

Case Report

**Fascioliasis Cases Recently Found
in the Southern Part of Kyushu District, Japan**

HARUHIKO MARUYAMA¹⁾, SHINICHI NODA²⁾, TATSUYUKI MIMORI³⁾ AND YUKIFUMI NAWA¹⁾

¹⁾Department of Parasitology, Miyazaki Medical College, Kiyotake, Miyazaki 889-16, Japan.

²⁾Department of Medical Zoology, Faculty of Medicine, Kagoshima University,
8-35-1 Sakuragaoga, Kagoshima 890, Japan.

³⁾Department of Tumor Genetics and Biology, Kumamoto University Medical School,
2-2-1 Honjo, Kumamoto 860, Japan.

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Adult *Fasciola* spp. live in the bile passages of the liver of many kinds of herbivorous animals, especially ruminants such as cattle and sheep. Thus, fascioliasis is primarily an important disease in veterinary medicine and livestock industry. However, infection with *Fasciola* spp. also occurs in human in farming areas. In Japan, 37 cases were recorded until 1985 (Yoshida, 1991) and a few additional cases (1 case from Oita: Maeo *et al.*, 1989; 3 cases from Hyogo: Kodama *et al.*, 1991; one case from Osaka: Hosoya *et al.*, 1992) were reported thereafter. Geographical distribution of fascioliasis in human is spread nation-wide except for Hokkaido. In Kyushu District, 8 cases were found in the northern part. However, so far we could gather, no cases have been reported from the southern Kyushu, though many cattle farms are located in this area. Here we report 7 cases of fascioliasis; 2 cases from Kagoshima in 1993, one case each from Miyazaki, Kumamoto and Kagoshima in 1995, and one case each from Kagoshima and Kumamoto on January 1996.

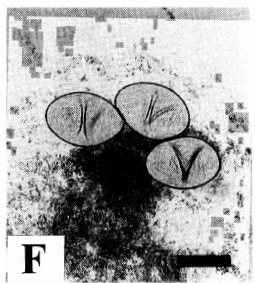
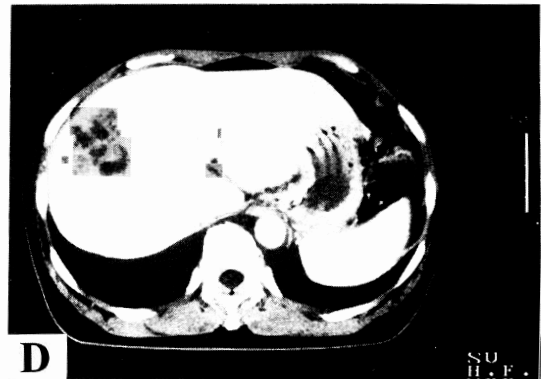
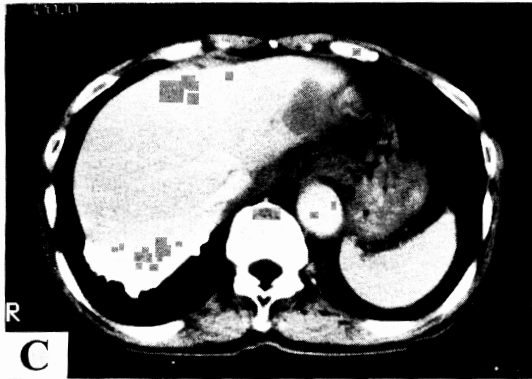
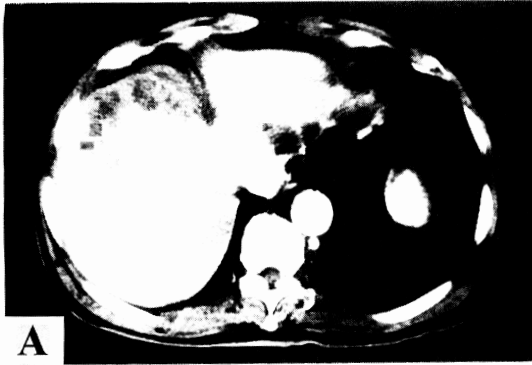
Case Report

Cases #1 and #2

The patients were a 70 year-old retired post officer (#1) and his wife (#2), a 62 year-old farmer, living in Sata-cho, Kimotsuki-gun, Kagoshima Prefecture. They have never been abroad except for the husband's military service in Philippines during 1943–1945. Neither cattle nor sheep were kept in their farm, though they are living in the area where many cattle farms are located. They frequently use bovine manure as a fertilizer in their garden. On October 1992, the wife was pointed out of having liver function disorder when she visited to their home doctor because of nausea, anorexia and headache. On November 1992, when she visited a local hospital because of right-lateral abdominal pain and back pain, a nodular lesion was found in the liver by ultrasonography (US). She was admitted to a regional central hospital for work-up. A few days later, her husband also complained of right upper abdominal discomfort and a nodular lesion was found in the liver by US when he visited a local hospital. Immediately he was admitted to the same hospital where his wife was in. By enhanced computed tomography (CT), low density areas of irregular shape were found in the right anterior segment of the liver of the husband (Fig. 1A) and in the right posterior segment of the liver of the wife (Fig. 1B).

Correspondence: Yukifumi Nawa, paras@macqm.miyazaki-med.ac.jp

丸山治彦¹, 野田伸一², 三森龍之³, 名和行文¹ (¹宮崎医科大学寄生虫学教室, ²鹿児島大学医動物学教室, ³熊本大学腫瘍学教室)



Their laboratory data were also similar in that they had marked eosinophilia (the husband's total WBC: 14,800/mm³ with 50% eosinophils; the wife's WBC: 10,200/mm³ with 27.7% eosinophils) and moderate liver dysfunctions [the husband's data (ZTT: 17.5 K-U; TTT: 6.8 K-U; ALP: 11.9 KAU; γ -GTP: 74.1 IU/ml; GPT: 29.5 KU; LDH: 469 WU); the wife's data (ZTT: 17.5 K-U; TTT: 6.8 K-U; alkaline phosphatase: 11.9 KAU; γ -GTP: 74.1 IU/ml; GPT: 29.5 K-U; LDH: 469 W-U)]. Since they were suspected to be suffering with some kind of parasitic infections, their sera were sent to Prof. M. Tsuji, the Department of Parasitology, Kyorin University, School of Medicine. By Ouchterlony's double diffusion test in agarose and immunoelectrophoresis, their sera were positive against *Fasciola* antigen. Figs. 2A and 2B are the multiple dot-enzyme-linked immunosorbent assay (dot-ELISA) done in the Department of Parasitology, Miyazaki Medical College. The husband's serum reacted with *Fasciola* and *Trichinella* antigens, whereas the wife's serum reacted nonspecifically with almost all antigens tested. However, when their sera were reexamined by double diffusion test (Fig. 3A), they reacted specifically with *Fasciola* antigen. The wife was treated with bithionol, whereas the husband refused to receive chemotherapy.

Case #3

The patient was a 75 year-old male farmer. He was born and grown up in Takaoka-cho, Miyazaki Prefecture, and has never been abroad. Several cattle are constantly raised in his farm and the manure is used as fertilizer. On January 1994, he was pointed out of having liver function disorders by regular checking-up. By US and CT at a local hospital, an irregular-shaped low density area was found in lt-lateral superior segment of the liver (Fig. 1C). In addition, a cystic lesion was found behind the caudal lobe and diffuse calcified lesions were found in between the liver and diaphragm. He was suspected to have cholangiocarcinoma and was ad-

mitted to the 1st Department of Surgery, Miyazaki Medical College, for work-up. Laboratory data revealed that tumor markers were almost within a normal range (CEA: 3.6 ng/ml; CA19-9: 39 U/ml; DUPAN-2: <25 U/ml; SPAN-1: 19 U/ml). During admission, he developed high fever with marked eosinophilia (total white blood cell count: 5,600/mm³ with 41.3% eosinophils). Because he was strongly suspected to have parasitic diseases, his serum was forwarded to the Department of Parasitology, Miyazaki Medical College, for immunoserological examinations. By dot-ELISA, his serum showed moderate binding to *Fasciola* sp. and *Gnathostoma* antigens (Fig. 2C). By double diffusion test, his serum formed a sharp precipitin band only against *Fasciola* antigen (Fig. 3B). Parasite eggs were negative in the stool nor duodenal fluid. He was treated successfully with praziquantel (PZQ: Biltricide[®], Bayer Yakuhin, Ltd) at a dose of 25 mg/kg/day for 3 days twice at a 2-week interval. (This case was reported orally by Naganuma *et al.* at the 65th Annual Meeting of the Japanese Society of Digestive Diseases, Kyushu branch, held in Kagoshima on June 1995.)

Case #4

The patient was a 52 year-old female farmer. She was born and grown up in Aso-gun, Kumamoto Prefecture, and has never been abroad. A few cattle were constantly raised in her farm and she often eats water plants. Bovine manure is used as fertilizer. In summer, 1994, she frequently felt dull epigastric and back pain. However, any particular lesions were noted by ultrasonography or gastrofiberscopy by the end of 1994. On January 1995, she was pointed out of having an elevation of alkaline phosphatase (422 U/l). An irregular shaped nodular lesion (about 5×5.5×8 cm) in the liver was found by US and CT (Fig. 1D). She was suspected to have a cholangiocellular carcinoma and was admitted to the 3rd Department of Internal Medicine, Kumamoto University Medical School, for work-up. Marked eosi-

Fig. 1 Radiologic findings of the patients.

Figs. 1A-1D, 1G, 1H: enhanced computed tomogram of the patients #1-4, 6 and 7 respectively.

Fig. 1E: ERCP showing the presence of parasite (arrow head).

Fig. 1F: parasite eggs found in the duodenal aspirate.

Scale bar = 100 μ m.

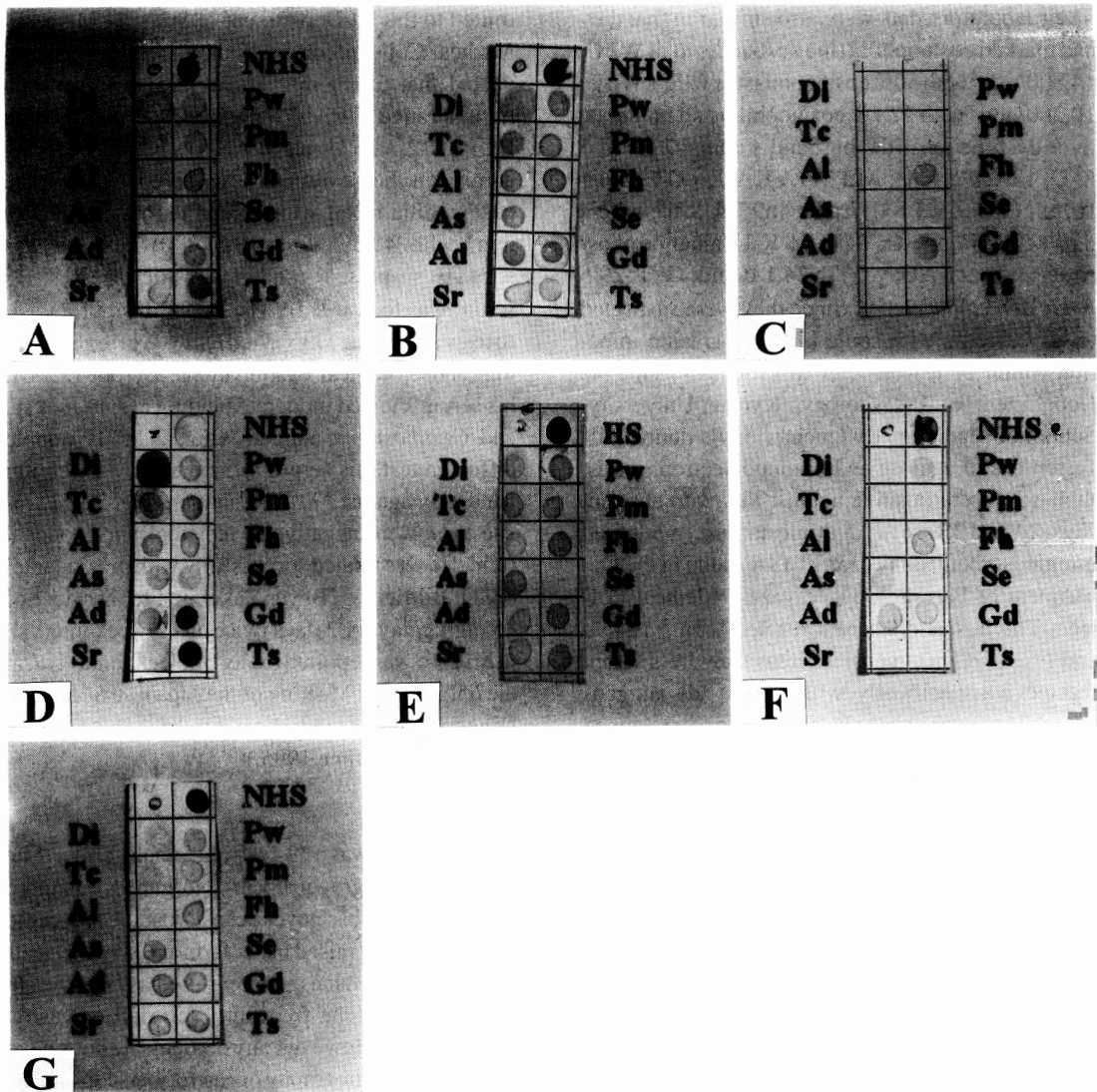


Fig. 2 Multiple dot-ELISA test of the patients' sera.

NHS or HS: normal human serum.

Pw: *Paragonimus westermanii*; Pm: *Paragonimus miyazakii*; Fh: *Fasciola* sp., Se: *Spirometra erinacei*, Gd: *Gnathostoma doloresi*, Ts: *Trichinella spiralis*, Di: *Dirofilaria immitis*, Tc: *Toxocara canis*, Al: *Ascaris lumbricoides suum*, As: *Anisakis simplex*, Ad: *Ancylostoma duodenale*, Sr: *Strongyloides ratti*.

Each spot was coated with 1 μ l of antigen solutions (Antigen concentration of Fh, Ad and Sr is 0.05 mg/ml, while that of others is 0.2 mg/ml).

nophilia (total WBC: 7,860/mm³ with 32.6% eosinophils) was noted by laboratory examinations and was increased up to 45.8% within a week. Liver biopsy revealed that the lesion was an eosinophilic

granuloma without any signs of malignancy. Since fascioliasis was strongly suspected, her serum was sent to the Department of Parasitology, Miyazaki Medical College, for examinations for parasitic dis-

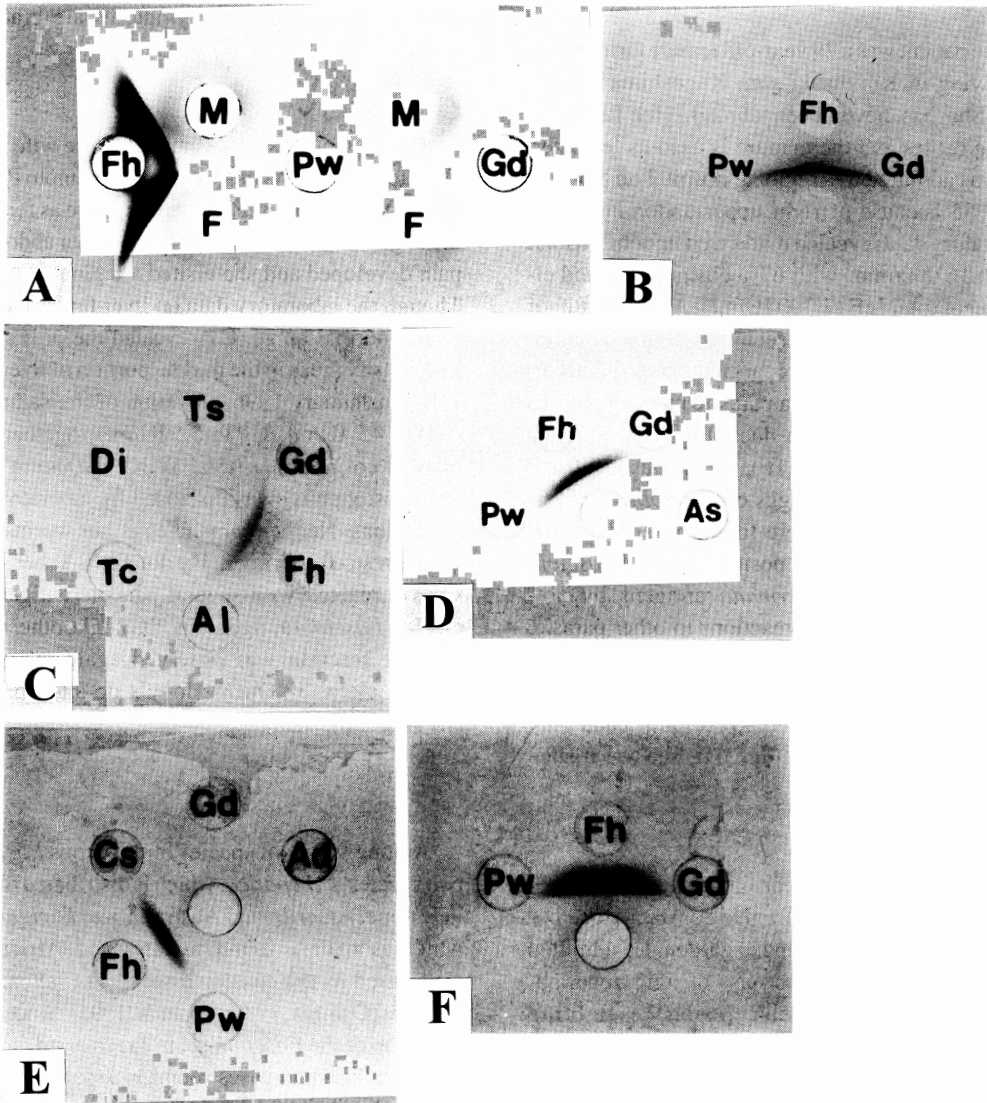


Fig. 3 Ouchterlony's double diffusion test in agarose.

Abbreviations for parasite antigens are the same as those listed in Fig. 2.

Except otherwise stated, patient's serum was placed in the center well of each plate. In Fig. 2A, M is the serum from the patient #1, whereas F is that from #2.

eases. By dot-ELISA screening, the patient's serum strongly reacted against *Gnathostoma*, *Trichinella*, and *Dirofilaria* antigens and moderately to weakly reacted with other parasite antigens (Fig. 2D). However, by Ouchterlony's method including three strongly reacted antigens, a sharp precipitin band was seen only against *Fasciola* antigen (Fig. 3C).

The patient was treated with PZQ at a dose of 75 mg/kg/day for 6 days. (This case was reported orally by Mizutari *et al.* at the 30th Annual meeting of the Western Branch of the Japanese Society of Hepatology held in Shimane on November 1995).

Case #5

The patient was a 79 year-old female farmer born and living in Kimotsuki-gun, Kagoshima Prefecture. She has never been abroad. Her family is raising cattles and the manure is used as fertilizer. She was admitted to a regional hospital on November 1995 because of right upper abdominal pain. Laboratory data revealed marked eosinophilia (total WBC: 16,000/mm³ with 67% eosinophils) and elevation of total IgE (7,300 IU/ml). Although tumor mass or abscess was not seen by US or CT, endoscopic retrograde cholangiopancreatography (ERCP) revealed the presence of a parasite moving alive in the dilated common bile duct (Fig. 1E). Furthermore, parasite eggs (Fig. 1F), which was identified morphologically as the eggs of *Fasciola* sp. (average length: 150 μ m), were found in the duodenal aspirate. Her serum was positive against *Fasciola* and *Paragonimus westermanii* antigens by dot-ELISA with weak cross reactions to other parasite antigens (Fig. 2E). Similar to the case #4, Ouchterlony's double diffusion test revealed that the patient's serum formed a precipitin band only against *Fasciola* antigen (Fig. 3D). She was treated with PZQ (75 mg/kg/day for 3 days).

Case #6

The patient was a 69 year-old male living in Soogun, Kagoshima Prefecture. He constantly use bovine manure as fertilizer in his garden. He admitted to the regional hospital because of right upper abdominal pain. A nodular lesion resembling cholangiocellular carcinoma was found by US and CT (Fig. 1G). Because of marked eosinophilia (total WBC 14,000/mm³ with 60–70% eosinophils) by laboratory examinations, his disease was suspected as parasitic infections in the liver, most likely clonorchiasis. Therefore, he was treated with PZQ (20 mg/kg/day) for 2 days. However, right upper abdominal pain rather increased in severity so that he was transferred to the 3rd Department of Internal Medicine, Faculty of Medicine, Kagoshima University, for work-up. His serum was positive against *Fasciola* sp. with weak cross reactions to some other antigens by dot-ELISA (Fig. 2F). Positive reaction of the serum against *Fasciola* antigen was confirmed by Ouchterlony's double diffusion test (Fig. 3E). He received higher dose of PZQ (75 mg/kg/

day) for 6 days and his symptoms are gradually improving.

Case #7

The patient was a 67 year-old house wife living close to cattle farms in Aso-gun, Kumamoto Prefecture. She sometimes use bovine manure as fertilizer in her garden. On November 1995, upper abdominal pain developed and she visited a regional hospital. Though the laboratory data on liver functions were within normal range, CT revealed the presence of low density areas in the middle portion of liver (Fig. 1H). On January 1996, elevation of transaminases (GOT: 45 IU/ml, GPT: 52 IU/ml) together with marked eosinophilia (total WBC: 4900/mm³ with 28% eosinophils) was disclosed by laboratory examinations. Neither parasite eggs nor worms were found in stool specimen. By dot-ELISA, her serum weakly reacted with several parasite antigens including *Fasciola* antigen (Fig. 2G). Like other cases, positive reaction was observed against *Fasciola* antigen alone by Ouchterlony's double diffusion test (Fig. 3F).

Discussion

Among 9 known species of the genus *Fasciola*, two species, *F. hepatica*, which is distributed mainly in Europe, America and Australia, and *F. gigantica*, which is mainly found in Asia and Africa, are considered as the causative species of human fascioliasis (Oshima, 1989; Yoshida, 1991). Since identification of the *Fasciola* sp. in Japan is still controversial, all human cases found in Japan have been recorded as infection with *Fasciola* sp. Therefore, all seven cases reported here are recorded as infection with *Fasciola* sp. The patients' data are summarized in Table 1.

Although cattle farming is popular all over Japan, the incidence of fascioliasis is rather low and sporadic compared to the high incidence and mass infections in European countries. Oshima (1989) considered that this difference is primarily due to different distributions of the intermediate snail host, *Lymnaea* spp. In Japan, *L. ollula* lives in the fresh water of hilly or mountainous areas so that fascioliasis occurs in and around small scale cattle farms in such areas. All patients reported here live in such

Table 1 Summary of the fascioliasis patients

No.	Case	Age	Sex	Diagnosed on	Lesion in liver	WBC /mm ³	Eo (%)	IgE IU/ml	Eggs
1	HA	70	M	Feb./'93	r-anterior	14,800	50.0	ND	-
2	YA	62	F	Feb./'93	r-posterior	10,200	27.7	ND	-
3	HH	75	M	Feb./'95	l-lateral	5,600	41.3	ND	-
4	YG	52	F	Mar./'95	r-anterior	7,860	32.6	666	-
5	SM	79	F	Nov./'95	(-)	16,000	67.0	7,300	+
6	YK	69	M	Jan./'96	r-anterior	14,000	60.0	ND	-
7	UH	67	F	Jan./'96	r-anterior	4,900	28.0	3,848	-

ND: not determined or data not reached in our hand.

places and either they themselves raise cattle in their farm or live close to cattle farm and frequently use bovine manure as fertilizer in their garden or farm. Since cattle farming is one of the major agricultural activities in the southern part of Kyushu and the incidence of fascioliasis in cattle remains to be around 2% or more for years (personal communication from Dr. Y. Murakumo, B.V.S., Kagoshima Meat Inspection Center, Kushikino branch), the reason why fascioliasis has never been reported in this area should be carefully studied in medical as well as veterinary aspects.

In the present study, all patients complained right upper abdominal pain or discomfort, and, except for the patient #5, US and CT of all patients revealed the presence of hepatic lesions similar to malignant tumors in the liver, especially to cholangiocellular carcinoma. In fact, the patients #3, 4 and 6 were initially suspected to have this type of malignancy. However, including these cases, all patients reported here showed eosinophilia of extremely high degree (30–70%; Table 1). Once attention is paid for the presence of such marked eosinophilia, parasitic diseases is the most probable diagnosis for the disease of the patients. Although not only fascioliasis (Oshima, 1989) but also some other parasitic diseases such as toxocariasis (Ishibashi *et al.*, 1992; Bhatia and Sarin, 1994) could cause hepatic lesions with eosinophilia, they can be easily discriminate from each other by rather simple immuno-serological diagnostic methods. We employed dot-ELISA for primary screening and Ouchterlony's double diffusion test in agarose for the identification the pathogens. Within the results of the present study,

dot-ELISA was not always informative for the diagnosis of fascioliasis, whereas double diffusion gave clear cut results. This is partly due to the concentration of antigens used. Because of high degree of nonspecific binding of *Fasciola* antigen to normal human IgG, we should reduce the concentration of *Fasciola* antigen compare to other antigens. Although usefulness of dot-ELISA for the diagnosis of fascioliasis was described by others (Shaheen *et al.*, 1989), they used this method only for titration but not for screening. Further modification of our dot-ELISA system is necessary to improve sensitivity and specificity to detect anti-*Fasciola* antibodies.

Concerning the chemotherapy of fascioliasis, Yoshida *et al.* (1962) first reported the efficacy of bithionol. Unfortunately bithionol is no longer available since 1994. Thus, PZQ is the only drug of choice for the treatment of fascioliasis in Japan, although it was reported to be less effective or variable compare to the efficacy of bithionol (Knobloch *et al.*, 1985; Farid *et al.*, 1989) or triclabendazole (Arjona *et al.*, 1995). In the present study, the patient #1 refused to take drugs, #2 treated with bithionol, and the patients #3–7 were treated with PZQ. Although the results of PZQ treatment was variable, clinical manifestations of the patients were improved to a certain degree without significant side effects. Recently Bhatia and Sarin (1994) reported the efficacy of high dose albendazole therapy for fascioliasis, and Arjona (1995) reported that triclabendazole was as effective as bithionol. These drugs should be included in the list of orphan drugs for the treatment of tropical diseases.

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