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EXPERIMENTALLY INDUCED *Fasciola hepatica* INFECTIONS IN BLACK-TAILED DEER

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Abstract: The susceptibility of black-tailed deer (*Odocoileus hemionus columbianus*) to the common liver fluke (*F. hepatica*) was studied. Two deer and one sheep comprised each of three experimental groups. Animals in each group were inoculated individually with 250, 500, or 1000 *F. hepatica* metacercariae. One deer and one sheep given 1000 metacercariae died with lesions consistent with black disease 7 weeks after inoculation. At necropsy 6 or 15 weeks postinoculation, the mean percentage recovery of the inoculum was 38.9% from the deer and 51.9% from the sheep. Fluke eggs recovered from the deer were viable and metacercariae cultured from the eggs were fully infective for sheep.

Pathologic changes associated with *F. hepatica* infection were more severe in the infected deer; consequently, the deer were less resistant to the lethal effects of the parasite than sheep. Considering the experimental results and the fact that naturally acquired common liver fluke infection has been reported infrequently from black-tailed deer, it was concluded that black-tailed deer do not constitute a significant reservoir for *F. hepatica* in domestic livestock.

INTRODUCTION

Although wild ruminants share much range with liver fluke (*F. hepatica*) infected cattle and sheep, this parasite has been reported infrequently from North American big game animals,[4,8,9,15,18,19] and specifically only from antelope,[23] black-tailed deer[3,7,11,20] and mule deer [Lang, B. Z. 1974. Pers. Comm.]. Despite intimate contact with infected domestic animals, *F. hepatica* has not been reported from white-tailed deer.[5,11,22] Patent infection, however, has been induced experimentally in white-tailed deer.[6,13]

It was decided to investigate concurrently the apparent natural resistance to *F. hepatica* in Oregon black-tailed deer and eastern white-tailed deer (*O. virginianus borealis*) by means of experimental inoculation with metacercariae. This paper reports the results of experimental infection in black-tailed deer. The results of the companion studies in white-tailed deer were reported independently.[13]

MATERIALS AND METHODS

Metacercariae

Sheep origin *F. hepatica* metacercariae were produced at this laboratory in the snail, *Lymnaea columella*. Snails were artificially exposed to miracidia hatched from eggs collected from the bile ducts and gallbladder of naturally infected sheep. A portion of the metacercariae was used in the black-tailed deer experiment; sufficient numbers of the same lot were air-shipped in ice to Guelph, Ontario, Canada, for a white-tailed deer study.[13]

Animals

Six 5-month old fluke-free black-tailed deer fawns were used in the experiment. The fawns were reared artificially on condensed cow milk, commercial pelleted ration[1] and alfalfa hay. Three fluke-free 9-month old sheep were used as controls.

Technical Paper No. 3879, Oregon Agricultural Experiment Station.

[1] Purina Calf Startena, Ralston Purina Company, St. Louis, Mo. 63188.

Procedure

Two fawns and one sheep were assigned to each of three groups. Each of the animals in group I was given 250 metacercariae; those in group II, 500 metacercariae; and in group III, 1000 metacercariae, via stomach tube. The dosage of metacercariae selected usually produced subclinical, chronic, and subacute fascioliasis in sheep.[2] Animals in each treatment group were maintained in bedded stalls after inoculation. One deer from each group was killed at 6 weeks post-inoculation (PI); the remaining deer and the sheep from group III died during the 7th week PI. The other animals were killed at 15 weeks PI.

Necropsy Procedure

At necropsy, the small intestine of each animal was ligated at the pylorus and 100 cm posterior to the bile duct. The liver and attached section of intestine were removed from the carcass and placed in a pan. The portion of small intestine that was removed was incised longitudinally and examined grossly for flukes. Each liver was examined grossly, photographed, and weighed after removal of attached diaphragm, lymph nodes and fat. Representative 0.5 mm thick samples of liver were collected and preserved in 10% buffered formalin for histopathologic examination. Tissue sections were prepared routinely and stained with hematoxylin-eosin and Masson's trichrome.

All visible bile ducts were incised and examined grossly for flukes. Scrapings from the major bile duct of deer and gallbladder of sheep were examined microscopically for fluke eggs. Each liver was then cut into 0.5 cm thick slices and soaked in warm tap water for 2 hours. Individual slices were squeezed between the fingers to express flukes, and then rinsed. The water containing the flukes was washed over a 100 mesh Tyler Sieve and the material remaining on the sieve was then examined under magnification (10-20x) for flukes and parts thereof. Entire flukes as well as head and tail portions were counted. Total counts comprised the number of entire flukes plus either the head or tail portions, whichever number was greater.

Flukes were refrigerated overnight in physiologic saline to allow uniform relaxation. The following morning, 30 flukes from each animal were randomly removed and measured after compression between two plates of glass (0.3 x 11 x 38 cm).[13]

Fluke eggs recovered from deer were pooled, embryonated, and miracidia hatched from these eggs were used to infect *L. columella*. The infectivity of metacercariae resulting from infection of the snails was evaluated in three fluke-free sheep (group IV). The sheep were killed at 8 weeks PI and the livers examined for flukes as described above.

RESULTS

Condition of Animals

The three fawns killed 6 weeks PI were clinically normal and could not be differentiated from pen mates. The fawn and lamb from group III which died suddenly at 7 weeks PI also appeared healthy until found dead in the stall. The remaining animals were normal until killed.

Necropsy Findings

The deer and sheep from group III died with signs of black disease (*Clostridium novyi* infection). Diagnosis was based on lesions at necropsy and confirmed by the fluorescent antibody technique.

The livers from all inoculated animals showed evidence of *F. hepatica* infection.[16] Numbers of flukes found, size, and percentage recovery of inoculated metacercariae are listed in Table 1. Livers were enlarged 30-40% in animals examined at 6-7 weeks PI, whereas the livers were only slightly larger than normal in animals killed at 15 weeks PI. At 6-7 weeks PI, gross lesions consisted of diaphragmatic hemorrhage, fibrinous peritonitis and perihepatitis, enlarged and edematous portal lymph nodes, increased liver friability, hemorrhagic tracks, and hematomas in the liver (Fig. 1). The

degree of traumatic hepatitis was in direct proportion to the metacercarial dosage, with the most severe lesions in deer 5 and 6 which each were given 1000 metacercariae. In addition, the thoracic and peritoneal cavities of deer 5 each contained 500 ml of blood-stained fluid. At 15 weeks PI, livers were mottled and scarred (Fig. 2 and 3). Organizing hematomas were more pronounced in deer than sheep livers, but hyperplasia of bile duct epithelium and thickening of Glisson's capsule was more prominent in sheep livers (Fig. 2 and 3). Edema and enlargement of portal lymph nodes was not as prominent at 15 weeks PI as at 6-7 weeks PI. Calcification of the bile ducts was not seen in any animal.

TABLE 1. Age of flukes, number found, size of flukes, and percentages of inoculum recovered from deer and sheep experimentally infected with sheep-strain **F. hepatica** metacercariae.

Group and Animal No. [1]		Dosage Inoculum	Age of Flukes (weeks)	No. Flukes Recovered	Size (mm) (Range)	Percentage Recovery
I	D1	250[2]	6	92	9.0 (6-14)	36.8
	D2	250[2]	15	135	27.3 (23-34)	54.0
	S 1	250[2]	15	100	25.9 (15-35)	40.0
II	D3	500[2]	6	128	10.7 (6-16)	25.6
	D4	500[2]	15	227	31.3 (19-33)	45.0
	S 2	500[2]	15	360	22.8 (12-35)	72.0
III	D5	1000[2]	6	369	10.7 (7-14)	36.9
	D6	1000[2]	7	352	7.9 (4-12)	35.2
	S 3	1000[2]	7	436	4.6 (2-8)	43.6
IV	S 4	250[3]	8	140	14.4 (6-21)	56.0
	S 5	250[3]	8	76	11.6 (6-16)	30.4
	S 6	250[3]	8	120	14.7 (9-27)	48.0

[1] D = Deer; S = Sheep
[2] Metacercariae cultured in *L. columella* from eggs of fluke-infected sheep.
[3] Metacercariae cultured in *L. columella* from eggs of fluke-infected black-tailed deer.

Flukes were found in both the hepatic parenchyma and bile ducts at 6-7 weeks PI. By 15 weeks PI, flukes were recovered primarily from the bile ducts. Numerous eggs were recovered from the major bile duct of both deer and from the gallbladder of both sheep. A few flukes were found encapsulated in the hepatic parenchyma of both deer and sheep at 15 weeks PI.

Table 1 also lists the results of experimental infection in sheep with eggs recovered from deer. Gross lesions in sheep from group IV consisted of moderate enlargement of portal lymph nodes, slight hepatic enlargement, major bile duct hypertrophy, healing fluke tracks, thickening of Glisson's capsule, and fibrous tags attached to the capsule. Numbers of flukes recovered from group IV sheep varied from 30.4-56.0% of the metacercarial dosage (Table 1).

Histopathologic Findings

The liver from deer 1, dosed with 250 metacercariae and killed at 6 weeks PI, had many tracks in the parenchyma that were filled with erythrocytes, fibrin, neutrophils, eosinophils, mononuclear cells and cellular debris. The portal triads were infiltrated with eosinophils, mononuclear cells (epithelial cells, histiocytes, lymphocytes, and plasma cells) and fibrous connective tissue. Many small collateral bile ducts, evidence of bile duct hyperplasia, were evident in the portal triad areas.

Deer 3, dosed with 500 metacercariae and killed at 6 weeks PI, had liver lesions similar to deer 1, but the structural damage was more extensive. Flukes were identified in the parenchyma, but not in the bile ducts. Large lymphoid aggregates were present in some portal triads.

Deer 2, given 250 metacercariae and killed 15 weeks PI, had liver lesions

FIGURE 1. Gross appearance of the visceral surface of the liver of a black-tailed deer infected with 1000 **F. hepatica** metacercariae and examined at 6 weeks PI.

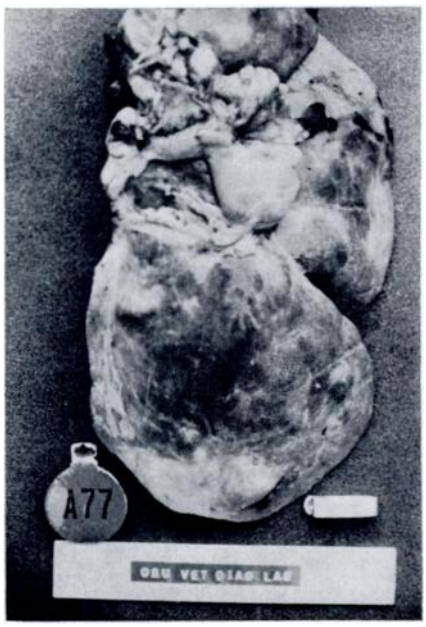

FIGURE 2. Gross appearance of the visceral surface of the liver of a black-tailed deer infected with 500 **F. hepatica** metacercariae and examined at 15 weeks PI.

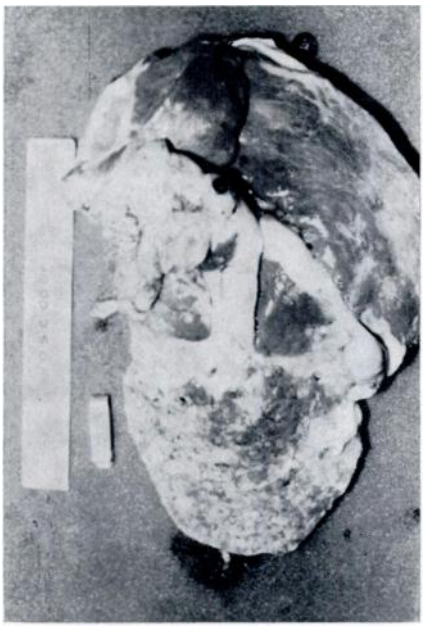

FIGURE 3. Gross appearance of the visceral surface of the liver of a sheep infected with 500 **F. hepatica** metacercariae and examined at 15 weeks PI.

similar to deer 1. However, there was hyperplasia of the epithelium in the larger bile ducts and enlarged ducts were surrounded by a moderately thickened fibrous capsule. One fluke was found in a bile duct. Also, a few portal triads contained extensive fibrous connective tissue.

Lesions in the liver of deer 4, exposed to 500 metacercariae and killed 15 weeks PI, were similar to deer 2. However, deer 4 showed marked hyperplasia of the epithelium in the larger bile ducts, and in some areas there was necrosis of the epithelial lining, which was infiltrated with eosinophils, neutrophils, mononuclear cells, and some fibrous connective tissue. A thick fibrous connective tissue capsule surrounded thickened bile ducts. In addition, many large lymphoid nodules were found in the portal triads.

Tissues from animals which were given 1000 metacercariae were misplaced and were not examined microscopically.

DISCUSSION

The death of deer 6 from black disease at 7 weeks PI was not anticipated, whereas death of sheep 3 at this time was not surprising. Sheep mortality from black disease in unvaccinated flocks in Oregon is a common sequel to traumatic hepatitis associated with migrating immature flukes.[16] Gross hepatic lesions in both the deer and sheep were typical of black disease.[10,16,21] The occurrence of black disease in this experiment demonstrated the susceptibility of black-tailed deer to *C. novyi*. A previous report indicated that black-tailed deer were not susceptible to this disease.[11]

At 6 weeks PI, gross hepatic lesions were similar in both deer and sheep. The excessive thoracic and peritoneal fluid in deer 5, however, exceeded that reported from sheep.[2] Boray[2] noted that excessive thoracic and peritoneal fluids were found at 4-8 weeks only in sheep whose liver contained more than 1000 flukes. At 15 weeks PI, bile ducts in the livers of deer were not as thickened as those in sheep.

The percentage recovery of metacercariae from deer, 25.6-54.0%, as immature or mature flukes did not differ greatly from the percentage recovery from our sheep, 30.4-72.0%, and was within the ranges reported from other sheep.[2] Fluke recoveries from black-tailed deer in this experiment were generally higher than that reported from experimentally induced *F. hepatica* infections in red and roe deer, 1.7-28.9%,[1] and were much higher than recoveries from white-tailed deer, 0.0-1.3%.[13] Presidente et al.[13] found that white-tailed deer were very resistant to experimentally induced fascioliasis with metacercariae from the same lot used in the present study. The larger size of the flukes recovered from black-tailed deer, as compared to those from the sheep with the same duration of infection and metacercarial dosage, suggested that black-tailed deer possessed less early resistance to *F. hepatica* than domestic sheep.[2]

The histopathologic lesions produced by *F. hepatica* in black-tailed deer were similar to those reported in sheep,[2,17] but some differences were evident. Black-

tailed deer with 6 week infections developed bile duct hyperplasia and moderate fibrosis, but hyperplasia of the bile duct epithelium was not evident. At 15 weeks PI, proliferation of bile duct epithelium with localized necrosis[17] was evident, as was an increased amount of fibrous connective tissue in the portal triad areas. Hyperplasia of bile duct epithelium, hepatic fibrosis, and bile duct enlargement at 15 weeks PI, however, were not as severe in deer as were these lesions in sheep infected with the same metacercarial dosage. Histologically, lesions produced by 250 and 500 metacercariae differed only in degree.

Gross hepatic lesions in sheep from group IV were similar to other 8 week old experimental *F. hepatica* infections induced in sheep at this laboratory with 250 metacercariae. The infection of snails and fluke recoveries from group IV sheep demonstrated that the fluke eggs from deer were fully viable.

Based on findings in this experiment, it appears that black-tailed deer are more susceptible to *F. hepatica* infection than domestic sheep. The larger size of similarly aged flukes recovered from deer as compared to those from sheep, excessive thoracic and peritoneal fluids at 6-7 weeks PI, and decreased fibrous tissue reaction in deer tend to support the premise that black-tailed deer are the more susceptible species.[2] Boray[2] stated that in highly susceptible hosts, *F. hepatica* is pathogenic in both the acute and chronic phases. The parasite is often destroyed with the host. Rapid mortality from acute or chronic fascioliasis and possibly from black disease may account for the apparent paradox that despite the susceptibility demonstrated in this experiment, very few black-tailed deer have been found naturally infected with *F. hepatica*.[3,7,11,20] Taber and Dasmann[20] reported that in Lake County, California, liver flukes are found in deer on sheep range fairly commonly (10% of collected deer were infected), but on range where livestock do not graze, infection is rarely found. This observation suggests that black-tailed deer are unable to maintain infections of *F. hepatica*, and hence do not serve as a significant natural reservoir for cattle and sheep. It also is possible that unless forced to graze,[11] the normal browsing and drinking habits of native deer may largely preclude exposure to *F. hepatica* metacercariae. Regardless of the apparent paradox, Boray[2] stated that ". . . the survival, the flourishing existence and distribution of the fasciolid species [*F. hepatica*] may be supported chiefly by bovine and ovine hosts, assisted by human interference."

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