INFECTION WITH LIVER FLUKES
(*Opisthorchis viverrini, Opisthorchis felineus* and *Clonorchis sinensis*)

1. Exposure Data

1.1 Structure and biology of liver flukes

1.1.1 *Taxonomy*

Three of the human liver flukes, *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*, are pathologically important food-borne members of the class Trematoda (Beaver *et al.*, 1984). These flukes establish a chronic infection within the smaller intrahepatic bile ducts and occasionally in the pancreas and gall-bladder of humans and other fish-eating mammals.

The life cycle of food-borne trematodes is complex, involving two more intermediate hosts (the first always a snail) and several morphological stages. The consumption of raw or incompletely cooked foods which contain the infective stages is the major risk factor for these infections. As a result, people who enjoy a variety of raw foods often harbour several trematodes (liver and intestinal flukes). Similarities in egg morphology and cross-reactive antigens complicate both parasitological and immunological diagnosis and may confound clinical and epidemiological research on the liver flukes.

Fish-eating mammals, for example dogs, cats, pigs, mink, weasels and civets, may become infected with human liver flukes, and some may act as reservoir hosts (Beaver *et al.*, 1984).

1.1.2 *Structure*

In humans, *Clonorchis* measures 8–15 mm long and 1.5–5 mm wide, while the two *Opisthorchis* species are somewhat smaller—3–12 mm by 1–3 mm. They are covered by a tegument and have an oral sucker at the anterior end and a ventral sucker or acetabulum located posterior at about one-third to one-fifth of the body length. Adult worms are hermaphroditic, with reproductive organs occupying much of the body (Sadun, 1955; Komiya, 1966; Beaver *et al.*, 1984; Rim, 1986).

The morphology of the adult worms of *O. viverrini*, *O. felineus* and *C. sinensis* is similar but can be distinguished at the cercarial stage by the bilateral pattern of excretory flame cells (Vajrasthira *et al.*, 1961; Wykoff *et al.*, 1965). The adults differ in the shape of testicular lobes, their location relative to the ovary and the appearance of vitelline glands (Sadun, 1955; Wykoff *et al.*, 1965). The metacercariae and juvenile worms bear spines.

The yellowish-brown eggs have a distinct operculum, which opens to release the miracidia when the eggs are ingested by an appropriate species of snail. The posterior end of the egg has a small protuberance, or knob. Eggs average 29 μm long by 15 μm wide for *C. sinensis* (Ditrich *et al.*, 1992), 27 by 15 μm for *O. viverrini* (Sadun, 1955; Kaewkes *et al.*, 1991) and 30 by 11 μm for *O. felineus* (Ditrich *et al.*, 1990), with differences in shape between species.

1.1.3 *Life cycle and biology of the adult worm*

The life cycle of liver flukes is illustrated in Figure 1.

Infection with *Opisthorchis* and *Clonorchis* is acquired through the consumption of raw or undercooked fish containing the infective stage, called metacercaria. The metacercariae leave the cyst in the duodenum and migrate through the ampulla of Vater via the common and extrahepatic bile ducts to the smaller, proximal bile ducts under the surface of the liver, where they mature. Although most adult worms are found in these ducts, in heavy infections they can be found in the extrahepatic bile ducts, pancreatic ducts and, rarely, the gall-bladder (Hou, 1955; Sithithaworn *et al.*, 1991a). Infection is confined to the lumen of the hepato-biliary tract; there is no phase of tissue migration, even when the common bile duct is severed (Sun *et al.*, 1968).

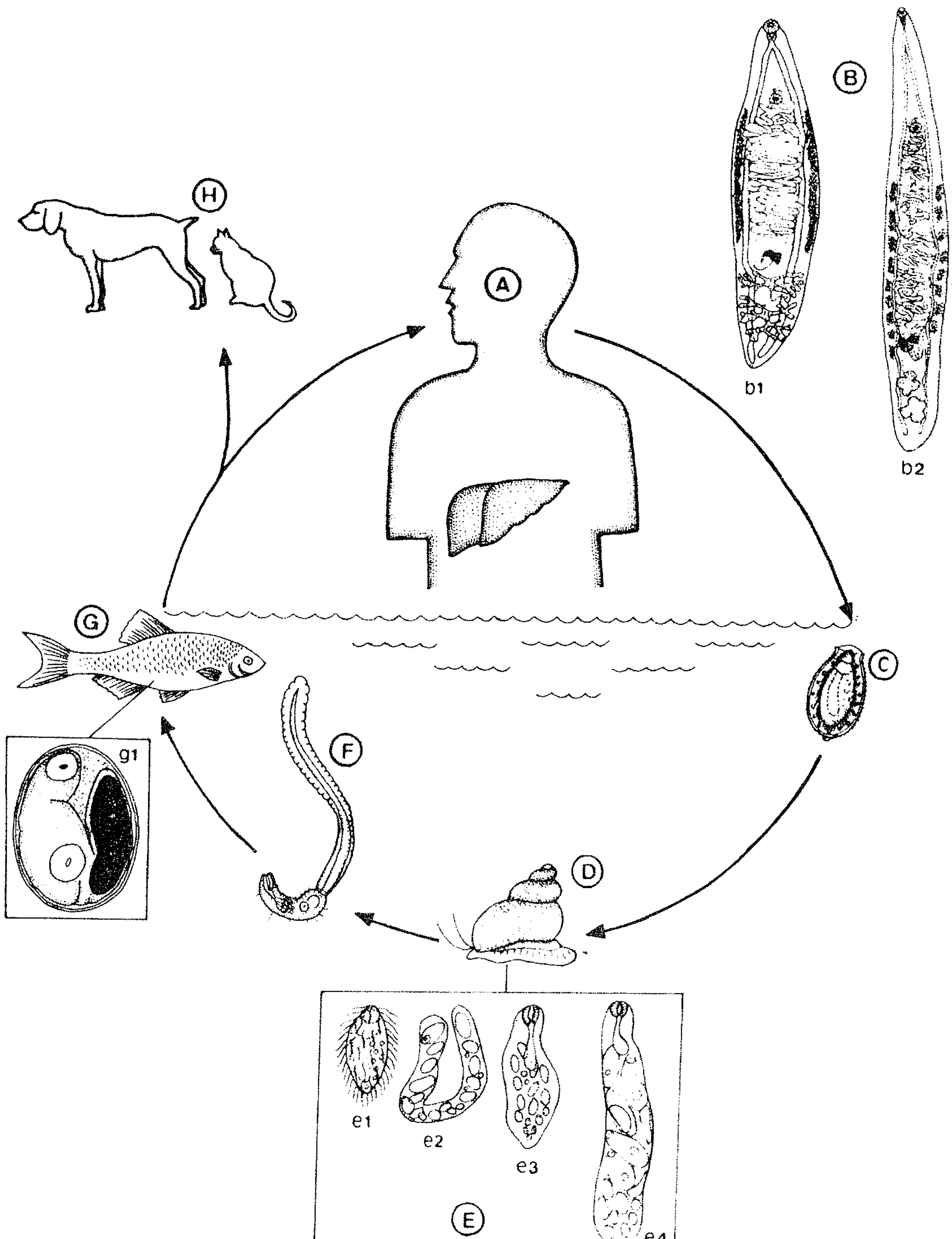
Clonorchis moves up the biliary tract by attaching and detaching its two suckers and extending and contracting its body. Its attachment to the wall of the bile duct may be secured by adherence of the ventral sucker to the biliary epithelium, leaving the oral sucker free for feeding (Hou, 1955).

About one month after the metacercariae have been ingested, adult worms begin producing eggs, which pass down the bile duct and are excreted in the faeces. Eggs can also be found in gall-bladder bile. The average egg output per gram of faeces per adult *Opisthorchis* worm ranges from 15 to 180 (Elkins *et al.*, 1991; Sithithaworn *et al.*, 1991b). Density-dependent decreases in fecundity have been documented, which partially explain the wide variation in estimates between these studies (Ramsay *et al.*, 1989). Estimated fecundity in infected people and animals is generally in the range of 1000–5000 eggs per day (Komiya, 1966; Wykoff & Ariyaprakai, 1966; Rim, 1986). The average lifespan of the worms, inferred from epidemiological data, is about 10 years, while the maximal lifespan in the absence of reinfection may exceed 25 years (Attwood & Chou, 1978; Zhu, 1984).

If the eggs reach a body of freshwater (small ponds, streams and rivers, flooded rice fields and large reservoirs), they are ingested by snails. Asexual reproduction in the snail results in the release daily of thousands of cercariae one to two months after infection of the snail. The free-swimming cercariae penetrate the tissue of fish and encyst, becoming fully infective metacercariae after 21 days. Over 80 species of the Cyprinidae family and some 13 species of other families, and possibly freshwater prawns, can serve as the second intermediate host (Komiya, 1966; Vichasri *et al.*, 1982; Rim, 1986; Joo, 1988).

In any defined freshwater body, only 1–3% of snails may be infected, while up to 100% of fish may contain metacercariae (Vichasri *et al.*, 1982; Rim, 1986; Brockelman *et al.*, 1986). The distribution patterns of metacercariae in fish determine patterns of human exposure in endemic areas. The intensity of liver fluke infection in fish varies from one to hundreds, depending on season, type of water body, species and individual. Transmission is seasonal, as a result of patterns of human faecal contamination, water temperature and snail or fish density.

Figure 1. Life cycle of liver flukes



A: definitive host, human; B: adult liver flukes in bile duct, *Clonorchis sinensis* (b1), *Opisthorchis viverrini* (b2); C: embryonated egg; D: first intermediate host, *Bithynia* sp.; E: intramolluscan stages, miracidium (e1), sporocyst (e2), mother redia (e3), daughter redia (e4); F: cercaria; G: second intemediate host (cyprinoid fish), metacercaria in fish muscle (g1); H: reservoir host, dog and cat

The prevalence of infection in reservoir hosts varies by area and is not closely associated with human infection patterns. In endemic areas, transmission to snails is due mainly to human eating patterns, poor sanitation and high egg excretion (Sadun, 1955; Rim, 1986); the importance of reservoir hosts is limited.

1.1.4 *Immune response to infection*

The existence of protective immune responses to liver fluke infections remains unclear (Sirisinha, 1984). Several experimental studies have suggested that small decreases in the establishment or fecundity of worms can be observed in animals that receive repeated infections, spleen cells or serum from infected donors and immunization with parasite antigens (Flavell *et al.*, 1980; Flavell, 1982; Sirisinha *et al.*, 1983; Sirisinha, 1984; Kwon *et al.*, 1987). Flavell and Flavell (1986) reported that animals deprived of T cells had similar worm burden and egg output to intact animals after an equivalent challenge. Wongratanacheewin *et al.* (1987) reported that *O. viverrini* infection was associated with reduced immuno-responsiveness to red blood cells and mitogens, an effect that was reversed by praziquantel treatment.

In humans, the parasites clearly survive high levels of parasite-specific immunoglobulins G, A and E in both serum and bile (Wongratanacheewin *et al.*, 1988). While experimental studies suggest that parasites may induce immunosuppression, no evidence of suppressed skin test reactivity or reduced responsiveness during infection has been observed in humans (Wongratanacheewin *et al.*, 1988; Haswell-Elkins *et al.*, 1991a). Epidemiological patterns reveal little evidence of, but do not rule out, protective immunity. There appears to be no decline in prevalence of infection among individuals exposed to infection for decades, and rapid rates of reinfection have been reported after treatment in areas of heavy infection (Upatham *et al.*, 1988).

High levels of parasite-specific antibodies have been reported in people with severe hepatobiliary disease and cholangiocarcinoma (Srivatanakul *et al.*, 1990; Haswell-Elkins *et al.*, 1991a). Antibody levels are correlated more closely with clinical indicators of infection, such as gall-bladder size and function, wall abnormalities and ultrasound echo-genicity of the portal triad, than is egg count (Haswell-Elkins *et al.*, 1991a; Mairiang *et al.*, 1992).

1.2 Methods for detection of infection

1.2.1 *Qualitative faecal examination for eggs*

Detection of liver fluke infection is most often based on the observation of eggs in faeces. The techniques that have been used, in increasing order of sensitivity, are: direct smear, sedimentation, Kato technique, Stoll's technique and formol–ethyl acetate/ether concentration (FECT) (Viyanant *et al.*, 1983; Feng & Chen, 1985; Zavoikin *et al.*, 1985, 1986; Sithithaworn *et al.*, 1991b; Chen *et al.*, 1994). FECT has been used for quantitative measurements, and the Kato technique in large-scale surveys.

Qualitative diagnosis based on a single reading with Stoll's technique and FECT is highly sensitive (nearly 100%) in people with 20 worms or more, but the sensitivity of a single reading drops to as low as 20% in people with fewer than five worms (Sithithaworn *et al.*, 1991b). Multiple reading of the same sample increases sensitivity up to 20% (Haswell-Elkins *et al.*, 1994a). The sensitivity of the diagnostic techniques used in epidemiological studies determines accurate assessment of prevalence and the effects of intervention.

In patients whose bile ducts are completely obstructed, eggs occur in the gall-bladder bile, and serological methods may be used to determine infection (Kurathong *et al.*, 1985; Pungpak *et al.*, 1985).

Eggs of minute intestinal flukes, e.g. species of *Heterophyes*, *Phaneropsolus* and *Haplorchis*, can be confused with those of *Opisthorchis* and *Clonorchis* (Ditrich *et al.*, 1990; Giboda *et al.*, 1991a; Kaewkes *et al.*, 1991; Ditrich *et al.*, 1992).

1.2.2 *Quantitative faecal assessment of intensity of infection*

The combination of egg counts with worm recovery after treatment is the optimal procedure for assessing intensity of infection (Haswell-Elkins *et al.*, 1994a), as there is a close relationship (Radomyos *et al.*, 1984; Sithithaworn *et al.*, 1991b). Studies of autopsy specimens show, however, that people with high egg counts sometimes do not expel eggs (Ramsay *et al.*, 1989; Elkins *et al.*, 1991).

Daily variation in faecal egg output appears to be minimal and does not greatly affect the accuracy of different techniques (Viyanant *et al.*, 1983; Kurathong *et al.*, 1984; Pungpak *et al.*, 1990).

1.2.3 *Serological tests for helminth-specific antibody and antigen*

Immunodiagnostic tests for liver fluke infections are considered to be supplementary tools rather than definitive diagnostic assays (Rim, 1986; Sirisinha, 1986). Their use in epidemiological surveys is limited, owing to lack of specificity, lack of differentiation of past and present infections and limited sensitivity (Viyanant *et al.*, 1985; Chen *et al.*, 1987; Hong, 1988; Thammapalerd *et al.*, 1988; Wongratanacheewin *et al.*, 1988).

Serological methods may be preferable in some circumstances, as they indicate exposure that occurred before antihelminthic treatment.

Comparisons between the enzyme-linked immunosorbent assay (ELISA), immunofluorescence, complement fixation and indirect haemagglutination for the detection of antibodies against *Opisthorchis* and *Clonorchis* show that ELISA is usually the most sensitive and specific. A sensitivity of 90.2% and a specificity of 84% was seen for *Clonorchis* in a comparison of infected people with people outside an endemic area (Chen *et al.*, 1988). Cross-reactions in crude ELISAs have been reported in sera from patients with intestinal nematodes, schistosomiasis, angiostrongyloidiasis, paragonimiasis and minute intestinal fluke infection (Chen *et al.*, 1988; Poopyruchpong *et al.*, 1990; Ditrich *et al.*, 1991).

Comparisons of antibody responses to crude somatic extracts among infected and uninfected individuals within endemic communities show significant associations between infection and the frequency and level of helminth-specific antibody (Janechaiwat *et al.*, 1980; Srivatanakul *et al.*, 1985; Poopyruchpong *et al.*, 1990; Haswell-Elkins *et al.*, 1991a). The sensitivity of antibody tests in light infections is limited (Haswell-Elkins *et al.*, 1991a), and intensity of infection cannot be inferred from antibody level (Rim, 1986; Haswell-Elkins *et al.*, 1991a).

Chen *et al.* (1987) described a sandwich ELISA for detecting circulating antigen in sera of people infected with *C. sinensis*. ELISAs that include a mixture of helminth-specific monoclonal antibodies can be used to detect *O. viverrini* antigens in faeces, while a 340-base-pair DNA probe can be used to detect helminthic DNA in faeces (Sirisinha *et al.*, 1991, 1992).

1.2.4 *Intradermal tests*

Skin testing with a diluted extract of adult *C. sinensis* antigens has been used widely in China and the Republic of Korea in epidemiological and surveillance studies, but this method is less commonly used today. The estimated specificity and sensitivity of the reaction was reported to be 98% by Komiya (1966), but lower values (83 and 78%) were reported subsequently (Rim, 1986).

1.3 Epidemiology of infection

1.3.1 *Geographical distribution*

The worldwide distribution of *O. viverrini*, *O. felineus* and *C. sinensis* is shown in Figures 2 and 3.

Countries in which human liver fluke infection is endemic are China, Japan, the Republic of Korea, Laos, Thailand, Viet Nam, the Russian Federation, the Ukraine and parts of eastern Europe. A very crude estimate of the global number of infections is of the order of 17 million, comprising seven million with *Clonorchis*, nine million with *O. viverrini* and 1.5 million with *O. felineus* (WHO, 1994). Regional and global migration of peoples has broadened the distribution of the helminths. Since their life cycles usually do not become established, this widened distribution has limited epidemiological relevance, but, given the potential severity of disease, it is of clinical importance (Chan & Lam, 1987). As details of the sampling methods and examination techniques used are sometimes omitted from survey reports, the sensitivity and representativeness of the measurements cannot be evaluated.

(*a*) *Opisthorchis viverrini*

The first studies of the epidemiology of *O. viverrini* in North-east Thailand, for which relatively insensitive diagnostic methods (simple smears and Kato technique) were used, suggested that about one-third of people in the region harboured infection (Sadun, 1955; Wykoff *et al.*, 1965; Harinasuta, 1969). A survey summarized by Harinasuta (1969), in which the FECT method was used, showed, however, that more than 60% of a sample drawn from 15 north-eastern provinces was infected. Higher prevalences (up to 92%) were seen in the northern provinces that border Laos, and lower prevalences (as low as 10%) in the southern provinces, which border Cambodia. More recent surveys have shown that *O. viverrini* still infects about 15% of the Thai population of 58 million, and about 24% of the North-east Thai population of 20 million (Jongsuksantigul *et al.*, 1992). The level of infection in northern Thailand is less clear, owing to its uneven distribution. Harinasuta (1969) reported prevalences of over 15% in three of 17 provinces (Chiang Mai, Prae and Lampang); Preuksaraj (1984) noted similar levels only in Sukhothai (22%) and Phetchabun (17%). The most recent survey in northern Thailand (Jongsuksantigul *et al.*, 1992) showed an overall average prevalence of 23% which, if substantiated, suggests an increase in prevalence.

Figure 2. Worldwide distribution of *Opisthorchis viverrini* and *O. felineus*

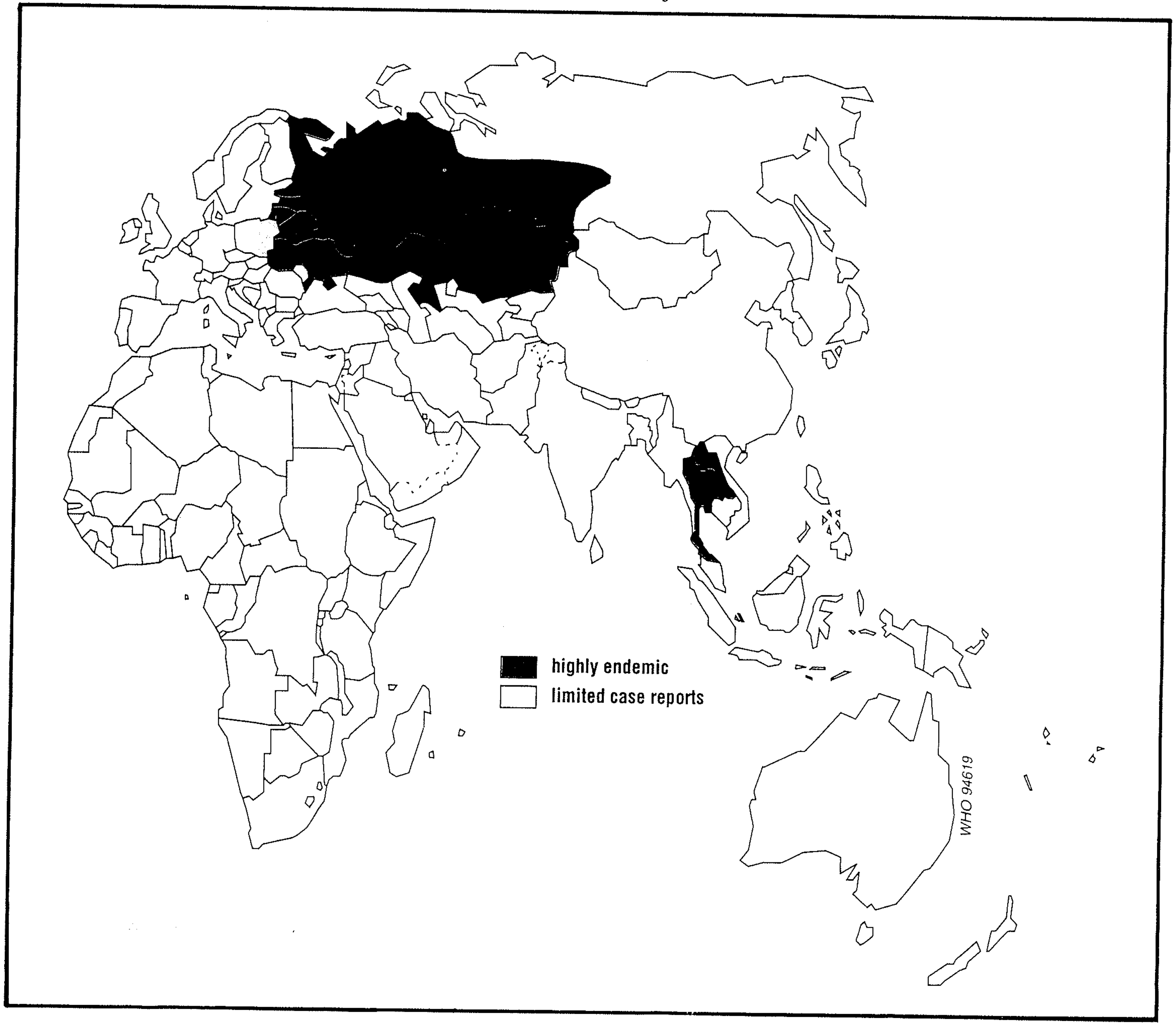
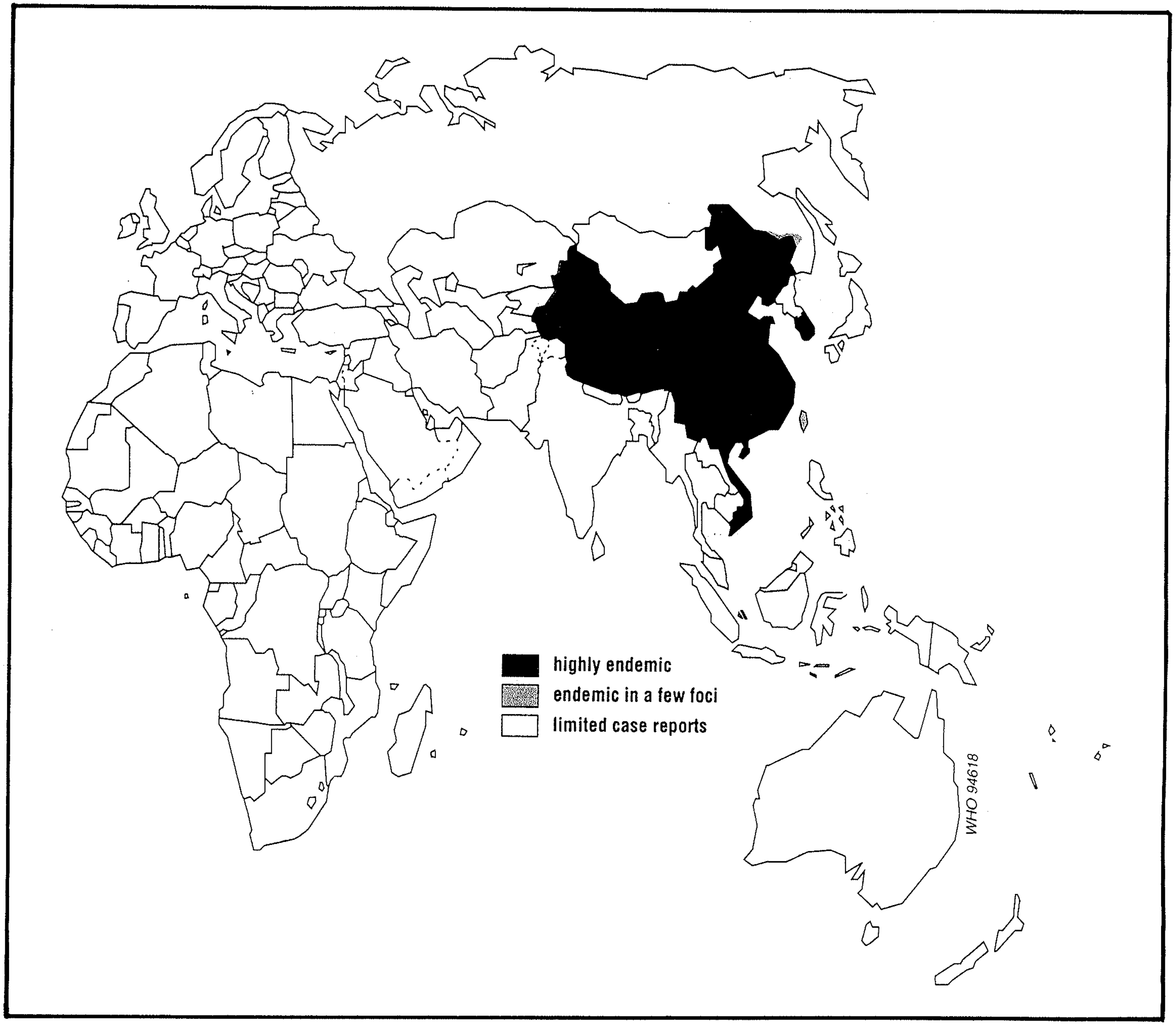


Figure 3. Worldwide distribution of *Clonorchis sinensis*



The helminth is common in the lowlands of Laos among people with close ethnic ties to the majority of the North-east Thai population; however, the total number of infections is not known (Giboda *et al.*, 1991b; Pholsena *et al.*, 1991).

(*b*) *Opisthorchis felineus*

About 1.5 million cases of *O. felineus* infection are seen in the former USSR, according to a tabulation of the results of surveys prepared in 1992 for WHO (Iarotski & Be'er, 1993). Some 1.2 million infections are estimated to occur in the Russian Federation, as projected from a total of 78 400 that were officially registered. Infections are registered in 24 of the 73 territories in the Federation, mostly in western Siberia and particularly along the valleys of the Ob' and Irtys' rivers and their tributaries; the largest number of registered infections (and over 900 000 extrapolated cases) were reported from the two districts of T'umen' and Tomsk. High prevalences were also observed in areas along the Volga–Kama river basin, and along river basins in the the Novosibirsk, Krasnojarsk, Kurgan, Kemerovo, Sverdlovsk, Omsk and Tomsk districts and the Altaj territory (Klimshin *et al.*, 1981; Iarotski & Be'er, 1993).

Infections also occur in the Ukraine and Kazachstan. Reports from the Ukraine indicate that infection is found in the Sumy, Poltava and Černigov districts within the Dnepr River basin, with prevalences of 5–40%. In Kazachstan, the average prevalence in six endemic districts was less than 10% (Iarotski & Be'er, 1993).

Eight percent of the population of one rural area in Germany was reported to be infected in 1929, but more recent surveys on parasitic zoonosis in this region indicated that the infection no longer persists (Hinz, 1991).

(*c*) *Clonorchis sinensis*

C. sinensis is distributed in reservoir hosts throughout China, but human infection is largely confined to 24 provinces and municipalities in the south and north-east, as delineated by the eating of raw or undercooked fish. This behaviour and the infection are ethnically and geographically associated; the most frequent consumers and infections in the south occur among the Cantonese, particularly the Hakka people (Rim, 1986), and those in the north-east occur among the Korean national minority who migrated there (Chen *et al.*, 1994).

Clonorchis is commonest in Guangdong and Guanxi Zhuang provinces in the south, where four million people are thought to be infected (Li, 1991; Chen *et al.*, 1994; Fang, 1994). The highest infection levels in Guangdong Province are observed in the Pearl River delta (with an estimated prevalence of 21.1% based on surveys between 1973 and 1991), the upper reaches of the Pearl River (4.4%) and the Han River drainage basin (5.1% infected) (Fang, 1994). The You River runs through the areas of highest prevalence of infection in Guanxi, where some 7.3% of inhabitants are infected. Other endemic provinces in China include Heilongjiang, Jilin and Liaoning in the north-east, Jiangsu along the Yangtze River and periurban areas of Beijing where fish are abundant in canals (Chen *et al.*, 1994).

Infection in Hong Kong is probably acquired by eating fish imported live from Guangdong Province in southern China, since no infection has been found among local snails. Estimated prevalences of infection in Hong Kong, with its large Cantonese population, range from as high as 46–65.6% (Hou & Pang, 1964; Belamaric, 1973) to 23% (Attwood & Chou, 1978). Eggs were found in 13.4% of simple faecal smears (an insensitive method) of Hong Kong residents applying to emigrate to Canada (Ko, 1991). The populations sampled in these surveys, however, are not random, so that the true prevalence may be overestimated. For example, since imported fish are expensive, the prevalence may be higher among wealthier residents (Chen *et al.*, 1994) who might be more likely to apply to go abroad.

Clonorchis infection is distributed throughout Taiwan, at prevalences ranging from < 1 to 57%. Heavy infection is frequent among Hakka people who emigrated from Guangdong Province to the Mei-Nung and Kaohsiung districts in southern Taiwan (Komiya, 1966; Hou *et al.*, 1989; Chen, 1991). The Miaoli district in the north and the Sun–Moon Lake area in the central part are also important endemic areas, where 20–50% of the population are infected (Chen, 1991). The endemic area may be increasing as new areas are reporting significant prevalences of infection.

Infections have largely been eliminated in Japan, where highly endemic areas were reported in the 1960s in several river basins (Chen *et al.*, 1994). The prevalence and intensity have since dropped steadily, and *Clonorchis* may now be almost eradicated (Rim, 1986), largely due to improvements in sanitation and health education.

Infection in the Republic of Korea has been documented extensively. In the past, both prevalence and intensity were high: in a nationwide survey in 1959, up to 15% of the population responded positively to skin testing (Chen *et al.*, 1994). The highly endemic areas occurred in seven river basins, in which community prevalences were 30–80% (Elkins *et al.*, 1994). Large-scale control activities under way since 1984 have decreased the prevalence to 2.2% (Ministry of Health and Social Affairs, 1992).

High prevalences of infection were also reported in the past in northern Viet Nam, in the Red River delta near Hai Phong and Ha Noi; however, *Clonorchis* infection was rare in the south (Rim, 1986). A survey among 968 inhabitants of Ha Nam Nin province showed a prevalence of 28.4% (Lam *et al.*, 1990).

Clonorchis has also been reported in the Amur River basin in the far eastern region of the Russian Federation, where it infects some 24% of the aboriginal population (the Nanaians) (Sergiev *et al.*, undated).

The prevalence of infection with *Opisthorchis* and *Clonorchis* in places like Hong Kong and Macao, where most freshwater fish is imported, depends on the origin of the fish.

1.3.2 *Risk factors for infection*

(*a*) *Opisthorchis viverrini*

In North-east Thailand, three types of preparations contain uncooked, usually small fish: fresh (*koi-pla*; eaten the same day), moderately fermented (*pla-som*, salted and stored for five days to three months) or completely fermented (*pla-ra*, highly salted, stored for two to three months to over one year) (Sadun, 1955). In the past, reported consumption frequencies of *koi-pla* were very high: up to 80% in some communities ate the dish on a weekly basis (Migasena, 1982). In a comparison of rural and urban dwellers, Kurathong *et al.* (1987) reported higher prevalences of liver fluke infection among rural than urban residents from the north-east region and among rural residents who reported having eaten *koi-pla* (87%) than among those who did not (61%). Upatham *et al.* (1984) reported a closer relationship with *koi-pla* consumption within a heavily infected village, with only 19% of uninfected people, 79% of infected people and > 90% of heavily infected people reporting consumption.

More recent surveys suggest that the frequency of *koi-pla* consumption has declined and is generally confined to special social occasions, while uncooked *pla-som* is generally eaten several times a week (Changbumrung *et al.*, 1989). Fully preserved fish (e.g. *pla-ra*) is an important staple food, consumed daily by 80–99% of north-eastern Thais descended from Laotians (Migasena, 1982; Changbumrung *et al.*, 1989). It is commonly believed that liver fluke infection can occur from eating any of these dishes, but the infectivity of the various preparations remains unclear. Several studies have indicated that survival of the infective stages depends on the concentration of salt and the degree of fermentation (Tesana *et al.*, 1986). These findings suggest that *koi-pla* is probably the most infective, followed by fish preserved for less than seven days, while viable metacercaria would be very rare in *pla-ra*.

(*b*) *Opisthorchis felineus*

Fish is a major source of food in western Siberia and other endemic areas of the former USSR, where people eat uncooked fish, frozen, salted and smoked; frozen fish is sliced and eaten with condiments. Aboriginal inhabitants (Ugro-Finn, Khanti, Mancy, Nencie) eat raw fish, as do 10–40% of migrants into the endemic areas, e.g. miners, geologists and labourers, who become infected with *O. felineus* within one to two years (Iarotski & Be'er, 1993).

(*c*) *Clonorchis sinensis*

In southern China and among the Cantonese of Hong Kong, raw fish is traditionally eaten after being dipped in rice porridge (Chen *et al.*, 1994). Alternatively, large fish are sliced and eaten with ginger and garlic. Higher levels of infection and poorer nutritional status were reported among children in hilly areas of Guangdong Province than among those living along rivers, while infection patterns among adults show the opposite trend. This observation led to the finding that children in the hilly areas often catch fish during play and roast them incompletely before eating. As they grow older, they catch fish less frequently than adults living on riversides, and the intensity of infection declines. Koreans eat raw fish soaked in vinegar, red-pepper mash or hot bean paste with rice wine at social gatherings (Choi, 1984). The fact that men do so more frequently than women has been given as an explanation for higher prevalences of infection among men; however, in heavily infected areas, there is often no difference between the sexes. When fish are abundant, raw fish is eaten commonly rather than being reserved for special occasions (Rim, 1986). Vietnamese people eat raw fish in salads (Kieu *et al.*, 1990).

Infection in Japan, which is now very rare, appeared to come from frequent consumption of slices of large, raw, freshwater fish with vinegar or soya bean paste (Chen *et al.*, 1994). In contrast, smaller co-existing species were rarely eaten uncooked. The large fish, namely *Cyprinus carpio* and *Carassius carassius*, were infrequently and lightly infected with metacercariae, possibly because of the presence of toxic components in their mucus (Rhee *et al.*, 1988). *Sushi* and other preparations of uncooked seafish eaten in Japan today do not carry *Clonorchis*.

1.3.3 *Age- and sex-related patterns of infection*

While the levels of *O. viverrini* infection vary considerably between villages in Thailand, the patterns of infection are fairly similar. In general, the youngest age groups (often 0–4 years) show low prevalence and intensity, while these increase in the pre- and early teens and often reach a plateau in late teenage groups (i.e. 15–19). In some areas, the intensity of egg excretion continues to increase with age (Upatham *et al.*, 1982), while the worm burden may decline (Haswell-Elkins *et al.*, 1991b; Sithithaworn *et al.*, 1991a).

Anecdotal descriptions have been reported of mothers in the Republic of Korea and Thailand feeding raw fish to their infants (Choi, 1984), and infections have been observed in young infants (Sadun, 1955; Harinasuta & Vajrasthira, 1960; Upatham *et al.*, 1982, 1984). The reported intensities of infection in children under the age of four are, however, invariably very low, and there is little evidence that young children have ever had frequent, intense exposure to infection.

The prevalence and average intensity of *O. viverrini* infection do not usually differ, or are slightly higher, among males than females (Wykoff *et al.*, 1966; Upatham *et al.*, 1982, 1984; Haswell-Elkins *et al.*, 1991b; Elkins *et al.*, 1994). Even in areas where these measurements do not differ significantly with sex, higher frequencies of heavy infection may be observed among males (Haswell-Elkins *et al.*, 1991b; Elkins *et al.*, 1994).

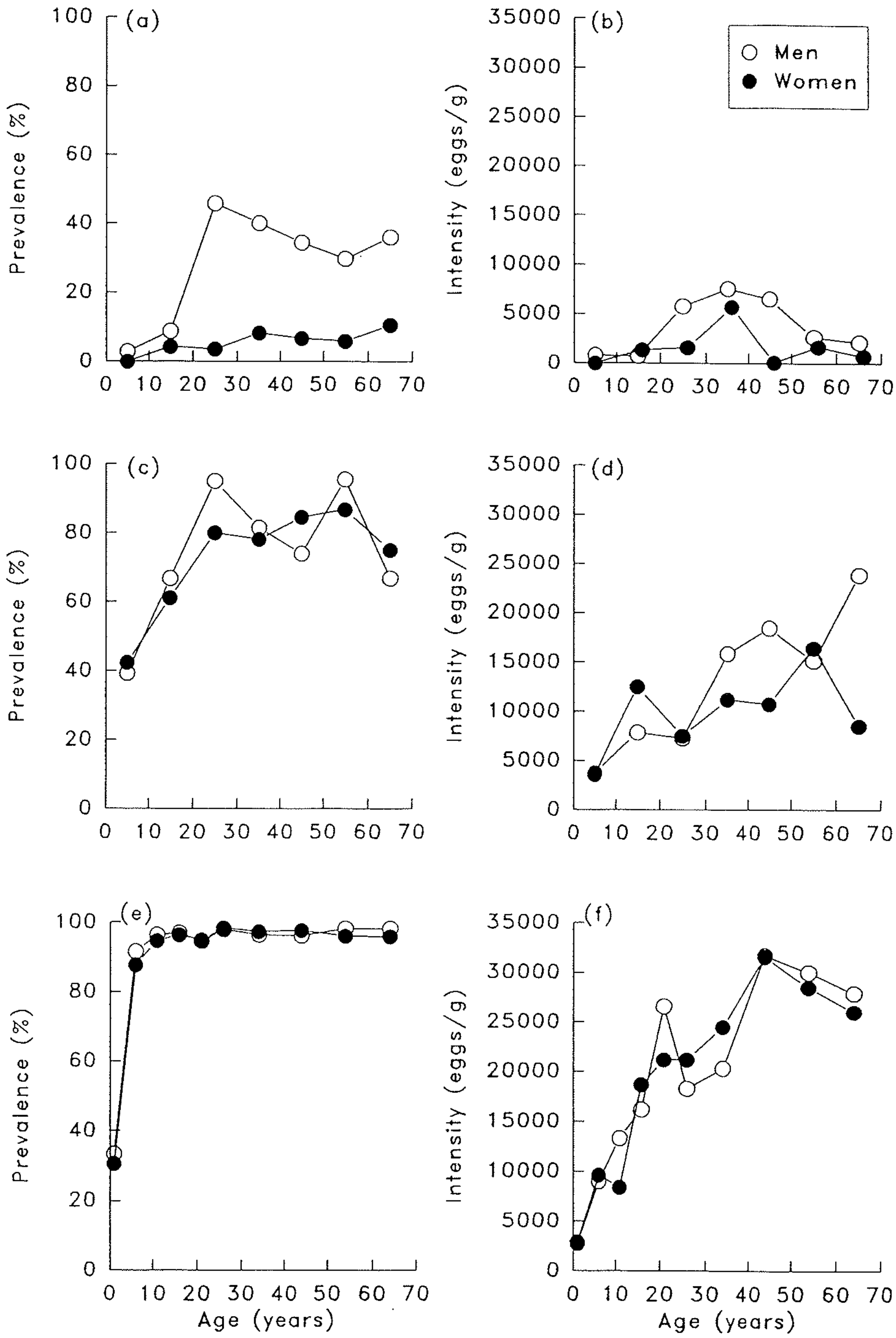
In general, the prevalence of *Clonorchis* infection appears to rise at later ages, and differences in prevalence and intensity between the sexes are more pronounced than those of *Opisthorchis* (Figure 4). For example, in several river basins in the Republic of Korea, large increases in prevalence are observed between the ages of 10–19 and 20–29. Sometimes this is apparent only in males, and females maintain relatively low prevalences throughout life, while in other areas the two sexes have virtually identical age-related patterns of infection. Most studies in Japan show maximal prevalences at 30–50 years of age (e.g. 39–67% and 8.8–46%) (Rim, 1986). This finding appears to be generally true in China, except in areas where children become infected by catching and eating undercooked fish during play (Chen *et al.*, 1994).

1.3.4 *Aggregation of infection*

The population of *O. viverrini*, and probably all three liver flukes, is highly aggregated within a small minority of people who are heavily infected. For example, Haswell-Elkins *et al.* (1991b) observed that 81% of 11 027 worms recovered after treatment of 246 village residents were expelled by just 27 individuals with burdens of over 100 worm Similarly, Sithithaworn *et al.* (1991a) reported that 30 of 181 cadavers examined contained 66% of all worms recovered at autopsy.

The levels of infection vary considerably between communities within the same province and district, for unknown reasons. Tesana *et al.* (1991) found higher prevalences of infection in six villages located far from a river than in villages situated along river banks. This observation is in contrast to the patterns usually reported for *Clonorchis* infection and may reflect variation in the habitats of infected fish.

Figure 4. Prevalence (a) and intensity (b) of infection with *Clonorchis sinensis* in an area of low intensity in the Republic of Korea; prevalence (c) and intensity (d) of infection with *C. sinensis* in an area of high intensity in the Republic of Korea; prevalence (e) and intensity (f) of infection with *Opisthorchis viverrini* in an area of high intensity in Thailand



Intensities are arithmetic means. (a)-(d) from Rim (1986); (e)-(f) from Upatham *et al.* (1994)

1.4 Clinical disease in humans (other than cancer)

The frequency and types of clinical disease appear to differ for the three human liver flukes. Most notably, reports in the Russian literature give specific signs and symptoms for well-defined clinical stages of opisthorchiasis, from acute to chronic (Bronshtein, 1986). Acute infection, characterized by high fever, hepatitis-like symptoms and eosinophilia, is frequently reported in *O. felineus* infections but has been documented infrequently for clonorchiasis (Rim, 1986) and for *O. viverrini* infections. This finding may be due to the fact that a large number of migrants enter the area endemic for *O. felineus* and become infected as adults; this pattern is unusual in infections with the other two liver flukes.

Much of the published information comes from uncontrolled clinical investigations, e.g. case studies and reviews of hospital records, which do not include a control group for comparison (Markell, 1966). Furthermore, since most of the studies have been hospital-based, the frequencies with which these clinical manifestations occur during the course of infection cannot be inferred. As a result, there has been a strong tendency to overestimate both the frequencies and strengths of association between the infections and various presentations (Markell, 1966; Woolf *et al.*, 1984).

Two large studies (Upatham *et al.*, 1982, 1984) within a heavily infected community reported significantly increased frequencies of abdominal pain in the right upper quadrant, flatulence or dyspepsia and weakness associated with increasing intensity of infection. They estimated that 5–10% of the community had symptoms attributable to the infection.

Most other clinical and laboratory assessments show little or no difference in liver function, nutritional status or clinical signs and symptoms between infected and uninfected individuals, and no difference following anthelminthic treatment (Pungpak *et al.*, 1990). Total serum IgE, white blood cell count and percentage of eosinophils are often elevated, but this finding may sometimes be confounded by other infections (Joo & Rim, 1982).

Increased levels of serum protease inhibitors (α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin) (Changbumrung *et al.*, 1982), of three serum bile acids (taurocholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid) (Migasena *et al.*, 1983) and of the activities of a number of hepatic enzymes (Pongpaew *et al.*, 1985) have been reported among people with *O. viverrini* infection. Migasena *et al.* (1983) reported an increase in the trihydroxy:dihydroxy ratio and in total bile acids with intensity of egg output, which is a stronger indication of association with infection.

Schelp *et al.* (1974) similarly observed no difference in nutritional, clinical or haematological status between infected and uninfected individuals in a village in North-east Thailand. They did, however, find an increase in the ceruloplasmin and haemopexin peak and in haptoglobin levels among infected people, which they suggested was due to bile retention in liver cells and inflammation. Analyses were not done after treatment.

Studies using ultrasonography have shown strong relationships between intensity of infection and gall-bladder enlargement, gall-bladder wall irregularities and sludge, and enhanced echogenicity of the portal triad (Dhiensiri *et al.*, 1984; Mairiang *et al.*, 1992). These abnormalities were reversible within 10 months after praziquantel treatment (Mairiang *et al.*, 1993).

The presence of stones in the gall-bladder, liver and bile ducts has frequently been linked to *Clonorchis* infection; the best evidence is the finding of eggs or worm fragments in the nidus (Teoh, 1963). Hou *et al.* (1989) reported a consistent increase in gallstone frequency (diagnosed by ultrasound) with increasing intensity of infection among Hakkanese people in Taiwan, from 4.2% in uninfected subjects to over 14% in those who excreted more than 5000 eggs/g. Similar clinical findings have been reported infrequently in cases of *Opisthorchis* infection (Riganti *et al.*, 1988).

Ascending cholangitis and obstructive jaundice are common complications of opisthorchiasis. Pungpak *et al.* (1985), however, reported only 88 cases of severe manifestations among 15 243 infected people who attended a hospital in Bangkok. These manifestions included obstructive jaundice and cholangitis; at least 16 patients had cholangiocarcinoma. Since radiological investigations were not performed, cholangiocarcinoma could not be ruled out as a cause of the manifestations.

1.5 Treatment and control

Clonorchis has been successfully controlled in Japan, and the current prevalence in the Republic of Korea is 2.2% (Ministry of Health and Social Affairs, 1992). In other areas, it is often difficult to assess the success of control efforts, owing to lack of epidemiological data. The main tools that have been used in control programmes have been anthelminthic treatment, improved sanitation and health education. The rationale is that treatment is required to eliminate the long-lived parasites immediately, sanitation interrupts transmission from human faeces to snails, and health education stops people from eating raw fish and becoming reinfected after treatment. A number of studies have suggested that control programmes involving treatment and health education are more effective in suppressing reinfection than treatment alone (Sornmani *et al.*, 1984; Saowakontha *et al.*, 1993). Community participation in the planning and implementation of control programmes is a vital element in their success (Keittivuti *et al.*, 1986; Sornmani, 1987).

Strategies that have been suggested but not widely implemented include destroying metacercaria in fish through irradiation (Lee *et al.*, 1989; Sornmani *et al.*, 1993) and deep-freezing (Song, 1987; Iarotski & Be'er, 1993), applying molluscicides, using biological agents (*Mesocyclops leuckari*) to destroy cercariae (Intapan *et al.*, 1992) and treating reservoir hosts. Improvements in sanitation, by supplying latrines and stopping the use of night-soil as fertilizer on fields and as food for fish, have been widely implemented. No progress has been reported towards development of a vaccine.

Control efforts are influenced by the massive environmental changes that are occurring in many endemic areas, notably China, Japan, the Repubic of Korea and Thailand. As natural aquatic life is affected by pollution, fish become less abundant and the life-cycle is disrupted (Choi, 1984; Joo, 1988).

The single dose of praziquantel generally used for *O. viverrini* and *C. sinensis* infections in the Republis of Korea and Thailand is 40 mg/kg bw, while higher, multiple doses (3 × 25 mg/kg bw for one to three days) have been used for treatment in China. Although the drug has a number of side-effects, these are transient and relatively minor. The published efficacy of this dosage is invariably over 90% (Vivatanasesth *et al.*, 1982; Chen *et al.*, 1983; Rim, 1986;

Viravan *et al.*, 1986). Reinfection can occur after treatment. Upatham *et al.* (1988) reported an extremely rapid return (less than one year) to pre-treatment levels of infection in a mass-treated community that had had an extremely high initial intensity of infection. Furthermore, these authors showed a significant association between pre- and post-treatment egg counts among individuals, indicating that stable, individual behavioural and immunological factors, as well as chance, determine levels of infection.