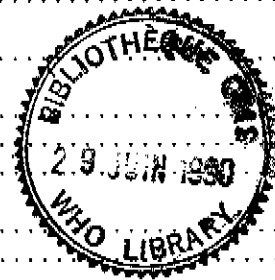




PROGRESS IN ASSESSMENT OF MORBIDITY DUE TO FASCIOLA HEPATICA  
 INFECTION: A REVIEW OF RECENT LITERATURE<sup>1</sup>

CONTENTS

	<u>Page</u>
1. Introduction .....	3
2. Etiology and life cycle of the parasite .....	3
2.1 The adult worms .....	3
2.2 The eggs .....	4
2.3 The miracidia and the intermediate snail hosts, sporocysts, rediae and cercariae .....	4
2.4 The metacercariae .....	4
3. Epidemiological situation in endemic countries .....	5
3.1 Cases reported from different countries during the past two decades ....	5
3.2 Epidemiological characteristics .....	8
4. Pathology .....	9
4.1 Pathogenesis .....	9
4.2 Immunity .....	10
4.3 Disease in different organs .....	10
4.3.1 The liver and biliary tract .....	10
4.3.2 Other lesions .....	11
5. Clinical presentations .....	11
5.1 Incubation period .....	11
5.2 Prepatent period .....	11
5.3 Phases of the infection .....	13
5.3.1 Invasive or acute phase .....	13
5.3.2 Latent phase .....	14
5.3.3 Obstructive phase .....	14



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	<u>Page</u>
5.4 Ectopic fascioliasis .....	15
5.5 Laboratory findings .....	16
5.5.1 Haematology .....	16
5.5.2 Erythrocyte sedimentation rate .....	17
5.5.3 Hepatic functions .....	17
5.5.4 Immunoglobins .....	17
5.6 Non-invasive diagnostic techniques .....	17
5.6.1 Radiology .....	17
5.6.2 Radioisotope scanning .....	18
5.6.3 Ultrasound .....	18
5.6.4 Computed tomography .....	18
5.7 Complications .....	19
5.7.1 Bleeding .....	19
5.7.2 Biliary cirrhosis .....	19
5.8 Causes of death .....	19
6. Diagnosis .....	19
6.1 Parasitological examinations .....	19
6.2 Immunological tests .....	20
6.3 Clinical diagnosis .....	21
7. Treatment .....	22
7.1 Emetine .....	22
7.2 Chloroquine .....	22
7.3 Hexachloro-para-xylol (Hetol) .....	22
7.4 Bithionol (Bitin) .....	23
7.5 Niclofolan .....	23
7.6 Metronidazole .....	23
7.7 Albendazole .....	23
7.8 Triclabendazole .....	24
7.9 Praziquantel .....	24
7.10 Other drugs .....	25
8. Conclusions .....	25
Acknowledgements .....	26
References .....	27
Annex .....	43

## 1. INTRODUCTION

Fasciola hepatica is a common parasite of ruminants, especially sheep, goats and cattle. It is the cause of economic losses in the animal husbandry industry (41, 93). A large variety of other domestic and wild animals may also be infected. In the final hosts, the adult worms of F. hepatica are usually found in the bile ducts of the liver. Juvenile and adult worms of F. hepatica cause destruction of the liver and the bile ducts, and, in heavy infections, death may ensue. The distribution of the parasite is mainly in temperate and subtropical zones. In comparison with animal infections, human infections are uncommon. However, clinical cases have been reported from more than 40 countries in Europe, the Americas, Asia, Africa and the western Pacific. Several epidemics have been recorded in the literature. As the infection may be asymptomatic, and the symptoms and signs are not pathognomonic, the actual number of human cases is undoubtedly much greater than that reported. Although disease sequelae due to F. hepatica infection in man are not as severe as those in sheep and cattle, they may cause considerable morbidity, and, infrequently, death.

This paper reviews the epidemiology and clinical aspects of human F. hepatica infection as reported in publications since 1970.

There are several classic investigations on animal fascioliasis published in English that should be consulted and that form a background to the life history of the parasite as well as to the epidemiology and clinical pathology of the disease as recorded in the current literature on human infection. These are: Dawes and Hughes, 1964 (67); Taylor, 1964 (220); Soulsby, 1965 (214); Lapage, 1968 (142); Boray, 1969 (40); Dawes and Hughes, 1970 (68); and Boray, 1981 (41). Human fascioliasis was reviewed by Facey and Marsden in 1960 (84).

Human disease due to F. gigantica infections has only been reported in comparatively limited geographical areas, mainly in Africa, the western Pacific and Hawaii, and is therefore not included in this review. The pathology and clinical presentation of F. gigantica and F. hepatica infections in man are similar.

## 2. ETIOLOGY AND LIFE CYCLE OF THE PARASITE

Since the classic investigations of Thomas (221) between 1870 and 1881 on F. hepatica infection in experimental and domestic animals, its life cycle is well understood. For the purpose of describing the epidemiological and human morbidity due to F. hepatica infection, a short review on the life history of the fluke is presented. The life cycle consists of 6 phases in the development of the fluke. They are:

- (1) passage of eggs from the host to the outside environment;
- (2) development of the eggs;
- (3) miracidia hatching from the eggs in water to enter intermediate snail hosts;
- (4) development and multiplication of the parasite in the snails;
- (5) emergence of the cercariae from the snails and encystment on aquatic plants;
- (6) ingestion of the infested aquatic plants by the final hosts and development to adult worms in them.

### 2.1 The adult worms

The adult F. hepatica is leaf shaped. When it is fully grown it may reach 30 mm long by 13 mm wide at its broadest section. The adult worms reside in the large biliary

passages and in the gall-bladder. The worm is hermaphroditic, but cross-fertilization between two flukes is thought to be the most common form of sexual reproduction (115).

The egg output of the adult flukes in sheep is relatively high. In two groups of the experimental animals it was shown that the daily egg output per fluke was dependent on the number of the flukes in the liver and varied from 4000 to 50 000, with the average numbers ranging from 8800 to 25 100 during 13 to 19 weeks after infection (40). These levels were much higher than those of an earlier observation by Taylor in 1951 (cited in 142) who estimated that in a lightly infected sheep or bovine the average daily production was 3000-3500 eggs per fluke. Daily egg output per adult fluke is generally inversely proportional to the intensity of the fluke burden. In moderate infections the daily egg output is usually constant. In heavy infections, as assessed by autopsy, the egg output varies considerably.

The life span of the parasite in sheep can be as long as 11 years (67, 142, 212). Cattle are more resistant to the infection and the parasites generally survive between 9 and 12 months (212). In man no reliable estimate of the life span of the fluke has been reported. Dan et al. (65) suggested, on the basis of imported cases from Afghanistan, that *F. hepatica* may survive in man for at least 9 years. These authors cited another report which suggested that the fluke may have survived 13.5 years. Chatterjee (52) estimated that the life span of the adult fluke in man was between 9 and 13 years.

## 2.2 The eggs

Eggs of *F. hepatica* are operculated and measure 130 to 150  $\mu\text{m}$  in length and 63 to 90  $\mu\text{m}$  in breadth with two characteristic yellow colours. They are not readily differentiated from those of *F. gigantica*. *Fasciolopsis* eggs have a clear, thin shell with a delicate operculum and *Echinostoma* eggs are smaller (88-111 x 53-74  $\mu\text{m}$ ) (153). The eggs mature in water. If the climatic conditions are suitable (15-25°C), the miracidia develop and hatch in about 9 days (142) to 21 days (40). If conditions are unfavourable, they may not mature but may remain viable for several months (142).

## 2.3 The miracidia and the intermediate snail hosts, sporocysts, rediae and cercariae

The miracidia hatch and swim rapidly by means of their cilia until they encounter an intermediate snail host. Those failing to penetrate an appropriate snail die within 24 hours (169).

The intermediate snail hosts of the flukes are amphibious. In Europe and some parts of Asia, the intermediate snail host is usually *Lymnaea truncatula* while elsewhere several other species of *Lymnaea* are implicated. Their habitat is along the margins of small pools and ditches, in marshes and in swamps. The development of the larval stages in the snails is inversely proportional to the ambient temperature (40). Development is arrested below 10°C or over 30°C (40, 169).

Upon contact with the snails, the miracidia penetrate and transform into sporocysts. Within each sporocyst mother, rediae form which further subdivide into daughter rediae. Each daughter redia produces many cercariae. The cercariae develop in the snails within 6 to 7 weeks, at 20-25°C. At lower temperatures the development is delayed. A single miracidium can thus give rise to a thousand or more cercariae (142). In the water the cercaria becomes infective upon encysting to transform into a metacercaria. Unlike *Schistosoma* cercariae which actively penetrate the skin of the host, the metacercariae of *Fasciola* can only infect the host after ingestion. A proportion of the larvae of *F. hepatica* die in the gastrointestinal tract of the hosts after being ingested; relatively few eventually develop into adults.

## 2.4 The metacercariae

The cercariae, emerging from the snails, lose their tails upon encystment on aquatic plants. Metacercariae are infective within 24 hours after encystment. They are round and about 0.2 mm in diameter. They can be found on the leaves of water plants above or below the water line. The metacercariae begin to excyst in the small intestine within an

hour after ingestion. After penetrating the wall of the host's intestine, they appear in the abdominal cavity at about 2 hours after ingestion. Within 24 hours after ingestion the majority become immature flukes. At 48 hours they begin to penetrate the liver capsule. Most reach the liver within 6 days after excystment. In the liver they migrate for 5 to 6 weeks, preferentially feeding directly on liver tissue. They eventually penetrate into the bile ducts where they become sexually mature. In sheep and cattle, egg laying begins about 2 months (6 to 13 weeks) after the metacercariae are ingested. This period, in addition to the 2 to 3 weeks required for the maturation of the eggs and 6 to 7 weeks for the development of the cercariae in the snails, brings the cycle from egg to egg to about 14 to 23 weeks (40, 142).

It has been speculated that the immature flukes may enter the blood stream and be carried to various parts of the body, or may reach the liver by travelling up the bile duct. In the case of failure to reach the biliary system of the liver, the immature flukes die in the abdominal cavity and other parts of the body.

### 3. EPIDEMIOLOGICAL SITUATION IN ENDEMIC COUNTRIES

#### 3.1 Cases reported from different countries during the past two decades

Human infections with F. hepatica have been reported from Europe, Latin America, North Africa, Asia and the western Pacific during the past two decades, as shown in Table 1. During this period 2594 persons with F. hepatica infection from 42 countries (areas) were reported: 1103 of the cases were detected by parasitological methods (either by finding the eggs in the stool or bile, or the adult worms on surgical operation or at autopsy); 778 persons were diagnosed by serological tests; 624 persons were diagnosed by parasitological and/or serological methods; and 28 patients were diagnosed after pathohistological examination of liver sections, or by ultrasound showing the adult worms, or again from their clinical presentation. In 61 cases the diagnostic technique was not mentioned in the report of the Ministry of Health of Cuba (174). Obviously, the list in Table 1 has inherent limitations, mainly for two reasons. The first is that different survey methodologies were used. Most publications have described small series of hospital inpatients with clinical symptoms; only a few community-based or epidemiological surveys have detected larger numbers of the infected persons as reported in Peru (216), Egypt (87), France (14, 189), Portugal (198, 199), and Puerto Rico (32). The second reason is that more cases have been diagnosed by serological methods than by finding eggs and/or adult flukes. For example, in papers published from Asian countries, all the infections were identified by parasitological techniques and no community-based survey has been found in this review.

More than half of the human infections were described in European countries, mainly in France, Portugal, Spain, the United Kingdom and the USSR. These data from scientific journals represent about 20% of the cumulative number of cases in reports from national sources.

In addition to the case reports summarized in Table 1, other public health reports also emphasize that France is an important endemic area for F. hepatica (140). The first large modern epidemic of human fascioliasis in France occurred in 1956 (61). Between 1950 and 1983, Gaillet et al. (94) catalogued 3297 cases from published reports: 788 cases from the reports of the Ministry of Health, and, between 1970 and 1982, 4813 cases from 23 hospital laboratories. Most cases were reported from four areas of France: Lyon, Bretagne Nord/Pas de Calais and Sud-Ouest. Wild watercress is the main source of human infection in these areas where fascioliasis in domestic animals is also highly endemic. Other recent reports contain detailed reviews on the situation in Sud-Ouest France, referring to 274 cases (140) and 37 cases (98), respectively. Most cases have been reported from France in part because serological tests have been widely used there, whereas in other countries diagnosis of the infection is mainly based on parasitological examinations.

TABLE 1. CASES OF FASCIOLA HEPATICA INFECTION REPORTED FROM DIFFERENT COUNTRIES (AREAS) DURING THE PAST TWO DECADES

Country (area)	No. of cases	Diagnostic method				References
		Parasitological	Serological	Clinical	Unknown	
Algeria	6	3		3		106,112,158
Egypt	125	125				87,88,89,91,152, 183,197
Morocco	1	1				210
Zimbabwe	1	1				26
Argentina	13	13				50
Brazil	14	14				10,59,72,104
Chile	4	4				16,141,203,204
Cuba	216	117		38	61*	82,83,101,105,159, 174,192
Mexico	5	5				2,127,164
Peru	163	163				135,136,216
Puerto Rico	18	18				31,32,147,190
Uruguay	16	8		8		1
United States of America	1			1		111
China	41	41				75,120,146,149,218, 228,233,244,245
India	1	1				36
Iran	16	16				90,108,133,166
Israel	2	2				65,205
Japan	5	5				7,129,219,240
Saudi Arabia	2	2				38
South Korea	3	3				54,144,172
Thailand	1	1				53
Turkey	8	8				23,79,131,227,241
Yemen	3	3				165,185,206
Austria	4	4				20,139,236
Belgium	3	2		1		64,143
Bulgaria	1	1				222
Czecho- slovakia	2	2				99
Federal Republic of Germany	3	3				150,207,236
France	963	121	562	16	264**	6,11,14,33,39,45, 48,62,73,76,116, 119,130,148,155, 180,181,182,186,188, 189,194,195,196,208, 226,231
France (Corsica)	2	2				69,77
Greece	1	1				17
Ireland	1	1				74

TABLE 1. CASES OF FASCIOLA HEPATICA INFECTION REPORTED FROM DIFFERENT COUNTRIES (AREAS) DURING THE PAST TWO DECADES (continued)

Country (area)	No. of cases	Diagnostic method				References
		Parasitological	Serological	Clinical	Unknown	
Italy	5	5				25,83,225
Poland	16	15	1			24,134,177,215,230
Portugal	538	121	97		320***	51,193,198,199,200
Spain	142	94	47	1		3,4,5,8,9,15,21,22,46,47,58,60,71,92,95,97,113,156,157,161,162,163,167,170,171,179,224,242
Sweden	2	1	1			85
Switzerland	13	12	1			13,100,154,209,217
United Kingdom	93	25	28		40**	18,19,37,80,109,121,126
Union of Soviet Socialist Republics	131	131				27,132,184
Yugoslavia	1		1			43
Australia	8	8				63,102,151,237,238
Total	2 594	1 103	778	28	685	

\* Data from a report of the Ministry of Health, method not mentioned.

\*\* By parasitological and/or serological tests.

\*\*\* 108 cases by parasitological and/or serological tests, 212 cases by serological and/or clinical diagnosis.

Northern Portugal is another endemic area. In addition to the case reports summarized in Table 1, a total of 561 persons have been diagnosed with F. hepatica infection in the National Laboratory in Porto between 1970 and 1985. Furthermore, the only reports of surveys of entire populations have been from this laboratory (see Annex). Cases reported by laboratories in Portugal included those in residents from two islands, Madeira and Cape Verde (193).

The epidemiology of animal F. hepatica and its geographical distribution in Switzerland was investigated and it seems that the veterinary disease is quite common, especially in the northern part of that country (78). However, human infection has only been occasionally reported (13, 100, 154, 209, 217).

Most of the reported cases (130 out of 131) in the Soviet Union were from its southern, Asian republic, Tadzhik, near the Afghanistan-Soviet border (128, 132, 184).

Several severe outbreaks of human F. hepatica infection were recorded in Hungary between 1959 and 1970, usually after heavy summer rainfall (137). No recent report on fascioliasis is available from that country.

In the Americas, Cuba (82, 83, 101, 105, 159, 174, 192) and Peru (135, 136, 216), respectively, have each reported more than 100 cases.

In Africa, most cases have been reported from Egypt (probably due to F. gigantica since it is the only species in domestic animals in that country) (87, 88, 89, 91, 152, 183, 197).

Fewer cases have been described in Asia, including China (75, 120, 146, 149, 218, 228, 233, 244, 245) and Iran (90, 108, 133, 166). In most case reports from Korea and Japan the authors gave the name of Fasciola spp., because it was not possible to identify the species accurately (7, 54, 129, 144, 187, 240). Furthermore, although the distribution of F. hepatica is considered not as wide as that of F. gigantica, both species are present in these two countries (56, 187).

Although Australia is one of the world leaders in livestock production, the temperature is much more favourable for the development of F. hepatica than in European countries, and the prevalences of F. hepatica in sheep and cattle are high (40); human infections have been infrequently reported (63, 102, 151, 237, 238). No human infection with this fluke was found in our review of the literature from New Zealand, with its flourishing animal husbandry and prevalent animal fascioliasis.

### 3.2 Epidemiological characteristics

The occurrence of human F. hepatica infection is determined by the presence of the intermediate snail hosts, herbivorous animals and dietary habits of man.

Climatic conditions are critical for the development of both the Lymnaea snails and the flukes. The snails are more resistant to low temperature compared with high temperature. They can survive through the winter although there is little or no development and multiplication (40). On the other hand, persistent high temperatures and dry conditions adversely influence snail populations. High temperature also impairs the development of the larval stages of the fluke. The metacercariae may survive for long periods at low temperatures if the level of moisture is sufficient. However, they are susceptible to desiccation and to temperatures over 25°C (40). In areas with high temperatures and low humidity like sub-Saharan Africa, few cases of F. hepatica infection have been reported. In contrast, high humidity associated with heavy rainfall and moderate temperatures may herald hyperendemicity of F. hepatica infection in herbivorous animals. Accordingly, human infection has been more frequently observed in the years with heavy rainfall in France (189).

Animal reservoirs, mainly sheep, goat and cattle, promote transmission to man. There is no evidence that sheep or goats acquire immunity against F. hepatica, whereas cattle are resistant to challenge after initial infections. The duration of egg production in cattle is short and high egg output in cattle lasts for only a few weeks (40). Most of the flukes in cattle are eliminated within 9-12 months (40, 67, 212). Thus, sheep play a more important role in contamination of the pastures and in human transmission. A large variety of domestic and wild animals as well as laboratory animals can be infected with F. hepatica, but they are usually not very important for transmission of the human disease.

Human dietary habits are related to the occurrence of the disease. Watercress and other aquatic vegetables serve as vehicles of the infection. The habit of eating raw watercress and other vegetables causes the metacercariae to enter the human alimentary tract. In some countries, such as China, where vegetables are always cooked for eating, infection may occur, though rarely, by ingestion of unboiled drinking water, or from the metacercariae on cutting boards and other kitchen utensils.

Other epidemiological characteristics are as follows.

(1) Seasonal incidence: Although human infections occur nearly throughout the year, most clinical cases have been observed during the third (89) or last quarters (189), or cooler seasons, of the year.

(2) Familial clustering: The incidence of infection is significantly aggregated within family groups because the family shares the same contaminated food (96). In a community-based survey in Egypt, Farag et al. (87) found that among 25 families with at least one infected person, 20% had 2 members infected and another 20% had 3 members infected.

(3) Professions: Fascioliasis is predominately a rural disease. Sheep- or cattle-herders were more frequently infected than those in other professions (216).

(4) Age and sex distributions: All age groups can be affected. However, those less than 5 years of age had the lowest prevalence as reported by Garcia-Rodriguez et al. (96). Distribution by sex was very similar in Spain (96), but in Egypt a higher prevalence was observed in women (10.3%) than in men (4.4%) (87).

(5) Sporadic infections: The scientific literature is mainly individual case reports. A large single outbreak of fascioliasis including 44 cases in one year in Monmouthshire, Britain, was reported by Hardman et al. (109). However, in community-based surveys in areas for *F. hepatica*, larger numbers of infected persons have been identified by stool examinations combined with serological tests (87, 216, Annex). The symptoms were not pathognomonic nor were they severe enough for most persons to seek medical attention.

#### 4. PATHOLOGY

Relatively little information is available on the pathology of fatal fascioliasis (1, 75) since death rarely occurs. However, the histopathology of surgical specimens and laparoscopic examination results have been reported by many investigators from different countries (1, 23, 26, 53, 74, 75, 102, 126, 172, 175, 224).

In contrast, the literature on the experimental pathology of fascioliasis is extensive and has contributed considerably to our understanding of the disease. This information, with the few publications on the pathology of the human disease, are reviewed below.

##### 4.1 Pathogenesis

The degree of pathological change depends primarily on the number of flukes that penetrate the wall of the small intestine and invade the liver. In animals the mortality rate is inversely proportional to the number of flukes in the liver. Penetration of the wall of the duodenum or jejunum by the metacercariae may cause focal haemorrhage and inflammation by the lesions are usually not clinically evident. The major pathological changes are seen during the migration of the immature flukes through the liver parenchyma for 4-6 weeks or longer before they enter the biliary tree. During their period of migration, the immature flukes digest hepatic tissue and cause extensive destruction of the liver parenchyma with intensive haemorrhagic lesions as well as immunological and inflammatory reactions. The tracks of migration may be observed in histological sections of the liver and other organs. The immature flukes are sometimes trapped in the organ and die leaving cavities filled with necrotic debris. When these heal, considerable areas of the liver may be replaced by scar tissue (212). In man, a small proportion of the flukes may reach the bile ducts where they live for months to years. When they are in the bile ducts, less pathogenic effects to the liver can be found, although inflammation of the bile ducts resulting in fibrosis, thickness and expansion is common.

In infected rats Isseroff et al. (122) observed that extensive hyperplasia of the main bile ducts occurs resulting in enlargement (up to 20 times) of the ducts. Hyperplasia of the bile ducts could also be provoked by infusion of proline into the abdominal cavity of uninfected rats. The authors suggested that *F. hepatica*, which synthesizes and releases large amounts of proline, induces enlargement of the bile ducts with a similar mechanism and that proline is the mediator of the hyperplastic response in *F. hepatica* infection.

Both in animal and human infections, anaemia is one of the most characteristic symptoms, especially in the heavier infections. The pathogenesis of the anaemia has been disputed and has been reviewed comprehensively by Boray (40) and Dawes and Hughes (68). The results of the investigations up to date can be summarized as follows: (1) the fluke is a blood feeder but may also feed on tissue; (2) haemorrhage may occur from the erosion of the biliary epithelium due to the infection; (3) there is no evidence of plasma iron or vitamin B<sub>12</sub> deficiencies; (4) reticulocytes are increased in the peripheral blood and the half-life of erythrocytes in the infected animal is shortened; (5) generalized haemolysis is absent. Blood loss into the bile seems, most probably, to be an important, if not the only, factor contributing to severe anaemia.

#### 4.2 Immunity

Immunity to reinfection after initial exposure to the metacercariae differs greatly from host to host. Dogs and cats may have a natural resistance to infection due to an early tissue reaction by these hosts which eliminates the flukes (40) whereas in rats, guinea pigs, rabbits and cattle, resistance is acquired during the primary infection. Although the disease is self-limiting in this latter group, severe hepatic lesions and high mortality may occur, particularly in young or debilitated animals. Low or no resistance is seen in sheep, goats, hamsters (J. C. Boray, personal communication), and mice and the infection is highly pathogenic in both the acute and chronic phases. In heavy infections, death is a usual sequela (40, 212). Studies on immunity to the infection in man are limited. However, it is generally believed that man is not a suitable host of F. hepatica and the most immature migrating flukes become trapped in the liver parenchyma and die without reaching the bile ducts (1). Considerable tissue reaction and calcification of the bile passages due to the flukes have been recorded (1) and spontaneous cure of the infection, as seen in cattle, is not uncommon (40).

Cross-resistance in mice between Schistosoma mansoni and F. hepatica infections was investigated. Whereas simultaneous infection with the two trematodes induced a statistically significant reduction in the number of schistosomes, no reduction of F. hepatica worm burden was observed (55). In another study, sheep with a primary infection of S. mansoni were found to show resistance to challenge with F. hepatica (110).

The mechanism of the immunity, i.e., cell- and/or antibody-mediated response to the flukes, varies from host to host, and in the same host, according to the phase of the infection (168). In mice, delayed hypersensitivity may play a major role in the immunity acquired during the primary infection (68) causing an earlier migration of the flukes to the common bile ducts whereas, in cattle, this mechanism may not have an important role (168). Although high antibody levels against the adult flukes can be found in experimentally infected sheep (232), there was no evidence of acquired resistance against F. hepatica in sheep whose chronic infection had been cured by treatment with an anthelmintic. After the secondary challenge the worm burden was similar to that of the primary infection, however, the pathological changes in the liver and biliary ducts were less serious (40).

#### 4.3 Disease in different organs

##### 4.3.1 The liver and biliary tract

Disease due to F. hepatica is chiefly confined to the liver. The most important pathogenic sequelae are hepatic lesions and fibrosis, and chronic inflammation of the bile ducts. Unlike clonorchiasis or opisthorchiasis, there have been no reported associations of fascioliasis hepatica with biliary carcinoma.

Human pathological studies were carried out by Acosta-Ferreira et al. (1) on 15 surgical specimens and one autopsy in Uruguay, by Uribarrena et al. (224) and Moretó et al. (163) from laparoscopic examinations in 8 and 18 patients, respectively, in Spain, and by Duan et al. (75) and Zhu et al. (245) in China from laparotomy in 4 patients and 2 autopsies. In all the above patients, F. hepatica adult worms were found. The livers were usually enlarged with a smooth or uneven surface. The most common macroscopic

lesions were multiple soft, yellowish or grey-white nodules ranging from 2 to 30 mm (mainly 5-20 mm) in diameter; 1-4 mm in diameter in the series reported by Uribarrena et al., or 3-4 mm in that reported by Moret6 et al. Microscopically these were found to be eosinophilic abscesses. At times they were confused with hepatic metastases. Nodules were also observed in the parietal peritoneum proximal to the liver and on the round ligament of the liver. Haemorrhagic stippling appeared at the margin of the nodules. White or yellow striae measuring 0.5 x 5-10 cm, surrounded by telangiectasia, were observed on the liver capsule (163). Close to the nodules, ribbed or vermiform formations with a colour and consistency similar to those of the nodules were observed in 5 out of 8 patients under laparoscopy (224). Hepatic capsular thickening of varying degree was observed in 7 out of 18 cases, and in a few cases the entire hepatic capsule was thickened (163). Subcapsular lymphatic vessels were dilated (163, 234). The lymph nodes near the porta hepatis may be markedly enlarged (165). In one patient who died, multiple subcapsular cavities filled with necrotic material, 5-10 mm in diameter, were observed below the liver capsule. Several reddish-purple tracks radiated from the nodules, whereas the others were greyish-white and fibrous. The tracks extended from the liver capsules and ended in subcapsular cavities. Most of the lesions were less than 20 mm below the capsules (1). In 4 cases with marked involvement of the peritoneal wall and the liver surfaces, yellow and opalescent ascites were present (163). On a single section of the liver 7 adult flukes were observed in dilated intrahepatic bile ducts by Acosta-Ferreira et al. (1) and more than 40 living flukes (the stage of development was not mentioned) were found in the liver of a patient also with ascites by Zhu et al. (245). Apart from mild splenomegaly in 2 patients, no significant portal hypertension was found in 18 cases upon laparoscopic examination (163).

The common bile ducts were usually large and dilated and the wall was thickened on palpation (26, 74, 75, 244). The gall-bladder wall was greatly thickened and oedematous (1, 26, 75, 102). Multiple, greyish-white subserous nodules were present and adhesions of the gall-bladder to adjacent structures were common (1). The mucosal folds of the gall-bladder were prominent (102). The wall of the gall-bladder was thickened owing to muscular hypertrophy and perimuscular fibrosis. There was glandular epithelial hyperplasia. All layers of the wall contained patchy infiltrates with lymphocytes, plasma cells and eosinophils (1, 126). Lithiasis, often multiple, in the common bile ducts and gall-bladders was very frequent. Living adult flukes and their eggs were often found within the bile duct and gall-bladder.

The microscopic changes may be specific or non-specific. The presence of the fluke in the liver parenchyma is of diagnostic value but is uncommon. Generally, the migration tracks can be found in the liver and other organs. The walls of the tracks in the liver often contain Charcot-Leyden crystals and eosinophils. The cavities of the tracks are filled with necrotic cellular debris, including hepatocytes, fibrin and red cells. A considerable eosinophilic infiltrate surrounds the tracks. Longer tracks can cross several hepatic lobules. The older lesions, macrophages, lymphocytes, eosinophils and fibrous tissue were observed. Focal calcification was sometimes seen in the margin of the necrotic debris. Calcification may form the outline of a dead fluke (1).

F. hepatica egg granulomas have been described (1, 102, 126). Multinuclear giant cells surround a single egg with subsequent layers of epithelioid cells, and fibrous tissue, plasma cells, lymphocytes and eosinophils. The portal triads are dilated and oedematous with infiltrates of lymphocytes and eosinophils. Bile duct proliferation, periductal fibrosis, necrotizing arterial vasculitis and portal venous thrombosis are frequent.

#### 4.3.2 Other lesions

Ectopic localization of F. hepatica occurs during the migratory phase. Among ectopic lesions, those of the gastrointestinal tract are most frequent.

Yellowish-white subserosal gastric nodules, 3-5 mm in diameter, were shown at autopsy (1). These nodules were granulomas putatively caused by F. hepatica as suggested by migratory tracts in the pyloric area of the stomach and the presence of adult flukes in the liver.

Surgical recovery of an F. hepatica adult worm from the pancreas of a Thai woman was reported by Chitchang et al. (53). At the time of the operation a firm 5 cm mass in the head of the pancreas was observed. On cut section, it was whitish-green and fibrotic with areas of multiple cystic degeneration. Microscopically, a fluke identified as F. hepatica was found in the mass with marked chronic inflammation, eosinophilic infiltrates and fibrosis surrounding the fluke and egg granulomas. Cholelithiasis of the gall-bladder and chronic liver lesions were also observed.

A caecal mass was surgically resected from a Korean woman by Park et al. (172). The mass was soft, 5 x 4 x 4 cm in size with several round to oval cystic cavities containing a dark brown exudate. These cavities were associated with the migratory tracts of a fluke. A Fasciola sp. fluke was found in a U-shaped tract which extended from the serosal surface to the submucosa of the caecum. Histological changes were typical of those of fascioliasis in other organs with granulomatous responses to the eggs.

Occasionally immature flukes may enter the blood stream and disseminate to other organs. In cattle F. hepatica is frequently observed in the lungs (214). Live flukes recovered from organs other than the liver in cattle or sheep were always stunted and their length was only 1-2.5 mm.

In man, other ectopic lesions were in: abdominal wall (222), spleen (233), subcutaneous tissue (4, 96, 172), blood vessels, the lung and pleural cavity (96, 172), brain, orbit (96), skeletal muscle, appendix (172) and epididymis (4). The flukes, in these circumstances, never achieve maturity. They may be calcified or become incorporated in a granuloma (84).

## 5. CLINICAL PRESENTATIONS

### 5.1 Incubation period

The period between the ingestion of the metacercariae into the gastrointestinal tract and the appearance of the first symptoms varies considerably depending on the number of the metacercariae ingested and the host's response. The period of incubation in man has not yet been accurately determined as eating raw vegetables or drinking contaminated water may occur repeatedly over a few days, weeks or even months. Furthermore, in light and chronic infections, the symptoms in man may be vague or absent. The incubation period has been estimated only in those with significant illness during the invasive phase of the infection.

In the Haute-Loire department of France, an outbreak of F. hepatica infection occurred in a closed community of 79 persons of whom 68 had eaten wild watercress over a period of 15 days. Within 6 weeks the first clinical symptoms appeared. The peak incidence of cases diagnosed occurred two to two and a half months after ingestion. Of the 68 persons who had a history of eating watercress, 54 (79.4%) were serologically positive whereas only 3 of the 68 persons had F. hepatica eggs in the stool which were detected during hospitalization of the patients (188).

The initial symptoms are fever, sweating, abdominal pain and urticaria. A 10-year-old Egyptian boy suffered from fever, shivering and sweating a few days after being rescued from drowning in a pond. Thereafter he developed typical symptoms as seen in the acute phase of F. hepatica infection and the eggs were found 4 months after this event when he was in hospital (183). The estimated incubation period in this boy was only "a few days". In the outbreak involving 44 cases of F. hepatica infection in Chepstow, United Kingdom, all the patients had histories of having eaten wild watercress during September-October, and their symptoms appeared some 2-3 months after eating the watercress (109).

### 5.2 Prepatent period

The period between ingestion of metacercariae and the presence of eggs in the faeces varies according to the host, and also depends on the number of adult flukes in the

liver. The greater the number of flukes invading the liver, the longer the time required for the juvenile flukes to mature in the bile ducts and to initiate egg laying (40). In mice, the first eggs appeared 35 to 42 days after ingestion; in guinea pigs, the prepatent period was 55 days. In sheep infected with 200 metacercariae, the prepatent period was 63 days, whereas in those with heavy infections (i.e., infected with 2000 metacercariae) eggs appeared 13-15 weeks after ingestion. In cattle, the prepatent periods varied between 56 and 61 days according to the age of the cattle (40, 70).

In man, a period of at least 3 to 4 months is necessary for the flukes to attain sexual maturity (84, 233).

### 5.3 Phases of the infection

Generally, there are three phases: invasive or acute phase, latent phase, and obstructive or chronic phase (84, 151). In some cases, the acute phase may be severe, but more commonly the acute phase passes without significant symptoms and infection is detected in routine faecal or serological examinations.

#### 5.3.1 Invasive or acute phase

This phase coincides with the period of time during which the immature flukes migrate through the peritoneal cavity, reach the liver by penetrating the liver capsule and then penetrate the hepatic parenchyma until they become mature and reach the bile ducts. The symptomatology of the acute disease is due mainly to the mechanical destruction of the liver tissue and of the abdominal peritoneum by the migrating larvae causing localized or generalized toxic and allergic reactions (84, 233) lasting 2 to 4 months. However, in endemic areas, the infection with F. hepatica is usually repetitive and the acute lesions are superimposed on chronic disease. Thus, the acute phase may be prolonged and overlap onto a latent or an obstructive phase.

The major clinical symptoms of the acute phase are fever, abdominal pain, gastrointestinal disturbances and urticaria.

(1) Fever: The onset of fever is usually the first symptom and is often abrupt, accompanied by shivering and sweating. The temperature is usually low or moderate but may reach 40°C, and in heavily infected cases as high as 42°C (245). In most cases pyrexia is remittent, intermittent or irregular with higher temperature in the evening. In some cases, a low, recurrent fever lasts for a long time (4 to 18 months) (183).

(2) Abdominal pain: The degree of the pain varies from mild to excruciating. Abdominal pain may be generalized at the outset, but usually becomes localized in the right hypochondrium or below the xyphoid. In some cases, vague abdominal discomfort is the only complaint.

(3) Gastrointestinal disturbances: Loss of appetite, abdominal flatulence, nausea and diarrhoea are common, whereas vomiting and constipation are infrequent.

(4) Urticaria: This manifestation, with dermatographia, is a distinctive feature in the early stage of invasion by the fluke (81, 109, 192). Out of 34 infected persons, 7 (20.6%) had urticaria, in either the acute or the chronic form, as reported by Rodriguez Barreras et al. (192) from Cuba. Repeated attacks of urticaria have occurred at intervals of 2-4 days during two and a half months of infection (108). The urticaria may be accompanied by bouts of bronchial asthma that are refractory to ordinary treatment (6) and that cease after specific treatment for F. hepatica infection (6, 108).

(5) Respiratory symptoms: Non-productive cough is common. Chest pain occurs occasionally (9, 17, 92, 228, 245).

On physical examination, the following signs may appear.

(1) Hepatomegaly and splenomegaly: The liver is usually enlarged and tender, sometimes reaching down to the right iliac fossa (84). The degree of hepatomegaly seems to increase during the course of the disease. Hepatic abscess was identified by ultrasound in 3 cases with the infection; these lesions regressed after treatment with dehydroemetine (130). In the acute phase, the liver is never hard. Splenomegaly is not common and has been reported in 12.5% (2 out of 16 cases) (183) and 25% (17, 84) of infected cases by different authors.

(2) Ascites: During the course of the acute phase, ascites has been reported in 14 infected persons (75, 141, 163, 218, 245). The ascites was yellow with a high leukocyte count: in 4 cases reported by Zhu et al. (245) the leukocyte counts were all over 1000/mm<sup>3</sup>, the highest being 6850/mm<sup>3</sup>. Eosinophils predominated (in 1 case they accounted for 92% of the total cells). The pathogenesis of the ascites was considered to be an inflammatory response to a large number of juvenile flukes penetrating the intestinal walls, irritating the peritoneum and penetrating through the liver capsule during their migration rather than to hepatic failure per se (245).

(3) Anaemia: Mild to moderate anaemia can be seen in the acute phase of the disease. Pallor of the skin and mucosa is commonly associated with lassitude, dizziness, palpitation and weakness (183, 228, 245).

(4) Chest signs: On auscultation, dry or moist rales can occasionally be elicited upon coughing at the base of the right lung probably due to migration of the juvenile flukes. Pleural rub with effusion and even spontaneous pneumothorax have been reported (245). A large pleural effusion due to the infection without any parenchymal pulmonary abnormality associated with increasing dyspnea on exertion was observed in another case (76). Pyopneumothorax was recorded in a 73-year-old patient due to F. hepatica infection (226). Pulmonary infiltrates on chest X-ray film in the acute phase of the disease disappeared after dehydroemetine treatment (92). Aliaga et al. (9) suggested that a "flecting right lung infiltrate" observed in their patient was probably due to dead immature flukes and/or to a hypersensitivity provoked by the flukes.

(5) Jaundice: Jaundice is infrequent in the acute phase of the infection; if the jaundice appears it is milder than that seen in the obstructive phase (109, 183, 245).

### 5.3.2 Latent phase

When the juvenile flukes become mature and enter the bile ducts and initiate oviposition, the latent phase begins. This phase can last for months or years. The proportion of asymptomatic persons in this phase is unknown. Diagnosis of infection may be confirmed after clinical suspicion or in epidemiological surveys (109) by finding the eggs in the duodenal fluid and/or in the stool. An unexplained, prominent eosinophilia may suggest a helminthic infection. These persons may have gastrointestinal complaints or one or more relapses of the acute symptoms during this phase (84, 151).

### 5.3.3 Obstructive phase

Adult flukes in the bile ducts cause inflammation and hyperplasia of the epithelium. Thickening and dilation of the ducts and the gall-bladder walls ensue. The resulting cholangitis and cholecystitis, combined with the large body of the flukes, are sufficient to cause mechanical obstruction of the biliary duct which is comparatively small in diameter.

Although biliary obstruction and inflammation due to F. hepatica have been reported in many cases from different parts of the world (1, 29, 31, 53, 54, 58, 60, 65, 74, 95, 102, 105, 111, 127, 129, 133, 146, 151, 165, 171, 177, 178, 179, 206, 225, 244, 245), the proportion of those whose infections develop into the obstructive phase and their prognosis have not been defined.

The clinical manifestations in this phase, such as biliary colic, epigastric pain, fatty food intolerance, nausea, jaundice, pruritus, right upper-quadrant abdominal tenderness, etc., are indistinguishable from cholangitis, cholecystitis, and cholelithiasis of origins other than *F. hepatica* infection. In chronic infection of long duration, the liver is usually enlarged with slight tenderness on palpation, but generally not hard. Unusually large hepatic enlargement with *F. hepatica* infection has been reported in China (218) and in the United States of America (111). Hepatic enlargement may be associated with an enlarged spleen or ascites (1, 75, 218).

During surgical exploration the common bile ducts are usually seen as distended and thickened. The diameters of the common bile ducts have been recorded as 1.5 cm (31, 244), 2 cm (65, 151) and 3 cm (146), which are 1.5-3.0 times normal size. The most frequent site of obstruction is the common bile duct (133). A diverticulum in the common bile duct was found during a surgical exploration in a patient with *F. hepatica* infection with a long history of angiocholitis and a recent attack of acute pancreatitis. The head of the pancreas was enlarged and firm. After dehydroemetine treatment the symptoms vanished (155).

If obstruction is present, the gall-bladder is usually enlarged and oedematous with thickening of the wall. With *F. hepatica* infection a gall-bladder measuring 12 x 7 x 7 cm was reported (244) and in another patient the lower edge of the gall-bladder reached the umbilicus (146). Fibrous adhesions of the gall-bladder to adjacent organs are common. Lithiasis of the bile duct or the gall-bladder is frequent (1, 31, 53, 54, 60, 65, 74, 179, 196, 203, 206, 210, 225). While stones may be single (206), usually they are small and multiple. In *F. hepatica* infection and cholelithiasis the bile duct and the gall-bladder may contain blood mixed with bile (haemobilia), blood clots and fibrinous plugs (2, 26, 102, 146).

The diagnosis has usually been confirmed at laparotomy by the finding of flukes in the common bile duct or in the gall-bladder, commonly associated with cholangitis and cholelithiasis. With proper clinical management - removal of the obstruction and temporary biliary drainage - the prognosis is good. Up to 12 flukes (26, 31, 60, 62, 65, 244) have been removed at laparotomy with clinical recovery. In one unusual case, during and after bithionol treatment, 19 dead flukes were found in T-tube drainage from the common bile duct (244).

#### 5.4 Ectopic fascioliasis

If the juvenile flukes deviate during normal migration to the liver they may enter other organs and cause ectopic fascioliasis. The usual pathological effects of ectopic lesions are due to the migratory tracks causing tissue damage with inflammation and fibrosis (see also 4.3.2).

Gastric fascioliasis has been confirmed by examination of surgical specimens following partial gastrectomy. In one case, although the clinical diagnosis of peptic ulcer of the stomach was confirmed at surgery, migratory tracks in the gastric wall associated with hepatic lesions typical of *F. hepatica* infection were observed. No causal relationship between peptic ulcer and *F. hepatica* infection was suggested by the authors (1). Another case of fascioliasis was initially diagnosed as acute cholecystitis. The surgical and pathological findings revealed many migratory tracks caused by the flukes in the gastric wall, as well as in the gall-bladder and in the hepatic parenchyma (1).

Similar migratory tracks and an adult *F. hepatica* were found in the head of the pancreas in a patient, associated with chronic cholelithiasis. Marked chronic inflammation, eosinophilic reaction and fibrosis surrounding the fluke and the eggs were seen (53).

At surgery, a mass in the right iliac fossa which was pre-operatively suggestive of malignancy was found to be a caecal mass surrounding dead *F. hepatica* flukes and numerous abscess-like cavities corresponding to the migratory tracks of the flukes (172). Another mass involving the caecum and ascending colon showing multifocal migratory tracks and a

juvenile worm of Fasciola species was reported in a Korean woman. In this case, colonic lymphoma had been the pre-operative diagnosis which led to an exploratory laparotomy (144).

Roentgenographic pulmonary infiltrates have been suggested to be related to the invasion of the immature flukes (9, 92). Although pulmonary infiltrates (9, 92), pleural effusion (8, 76) and pyopneumothorax (226) in different case reports seem to have been etiologically related to F. hepatica, as indicated by the excellent clinical responses after treatment for the infection, there was no histopathological confirmation.

A causal relationship between endomyocardial fibrosis and F. hepatica infection has been suggested by Potier et al. (181, 182). The authors reviewed 5 published cases of the association between the two diseases and reported 3 new cases. In one case, mitral incompetence was due to biventricular endomyocardial fibrosis (181). In the other 2 cases, the main changes were biventricular endocardial fibrosis and cardiomyopathy with atrial endocardial fibrosis in the left ventricle, respectively (182). All three cases had parasitological (1 case) and/or serological (3 cases) evidence of F. hepatica infections. The pathogenesis of the cardiac changes was suggested to be immunological in nature.

Generalized lymphadenopathy related to F. hepatica infection with recovery following treatment with dehydroemetine has been reported (208).

Subcutaneous nodules and hydrocele due to epididymitis were reported in association with F. hepatica infection. The lesions and symptoms disappeared after dehydroemetine treatment (4).

Cerebral symptoms or signs in association with F. hepatica infections have been rarely reported. In the acute phase, right hemiplegia ensued in a boy after recovery from generalized grand mal seizure. The F. hepatica infection was treated with emetine hydrochloride and, apart from general improvement, the hemiplegia in the boy regressed remarkably (183). In another child in this series, treatment with emetine hydrochloride relieved all symptoms but pathological obesity appeared and the authors suggested the obesity was probably due to a hypothalamic lesion caused by the fluke (183).

Polyradiculoneuritis and encephalopathy (febrile coma) developed in the course of F. hepatica infection with urticaria and asthma. The disease regressed spontaneously and the authors suggested that these symptoms were related to an immunological response to the infection (6). In another case report the initial clinical presentation included facial paraesthesia and paresis with generalized convulsions, hepatomegaly and a high eosinophil count in the cerebrospinal fluid. Complete clinical recovery occurred following dehydroemetine treatment (4). A French woman with F. hepatica infection showing the eggs in duodenal drainage and a positive serological test was admitted to hospital because of paraplegia and temporary diplopia. On physical examination a positive bilateral Babinski's sign was shown. After treatment with dehydroemetine and prednisone, all the symptoms and signs disappeared (73).

## 5.5 Laboratory findings

The outstanding abnormal laboratory finding in all phases of F. hepatica infection is eosinophilia, accompanied by leukocytosis, especially in the acute phase. Anaemia is common, but usually not very severe. The erythrocyte sedimentation rate (ESR) may be high in the acute phase. Abnormal liver function tests may be seen both in the acute and in the obstructive phases, but high serum bilirubin levels are associated with the obstructive phase.

### 5.5.1 Haematology

(1) Leukocytosis and eosinophilia: In F. hepatica infection, especially in the acute phase, the leukocyte counts are usually over  $10\ 000/\text{mm}^3$  up to  $43\ 000/\text{mm}^3$ . The eosinophil count is nearly always greater than 5% of the total leukocytes and may be as

high as 79% (2, 8, 17, 18, 23, 26, 31, 37, 46, 73, 75, 102, 108, 126, 133, 146, 151, 161, 206, 218, 228, 244, 245). In an outbreak in Monmouthshire in Britain (109), most infected persons had eosinophilia greater than 5%, the highest being 83%.

(2) Anaemia: Mild to moderate anaemia between 7.0 g and 11.0 g/dl haemoglobin had been described in about half of the infected persons. In individual cases, haemoglobin levels as low as 2.8 g/dl (218) and 4.0 g/dl (146) have been reported. The decrease of the concentration of erythrocytes in the peripheral blood was proportional to their haemoglobin levels. The anaemias in experimental *F. hepatica* infections were normocytic and normochromic, but erythropoiesis was suppressed (67).

#### 5.5.2 Erythrocyte sedimentation rate

Generally, the ESR was higher in the acute phase, normal in the latent phase and normal or only moderately high in the obstructive phase (96, 109, 183). The ESR may reach 165 mm in an hour in the acute phase (75).

#### 5.5.3 Hepatic functions

(1) Acute phase: The data on hepatic function in the acute phase of the infection have been inconsistent. In two series of patients (75, 109), abnormal liver function tests were reported, including elevated serum glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), thymol turbidity, zinc sulfate turbidity, and serum globulin. In individual patients, elevated serum bilirubin has been reported (63, 228). The most recent report of 16 patients with fascioliasis, of whom the majority were in the acute phase, showed that liver function tests were usually normal with the exception of alkaline phosphatase (AKP). Serum electrophoresis showed an increase of  $\alpha_2$  and gamma globulins (17).

(2) Obstructive phase: Jaundice is a prominent feature in this phase. Serum bilirubin was often slightly elevated to 2-3 mg/dl (133, 146), higher levels of 7.5-8.6 mg/dl were also reported (102, 126, 151). Biliary colic was usually followed by a higher level of serum bilirubin as well as dark urine positive for bilirubin (151). Serum bilirubin may be normal in the obstructive phase and between attacks of biliary colic (31, 74, 206). AKP, GPT, GOT and serum globulin (mainly gamma globulin) were often elevated in this phase (26, 31, 126, 133, 151, 206), while albumin was decreased (126, 133).

#### 5.5.4 Immunoglobulins

Serum immunoglobulin (Ig) levels for IgG, IgM and IgE are usually elevated in *F. hepatica* infections (126, 197, 200). Specific IgE antibodies were detected in 48% of the patients. Moreover, total and specific IgE levels have been shown to be positively correlated with the egg burden, age, clinical features and degree of eosinophilia (200). IgA levels are usually normal (126, 200).

#### 5.6 Non-invasive diagnostic techniques

##### 5.6.1 Radiology

Fascioliasis has been diagnosed by abdominal and chest X-ray examination; oral, percutaneous and intravenous cholangiography; as well as endoscopic retrograde cholangio-pancreatography (ERCP) (9, 31, 45, 58, 74, 111, 113, 127, 155, 171, 177, 204, 225, 238). However, the findings are not pathognomonic of *F. hepatica* infection. Dilated and sacculated bile ducts (74), multiple filling defects consistent with calculi in the bile duct and/or in the gall-bladder (31, 64, 111) and multiple areas of alternating narrowing and fusiform dilation in the intrahepatic radicals (111) have been shown by different types of cholangiography. Inside a dilated common duct ovoid radiolucent images (about 1 x 3 cm) suggestive of adult flukes have been observed by percutaneous cholangiography (58). Typical radiolucent shadows of *F. hepatica* in the common bile duct were shown by ERCP (204) and by cholangiography (225).

### 5.6.2 Radioisotope scanning

Radioisotope liver scan may be useful in the diagnosis of fascioliasis (3, 5, 95, 96, 156, 190, 238); patterns observed however are not specific. Aguirre Errasti et al. (3) reported a radiocolloid demonstration of the presence of cold areas in the liver in 18 out of 23 cases (78%) with F. hepatica infection. Among them, 13 showed positive uptake with  $^{67}\text{Ga}$  in the cold areas in a radiocolloid scan. None of the patients with a  $^{67}\text{Ga}$ -positive scan had a normal radiocolloid scan. F. hepatica infection is one of the causes of "cold areas" in traditional liver scan and of positive  $^{67}\text{Ga}$  uptake. Similar scintigraphic images using radiocolloid were reported by Rivera and Bermúdez (190) in 4 persons with fascioliasis in whom the differential diagnosis had included liver metastatic cancer, hydatid disease or another parasitic infection. Increased liver uptake of  $^{67}\text{Ga}$  was observed in 2 patients and focal defects were demonstrated by Tc-99m sulfur colloid in 2 out of 4 patients as well as hepatomegaly (4 cases) and splenomegaly (3 cases). Filling defects in the right lobe and in the porta hepatitis area on liver scan have also been observed.

### 5.6.3 Ultrasound

Ultrasound has proved useful in the diagnosis of the pathological lesions secondary to F. hepatica infection in the liver and the biliary tract (26a). In fascioliasis, the ultrasound image is usually normal and the individual adult flukes are not visualized by the current ultrasound technology. Among the lesions that have been reported are:

- (1) an echogenic defect which corresponded with a defect on radiocolloid scan (190);
- (2) cyst in the left lobe with patchy focal echogenicity in the liver parenchyma (similar to the image observed by ERCP) (111);
- (3) liver abscess with regression after specific treatment (130, 217);
- (4) intrahepatic hypoechogenic nodule with extension to a thickened liver capsule (51);
- (5) echodense mass in the gall-bladder confirmed by operation as F. hepatica (119);
- (6) heterogeneous echogenic subcapsular lesion diagnosed as a subcapsular haematoma confirmed by computed tomography (CT) and arteriography (180).

### 5.6.4 Computed tomography

CT has a high level of resolution for auxiliary and pathological diagnosis of F. hepatica infection. Pagola Serrano et al. (170) reported 8 cases of infection that were examined by CT. In the 7 cases in the invasive phase, intrahepatic nodular lesions of diminished attenuation, 4 to 10 mm in size, were observed. In 2 of them, the hypodense nodules were larger than 20 mm. Peripheral branching lesions were observed in 2 cases. The morphology and location of the lesions were defined with the intravenous contrast medium given before CT scans. However, in one case in the latent phase, CT showed a negative result. Other reports also showed multiple, small, hypodense areas (nodules) in the centre and periphery of the liver (71, 100, 219). The abnormalities found initially were small areas of decreased attenuation in the periphery of the liver which sometimes could not be detected by ultrasound (71). Larger cystic lesions in the liver with low echogenic areas were shown in one case (219). A linear image with high density which showed calcification in the proximity of the lesions has been reported (71, 170).

Several months after treatment for F. hepatica infection, a marked improvement in the CT images has been shown by different investigators. The multiple hypodense areas in the liver were reduced significantly in number and size (71, 100, 170, 219). CT scan can be a useful tool for diagnosis of the disease and its possible complications as well as follow-up of the patient's response to the treatment.

## 5.7 Complications

### 5.7.1 Bleeding

Mechanical injury to the biliary epithelium and surrounding tissues by the fluke in experimental infections can cause extensive haemorrhage in the bile ducts (40). The primary cause of anaemia in infected animals is the loss of blood into the bile (67). This is associated with shorter erythrocyte half-life.

In human infections, a subcapsular haematoma was identified by ultrasound and CT (180). Severe haemobilia was reported in 4 children with a high rate of mortality (2, 26, 102, 146). In these children haematemesis and melaena were associated with obstructive jaundice, epigastric pain and severe anaemia. The sources of the bleeding were all identified from laparotomy, and other causes of upper gastrointestinal bleeding were carefully ruled out. Bleeding occurred due to an ulcer in the common bile duct in one child (2). In the other children no single bleeding point was detected in the common bile ducts and/or in the gall-bladder (26, 102, 146).

### 5.7.2 Biliary cirrhosis

During the course of the infection, inflammation, hyperplasia and hypertrophy of the bile duct epithelia may induce periductal fibrosis. Prolonged heavy infection rarely leads to biliary cirrhosis (84, 111, 218). The outstanding findings on physical examination were firm untender hepatomegaly with or without jaundice and ascites. Splenomegaly was not prominent (218). Sclerosing cholangitis with biliary cirrhosis was detected by ERCP (111).

## 5.8 Causes of death

Death is rare as the infection is usually sporadic and the overall prevalence is low. Only 8 deaths related to fascioliasis were reported in the recent literature. Uncontrollable massive bleeding from the bile ducts (haemobilia) was the cause of death in 3 patients (2, 26, 146). In the other 5 patients the causes of death were not documented (75, 149, 245). In these 5 patients the clinical presentation included high fever up to 40-42°C, ascites, biliary colic and extreme fatigue. It is speculated that all the 5 patients were in the invasive phase of heavy infection with serious liver damage. More than 40 flukes were detected from the liver of one patient at autopsy (245).

## 6. DIAGNOSIS

### 6.1 Parasitological examinations

Parasitological diagnosis is based on the identification of F. hepatica eggs in the stool or in duodenal or biliary drainage. Adult flukes and/or eggs may be found in the biliary tract or in the bile at exploratory laparotomy in patients suspected of F. hepatica infection or in patients with cholelithiasis or obstructive jaundice of unknown cause. Histological examination of liver biopsy material may occasionally reveal an egg granuloma or sections of the fluke.

The flukes become mature and oviposition begins usually 3-4 months after infection; at that point the diagnosis may be made by stool examination. Stool examination techniques, ranging from a simple direct smear to different concentration methods, have been used for the diagnosis of chronic F. hepatica infection. Egg concentration has been achieved by flotation, sedimentation (19, 32, 38, 40, 70, 87, 136, 216) and the cellophane faecal thick-smear techniques (Kato, Kato-Katz) (138). The sedimentation technique is more accurate and sensitive than flotation techniques as most of the hyperosmotic flotation solutions distort the eggs (40). The Kato cellophane faecal thick-smear technique has the advantages of being rapid, inexpensive, reproducible and quantitative and it has been widely used in the diagnosis of Schistosoma and other

helminthic infections. The Kato technique has been used in the diagnosis of experimental F. hepatica infection (145) but, although it may be useful in epidemiological studies, its relatively low sensitivity limits its clinical application.

A comparative study (136) was done on three techniques, i.e., merthiolate-iodine-formaldehyde concentration method (MIFC) using 1 gram of faeces in a single examination; rapid sedimentation introduced by Lumbreas et al. in 1962 (cited by Knobloch et al.) using 20 g faeces on each of three consecutive days; and a single examination of duodenal fluid by the Enterotest according to Beal et al. (28). In 16 persons infected with F. hepatica identified by the rapid sedimentation method, only 2 and 6 were positive for the eggs by the MIFC and the Enterotest, respectively. Rapid sedimentation, although inconvenient, seemed to be more sensitive than the other two techniques in the diagnosis of F. hepatica infections (136). Five concentration techniques were compared by Akahane et al. (7) for the recovery rates of Fasciola eggs in a patient with 440 eggs/g faeces. The recovery rates for each technique were: formalin-ether method, 5.3%; HCl-ether method, 7.8%; Weller-Darmin's modification method, 37.7%; citrate buffer-Tween 80-ether method, 25.3%; and AMS III (Tween 80) method, 30.5%. Weller-Darmin's modification method and AMS III method seemed to be preferable.

F. hepatica eggs in the stool may originate from ingested livers of infected animals (183, 216). When this is suspected, stool examination should be repeated after a few days of a liver-free diet.

## 6.2 Immunological tests

Because the prepatent period of fascioliasis is long (3-4 months), early diagnosis of the acute phase may be achieved by immunological techniques. In the past, diagnosis of the infection could not be confirmed until the eggs were observed.

Skin tests employing an antigen prepared from the adult flukes (212) or purified fraction of F. hepatica (216) have been used occasionally since the early 1960s. The tests were simple and sufficiently sensitive to propose a diagnosis of the infection (49) but not very specific (216). The technique is rarely used nowadays.

During the past two decades, with the development of new technology, different serological tests have been used both in experimental infections (11, 66, 114, 115, 117, 135, 145, 176, 202) and in humans (11, 33, 43, 49, 82, 96, 97, 114, 115, 116, 135, 152, 162, 191, 198, 199, 210, 216, 231, 236). These include:

- complement fixation (CF) (43, 49, 216, 236),
- immunofluorescence assay (IFA) (43, 49, 198, 216, 236),
- counter-electrophoresis (CEP) (66, 96, 97, 114, 115, 116, 118, 145, 198, 216, 231),
- enzyme-linked immunosorbent assay (ELISA) (11, 82, 115, 117, 135, 145, 202, 236),
- kinetic-dependent ELISA (239),
- double diffusion (43, 96, 97),
- indirect haemagglutination (IHA) (49, 96, 97, 152, 162, 191, 198),
- enzyme-linked immuno-electrotransfer blot (EITB) (202),
- automated assay of anti-P<sub>1</sub> antibodies (33),
- circulating antigen (11), and
- circulating immune complex (CIC) (199).

Almost all the serological tests are highly sensitive. With partially purified somatic or excretory-secretory products of adult F. hepatica as antigen, ELISA (82, 115, 117, 145), IFA (43, 49) and CEP (115, 118) have been reported to have the highest sensitivity and specificity. IFA was reported to have 92-96% sensitivity in the acute phase of the infection by Capron et al (49). However, cross-reactions in other helminthic infections such as schistosomiasis, ascariasis and filariasis have been reported. In chronic infection with F. hepatica, IFA and CEP may not be positive (49). Comparative studies have not yet been completed and no consensus as to the optimal antigen or test system has been reached (216).

Crude F. hepatica antigen may have cross-reactivity with other trematodes (115). The specificity of the serological tests may be improved by elimination of cross-reactivity with antibodies to Schistosoma and other trematodes through partial purification of the antigen (117). A genus-specific antigen, which was isolated from adult F. hepatica and applied to ELISA to detect and monitor a primary F. hepatica infection in rabbits, showed good sensitivity and specificity (201).

Serological tests have an early diagnostic value in experimental infections. For example, precipitins monitored by the CEP test appeared as early as 2 weeks after F. hepatica infection in mice (114, 145), and 4 weeks after the infection in mice, rats and rabbits the CEP test was positive (118). In contrast, F. hepatica eggs were detected in the stool only 6-8 weeks after the infection in mice and 8-10 weeks after the infection in rabbits (145). By ELISA, a significant increase in specific antibody levels has been observed 2 weeks after the infection in cattle (202, 239), 4 weeks in rats (117) and in sheep (202), and 6 weeks in rabbits (117).

CIC was detected in only 36% of 291 infected persons compared with 11% of 18 uninfected control subjects. However, 74.2% of 31 patients with acute F. hepatica infection were positive for CIC, and a close relationship was observed between F. hepatica egg output and the detection rate of CIC of F. hepatica (199).

Several experimental studies have shown that after effective chemotherapy, anti-F. hepatica antibodies became undetectable (117, 118, 145). Hillyer and Santiago de Weil (117) reported that titres of ELISA dropped rapidly when rats and rabbits were successfully treated, and the test was useful for the evaluation of chemotherapeutic success. The same authors also reported that precipitins in the CEP test disappeared by 4 weeks after effective treatment (118). When mice were treated with rafoxanide, both CEP and ELISA, as well as search for eggs in the Kato stool specimen, became negative 3-4 weeks later (145). However, double diffusion and IHA became negative after a longer period, i.e., 1-2 years after treatment, according to Garcia-Rodriguez et al. (97).

### 6.3 Clinical diagnosis

Although parasitological findings can confirm the diagnosis of the infection and a positive serological test permits a presumptive diagnosis, the clinical presentation may be suggestive of the diagnosis.

Chronic fascioliasis is frequently considered among the differential diagnoses in a well-known endemic area. However, in areas where the disease is rarely reported or absent, physicians may not consider this diagnostic possibility. In support of the clinical diagnosis, a knowledge of the epidemiology of the disease is important. History of ingestion of raw wild or cultivated watercress or other vegetables, or of other contaminated food or water may be suggestive of the infection. In the acute phase the clinical presentation includes fever, pain in the right hypochondrium, prominent eosinophilia with leukocytosis, anaemia and a moderately to significantly high ESR. Increases in AKP, GPT, GOT and gamma globulin may or may not be present. In this phase a positive serological reaction against F. hepatica antigen is most suggestive of the diagnosis.

In the chronic (latent and obstructive) phase, the clinical picture is attenuated and easily confused with other diseases. The classic pattern includes: vague gastrointestinal complaints, pain in the right hypochondrium or epigastrium, cholecystitis, cholangitis and bile duct or gall-bladder stones. The liver is usually enlarged with or without pain on palpation. Ascites may appear in advanced cases. Radiology, radioisotope, ultrasound and computed tomography are of value in confirming the diagnosis. Definitive diagnosis can be made by finding eggs in the stool or biliary drainage, or by finding egg granulomas in the liver tissue sections, adult worms in the bile ducts or eggs in the bile through exploratory laparotomy.

In both the acute and chronic infections, ectopic localization of the parasite may cause a confusing clinical presentation. In the differential diagnosis, febrile diseases such as typhoid, brucellosis, acute schistosomiasis, hepatitis and hepatic abscess should be ruled out. Other parasitic infections causing eosinophilia: schistosomiasis, clonorchiasis, trichinellosis, hydatid disease, visceral larva migration and Loeffler's syndrome, as well as eosinophilic leukaemia, must be excluded (84, 109, 233).

"Halzoun", an acute dysphagia and laryngeal obstruction after ingestion of raw liver of sheep or goats, was formerly considered to be due to invasion of immature F. hepatica and was known as pharyngeal fascioliasis (84). Now it is attributed to the ingestion of nymphs of Linguatula serrata, a pentostomid parasite (52).

## 7. TREATMENT

The classic drugs for the treatment of F. hepatica infection are emetine and dehydroemetine. They are effective and are still being used. However, owing to their side effects, several other types of drugs have been developed during the past decade. The following is a general review of the chemotherapeutic agents against F. hepatica being used in man and/or domestic animals.

### 7.1 Emetine ( $C_{29}H_{40}N_2O_4$ )

Emetine is an alkaloid obtained from ipecacuanha, or prepared by methylation of cephaeline, or prepared synthetically. Both emetine and dehydroemetine ( $C_{29}H_{38}N_2O_4$ . INN<sup>1</sup>) have been used widely (5, 8, 17, 18, 35, 46, 50, 59, 71, 73, 84, 92, 102, 104, 109, 147, 161, 183, 188, 209). The usual dose is 1 mg/kg daily for 10 days given intramuscularly or subcutaneously. Their therapeutic effects in eliminating the infection as well as in improving the symptoms are well known, but they cause a variety of toxic manifestations involving the heart, liver and digestive tract. Flattening and inversion of the T waves and prolongation of the Q-T intervals are frequent changes seen in the electrocardiogram (ECG). Hypotension sometimes occurs during treatment. Dehydroemetine is an analogue of emetine and it has a shorter tissue half-life and disappears more rapidly from the heart and liver as compared with emetine (102). No deaths have been reported due to emetine or dehydroemetine treatment of F. hepatica infections.

### 7.2 Chloroquine ( $C_{18}H_{26}ClN_3$ . INN)

Chloroquine is a 4-aminoquinoline which has been used to treat F. hepatica infection although no cidal effects on the flukes have been shown. However, in the acute phase, chloroquine treatment improved the symptoms dramatically; with the disappearance of fever, the patients' general condition improved, and hepatomegaly, eosinophilia and ESR were reduced. Cure of the infection has not been documented (84, 109). Chloroquine may act via immune suppression rather than an anti-parasite effect.

### 7.3 Hexachloro-para-xylol (Hetol) ( $C_8H_4Cl_6$ )

The drug has been used in China and in the Soviet Union for F. hepatica and some other trematode infections. The usual dosage schedule used in China is 50-80 mg/kg body

<sup>1</sup> INN International nonproprietary names.

weight daily divided into 3 doses given orally for 7 consecutive days (218, 229), and in the Soviet Union 60 mg/kg daily for 5 days (132, 184). The therapeutic effect is good. The side effects include gastrointestinal complaints and dizziness.

#### 7.4 Bithionol (Bitin) ( $C_{12}H_6Cl_4O_2S$ . INN)

Bithionol is a halogenated phenol derivative with cidal effects on F. hepatica in man (37, 89, 99, 104, 126, 133, 134, 240, 244) and in buffaloes (243). In 4 cases of fascioliasis resistant to emetine treatment (104) and in one case treated with praziquantel without effect (37), bithionol achieved cure in dosages of 50 mg/kg daily for 10 alternate days (104) or 40 mg/kg daily for 15 alternate days (37). The usual dosage schedule is 50 mg/kg daily, divided into 3 oral doses on alternate days for 15 treatment days. The side effects are mild, are related to the gastrointestinal tract, and include anorexia, nausea, vomiting and abdominal pain.

#### 7.5 Niclofolan ( $C_{12}H_6Cl_2O_6$ . INN)

Niclofolan, a nitro-derivative of a halogenated phenol type substance, has been widely used in treating F. hepatica infections in domestic animals in China. In clinical trials, 146 cattle (123, 213), 94 buffaloes (123), 20 goats (216), 12 yaks (211) and 466 sika (a type of deer) (107) have been treated. The effective total doses were: 6 mg/kg in cattle, 4 mg/kg in buffaloes, 2 mg/kg in goats, 3-5 mg/kg in yaks, and 3 mg/kg in sika. The cure rates at these doses were 90-100% in cattle, 98% in buffaloes, 80% in goats, 50% and 100% in yaks with 3 mg/kg and 5 mg/kg of the drug, respectively, and 100% in sika, as confirmed by stool examinations and/or at autopsy.

There are two reports of treatment of human F. hepatica infection with this drug (79, 185). Two oral doses of niclofolan at 2 mg/kg body weight were given 3 days apart (79). During and for a few days after the treatment, the patient suffered from sweating, palpitation, nausea, and diffuse upper abdominal pain. Repeated stool examinations up to 6 months after treatment were negative. Eosinophil counts and liver enzymes returned to normal levels. At a 6-month follow-up, as the high titres of antibody by IHA and CF before treatment had returned to normal, it was concluded that the infection had been cured. In the second report (185), niclofolan was given at 0.5 mg/kg twice a day for 3 days to a woman. The patient experienced frequent vomiting and abdominal pain during the treatment course. One week after starting niclofolan, she developed generalized itching and jaundice with dark urine. Serum GOT, AKP and bilirubin were elevated. Three weeks after treatment, the jaundice disappeared and liver functions returned to normal. The toxicity of niclofolan is such that clinical use cannot be recommended.

#### 7.6 Metronidazole ( $C_6H_9N_3O_3$ . INN)

Metronidazole is a nitro-imidazole compound which is effective against a wide range of protozoa. In 4 cases of fascioliasis, 3 in the acute phase and one in the obstructive phase, with eggs in the stool and duodenal fluid, daily oral doses of 1.5 g metronidazole were given for 13, 14, 21 and 28 days. In all patients the eggs were no longer detected in stools and duodenal fluid. In the 3 acute cases, fever, urticaria and abdominal pain disappeared (166). A smaller total dose of 4 g metronidazole was reported to have failed to cure a chronic infection (79).

#### 7.7 Albendazole ( $C_{12}H_{15}N_3O_2S$ . INN)

Albendazole is a broad-spectrum benzimidazole anthelmintic. F. hepatica infection in cattle was treated with albendazole at a single oral dose of 15 mg/kg body weight (160). After the treatment, most of the cattle experienced weight gain, increase in appetite and disappearance of diarrhoea. Fifty days after treatment, eggs were absent from the stool of 34 out of 36 (94.4%) cattle. When the drug was administered to experimentally infected sheep at a single dose ranging from 3.8 to 7.5 mg/kg body weight, worm reduction rates were from 83.3% to 94.5% against mature F. hepatica compared with untreated control groups at slaughter. However, the efficacy of the drug against the immature flukes was lower. When the sheep were treated 6 and 7 weeks after the infection, worm reduction rates were 16.0% and 43.6%, respectively (125).

### 7.8 Triclabendazole (C<sub>14</sub>H<sub>9</sub>Cl<sub>3</sub>N<sub>2</sub>OS. INN)

Triclabendazole is a benzimidazole compound used in veterinary medicine which is effective against both adult and immature F. hepatica. In experimental studies in sheep, Boray et al. (42) and Turner et al. (223) showed that doses ranging from 2.5 to 5.0 mg/kg body weight could eliminate almost all the flukes (98.1-100% reductions) 12 weeks after infection. One week after infection, a higher dose of 10 mg/kg body weight achieved reductions of between 93% and 98% of the flukes (42). As the age of the flukes increases, the dosage per kg body weight decreases to achieve high reduction of the flukes (42, 223). A single oral dose of 40 mg/kg body weight killed 99% of the adult flukes in rats (57). In vitro, triclabendazole causes degenerative changes in the tegument and gut surface of the mature flukes (103) and biochemical alterations of the immature and mature flukes (34).

Although this drug is not yet registered for human use, some preliminary clinical data have been published and clinical trials are anticipated in the near future. Three patients with chronic fascioliasis were effectively treated with triclabendazole (12 mg/kg single dose for the first patient; 5 mg/kg first dose, followed by 10 mg/kg second dose on the next day for the second patient; 10 mg/kg single dose for the third patient). The liver flukes had survived prior treatment with albendazole, praziquantel, dehydroemetine or fenbendazole as shown by persistent egg excretion. Clinical tolerability was excellent in one of these patients while in the other 2 patients after a single dose a transient febrile episode with reversible liver function alteration was observed (154, 235, 236). Four patients, whose diagnosis was confirmed by specific immunoelectrophoresis and indirect haemagglutination, were also successfully treated with a single dose of 10 mg/kg (143a).

Most recently, 3 other patients have been treated in Switzerland with a single oral dose of 10 mg/kg body weight in the fasting state (148). The drug was well tolerated, as evidenced by close clinical and laboratory monitoring. The 19-year-old man and his mother recovered steadily over 3 weeks. However, after a month of slight improvement, the 57-year-old man again felt ill. Very few eggs were found in subsequent stool examinations. He was given a second treatment of triclabendazole in 2 successive postprandial doses (10 mg/kg) 12 hours apart.

Levels of unchanged drug as well as the major metabolites (the antiparasitically active triclabendazole sulfoxide and the sulfone) were assessed in one uninfected volunteer and in 5 patients. These human data were comparable with those found in pigs (monogaster), however, with a biological half-life of the sulfoxide metabolite of about 6 hours as compared with 22-24 hours in sheep.

In the 3 patients from Switzerland but infected in France, plasma concentrations of the unchanged drug and its two main metabolites (the sulfoxide and sulfone) were measured for all 3 patients after the first treatment (fasting) and in the patient given 2 postprandial doses. Fasting plasma concentrations of drug and metabolites were about 3 times lower in the 57-year-old man than in the other 2 patients. Ratios of peak plasma concentrations of postprandial versus fasting administration were: unchanged drug 4.3; sulfoxide 2.5; sulfone 2.8. Ratios for the area under the curve were 5.1, 2.6 and 3.0, respectively (148). It seems that postprandial administration influenced the absorption of triclabendazole significantly in this man.

### 7.9 Praziquantel (C<sub>19</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>. INN)

Praziquantel is an isoquinoline-pyrazine derivative. Whether the drug is effective against human infections with F. hepatica remains doubtful (12, 173); most probably it is not effective. Fasciola may be the only genus of trematode that has practically no response to praziquantel.

Most clinical reports have shown that praziquantel failed to cure F. hepatica infections (37, 88, 91, 135, 136, 235, 236), even at higher doses, i.e., 75 mg/kg daily for 5 days (91, 236). Post-operatively, Schiappacasse et al. (206) used a total dose of 75 mg/kg body weight divided into 3 doses over a one-day period. Although the flukes and eggs were found in the bile ducts at surgery, after treatment repeated stool examinations were negative. Wahn and Mehlhorn (227) reported the cure of an 8-year-old boy treated with praziquantel at 15 mg/kg body weight, 5 times daily (75 mg/kg daily) for 5 days.

In vitro and in mice, rats and sheep, F. hepatica is refractory to praziquantel (12, 173). No changes were found in the tegument of F. hepatica after treatment with praziquantel (30).

#### 7.10 Other drugs

##### Mebendazole ( $C_{28}H_{32}O_6$ . INN)

Mebendazole, a benzimidazole derivative, in a daily dose of 4 g for 3 weeks was reported to have cured a F. hepatica infection diagnosed clinically and serologically in the invasive phase (76).

##### Diamphenetide ( $C_{20}H_{24}N_2O_5$ . INN)

Diamphenetide, an acetanilide derivative, was effective both in vitro (86) and in experimentally infected sheep at 120 mg/kg body weight (124).

##### Rafoxanide, or penta-chlorosalicylanilide

( $C_{19}H_{11}Cl_2I_2NO_3$ . INN)

Rafoxanide, or penta-chlorosalicylanilide, a salicylanilide derivative, was reported to be effective against F. hepatica in sheep and cattle (44) and was used in the treatment of a child with fascioliasis (241).

Among the drugs currently available, and until triclabendazole becomes available, bithionol seems to be the drug of choice although its treatment course is comparatively long. In the hands of experienced clinicians, emetine and dehydroemetine are still effective drugs. In China and in the Soviet Union, hexachloro-para-xylol has been used against F. hepatica. Triclabendazole, albendazole and niclofolan are quite effective in veterinary infections. In the absence of toxicological information required for registration or clinical trials in man, their use cannot yet be recommended.

#### 8. CONCLUSIONS

The numbers of clinical cases of F. hepatica reported as well as of infected persons identified during epidemiological surveys have been increasing since 1970. These increases may be due to a better understanding of the disease and the improvement of the diagnostic methods especially in areas where serological tests have been used. The major sources of the infection, the domestic herbivorous animals, are widely distributed in the world and human infection is not rare in these areas. During the past two decades, more than 2500 cases have been documented in the scientific literature from 42 countries (areas). The actual number of human infections is probably much greater than this figure. The disease is mainly endemic in the temperate and subtropical zones. A moderate temperature and a high humidity are necessary for the development and multiplication of the intermediate snail hosts and the flukes in various developmental stages. A prolonged and wet summer in Europe has often been followed by an outbreak of the disease.

The severity of the disease depends mainly on the intensity of the infection. In man, only a small proportion of the flukes may reach the bile ducts whereas the others die in the abdominal cavity and in the liver during their migration. Extensive destruction of the liver parenchyma and intense immunological and inflammatory reactions occur during the fluke migration; this is consistent with the acute phase in clinical observation. When the flukes reach the bile ducts they live for months to years with less liver damage, although inflammation of the biliary passage is prominent and biliary obstruction is common. In the acute phase, infected persons usually have fever and complain of pain in the right hypochondrium, of gastrointestinal disturbances and of urticaria; eosinophilia is the most striking laboratory finding. After months to years of infection, an obstructive phase may develop. The clinical manifestations in the obstructive phase are indistinguishable from cholangitis, cholecystitis and cholelithiasis of other origins; discovery of the eggs in the stool or duodenal drainage or of the adult flukes in the biliary passage during operation confirm the diagnosis. Death due to the disease is rare; biliary bleeding and biliary cirrhosis may be the major causes of death. Parasitological examination is still the main method of diagnosis. However, serological methods have been developed and have confirmed the diagnosis in the acute phase of the disease; they are also useful for monitoring post-treatment evolution. Safe and effective drugs for treatment of the disease are now available. The challenges to the control of this endemic disease are mainly its wide distribution in domestic animals and the control of the intermediate snail hosts. This latter aspect has not received the attention from public health officials that would be required to eliminate definitively transmission of the disease.

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## ANNEX

SUMMARY OF A REPORT ON FASCIOLA HEPATICA INFECTION IN NORTHERN PORTUGAL  
FROM THE INSTITUTO NACIONAL DA SAUDE, PORTO, PORTUGAL, IN 1986

Between 1970 and 1985, 561 persons were diagnosed with Fasciola hepatica infection either by stool examination and/or serology by the Instituto Nacional da Saúde.

Three communities in northern Portugal were intensively surveyed: 207 of 6370 persons examined (3.3%) had F. hepatica eggs in their stools. In addition 128 others had serological evidence of infection by indirect haemagglutination or immunoelectrophoresis. The stool examination data from these population-based investigations are shown in the following tables.

TABLE 1. PREVALENCE OF F. HEPATICA INFECTION IN THREE LOCALITIES IN NORTHERN PORTUGAL: VIZELA (1970-1974), AMARES (1974-1978) AND FAPE (1979-1982)

Locality	Number examined	Number positive for eggs	Eggs per gram faeces	Prevalence (%)
			Mean (range)	
Vizela	951	69	308 (25-950)	7.3
Amares	1 782	64	223 (50-900)	3.6
Fafe	3 637	74	173 (50-2100)	2.0
Total	6 370	207	233 (25-2100)	3.2

TABLE 2. PREVALENCE AND INTENSITY OF INFECTION ACCORDING TO AGE IN THREE COMMUNITIES IN NORTHERN PORTUGAL

Age (years)	Number examined	Number positive for eggs	Eggs per gram faeces mean*	Prevalence (%)
0-6	904	21	124.0	2.3
7-13	1 586	47	220.4	3.2
>13	3 880	139	254.2	3.7
Total	6 370	207	233.4	3.2

\* Arithmetic mean; geometric mean and range not cited.

Annex

TABLE 3. DISTRIBUTION OF INTENSITY OF INFECTION DUE TO F. HEPATICA  
IN 207 INFECTED PERSONS IN THREE COMMUNITIES IN NORTHERN PORTUGAL

Eggs per gram faeces	Number of persons	Percentage of total
0-100	93	44.9
101-200	32	15.5
201-300	35	16.9
301-400	18	8.7
401-500	10	4.8
501-800	13	6.3
801-1000	5	2.4
>1000	1	0.5
Total	207	100.0