Experimentally Induced Fasciola hepatica Infection in White-tailed Deer II. Pathological Features

P. J. A. Presidente, B. M. McCraw and J. H. Lumsden*

ABSTRACT

Six white-tailed deer (Odocoileus virginianus) and six sheep were inoculated with metacercariae of Fasciola hepatica. Two animals of each species were given 100, 500 or 2500 metacercariae. One animal in each inoculated group was killed and examined at six weeks postinoculation and the remainder at 15 weeks postinoculation.

At six weeks postinoculation the parietal surface of the livers from inoculated deer was covered with gray fibrous plaques and rust colored patches. Fibroplasia with mononuclear cell infiltration characterized Glisson's capsule on the parietal surface. Granulomas were found in the hepatic parenchyma and on the dorsal surface of the lung. Fresh and healing tracks were occasionally found in the liver. In the sheep fibrinous exudate and numerous subcapsular tracks were found on both surfaces of the liver. Inflammatory changes in portal areas and numerous fresh and healing tracks in the hepatic parenchyma were prominent features.

At 15 weeks postinoculation inflammatory changes in Glisson's capsule of inoculated deer were less marked than at six weeks but portal fibrosis and hyperplasia of bile duct epithelium were more advanced. A zone of hemorrhage surrounded ducts that contained mature F. hepatica in one deer. The livers from the sheep were rough, pitted and covered with fibrous tags and adhesions to the diaphragm and greater omentum were common. Hemorrhagic tracks were common in the sheep given 500 and 2500 metacercariae. Portal fibrosis and hyperplasia of bile duct epithelium were seen in the sheep (100 metacercariae) that harbored mature F. hepatica.

RÉSUMÉ

On a administré des métacercaires de Fasciola hepatica à six cerfs de Virginie (Odocoileus virginianus) et à six agneaux. Deux sujets de chacune de ces deux espèces reçurent respectivement: 100, 500 et 2500 métacercaires. On procéda à l'euthanasie et à la nécropsie d'un sujet de chacun des groupes expérimentaux, au bout de six semaines; on fit la même chose avec le restant des sujets, neuf semaines plus tard.

Au bout de six semaines, la surface pariétale du foie des cerfs expérimentaux était recouverte de plaques fibreuses et de foyers de couleur rouille. Quant à la capsule de Glisson de cette surface, elle présentait de la fibroplasie et une infiltration de cellules mononucléaires. On décela des granulomes au sein du parenchyme hépatique et sur la surface dorsale des poumons et, occasionnellement, des sillons frais ou en voie de cicatrisation. Chez les agneaux, on nota la présence d'un exsudat fibrineux et de plusieurs sillons sous la capsule de Glisson, sur les deux surfaces du foie. Les lésions prédominantes comprenaient une réaction inflammatoire, dans les espaces portobiliaires, et plusieurs sillons frais ou en voie de cicatrisation, au sein du parenchyme hépatique.

Au bout de 15 semaines, la réaction inflammatoire au sein de la capsule de Glisson des cerfs expérimentaux s'avéra moins intense qu'après six semaines; la fibrose portale et l'hyperplasie des canaux biliaires étaient cependant plus marquées. Chez un cerf, une zone hémorragique entourait les canaux bili-

*Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

This work was supported by the Ontario Ministry of Agriculture and Food, Grant No. 459 from the Canadian National Sportsmen's Show and the National Research Council of Canada which awarded a Post Doctoral Fellowship to the senior author.

Submitted June 5, 1974.

aires qui contenaient des douves adultes. Le foie des agneaux était rugueux, bosselé et recouvert de taches fibreuses; on observa aussi, souvent, des adhérences avec le diaphragme et l'épiploon. Les agneaux auxquels on avait administré 500 et 2500 métacercaires présentaient souvent des sillons hépatiques hémorragiques. Quant à ceux qui n'avaient reçu que 100 métacercaires et qui hébergaient des douves adultes, ils présentaient de la fibrose portale et une hyperplasie de l'épithélium des canaux biliaires.

INTRODUCTION

Experimental infection studies in sheep, cattle and swine have shown that there are differences in host susceptibility to *Fasciola hepatica* infection (3, 4, 5, 21). In sheep there is little resistance to fluke migration and the pathogenic effect is determined by the fluke burden (2, 3, 7, 15, 18, 20, 22). In cattle a smaller percent of the inoculum is recovered, fluke maturation is inhibited and death is not commonly associated with this infection (3, 6, 16, 19). In swine there is strong resistance to *F. hepatica* and most flukes are killed and destroyed within six weeks after inoculation (4, 8, 11, 17).

Recent experiments with *F. hepatica* in cervid hosts indicate a similar pattern of susceptibility and resistance to infection. Black-tailed deer (*Odocoileus hemionus columbianus*) in North America (9) and roe deer (*Capreolus capreolus*) in Europe (1) are highly susceptible to infection and acute fascioliasis has been demonstrated. Roe deer are not entirely suitable hosts because *F. hepatica* were spontaneously cleared at approximately 18 weeks postinoculation (PI) (1). Maturation of many *F. hepatica* in red deer (*Cervus elaphus*) was inhibited and host response was similar to that in cattle except that calcification of bile ducts did not occur (1). The marked resistance of white-tailed deer to *F. hepatica* infection has recently been demonstrated (12, 13).

In previous publications we reported the early pathological changes (12) and clinicopathological and parasitological features of later stages of *F. hepatica* infection (13) in white-tailed deer. A comparison of the pathological features in white-tailed deer and sheep at six and 15 weeks PI is presented herein.

MATERIALS AND METHODS

The design for this experiment and data pertaining to the innoculum and fawns and lambs used were previously given (12, 13). Formalin-fixed tissues were embedded in paraffin and stained with hematoxylin and eosin (H & E). Periodic acid-Schiff (PAS) and Glenner's trichrome stains were used to characterize the pigment seen in macrophages.

RESULTS

The major gross and histopathological features of *F. hepatica* infection in the fawns and lambs are summarized in Table I. Significant findings in smears of the peritoneal fluid recovered from these animals are given in Table II.

Six Weeks Postinoculation

Fawn No. 3, 100 MC; No. 5, 500 MC; No. 7, 2500 MC

An increase in the intensity and extent of the capsular reaction on the liver was associated with the increased doses of metacercariae (MC) given (Table I). The parietal surface of the left and middle lobes was primarily involved whereas the right lobe and visceral surface were mostly normal. In fawn No. 3 there were a few gray areas, irregular in shape and 2 to 6 mm in size, on the left and middle lobes (Fig. 1). A tortuous track 30 mm long and 4 mm wide was found on the middle lobe. In fawn No. 5 and 7 most of the partial surface was pale, rough and covered with gray plaques and rust colored areas (Fig. 2). On the cut surface the capsule on the parietal surface of the liver from fawn No. 7 was greatly thickened (Fig. 3). Several granulomas 4 to 10 mm in diameter were found beneath the capsule.

A similar degree of cellular infiltration and fibroplasia was seen in the diaphragm from these fawns (Table I). The serosa on Glisson's capsule in fawn No. 5 was disposed into filaments up to 400 μ long in some areas. They contained a core of fine collagen and eosinophils and capillaries were occasionally seen (Fig. 4). In fawn No. 7 Glisson's capsule was up to 1200 μ thick. There was a thick inner layer of collagen and an outer cellular one (Fig.

TABLE I. Gross and Histopathological Features of Fawns and Lambs Inoculated with Metacercariae (MC) of *Fasciola hepatica* and Examined Six and 15 Weeks Postinoculation (PI)

	Fawns						Lambs					
	6 weeks PI			15 weeks PI			6 weeks PI			15 weeks PI		
Item — No. of MC = / Animal No. =	100 / 3	500 / 5	2500 / 7	100 / 4[a]	500 / 6	2500 / 8	100 / 3	500 / 5	2500 / 7	100 / 4	500 / 6	2500 / 8
Fibrinous peritonitis	+	++	++	+	+	+	+	++	+++	+	+	++
Ascites	− − −	+	+	− − −	− − −	− − −	+	++	+++	− − −	− − −	+++
(ml)		(10)	(15)				(10)	(40)	(140)			(770)
Diaphragm, abdominal surface												
fibrinous exudate	− − −	− − −	− − −	− − −	+	− − −	AN[b]	AN	AN	++	++	− − −
hyperplasia of the serosa	+	++	++	+	+	+				++	+	+
eosinophil infiltration	+	+	+	− − −	− − −	+				++	+	− − −
mononuclear cell infiltration	+	++	++	+	+	++				++	++	+
fibroplasia	++	++	++	++	+	++				++	+	+
Fibrous adhesions												
liver to diaphragm	− − −	+	+	− − −	+	++	− − −	− − −	− − −	+	++	++
liver to omentum	− − −	− − −	− − −	− − −	− − −	+	− − −	− − −	− − −	− − −	+++	+++
Liver, Glisson's capsule												
hyperplasia of the serosa	+	+++	++	+	+	+	− − −	− − −	+	++	+	+
eosinophil infiltration	+++	++	+	− − −	− − −	− − −	− − −	+	++	− − −	− − −	+++
mononuclear cell infiltration	+	+	+++	+	+	+	+	+	++	++	+	+
fibroplasia	+	++	+++	+	++	++	− − −	− − −	++	++	++	+++
hemmorrhage	+	++	++	+	− − −	− − −	+	+	+	+	− − −	− − −
Liver, portal areas												
bile duct hyperplasia	++	+	+	+	++	++	++	+++	+++	++	+++	++
hyperplasia of bile duct epithelium	+	− − −	− − −	− − −	+	++	+	+	++	+++	+++	++
eosinophil infiltration	++	++	+	− − −	− − −	− − −	++	+	+++	+	− − −	+++
mononuclear cell infiltration	+++	++	++	++	++	+	++	++	++	++	++	++
fibroplasia	++	+	+	+	++	+++	− − −	++	+++	+++	+++	++
mature flukes in ducts	− − −	− − −	− − −	− − −	− − −	+	− − −	− − −	− − −	++	+	+
Liver, parenchyma												
fluke tracks, hemorrhagic	+	− − −	− − −	− − −	− − −	+	++	+++	++++	+	++	+++
fluke tracks, healed	+	+	− − −	− − −	− − −	+	++	+++	++++	++	+++	++
immature flukes	− − −	− − −	− − −	− − −	− − −	− − −	+	++	+++	− − −	+	+++
subcapsular granulomas	+	++	++	++	++	++	− − −	− − −	− − −	+	++	++
hepatocytes contain fat	− − −	− − −	− − −	− − −	+++	++	− − −	+	+	+	++	+

TABLE I. (continued)

Item		Fawns						Lambs					
		6 weeks PI			15 weeks PI			6 weeks PI			15 weeks PI		
No. of MC =		100	500	2500	100	500	2500	100	500	2500	100	500	2500
Animal No. =		3	5	7	4[a]	6	8	3	5	7	4	6	8
Portal lymph nodes													
enlarged		− − −	− − −	− − −	+	+	++	+	++	++	+++	++	+++
cortical hyperplasia		+	++	++	++	++	+++	+	++	+++	+++	++	+++
$\overline{X}$ no. of follicles[c]		27	18	16	17	22	55	30	20	15	34	17	21
$\overline{X}$ diameter of follicles, μ[d]		398	470	408	600	355	499	431	542	691	629	480	605
germinal centers, pale		++	+	+	++	++	+++	+++	+	+	+++	++	+
germinal centers, diffuse		++	++	++	+	++	++	++	+++	+	++	+	+
germinal centers, "starry sky"		− − −	+	++	+	+	+	+	+	+++	+	+	++
medullary hyperplasia		− − −	+	+	+	++	++	+	+++	++	+++	+++	+++
Hydrothorax		− − −	− − −	− − −	− − −	+	− − −	− − −	− − −	− − −	− − −	− − −	+++
(ml)						(20)							(400)
Lung, dorsal surface													
granulomas		− − −	− − −	+	− − −	+	− − −	− − −	− − −	− − −	− − −	− − −	− − −
hemorrhagic nodules		− − −	− − −	++	− − −	+	− − −	+	+	+	− − −	+	+
Heart, myocardium													
fluke track		− − −	− − −	+	− − −	− − −	− − −	− − −	− − −	− − −	− − −	− − −	− − −

[a]Died with signs of acute hemorrhagic enteritis on day 62 PI

[b]Appeared normal on gross examination so tissues were not taken for histopathological examination

[c]Calculated from examination of five low-power fields (25X) through the cortices. Individual counts for the number of follicles from eight of the animals were ± 2 to ± 5 of the mean; and for one fawn(No. 8) it was ± 11

[d]Calculated from the measurement of the greatest diameter of 10 follicles selected at random. The minimal-maximal size was considerable: 240-530 μ for two fawns (No. 7 and 6); 290-960 μ for three animals (fawn No. 4 and 8, Lamb No. 6); 340-770 μ for four animals (fawn No. 3 and 5, lamb No. 3 and 5); and 430-860 μ for three lambs (No. 7, 4 and 8)

− − − indicated no change; +, a slight change; ++, a moderate change; +++, a severe change; and ++++. a very severe change

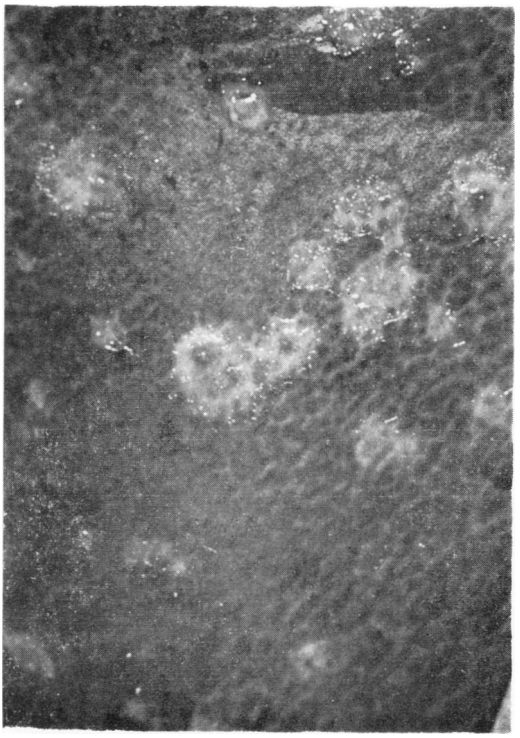

Fig. 1. Irregularly shaped gray plaques 2 to 6 mm in size on the parietal surface of the middle lobe of the liver. Fawn No. 3, 6 weeks PI, 100 MC.

5). In the cellular portion plasma cells predominated although some eosinophils were seen. Fluke tracks were occasionally found in the hepatic parenchyma of fawn No. 3. These consisted of a core of necrotic hepatocytes and eosinophils surrounded by a zone of collagen, numerous small bile ducts and a heavy infiltration of mononuclear cells (Fig. 6). A hemorrhagic track was found in one area. Bile duct hyperplasia was evident in portal areas of these livers and also in healed tracks from fawn No. 5. Granulomas in the livers from fawn No. 5 and 7 and lung from No. 7 (Table I) had a core of necrotic debris surrounded by a wall of dense connective tissue that was calcified and contained lymphoid aggregations. A zone of hemorrhage was commonly found around these granulomas. A track was found in section through the epicardial surface of the left ventricle from fawn No. 7 (Fig. 7). In one area only fibrous tissue remained, while in two others there were infiltrations of eosinophils and plasma cells. Giant cells were occasionally seen in this lesion. The mean number, size and characteristics of the germinal centers in portal lymph nodes of these deer are given in Table I. Eosinophils were the predominant cell in the peritoneal fluid recovered from fawn No. 7 (Table II).

Lamb No. 3, 100 MC; No. 5, 500 MC; No. 7, 2500 MC

The severity and extent of the lesions found in these lambs were directly related to the dose of MC (Table I). Both parietal and visceral surfaces of these livers were involved, especially the left lobe which was rough and covered with fibrinous exudate. Numerous tortuous subcapsular hemorrhagic tracks were seen on both surfaces (Fig. 8). The left lobe of the livers from lamb No. 5 and 7 was firm and smaller than normal.

Only minor changes were seen in Glisson's capsule on the parietal surface of the liver. The capsule was 100 μ thick in lamb No. 5 and 250 μ with mild hyperplasia of the serosa in No. 7 (Table I). Portal areas were characterized by a heavy infiltration

Fig. 2. Extensive rough rust colored areas on the parietal surface of the liver. The capsule was gray and thickened. Fawn No. 5, 6 weeks PI, 500 MC.

TABLE II. Characteristics of the Peritoneal Fluid Recovered from Fawns and Lambs Inoculated with Metacercariae (MC) of *Fasciola hepatica* and Examined at Six and 15 Weeks Postinoculation (PI). Comparison with Values Obtained from the Circulating Blood at the Time of Necropsy

	Fawns	Lambs			
	6 weeks PI	6 weeks PI			15 weeks PI
No. of MC =	2500	100	500	2500	2500
Item Animal No. =	7	3	5	7	8
Peritoneal Fluid					
Appearance	bloody purulent	amber cloudy	pale cloudy	amber cloudy	amber cloudy
Total protein g/100 ml[a]	7.4	2.3	4.2	7.2	8.5
Leukocytes/mm³	151000	7500	8400	14600	5900
eosinophils/mm³[b]	63420 (42)[c]	1950 (26)	2690 (32)	5110 (35)	4660 (79)
Erythrocytes	+++	++ crenated	—	—	—
Macrophages containing erythrocytes or hemosiderin	++	+	+	+	—
Circulating Blood					
Total protein g/100 ml[d]	8.0	5.8	6.8	7.2	9.1
Leukocytes/mm³	3000	9200	8800	8400	14600
eosinophils/mm³[e]	603 (20)	744 (8)	1134 (13)	666 (8)	6803 (47)

[a]Values determined with an AO TS Meter, model 10400, American Optical Instrument Company, Buffalo, New York
[b]Values calculated from the percent recorded in a differential count
[c]Values in parentheses are the percents that eosinophils made up of the total leukocyte counts
[d]Values determined by the biuret method
[e]Absolute counts made on blood collected in EDTA

of mononuclear cells and macrophages that contained hemosiderin. Hyperplasia of bile duct epithelium was seen in portal areas that contained large ducts. The hepatic parenchyma was disrupted by numerous fresh tracks with cores of necrotic hepatocytes and eosinophils surrounded by mononuclear cells and a zone of hemorrhage. There was little evidence of fibroplastic activity around these tracks. In healing tracks macrophages containing hemosiderin and bile duct hyperplasia were common. In lamb No. 7 extensive hemorrhage and severe destruction of the hepatic parenchyma was caused by migrating flukes and sections through immature flukes were found (Table I).

The hemorrhagic nodules on the dorsal surface of the lungs from lamb No. 5 and 7 (Table I) were either small granulomas or hemorrhagic tracks containing eosinophils and mononuclear cells. Proteinaceous fluid was seen in alveoli surrounding these areas. A hemosiderin-like pigment was found in older fibrosed tracks. The pleura was slightly thickened in areas adjacent to the granulomas. Characteristics of the portal lymph nodes in these lambs are given in Table I.

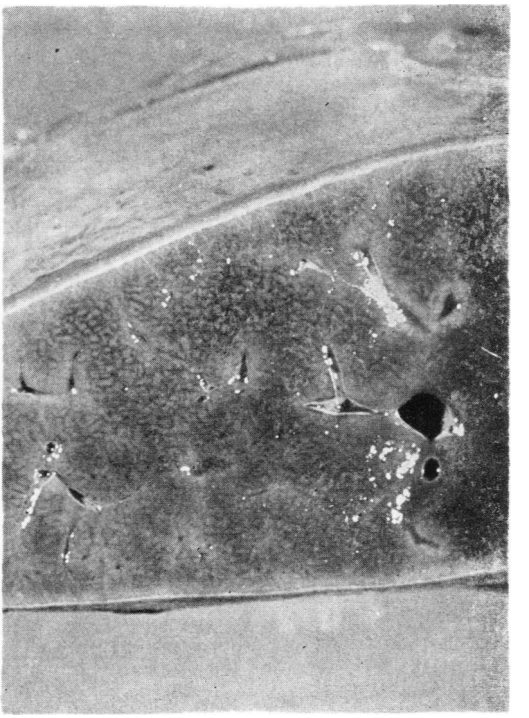

Fig. 3. Fibrous thickening of Glisson's capsule on the parietal surface. Cut surface of the liver from fawn. No. 7, 6 weeks PI, 2500 MC.

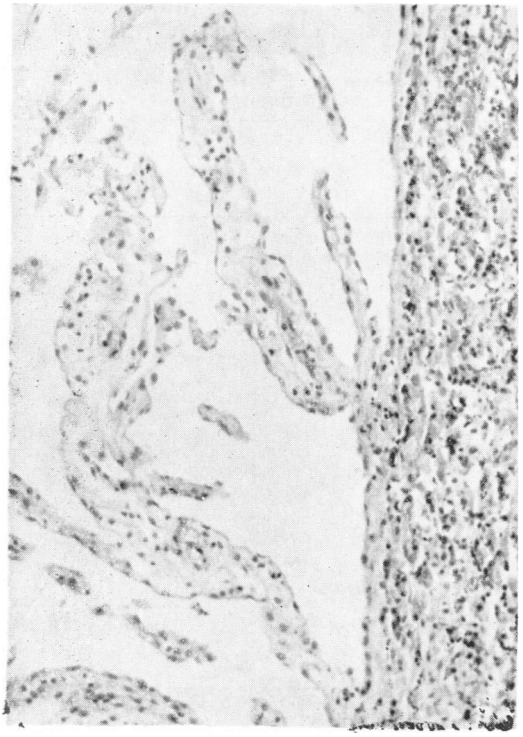

Fig. 4. The serosa on Glisson's capsule on the parietal surface of the liver. It was disposed into filaments up to 400 μ long that contained a core of fine collagen; eosinophils and capillaries were occasionally seen. Fawn No. 5, 6 weeks PI, 500 MC. H & E. X380.

The total leukocyte counts, percent of eosinophils and protein concentration in the peritoneal fluid from these lambs correlated with the dose of MC given (Table II). Eosinophils made up a significant proportion of the total leukocytes in the peritoneal fluid. Lymphocytes and monocytes were also present in large numbers. In the circulating blood the percent of eosinophils was relatively low and lymphocytes and neutrophils were the major types. The protein concentration of the blood was greater than that found in the peritoneal fluid (Table II).

Fifteen Weeks Postinoculation

Fawn No. 6, 500 MC; No. 8, 2500 MC

The parietal surface of the livers from fawns No. 6 and 8 was very rough and covered with gray plaques over the left and middle lobes (Fig. 9). A red area 15 mm in diameter in the central region of the middle lobe from fawn No. 8 was adherent to the overlying diaphragm. On the cut surface several hemorrhagic tracks were evident beneath a thick fibrous layer. The visceral surface of both livers was pale and mottled in appearance. In fawn No. 8 there was adhesion of the small intestine to the middle lobe. In fawn No. 6 a white fibrous area 10 mm in diameter was found on the middle lobe. On the cut surface it was a large granuloma with three necrotic foci (Fig. 10). Several granulomas 5 to 10 mm in diameter were also seen in subcapsular locations and one was found on the anteroventral border of the right cardiac lobe of the lung. There was a zone of hemorrhage around thickened bile ducts in the fawn (No. 8) that harbored mature *F. hepatica.*

There was only moderate fibrosis and slight cellular infiltration in Glisson's capsule on the parietal surface of the liver (Table I). In portal areas with large bile ducts moderate hyperplasia of bile duct epithelium and fibrosis among triad components were seen, particularly in fawn No. 8. Some of these ducts contained mature

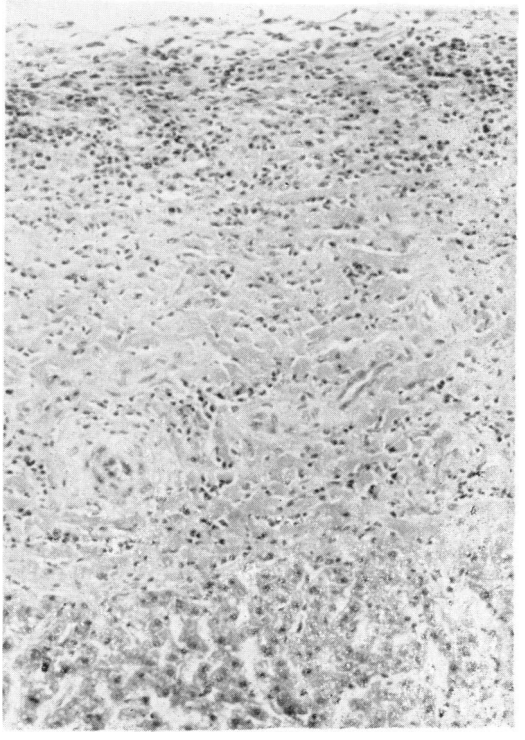

Fig. 5. The thickened Glisson's capsule on the parietal surface of the liver. There was a thick inner layer of collagen and an outer cellular one in which plasma cells predominated. Fawn No. 7, 6 weeks PI, 2500 MC. H & E. X380.

F. hepatica and these were surrounded by a zone of hemorrhage (Fig. 11). Fluke eggs and cellular debris were found in the lumina of some ducts. A granuloma, similar in structure to those described previously, was found in section through the nodule on the cardiac lobe of the lung from fawn No. 6. Variation in the degree of cortical and medullary hyperplasia of portal lymph nodes was seen between fawn No. 4 that died on day 62 PI and those examined at 15 weeks (Table I).

Lamb No. 4, 100 MC; No. 6, 500 MC; No. 8, 2500 MC

The severity of the lesions associated with *F. hepatica* infection in these lambs was directly related to the dose of MC given (Table I). The entire parietal surface of these livers was pale, very rough, pitted and covered with fibrous tags (Fig. 12). The visceral surface of the left lobes was similarly affected and in lamb No. 4 and 8 this lobe was small and firm. Adhesion of the liver to the diaphragm and greater omentum was most extensive in lamb No. 6 while ascites and hydrothorax were most severe in No. 8.

Fig. 7. Infiltration of inflammatory cells in a track associated with a migrating fluke in the epicardium. Left ventricle of the heart from fawn No. 7, 6 weeks PI, 2500 MC. H & E. X380.

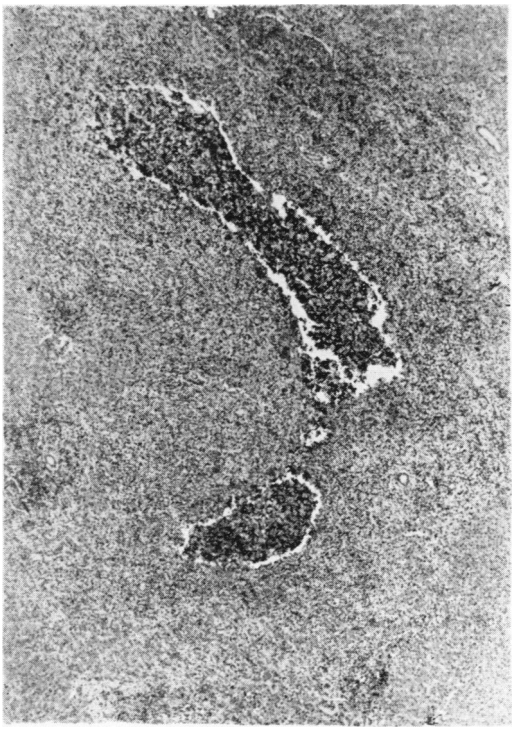

Fig. 6. A fresh track associated with a migrating fluke in the hepatic parenchyma. Necrotic hepatocytes and eosinophils were surrounded by heavy infiltrations of mononuclear cells and eosinophils. Fawn No. 3, 6 weeks PI, 100 MC. H & E. X380.

Fibrosis of Glisson's capsule was evident in these lambs. The capsule was 200 μ thick in No. 4 and up to 900 μ in No. 8. Mild hyperplasia of the serosa was seen and cellular infiltration was light (Table I). Marked fibrosis with hyperplasia of bile duct epithelium characterized portal areas, especially in lamb No. 4. The bile in lamb No. 8 was very dark, thick and contained an excessive quantity of mucus, necrotic debris and erythrocytes. Fluke tracks in lamb No. 4 were mostly healed, whereas in No. 6 and 8 they were fresh and often hemorrhagic. Sections of large immature flukes were found in the hepatic parenchyma of lamb No. 8 (Table I). Massive bile duct hyperplasia and lymphoid aggregations were seen in healed tracks and there were small granulomas in some areas. In healing tracks and in the portal lymph nodes from lamb No. 6 and 8 there was an orange-brown pigment in macrophages in H & E stained tissues. The pig-

ment was PAS positive and in Glenner's trichrome stained red or dull brown-green. Characteristics of the portal lymph nodes in these lambs are given in Table I.

Eosinophils predominated in the peritoneal fluid from lamb No. 8 and protein concentration was similar to that in the circulating blood (Table II). Approximately 400 ml of an amber colored fluid (protein concentration 8.0 g/100 ml) was recovered from the thoracic cavity from this lamb.

DISCUSSION

Hyperplasia of the serosa on the parietal surface, fibroplasia and inflammation of the capsule and portal areas and subcapsular granulomas were the major features of the liver in *F. hepatica* infected fawns. At six weeks PI inflammatory and proliferative changes in the diaphragm and Glisson's capsule were greater than at 15 weeks. These changes were similar to, but more advanced, than those seen in white-tailed deer at 28 days PI (12). In the fawn given 100 MC, tracks associated with migrating flukes were found and there was greater proliferation of lymphoid follicles in portal lymph nodes (Table I). In fawns given 500 or 2500 MC the reaction of Glisson's capsule was more intense and extensive. At 15 weeks PI portal fibrosis with hyperplasia of bile duct epithelium was more evident in the fawn (No. 8) that harbored mature *F. hepatica*. Proliferative changes in the portal lymph nodes were also greater than in the other fawn (No. 6) (Table I). It was evident that by 15 weeks PI the inflammatory response to *F. hepatica* infection had diminished. Granulomas associated with sites where immature flukes had been destroyed were similar in structure at both times that fawns were exam-

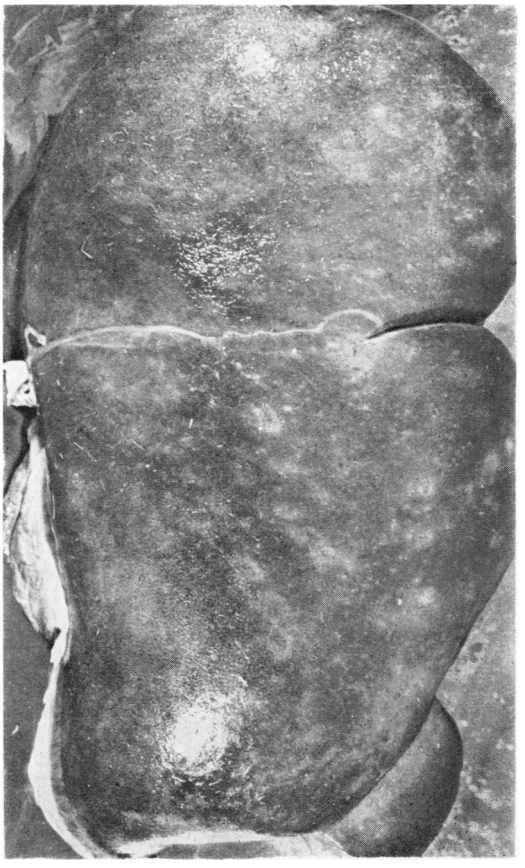

Fig. 9. Numerous white plaques distributed generally over the rough parietal surface of the liver. Fawn No. 6, 15 weeks PI, 500 MC.

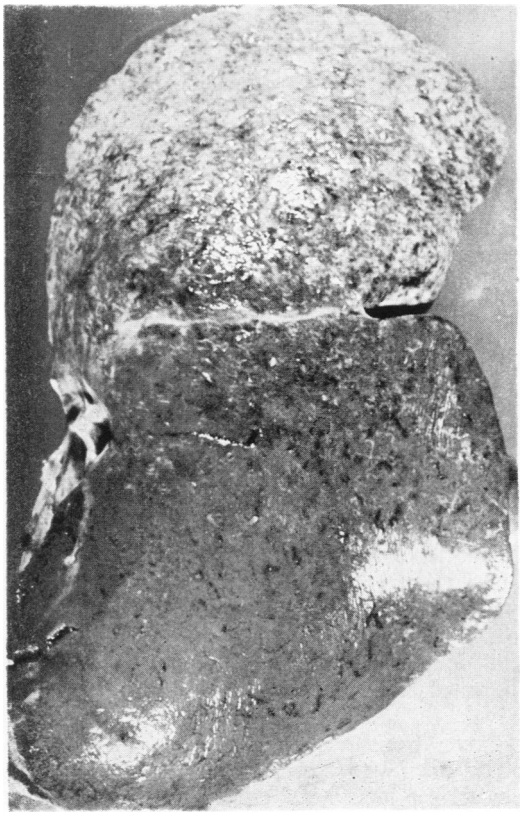

Fig. 8. Subcapsular tracks on the parietal surface of the middle lobe of the liver. The left lobe was small, firm and covered with fibrinous exudate. Lamb No. 7, 6 weeks PI, 2500 MC.

ined. These lesions must therefore persist for some time before they are completely resolved.

In *F. hepatica* infected lambs ascites, hepatic adhesions, inflammatory and proliferative changes in portal areas and extensive hemorrhagic, healing and healed tracks in the hepatic parenchyma were the principal changes observed. Ascites was common at six weeks PI, whereas adhesions of the liver to adjacent structures were greater at 15 weeks. When eosinophil counts in the peritoneal fluid were compared with those from the circulating blood it was evident that these cells accumulate in the exudate while counts in the blood remain relatively low. Only mild inflammation and fibroplasia of Glisson's capsule was seen at six weeks PI and hyperplasia of the serosa was observed at 15 weeks. The severe reaction in one lamb (No. 8) was attributed to many large immature *F. hepatica* still migrating in the hepatic parenchyma at 15 weeks PI. The pigment seen in macrophages in portal areas, healing tracks in the liver and lung as well as in portal lymph nodes was associated with

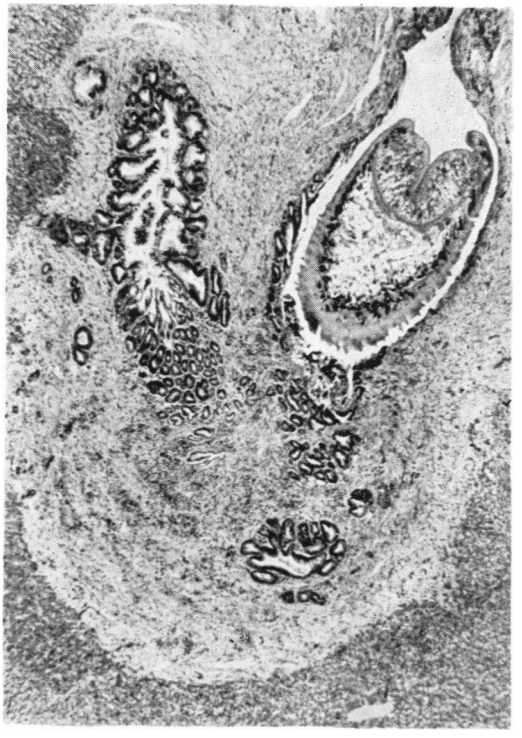

Fig. 11. Fibrosis, hyperplasia of bile duct epithelium and hemorrhage around a portal area. The bile duct contains a mature F. hepatica. Fawn No. 8, 15 weeks PI, 2500 MC. H & E. X125.

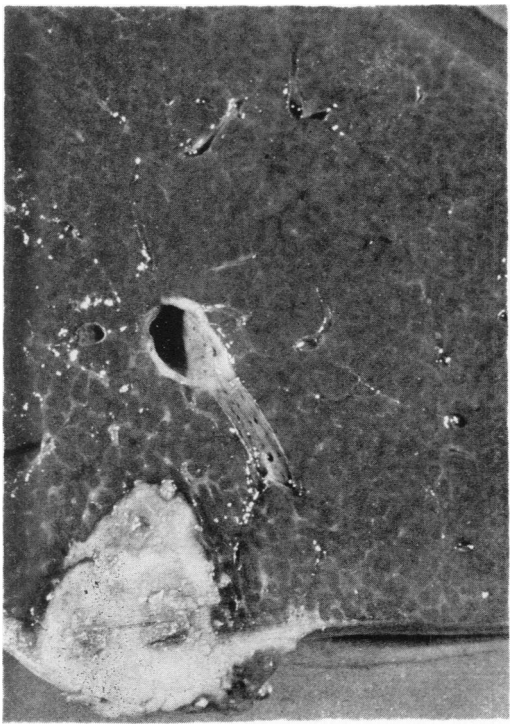

Fig. 10. A large granuloma with three necrotic foci on the cut surface through the middle lobe of the liver. Fawn No. 6, 15 weeks PI, 500 MC.

the removal of tissues damaged by migrating flukes. At six weeks PI the pigment was mostly hemosiderin, while at 15 weeks it was probably a mixture of hemosiderin, ceroid and bile. At both times greater numbers of lymphoid follicles were present in portal lymph nodes of lambs given smaller numbers of MC but follicles were usually larger in those given the high dose (2500 MC) (Table I). Medullary hyperplasia was more evident at 15 weeks PI. The pathological changes in these infected lambs were typical for sheep with subacute (2, 15, 19, 22) and early chronic or subclinical fascioliasis (3, 7, 17, 18, 20).

The severe and extensive pathological changes associated with *F. hepatica* infection in the lamb were not seen in infected fawns. The parietal surface of the left and middle lobes was mostly affected in the fawns, whereas both surfaces, particularily the left lobe, were involved in the lambs. The hepatic parenchyma in lambs was severely disrupted by migrating flukes but tracks were found only in three fawns (No. 3, 4 and 8) and these were not exten-

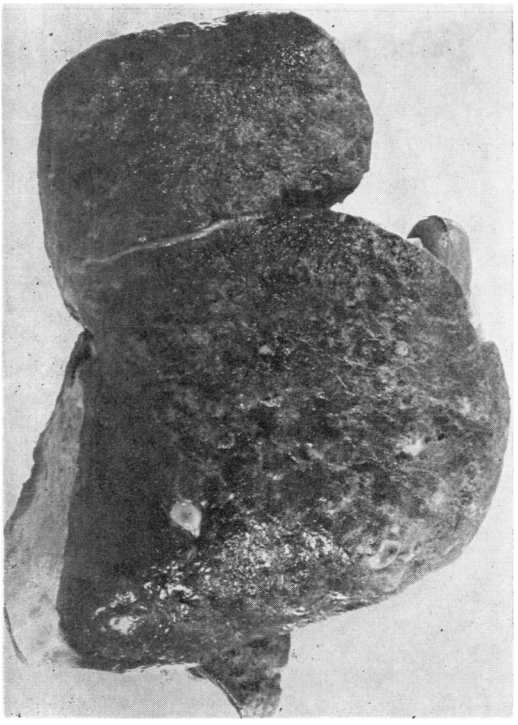

Fig. 12. Severe hepatitis and atrophy of the left lobe of the liver. The parietal surface was rough, pitted and covered with fibrous tags. Lamb No. 8, 15 weeks PI, 2500 MC.

sive. Numerous macrophages containing pigment indicated the extensive hemorrhage and tissue destruction in lambs but these were not seen in infected fawns. Greater exudation of leukocytes, erythrocytes, serum and fibrin through Glisson's capsule probably accounted for the greater prevalence of ascites, fibrinous peritonitis and fibrous adhesions seen in the lambs. In both hosts given 500 or 2500 MC lesions associated with *F. hepatica* were found in the lungs. The nature of these lesions was different and suggested that the flukes were able to migrate for some time in lambs but were quickly destroyed in the fawns.

The present findings reinforce our earlier observations that the white-tailed deer is an abnormal host for *F. hepatica* (12, 13). White-tailed deer are evidently considerably more resistant to *F. hepatica* than black-tailed deer (9), red and roe deer (1) or domestic livestock (2, 3, 4, 5, 17, 21). Their response to infection is most similar to that described for swine (8, 11, 17) but differs in that *F. hepatica* are more rapidly eliminated (12). Residual signs of infection remain but the lesions observed are not specific enough to associate them with *F. hepatica,* unless the flukes are also recovered.

ACKNOWLEDGMENTS

The authors wish to thank Dr. T. P. Kistner, Department of Veterinary Medicine, Oregon State University for providing the metacercariae used in this investigation. The technical assistance of Messrs. Ted Eaton, Department of Pathology and Hugh Belcher, animal technician, Edinburgh Road Research Station, Ontario Veterinary College is gratefully acknowledged.

REFERENCES

1. **BARTH, D. and K. SCHAICH.** Untersuchungen zur experimentellen Fasciolose bei Reh-(**Capreolus capreolus**) und Rotwild (**Cervus elaphus**). Z. Jagdwiss. 19: 183-197. 1973.
2. **BORAY, J. C.** Studies on experimental infections with **Fasciola hepatica,** with particular reference to acute fascioliasis in sheep. Ann. trop. Med. Parasit. 61: 439-450. 1967.
3. **BORAY, J. C.** Experimental fascioliasis in Australia. Adv. Parasit. 7: 95-210. 1969.
4. **DALCHOW, W. and F. HÖRCHNER.** [Experimental infection with **Fasciola hepatica** in different animals]. Berl. Münch. tierärztl. Wschr. 85: 271-274. 1972.
5. **DIXON, K. E.** The relative suitability of sheep and cattle as hosts for the liver fluke, **Fasciola hepatica** L. J. Helminth. 38: 203-212. 1964.
6. **DOW, C., J. G. ROSS and J. R. TODD.** The pathology of experimental fascioliasis in calves. J. comp. Path. 77: 377-385. 1967.
7. **DOW, C., J. G. ROSS and J. R. TODD.** The histopathology of **Fasciola hepatica** infections in sheep. Parasitology 58: 129-135. 1968.
8. **HÖRCHNER, F. and W. DALCHOW.** [Experimental **Fasciola hepatica** infection in pigs]. Berl. Münch. tierärztl. Wschr. 85: 184-188. 1972.
9. **KISTNER, T. P and L. D. KOLLER.** Experimentally induced **Fasciola hepatica** infections in black-tailed deer. J. Wildl. Dis. 11 214-220. 1975.
10. **MORRILL, O. R and J. N. SHAW.** Studies of pathology in cattle produced by liver fluke (**Fasciola hepatica**). Oregon State University Agr. Exp. Sta. Bull. 408. 30 pp. 1942.
11. **NANSEN, P., S. ANDERSEN, E. HARMER and H.-J. RIISING.** Experimental fascioliasis in the pig. Expl Parasit. 31: 247-254. 1972.
12. **PRESIDENTE, P. J. A., B. M. McCRAW and J. H. LUMSDEN.** Early pathological changes associated with **Fasciola hepatica** infection in white-tailed deer. Can. J. comp. Med. 38: 271-279. 1974.
13. **PRESIDENTE, P. J. A., B. M. McCRAW and J. H. LUMSDEN.** Experimentally induced **Fasciola hepatica** infection in white-tailed deer. I. Clinicopathological and parasitological features. Can. J. comp. Med. 39: 155-165. 1975.
14. **RAHKO, T.** The pathology of natural **Fasciola hepatica** infection in cattle. Pathologia vet. 6: 244-256. 1969.
15. **ROBERTS, H. E.** Observations on experimental acute fascioliasis in sheep. Br. vet. J. 124: 433-450. 1968.

16. **ROSS, J. G.** Experimental infection of cattle with **Fasciola hepatica**: High level single infections in calves. J. Helminth. **41**: 217-222. 1967.

17. **ROSS J. G., C. DOW and J. R. TODD.** The pathology of **Fasciola hepatica** infection in pigs and other hosts. Br. vet. J. **123**: 317-321. 1967.

18. **ROSS, J. G., C. DOW and J. R. TODD.** A study of **Fasciola hepatica** infections in sheep. Vet. Rec. **80**: 543-546. 1967.

19. **ROSS, J. G., J. R. TODD and C. DOW.** Single experimental infections of calves with the liver fluke, **Fasciola hepatica** (Linnaeus 1758). J. comp. Path. **76**: 67-81. 1966.

20. **SIMESEN, M. G., L. ERIKSEN, P. NANSEN, S. ANDERSEN and K. NIELSEN.** Chronic fascioliasis in sheep. 1. Clinical, clinical-pathological and histological studies. Nord. VetMed. **20**: 638-650. 1968.

21. **SINCLAIR, K. B.** Pathogenesis of Fasciola and other liver-flukes. Helminth. Abstr. **36**: 115-134. 1967.

22. **THORPE, E. and E. J. H. FORD.** Serum enzyme and hepatic changes in sheep infested with Fasciola hepatica. J. Path. **97**: 619-629. 1969.

Book Review

LIVING CLOCKS IN THE ANIMAL WORLD. *Miriam F. Bennet. Published by Charles C. Thomas, Springfield, Illinois. 1974. 221 pages. Price $11.75.*

This short, informative book discusses biological rhythms, their various forms, their interactions and their adaptive value to groups of animals. The scope is restricted to cover invertebrate forms and some lower vertebrates.

The author has chosen five Phyla and in general zooms in on one or two members of each. Two chapters are devoted to crabs, primarily *Uca* sp. The intricacies of colour changes, and metabolism and activity rates that link up with endogenous clocks and exogenous cues are discussed in the light of ongoing research. Honey bees and the now classical works of von Frisch and his students are the subject of another chapter. The earthworm and its nocturnal and yearly activities are also described. Dr. Bennett's own research, both past and present, enables her to add personal notes to all of the above topics and to be able to critically evaluate the experimental data in nearly all of the Phyla included in her book. Her approach to each animal group is consistent. The reader is first introduced to the historical background relating to early discoveries of predictable, repetitive, daily, monthly or yearly behaviour patterns. She brings the reader through to the present in each chapter by adding one worker's research to another's until a web begins to take shape. This web is supplemented with additional fibres of hypothesis, of ongoing research, of required research, and of comparative and conflicting data.

Dr. Bennett's chapter on the Molusca is probably the weakest in the book. It is necessary at times to go back several pages to reintegrate the author's expanding examples of biochronometry. This particular chapter suggests that the author might better have organized this text in terms of the various kinds of biological rhythms (circadian, lunar, annual etc.) as they relate to animal clocks. One significant omission in *Living Clocks in the Animal World* is a discussion of the molecular basis of these fascinating pacemakers. Ehret's chronon hypothesis is mentioned at the end of Chapter 6. However, more emphasis on this rapidly expanding area is warranted, and from a cursory survey of the literature available, would be most timely.

The greatest strength of this book lies in its usefulness as a reference text. Any student of biochromometry will appreciate the detailed presentation and analysis of data especially in those chapters dealing with the crab, honey bee and earthworm. Dr. Bennett has included both an author and subject index and a bibliography that contains over 225 references. The descriptions of experimental designs, often so simple yet so definitive, and the time consuming accumulation of data necessary for statistical analysis should provide every reader with a realistic appreciation of modern research. Dr. Bennett clearly emphasizes the tedious and laborious work required to unravel the complicated and exciting mechanisms of biological time clocks. — *A. P. Gilman.*