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).

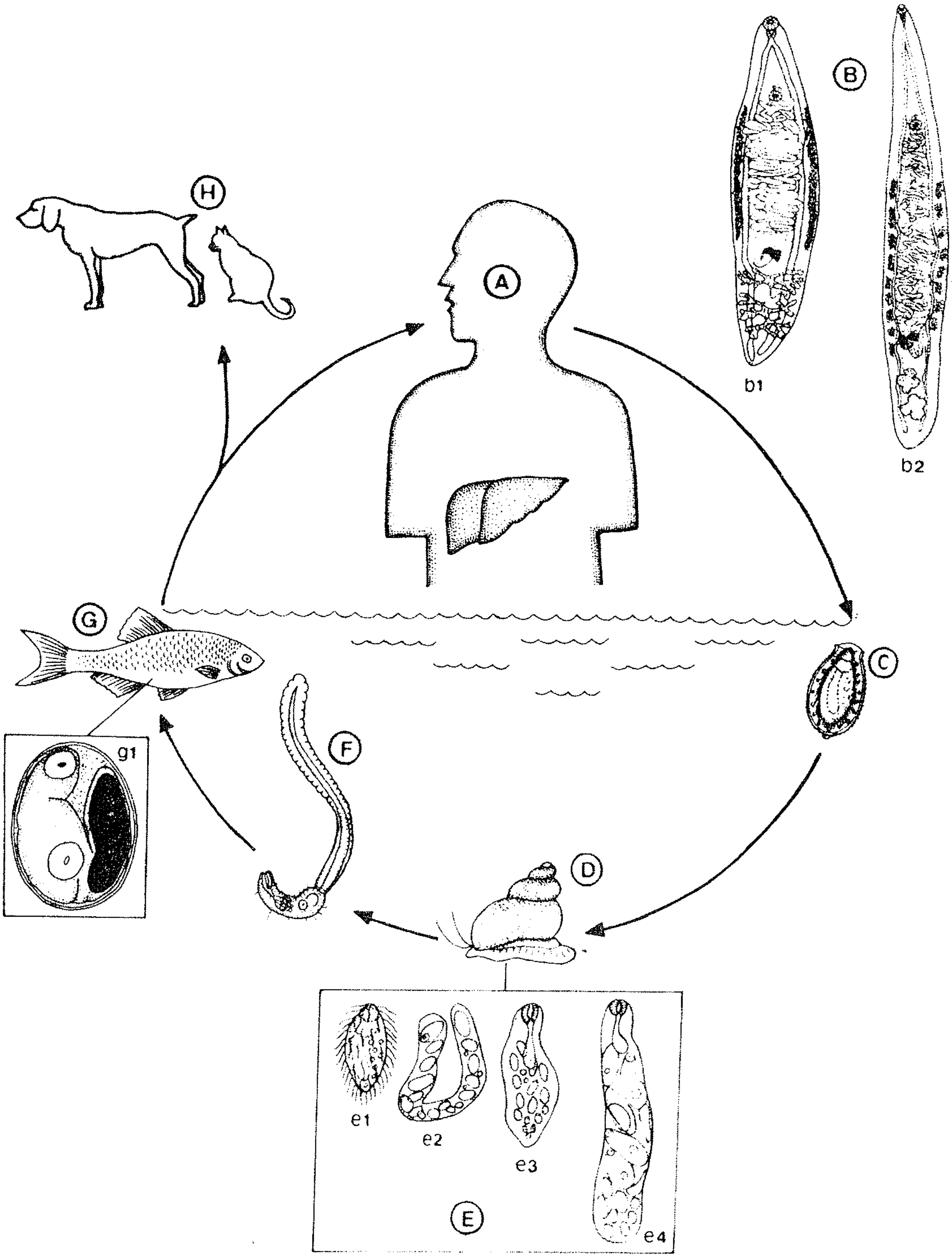
Clonorchis moves up the biliary tract by attaching and detaching its two suckers and extending and contracting its body. Its attachment to the wall of the bile duct may be secured by adherence of the ventral sucker to the biliary epithelium, leaving the oral sucker free for feeding (Hou, 1955).

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*

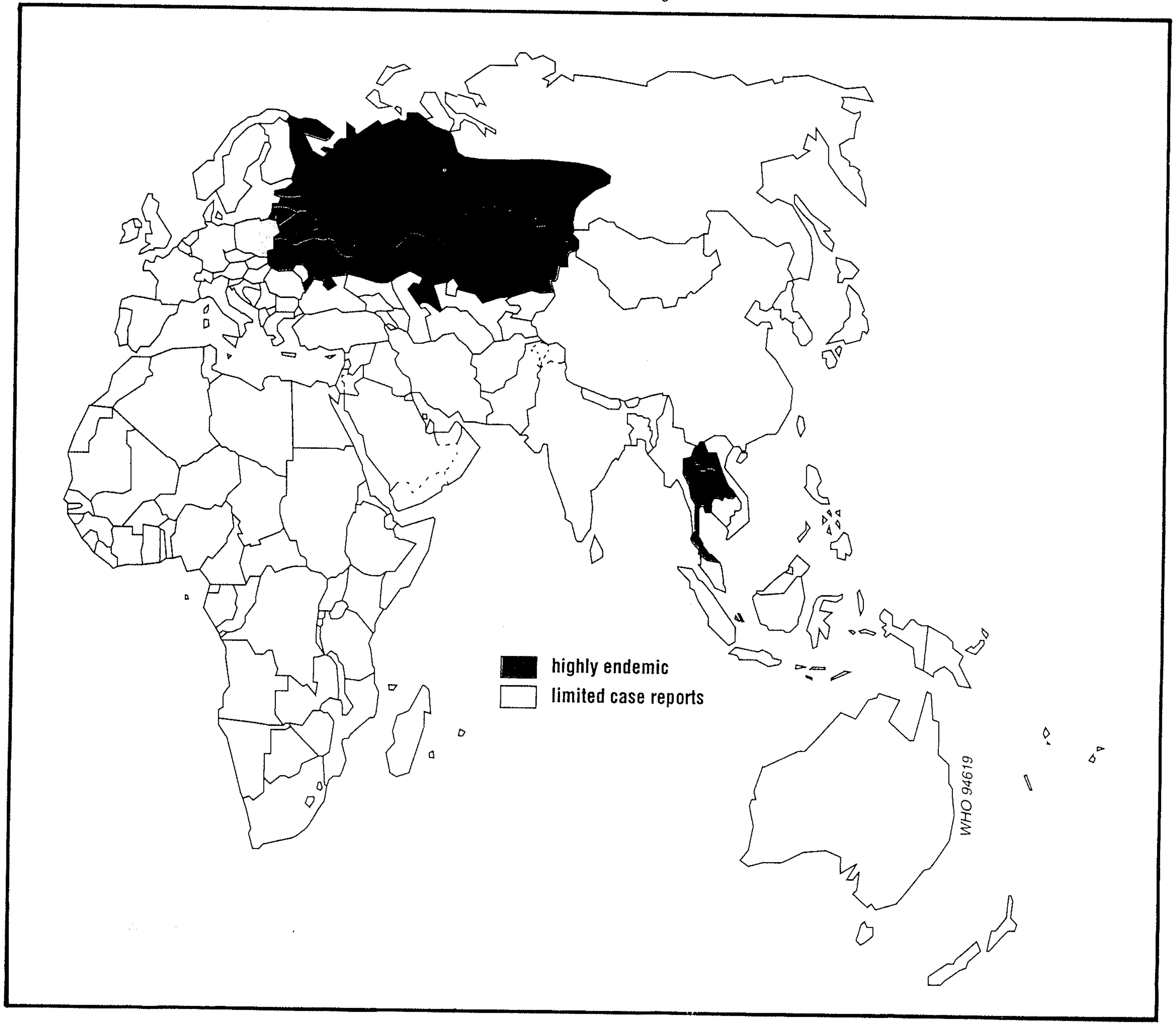
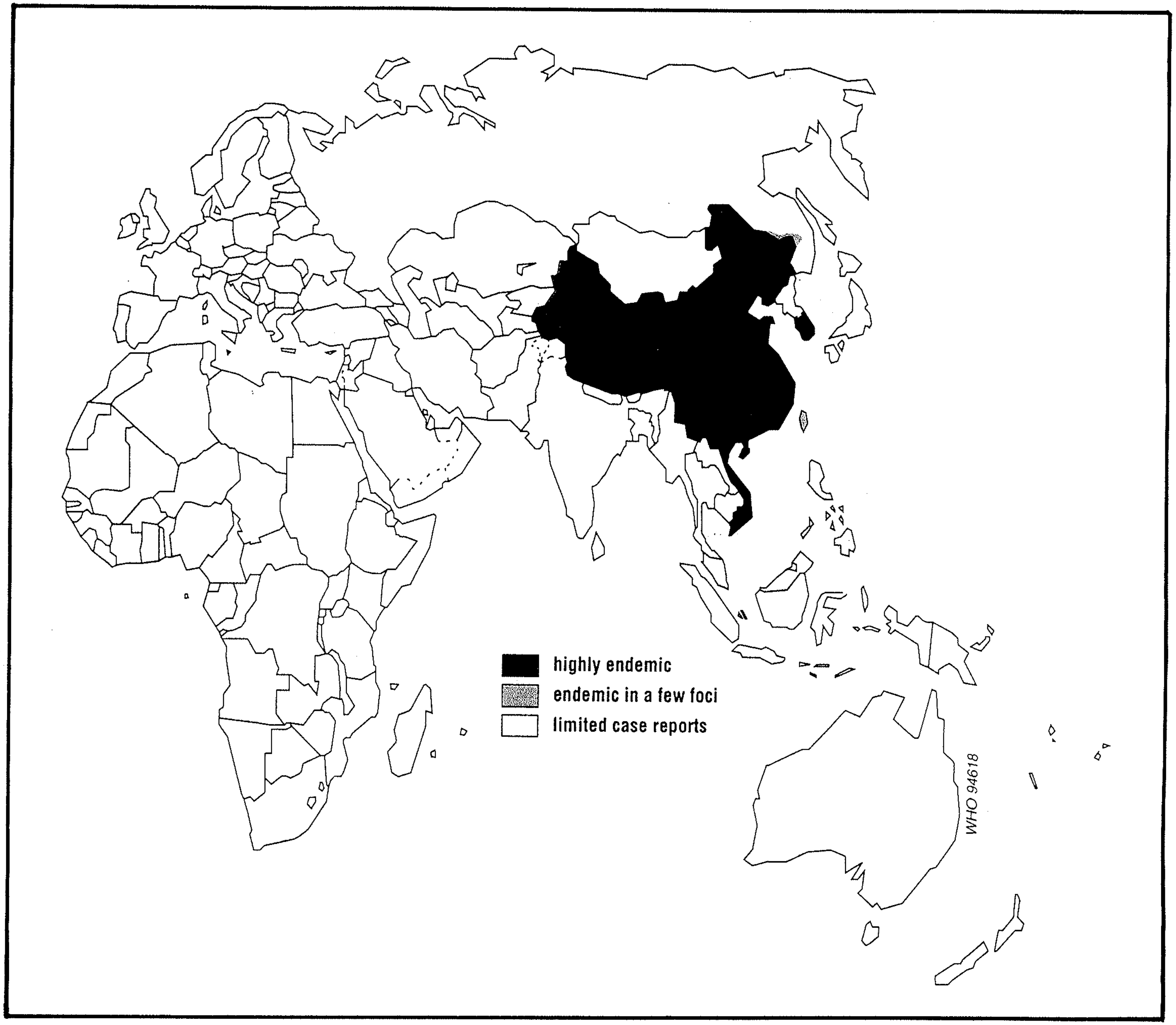


Figure 3. Worldwide distribution of *Clonorchis sinensis*



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).

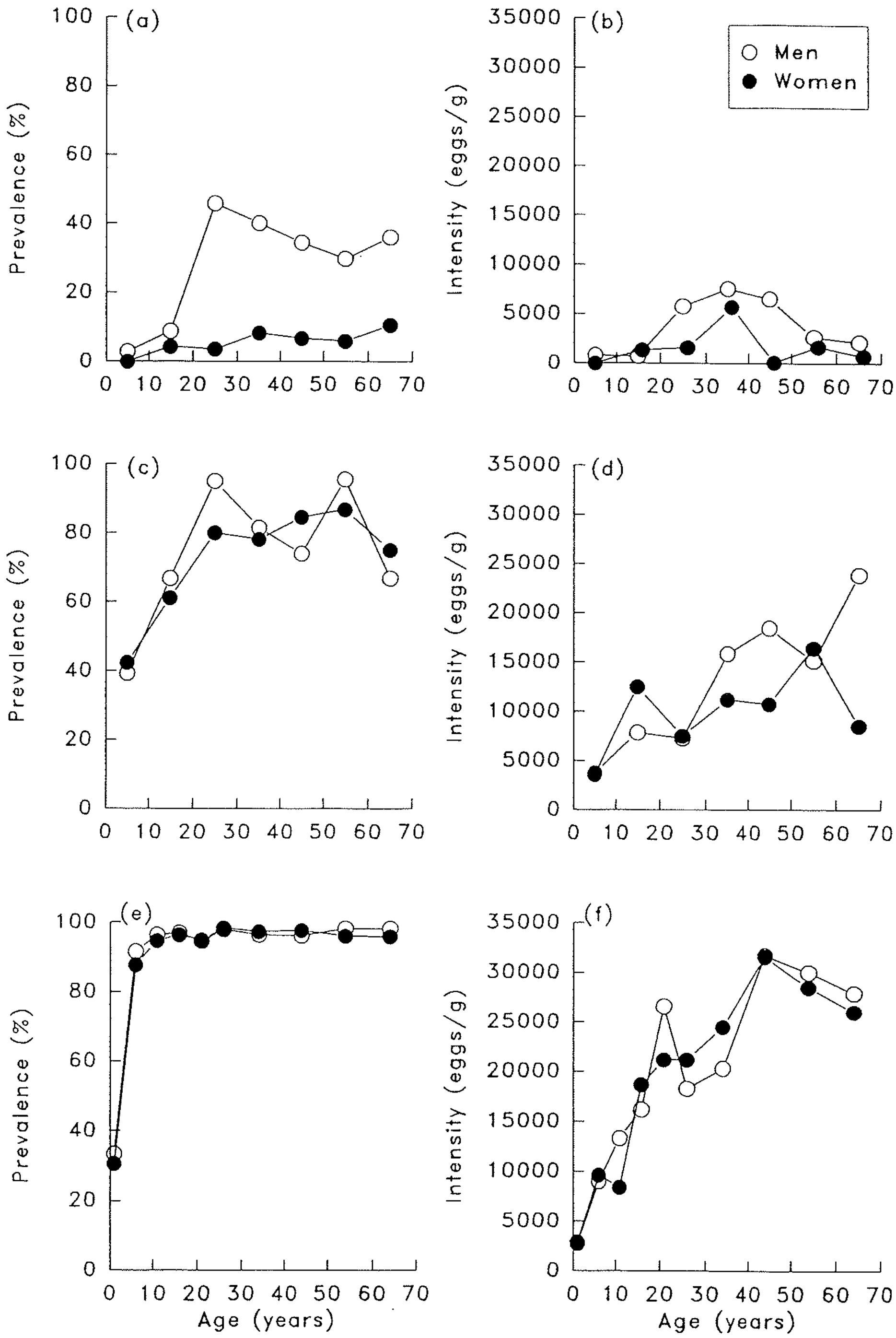
In general, the prevalence of *Clonorchis* infection appears to rise at later ages, and differences in prevalence and intensity between the sexes are more pronounced than those of *Opisthorchis* (Figure 4). For example, in several river basins in the Republic of Korea, large increases in prevalence are 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.

2. Studies of Cancer in Humans

2.1 Descriptive studies

The association between liver fluke infection and the occurrence of cancer in humans has been reviewed extensively (Stewart, 1931; Higginson, 1955; Yamagata & Yaegashi, 1964; Gibson, 1971; Tansurat, 1971; Viranuvatti & Stitnimankarn, 1972; Schwartz, 1980; Flavell, 1981; Juttijudata *et al.*, 1984; Kim, 1984; Chan & Lam, 1987; Haswell-Elkins *et al.*, 1992a,b; Parkin *et al.*, 1993; Sithithaworn *et al.*, 1994).

2.1.1 *Opisthorchis viverrini*

All of the available studies are from Thailand, where there is substantial geographical variation in the prevalence of infection, increasing from the south to the north, the highest rates being observed in Khon Kaen Province in North-east Thailand (see section 1.3.1a). In incidence data from the national cancer registry, the highest frequency was observed in North-east Thailand in 1980–82 (Srivatanakul *et al.*, 1988) and again, especially in Khon Kaen Province, in 1988–91 (Vatanasapt *et al.*, 1993). In the earlier period, the proportionate incidence ratio was 3.1 (95% confidence interval [CI], 2.8–3.5) for cholangiocarcinoma and was 1.2 (95% CI, 1.1–1.4) for hepatocellular carcinoma (Srivatanakul *et al.*, 1988). In Khon Kaen Province around 1985, the age-standardized incidence rate of cholangiocarcinoma was 84.6 per 100 000 per year in men and 36.8 per 100 000 per year in women. Outside of Thailand, the incidence of cholangiocarcinoma shows little variation (range, 0.2–2.8 per 100 000 per year in men, and 0.1–4.8 per 100 000 per year in women) (Parkin *et al.*, 1993). Thus, the incidence in the area of highest incidence in Thailand is at least 40 times as high as that in the area of highest incidence elsewhere.

Within Khon Kaen Province, during the period 1985–88, Vatanasapt *et al.* (1990) observed the highest incidence and mortality rates of liver cancer in three adjacent districts; studies in two of the districts had shown high prevalences of infection and heavy infection (Upatham *et al.*, 1984). Subsequently, Sriamporn *et al.* (1993) showed that there was no difference in the overall prevalence of infection between the districts of highest and lowest incidence of liver cancer within the Province during the period 1988–90; however, 9% of 331 subjects from randomly selected villages in the district of highest incidence had > 10 000 fluke eggs/g of stool, while only 3.7% of 296 subjects in villages in the district of low incidence had the same level of infection.

Srivatanakul *et al.* (1991a) carried out a correlation analysis of liver cancer incidence, titre of antobodies to *O. viverrini* and faecal egg count (determined in healthy volunteers who had been born and resided in the area) in five regions with different frequencies of the two main histological types of liver cancer: Chiang Mai in the north, Nakon Ratchasima and Ubon Ratchathani in the north-east (but not in Khon Kaen Province), Bangkok in the centre and Songkhla in the south. The correlation between the incidence of cholangiocarcinoma and the proportion of subjects with an antibody titre ≥ 1:40 was 0.98 (p = 0.004), and that with faecal egg count was 0.53 (p = 0.35). For hepatocellular carcinoma, which showed little geographical variation in incidence, the correlations were –0.37 (p = 0.54) and 0.02 (p = 0.96), respectively. [The weaker association between cholangiocarcinoma and faecal egg count may reflect the introduction of effective therapy; antibody titre is thought to provide a more valid indicator of past infection, but cross-reactivity with other parasites common in the region may have been involved.]

These studies are summarized in Table 1.

2.1.2 *Opisthorchis felineus*

In the T'umen' region in western Siberia (an area of *O. felineus* endemicity), Shain (1971) related the prevalence of infected people in four subregions as reported by local health centres with the incidence of liver cancer observed in the same period, 1960–69. The correlation computed by the Working Group from the tabulated data was [0.98; $p < 0.05$]. A similar analysis in seven cities within one of the regions confirmed this correlation [0.77]. No information was given on the relative frequency of histological types.

2.2 Case reports and case series

2.2.1 *Opisthorchis viverrini*

All of the available reports are from Thailand. The earliest case reports are of a papillary adenocarcinoma of the liver and an adenocarcinoma of the bile duct (Viranuvatti & Mettiyawongse, 1953) and a retention cyst of the liver caused by opisthorchiasis associated with carcinoma of the liver (Viranuvatti *et al.*, 1955); *O. viverrini* infection was detected at autopsy in each case. Subsequent case series are summarized in Table 2. Among patients from the area in which *O. viverrini* is endemic, cases of cholangiocarcinoma outnumber cases of hepatocellular carcinoma, in contrast to other series.

Cancers other than of the liver have been reported in association with this infection, but no particular type has predominated (Koompirochana *et al.*, 1978; Pungpak *et al.*, 1985).

2.2.2 *Opisthorchis felineus*

Three studies on the presence of *O. felineus* infection in liver cancer cases were conducted in western Siberia (Table 3). One of the regions, T'umen', is reported to be an area of high endemicity. The prevalence of infection in 250 histologically verified cases of liver cancer was 52% in the study of Shain *et al.* (1971). The prevalence of infection in 44 cases of liver cancer detected in 657 autopsies performed in the same region was 95% (Glumov *et al.*, 1974). The first study also reported a higher frequency of cholangiocarcinoma among infected liver cancer cases and a difference in the sex ratios between the two main histological types [no information was provided about the sex ratio of infection].

Table 1. Descriptive studies of *Opisthorchis viverrini* and liver cancer in Thailand

Reference	Area and period of study	Details of cases of liver cancer: Deaths or incidence	Type	Number	Measure of exposure to *O. viverrini*	Number of geographical units	Association	Comments
Srivatanakul *et al.* (1988)	Whole country, 1980–82	Incidence	Liver cancer CCA HCC	3820 523 779	-	10 9 9	Highest PIR for liver cancer (men, 2.0; 95% CI, 1.9–2.2; women, 2.7; 95% CI, 2.4–3.0) observed in Khon Kaen Province in North-east Thailand. Highest PIR (3.1, 95% CI, 2.8–3.5) for CCA observed in North-east Thailand. Corresponding PIR for HCC was 1.2 (95% CI, 1.1–1.4).	
Vatanasapt *et al.* (1993)	Four population-based cancer incidence registries, 1988–91	Incidence	Liver cancer	4314	-	4	Highest incidence for CCA in Khon Kaen Province in North-east Thailand	
Vatanasapt *et al.* (1990)	Khon Kaen Province, 1985–88	Incidence Deaths	Liver cancer Liver cancer	1338 NR	-	20	Highest incidence and mortality rates in three adjacent districts (Chonnabot, Nong Rua and Muncha Khiri), in which other studies showed high prevalences of infection and heavy infection	Rate of total cancers in these areas very high
Sriamporn *et al.* (1993)	Districts with highest (Chonnabot) and lowest (Ban Phang) incidence of liver cancer in Khon Kaen Province, 1988–90	Incidence	Liver cancer	140	Eggs/gram in stool samples from 627 subjects aged ≥30 from randomly selected villages in each district	2	No difference in overall prevalence of infection; 9% of subjects from district in high-incidence area had > 10 000 eggs/g, compared with 3.7% in the other district	No significant difference in age and sex distribution of subjects
Srivatanakul *et al.* (1991a)	Five areas with different frequencies of CCA and HCC, 1980–82, 1983–87, 1988, depending on area	Incidence	CCA HCC		Antibody titre and faecal egg count in about 100 volunteers aged 30–40 in each area	5	Positive correlation between proportion of subjects with antibody titre ≥1:40 and CCA (r = 0.98, p = 0.004). Correlation between eggs/g and CCA was 0.53 (p = 0.35). Corresponding correlations with HCC -0.37 (p = 0.54) and 0.02 (p = 0.96)	No strong or significant correlations between CCA and HBV infection, prevalence of HBsAg carriers, and aflatoxin levels in serum or urine

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; PIR, proportionate incidence ratio; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; NR, not reported

Table 2. Case series of patients with liver cancer associated with *Opisthorchis viverrini* infection in Thailand

Reference	Patients specified as coming from endemic area	Period of study	Cases				
			Method of ascertainment	Type	Number	*O. viverrini* infection	
						No.	%
Bhamrapravati & Virranuvatti (1966)	No	1960–62	Liver biopsy	HCC	251	5	2
				CCA	61	11	18
		1959–61	Autopsy	HCC	33	0	0
				CCA	14	11	79
Chainuvati *et al.* (1976)	Yes	NR	NR	Adenocarcinoma of cystic duct	4	3[a]	75
Koompirochana *et al.* (1978)	No	1954–74	Autopsy	HCC	266[b]	9	3.4
				CCA	108[b]	67	62
Sonakul *et al.* (1978)	No	17 years	Autopsy	HCC	9	From case series with *O. viverrini*	
				CCA	67		
	Yes	3 years	Autopsy	HCC	3	3	100
				CCA	8	8	100
Stitnimankarn *et al.* (1978)	Yes	NR	Liver biopsy	CCA	11	11	100
Pungpak *et al.* (1985)	No	1982–84	Autopsy, liver biopsy, surgery, ascitic fluids	Adenocarcinoma of liver	16	From case series with severe *O. viverrini*	
Riganti *et al.* (1989)	Yes	1969–88	Autopsy	Adenocarcinoma of bile duct	8	From case series with *O. viverrini*	
				HCC	2		

NR, not reported; HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma
[a]By stool examination; all were found to have infection when the ducts were examined histologically.
[b]Combining cases reported to have *O. viverrini* infection and those reported to be without the fluke

Table 3. Prevalence of *Opisthorchis felineus* in case series of liver cancer in western Siberia in the Russian Federation

Reference	Region	Endemicity	Cases				Results
			Method of ascertainment	Total no.	*O. felineus* infection		
					No.	%	
Shain *et al.* (1971)	T'umen'	High	Clinical	250	130	52	Sex ratio (M/F) in uninfected same as expected from literature, i.e. 2–6; sex ratio in infected was reversed [figures not given]. Cancers in uninfected patients mainly HCC; those in infected patients CCA: 4–5 times more frequent than HCC
Glumov *et al.* (1974)	T'umen'	High	Autopsy	44	42	95	35/44 CCA, frequency in infected not given. Prevalence of liver cancer at autopsy 6.7%; 0.7% in another pathology department
Iablokov *et al.* (1980)	Tomsk	Intermediate	Autopsy	103	7	7	In the whole series, 54% HCC and 46% CCA. Four infected cases had CCA; 3 had HCC.

HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma

In a similar study conducted in a region of intermediate endemicity, 7 liver cancers out of 103 detected at autopsy were infected with *O. felineus* (Iablokov *et al.*, 1980). Similar proportions of cases of cholangiocarcinoma (4/47) and hepatocellular carcinoma (3/56) were infected.

2.2.3 *Clonorchis sinensis*

The earliest case reports of primary liver cancer concerned Chinese subjects (Watson-Wemyss, 1919; Bentham, 1920; Nauck & Liang, 1928; Ch'in *et al.*, 1955). Subsequent case series, from Hong Kong and the Republic of Korea and among Asian subjects in the USA, are summarized in Table 4. Cases have also been described in immigrants to North America from China (Schwartz, 1986; Colquhoun & Visvanathan, 1987) and Laos (Drinka & Sheehy, 1985; Sher *et al.*, 1989; Ona & Dytoc, 1991). The only other population in which cases have been reported is that of Japan (Nakashima *et al.*, 1977).

2.3 Case–control studies

2.3.1 *Opisthorchis viverrini*

Kurathong *et al.* (1985) assessed the prevalence of cholangiocarcinoma and hepatocellular carcinoma during 1981–83 in 551 (47%) patients from the north-east (49.8% of those attending a hospital in Bangkok) who agreed to provide stool specimens, on the basis of which they were characterized for the presence of *O. viverrini* eggs. All 551 were screened for hepatobiliary tract diseases. Nineteen of 25 cases of cholangiocarcinoma and 9 of 12 of hepatocellular carcinoma had ova in the stools. The cases were diagnosed by a variety of methods, including ultrasound biopsy and hepatic angiography. The crude prevalence odds ratios were [1.3 (0.5–3.6)] for cholangiocarcinoma and [1.3 (0.3–4.7)] for hepatocellular carcinoma. [Use of controls with other hepatobiliary disease may have biased the results.]

A hospital-based case–control study of cholangiocarcinoma (Parkin *et al.*, 1991) and hepatocellular carcinoma (Srivatanakul *et al.*, 1991b) was carried out in Thailand, in which 103 cholangiocarcinoma patients and 65 hepatocellular carcinoma patients living in and originating from North-east Thailand were recruited in 1987–88 from among patients whose disease was diagnosed sequentially in three hospitals. One control was matched to each case for sex, age (within five years), residence and hospital of recruitment. Controls were selected from among patients affected by a variety of non-malignant diseases, considered not to be related to the consumption of alcohol or tobacco. Infection with *O. viverrini* was assessed in terms of an increase in titre of antibodies to *O. viverrini* in serum as observed by ELISA (Srivatanakul *et al.*, 1985). For cholangiocarcinoma, the matched estimate of the odds ratio obtained from the final multivariate model, including adjustment for consumption of 'sticky' rice and betel-nut chewing, was 5.0 (95% CI, 2.3–11.0). No association was seen with chronic carriage of hepatitis B virus nor with recent aflatoxin intake (Parkin *et al.*, 1991). *O. viverrini* infection was not significantly associated with the risk of developing a hepatocellular carcinoma. The observed odds ratio was 1.7 (0.8–3.7). In a multivariate analysis, there was a strong association with chronic carriage of hepatitis B virus (Srivatanakul *et al.*, 1991b).

Haswell-Elkins *et al.* (1994a) conducted a cross-sectional population-based survey in 1990–91 of subjects aged 25 or more from 46 villages in two districts of Khon Kaen Province

Table 4. Case series of patients with cancer of the liver associated with Chlonorchis sinensis infection

Reference	Location	Period of study	Cases				
			Method of ascertainment	Type	No.	*C. sinensis* infection	
						No.	%
Hou (1956)	Hong Kong	7 years	Autopsy	Adenocarcinoma (21) and mixed type of intrahepatic second-order bile duct tumours	30	30[a]	100
Belamaric (1973)	Hong Kong	1961–66	Autopsy	Adenocarcinoma of intrahepatic bile duct	19	18	95
Chou & Chan (1976)	Hong Kong	1964–73	Autopsy	CCA	50	46	92
Purtilo (1976)	Hong Kong	NR	Autopsy	CCA HCC	7 10	From series of subjects with *C. sinensis* infection	
Ho (1980)	Hong Kong	Before 1976[b]	Autopsy	Mucoepidermoid carcinoma of the liver	2	0	0
Koo *et al.* (1982)	Hong Kong	1976–80	Laparotomy	Mucoepidermoid carcinoma of the bile duct	3	3	100
Kim *et al.* (1974)	Republic of Korea	1962–72	Autopsy				
	Seoul			HCC	339	28	8.3
				CCA	33	8	24.2
	Pusan			HCC	84	15	17.9
				CCA	21	13	61.9
Choi *et al.* (1988)	Republic of Korea	7 years	Surgery	CCA	16	10	62.5
Choi *et al.* (1989)	Republic of Korea	4 years	Surgery	CCA HCC	20 4	From series of subjects with *C. sinensis* infection	
Strauss (1962)	USA, Asian subjects	1945–60	Surgery	Hepatomas	5	From series of subjects with *C. sinensis* infection	

NR, not reported; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma
[a]Clonorchiasis; 28 (93%) cases were found to have flukes in the bile duct.
[b]Koo *et al.* (1982)

and 39 villages in Maha Sarakham Province, within the endemic area of *O. viverrini* infection in North-east Thailand. Stool specimens were obtained from 7727 subjects (participation rate, 72%) in Khon Kaen Province and 4585 subjects (participation rate, 79%) in Maha Sarakham Province after a health education programme about liver fluke infection. A 15% random sample of 1807 uninfected and lightly infected (< 3000 fluke eggs/g) subjects and all subjects with higher intensities of infection were invited to undergo an ultrasound examination. Among the 78% of subjects who complied, 44 had evidence of cholangiocarcinoma without overt symptoms. In nine of these, the diagnosis was corroborated by endoscopic retrograde cholangiopancreatography; a further six who died before they could undergo the procedure or who declined it were strongly suspected to have cholangiocarcinoma. Thus, there was a total of 15 cases, seven in patients who died with jaundice and hepatomegaly in 1991–92. Among 410 uninfected subjects, one case occurred. The multivariate prevalence odds ratios, accounting for age, sex and district of residence, were 1.7 (95% CI, 0.2–16.3) for subjects with up to 1500 fluke eggs/g, 3.2 (0.4–30) for subjects with 1501–6000 eggs/g and 14 (1.7–119) for more heavily infected subjects.

2.3.2 *Clonorchis sinensis*

In a consecutive series of 1484 autopsies in a single hospital in Hong Kong during the period 1964–66, clonorchiasis was found on gross examination in 11 of 17 (65%) cases of cholangiocarcinoma and in 24 of 83 (29%) cases of hepatocellular carcinoma. The expected proportions infected, on the basis of the whole series and adjusted for age and sex, were 38 and 35%, respectively. [The odds ratios, adjusted for age and sex, calculated by the Working Group, were 3.1 (95% CI, 1.1–8.4) for cholangiocarcinoma and 0.73 (0.45–1.2) for hepatocellular carcinoma] (Gibson, 1971).

Kim *et al.* (1974) studied records of autopsy and surgical specimens from one hospital in an area of low prevalence of *C. sinensis* (Seoul) and one hospital in an area of high prevalence (Pusan) in the Republic of Korea during the period 1961–72. In the area of low prevalence, a total of 386 histologically proven cases of primary liver cancer were identified among 1447 subjects with liver disease, and in the area of high prevalence, there were 109 cases of primary liver cancer among 396 subjects with liver disease. *C. sinensis* infection was determined by examination of liver tissue or stool samples. Comparison of cases of liver cancer with subjects with liver disease in whom cancer was not found showed a weak positive association between the cancer and *C. sinensis* infection [odds ratio, 1.7; 95% CI, 1.2–2.3]. The corresponding odds ratio for cholangiocarcinoma, based on 54 cases, was [6.5 (95% CI, 3.7–12)] and that for hepatocellular cancer, based on 423 cases, was [1.2, 0.80–1.7].

In Pusan, Republic of Korea, one of the areas of highest prevalence of *C. sinensis* infection, the occurrence of clonorchiasis was determined in stool specimens from 206 of a consecutive series of 368 cases of primary liver carcinoma diagnosed mainly in two hospitals during the period 1963–74 (Chung & Lee, 1976). [The Working Group noted that as one of these hospitals had been included in the study of Kim *et al.* (1974), there is some overlap with that study.] The control series comprised 559 subjects admitted to these hospitals with diseases other than of the liver; again, the presence of clonorchiasis was determined from stool specimens [no further details]. The crude odds ratio for cholangiocarcinoma, based on 36 cases, was [6.0 (95% CI, 2.8–13)]; the odds ratio was unchanged after adjustment for age and sex. The crude odds ratio for hepatocellular carcinoma, based on 170 cases, was 1.1 (95% CI, 0.65–1.7).

These studies are summarized in Table 5.

3. Studies of Cancer in Animals

3.1 Infection with *Opisthorchis viverrini* alone

Hamster: In a histopathological study, a group of 30 male Syrian golden hamsters, three to four weeks of age, were infected with 100 metacercariae of *O. viverrini* by intragastric intubation. A group of 18 untreated hamsters served as controls. Five treated and three control animals were killed at 3, 7, 15, 30, 45 and 154 days after infection. The early pathological changes consisted of an acute inflammatory reaction involving the second-order bile ducts and portal connective tissue as well as focal coagulation necrosis of the liver lobules. As the liver flukes developed into adults (after 28 days), they induced hyperplasia, 'adenomatous formation' of the bile-duct epithelium, ductular proliferation and multilobular cirrhosis (Bhamarapravati *et al.*, 1978). [The Working Group noted the short duration of the study in relation to the lifespan of the animals, as it is possible that tumours could have developed in the animals if they had been allowed to live.]

As part of combination experiments (see section 3.2), a control group of 50 male Syrian golden hamsters, six to eight weeks of age, was given 50 *O. viverrini* metacercariae intragastrically and followed for 76 weeks. No bile-duct carcinoma was found (Flavell & Lucas, 1982, 1983).

Other groups of hamsters administered *O. viverrini* metacercariae alone as controls in combination experiments also had no bile-duct tumours after observation periods ranging from 22 to 45 weeks (Thamavit *et al.*, 1978, 1987a,b, 1988a,b, 1992a,b, 1994). In a further study (Thamavit *et al.*, 1993), a group of 18 female Syrian golden hamsters, six to eight weeks of age, received 60 *O. viverrini* metacercariae by intragastric intubation; 15 females received no treatment. Ten treated animals developed cholangiofibrosis and two developed cholangiocarcinomas within 38 weeks. No tumour was observed among controls. The difference in tumour rate was not significant.

A total of 150 male and 150 female Syrian hamsters, six to eight weeks of age, were divided into four groups and were infected monthly for 10 months with 0 (20 males and 20 females), 13 (40 males and 40 females), 25 (40 males and 40 females) or 50 (50 males and 50 females) *O. viverrini* metacercariae per intragastric intubation. Animals were then maintained on basal diet until they were killed at the end of week 52. Ten monthly intragastric applications of 0, 13, 25 or 50 metacercariae resulted in pronounced proliferative and inflammatory lesions involving the first- and second-order ducts, in response to the presence of adult worms. Cholangiofibrosis was seen, but no neoplastic lesion was evident after one year (Thamavit *et al.*, 1995). [The Working Group noted the short duration of the study.]

Table 5. Case–control studies of the association between *Chlonorchis sinensis* infection and cholangiocarcinoma and hepatocellular carcinoma

Location	Period of study	Type of cancer	Cases: Method of ascertainment	Cases: No.	Controls: Definition	Controls: No.	Method of assessing *O. sinensis* infection	RR	95% CI	Reference
Hong Kong	1964–66	CCA HCC	Autopsy	17 83	Autopsied subjects without CCA or HCC	1384	Gross examination at autopsy	3.1[a] 0.73[a]	0.13–8.4 0.45–1.2	Gibson (1971)
Republic of Korea, Seoul and Pusan	1961–72	CCA HCC	Autopsy and surgery of subjects with liver disease	54 423	Subjects coming to autopsy or surgery with liver disease in whom cancer was not found	1348	Examination of liver tissue or stool samples	6.5 1.2	3.7–12 0.80–1.7	Kim *et al.* (1974)
Republic of Korea, Pusan	1963–74	CCA HCC	Consecutive series of patients diagnosed mainly in two hospitals	36 170	Subjects admitted to these hospitals with diseases other than of the liver	559	Examination of stool samples	6.0 1.1	2.8–13 0.65–1.7	Chung & Lee (1976)

Relative risks and 95% confidence intervals calculated by the Working Group. CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma. The two last studies partially overlap.
[a]Adjusted for age and sex

3.2 Infection with *Opisthorchis viverrini* in combination with administration of known carcinogens

3.2.1 N-*Nitrosodimethylamine*

Hamster: Male Syrian golden hamsters, aged three to four weeks, were divided into four groups: 18 animals served as untreated controls; 21 animals received 0.0025% [25 mg/L] *N*-nitrosodimethylamine (NDMA) in the drinking-water starting from seven to eight weeks of age; 18 animals were infected with 100 *O. viverrini* metacercariae by intragastric intubation; and 21 animals were infected with *O. viverrini* and, four weeks later (as soon as the parasitic eggs were detected in faeces), received NDMA in the drinking-water. NDMA treatment was discontinued after 10 weeks, and animals were killed eight weeks thereafter (at 23 weeks). All of the animals that received NDMA and were infected developed cholangiocarcinoma and cholangiofibrosis. No such tumour was observed in the group that received either NDMA or parasite alone [$p < 0.001$; Fisher exact test], although cholangiofibrosis was found in some NDMA-treated animals (Thamavit *et al.*, 1978).

A total of 130 male Syrian golden hamsters, six to eight weeks of age, were divided into three groups: 50 animals were infected with 50 *O. viverrini* metacercariae by intragastric intubation, followed 41 days later by a single oral dose of 1.6 mg NDMA; 30 animals received a single oral dose of 1.6 mg NDMA on day 41; and 50 animals were infected with 50 *O. viverrini* metacercariae. Animals were maintained for 70 weeks or were killed when moribund. Cholangiocarcinomas developed in 5/50 infected animals given NDMA at latent periods of 18, 21, 29 (two animals) and 42 weeks after NDMA treatment. No malignant bile-duct tumour was found in any of the hamsters given either NDMA or metacercariae alone, but benign cystic cholangiomas [numbers not specified] were found commonly in these animals (Flavell & Lucas, 1982). [The Working Group noted that the authors did not report cholangiofibrosis in any of the groups. They also noted the single treatment and small dose of the carcinogen.]

A total of 176 male Syrian golden hamsters, six to eight weeks of age, were divided into four groups: 50 animals were infected with 50 *O. viverrini* metacercariae by intragastric intubation, followed 41 days later by a single oral dose of 1.6 mg NDMA; 46 animals received a single oral dose of 1.6 mg NDMA, followed 96 h later by infection with 50 *O. viverrini* metacercariae; 30 animals received a single oral dose of 1.6 mg NDMA; and 50 animals were infected with 50 *O. viverrini* metacercariae. Animals were killed when in poor condition or at the end of the 490-day experimental period. Mortality was highest in infected animals that received NDMA. Cholangiocarcinomas were observed in 5/50 animals (10%) that were first infected and then received NDMA and in 9/46 animals (20%) that received NDMA and were then infected. The difference between these two groups was not significant [Fisher's exact test]. None of the animals given NDMA alone or only infected with parasites developed malignant bile-duct tumours. The mean tumour latency was 249 days (range, 124–346 days) for the group that was first infected and then received NDMA, and that for the group that first received NDMA and were then infected was 308 days (range, 184–393 days); the difference was not significant. Tumours were most frequently found in the right liver lobe, the lobe in the hamster that also contains the largest proportion of *O. viverrini* worms (Flavell & Lucas, 1983).

A total of 280 male Syrian golden hamsters, three to four weeks of age, were divided into four main groups: one remained untreated; others were infected with 12, 25, 50 or 100 *O. viverrini* metacercariae by intragastric intubation; further groups were administered NDMA at 3, 6 or 12 mg/L in the drinking-water at four to five weeks of age for 10 weeks; and others wre infected with 12, 25, 50 or 100 metacercariae two weeks before administration of NDMA at 3, 6 or 12 mg/L in the drinking-water for 10 weeks. All animals were then maintained on basal diet until the end of the experiment at week 40, at which time they were killed. Only 2/17 animals (12%) in the group that received NDMA at 12 mg/L had detectable cholangiocarcinomas. No neoplastic lesion was seen in those that received NDMA at 6 mg/L or 3 mg/L, in those only infected or in untreated controls. In contrast, significant increases in the incidence of cholangiocarcinomas were seen in animals given both NDMA and metacercariae: 14/15, 10/17, 13/19, 7/10 [$p < 0.01$; Fisher's exact test]; and cholangiofibrotic lesions were observed (Thamavit *et al.*, 1987a).

Nitrite and aminopyrine can form NDMA in the stomach under certain conditions. A total of 150 male Syrian hamsters, three to four weeks of age, were divided into eight groups: one group was untreated; a second received 0.1% sodium nitrite in the drinking-water; one received 0.1% aminopyrine in the drinking-water; one received sodium nitrite and aminopyrine in the drinking-water; one was infected with 100 *O. viverrini* metacercariae by a single intragastric intubation; one was similarly infected and four weeks later received sodium nitrite in the drinking-water for 8 or 10 weeks; one was infected and four weeks later received aminopyrine in the drinking-water for 8 or 10 weeks; and the last was infected and four weeks later received sodium nitrite and aminopyrine in the drinking-water for 8 or 10 weeks. Hamsters that received the eight-week drinking-water treatment were killed 12 weeks later, and animals that received the treatment for 10 weeks were killed 20 weeks later. Combined administration of nitrite and aminopyrine for 8–10 weeks resulted in development of two hepatocellular nodules, seven cholangiofibrotic lesions and three cholangiocellular carcinomas. Prior infection with *O. viverrini* metacercariae induced inflammatory and proliferative changes in the livers of infected hamsters and was associated with a significant increase in the incidences of hepatocellular nodules (8; $p < 0.05$), cholangiofibrosis (18; $p < 0.05$) and cholangiocarcinomas (14; $p < 0.01$) (Thamavit *et al.*, 1988a).

A total of 105 male Syrian hamsters, six to eight weeks of age, were divided into four groups: 50 animals received a single intraperitoneal injection of 20 mg/kg bw NDMA, followed 19 days later by infection with 80 *O. viverrini* metacercariae by single intragastric intubation; 25 animals received the intraperitoneal dose of NDMA only; 15 animals were infected with *O. viverrini* only; and 15 animals served as untreated controls. Hamsters were killed when they became moribund or at the end of the experiment at 45 weeks. Among the 43 animals treated with both NDMA and *O. viverrini*, 19 developed cholangiocarcinomas, 40 developed cholangiofibrosis, 15 developed mucinous cystadenomas, 2 developed hepatocellular carcinomas and 42 developed hepatocellular nodules. Although 17/20 (85%) of the hamsters treated with NDMA alone developed hepatocellular nodules, with an average of 3.0 nodules per animal, there was an average of 9.5 nodules per animal in the combined treatment group. No lesion was observed in untreated controls, and 2/15 animals only infected with the parasite developed cholangiofibrosis. The difference in incidence of cholangiocarcinomas between the combined group (19/45) and the group only infected with *O. viverrini* (0/20) was significant ($p < 0.001$; Fisher's exact test) (Thamavit *et al.*, 1994).

3.2.2 N-*Nitrosodiethylamine*

Hamster: A total of 180 female Syrian hamsters, three to four weeks of age, were divided into eight groups: 20 animals served as untreated controls; 20 animals were infected by gastric intubation with 60 *O. viverrini* metacercariae only; groups of 20–30 animals were infected with 60 *O. viverrini* metacercariae, followed four weeks later by administration of 10, 20 or 40 mg/L *N*-nitrosodiethylamine (NDEA) in the drinking-water for 12 weeks; and groups of 20–25 animals were administered only 10, 20 or 40 mg/L NDEA in the drinking-water for 12 weeks. The animals were killed at week 32. Infection with 60 metacercariae four weeks before administration of 20 or 40 mg/L NDEA resulted in significantly ($p < 0.01$) increased incidences of hepatocellular nodules in the groups also receiving NDEA (12/19 and 23/25, with 2.5 and 7.1 nodules/animal) when compared with the groups that received NDEA alone (3/19 and 9/21 with 0.2 and 0.9 nodules/animal). A high incidence of cholangiofibrosis was seen in animals receiving the combined treatment (Thamavit *et al.*, 1987b).

In a further study, 95 female Syrian golden hamsters, six to eight weeks of age, were divided into five groups: a group of 20 animals received a single intraperitoneal injection of 150 mg/kg bw NDEA dissolved in saline, and two groups of 20 animals each received NDEA followed 18 days later by infection with 50 or 100 *O. viverrini* metacercariae by intragastric intubation; 20 animals received 100 metacercariae without prior treatment with NDEA, and 15 animals were untreated. The animals were killed at the end of week 41. Infection with either 50 or 100 metacercariae of *O. viverrini* after NDEA injection resulted in significantly ($p < 0.01$) enhanced incidences of hepatocellular nodules/animal: 4.3 and 6.8 *versus* 1.4 in animals treated with NDEA alone (Thamavit *et al.*, 1992a).

3.2.3 N-*Nitrosodihydroxydi*-n-*propylamine*

Hamster: A total of 75 male Syrian golden hamsters, three to four weeks of age, were divided into four groups: 25 animals were infected with 100 metacercariae of *O. viverrini* per animal by gastric intubation and two and four weeks later received intraperitoneal injections of 1000 mg/kg bw *N*-nitrosodihydroxydi-*n*-propylamine (NDHDPA); 20 animals were treated with NDHDPA alone; 15 animals were infected with *O. viverrini* alone; and 15 animals served as untreated controls. Animals were killed at week 22. In the group treated only with NDHDPA, 2/20 animals had basophilic hepatocellular foci. Among 19 animals receiving combined treatment with NDHDPA and *O. viverrini*, six developed cholangiocarcinomas [$p = 0.02$], 18 developed cholangiofibrosis [$p = 0.001$] and nine developed hepatocellular nodules [$p = 0.002$] [all Fisher's exact test]; all 19 had hepatocellular basophilic foci, and eight had atypical proliferation of the pancreatic duct. Two of 20 animals given NDHDPA alone had hepatocellular basophilic foci (Thamavit *et al.*, 1988b).

A total of 100 male Syrian hamsters, three to four weeks of age, were divided into four groups: 10 animals served as untreated controls; 20 animals were infected with 80 *O. viverrini* metacercariae by intragastric intubation; 30 animals received three intraperitoneal injections of 500 mg/kg bw NDHDPA at weeks 16, 17 and 18; and 40 animals were infected with 80 *O. viverrini* and received similar NDHDPA treatment. Animals were maintained on basal diet until they were killed, at week 52, when they were examined histologically. Cholangiocarcinomas occurred in 8/16 animals in the combined treatment group and 0/16 in that receiving NDHDPA alone [$p = 0.001$; Fisher's exact test]. Liver foci were seen in 16/16 hamsters in the combined treatment group and in 14/16 of those given NDHDPA, but the group receiving the combined treatment had a significantly increased number of foci per cm^2 (23.4 ± 7.5 *versus* 3.5 ± 2.6; $p < 0.001$) (Moore *et al.*, 1991).

3.3 Infection with *Opisthorchis viverrini* in combination with administration of other modifying factors

Hamster: A total of 115 male Syrian golden hamsters, six to eight weeks of age, were divided into four groups: 50 animals received five administrations of 60–80 *O. viverrini* metacercariae by intragastric intubation at weeks 0, 8, 16, 24 and 32 and 300 mg/kg bw praziquantel suspended in corn oil five weeks after the time of each administration; 30 animals were given praziquantel alone; and 20 animals received parasites alone, each by the above schedule; 15 animals served as untreated controls. Many of the animals infected with *O. viverrini* metacercariae became moribund and died (16/50 in the combined group; 8/20 in the group receiving infection alone). Surviving animals were killed at the end of week 40. Of the 34 surviving hamsters that received the combined treatment, one developed a cholangiocarcinoma, seven had cholangiofibrosis and one had a hepatocellular nodule. No such lesions were found in hamsters that received the drug alone, but 6/12 surviving hamsters that received infection alone developed cholangiofibrosis (Thamavit *et al.*, 1992b). [The Working Group noted the high mortality in the groups administered *O. viverrini* and the large total number of metacercariae administered.]

A total of 205 female Syrian golden hamsters, six to eight weeks old, were divided into seven groups of 25–40 animals each: three groups received two intraperitoneal injections of 1000 mg/kg bw NDHDPA dissolved in saline at two-week intervals; two weeks later, they were infected with 60 *O. viverrini* metacercariae by intragastric intubation and, at 4, 12 or 20 weeks, received a single dose of 250 mg/kg bw praziquantel suspended in corn oil by intragastric intubation. Two further groups received NDHDPA and *O. viverrini* by the same schedule, but with no praziquantel. One group received injections of saline at two-week intervals, followed two weeks later by infection with *O. viverrini*; another received the saline injections alone. The animals were maintained on basal diet and killed at the end of week 38. Of infected animals given NDHDPA, 16/16 developed cholangiofibrosis, 8/16 developed cholangiocarcinomas (2/18 in the group treated only with *O. viverrini* [$p = 0.015$; Fisher's exact test]) and 16/16 developed hepatic nodules with a multiplicity of 13.6 nodules/cm^2. Praziquantel administration at 4 or 12 weeks reduced the incidences of cholangiocarcinoma to 4/22 and 6/22, respectively. Praziquantal also reduced the multiplicity but not the incidence of hepatocellular nodules (3.6 nodules/cm^2 and 7.4 nodules/cm^2, respectively), but one animal in each of these groups also had a hepatocellular carcinoma. Cholangiofibrosis occurred in all animals treated with NDHDPA and *O. viverrini* plus praziquantel, except in those treated four weeks after infection, of which only 8/22 had cholangiofibrosis (Thamavit *et al.*, 1993).

3.4 Infection with *Opisthorchis felineus*

No data were available to the Working Group.

3.5 Infection with *Clonorchis sinensis* alone

3.5.1 *Rat*

As part of a combination study (see section 3.6.1), a control group of 25 male Wistar albino rats, 8–10 weeks of age, was administered 50 *Clonorchis sinensis* metacercariae by intragastric intubation. A few hepatic necrotic foci and mild inflammatory cell changes were seen in animals from each group killed at 4, 8, 12, 16, 20, 24 and 28 weeks after infection. Neither bile-duct lesions nor liver tumours were observed (Park, 1989). [The Working Group noted the short duration of the study and the inadequate reporting.]

In a further combination study, a control group of 10 male Fischer 344 rats, six weeks old, were each infected with 60 *C. sinensis* metacercariae by intragastric intubation and killed after 40 weeks. The infected animals developed cholangiocellular lesions, including bile-duct proliferation, periductal inflammation, fibrosis with occasional mucinous metaplasia, particularly at the main duct, and extensive areas of ductular proliferation. No tumour was observed (Jang *et al.*, 1990). [The Working Group noted the short duration of the study.]

3.5.2 *Cat*

Three cases of cholangiocarcinoma associated with *C. sinensis* infection were reported in cats (*Felis catus*) (Hou, 1964). Two of the cases were found at necropsy in two approximately four-year-old, well-developed, well-nourished cats out of a total of 215 obtained at random. The two cats harboured 150 and 200 adult *C. sinensis* in the liver. The third case was also in a four-year-old cat, which was one of 26 infected experimentally by feeding a diet of fish (*Ctenopharyngodon idellus*, *Hypophthalmichthys nobilis* and *Mylopharyngodon aethiops*) flesh containing metacercarial cysts of *C. sinensis* for 28 feedings. The animal died of bronchopneumonia; 105 *C. sinensis* were recovered from the bile ducts. The authors reported that the histopathological features of cholangiocarcinoma in the three cats were similar to those of many forms of bile-duct cancer found in humans infected with *C. sinensis* (Hou, 1956).

3.5.3 *Dog*

Cholangiocarcinoma associated with *C. sinensis* infection was also reported in one well-developed, well-nourished eight-year-old female chow dog, which had suffered from abdominal enlargement for an unknown period before death (Hou, 1965a). The histopathological features of the cholangiocarcinoma were reported to be similar to those of a form of bile-duct cancer found in humans infected with *C. sinensis* (Hou, 1956).

3.6 Infection with *Clonorchis sinensis* in combination with administration of known carcinogens

3.6.1 *Aflatoxin B_1*

A total of 75 male Wistar albino rats, 8–10 weeks old were divided into three groups: 25 rats were fed aflatoxin B_1 at 1 mg/kg diet for 12 weeks; 25 rats were infected by administration of 50 *C. sinensis* metacercariae by intragastric intubation; and 25 animals were infected with *C. sinensis* and fed aflatoxin B_1 in the diet concomitantly. Three rats from each group were killed at four-week intervals up to 28 weeks after the beginning of treatment. Well-differentiated hepatocellular carcinomas were detected in two of three rats given the combined treatment and alive at 28 weeks; such tumours were not seen in rats treated with aflatoxin B_1 alone and killed at the same intervals (Park, 1989). [The Working Group noted the inadequate reporting of the study and the small comparison groups in the serial killings.]

3.6.2 N-*Nitrosodimethylamine*

Rat: A total of 101 male Fischer 344 rats, six weeks of age, were divided into six groups: 20 animals were each infected with 60 *C. sinensis* metacercariae by single intragastric intubation four weeks before receiving NDMA at 25 mg/L in the drinking-water for eight weeks; 20 animals were infected with *C. sinensis* while receiving NDMA at 25 mg/L in the drinking-water for eight weeks; 20 animals were infected with *C. sinensis* one week after NDMA treatment; 19 animals received NDMA in the drinking-water alone for eight weeks; 10 animals were infected with *C. sinensis* alone; and 12 animals served as untreated controls. The animals were killed at week 40, and all were found to have heavy helminthic loads. Livers were examined immunohistochemically for foci of the placental form of glutathione *S*-transferase. Animals infected before NDMA administration had significantly ($p < 0.05$) increased numbers of foci. No such effect was seen when animals were infected with *C. sinensis* during or after exposure to NDMA (Jang *et al*., 1990).

Hamster: A total of 48 Syrian golden hamsters [sex unspecified], three to four weeks old, were divided into four groups: 12 animals received NDMA at 15 mg/L in the drinking-water for eight weeks and were given 10 metacercariae of *C. sinensis* suspended in saline by intragastric intubation seven days after the beginning of NDMA administration; 12 animals received the NDMA treatment alone; 12 received the helminthic treatment alone; and 12 animals served as untreated controls. After 11 weeks, 6/8 (75%) infected animals given NDMA developed cholangiocarcinomas, 8/8 developed cholangiofibrosis and 8/8 developed cholangiofibroma. Of the 12 animals given NDMA alone, two developed cholangiofibrosis and cholangiofibroma; of those given the helminth alone, 5/12 developed cholangiofibrosis. No lesions were observed in the 12 untreated controls (Lee *et al*., 1993).

A total of 90 Syrian golden hamsters [sex unspecified], weighing 50–60 g, were divided into six groups of 15 animals each: one group received NDMA at 15 mg/L in the drinking-water for four weeks, followed one week later by administration of 15 metacercariae of *C. sinensis* suspended in saline by intragastric intubation; five weeks later the animals received oral administrations of 200 mg/kg bw praziquantel daily for three days. Another group was similarly infected with *C. sinensis* metacercariae but was treated with praziquantel for three days before treatment with NDMA. A further group received concomitant administration of NDMA and infection with *C. sinensis*. One control group received NDMA and another was infected with the helminth only. A final group served as untreated controls. At the end of 13 weeks, the group that had received concomitant treatment with NDMA and *C. sinensis* had 11/15 cholangiocarcinomas, 3/15 cholangiofibromas and 1/15 cholangiofibroses. In the group infected one week after NDMA treatment and given praziquantel, 3/15 had cholangiocarcinomas, 3/15 had cholangiofibromas and 6/15 had cholangiofibroses. In the group with combined treatment but given praziquantel three days before NDMA, 11/15 animals developed cholangiofibroses. In the group given NDMA alone, 4/15 animals had cholangiofibroma and 5/15 had cholangiofibroses; and in the group receiving only helminthic infection, 12/15 animals developed cholangiofibroses. No cancerous or precancerous lesion of the bile duct was found in the untreated control group (Lee *et al.*, 1994).

3.6.3 *2-Acetylaminofluorene*

Hamster: Groups of 50 and 60 female Syrian golden hamsters, 8–10 weeks old, received 0 or 40 *C. sinensis* metacercariae per animal orally and were fed diets containing 0.03% 2-acetylaminofluorene for 40 weeks. After this time, all surviving animals were fed normal diets without carcinogen. Small numbers of animals from both groups were killed every three to four weeks from 0 up to 54 weeks, at which time the experiment was terminated. In animals that lived beyond 25 weeks, the incidence of cholangiocarcinomas was significantly ($p < 0.05$) higher in the infected group (11/14 animals) than in the uninfected group (6/17 animals). Metastases to other organs were observed only in infected animals with cholangiocarcinomas. The first bile-duct tumours were noted at 25 weeks in the infected group and at 35 weeks in the uninfected group (Iida, 1985).

4. Other Data Relevant for Evaluation of Carcinogenicity and its Mechanisms

4.1 Pathology of infection

4.1.1 *Humans*

(*a*) *Opisthorchis viverrini*

Tansurat (1971) described the detailed pathological features of infection with *O. viverrini* on the basis of 70 autopsied cases in Thailand. In early infections, there was no epithelial hyperplasia or fibrous proliferation. In chronic infections, there was proliferation of epithelial cells with formation of glandular acini, similar to the adenomatous changes in clonorchiasis, and there were varying degrees of periductal fibrosis. Enlargement of the liver is observed in most cases of opisthorchiasis, especially in cases of massive infection. The weight of the liver in massive infections is more than double the normal (3000–3500 g); the maximal weight recorded was 4000 g.

The major microscopic changes (Riganti *et al.*, 1989) are confined to the large and medium-sized bile ducts where the flukes are harboured. The cellular infiltrates consist of lymphocytes, monocytes, eosinophils and some plasma cells. Dilatation of the bile ducts, hyperplasia, desquamation and proliferation of the bile-duct lining cells, glandular formation and fibrosis of the periductal connective tissue of the walls are the commonest features. The gross and microscopic characteristics of human opisthorchiasis in 22 adults and seven children were similar, and the pathological changes were well established within 7–15 years after *O. viverrini* infection; however, dilatation of the gall-bladder, chronic cholecystitis and carcinoma were found only in adults.

In chronic and heavy infections, various degrees of cellular infiltration are caused by superimposed bacterial infection. This may result in suppurative cholangitis, and the infection may extend into the parenchyma of the liver tissue, causing cholangiohepatitis with abscess formation. Of 70 cases of advanced opisthorchiasis seen at autopsy, 10 showed multiple abscesses in the liver. The abscesses varied in diameter, from 5 to 10 mm; some ruptured into the right pleural cavity, and in some infections the lower lobe of the right lung was involved (Prijyanonda & Tandhanand, 1961).

In heavy infections with *Opisthorchis*, adult parasites are always discovered in the gall-bladder, the common bile duct and the pancreatic duct (Pungpak *et al.*, 1985, 1987). As in the large and medium-sized bile ducts, the parasites give rise to chronic cholecystitis. When there is superimposed bacterial infection, empyema of the gall-bladder may result. No stone formation was seen, however, either in the bile ducts or in the gall-bladder in one series of 70 cases at autopsy (Tansurat, 1971) or in another series of 154 cases (Koompirochana *et al.*, 1978). This finding is in contrast to that seen for clonorchiasis, in which cholelithiasis is one of the most serious complications (Rim, 1986). A number of biliary tract abnormalities associated with moderate to heavy *O. viverrini* infection were demonstrated by ultrasonography (Elkins *et al.*, 1990; Mairiang *et al.*, 1992). According to Mairiang *et al.* (1993), abnormal findings seen at ultrasonography improved dramatically after treatment with praziquantel.

(*b*) *Opisthorchis felineus*

Hepatic lesions produced by *O. felineus* are similar to those caused by *O. viverrini*. In the course of their development, they initiate inflammatory and proliferative changes of the biliary epithelium, which continue after the worms have matured and are accompanied by fibrosis of the distal biliary ducts. If the infection is intensified by continued exposure, the pathological process may extend to the bile ducts and gall-bladder and result in cirrhosis. The degree of pathogenicity and clinical involvement depends largely on the number of parasites and the duration of infection. Usually, small numbers of worms do not cause serious damage and do not give rise to clinical signs. In the Russian Federation, many apparently healthy people have been found to be infected; however, their worm burden was light, with an average of no more than 200 eggs/g faeces (Bronshtein *et al.*, 1991). When several hundred or thousand worms are present, severe damage to the liver and pancreas can occur (Rim, 1982a).

Hyperplasia of the epithelium of the larger bile ducts with cholangitis is much commoner. Advanced hepatic cirrhosis is rare. Occasionally, carcinoma of the bile ducts or of the pancreas, with metastases into the epigastric lymph nodes, is responsible for death (Faust *et al.*, 1970).

(*c*) *Clonorchis sinensis*

Most of the information on the pathological manifestations of *C. sinensis* comes from Hou's (1955) study of 500 autopsy cases. The liver appears grossly normal in light infections, but in heavy infections there is localized dilatation of the slightly thickened peripheral bile ducts (which can be seen on the surface beneath Glisson's capsule as pale-blue or greenish-blue blobs) and some atrophy of the parenchymal cells. The dilatation of bile ducts is invariably caused by obstruction of the common bile duct by a stone, a tumour or inflammatory stricture resulting from cholangitis. Under these circumstances, nearly all the medium-sized bile ducts are dilated and filled with clear or turbid bile, with or without worms.

The major microscopic findings in the early stage of clonorchiasis are periductal oedema and acute inflammatory cellular responses in the bile duct walls. The bile ducts show not only desquamation but also marked hyperplasia of epithelial cells. Subsequently, marked goblet-cell metaplasia of ductal epithelial cells is seen, and remarkable adenomatous hyperplasia appears in the mucosa. Periductal connective tissue is increased around the biliary passages and the portal tract. In the chronic stage of infection, the ductal tissue is gradually replaced by fibrous tissue (sometimes described as cholangiofibrosis), which causes thickening of the bile duct wall (Hou, 1955).

The microscopic changes vary with the intensity and duration of infection and the coexistence of bacterial infections. Without secondary bacterial infection, the genuine histological changes are usually represented by a characteristic adenomatous formation, periductal fibrosis and heavy eosinophilic infiltration. With secondary bacterial infection, however, biliary obstruction is common and is due to adenomatous proliferation, calculi and cholangitis (Hou, 1955).

Extrahepatic involvement is relatively common in *C. sinensis* infection. Hou and Pang (1964) reported that 19/300 clonorchiasis patients had pancreatic involvement; Chan and Teoh (1967) found *C. sinensis* in 24 of 64 cases seen at autopsy. Adult fluke invasion of the pancreatic ducts occurs most frequently in heavy infections, but the pathological changes are usually less extensive than those in the intrahepatic bile ducts. The flukes reside in the main pancreatic duct and its tributary ducts. The changes are similar to those seen in the hepatic lesions: namely, adenomatous hyperplasia of ductal epithelium and, sometimes, squamous metaplasia (Chen *et al.*, 1994).

One of the most characteristic complications of clonorchiasis is formation of calculi in the intrahepatic biliary passages. It is sometimes accompanied by suppurative cholangitis, cholecystitis and biliary abscesses or so-called cholangiohepatitis and, ultimately, cholangiocarcinoma (Rim, 1986). The occurrence of calculi in clonorchiasis is due to bile stagnation caused by mechanical obstruction and the presence of worms and ova, which become nuclei for hepatolithiasis. Intra- and extrahepatic bile-duct calculi are composed almost entirely of bilirubin carbonate. According to Chen *et al.* (1994), the formation of pigmented stones in clonorchiasis can be attributed to changes in the concentrations of bilirubin, cholesterol, phospholipids and bile acids and the activity of bacterial glucuronidase in bile stagnation caused by mechanical obstruction. An increase in bacterial glucuronidase activity following *Escherichia coli* infection and glycoprotein in the bile favours the formation of pigmented stones (Guo *et al.*, 1990).

With goblet-cell metaplasia of the bile-duct epithelium, the bile has a high content of mucin, which combines with the presence of the helminth and its ova in the bile duct to cause cholestasis and to furnish a favourable environment for secondary bacterial infection. The most frequent infection is with *E. coli*, which induces ascending cholangitis from the intestine. Chou *et al.* (1976) studied mucin from 17 cases of clonorchiasis-associated cholangiocarcinoma seen at autopsy. Histochemically, the mucins were qualitatively similar to those secreted by normal and *C. sinensis*-infected bile ducts, but the concentration of carboxymucins was reduced and sulfomucins were absent or present in only trace amounts in the neoplastic epithelium. Sulfomucins were abundant, however, in the hyperplastic epithelium of patients with clonorchiasis. The authors concluded that sulfomucins are valuable in differentiating hyperplastic bile ducts from cholangiocarcinoma.

Acute suppurative cholangitis may be caused by blockage of extrahepatic biliary ducts by masses of dead worms. Gallstones and the results of inflammation by bacterial infection often cause recurrent pyogenic cholangitis (Hou, 1955; Ong, 1962; Teoh, 1963). In a study of 525 *Clonorchis*-infected patients, only three had egg-induced lesions: an eosinophilic granuloma in the gall-bladder, a giant-cell reaction in the liver and pulmonary embolism (Sun, 1984). Periductal egg granulomas are rarely found (Sun, 1980).

Morphological studies by many investigators in Hong Kong and the Republic of Korea (Hou, 1956; Chou & Gibson, 1970; Kim *et al.*, 1974) indicate that carcinomas usually arise in association with pre-existing epithelial changes, which vary from hyperplasia to dysplasia and adenomatous formation in the secondary intrahepatic bile ducts.

Human cholangiocarcinoma can be divided into two macroscopic types according to the site of involvement, peripheral (intrahepatic) and hilar (extrahepatic). The peripheral type has multicentric growth as seen most frequently in *Clonorchis*-related neoplasms in patients, all of whom had histories of recurrent pyogenic cholangitis (Parkin *et al.*, 1993).

Of 38 subjects from Hong Kong chronically infected with *C. sinensis*, only one patient with cholangiocarcinoma had cirrhosis, whereas all but one patient with hepatocellular carcinoma had cirrhosis (Purtilo, 1976).

4.1.2 *Experimental systems*

(*a*) *Opisthorchis viverrini*

The pathological changes seen in the livers of cats, rabbits, guinea-pigs, hamsters and albino rats, which are considered to be suitable hosts, are grossly similar to those seen in man. After metacercariae are fed to animals, they grow into adult worms in the liver within about 30 days. The size of the worms found differs with species and is dependent on their size (Wykoff, 1958). Most studies of carcinogenesis have been conducted in Syrian hamsters, as the other species do not develop cholangiocarcinoma.

Bhamarapravati *et al.* (1978) described the histopathological response of Syrian hamsters to *O. viverrini* infection. The early changes consisted of an acute inflammatory reaction involving the second-order bile ducts and partial flattening of the epithelial cells, especially those in contact with the flukes. The main finding was foci of varying size consisting of liver cells that had undergone haemorrhagic and coagulation necrosis. Some multinucleated, foreign body-type giant cells were seen at the edge of the necrotic areas, but flukes were not found in these foci. The inflammatory reaction in the early stage of infection was predominantly eosinophilic infiltration of the portal areas, with some neutrophils and mononuclear cells. The dilated ducts showed hyperplasia and an atypical epithelial lining, which was piled up in places. An increase in the number of goblet cells was also evident. As the flukes developed into adults, they induced hyperplasia and adenomatous formations of the bile-duct epithelium. There was also a granulomatous response to adult flukes and eggs. Resolution of the granulomas around eggs led to periductal and portal scarring and fibrosis. The major findings were two types of granuloma—one in response to the dying adult flukes and the other to the eggs. Dead or dying worms lying in bile ducts were surrounded by a granulomatous mass which consisted of eosinophilic, homogeneous, foamy material and various numbers of neutrophils, eosinophils and foamy macrophages. Granulomatous masses in the lumina of the ducts were usually connected to granulomatous masses in the periductal tissue through ulcerated areas of the mucosa. Numerous epithelioid granulomas containing eggs were seen in the periductal areas, occasionally extending into the lumen through the mucosal ulcers to connect with other granulomatous masses. The centres of the granulomas consisted of homogeneous eosinophilic precipitates and necrotic cellular debris. The shells of the eggs in some of the granulomas had been ingested by multinucleated giant cells, and in some granulomas the eggs were calcified.

(*b*) *Opisthorchis felineus*

The presence of *O. felineus* causes irritation of the intrahepatic bile ducts and pancreatic ducts, leading to a catarrhal inflammation and desquamation of the epithelium (Soulsby, 1965). As seen in *O. viverrini* and *C. sinensis* infections, extensive hyperplasia of the biliary system, papillomatous and adenomatous changes in bile ducts, cystic dilatation, necrosis and secondary atrophy of the hepatic cells, and extensive fibrosis occur in experimental animals (Rim, 1982b).

Formation of granulomas in the walls of bile ducts around *O. felineus* eggs was observed at days 20–25 of experiments in Syrian hamsters (Zubov & Mukanov, 1976).

(*c*) *Clonorchis sinensis*

Many laboratory animals are sensitive to *C. sinensis*. Rabbits and guinea-pigs are the most susceptible; rats, Syrian hamsters and dogs are relatively susceptible; and mice are the least susceptible of these species. The degree of pathological change depends on both the intensity and the duration of infection. The major pathological findings in the livers of animals with clonorchiasis are in the biliary system, which the helminths inhabit. The most characteristic pathological change in infection is diffuse adenomatous tissue formation in the secondary bile ducts. Desquamation, hyperplasia of lining epithelial cells, regeneration and adenomatous hyperplasia are seen (Hou, 1965b; Kim *et al.*, 1974).

Microscopically, periductal and ductal aggregations of inflammatory cell infiltrates are usually profound in the acute stage and consist of lymphocytes, plasma cells, histiocytes and fibrosis. Hyperplasia of epithelial cells is frequent (Rim, 1982b). Small eosinophilic abscesses and focal liver cell necrosis may be present, but the hepatic lobular structure remains intact (Chen *et al.*, 1994).

Cha *et al.* (1991) noted in rats infected repeatedly with *C. sinensis* that a heavy eosinophilic infiltration appeared around the bile duct after two to four weeks. The cells were then replaced by massive mononuclear cells, which often formed lymphoid follicles. In similarly infected mice, the epithelial cells of the bile duct were changed to secretory cells, which secreted hyalinized materials into the lumen of the bile duct. Inflammatory cells infiltrated the adjacent hepatic parenchyma and formed microabscesses.

Clonorchis infection induces severe hyperplasia of epithelial cells and metaplasia of mucopolysaccharide producing cells in the biliary epithelium (Lee, S.H. *et al.*, 1978; Song *et al.*, 1989; Hong *et al.*, 1990). In a study of the proliferative activity of bile-duct epithelial cells in clonorchiasis by immunostaining bromodeoxyuridine incorporated into the DNA of cells in the S phase of division (Risio *et al.*, 1988), the greatest rate was found mainly in cells located at the base of the mucosal layer (Hong *et al.*, 1993). The authors suggested that mucosal epithelial cells of bile ducts infected with *C. sinensis* become hyperplastic mainly by direct and local stimulation by the worms.

Hepatic changes in rabbits in the early stage (first two weeks) of infection were reversible after treatment with praziquantel; however, some of the biliary epithelial changes that occurred in the chronic stage (12 weeks) of infection were irreversible (Lee *et al.*, 1989).

In guinea-pigs infected with *C. sinensis*, the biliary epithelium had an increased prevalence of mucin granules, cytoplasmic projection into the lumen, decreased numbers of microvilli and obstruction of the bile canaliculi. Blurring or irregularity of intercellular lateral interdigitation was observed in most of the biliary epithelium. Hepatocytes showed dilatation of endoplasmic reticulum and destruction of cristae in some mitochondria (Lee, Y.S. *et al.*, 1978).

4.1.3 *Comparison of humans and experimental animals*

The general pathological features of clonorchiasis and opisthorchiasis are similar in human cases and experimental animals, including the Syrian hamster, which is the most commonly used species in carcinogenicity studies. The changes involve predominantly the intrahepatic bile ducts and pancreatic ducts. The initial changes, such as desquamation of the bile-duct lining cells, are followed by hyperplasia of the cells lining the intrahepatic bile ducts and are identical in humans and in the acute stage of experimental infections. In chronic infections in humans and experimental animals, adenomatous hyperplasia of the bile ducts, heavy eosinophilic infiltration and periductal fibrosis occur. Secondary bacterial infections, especially ascending infection with *E. coli*, result in multiple hepatic abscesses and cholangiohepatitis in livers infected by both *Opisthorchis* and *Clonorchis*.

4.2 Other observations relevant to the interpretation of carcinogenicity and mechanisms of carcinogenesis

4.2.1 *Humans*

Inflammatory responses in host tissues challenged by infections or inflammatory agents have been postulated to play a role in the development of cancers which arise in infected organisms (for reviews, see Gentile & Gentile, 1994; Ohshima & Bartsch, 1994). Reactive oxygen species and nitrates, nitrites and various nitrosating agents are produced to kill invading microorganisms and helminths. Polymorphonuclear leukocytes play a prominent role in the production of these host defence agents (for reviews, see Preussmann & Eisenbrand, 1984; Shepard *et al.*, 1987). The radicals have been shown to induce genetic damage in normal host tissues adjacent to the site of inflammation, producing DNA strand breaks, mutations and chromosomal aberrations (Weitzman & Stossel, 1981; Birnboim, 1982). While no data on humans are available to verify these observations, increased levels of urinary nitrates and salivary nitrites are found in *O. viverrini*-infected individuals.

Srianujata *et al.* (1984) reported significantly higher concentrations of nitrate (2–2.8 times) and nitrite (2–5.6 times) in the saliva of inhabitants in a high-risk area for cholangiocarcinoma in North-east Thailand than in subjects in Bangkok (low-risk area). Nitrate levels in urine were also significantly higher (1.5–3 times) in the subjects from the high-risk areas. Srianujata *et al.* (1987) also reported higher levels of nitrite (1.8 times) and *N*-nitrosoproline (2.6 times) in the urine of subjects infected with *O. viverrini* than in uninfected subjects from the same area of North-east Thailand. Haswell-Elkins *et al.* (1994b) confirmed these observations in a study in North-east Thailand in which diet and smoking were controlled for; they also demonstrated decreased concentrations of nitrates and nitrites in these subjects after treatment with praziquantel. Srivatanakul *et al.* (1991c), in a study in which diet and smoking were not controlled for, reported that subjects living in high-risk areas for fluke infection who had antibodies to *O. viverrini* had a 10-fold greater potential for endogenous nitrosation, measured on the basis of urinary levels of *N*-nitrosoproline after proline ingestion, than individuals who did not have antibodies.

Cholangiocarcinomas from *O. viverrini*-infected patients differed from those in uninfected patients with respect to point mutations in the c-Ki-*ras* proto-oncogene: mutations were found at codon 12 of this gene in five of nine individuals in Japan who had cholangiocarcinoma but no concomitant fluke infection, but not in six patients from Thailand who harboured both cholangiocarcinoma and fluke infection (Tsuda *et al.*, 1992). Similar results were reported by Kiba *et al.* (1993), who found, however, that a mutation at the *p53* tumour suppressor gene was similar in the two sets of cholangiocarcinoma patients, all but one being GC→AT transitions in a highly conserved GpG site.

4.2.2 *Experimental systems*

In male Syrian hamsters and jirds (*Meriones unguiculatus*), 220 days after experimental infection with *O. viverrini*, marked proliferation of smooth endoplasmic reticulum was observed in hepatocytes, and lobed and enlarged nuclei and mitochondria were seen which showed significant pathological degeneration, up to lysis. There was also accumulation of intermediate filaments in adjacent bile-duct epithelia and in the epithelium of the gall-bladder (Adam *et al.*, 1993). Depressed lymphoproliferative response to phytohaemagglutinin stimulation has also been described in Syrian hamsters infected with *O. viverrini*, suggesting an immunodepressive effect (Wongratanacheewin *et al.*, 1987).

The role of *O. viverrini* in enhancing host response to chemical carcinogens (particularly nitrosamines) has been well documented in Syrian hamster models (see section 3.2). A significant increase in the proportion of water-soluble aflatoxin B_1 metabolites was found in hamsters infected with liver flukes over that measured in uninfected animals (Makarananda *et al.*, 1991), suggesting increased expression of enzymes that metabolize aflatoxin B_1. A cytochrome P450 isozyme(s) (CYP2A) has been identified in the livers of hamsters infected with *O. viverrini*, the activity of which increased nonuniformly in male but not female animals, the highest levels of activity occurring in hepatocytes immediately adjacent to areas of inflammation. This increase occurred in spite of a decrease in the total hepatic P450 content. The enzyme was shown to contribute up to 50–60% of the metabolism of hepatic aflatoxin B_1 and *N*-nitrosodiethylamine in infected males and 20–40% in infected females (Kirby *et al.*, 1994).

Immunohistochemical analysis of aflatoxin B_1–DNA adducts in parasitized animals indicated that the greatest numbers of adducts occurred in the regions of highest CYP2A activity. Studies with a related liver fluke, *Fasciola hepatica*, also showed enhanced cytochrome P450-related activation of aflatoxin B_1 into a mutagen by liver extracts from fluke-infected mice over that with extracts prepared from livers of uninfected animals (Gentile & DeRuiter, 1981).

Nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase was found to be increased in *O. viverrini*-infected Syrian hamsters, and nitric oxide synthase activity in liver cytosol was twice as high in infected as in untreated hamsters The enzyme was located in macrophages and eosinophils which accumulated at the site of the infection (Ohshima *et al.*, 1994).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The liver flukes, *Opisthorchis viverrini*, *O. felineus* and *Clonorchis sinensis*, are biologically similar, food-borne trematodes which chronically infect the bile ducts and, more rarely, the pancreatic duct and gall-bladder of human beings and other mammals. Infection is acquired by eating raw or undercooked freshwater fish which contain the infective stage (metacercaria) of flukes. Immature flukes migrate up through the ampulla of Vater to the biliary tree, mature in the small intrahepatic ducts and produce eggs, which are passed in the faeces. If the eggs reach a water body and are consumed by an appropriate species of snail, they hatch and undergo asexual multiplication to produce free-swimming larvae, which can penetrate freshwater fish and become encysted metacercariae.

Liver fluke infections are best detected by identification of eggs in the faeces. In light infections and severe disease with obstruction, eggs may not be found. There is a close quantitative relationship between the number of eggs per gram of faeces and the number of adult worms. Immunodiagnostic techniques cannot be used reliably to detect active infections.

Nine million people are infected with *O. viverrini*, which is common in North-east Thailand, at least one-third of the population being infected, and in North Thailand and Laos. *O. felineus* affects 1.5 million people, mainly in the central part of the Russian Federation. An estimated 7 million people are infected with *C. sinensis* in the Republic of Korea, southern China, Hong Kong, Macao and Viet Nam. The distribution of human infection is determined primarily by the distribution of the habit of eating raw freshwater fish; heterogeneity within endemic areas is probably due to environmental factors and control. Infection generally occurs during the first decade of life, often with a similar pattern in men and women, although men may be more frequently and heavily infected than women.

Most liver fluke infections lead to local inflammation, and they are associated with specific clinical signs and symptoms in 5–10% of infected people. The intensity of infection is correlated with hepatobiliary tract abnormalities visualized by ultrasound. Biliary and gall-bladder stones are commoner among individuals heavily infected with *Clonorchis* than among those infected with the other liver flukes. Treatment with praziquantel is highly effective and also leads to reversal of biliary tract abnormalities. Control of infection has been achieved in some areas by a combination of chemotherapy, health education and improved sanitation.

5.2 Human carcinogenicity data

Opisthorchis viverrini

Within Thailand, the highest proportional incidence rate of cholangiocarcinoma is observed in the north-east region of the country where the prevalence of infection with *O. viverrini* is also highest. In this region, the incidence of cholangiocarcinoma is about 40 times the highest incidence outside Thailand. A formal analysis across five regions of the country showed a strong correlation between proportional incidence of cholangiocarcinoma and estimated average titres of antibodies to *O. viverrini* and, to a lesser degree, faecal egg count. Correlations with proportional incidence rates of hepatocellular carcinoma were much weaker.

Many cases of liver cancer arising in patients with *O. viverrini* infection have been reported from Thailand. In most regions of the world, cholangiocarcinoma is a very rare tumour. In areas where *O. viverrini* is endemic, however, the numbers of cases of cholangiocarcinoma generally outnumber those of hepatocellular carcinoma.

Three cross-sectional or case–control studies of the association between infection with *O. viverrini* and cancer of the liver have been reported from Thailand. In the earliest and smallest of these studies, the estimated relative risks for cholangiocarcinoma and hepatocellular carcinoma in association with the presence of *O. viverrini* eggs in faeces were each 1.3. In the second study, the estimated relative risk for the association between cholangiocarcinoma and the presence of *O. viverrini* antibodies in serum was 5.0, which was significant. The association was not explained by possible confounding with hepatitis B virus infection or estimated recent intake of aflatoxins. The estimated relative risk for the association with hepatocellular carcinoma was 1.7 (not significant). In the third study, based on 15 cases of cholangiocarcinoma, estimated relative risks of 1.7, 3.2 and 14.1 were calculated for categories of faecal excretion of increasing numbers of *O. viverrini* eggs. This trend was highly significant.

Opisthorchis felineus

The incidence of liver cancer was observed to be correlated with the prevalence of infection with *O. felineus* across four areas in the T'umen' region of north-west Siberia. Cases of both cholangiocarcinoma and hepatocellular carcinoma have been reported in people infected with *O. felineus*.

Clonorchis sinensis

Cases of cancer of the liver in association with infection with *C. sinensis* have been reported from China, Hong Kong, the Republic of Korea and Japan and in immigrants to North America from China and Laos.

Two case–control studies of the relationship between *C. sinensis* infection and liver cancer, with partially overlapping case series, have been carried out in the Republic of Korea. Significantly increased estimated relative risks of 6.5 and 6.0 were seen for an association with cholangiocarcinoma, but no significant association was seen with the occurrence of hepatocellular carcinoma. In a third case–control study, in Hong Kong, the estimated relative risk for cholangiocarcinoma, after adjustment for age and sex, was 3.1, while that for hepatocellular carcinoma was 0.7.

5.3 Animal carcinogenicity data

Infection with *O. viverrini* alone was evaluated in hamsters in several studies that were not designed specifically as long-term carcinogenicity studies. Two cholangiocarcinomas were found in one of these studies. In several studies in hamsters infected with *O. viverrini* and treated with various carcinogenic *N*-nitrosamines, induction of cholangiocarcinomas and of hepatocellular nodules was enhanced.

No study of the carcinogenicity of *O. felineus* was available.

Infection with *C. sinensis* was associated with the presence of a few cholangiocarcinomas in cats and one in a dog. Two experiments in rats were inadequate for evaluation. Infection with *C. sinensis* increased the incidence of cholangiocarcinomas in hamsters treated with 2-acetylaminofluorene or *N*-nitrosodimethylamine.

5.4 Other relevant data

The general pathological features of infection with liver flukes are similar in humans and animals. The changes are characterized by oedema, desquamation and acute inflammatory cellular responses in the bile ducts in the early stage; in the chronic stage, the bile ducts show marked goblet-cell metaplasia, adenomatous hyperplasia and thickening of the walls. Complications may include calculi, suppurative cholangitis and biliary abscess caused by bile stagnation due to mechanical obstruction.

Cholangiocarcinomas appear to arise from pre-existing adenomatous changes in the bile ducts through the phase of intestinal metaplasia or dysplastic change.

The expression of CYP2A isozymes that catalyse the metabolism of aflatoxin and nitrosamines in the liver is increased in *O. viverrini*-infected hamsters. The increased expression is located in regions of the liver adjacent to the site of inflammation. The activity of macrophage-associated nitric oxide synthase is also increased in these animals. No information was available about the effects of liver fluke infection on carcinogen metabolism in humans. Increased urinary levels of nitrate and certain nitrosamines are detected in people infected with *O. viverrini*.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in humans for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in humans for the carcinogenicity of infection with *Clonorchis sinensis*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *inadequate evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis felineus*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Clonorchis sinensis*.

In making the overall evaluation, the Working Group noted that experimental and epidemiological studies on *Clonorchis sinensis* confirm:

(i) that the biological and pathological characteristics of *Opisthorchis* and *Clonorchis* are similar;

(ii) that cholangiocarcinoma occurs in infected animals, especially when infection is combined with administration of known carcinogens; and

(iii) that the relative risks for cholangiocarcinoma, and not for hepatocellular carcinoma, are consistently increased in people infected with this organism.

Overall evaluations

Infection with *Opisthorchis viverrini* is carcinogenic to humans *(Group 1)*.

Infection with *Opisthorchis felineus* is not classifiable as to its carcinogenicity to humans (Group 3).

Infection with *Clonorchis sinensis* is probably carcinogenic to humans (Group 2A).

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