Clonorchiasis is an infectious disease of the biliary passages caused by the Chinese or oriental liver fluke, *Clonorchis sinensis*, the most important liver fluke of humans. This infection is common in the Far East, especially in southern Korea, China, Taiwan, Japan, the Far East part of Russia and northern Vietnam. Clonorchiasis occurs in all parts of the world where there are Asian immigrants from endemic areas. Dogs, hogs, cats, and rats are reservoir hosts. The human and animal reservoir hosts acquire the infection from the ingestion of raw, inadequately cooked, or even dried, salted, or pickled flesh of infected freshwater fish. The flukes reach maturity within 16 to 25 days after encysted larvae are infested. The infected individuals may pass viable eggs for as long as 30 years. The first intermediate small hosts are mainly the species of *Parafossarulus* and *Bithynia*. Numerous species of freshwater fishes serve as the second intermediate hosts of *C. sinensis*. The majority of fish intermediate hosts are confined to Cyprinidae.

The morphologic features of the adult worm, larval stages and egg as well as the life cycle and the development on final host are well known. The parasitologic diagnosis is based on finding the characteristic eggs in the feces or duodenal bile drainage. The eggs require differentiation from those of *Opisthorchis* and heterod跌ry flukes. A number of immunological techniques have currently been applied for the diagnosis of this infection. However, immunodiagnosis is still regarded as a supplementary diagnostic method.

Clinical manifestations result from the number of worms, infection period, and complications in the chances of heavy infection due to the long infection period. As for the complications of clonorchiasis, formation of calculi in the intrahepatic biliary passages is one of the most characteristic pathologic features. It is sometimes accompanied by suppurative cholangitis, cholecystitis, and biliary abscesses or so-called cholangiohepatitis and ultimately can cause primary liver cancer, especially cholangiocarcinoma. The experimental result on the relationship to the occurrence of cholangiocarcinoma is presented. The clinical diagnosis by radiologic findings including cholangiography, sonography, and computerized tomography (CT) as well as MRI for biliary or pancreatic ducts are outlined.

A quantitative epidemiological analysis on the levels of intensity or endemicity, age, sex and age distribution, the features of transmitting *C. sinensis* infection, as well as the epidemiological changes of clonorchiasis after control measure are applied by the Muench's catalytic model.

Praziquantel is the treatment of choice for clonorchiasis. The most effective regimen is 25 mg/kg three times daily (total dose, 75 mg/kg) administered orally at 5- to 6-hours intervals over a single day. The modes of action to *C. sinensis* by praziquantel are shown by transmission and scanning electron microscopy. The side effects and the tolerance of praziquantel to clonorchiasis patients are noted. The results of large scale treatment as a mass treatment project are also analysed. The prevention and control measures are discussed.

**Dicrocoelium dendriticum and dicrocoeliosis: a review of our research**

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Dicrocoeliosis, caused by *D. dendriticum*, is an important parasitic disease from an economic and health viewpoint. This trematode, whose adults live in the liver and bile ducts of numerous mammal species, mainly ruminant, which act as definitive hosts in several countries in Europe, Asia, America and North Africa, needs land molluscs and ants as first and second intermediate hosts, respectively, to complete its life cycle. *D. dendriticum* occasionally affects humans. The application of efficacious prophylactic and control measures against dicrocoeliosis -which have not been satisfactory so far-, requires precise early diagnosis, a previous epidemiological study and experimental research to help interpret field observations and extend knowledge of the parasite/host relationships. We therefore decided to carry out studies on: epidemiology; strategic treatments; experimental infection in molluscs, hamsters and lambs; parasite isoenzymatic characterisation and genetic variability; and antigenic molecules as targets for specific diagnosis and protection.

Our research on *D. dendriticum* started 30 years ago, mainly focusing on the specific determination, distribution and natural infection by trematodes of 23 species of Helicidae (Mollusca) collected over 12 years in more than 350 villages throughout the province of León (NW Spain). This parasite was detected in 11 mollusc species collected in 95 of these villages. Likewise, the monthly kinetic of *D. dendriticum* egg elimination in faeces of sheep and cattle chosen at random in 5 localities in the upper and middle Porma river basin was followed for one year. We found *D. dendriticum* eggs in 63.6% and 37.6% of the 995 and 1251 sheep and cattle samples examined, respectively. The highest egg-elimination period in both was the end of autumn-winter. Moreover, simultaneous and integrated studies concerning the transmission of *D. dendriticum* were carried out on 81 labelled sheep and lambs, molluscs and ants, over two consecutive years in one of the mountain localities with the highest infection prevalence detected (73.7%) and egg (x 398.8) in randomly sampled sheep. *Two Helicella* of the 29 Gastropoda species identified contained *D. dendriticum*. Daughter sporocysts with well-developed cercariae predominated in spring and autumn. *Four Formica* of the 21 ant species studied harboured metacercariae in the abdomen. Infected ants were detected from April to November and in tetania from May to October (from 06.00-14.10 and 18-21.5 hours, at 7.5-26.9 °C). The highest values of egg elimination in all the animals were detected in January-February. According to our results, shedding of *D. dendriticum* eggs with the faeces of the ruminants occurs uninterruptedly throughout the year, but the highest values are recorded at the end of autumn-winter. At these times survival of *D. dendriticum* eggs is very high (low temperatures do not affect egg survival), so pasture contamination by viable eggs is very great in spring, when the molluscs are very abundant and active. Those infected at the beginning of this period could shed slimeballs with cercariae at the end of summer and during autumn, whilst those infected later can shed slimeballs the following year, beginning in spring, if they survive the harsh winter. Approximately 45 days later the cercariae ingested by ants will have become infected metacercariae for the definitive hosts. This will allow the parasite cycle to be completed when the ants are ingested by ruminants on grazing, during the active period of the ants (March-November). Nevertheless, some infected ants survive in their nest during the winter, and they are responsible for definitive host infection at the beginning of spring. As the ant activity increases, so does metacercaria ingestion by the definitive hosts and their *D. dendriticum* worms increases. Thus, egg elimination also increases during this period, reaching the highest values in January-February (Manga-González et al., 2001). Two treatments applied in November and January were the most effective to reduce egg shedding by natural infected sheep.
The ovine experimental dicrococaliosis studies were carried out on 32 lambs: 12 infected with 1000 *D. dendriticum* metacercariae, 12 with 3000 and 8 controls. Half the lambs in each group were slaughtered on day 60 and 180 p.i., respectively. The percentage of metacercariae established as adult worms in the total of infected lambs was 17%. The worms recovered on necropsy of each animal was 110-2063 worms (dose 3000) and 30-437 worms (dose 1000). Egg elimination was first detected between days 49 and 79 p.i. The highest epg values were observed 180 days p.i. (Campos et al., 2000). The increased AST and ALT enzyme values were significant 60 days p.i., when the IgG antibody response against excretory-secretory and somatic antigens was highest and the greatest decrease in the weight of the lambs took place. Severity of the lesions observed in experimental dicrococaliosis, affecting mainly the biliary system but also hepatocytes, was closely associated with the parasitic burden. The best enzymatic systems for the characterization of the *D. dendriticum* adults and larval stages were LDH, GPI, and PGM.

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### 459 (KS) Food-borne intestinal trematodiasis in Korea and Asia

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Food-borne intestinal trematode infections of man in Asia are briefly reviewed. More than 50 species are known to occur as human infections around Asia. They include more than 20 species of Heterophyidae, more than 15 species of Echinostomatidae, and one or a few species each of the family Plagiorchiidae, Neodiplodistomidae, Strigeidae, Gymnophallidae, Clinostomatidae, Dicrocoeliidae, Microphallidae, Lecithodendridae, Nanophyetidae, Fasciulidae, and Gastrodiscidae. In the Republic of Korea, 17 species are known to occur indigenously; Heterophyidae (10 species), Echinostomatidae (4), Neodiplodistomidae (1), Plagiorchiidae (1), and Gymnophallidae (1). The second intermediate hosts (food; the infection source) are fishes (Heterophyidae, Echinostomatidae, Nanophyetidae, Clinostomatidae), crustaceae (Microphallidae), molluscs (Echinostomatidae, Gymnophallidae), plants (Fasciulidae), amphibia and reptiles (Echinostomatidae, Neodiplodistomidae, Strigeidae), and insects (Plagiorchiidae, Lecithodendridae). These parasites can make mucosal inflammation and destruction in the intestinal tract, and cause abdominal pain, diarrhea, and weakness. Differential diagnosis by discovery of eggs in the feces is often impossible or needs much experience. Management can be done successfully by prescribing praziquantel at the dose of 10 mg/kg in a single dose in most cases. For prevention, consumption of improperly cooked food (the infection source) should be avoided.


### 459 (O) Protective responses against reinfection with *Clonorchis sinensis* in rats

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In the present study, the protective response against reinfection with *Clonorchis sinensis* in rats was characterized. Animals were challenged with *C. sinensis* metacercariae, then treated with praziquantel and reinjected. Worm recovery rate and development in reinjected animals was used to estimate resistance to reinfection. The determined resistance rate to reinfection in 4 weeks primary infected and cured rats was 97.7%. The greater the worm burden and the longer the duration of primary infection, the higher was the resistance rate. For primary infection doses of 10, 40 and 100 metacercariae per rat, the resistance rates were 87.4%, 93.8% and 98.4%, respectively. The resistance rates in rats after 2 or 8-week primary infection were 78.7% and 95.3%, respectively. The reinjected rats showed the resistance during the observation period from 5 days through 11 months after treatment, however, the resistance decreased gradually as the time past. There was no difference in worm recovery rate at 1, 2, 3 and 4 weeks after reinfection. All worms recovered from reinjected rats were immature and smaller than those from primary infected rats. Methylprednisolone suppressed antibody responses and totally impaired the resistance, suggesting a possible role of immune response in the resistance to reinfection. It is demonstrated that rats develop resistance to reinfection with *C. sinensis* and the resistance is related with the immune response.

### 460 (O) Sustained-release praziquantel tablet: pharmacokinetics and the treatment of clonorchiasis in beagle dogs

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