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EXPERIMENTAL *FASCIOLOIDES MAGNA* INFECTIONS OF MULE DEER (*ODOCOILEUS HEMIONUS HEMIONUS*)

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ABSTRACT: Six mule deer (*Odocoileus hemionus hemionus*) and one white-tailed deer (*Odocoileus virginianus*), approximately 5-mo-old, each were inoculated orally with 500 metacercariae of *Fascioloides magna*. All mule deer died from liver fluke infection between 69 and 134 days (mean = 114, SE = 9.9) after inoculation. Between 38 and 326 immature *F. magna* (mean = 102, SE = 45.5) were recovered from each deer at necropsy. Flukes were present in livers, lungs, and free in pleural and peritoneal spaces. Infection was characterized by necrotizing hepatitis, fibrosing peritonitis and pleuritis, and hematin pigment accumulation in liver, lung, and many other internal organs. Eggs of *F. magna* first were detected in feces of the white-tailed deer 28 wk after inoculation, and weekly thereafter until the healthy deer was euthanized at 31 wk. At necropsy, 205 *F. magna*, including 12 encapsulated mature and 193 nonencapsulated immature flukes were recovered from liver, lungs, and free in abdominal and thoracic spaces of the white-tailed deer. Based on these results, *F. magna* may be fatal to mule deer within 5 mo of infection. Like domestic sheep and goats, mule deer may be highly susceptible to infection, and it is unlikely mule deer can survive infection with large numbers of *F. magna*.

Key words: *Fascioloides magna*, liver flukes, trematode, *Odocoileus hemionus hemionus*, *Odocoileus virginianus*, experimental infection.

INTRODUCTION

Fascioloides magna is a large liver fluke commonly found in white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) in several areas in North America and Europe (Erhardova-Kotrla, 1971; Foreyt, 1981). In definitive hosts, *F. magna* matures in hepatic tissue approximately 30 wk after infection, and eggs are passed in feces (Foreyt and Todd, 1976). Aberrant hosts, such as domestic sheep and goats, usually die from infection because *F. magna* migrates quickly and severely damages hepatic tissue. In a third group of hosts, such as cattle, moose (*Alces alces*), and llamas (*Lama glama*), flukes can mature in the liver and produce eggs that remain trapped in hepatic tissue, but the infection usually is not lethal (Foreyt and Todd, 1976; Lankester, 1974; Foreyt and Parish, 1990). Mule deer (*Odocoileus hemionus hemionus*) are rare hosts for *F. magna*, and in this study we evaluated the pathogenicity of an experimental *F. magna* infection in mule deer.

MATERIALS AND METHODS

Six mule deer fawns and one white-tailed deer fawn, <1 wk old, were obtained from the wild in Oregon and Washington. They were bottle-fed goat milk until weaning at 15 wk of age. Approximately 5 wk later, each deer was inoculated orally with 500 viable metacercariae of *F. magna* in a gelatin capsule with a balling gun. Metacercariae were obtained from Baldwin Enterprises (Monmouth, Oregon, USA). Viability was determined microscopically by movement of flame cells. Deer were maintained together on a 3.2 ha pasture with natural grasses. Supplemental alfalfa pellets, mineralized salt, and fresh water were given daily and available at all times.

Fecal samples were collected weekly from each deer starting at 20 wk after inoculation. A modified fecal sedimentation technique (Flukefinder®, Visual Difference, 5051C Old Pullman Road, Moscow, Idaho, USA) was used to isolate fluke eggs in feces. Eggs were counted under a dissecting microscope (30×), and an estimate was made of eggs per gram of feces.

Deer that died were submitted to the Washington Animal Disease Diagnostic Laboratory (WADDL) (Pullman, Washington, USA). Standard necropsy, histopathologic, parasitologic, and microbiologic techniques were used by WADDL personnel. All internal organs were

examined for *F. magna*. Livers and lungs were sliced at approximately 1-cm intervals and grossly visible flukes were removed. Tissue slices were soaked in warm water for approximately 2 hr; slices and sediment from the washing were examined grossly. All recovered flukes were counted and placed between two glass slides; lengths of intact flukes were measured to the nearest mm. If flukes were severed, only heads were counted. Mature flukes were identified by presence of eggs in the uterus.

RESULTS

All six mule deer died from *F. magna* infection between 69 and 134 days (mean = 114, SE = 9.95) after inoculation (Table 1). Deer became depressed with droopy ears, poor appetite, and weakness, 1 to 4 wk before death. Between 38 and 326 immature *F. magna* (mean = 102, SE = 45.5) were recovered from each deer (Table 1). A majority of flukes were recovered from liver, but flukes also were recovered from lungs (n = 0 to 3 flukes per lung), and free in peritoneal (n = 5 to 16) and pleural spaces (n = 2 to 8) of the mule deer. Eggs were not recovered from feces of any mule deer.

All mule deer weighed between 31 and 40 kg and were in moderate body condition at the time of death. Infection was similar in all mule deer and was characterized by necrotizing hepatitis, fibrosing peritonitis and pleuritis, and hematin pigment accumulations in liver and other internal organs. Between 50 and 1,000 ml of serosanguinous fluid were present in thoracic cavities of three mule deer. Fibrin strands and tags were attached to visceral pleura, and occasional black fluke migration tracks were present in lungs. From 0.5 to 4 l of yellow to red fibrinous fluid were in peritoneal cavities. Fibrinous tags covered most abdominal serosa, and some organ surfaces were adhered. Liver was the most severely affected organ, being swollen to approximately twice normal size. Coagulative hepatic necrosis was common, and friable parenchyma was infiltrated by numerous tortuous tunnels filled with blood, black pigment, and immature flukes. Blood clots were closely associated with hepatic lesions in 3 of 6 mule deer. Thoracic and abdominal lymph nodes were greatly enlarged, dark grey to black, and often focally necrotic.

Histologically, 50 to 75% of each mule deer liver was replaced by anastomosing cords of collagen, macrophages, lymphocytes, fibroblasts, clusters of eosinophils, and hemorrhage in areas of fluke migratory tracks. Hepatocytes adjacent to fluke migration tracks often were degenerate to necrotic. Bile ducts and ductules often were moderately hyperplastic, and associated vessels often were thrombosed. Most phagocytic cells contained hematin pigment. The hepatic capsule usually had a thick coat of fibrin mixed with collagen, hemorrhage, macrophages, plasma cells, lymphocytes, and occasional clusters of eosinophils. Serosa of most abdominal organs was coated with a similar layer of fibrin, collagen, and inflammatory cells. Mesenteric lymph nodes usually were depleted of lymphocytes, but contained numerous macrophages filled with hematin pigment.

Severely affected portions of lung were characterized by increased numbers of type II pneumocytes, and prominent smooth muscle hyperplasia. Vessels were severely congested, and alveolar macrophages contained hematin pigment. Other organs had no significant changes associated with the parasitism.

The white-tailed deer remained clinically healthy throughout the infection period and was euthanized 31 wk after inoculation. Approximately 10 *F. magna* eggs per gram were detected in the feces 28 wk after inoculation, and weekly thereafter, until euthanasia. Approximately 100 eggs per gram of feces (range 98–124) were present in three replicate 1-g fecal samples examined on the day the deer was euthanized. At necropsy, 205 *F. magna*, including 12 mature (mean length = 44.4 mm) and 193 immature flukes (mean length = 20.3 mm, n = 125 measured) were detected (Table 1). All mature flukes and a majority of the immature flukes were

TABLE 1. *Fascioloides magna* from mule deer and a white-tailed deer, each inoculated with 500 metacercariae.

Deer no.	Sex	Post-inoculation day of death	*Fascioloides magna* recovered			
			Immature	Length ± SD (mm)	Mature	Length ± SD (mm)
Mule deer						
1	F	69	326	12.6 ± 3.3 range = 7 to 25 (n = 322)[a]	0	NA[b]
2	F	103	56	26.0 ± 8.8 range = 10 to 45 (n = 47)	0	NA
3	M	121	56	35.8 ± 6.5 range = 17 to 48 (n = 51)	0	NA
4	M	124	92	24.0 ± 3.6 range = 17 to 32 (n = 82)	0	NA
5	M	130	44	25.4 ± 6.4 range = 14 to 35 (n = 42)	0	NA
6	F	134	38	35.6 ± 7.2 range = 18 to 45 (n = 24)	0	NA
White-tailed deer						
7	F	218[c]	193	20.3 ± 11.9 range = 5 to 43 (n = 125)	12	44.4 ± 4.3 range = 37 to 51 (n = 12)

[a] n = Number of intact flukes measured.
[b] NA = None available.
[c] White-tailed deer was euthanized.

in hepatic parenchyma. Other immature flukes were found in the lung (n = 1 present), thoracic fluid (n = 3), and peritoneal cavity (n = approximately 35). Mature *F. magna* were encapsulated in pairs within six fibrous capsules, also containing thousands of eggs in black, viscous liquid.

At necropsy, the white-tailed deer weighed 37 kg and the body condition was moderate with adequate stores of fat. Approximately 50 ml and 200 ml of serosanguinous fluid were present in thoracic and peritoneal cavities, respectively. The lungs had one obvious fluke migratory track, and one immature *F. magna* was recovered from this area. Fibrin strands were common on pleural and peritoneal surfaces, and the diaphragm was adhered to the hepatic capsule. The liver was enlarged, with prominent bosselations and fissures. Three white fibrous capsules, each approximately 3 to 4 cm in diameter, were prominent on the hepatic surface. Tortuous tracks filled with blood, dark fluid, and numerous flukes were common on the cut surface.

Histologically, hepatic tissue was characterized by parenchymal cavitations up to 1 cm in diameter. These areas were rimmed by 1- to 2-mm layers of fibroblasts arranged perpendicularly to small blood vessels (granulation tissue), mixed with numerous macrophages filled with hematin pigment. Hemorrhages were in and near cavitations, and were accompanied by loose deposits of fibrin, neutrophils, and hematin-filled macrophages. Fibrous tissue extended throughout the sections, subdividing lobules, and formed a thickened organ capsule. Hematin pigment deposits were

dense along the fibrous tracts, and occasional degenerate trematode ova were embedded in granulation tissue margins. Hematin pigment was common in Kupffer cells. Nearly all periportal regions were characterized by fibrosis and biliary hyperplasia. Sections of lung tissue were altered by focal pleural thickening up to 3 mm, composed of well vascularized loose connective tissue, mixed with scattered macrophages containing hematin pigment. Macrophages throughout the lung also contained hematin pigment, and alveolar capillaries were focally hyperemic. One lung section contained a 2-mm cavitation which represented a fluke migratory track. The wall was lined by loose connective tissue, hematin-filled macrophages and acute hemorrhage. Bronchioles and bronchi were histologically normal.

DISCUSSION

Fascioloides magna has been reported from mule deer in the Canadian provinces of Alberta and British Columbia, but experimental infections have not been reported previously. Cowan (1946) reported one dead *F. magna* in 1 of 40 Columbian black-tailed deer (*O. hemionus columbianus*) in British Columbia, and Hadwen (1916) reported 18 *F. magna* from the liver of *O. hemionus columbianus* from Texada Island in British Columbia. Pybus (1990) found *F. magna* in 21 of 87 yearling and adult elk, 6 of 161 adult moose, and 2 of 97 adult white-tailed deer in Alberta, but did not find *F. magna* in lungs or livers of 247 mule deer from the same area. She also reported infection in 2 of 22 mule deer from Banff and Kootenay National Parks, but gave no further information regarding the infection.

Based on the results of this experiment, mule deer are highly susceptible to infection with large numbers of *F. magna*, and infection with these numbers of flukes is likely to be lethal. The infective dose of 500 metacercariae used in this experiment has been used in previous studies in white-tailed deer which resulted in a lower percentage recovery of flukes. Foreyt and Todd (1976, 1979) reported mean fluke recovery rates of 16 flukes (3.2%) per deer in one study of seven deer given 500 metacercariae, and 13 flukes (2.6%) per deer in a study of two deer. Mean numbers of flukes recovered in this experiment were 102 (20.4%) in the six mule deer, and 205 (41%) in the one white-tailed deer. The difference in fluke recovery rates in this study may have been the increased vitality of the metacercariae. In the present study, metacercariae were stored <1 wk after collection from infected snails before inoculation of deer, whereas in previous studies, metacercariae were stored up to several months before use. Free ranging white-tailed deer rarely are infected with more than 100 *F. magna* (Foreyt et al., 1977; Pursglove et al., 1977; Addison et al., 1988; Pybus, 1990), indicating that the 215 *F. magna* recovered from the experimentally infected white-tailed deer was excessive, and probably resulted from the extreme vitality of the metacercariae. However, the deer remained clinically healthy during the experiment, and 12 flukes had encapsulated, matured, and were passing eggs in feces at the time of euthanasia. Although all metacercariae used in previous experimental studies were grossly viable, as indicated by movement in their flame cells, metacercariae stored for the shortest period of time are likely to grow most vigorously in the host. No dead flukes were detected in any of the deer, indicating that little host resistance was present. Percentage recovery of *F. magna* administered as a single inoculum of 250 to 600 metacercariae in cattle, sheep, and goats has been variable, but less than 10% is routine (Foreyt and Todd, 1976; Foreyt and Leathers, 1980; Foreyt, 1989).

Mean lengths of *F. magna* recovered from the mule deer were much greater than mean lengths of flukes recovered from experimentally infected white-tailed deer, cattle, sheep, and goats on comparable days after inoculation (Foreyt and Todd, 1976;

Foreyt and Leathers, 1980). However, the mean length of flukes recovered from the white-tailed deer in the present study was less than expected. The 12 mature encapsulated flukes were of predictable size (mean = 44.4 mm), but it was surprising to find such a large number of very small immature flukes (mean length = 20.3 mm). Many immature flukes were less than 10 mm long even though all flukes were 31-wk-old. Foreyt and Todd (1976) reported that some *F. magna* remain small and immature well beyond the normal prepatent period of approximately 30 wk in experimental infections of white-tailed deer. The large number of flukes in the white-tailed deer in this study likely contributed to substantial retardation in development of flukes. The retardation phenomenon may last for years in massive infections such as this.

Infection with even a few *F. magna* usually is lethal to domestic sheep and goats within 5 mo because immature flukes migrate continually in hepatic and other tissues (Foreyt and Todd, 1976; Foreyt, 1989). Death usually is attributed to massive hemorrhage resulting from blood vessel rupture by migrating flukes. All six mule deer in this experiment died within 5 mo of infection and had lesions similar to those observed in sheep and goats. It is therefore likely that mule deer are highly susceptible to large numbers of *F. magna* and can die from infection. It is not known whether fewer flukes cause a similar response.

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