).

1. Selection for sexual reproduction:

Under the Red Queen hypothesis it is expected that both host and parasite will increase their genetic variability. In this context, if parasites induce a high selective pressure (i.e. by castrating or by killing their hosts), cross-fertilization will become advantageous for the host, since it produces genetically variable offspring. Some of these variants will therefore escape infection by parasites adapted to the most common genotypes.

2. Local adaptation:

Several experimental studies have shown that parasites are more infective (i.e. increased virulence and transmissibility) against the local hosts (sympatric hosts) than against hosts of a foreign population (allopatric). This phenomenon has received the name of local adaptation. Taking into account the population dynamics of hosts and parasites, Gandon et al. [39] have proposed a theoretical model for local adaptation. This model shows that local adaptation takes place when the migration rate is high for the parasite but low for the host. By contrast, if migration is

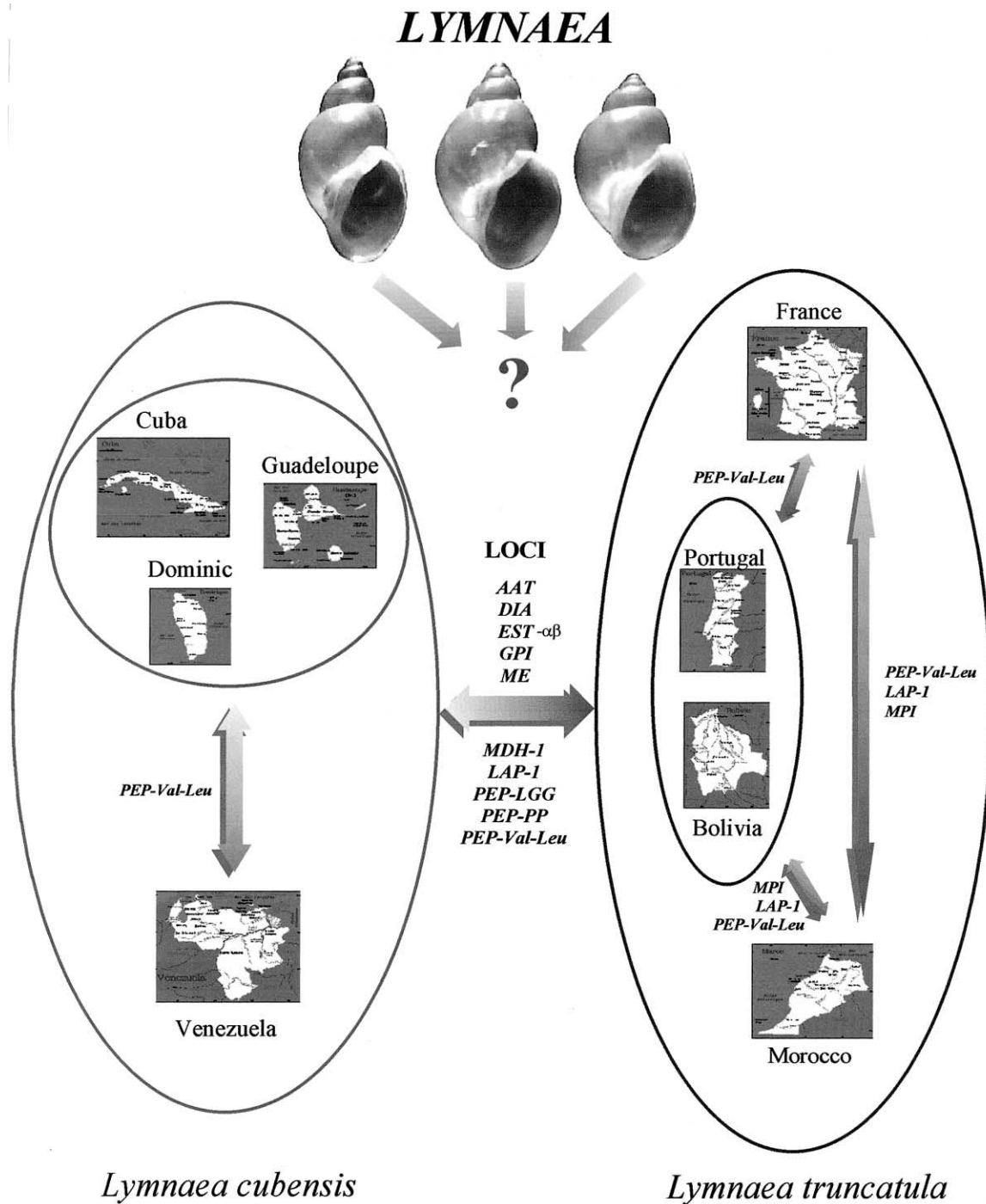


Figure 2. Phylogeography and genetic divergences in *L. truncatula*. We summarize here our knowledge of the distribution of genetic entities obtained by multilocus enzyme electrophoresis at 18 putative loci for the two species *L. truncatula* and *L. cubensis* (from [21]). These results reveal the following:

1. Between the two *Lymnaea* species: strong genetic divergences characterized by at least ten diagnostic loci. 2. Within the two *Lymnaea* species: slight genetic divergences between geographic sites. Moreover, the observed lack of heterozygotes within the different populations analyzed suggested a predominant selfing mode of reproduction for these species. 3. European origin of *Lymnaea* from Bolivian altiplano: indeed, although the literature suggested that snails from Bolivia belong to the South American *Lymnaea* group, results showed no genetic differences between *Lymnaea* collected in Portugal and Bolivia. Thus, the European origin of the Bolivian snails was clearly demonstrated. Names of the loci, abbreviation, full name of the enzyme, and EC number:

AAT: Aspartate amino transferase, 2.6.1.1; DIA: Diaphorase, 1.6.99.2; EST: Esterase, 3.1.1.1; GPI: Glucose phosphate isomerase, 5.3.1.9; LAP: Leucine aminopeptidase, 3.4.11.1; MDH: Malate dehydrogenase, 1.1.1.37; ME: Malic enzyme, 1.1.1.40; PEP LGG: Peptidase leu-gly-gly, 3.4.11.-; PEP PP: Peptidase phe-pro, 3.4.11.-; PEP VAL-LEU: Peptidase val-leu, 3.4.11.-.

Microsatellite Analysis of *Lymnaea truncatula*
(Locus 16)

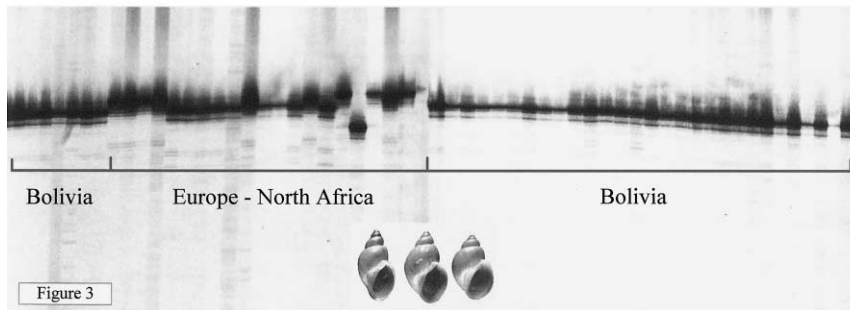


Figure 3. Example of microsatellite analysis of *L. truncatula* (Genbank accession number of this locus: AF 226978). PCR products are separated on a 5% polyacrylamide and 7 M urea gel. Individuals analyzed on this gel were sampled in the Bolivian altiplano (37 individuals) and in Europe and North Africa (21 individuals). Similar results were obtained for the complete analysis conducted by Meunier et al. [27], who studied 245 snails from Bolivia and 122 from Europe and North Africa at six microsatellite loci. Two main results were obtained:

1. An important deficit in heterozygotes (Fis values: 0.69–1) revealed that these lymnaeid snails reproduce mainly by selfing (estimated selfing rates: 0.82–1).
2. Although some genetic variability was detected in European and North-African populations (two to nine alleles per locus), all the Bolivian snails analyzed have exactly the same multi-locus genotype.

higher for the host than for the parasite, parasites are expected to develop better in allopatric hosts than in sympatric ones. If both host and parasite populations have high migration rates, similar parasite effects are expected against allopatric and sympatric hosts.

4. Relationships between liver flukes and vertebrates (definitive hosts)

The liver fluke has a very large spectrum of definitive hosts: humans, livestock and wild animals (reviews in [15, 28]). Infestation levels vary among sites and among species (see review in [10]). In humans, high variability in



Figure 4. Alice and the Red Queen.

infestation levels may be partly explained by behavioural and dietary habits (e.g., consumption of aquatic plants, living close to livestock etc., see [15]).

The host spectra differs strongly among regions. For instance, pigs are very occasional hosts in Europe, whereas they represent a secondary reservoir host in the Bolivian altiplano [15]. Similarly, although rats are only occasionally infected by the liver fluke in France (e.g., see [40]), a prevalence up to 45% had been reported in black rats on the nearest island of Corsica [15, 41]. Moreover, Menard et al. [40] have shown that the introduced coypu (*Myocastor coypus*) is a suitable host for the liver fluke in Europe, with a prevalence reaching 55%.

Among the definitive host species there is a considerable variation in susceptibility to infection and in immune responses against liver fluke [42]. For instance, cattle have proved to develop acquired immunological resistance against *F. hepatica* (review in [42]), whereas sheep develop little or no acquired protective immunity [43]. Panaccio and Trudgett [44] suggested that such variation in host immune systems would result in various selective pressures which can lead to sympatric speciation of different liver fluke strains. This hypothesis is supported by: (1) morphological; (2) genetic; (3) physiological; and (4) epidemiological arguments.

Indeed, (1) Morphology of adults and eggs of liver fluke varies across the different definitive hosts [45, 46]. (2) Miller et al. [47] have reported that the glutathione S-transferase presents higher variability in cattle flukes than in sheep or rat flukes. (3) Caseby et al. [48] have shown that the ionic composition of liver flukes infesting cattle differs from that of sheep flukes. (4) Interestingly, Rondelaud and Dreyfuss [49] have revealed that the success of experimental infestation of snails strongly differs between liver fluke miracidia obtained from different

definitive hosts. Indeed, the estimated prevalences were 35, 66 and 86% with liver fluke miracidia obtained from eggs collected, respectively, in rabbit, sheep and cattle.

This variability can result from phenotypic plasticity or from genetic differences between liver flukes infecting different definitive hosts. Dixon [50] has shown that whatever the definitive host in which liver fluke eggs have been initially sampled (sheep or cattle), liver flukes grow faster but have a lower fecundity in sheep than in cattle, suggesting that the differences might be due to environmental effects.

A better understanding of the relationships between the liver fluke and its definitive hosts is required to elaborate control programs (figure 1).

5. Concluding remarks and perspectives

Different antihelminthic drugs have proved to be effective against fasciolosis (review in [51]). However, cases of

resistance of the liver fluke to treatments have been reported [52, 53]. The development of vaccines is a new challenge in the control of fasciolosis [53–55], but the variability in host immune responsiveness is a serious problem [19, 56]. Moreover, candidate antigens for vaccine proved to be highly polymorphic [53], and they only induce partial protection. Other control strategies involve the management of mollusc populations (application of molluscicides, biological control). Another perspective is the selection of resistant definitive hosts. To achieve such control programs, it is necessary to assess the genetic variability of liver fluke, in relation to the diversity of its intermediate and definitive hosts (see figure 1).

As exemplified by studies on the liver fluke and its hosts, a better knowledge of genetic relationships between parasites and hosts will certainly provide a good framework for the control of diseases (figure 5).

Acknowledgments

Part of this work is supported by the University Montpellier II (grant "Jeune chercheur") to S. Hurtrez-Boussès. C. Meunier is supported by the French Ministère de l'Éducation Nationale (allocation de recherche).

References

- [1] Cheng T.C., in: Toft C.A., Aeschlimann A., Bolis L. (Eds.), *Is Parasitism Symbiosis? A Definition of Terms and the Evolution of Concepts*, Oxford University Press, Oxford, 1991, pp. 15–36.
- [2] Barbault R., Body size, ecological constraints and the evolution of life-history strategies, *Evol. Biol.* 22 (1988) 261–286.
- [3] Price P.W., *Evolutionary Biology of Parasites*, Princeton University Press, Princeton, 1980.
- [4] Rim H.J., Farag H.F., Sornmani S., Cross J.H., Food-borne trematodes: ignored or emerging? *Parasitol. Today* 10 (1994) 207–209.
- [5] McCarthy J., Moore T.A., Emerging helminth zoonoses, *Int. J. Parasitol.* 30 (2000) 1351–1360.
- [6] Despommier D.D., Karapelou J.W., *Parasite Life Cycles*, Springer-Verlag, New York, 1987.
- [7] Andrews S.J., in: Dalton J.P. (Ed.), *The Life Cycle of Fasciola hepatica*, CAB International, Oxon, 1999, pp. 1–29.
- [8] Vaughan J.L., Charles J.A., Boray J.C., *Fasciola hepatica* infection in farmed emus (*Dromaius novaehollandiae*), *Aust. Vet. J.* 75 (1997) 811–813.
- [9] Rondelaud D., Dreyfuss G., Bouteille B., Dardé M.L., Changes in human fasciolosis in a temperate area: about some observations over a 28-year period in central France, *Parasitol. Res.* 86 (2000) 753–757.
- [10] Torgerson P., Claxton J., in: Dalton J.P. (Ed.), *Epidemiology and Control*, CAB International, Oxon, 1999, pp. 113–149.
- [11] Hillyer G.V., Apt W., Food-borne trematode infections in the Americas, *Parasitol. Today* 13 (1997) 87–88.

EVOLUTIONARY DYNAMICS OF DISEASES

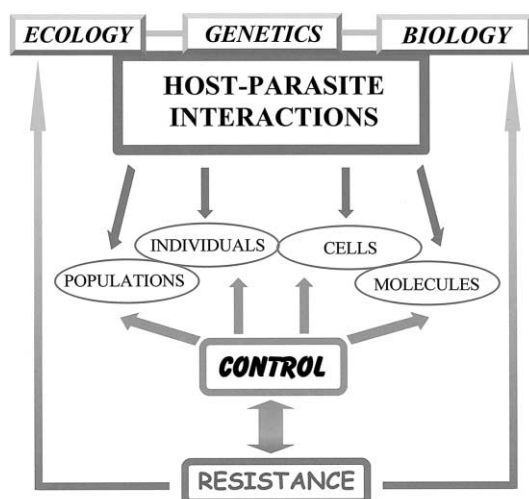


Figure 5. Summary of the host–parasite interaction studies. Ecology, genetics and biology are three fundamental parameters which characterize the evolution of host–parasite interactions through generations. These interactions act at hierarchical levels (i.e. from molecules to populations) for both host and parasite, and constitute the 'host–parasite coevolution'. This figure summarizes the general context that should be developed in order to better understand the epidemiology of diseases (from molecules to ecosystems), taking into account the role played by human societies. Indeed, control programs undoubtedly induce resistance in both parasite and vector populations. In turn, such resistance modifies the three fundamental parameters of host–parasite interactions, and thus their evolution. Because we are always confronted with new and different situations, the main epidemiological question is: 'Who transmits who, where and when?' The answer to this question will enable us to understand the epidemiology of diseases. To reach this goal, we must now combine complementary approaches (i.e. molecular, cell and population biology) in order to act efficiently against human, plant and animal diseases.

- [12] Chen M.G., Mott K.E., Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature, *Trop. Dis. Bull.* 87 (1990) R1–R38.
- [13] Hopkins D.R., Homing in on helminths, *Am. J. Trop. Med. Hyg.* 46 (1992) 626–634.
- [14] Anonymous, Control of foodborne trematode infections, World Health Organization, Technical Report Series 849 (1995).
- [15] Mas-Coma S., in: Angelico M. (Ed.), Human fascioliasis in Europe and Latin America, Balaban Publishers, Rehovot, Israel, 1998, pp. 1–17.
- [16] Mas-Coma S., Anglés R., Esteban J.G., Bargues M.D., Buchon P., Franken M., Strauss W., The Northern Bolivian altiplano: a region highly endemic for human fasciolosis, *Trop. Med. Int. Health* 4 (1999) 454–467.
- [17] Esteban J.G., Flores A., Anglés R., Mas-Coma S., High endemicity of human fascioliasis between lake Titicaca and La Paz valley, Bolivia, *Trans. R. Soc. Trop. Med. Hyg.* 93 (1999) 151–156.
- [18] Esteban J.G., Bargues M.D., Mas-Coma S., Geographical distribution, diagnosis and treatment of human fascioliasis: a review, *Res. Rev. Parasitol.* 58 (1998) 13–42.
- [19] Spithill T.W., Smooker P.M., Sexton J.L., Bozas E., Morrison C.A., Creaney J., Parsons J.C., in: Dalton J.P. (Ed.), Development of vaccines against *Fasciola hepatica*, CAB International, Oxon, 1999, pp. 377–410.
- [20] Boray J.C., Studies on the relative susceptibility of some lymnaeids to infection with *Fasciola hepatica* and *F. gigantica* and on adaptation of *Fasciola* spp., *Ann. Trop. Med. Parasitol.* 60 (1966) 114–124.
- [21] Jabbour-Zahab R., Pointier J.P., Jourdane J., Jarne P., Oviedo J.A., Bargues M.D., Mas-Coma S., Anglés R., Perera G., Balzan C., Khallayoune K., Renaud F., Phylogeography and genetic divergence of some lymnaeid snails, intermediate hosts of human and animal fascioliasis with special reference to lymnaeids from the Bolivian altiplano, *Acta Trop.* 64 (1997) 191–203.
- [22] Bargues M.D., Mangold A.J., Munoz-Antoli C., Pointier J.P., Mas-Coma S., SU rDNA characterization of lymnaeid snails transmitting human fascioliasis in South and Central America, *J. Parasitol.* 83 (1997) 1086–1092.
- [23] Samadi S., Roumegoux A., Bargues M.D., Mas-Coma S., Yong M., Pointier J.P., Morphological studies of lymnaeid snails from the human fascioliasis endemic zone of Bolivia, *J. Molluscan Stud.* 66 (2000) 31–44.
- [24] Graczyk T.K., Fried B., in: Dalton J.P. (Ed.), Development of *Fasciola hepatica* in the intermediate host, CAB International, Oxon, 1999, pp. 31–46.
- [25] Gutierrez A., Perera G., Yong M., Sanchez J., Lin W., Life-history traits of *Fossaria cubensis* (Gastropoda: Lymnaeidae) under experimental exposure to *Fasciola hepatica* (Trematoda: Digenea), *Mem. Inst. Oswaldo Cruz* 95 (2000) 747–752.
- [26] Lively C.M., Evidence from a New Zealand snail for the maintenance of sex by parasitism, *Nature* 328 (1987) 519–521.
- [27] Meunier C., Tirard C., Hurtrez-Boussès S., Durand P., Bargues M.D., Mas-Coma S., Pointier J.P., Jourdane J., Renaud F., Lack of molluscan host diversity and the transmission of an emerging parasitic disease in Bolivia, *Mol. Ecol.* 10 (2001) 11333–1340.
- [28] Boray J.C., CRC Handbook Series in Zoonoses. Section C: Parasitic Zoonoses, Fascioliasis, 1982, pp. 71–88.
- [29] Rondelaud D., Variabilité interpopulationnelle de l'infestation fasciolienne chez le mollusque *Lymnaea truncatula* Müller. Influence du contact préalable de la population avec le parasite, *Bull. Soc. Zool. Fr.* 118 (1993) 185–193.
- [30] Rondelaud D., Dreyfuss G., Variabilité de l'infestation fasciolienne chez *Lymnaea truncatula* Müller par rapport à la localisation de ses gîtes sur les réseaux hydrographiques, *Bull. Soc. F. Parasitol.* 14 (1996) 189–194.
- [31] Kaplan R.M., Dame J.B., Reddy G.R., Courtney C.H., The prevalence of *Fasciola hepatica* in its snail intermediate host determined by DNA probe assay, *Int. J. Parasitol.* 27 (1997) 1585–1593.
- [32] Rondelaud D., Dreyfuss G., Vareille-Morel C., Moukrim A., Les populations de *Lymnaea truncatula* Müller vivant sur les berges de rivières. Etude expérimentale de leur aptitude à l'infestation par *Fasciola hepatica* Linné, *Rev. Méd. Vét.* 148 (1997) 329–332.
- [33] Bargues M.D., Oviedo J.A., Funatsu I.R., Rodriguez A., Mas-Coma S., Survival of lymnaeid snails from the Bolivian northern altiplano after the parasitization by different Bolivian isolates of *Fasciola hepatica*, in: Guerra A., Rolan E., Rocha F. (Eds.), 12th International Malacological Congress, Instituto de Investigaciones Marinas (CSIC), Vigo, 1995, pp. 443–445.
- [34] Gasnier N., Rondelaud D., Abrous M., Carreras F., Bouldard C., Diez-Banos P., Cabaret J., Allopatric combination of *Fasciola hepatica* and *Lymnaea truncatula* is more efficient than sympatric ones, *Int. J. Parasitol.* 30 (2000) 573–578.
- [35] Van der Knaap W.P.W., Loker E.S., Immune mechanisms in Trematode-snail interactions, *Parasitol. Today* 6 (1990) 175–182.
- [36] van Valen L., A new evolutionary law, *Evol. Theory* 1 (1973) 1–30.
- [37] Hamilton W.D., Axelrod R., Tanese R., Sexual reproduction as an adaptation to resist parasites (a review), *Proc. Natl. Acad. Sci. USA* 87 (1990) 3566–3573.
- [38] Lively C.M., Dybdahl M.F., Parasite adaptation to locally common host genotypes, *Nature* 405 (2000) 679–681.
- [39] Gandon S., Capowiez Y., Dubois Y., Michalakis Y., Olivieri I., Local adaptation and gene for gene coevolution in a metapopulation model, *Proc. R. Soc. London B* 263 (1996) 1003–1009.
- [40] Menard A., L'-Hostis M., Leray G., Marchandeau S., Pascal M., Roudot N., Michel V., Chauvin A., Inventory of wild rodents and lagomorphs as natural hosts of *Fasciola hepatica* on a farm located in a humid area in Loire Atlantique (France), *Parasite* 7 (2000) 77–82.
- [41] Mas-Coma S., Fons R., Feliu C., Bargues M.D., Valero M.A., Galan-Puchades M.T., Conséquences des phénomènes liés à l'insularité dans les maladies parasitaires. La grande Douve du foie (*Fasciola hepatica*) et les Muridiés en Corse, *Bull. Soc. Neuch. Sci. Nat.* 110 (1987) 57–62.
- [42] Mulcahy G., Joyce P., Dalton J.P., in: Dalton J.P. (Ed.), Immunology of *Fasciola hepatica* infection, CAB International, Oxon, 1999, pp. 341–375.
- [43] Haroun E.M., Hillyer G.V., Resistance to fascioliasis - a review, *Vet. Parasitol.* 20 (1986) 63–93.
- [44] Panaccio M., Trudgett A., in: Dalton J.P. (Ed.), Molecular Biology, CAB International, Oxon, 1999, pp. 449–464.

- [45] Stunkard H.W., Intraspecific variation in parasitic flatworms, *Syst. Zool.* 6 (1957) 7–18.
- [46] Abrous M., Comes A.M., Gasnier N., Rondelaud D., Dreyfuss G., Chauvin A., Menard A., Agoulon A., Cabaret J., Morphological variability in *Fasciola hepatica* eggs in ruminants, rodents and lagomorphs, *J. Helminthol.* 72 (1998) 313–317.
- [47] Miller C.M.D., Howell M.J., Boray J.C., Host effects of glutathione s-transferase activity in *Fasciola hepatica*, *Int. J. Parasitol.* 23 (1993) 1073–1076.
- [48] Caseby R.H., Harriot M., Fairweather I., Ionic composition of the liver fluke *Fasciola hepatica* from different mammalian hosts and comparison with host bile, *Parasitol. Res.* 81 (1995) 394–397.
- [49] Rondelaud D., Dreyfuss G., *Fasciola hepatica*: the influence of the definitive host on the characteristics of infection in the snail *Lymnaea truncatula*, *Parasite* 2 (1995) 275–280.
- [50] Dixon K.E., The relative suitability of sheep and cattle as hosts for the liver fluke, *Fasciola hepatica* L., *J. Helminthol.* 38 (1964) 203–212.
- [51] Fairweather I., Boray J.C., in: Dalton J.P. (Ed.), *Mechanisms of Fasciolicide Action and Drug Resistance in Fasciola hepatica*, CAB International, Oxon, 1999, pp. 225–276.
- [52] Overend D.J., Bowen F.L., Resistance of *Fasciola hepatica* to triclabendazole, *Aust. Vet. Parasitol.* 72 (1995) 275–276.
- [53] Spithill T.W., Dalton J.P., Progress in development of liver fluke vaccines, *Parasitol. Today* 14 (1998) 224–227.
- [54] Smith W.D., Prospects for vaccines of helminth parasites of grazing ruminants, *Int. J. Parasitol.* 29 (1999) 17–24.
- [55] Meeusen E.N.T., Maddox J.F., Progress and expectations for helminth vaccines, *Adv. Vet. Med.* 41 (1999) 241–256.
- [56] Ortiz P., Cabrera M., Jave J., Claxton J., Williams D., Human fascioliasis: prevalence and treatment in a rural area of Peru, *Infect. Dis. Rev.* 2 (2000) 42–46.