The Journal of Veterinary Medical Science

FULL PAPER

Pathology

Gross and histological lesions in the livers of sika deer with particular emphasis on fascioliasis

Kazuya MATSUDA[1)]*, Shun KOGAME[1)], Hinako NIKI[1)], Moe SAITO[1)], Yuki ISHIGURO[1)] and Yuto SANO[1)]

[1)]Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

ABSTRACT. We performed gross and histological examinations of the livers of sika deer (*Cervus nippon yesoensis*) in Hokkaido, Japan. Out of 1,381 deer slaughtered for venison production, thickening and dilation of the large intrahepatic bile ducts and *Fasciola* flukes in the duct lumens were detected in 621 deer (45.0%). Furthermore, 107 non-bile lesions (75 intrahepatic and 32 capsular lesions) were detected during gross examinations. Histologically, the bile duct lesions included chronic proliferative cholangitis, papillary hyperplasia, goblet cell and pyloric gland metaplasia, and periductal fibrosis. Many of the intrahepatic non-bile duct lesions (53/75, 71%) were considered to be *Fasciola* fluke migration-associated lesions, including two lesion types: necrosis, hemorrhage, and eosinophilic granuloma formation (29 lesions), and lymphoid tissue formation (24 lesions). Lymphoid tissue formation was considered to result from the persistent immune responses against dead *Fasciola* flukes. An epidermoid liver cyst was found incidentally, which has not been reported in the veterinary literature. In summary, this study demonstrated the predominance of fascioliasis-associated lesions in sika deer livers. The gross and histological lesions caused by *Fasciola* flukes in sika deer were similar to fascioliasis in other animals. Moreover, we described lymphoid tissue formation as a fascioliasis-associated lesion for the first time. The fact that bile duct lesions (45.0%) had a markedly higher prevalence than fascioliasis-associated parenchymal lesions (53/1,381, 3.8%) indicated that sika deer are a permissive host for fascioliasis. Our results provide information that will aid pathological examinations of sika deer.

KEY WORDS: *Cervus nippon yesoensis*, *Fasciola*, liver, pathology, sika deer

J. Vet. Med. Sci.
82(2): 125–134, 2020
doi: 10.1292/jvms.19-0544

Received: 3 October 2019
Accepted: 4 December 2019
Advanced Epub:
13 December 2019

As the number of wild sika deer has increased in Japan, the numbers of deer that are captured and utilized for venison production have also risen [8]. During fiscal year 2017, over 64,000 sika deer were used to produce venison in Japan, and about 50% of these deer were slaughtered on Hokkaido, the northern island of Japan [8, 10]. Information about a broad range of deer diseases is required for safety and hygiene inspections relating to venison production; however, to the best of our knowledge, there have not been any comprehensive studies of the macroscopic or microscopic lesions found in the organs of sika deer.

A previous study based on fecal testing detected fascioliasis in sika deer on Hokkaido [11]. Fascioliasis is an important foodborne disease caused by *Fasciola* flukes [1, 2]. It mainly affects domestic livestock, particularly cattle and sheep, and occasionally arises in wildlife, zoo animals, and humans. The life cycle of *Fasciola* species involve snails and animals as intermediate and final hosts, respectively [1]. Animals and humans are infected via the ingestion of infective metacercariae, which emerge from their snail hosts and become encysted in the exterior environment. Ingested metacercariae excyst in the small intestine, penetrate the intestinal wall, move into the abdominal cavity, and invade the liver. The juvenile flukes wander and grow in the liver parenchyma and eventually reach the bile duct, where they grow into adults and produce eggs. The disease occurs in two phases: the migratory phase, which involves the migration of flukes through the liver parenchyma, and the biliary phase, during which mature flukes reside in the bile ducts [2]. Fascioliasis-associated gross lesions in sika deer were reported in a Japanese article, which dealt with 8 heads of deer inhabiting in Nara Park, Japan; however, a large scale pathological study on fascioliasis in sika deer was not found in the English literature [6].

In the present study, we focused on the liver, which is the main organ damaged in fascioliasis and is obviously an important organ that must be inspected during the evaluations conducted at slaughter in deer used for venison production. To provide

*Correspondence to: Matsuda, K.: kmatsuda@rakuno.ac.jp

(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

information about the pathological changes in the liver of sika deer, we performed gross and histological examinations and summarized the liver pathology of sika deer that were used for venison production.

MATERIALS AND METHODS

Animals and slaughter inspections

In total, 1,381 sika deer (*Cervus nippon yesoensis*), which were captured in the eastern district of Hokkaido in winter and had been farmed until slaughter for <1 year, were examined in the present study during a period from September, 2013 to February, 2017. They included 1,365 female and 16 male deer and weighed 26 to 102 kg (mean weight: 56.4 kg) at slaughter. The deer were stunned by electrocution and exsanguinated according to the Yeso Sika Deer Hygienic and Processing Manual by Hokkaido prefecture (2015). All deer were grossly inspected by veterinarians. To evaluate the deer for *Fasciola* infections, cut sections of the liver containing large intrahepatic bile ducts (IHBD) were produced and livers with thickened and dilated bile ducts and *Fasciola* flukes in the lumens of the bile ducts were diagnosed with fascioliasis.

Gross and histological examinations

A total of 302 livers were sent to our laboratory for detailed gross and histological examinations, which included all of the livers in which non-bile duct lesions were detected during slaughter inspections (85 livers), randomly selected livers with bile duct lesions (156 livers), and randomly selected livers without grossly detectable lesions (61 livers) (Supplementary Table 1). First, we examined the external surface of the whole liver. Then, we obtained a tissue slice at the porta hepatis and obtained further slices at 3-cm intervals, and examined their cut surfaces. For the histological examinations, we collected tissue slices at the porta hepatis (which included the left main hepatic duct just near its junction with the right hepatic duct), in the left lobe (which included the left main hepatic duct) and in the quadrate lobe (which included the branch of the right hepatic duct). If the livers had lesions other than fascioliasis-associated bile duct lesions, we also inspected and collected the lesions. The collected tissues were fixed in 10% formalin or Bouin's fixative, embedded in paraffin wax, sectioned into 4-μm slices, and stained with hematoxylin and eosin. To detect microorganisms, we performed Gram staining, Brown-Hopps method, on sections from selected lesions. The histological examinations were performed using a light microscope.

Bacteriological examinations

For bacterial isolation, swab samples from selected lesions were inoculated on the 5% horse blood agar plates and cultivated anaerobically at 37°C. After 48 hr of incubation, individual colonies were subjected to Gram staining. A single colony of a suspicious isolate from a pure culture plate was analyzed by the MALDI-Biotyper 3.1 (Bruker Daltonics, Bremen, Germany) [7]. Bacterial species was identified by MALDI-TOF MS.

Statistical analysis

To clarify the relationship between each non-bile duct lesion and *Fasciola* infection, the prevalence of each lesion was compared between deer with and without fascioliasis-associated bile duct lesions using the χ^2 test.

RESULTS

Bile duct lesions associated with Fasciola infections

Out of 1,381 deer that were inspected by veterinarians at slaughter, thickening and dilation of the large IHBD and *Fasciola* flukes in the lumens of the ducts; i.e., *Fasciola* infections, were detected in 621 (45.0%) (Fig. 1a and 1b). A summary of the lesions detected during the gross examinations is provided in Table 1. Severely affected bile duct segments were prominent on the surface of the liver and appeared as white foci (Supplementary Fig. 1a and 1b).

Histologically, in the livers that had not been infected by *Fasciola*, the mucosal epithelia of the large IHBD were almost flat, and peribiliary glands were located under the mucosal epithelial cells (Fig. 1c). In contrast, the affected bile ducts displayed hyperplastic and chronic inflammatory changes (chronic proliferative cholangitis) (Fig. 1d–g). In these cases, the mucosal epithelia were hyperplastic and formed deep crypts. In addition, the highly hyperplastic mucosa exhibited papillary projection into the lumen (papillary hyperplasia) with increased numbers of peribiliary glands (Fig. 1e). Eosinophils, lymphocytes and plasma cells had infiltrated into the lamina propria and periductal fibrous tissue (Fig. 1f). Furthermore, goblet cells were often seen in the hyperplastic epithelia (goblet cell metaplasia), and the bottoms of the crypts and the peribiliary glands frequently displayed a pyloric gland cell appearance (pyloric gland metaplasia) (Fig. 1g and 1h). Periductal fibrous connective tissue proliferated and increased in thickness (periductal fibrosis) (Fig. 1d and 1e). In most cases, one to several lymph follicles formed in the lamina propria and/or just below the mucosa (Fig. 1d). In such ducts, plasma cells were the most dominant cell type among the infiltrating leukocytes. In the markedly affected bile ducts, the hyperplastic and inflammatory changes extended to the small-caliber bile ducts around the affected large IHBD. Focal erosion or ulcers and the proliferation of granulation tissue were occasionally observed in the mucosa (Supplementary Fig. 2a and 2b). Of the 302 deer that were examined histologically, 203 had fascioliasis-associated bile duct lesions (Supplementary Table 1).

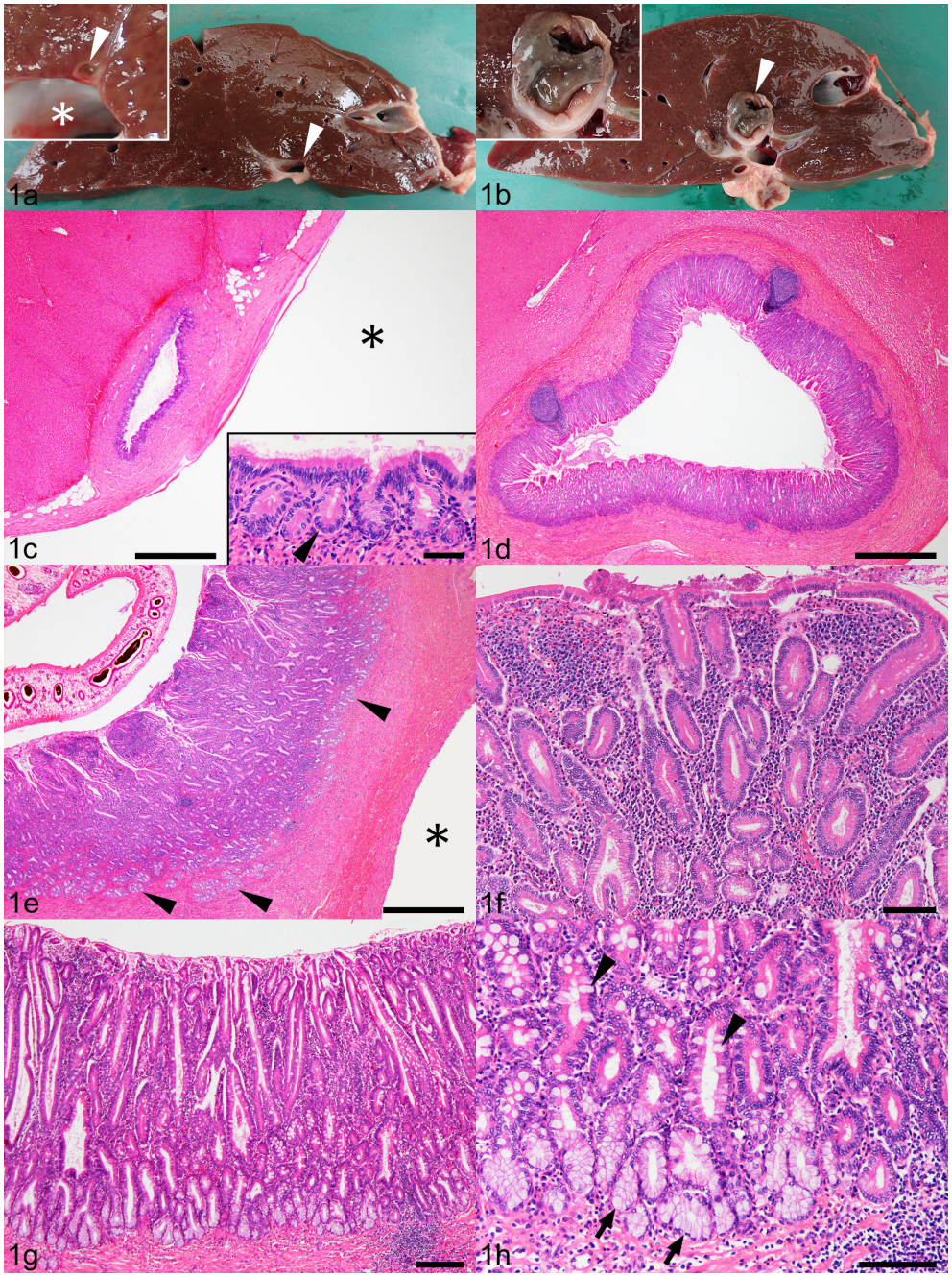

Fig. 1. Bile duct lesions associated with *Fasciola* infections. a. Cut surface of a section obtained at the porta hepatis of a liver without *Fasciola* infection. The left main hepatic duct (cross-section; arrowhead) is indistinct by the naked eye. Inset. Magnified photograph of the left main hepatic duct (arrowhead). The left main hepatic duct runs alongside a branch of the portal venule (asterisk). b. Cut surface of a section obtained at the porta hepatis of a liver with *Fasciola* infection. The left main hepatic duct (cross-section; arrowhead) is severely thickened and dilated, and *Fasciola* flukes are present in the lumen of the duct. Inset. Magnified photograph of the left main hepatic duct showing *Fasciola* flukes in the duct lumen. c. Cross-section of the left main hepatic duct obtained at the porta hepatis of a liver without *Fasciola* infection. Asterisk indicates the lumen of a branch of the portal vein. Hematoxylin and eosin (HE). Bar=1 mm. Inset. Higher magnification image of the biliary mucosa. The mucosal epithelial layer is almost flat and accompanied by peribiliary glands (arrowhead). HE. Bar=50 μm. d. Cross-section of the left main hepatic duct obtained at the porta hepatis of a liver with *Fasciola* infection. The cross-section displays moderate hyperplasia of the biliary epithelium, lymphoid follicle formation, and thickening of the periductal fibrous layer. HE. Bar=1 mm (same magnification as in Fig. 1c). e. Cross-section of the left main hepatic duct obtained at the porta hepatis of a liver with *Fasciola* infection. The cross-section displays papillary projection of the severely hyperplastic biliary epithelium, hyperplastic peribiliary glands (arrowheads), and thickening of the periductal fibrous layer. Asterisk indicates the lumen of a branch of the portal vein. HE. Bar=1 mm (same magnification as in Fig. 1c and 1d). f. Higher magnification of the tip of the hyperplastic biliary epithelium shown in Fig. 1e. The biliary epithelial cells proliferate to form crypts, and eosinophils, lymphocytes, and plasma cells infiltrate into the lamina propria. HE. Bar=100 μm. g. A moderately hyperplastic biliary epithelium with deep crypt formation. HE. Bar=200 μm. h. Higher magnification of the bottom of the hyperplastic biliary epithelium shown in Fig. 1g. The epithelial cells exhibit goblet cell metaplasia (arrowheads) and pyloric gland metaplasia (arrows). HE. Bar=100 μm.

Table 1. Summary of the liver lesions detected in the livers of 1,381 sika deer during gross examinations

Groups of lesions	Gross findings	Histological lesions	No. of deer with each type of lesion (%)			
			Total[a] [n=1,381]	*Fasciola* infection[b] (Fascioliasis-associated bile duct lesions)		
				+ [n=621]	– [n=760]	*P*-value
Bile duct lesions associated with *Fasciola* infections	Thickening and dilation of the large IHBD, *Fasciola* flukes residing in the lumens of the large IHBD	Chronic proliferative cholangitis, papillary hyperplasia, goblet cell metaplasia, pyloric gland metaplasia, periductal fibrosis	621 (45.0)	NA	NA	NA
Intrahepatic lesions associated with *Fasciola* fluke migration	Subcapsular, focal, multifocal, or locally extensive, hemorrhagic or discolored lesions	Necrosis, hemorrhage, eosinophilic granuloma formation, periportal eosinophilic hepatitis	29 (2.1)	19 (3.1)	10 (1.3)	0.025*
	Subcapsular, well-demarcated, translucent white nodules	Lymphoid tissue formation	24 (1.7)	11 (1.8)	13 (1.7)	0.931
Miscellaneous intrahepatic lesions	Subcapusular, well-demarcated, white or yellow foci	Subcapsular hepatocyte lipidosis	15 (1.1)	9 (1.4)	6 (0.8)	0.239
	Abscesses	Hepatic necrobacillosis	5 (0.4)	1 (0.2)	4 (0.5)	0.261
	Pinpoint white foci throughout the liver	Periportal suppurative hepatitis	1 (0.1)	1 (0.2)	0 (0.0)	0.268
	Thin-walled cyst	Solitary bile duct cyst	1 (0.1)	1 (0.2)	0 (0.0)	0.268
Capsular lesions	Focal, multifocal, or locally extensive thickening of the capusle	Hepatic capsulitis	17 (1.2)	11 (1.8)	6 (0.8)	0.100
	Adhesion between the liver and other organs	Fibrous adhesion to other organs	15 (1.1)	8 (1.3)	7 (0.9)	0.513

a) Number of deer with each type of lesion and prevalence rates (shown as percentages in parentheses) in all deer (1,381 heads). b) Number of deer with each type of lesion and their prevalence rates (shown as percentages in parentheses) in deer with (621 heads; column '+') and without (760 heads; column '–') fascioliasis-associated bile duct lesions. In each type of lesion (row), number of total deer with the lesion (number in column 'Total') is sum of numbers of deer with (number in column '+') and without (number in column '-') fascioliasis-associated bile duct lesions. *P*-values were obtained using the χ^2 test. NA, not applicable; *, Statisitically significant difference in the prevalence of the lesion between deer with and without fascioliasis-associated bile duct lesions ($P<0.05$); IHBD, intrahepatic bile ducts.

Intrahepatic lesions associated with Fasciola fluke migration

a) Necrosis, hemorrhage, and eosinophilic granuloma formation: Among the 1,381 slaughtered deer, 107 non-bile duct lesions, including 75 intrahepatic and 32 capsular lesions, were detected in 85 deer during gross examinations. Of the 75 intrahepatic lesions, 53 were considered to be associated with *Fasciola* fluke migration in the liver parenchyma. In this study, we grouped the lesions into two types. The first type of lesions were observed in 29 livers and grossly appeared as focal or multifocal, subcapsular, hemorrhagic or discolored (pale tan to yellow colored) foci, which sometimes showed a tortuous appearance (Fig. 2a and 2b and Supplementary Fig. 3a and 3b**)**. In some cases, the lesions were locally extensive and accompanied by indentation of the capsular surface (cicatricial contraction) (Supplementary Fig. 3c).

Histologically, the hemorrhagic lesions were composed of irregularly shaped, multifocal to coalescing regions of necrotic liver parenchyma with hemorrhage (Fig. 2c). In addition to the destructive necrosis with hemorrhage, focal coagulation necrosis was also observed (Fig. 2d). As the lesions became grossly discolored, large numbers of eosinophils infiltrated into the necrotic foci (they appeared as numerous degenerated eosinophils in the necrotic foci), and the necrotic foci were surrounded by palisading multinucleated giant cells admixed with eosinophils, lymphocytes, and plasma cells (eosinophilic granulomas) (Fig. 2e and 2f). Granulomatous reactions formed around the necrotic tissue of the liver parenchyma, and no flukes or fluke remnants were found in the granulomas. These changes were accompanied by capsular and periportal infiltration of eosinophils, lymphocytes, and plasma cells combined with fibrosis in the surrounding liver parenchyma (periportal eosinophilic hepatitis). In some cases, the necrosis was massive and transitioned to locally extensive eosinophilic hepatitis with fibrosis. No flukes or fluke remnants were found in these lesions.

In one case, a cyst lined by a keratinizing stratified squamous epithelium and surrounded by a smooth muscle layer was found in the vicinity of a hemorrhagic lesion (Supplementary Fig. 4a and 4b). Examinations of serial histological sections demonstrated that the cyst was branched and had blind ends.

b) Lymphoid tissue formation: Out of the 53 lesions associated with *Fasciola* fluke migration in the liver parenchyma, we classified 24 lesions as the second type of lesions. The lesions had an identical gross appearance. They appeared as white foci, which were usually 3 to 5 mm in diameter, on the surface of the liver and as subcapsular, solitary or closely arranged, well-demarcated, spherical to spheroidal, translucent white nodules on cut sections (Fig. 3a and 3b).

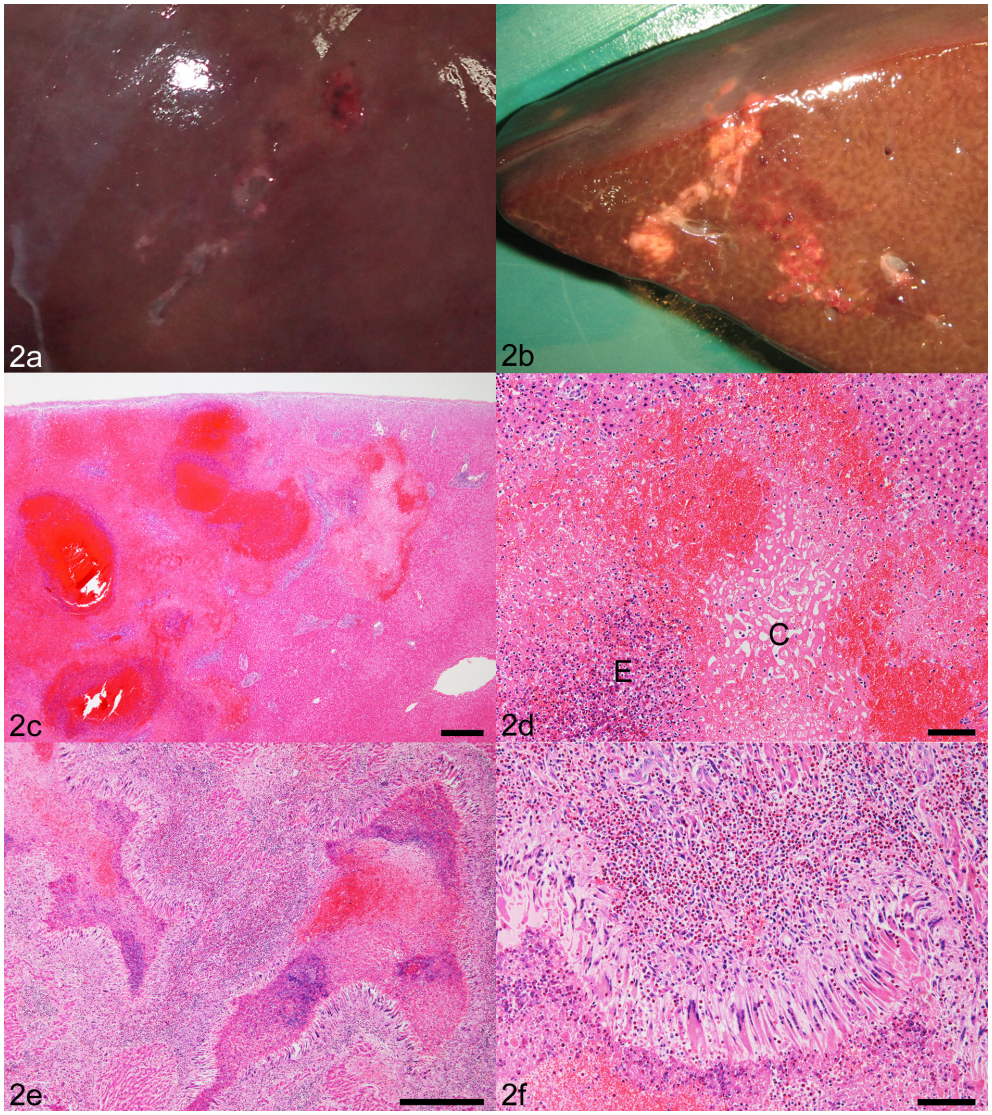

Fig. 2. Necrosis, hemorrhage, and eosinophilic granuloma formation associated with *Fasciola* fluke migration. a. Subcapsular hemorrhagic and discolored lesions with a patchy and tortuous appearance. b. Tortuous yellow lesions on the cut surface. c. Widespread subcapsular hemorrhage and necrosis. HE. Bar=500 μm. d. Higher magnification of the hemorrhage and necrosis of the liver parenchyma shown in Fig. 2c. Coagulation necrosis (C) of hepatocytes is evident. Eosinophils (E) infiltrate into the necrotic focus. HE. Bar=100 μm. e. An irregularly shaped eosinophilic granuloma centered on a region of necrosis with hemorrhage and cell debris and surrounded by many inflammatory cells. HE. Bar=500 μm. f. Higher magnification of the periphery of the eosinophilic granuloma shown in Fig. 2e. The necrotic focus (lower) is lined by palisading multinucleated giant cells and surrounded by eosinophils, lymphocytes, and plasma cells. HE. Bar=100 μm.

Histologically, the lesions were composed of aggregated lymphoid follicles (lymphoid tissue formation) (Fig. 3c). In some lesions, eosinophilic granulomas, which centered on eosinophilic and/or mineralized debris, were present within the lymphoid tissue (Fig. 3c–f and Supplementary Fig. 5a–f). In other cases, the infiltration of lymphocytes, eosinophils, and multinucleated giant cells was observed between the lymphoid follicles (Fig. 3g and 3h and Supplementary Fig. 5g and 5h).

Miscellaneous intrahepatic lesions

a) Subcapsular hepatocyte lipidosis: Out of the 75 intrahepatic, non-bile duct lesions detected during gross examinations, 15 were caused by subcapsular hepatocyte lipidosis. They appeared as subcapsular, smooth or irregularly lined, well-demarcated, white or yellow foci, measuring up to 2 cm in maximum diameter (Supplementary Fig. 6a). Some lesions formed at sites at which the liver adhered to other organs (described later). Histologically, the hepatocytes diffusely presented with fatty changes (Supplementary Fig. 6b).

b) Hepatic necrobacillosis: Out of the 75 intrahepatic, non-bile duct lesions, 5 were considered to have been caused by necrobacillosis. These lesions were abscesses, measuring up to 2 cm in diameter (Supplementary Fig. 7a and 7b). Histologically, the lesions were composed of amorphous necrotic material surrounded by neutrophils and accompanied by suppurative hepatitis in

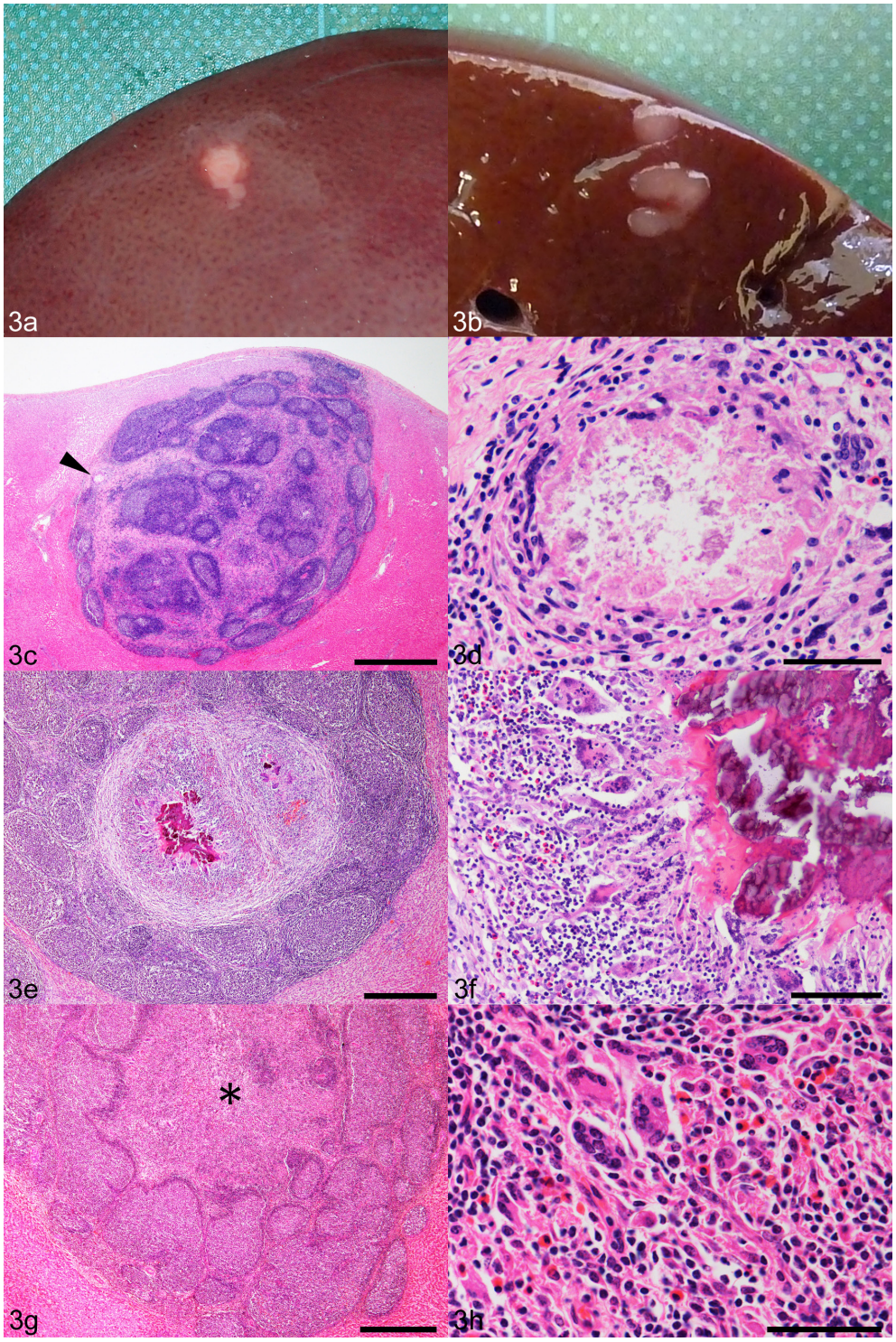

Fig. 3. Lymphoid tissue formation associated with *Fasciola* fluke migration. a. A white, round focus on the liver surface. b. Subcapsular, closely arranged and partially jointed, spherical to spheroidal, translucent nodules on the cut section. c. Subcapsular nodular lymphoid tissue composed of aggregated lymphoid follicles. A small granuloma is present at the periphery of the lymphoid tissue (arrowhead). HE. Bar=1 mm. d. Higher magnification of the granuloma shown in Fig. 3c. Collapsed debris is surrounded by multinucleated giant cells admixed with fibroblasts, lymphocytes, and a few eosinophils. HE. Bar=50 μm. e. Two encapsulated granulomas located at the center of lymphoid tissue. HE. Bar=500 μm. f. Higher magnification of the granuloma shown in Fig. 3e. Multinucleated giant cells admixed with lymphocytes and eosinophils infiltrate around mineralized debris. HE. Bar=100 μm. g. Lymphoid tissue with a central region (asterisk), where lymphocytes and eosinophils diffusely infiltrate (invisible at this magnification). HE. Bar=500 μm. h. Higher magnification of the central region shown in Fig. 3g. Infiltration of lymphocytes, eosinophils, and multinucleated giant cells are evident. HE. Bar=50 μm.

the surrounding parenchyma (Supplementary Fig. 7c). Gram staining revealed filamentous gram-negative bacilli at the periphery of the necrotic regions (Supplementary Fig. 7d). *Fusobacterium necrophorum* was identified in a case that was examined bacteriologically.
c) Other lesions: Periportal suppurative hepatitis was detected in one case. Many pinpoint white foci were distributed throughout the liver, and histologically they were characterized by the periportal infiltration of neutrophils. In one case, a thin-walled cyst, which measured 5 mm in diameter, was found on the cut surface of the liver and it was histologically lined with a single layer of cuboidal epithelial cells.

Capsular lesions

Out of the 107 non-bile duct lesions detected during gross examinations, 32 were classified as capsular lesions, which included 17 cases of hepatic capsulitis and 15 cases of adhesion between the liver and other organs. Focal, multifocal, or locally extensive capsular thickening with or without fibrous tags on the liver surface, was diagnosed as hepatic capsulitis (Supplementary Fig. 8a). Histologically, the capsular fibrous tissue proliferated and increased in thickness. In some cases, lymphocytes, hemosiderin-laden macrophages, and/or multinucleated giant cells infiltrated into the capsule. Fibrous adhesion of the liver to the diaphragm, rumen, reticulum, or omentum was observed in 15 deer (Supplementary Fig. 8b). In one case, an encapsulated abscess was found at the site of adhesion between the liver and the reticulum. Some cases exhibited focal lipidosis of the subcapsular hepatocytes (described above).

Microscopically detected lesions

In addition to the microscopic lesions described above, the following findings were detected in the 302 livers that were examined histologically. A summary of the characteristics and prevalence rates of each histological lesion is provided in Table 2.
a) Fasciola flukes residing in venules: In 8 livers, we detected *Fasciola* flukes in the lumens of venules, which were presumed to be branches of the portal vein because they ran alongside the branches of the hepatic ducts (Fig. 4).
b) Egg granuloma formation: Egg granulomas were histologically detected in 9 livers, most of which were located at the portal triads (Fig. 5).
c) Other microscopic findings: Diffuse, mild, centrilobular fatty changes were observed in the hepatocytes of 27 livers. Focal or multifocal accumulation of mononuclear cells, which were rarely admixed with multinucleated giant cells or neutrophils, was observed in the hepatic sinusoids in 104 livers. Mild infiltration of mononuclear cells or solitary lymphoid follicle formation at the portal triad was detected in 65 livers. These microscopic findings seemed to have no distributional association with the lesions detected during the gross examinations.

Statistical analysis

We compared the prevalence of gross or histological lesions between deer with and without *Fasciola* infections using the χ^2 test. First, the prevalence of each type of non-bile duct lesion detected during gross examinations was compared between the deer with (621 heads) and without (760 heads) fascioliasis-associated bile duct lesions (Table 1). As a result, intrahepatic lesions associated with *Fasciola* fluke migration, which consist of necrosis, hemorrhage, and eosinophilic granuloma formation, were detected at a significantly higher rate in the deer with *Fasciola* infections ($P<0.05$). Next, the prevalence of each type of microscopically detected lesion was compared between the deer with (203 heads) and without (99 heads) fascioliasis-associated bile duct lesions (Table 2). As a result, egg granulomas and *Fasciola* flukes in venules were detected at significantly higher rates in deer with *Fasciola* infections ($P<0.05$). Conversely, focal or multifocal accumulation of mononuclear cells in the hepatic sinusoids was detected at a significantly higher rate in the deer without *Fasciola* infections ($P<0.05$).

Table 2. Summary of the liver lesions detected in the livers of 302 sika deer during histological examinations

Histological lesions[a)]	No. of deer with each type of lesion (%)			
	Total[b)] [n=302]	*Fasciola* infection[c)] (Fascioliasis-associated bile duct lesions)		
		+ [n=203]	− [n=99]	*P*-value
Fasciola flukes residing within venules	8 (2.6)	8 (3.9)	0 (0.0)	0.045*
Egg granuloma formation	9 (3.0)	9 (4.4)	0 (0.0)	0.033*
Diffuse mild centrilobular fatty changes of hepatocytes	27 (8.9)	18 (8.9)	9 (9.1)	0.949
Focal or multifocal accumulation of mononuclear cells in hepatic sinusoids	104 (34.4)	56 (27.6)	48 (48.5)	0.0003*
Periportal mild mononuclear cell infiltration or solitary lymphoid follicle formation	65 (21.5)	44 (21.7)	21 (21.2)	0.927

a) Lesions exclude those associated with grossly detected lesions. b) Number of deer with each type of lesion and their prevalence rates (shown as percentages in parentheses) in all deer (302 heads). c) Number of deer with each type of lesion and their prevalence rates (shown as percentages in parentheses) in deer with (203 heads; column '+') and without (99 heads; column '−') fascioliasis-associated bile duct lesions. In each type of lesion (row), number of total deer with the lesion (number in column 'Total') is sum of numbers of deer with (number in column '+') and without (number in column '−') fascioliasis-associated bile duct lesions. *P*-values were obtained using the χ^2 test. *, Statisitically significant difference in the prevalence of the lesion between deer with and without fascioliasis-associated bile duct lesions ($P<0.05$).

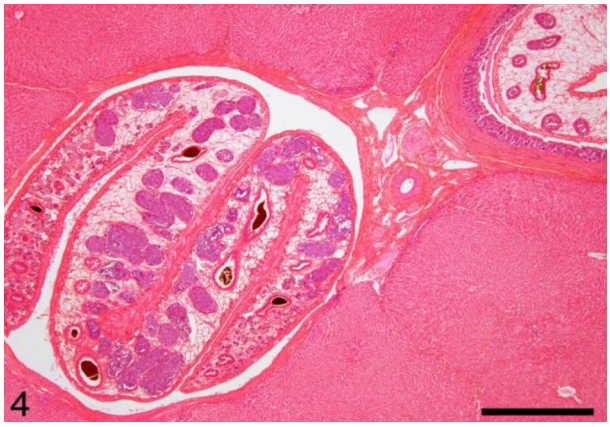

Fig. 4. A *Fasciola* fluke residing within a venule. The venule runs along the branch of the hepatic duct (upper right). HE. Bar=1 mm.

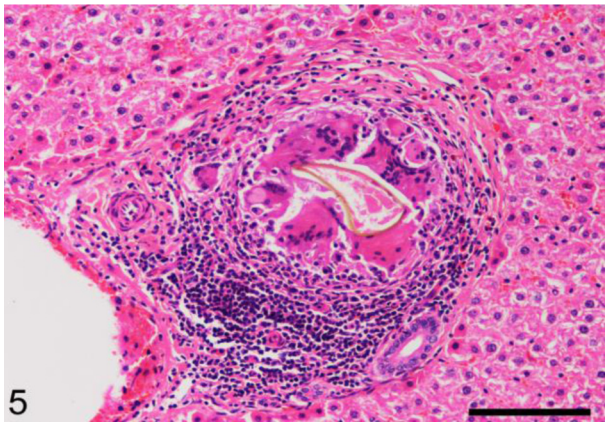

Fig. 5. An egg granuloma that formed at the portal triad. HE. Bar=100 μm.

DISCUSSION

In this study, we have described the gross and histological lesions affecting the livers of the sika deer inhabiting Hokkaido, Japan, and the prevalence of such lesions. As a result, we found that *Fasciola*-associated lesions predominated in the livers of the sika deer on Hokkaido. Bile duct lesions that were consistent with biliary-phase fascioliasis were evident in 621 out of 1,381 deer (45.0%). The large IHBD were markedly thickened and dilated, and histologically the affected bile ducts showed hyperplasia of the mucosal epithelium and peribiliary glands, chronic inflammation, goblet cell and pyloric gland metaplasia, and periductal fibrosis. These findings were similar to the bile duct lesions seen in animal fascioliasis, as well as those seen in humans with liver fluke infections (family *Opisthorchiidae*) or hepatolithiasis [2, 9, 15, 17, 18]. In human pathology, the term 'chronic proliferative cholangitis' or 'adenomatous hyperplasia' is applied to such hyperplastic and chronic inflammatory conditions. In experimental *Fasciola hepatica* infections in calves, the cells in the hyperplastic epithelium were reported to have cytoplasmic mucin, which may correspond to goblet cell and/or pyloric gland metaplasia detected in the hyperplastic bile duct epithelia of the affected deer in the present study [4]. In the current study, erosion and ulceration of the biliary epithelium were localized lesions and were not usually observed at sites where the tissue surface came into contact with the spiny bodies of flukes; therefore, it was suspected to have been caused by disruption due to the prehensile action of the flukes' suckers rather than abrasion caused by the flukes' spines.

The intrahepatic lesions composed of necrosis, hemorrhage, and sequential eosinophilic granuloma formation, were consistent with migratory-phase fascioliasis lesions [2, 3, 5]. The necrosis seen in the liver parenchyma of the infected deer included destructive necrosis with hemorrhage, which was caused by the migration of immature flukes, and coagulation necrosis, which was considered to be related to toxic excretions by the flukes [3]. The lymphoid tissue that formed in the subcapsular liver parenchyma characteristically appeared as translucent white, spherical or spheroidal nodules. Eosinophilic granulomas, which centered on eosinophilic and/or mineralized debris, or the infiltration of lymphocytes, eosinophils, and multinucleated giant cells was histologically detected in the lymphoid tissue; therefore, we considered that the lymphoid tissue formation resulted from the persistent immune responses against *Fasciola* flukes, which died during migration in the liver parenchyma. To the best of our knowledge, lymphoid tissue formation has not been reported in livers with *Fasciola* infection, and we described this finding as a fascioliasis-associated lesion for the first time. Overall, *Fasciola* fluke migration-associated lesions accounted for 71% (53/75) of the intrahepatic, non-bile duct lesions detected during gross examinations. Thus, fascioliasis-associated lesions accounted for a large proportion of the intrahepatic lesions detected in the sika deer.

Among all of the examined sika deer, the prevalence of fascioliasis-associated parenchymal lesions (53/1,381, 3.8%) was markedly lower than that of bile duct lesions (621/1,381, 45.0%). In addition, no flukes or fluke remnants were histologically detected in the parenchymal lesions. Based on these observations, it seems quite probable that migrating flukes seldom die in the liver parenchyma and that they efficiently reach the bile ducts. Most of the migration-associated lesions that arose in the liver parenchyma probably resolved without causing obvious gross lesions. Some animals, including sheep, rabbits, rats, and mice are permissive hosts for *F. hepatica* infections, and biliary-phase disease is common during such infections [2]. In contrast, in non-permissive hosts, such as cattle and humans, few flukes survive beyond the migratory phase, and biliary disease is relatively rare [2]. The results of the present study indicate that sika deer are permissive hosts for *Fasciola* infections.

The prevalence of intrahepatic lesions associated with *Fasciola* fluke migration, which consist of necrosis, hemorrhage, and eosinophilic granuloma formation, was significantly higher in the deer with fascioliasis-associated bile duct lesions than in the deer without such bile duct lesions (Table 1). This indicated that repeated infections occurred, and reinfection was more likely to occur than initial infections, but we could not explain the reason for this. The morbidity rate of fasciolosis in deer in the eastern district of Hokkaido (45.0%), which was confirmed pathologically in the present study, was higher than that detected in the Tokachi district

of Hokkaido (14.2%) in a previous fecal test-based study [11]. We could not determine whether these discrepancies should be attributed to differences in the test area or method; therefore we should test the same animal group using both methods in order to obtain exact estimates of the infection rates in sika deer.

In one case in the present study, a microscopically detected cyst lined by a keratinizing stratified squamous epithelium was shown to branch and to have blind ends. Based on these histological findings, the cyst was consistent with an epidermoid cyst, which is an extremely rare lesion in humans [13, 14]. The origin of epidermoid liver cysts is unknown, but it has been suggested that they might originate from accessory foregut remnants that give rise to the squamous epithelium, as occurs in the esophagus [14]. As far as we know, this is the first description of an epidermal liver cyst in the veterinary literature. The cyst was found in the vicinity of hemorrhagic lesions associated with *Fasciola* fluke migration, but the relationship between these lesions remains unclear.

In the present study, subcapsular hepatocyte lipidosis was detected in 15 out of 1,381 deer (1.1%) and accounted for 20.0% (15/75) of intrahepatic non-bile duct lesions. Such lesions have also been observed in other animal species and are probably caused by focal hypoxia induced by congenital vascular insufficiency or tension due to adhesion [3]. In addition, hepatic necrobacillosis was observed in 5 deer. These lesions were similar to the hepatic necrobacillosis lesions seen in cattle and might have been derived from ruminal lesions via the portal circulation [16]. The periportal suppurative hepatitis found in one case might have been derived from a suppurative condition in the digestive tract. In addition, a cyst lined by a single layer of cuboidal epithelial cells was diagnosed as a solitary bile duct cyst [12].

Capsular lesions, including hepatic capsulitis and adhesion between the liver and other organs, were detected in 32 out of 1,381 deer (2.3%) and accounted for 30.0% (32/107) of the non-bile duct lesions detected during the gross examinations of the liver. The associated disease in each case was unknown; however, some of the capsular lesions might have been associated with fascioliasis because juvenile *Fasciola* flukes wander across the peritoneal surface and cause peritonitis [3].

Microscopically, *Fasciola* flukes were detected in venules in a few deer with fascioliasis-associated bile duct lesions. Flukes that accidentally enter the blood vessels can become lodged at unusual sites via systemic circulation, and this might occur in sika deer with fascioliasis [3]. Egg granulomas seen in the liver parenchyma in some deer were considered to have formed around eggs that lodged in small bile ducts [5]. Mild fatty changes detected in centrilobular hepatocytes could have resulted from mild hypoxia, and mild inflammatory changes might be a non-specific reaction to inflammation in the digestive tract [3]. The etiology of these changes was not determined in the present study, but none of these lesions were considered to be life-threatening. These findings were not associated with fascioliasis-associated bile duct lesions (Table 2). On the other hand, mononuclear cell accumulation in sinusoids was observed more often in deer that had not been infected with *Fasciola* (Table 2), although the reasons for this are unknown.

In summary, this study demonstrated that fascioliasis-associated lesions were the predominant lesions in the livers of the sika deer inhabiting Hokkaido, Japan. The gross and histological lesions of fascioliasis in sika deer were similar to those in other animal species. In addition, this study described lymphoid tissue formation as a fascioliasis-associated lesion for the first time. Compared with the high morbidity rate of bile duct lesions, the prevalence of fascioliasis-associated parenchymal lesions was markedly lower, which indicated that sika deer are permissive hosts for fascioliasis. We also described the detection of an epidermoid liver cyst in an animal for the first time in the veterinary literature.

In Japan, a number of sika deer are used for venison production, and appropriate information about deer diseases is required during venison production. This study involved temporarily farmed deer, and most of the deer were female; therefore, the results might not completely reflect the condition of wild deer. However, our findings were obtained from a large number of deer that were utilized for venison production; therefore, the present results must provide useful information that will aid pathological examinations of sika deer.

ACKOWLEDGMENTS. We would like to thank the Biodiversity Conservation Division, Bureau of Environmental Affairs, Department of Environment and Lifestyle, Hokkaido, Japan, and the Hokkaido Sika Deer Meat Inspection Adviser Council for providing us with the inspection data obtained at slaughter and a chance to examine the present cases. We also thank Dr. R. Murata, Department of Veterinary Bacteriology, School of Veterinary Medicine, Rakuno Gakuen University, for bacteriological examinations of selected specimens. A part of this research was supported by the Akiyama Life Science Foundation (2019).

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