Eosinophilic Proliferative Pylephlebitis in the Liver of Japanese Beef Cattle with Fascioliasis

Tadashi TANIMOTO*, Kinji SHIROTA[1)], Yuji OHTSUKI[2)]** and Kunioki ARAKI[3)]

Chuo Meat Inspection Center, Kochi Prefectural Government, Ebinomaru, Kochi 780–0086, [1)]Research Institute of Biosciences, Azabu University, Fuchinobe, Sagamihara-shi, Kanagawa 229–8501, [2)]Department of Pathology II, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783–8505, and [3)]Department of Microbiology, National Institute of Public Health, Shirokanedai, Minato-ku, Tokyo 108-8638, Japan

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ABSTRACT. Intrahepatic pylephlebitis was detected in 17 Japanese beef cattle. Grossly, the intrahepatic vessels in the caudate lobe and/or in the periphery of the other hepatic lobes were thickened and protruded above the lobar surface. The vessel lumina were packed with white to red, waste thread-like contents. A few immature flukes were found in the bile ducts in 3 of the 7 cases with biliary thickening. Foci of hepatic necrosis and hemorrhage were scattered around the thickened vessels in 8 cases. Histologically, the interlobular veins were thickened due to severe intimal hyperplasia with endothelial proliferation and eosinophilic accumulation and medial hypertrophy, accompanied by fibrosis and eosinophilic infiltration in the portal areas. Hepatic tissues with necrosis and hemorrhage were surrounded by eosinophils and histiocytes including a granulomatous reaction. One immature fluke was detected in one of these regions of necrosis. Immunoperoxidase staining revealed that the small fluke, Kupffer cells, and histocytes in the liver of all cases were positively stained with anti-Japanese *Fasciola* sp. antiserum. Enzyme-linked immunosorbent assay of the sera of 15 cases revealed that all were positive for the anti-*Fasciola* antibody. On the basis of these findings, the present cases were regarded as an atypical form of fascioliasis, characterized by eosinophilic proliferative pylephlebitis of the liver.—KEY WORDS: cattle, eosinophils, fascioliasis, liver, pylephlebitis.

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Cases of intrahepatic vasculitis characterized by vascular thickening and eosinophilic infiltration, have recently been detected in Japanese beef cattle during routine meat inspection [15, 23]. The eosinophilic infiltration suggests that these lesions were caused by parasitic infection, but no parasites or eggs have been detected in the liver or stool [23].

In domestic animals, phlebitis is often secondary to systemic infection (e.g., salmonellosis, feline infectious peritonitis, or various septicemias), local extension of infection (e.g., metritis, hepatitis, or infection of the uncicatrized umbilical cord), and faulty intravenous injection procedures [18]. Primary phlebitis is rare, but can occur in association with various parasitoses in tropical regions of the world, e.g., eosinophilic endophlebitis of the intrahepatic portal vein in ruminants with schistosomiasis [8, 18] or with fascioliasis [1], portal phlebitis in pigs infected with *Stephanurus dentatus* [13], and thrombophlebitis of the lumbar vein in cats infected with *Gurltia paralysans* [18]. Among the hepatic parasitoses in ruminants, schistosomiasis was eradicated about 20 years ago in Japan [22] but fascioliasis has been epidemic, resulting in serious economic losses [16].

* PRESENT ADDRESS: TANIMOTO, T., Chuo Livestock Hygiene Service Center, Kochi Prefectural Government, 1879–1 Tairyonishi, Ino-cho, Agawa-gun, Kochi 781–2100, Japan.

** CORRESPONDENCE TO: Prof. OHTSUKI, Y., Department of Pathology II, Kochi Medical School, Kohasu, Oko-cho, Nankoku-shi, Kochi 783–8505, Japan.

Therefore, in this study we attempted to characterize the intrahepatic phlebitis detected in Japanese beef cattle, to determine whether the cases encountered were in fact cases of fascioliasis, and to compare the hepatic lesions observed in the present cases with those in bovine fascioliasis.

MATERIALS AND METHODS

Despite a number of studies, the species of Japanese *Fasciola* has not yet been determined, due to their variations in shape, size, and chromosome number [16, 21]. A recent study suggests that Japanese *Fasciola* is *Fasciola gigantica* on the basis of similar restriction-fragment-length polymorphism patterns of the mitochondrial DNA and the nucleotide sequences of some regions of the nuclear DNA [7]. In the present paper, however, the term Japanese *Fasciola* sp. is used for Japanese *Fasciola.*

Positive control sera and tissues of fascioliasis used in this study were obtained from Japanese beef cattle with thickening of the main bile ducts containing mature flukes and no vascular thickening.

Animals: Seventeen Japanese beef cattle with vascular thickening of the liver from two meat inspection facilities in Kochi Prefecture, Japan, between 1992 and 1996 were used in this study (Table 1).

Histology: Tissue samples, including liver and hepatic lymph nodes, were fixed in 20% neutral-buffered formalin solution for 48 hr at room temperature and routinely processed for paraffin-embedding. Deparaffinized sections were stained with hematoxylin and eosin (HE), periodic

Table 1. Gross findings of the livers in 17 Japanese beef cattle

Cattle No.	Breed[a)]	Age (years)	Sex[b)]	Vascular thickening[c)]		Biliary lesions	
				Location[d)]	Distribution[e)]	Biliary thickening	Flukes
1	JBL	3	M/c	ALs	D	–	–
2	JBR	3	F	CL	D	–	–
3	JBR	3	M/c	CL, RL	D	–	–
4	JBR	3	M/c	CL	D	–	–
5	JBL	3	F	RL	D	–	–
6	JBR	3	F	LL	D	+	–
7	JBR	3	F	RL	D	+	+[f)]
8	JBR	3	M/c	ALs	S	–	–
9	JBL	3	M/c	ALs	D	–	–
10	JBR	3	M/c	LL	D	+	–
11	JBL	3	M/c	ALs	D	+	–
12	JBL	3	M/c	CL, RL	D	–	–
13	JBL	6	F	LL	S	–	–
14	JBR	3	M/c	CL	S	+	–
15	JBR	3	M/c	ALs	D	+	+[f)]
16	JBL	3	F	CL, LL	D	–	–
17	JBR	3	M/c	ALs	D	+	+[f)]

a) JBL=Japanese Black, JBR=Janapese Brown.
b) F=female, M/c=male castrated.
c) Lesions were more prominent at the periphery of the hepatic lobes.
d) ALs=all the lobes, CL=caudate lobe, RL=right lobe, LL=left lobe.
e) D=diffuse, S=scattered.
f) A few immature flukes were present.

acid-Schiff with diastase digestion (D-PAS) for parasitic eggs, May-Giemsa (MG) for eosinophils and mast cells, Masson's trichrome for collagen fibers, and elastica van Gieson (EVG) for elastic fibers.

Immunoperoxidase staining with an anti-Japanese Fasciola sp. antiserum: To detect *Fasciola* antigens in tissue samples, we did immunoperoxidase staining of formalin-fixed, paraffin-embedded tissue sections with a rabbit anti-Japanese *Fasciola* sp. (*F.* sp.) antiserum (provided by Dr. Yoshifumi Ikeda, Hiroshima City Institute of Public Health, Nishi-ku, Hiroshima-shi, Hiroshima, Japan) [9]. For immunostaining, deparaffinized sections were incubated with 1% H_2O_2 in methanol for 30 min to inhibit endogenous peroxidase and then rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.4). To enhance immunoreactivity of the primary antibody, the sections were predigested with 0.1% pronase (Dako, Kyoto, Japan) in PBS at 37°C for 30 min. Nonspecific staining was blocked by incubation with 10% normal rabbit serum for 10 min, and then the rabbit anti-*F.* sp. antiserum (diluted 1:20,000) was applied for 24 hr at 4°C. This was followed by processing with a labeled streptoavidin-biotin kit (LSAB-PO Kit, Dako, Kyoto, Japan) [6], which uses incubations with biotinylated goat anti-rabbit antibody and peroxidase-conjugated streptoavidin for 10 min each at room temperature. Each staining step was done after washing three times with PBS for 5 min. Sites of specific reaction were visualized as dark brown following incubation of the sections for 3 min in the substrate solution (0.05% 3, 3'-diaminobenzidine tetrahydrochloride and 0.02% H_2O_2 in PBS). The sections were then counterstained with Mayer's hematoxylin for 1 min, dehydrated, and mounted. Formalin-fixed, paraffin-embedded tissue sections of 10 cattle with fascioliasis and those of 5 normal cattle served as positive and negative controls, respectively. Specificity of the antiserum for *F.* sp. was confirmed by Ouchterlony's double immunodiffusion test [10], using antigens of *F.* sp., *Schistosoma japonicum (S. japonicum), Clonorchis sinensis, Cysticercus bovis, Echinococcus multilocularis*, and *Toxocara canis*. In addition, specificities of antigens of *F.* sp. and *S. japonicum* were ascertained in the same manner with sera from a cow with fascioliasis and two Philippines with schistosomiasis, respectively.

Enzyme-linked immunosorbent assay (ELISA): For serological detection of the anti-*Fasciola* antibody, we did ELISA [10] on the sera of 15 cases (cattle Nos. 2 and 4–17). *Fasciola* antigen was prepared from adult *F.* sp. and adjusted into 3 mg protein/m*l* in 0.05 M carbonate buffer, pH 9.6 [10]. The antigen was coated onto microtiter plates (M129A; Dynatech, Alexandria, VA) by incubation of the dilution (100 μl/well) for 2 hr at 37°C. The sera of the cases (diluted 1:400) were then incubated for 40 min at 37°C, followed by incubation with peroxidase-conjugated rabbit anti-bovine IgG (Cappel Research Products, Organon Teknica, Durham, NC, diluted 1:12,000) for 40 min at 37°C. As a dilution buffer, 0.15 M PBS (pH 7.0) containing 0.05% Tween-20 and 1% gelatin was used. Between the reaction steps, sections were washed three times with 0.15 M PBS (pH 7.0) containing 0.05% Tween-20. The labeled peroxidase activity was colored for 5 min with 0.03% 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid)

diammonium salt (ABTS) in 0.05 M citrate phosphate buffer (pH 5.0) containing 0.01% H_2O_2. ABTS is one of the most sensitive substrates for peroxidase [14]. The reaction was stopped by adding 0.05% sodium fluoride to the substrate solution, and the optical density (OD) was read at 405 nm with an ELISA plate reader (MTP-32; Corona Electric, Ibaraki, Japan). Sera from 10 cattle with fascioliasis and those from 10 healthy cattle served as positive and negative controls, respectively. Sera of the cases were regarded as positive when the ODs exceeded the mean OD plus three standard deviations of sera from healthy cattle. In addition, ELISA was done with *S. japonicum* antigen in the same manner described above.

RESULTS

Clinical findings: The 17 affected Japanese beef cattle were born and fed in 17 different farms in Kochi Prefecture, Japan. Antemortem inspection before slaughter revealed no conspicuous abnormalities.

Gross findings: Gross findings of the 17 cases are summarized in Table 1. Intrahepatic vessels with thick walls, up to 1.0 cm in maximum diameter, diffused particularly in the caudate lobe and/or at the periphery of the other hepatic lobes, and often protruded above the lobar surface (Fig. 1). Characteristically, the vessel lumina were packed with white to red, waste thread-like contents. In seven cases, the main bile ducts in the left lobe were dilated and/or had thick walls (up to 0.5 cm in diameter). A few immature flukes (10 mm in maximum length) were present in the bile ducts in three cases. In eight cases (cattle Nos. 3–7, 11, 15, and 17), foci of hepatic necrosis and hemorrhage were scattered around the thickened vessels. Hepatic lymph nodes were slightly enlarged. No conspicuous abnormalities were noted in other organs, including intestine, mesentery, spleen, and lung.

Histologic findings: The thickened vessels were intrahepatic portal veins, mostly interlobular veins but occasionally interlobular arteries, and the thickening was due to hyperplasia of the endothelial cells and medial smooth muscle cells that accompanied severe eosinophilic infiltration and fibrotic expansion of the portal areas (Fig. 2). The lumina of the interlobular veins were occluded by papilliform endothelial hyperplasia and intimal edema, fibrosis, or hyalinization, accompanying severe intimal eosinophilic infiltration (Fig. 3). MG staining revealed many mast cells intermingled in the eosinophilic infiltrates. Lymphocytes, plasma cells, histiocytes, and neutrophils also infiltrated into the portal areas, and the numbers of each cell type varied from site to site even within the same specimen. Surprisingly, vascular smooth muscle cells emerged as a medial layer around the affected interlobular veins, and encircled or ran through their intima in a fashion similar to the circular or longitudinal layers of the tunica muscularis of the intestine (Fig. 2). The hyperplastic medial layer was irregular in thickness even within a given vein and often dispersed into adventitial portions of the vessels (Fig. 2). EVG staining revealed that elastic fibers of the affected veins were thin and fragmented in the intima and hyperplastic media. A variable number of branching veins, small to medium in size, appeared around the affected veins. Hyperplastic intimal lesions also developed in branching veins, and were much more severe than those in the interlobular veins (Fig. 2). Generally, as intimal fibrosis or hyalinization, hyperplasia of medial smooth muscle cells, and fibrotic expansion of the portal areas was more severe, endothelial hyperplasia and cellular infiltration were milder. In seven cases (cattle Nos. 1, 2, 4, 10, 11, 15, and 17), the venous lesions were sometimes accompanied by thrombi, which varied in degree of organization.

Occasionally, the interlobular arteries had medial hypertrophy and adventitial edema to a variable degree (Fig. 2). Intimal infiltration by eosinophils and neutrophils was occasionally observed, as in venous lesions. Interestingly, nerve fascicles consisting of unmyelinated nerve fibers with a Schwann cell sheath appeared around the hypertrophic interlobular arteries. Small arteries appeared around the enlarged interlobular arteries. In three cases (cattle Nos. 1, 7, and 10), fibrinoid necrosis was observed in some small arteries.

The central veins sometimes showed dilatation, perivascular fibrosis, and medial muscular hyperplasia.

In seven cases (cattle Nos. 6, 7, 10, 11, 14, 15, and 17), intrahepatic bile ducts in the left lobe were enlarged, owing to papillary and/or glandular hyperplasia of the biliary epithelium, accompanied by infiltration of eosinophils and lymphocytes and surrounding granulation tissue. The biliary epithelium was intermingled with variable numbers of globule leukocytes.

In eight cases (cattle Nos. 3–7, 11, 15, and 17), necrotic and hemorrhagic foci of hepatocytes surrounded by various numbers of eosinophils, neutrophils, and histiocytes were scattered around the portal lesions (Fig. 4). In such foci, often in the smaller ones, granulomatous reaction of epithelioid cells and multinucleated giant cells to eosinophilic matter, which were negative on D-PAS stain, was observed (Fig. 5). In cattle No. 3, one immature fluke was detected among the necrotic hepatic tissues. No parasitic eggs were detected in the hepatic tissues of any of the cases.

Immunoperoxidase staining and ELISA: We found a specific precipitin line between the antiserum used in this study and *F.* sp. antigen with Ouchterlony's double immunodiffusion test (Fig. 6). In the study for antigen specificities, *F.* sp. and *S. japonicum* antigens formed precipitin lines with sera from a cow with fascioliasis and two Philippines with schistosomiasis, respectively (Fig. 6). A whole immature fluke, seen in the necrotic hepatic tissue of cattle No. 3, was strongly positive for anti-*F.* sp. antiserum with immunoperoxidase staining (Fig. 7). Histiocytes in the interlobular lesions and the necrotic foci of the hepatocytes, and Kupffer cells had variable degrees of reactivity for the antiserum (Fig. 8). The reactivity and the intensity of staining of Kupffer cells, however, were

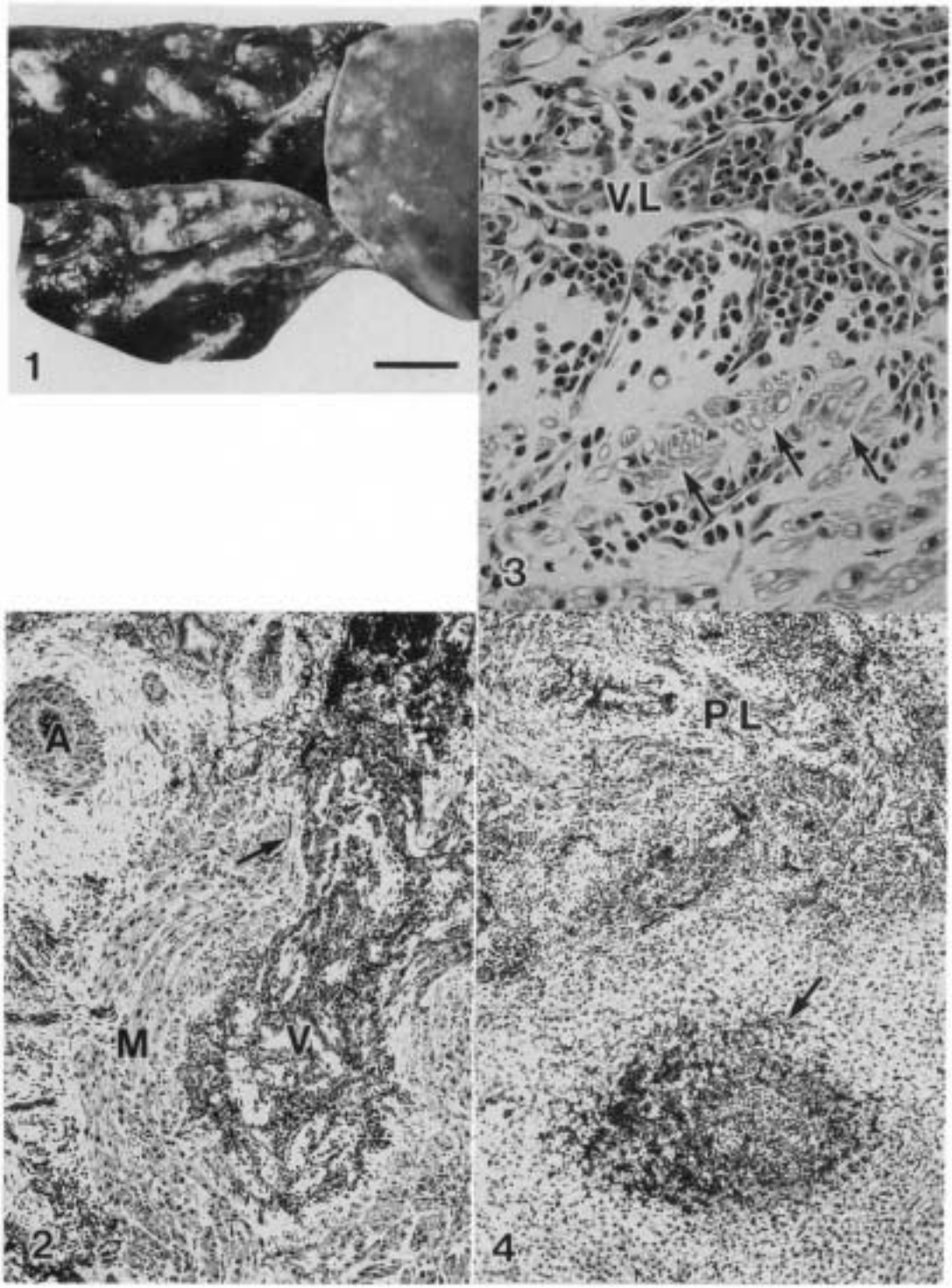

Fig. 1. Liver; cattle No. 9. Prominently thickened intrahepatic vessels. Bar = 2 cm.

Fig. 2. Liver; cattle No.1. Low power view of a portal area. An affected interlobular vein (V) showing intimal hyperplasia occupying the lumen, intimal cellular infiltrates, and hyperplastic media (M). Intimal lesions also developed in branching veins (arrow). Note a hypertrophic interlobular artery (A) with adventitial edema. HE. × 55.

Fig. 3. Liver; cattle No. 1. High power view of an affected interlobular vein showing papillary intimal hyperplasia occupying the lumen (VL), intimal eosinophilic infiltration, edema, and hyalinization. Note cytoplasmic vacuolation in hyperplastic medial cells (arrows). HE. × 349.

Fig. 4. Liver, cattle No. 7. An focal hepatic necrosis (arrow) near the portal lesion (PL). HE. × 44.

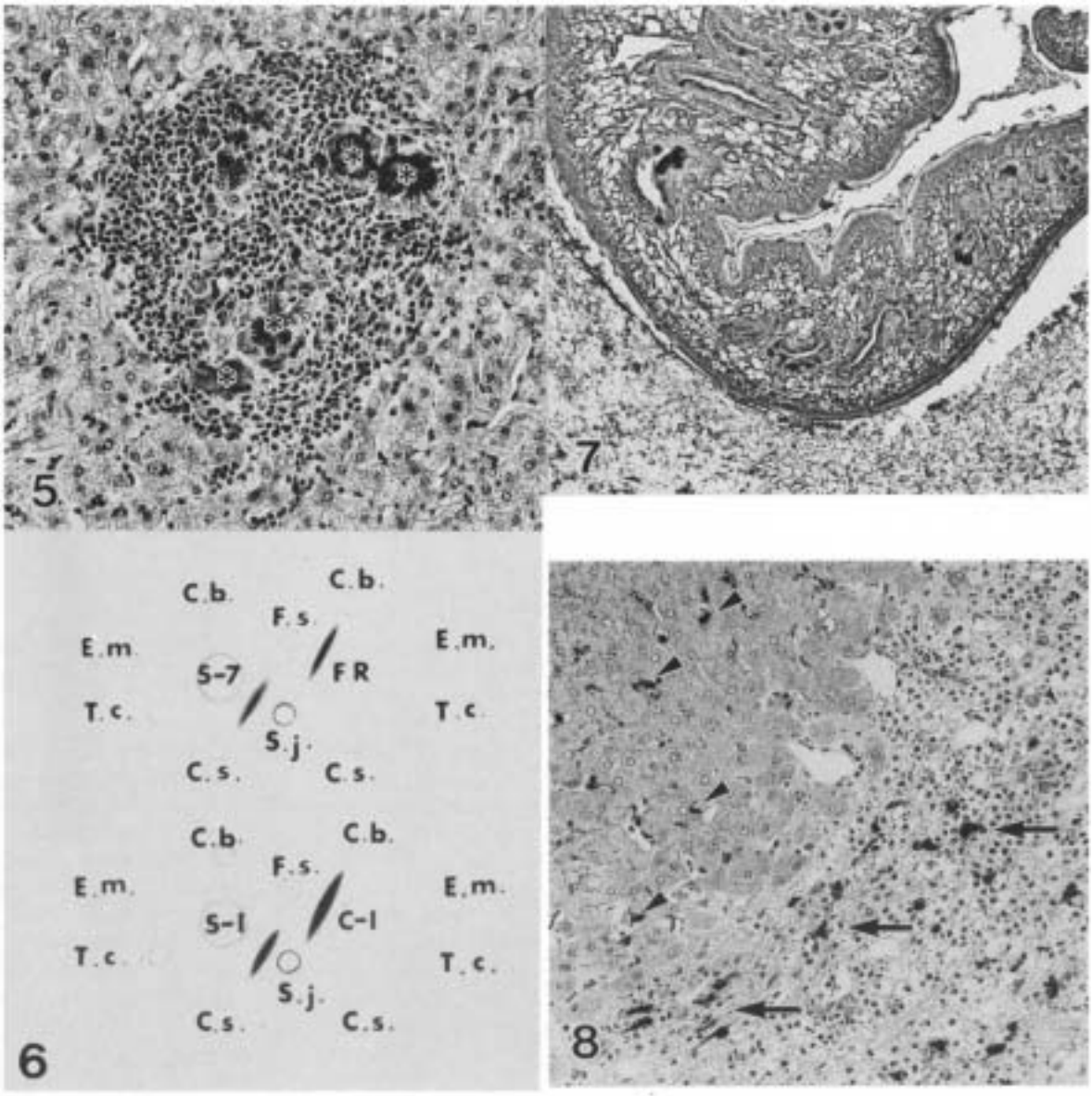

Fig. 5. Liver; cattle No. 7. An area of granulomatous reaction to the eosinophilic matter (asterisks) near the portal lesion. HE.× 175.

Fig. 6. Ouchterlony's double immunodiffusion test. A specific precipitin line between the anti-Japanese *Fasciola* sp. antiserum (FR) and Japanese *Fasciola* sp. antigen (F.s.). Note distinct precipitin lines in antigen control study: between F.s. antigen and the serum of a cow with fascioliasis (C-1), between *Schistosoma japonicum* (S.j.) antigen and two Philippines with schistosomiasis (S-1 and S-7). C.s. = *Clonorchis sinensis*; E.m. = *Echinococcus multilocularis*; C.b. = *Cysticercus bovis*; T.c. = *Toxocara canis*.

Fig. 7. Liver; cattle No. 3. Most parts of an immature fluke in a necrotic hepatic tissue were strongly positive for anti-Japanese *Fasciola* sp. antiserum. Labeled streptoavidin-biotin technique with a rabbit anti-Japanese *Fasciola* sp. antiserum, Mayer's hematoxylin counterstain.× 74.

Fig. 8. Liver; cattle No. 10. Positive reactions of histiocytes (arrows) in an portal lesion, and of Kupffer cells (arrowheads). Labeled streptoavidin-biotin technique with a rabbit anti-*Fasciola* sp. antiserum, Mayer's hematoxylin counterstain.× 175.

usually greater than those of infiltrating histiocytes. The eosinophilic matter in the granulomas was not stained with the antiserum. Histiocytes in the sinus and in the germinal center of the hepatic lymph nodes, and in the red pulp of the spleen also had variable degrees of reactivity for the antiserum. The results of staining in all of the cases were identical with those of bovine fascioliasis (positive controls). No positive reactions were observed in negative control

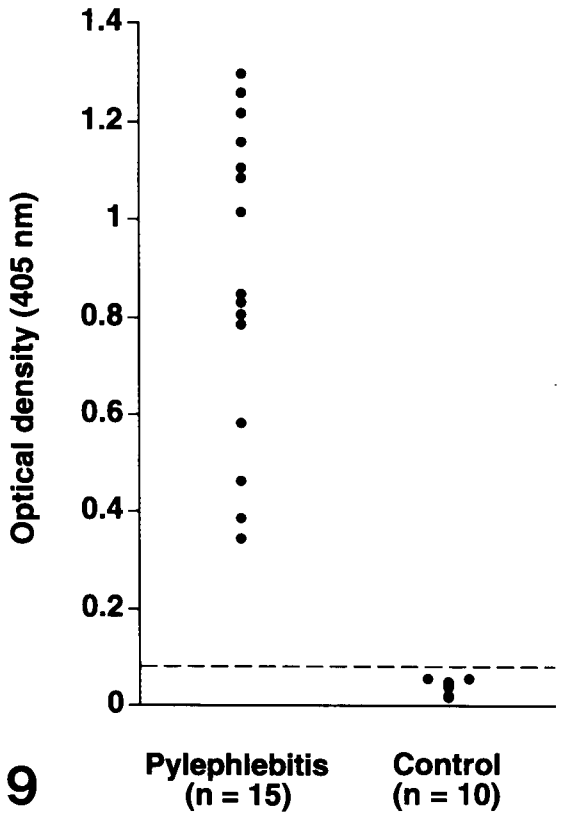

Fig. 9. Detection of anti-Fasciola antibody in the serum of 15 Japanese beef cattle with pylephlebitis. Horizontal dashed line represents threshold of positivity, determined by mean optical density plus three standard deviations of sera from 10 normal cattle (control).

tissues.

On ELISA, 15 cases tested, together with positive control sera, were considered positive for anti-*Fasciola* antibody (Fig. 9) and negative for anti-*Schistosoma* antibody.

DISCUSSION

The results of macropathological, histological, and immunohistochemical studies, as well as ELISA, are sufficient to determine that the present cases of intrahepatic pylephlebitis are fascioliasis. On the basis of the histological characteristics of the present cases, we designated the intrahepatic vascular lesions as "eosinophilic proliferative intrahepatic pylephlebitis". As for the lesions of bovine fascioliasis, intrahepatic pylephlebitis has not yet been recognized in Japan [2], but in other countries it has been reported to occur in *Fasciola hepatica* infections in cattle and sheep as only a microscopic focal lesion [1, 3, 17]. In contrast to the previous reports [1, 3, 17], the intrahepatic portal lesions observed in the present cases were so diffuse and severe that they could be found macroscopically as vascular thickening, and were not accompanied by the typical hepatic lesions of fascioliasis.

Generally in fascioliasis, mature flukes in the bile ducts, hypertrophic cholangitis, and interlobular fibrosis are observed in the liver, frequently in the left lobe [2, 11]. Although 7 cases possessed biliary lesions typical of fascioliasis, the other 10 cases had a form of fascioliasis that was unusual in some respects: 1) no flukes were detected on routine pathological examination, 2) visibly thickened veins were noted in the periphery of all the hepatic lobes that had severe proliferative intrahepatic pylephlebitis with infiltration by many eosinophils, and 3) fibrotic expansion of portal areas was present without severe interlobular fibrosis, hypertrophic cholangitis, or reactive lymphoid hyperplasia.

The precise reason why intrahepatic pylephlebitis occurs in Japanese beef cattle with or without the typical hepatic lesions of fascioliasis is unclear. Some of the following, however, might be related to the conditions that can induce intrahepatic pylephlebitis in ruminants with fascioliasis: the maturity of intrahepatic flukes [1, 17], the number of infected metacercariae [4, 12], the route of metacercarial infection [12], interspecies differences in pathogenicity of *Fasciola* [12], and hypersensitive host immune responses [20].

Previous studies have shown that intrahepatic pylephlebitis is frequently observed during intrahepatic migration of immature flukes in cases of spontaneous fascioliasis [1, 17] or in cases experimentally infected with a few metacercariae [4, 12]. The scattered necrotic and hemorrhagic foci in the hepatic tissues with inflammation, including the eosinophilic or granulomatous reaction observed in eight cases of the present study, resembled those caused by fluke infestation as described for fascioliasis [11]. Immunohistochemical detection of the *Fasciola* antigen in these sites is evidence for fluke infestation in the hepatic parenchyma. In addition, an immature fluke was detected in the necrotic focus in only one case and a few immature flukes were present in the bile ducts of three cases. These findings suggest that small numbers of immature flukes can induce intrahepatic pylephlebitis during migration. To confirm these findings, sieving of fresh hepatic tissues [19] would be helpful in detecting the number of young flukes in cattle with intrahepatic pylephlebitis.

As for the route of *Fasciola* infection, some investigators have proposed that young flukes rarely enter the liver parenchyma through the portal system [12, 13]. An experimental study with rabbits demonstrated that severe intrahepatic pylephlebitis could develop in all the lobes when excysted metacercariae of *F.* sp. were injected through the portal vein [12]. Most of the metacercariae entered the liver parenchyma within 24 hr after injection and remained there throughout the duration of the experiment (77 days after injection). This might be the reason for the absence of flukes within the intrahepatic portal veins and bile ducts, and fluke eggs in the feces of the present cases as well as previously reported cases [23]. In addition, similarly injected *Fasciola hepatica* did not cause intrahepatic pylephlebitis but did cause severe hypertrophic cholangitis.

Thus, there appears to be an interspecies difference in the pathogenicity of *Fasciola*.

The immunologic mechanism of the intrahepatic pylephlebitis in the present cases is unclear; however, in fascioliasis, local hypersensitive or immune complex formation might result in accumulation of eosinophils, mast cells, and lymphocytes [20].

The hyperplastic endothelial proliferation characteristically observed in the present cases was identical to papillary endothelial hyperplasia (PEH) [5]. PEH is considered an unusual form of organizing thrombus that probably originates from preexisting vascular lesions such as hemangiomas, pyogenic granulomas, arteriovenous malformations, or vascular hamartomas, although its pathogenesis is unclear [5]. This intimal lesion has been briefly described as a feature of intrahepatic pylephlebitis in fascioliasis [4, 20] and in schistosomiasis of cattle [8, 18]. In schistosomiasis, it has been supposed that adult schistosomes within intrahepatic portal veins irritate the endothelium and that dead ones release foreign proteins, inducing intimal hyperplasia and intimal eosinophilic infiltration [8, 18].

Surprisingly, medial layers surrounding the affected interlobular veins and nerve fascicles scattered around the hypertrophic interlobular arteries were observed in the present cases; these are not normally present in the bovine liver. These heterotopic hyperplastic changes have not been previously recognized in domestic animals and humans. We consider them to be unique adaptive or progressive changes to altered portal hemodynamics in the liver, resulting from intrahepatic pylephlebitis.

In the present cases, medial hypertrophy and adventitial edema of the interlobular arteries were accompanied by intrahepatic pylephlebitis. These arterial changes were similar to those observed in arteriovenous anastomosis of ovine fascioliasis resulting from veno-occlusion due to intrahepatic pylephlebitis [20].

From our histologic findings, we conjecture the pathogenesis of the intrahepatic vascular lesions to be as follows: migrating young flukes affect the endothelium of the intrahepatic portal veins causing severe proliferative pylephlebitis with occasional thrombosis and intimal infiltration of many eosinophils and mast cells and variable numbers of lymphocytes, histiocytes, and neutrophils. The venous lumina were frequently occluded by proliferative intimal lesions, resulting in portal hypertension, leading to irregular proliferation of medial vascular smooth muscle cells, arteriovenous anastomoses, and interlobular fibrosis.

In conclusion, we designated intrahepatic vascular lesions with unique pathological features seen in the 17 Japanese beef cattle as “eosinophilic proliferative intrahepatic pylephlebitis” and regard it as an atypical form of fascioliasis.

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REFERENCES

1. Anon. 1974. Hepatic vascular pathology in ovine fascioliasis. *Vet. Rec.* 95: 572–573.
2. Ashizawa, H. 1964. Pathological studies on fascioliasis. III. Pathological changes of liver of cattle invaded by *Fasciola sp. Bull. Fac. Agrc. Univ. Miyazaki* 10: 1–40 (in Japanese with English summary).
3. Dargie, J. D., Armour, J., Rushton, B. and Murray, M. 1974. Immune mechanisms and hepatic fibrosis in fascioliasis. pp. 249–271. *In*: Parasitic Zoonosis; Clinical and Experimental Studies (Soulsby, E. J. L. ed.), Academic Press, London, England.
4. Dow, C., Ross, J. G. and Todd, J. R. 1967. The pathology of experimental fascioliasis in calves. *J. Comp. Pathol.* 77: 377–385.
5. Enzinger, F. M. and Weiss, S. W. 1995. Benign tumors and tumorlike lesions of blood vessels. pp. 614–619. *In*: Soft Tissue Tumors, 3rd ed., Mosby, St Louis, MO.
6. Guesdon, J-L., Ternynck, T. and Avrameas, S. 1979. The use of avidin-biotin interaction in immunoenzymatic techniques. *J. Histochem. Cytochem.* 27: 1131–1139.
7. Hashimoto, K., Watanobe, T., Liu, C. X., Init, I., Blair, D., Ohnishi, S. and Agatsuma, T. 1997. Mitochondrial DNA and nuclear DNA indicate that the Japanese *Fasciola* species is *F. gigantica*. *Parasitol. Res.* 83: 220–225.
8. Hussein, M. F. 1971. The pathology of experimental schistosomiasis in calves. *Res. Vet . Sci.* 12: 246–252.
9. Ikeda, Y., Miyamoto, Y., Noda, M., Yamaoka, K., Matsuishi, T. and Ogino, T. 1992. Antigenic analysis of *Fasciola hepatica* with mouse monoclonal antibody. *Hiroshima J. Vet. Med.* 7: 49–52 (in Japanese with English summary).
10. Itagaki, T., Ohta, N., Hosaka, Y., Iso, H., Konishi, M., Chinone, S. and Itagaki H. 1989. Diagnosis of *Fasciola* sp. infections in cattle by enzyme-linked immunosorbent assay. *J. Vet. Med. Sci.* 51: 757–764.
11. Jones, T. C. and Hunt, R. D. 1983. Diseases caused by parasitic helminths and arthropods. pp. 851–856. *In*: Veterinary Pathology, 5th ed., Lea and Febiger, Philadelphia, PA.
12. Kawano, J., Simizu, A., Ishimaru, T., Umeda, S. and Kimura, S. 1990. Experimental infection of rabbits with newly excysted metacercariae of Japanese *Fasciola* sp. and American *Fasciola hepatica* by portal vein route. *Sci. Rep. Fac. Agric. Kobe Univ.* 19: 77–83 (in Japanese with English summary).
13. Kelly, W. R. 1993. The liver and biliary system. pp. 319–406. *In*: Pathology of Domestic Animals, 4th ed., vol. 2 (Jubb, K. V. F., Kennedy, P. C. and Palmer, N. eds.), Academic Press, New York, NY.
14. Matsuda, H., Tanaka, H., Blas, B. L., Nosenas, J. S,. Tokawa, T. and Ohsawa, S. 1984. Evaluation of ELISA with ABTS, 2-2,-azino-di-(3-ethylbenzthiazoline sulfonic acid), as the substrate of peroxidase and its application to the diagnosis of Schistosomiasis. *Jpn. J. Exp. Med.* 54: 131–138.
15. Nomura, Y., Une, Y. and Shirota, K. 1994. Vasculitis with intimal proliferation and eosinophil reaction in bovine liver. *Vet . Pathol.* 31: 595.

16. Oshima, T. 1983. Fascioliasis. pp. 152–162. *In*: Parasitic Zoonosis in Japan (Hayashi, S., Ishii, T., Oshio, Y., Koyama, T. and Kondo, S. eds.), Buneido, Tokyo (in Japanese).
17. Rahko, T. 1969. The pathology of natural *Fasciola hepatica* infection in cattle. *Vet. Pathol.* 6: 244–256.
18. Robinson, W. F. and Maxie, M. G. 1993. The cardiovascular system. pp. 51–79. *In*: Pathology of Domestic Animals, 4th ed., vol. 3 (Jubb, K. V. F., Kennedy, P. C. and Palmer, N. eds.), Academic Press, New York, NY.
19. Ross, J. G., Todd, J. R. and Dow, C. 1966. Single experimental infections of calves with the liver fluke, *Fasciola hepatica* (Linnaeus 1758). *J. Comp. Pathol.* 76: 67–81.
20. Rushton, B. and Murray, M. 1978. Intrahepatic vascular lesions in experimental and natural ovine fascioliasis. *J. Pathol.* 125: 11–20.
21. Watanabe S. 1965. A revision of genus *Fasciola* in Japan, with particular reference to *F. hepatica* and *F. gigantica*. pp. 361–381. *In*: Progress of Medical Parasitology in Japan, vol. 2 (Morishita, K., Komiya, Y. and Matsubayashi, H. eds.), Meguro Parasitological Museum, Tokyo.
22. Yokogawa, M. 1976. Review of prevalence and distribution of schistosomiasis in Japan. *Southeast Asian J. Trop. Med. Public Health* 7: 137–143.
23. Yoshida, T., Nagahama, K., Miyanowaki, K., Ogawa, T., Mizoguchi, T., Akagi, R. and Higuchi, T. 1996. Pathology of eosinophilic phlebitis in the liver of Japanese black cattle. *J. Jpn. Vet. Med. Assoc.* 49: 751–754 (in Japanese with English summary).