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MULE DEER (*ODOCOILEUS HEMIONUS*) AND ELK (*CERVUS ELAPHUS*) AS EXPERIMENTAL DEFINITIVE HOSTS FOR *FASCIOLOIDES MAGNA*

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ABSTRACT: In August 1992, six mule deer (*Odocoileus hemionus hemionus*) fawns and four elk (*Cervus elaphus*) calves ($n = 2$) or yearlings ($n = 2$) each were inoculated orally with 50, 250, or 2,000 metacercariae of the liver fluke *Fascioloides magna* to evaluate their potential to serve as definitive hosts. Animals were maintained for up to 403 days. Three mule deer each inoculated with 50 metacercariae survived the infection and shed eggs in feces; thus mule deer can function as definitive hosts for *F. magna*. The other three mule deer inoculated with 50 ($n = 1$) or 250 ($n = 2$) metacercariae died from fluke infection on days 91, 150, and 162 days postinoculation, respectively, and only immature *F. magna* were recovered. One elk calf inoculated with 2,000 metacercariae died from fluke infection 44 days after inoculation. The remaining three elk, each inoculated with 250 metacercariae, survived infection, and two of the three shed eggs in feces. The third elk contained only one immature *F. magna* at necropsy. The prepatent period in mule deer and elk was approximately 6 to 7 months.

Key words: *Fascioloides magna*, liver fluke, experimental infection, mule deer, *Odocoileus hemionus*, elk, *Cervus elaphus*.

INTRODUCTION

Fascioloides magna, the large American liver fluke or the deer liver fluke, commonly parasitizes white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) in North America (Foreyt, 1981; Wobeser et al., 1985) and Europe (Erhardova-Kotrla, 1971). Although *F. magna* has been reported to affect reproductive traits in white-tailed deer (Mulvey et al., 1994), infection is usually asymptomatic in these definitive hosts (Foreyt and Todd, 1976; Pybus, 1990). Experimental infections of *F. magna* in white-tailed deer have a documented prepatent period of approximately 6 mo (Foreyt and Todd, 1976), but the prepatent period in elk has not been established. Mule deer (*Odocoileus hemionus hemionus*) inoculated with 250 metacercariae died within 6 mo of infection; therefore, mule deer, like domestic sheep and goats, were considered poor hosts for *F. magna* and would likely die if infected (Foreyt, 1992). My purpose in this research was to determine the prepatent period of *F. magna* in elk, and to determine the importance of exposure intensity on the ability of mule deer to serve as definitive hosts for *F. magna* if given 50 or 250 metacercariae.

MATERIALS AND METHODS

In June 1992, six orphaned mule deer fawns, three males and three females, were obtained from the wild in eastern Washington (USA) (46°30′N, 117°10′W) when <1 wk old, and fed goat milk at a private holding facility, until weaned at approximately 3 mo of age. In June 1992, two male elk calves were captured from the wild in eastern Washington (46°02′N, 118°08′W) when <1-wk old, maintained in the same facility as the deer fawns, and fed goat milk until weaned at approximately 3-mo of age. In August 1992, two male yearling elk were obtained from a zoo in Spokane, Washington, and transported to Washington State University (WSU), Pullman, Washington. All animals were obtained from areas where *F. magna* has not been identified in wild animals. After weaning, deer fawns and elk calves were transferred from the holding facility to WSU and maintained with the yearling elk on a 0.4 ha pasture with natural grasses. Supplemental alfalfa pellets, mineralized salt, and fresh water were provided ad libitum. For the entire experiment, the six mule deer fawns and the four elk were maintained together in the same 0.4 ha pen.

On 31 August 1992, each deer fawn and elk calf (numbers 1 and 2) or elk yearling (numbers 3 and 4) was inoculated orally with viable me-

TABLE 1. *Fascioloides magna* recovered from experimentally inoculated mule deer and elk.

Animal number	Sex	Metacercariae given	Days post-inoculation	*F. magna* recovered		Prepatent period (days)
				Mature	Immature	
Elk						
1	M[a]	2,000	44[b]	0	198	NA[c]
2	M	250	253	9	0	201
3	M	250	253	0	1	NA
4	M	250	403	22	0	235
Mule deer						
1	M	250	91[b]	0	152	NA
2	F	250	150[b]	0	119	NA
3	M	50	326	12	0	203
4	F	50	326	2	0	179
5	M	50	162[b]	0	19	NA
6	F	50	326	12	0	217

[a] M, male; F, female.
[b] Died.
[c] Not applicable.

tacercariae of *F. magna* in a gelatin capsule with a balling gun (Table 1). Metacercariae were obtained from Baldwin Enterprises (Monmouth, Oregon, USA). Viability of metacercariae was determined microscopically by movement of flame cells. One elk calf received 2,000 metacercariae; three elk calves and two mule deer fawns received 250 metacercariae, and four deer fawns received 50 metacercariae. Deer fawns and elk calves were restrained physically during administration of metacercariae, and elk yearlings were injected intramuscularly with 100 mg (in volume) of xylazine (Rompun®, Mobay Corporation, Shawnee, Kansas, USA) for restraint. Fecal samples were collected every 10 to 14 days beginning 25 wk after inoculation. A modified sedimentation technique (Flukefinder®, Visual Difference, Moscow, Idaho, USA) was used to isolate fluke eggs from feces. Eggs were identified using a dissecting microscope (30×).

Between 250 and 403 days after inoculation, surviving animals were restrained physically and then euthanized with 30 grams of pentobarbital sodium (Anthony Products Company, Arcadia, California, USA) given intravenously. Following submission to the Washington Animal Disease Diagnostic Laboratory (WADDL) (Pullman, Washington), animals were evaluated by standard necropsy, histopathologic and parasitologic techniques as described by Foreyt (1992). Briefly, all internal organs were examined grossly for *F. magna* and for lesions compatible with migratory tracts of *F. magna*. Livers and lungs were sliced at approximately 1-cm intervals and visible flukes were removed. Tissue slices were soaked in warm water for approximately 2 hr; slices and sediment from the washing were examined grossly. Recovered flukes were counted and placed between two glass slides for observation. If flukes were severed only heads were counted. Mature flukes were identified by presence of eggs in the uterus. The following tissues were collected for histological evaluation: liver, lung, kidney, skeletal muscle, brain, adrenal gland, brain, ovary, testicle, mesenteric lymph node, intestine, urinary bladder and pancreas. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

RESULTS

Fascioloides magna were recovered from all animals at necropsy (Table 1). Three mule deer given 250 (n = 2) or 50 (n = 1) metacercariae died from fluke infection 162, 150 or 91 days after inoculation, respectively (Table 1). The deer were depressed, had droopy ears, and were anorectic and weak for 1 to 2 wk before death. At necropsy, 152, 119 and 19 *F. magna* were recovered from each deer, respectively (Table 1). Three deer survived the experiment for 326 days, and two, 12 and 12 *F. magna* were recovered from each deer at necropsy, respectively (Table 1). All flukes were mature and encapsulated in pairs in the livers of the surviving deer. Fluke eggs were first detected from these mule deer on day 179, 203 and 217 after inoculation, respectively, and all three continued to shed eggs until the experiment was terminated.

At necropsy, the three deer that died during the experiment weighed 24, 27 and 32 kg, respectively, and seemed to be in fair to good condition. Gross findings were similar in all three deer: numerous thick fibrin tags were adhered to the serosal surface of the liver, gastrointestinal tract, mesentery, spleen, lungs, pericardium, and thoracic walls. Approximately 300 ml of dark red fluid was in the peritoneal and thoracic cavities, and numerous immature *F. magna* were found in the fluid. The livers were enlarged, had a roughened sur-

face, and numerous 1 to 3 mm holes were in hepatic capsules. Numerous immature *F. magna* in necrotic black tracts were in all lobes. Black pigmented areas, up to 6 cm in diameter, were on numerous internal organs including liver, lungs, kidney and omentum. Lungs were collapsed, firm, and mottled brown and red. On cut surfaces, thick dark reddish-brown liquid oozed from multiple areas of the lung.

Histologically, sections of liver were characterized by periportal fibrosis, bile duct proliferation, and multifocal areas of hemorrhage and necrosis. Multiple tracts lined by hemosiderin-laden macrophages were surrounded by fibrous connective tissue throughout the parenchyma. Mineral deposits were present in some of the tracts. Focal accumulations of lymphocytes, eosinophils and macrophages were adjacent to the tracts. In several areas, there were multiple focal hemorrhages surrounded by necrotic hepatocytes. In lungs, alveolar septa were thickened with fibrous connective tissue and numerous eosinophils, and alveoli in lobules were small (atalectasis). Hemosiderin-filled macrophages were scattered throughout the interlobular septa. Widespread areas of hemorrhage with associated fibrin deposition, eosinophil infiltration, and hemosiderin pigment in accompanying histiocytes were also present.

The elk given 2,000 metacercariae died 44 days after inoculation, and 198 immature *F. magna* were recovered from the liver (n = 100) and peritoneal cavity (n = 98). The other three elk receiving 250 metacercariae survived the experimental period and one, nine and 22 *F. magna* were recovered, respectively (Table 1). All flukes were mature and encapsulated in fibrous hepatic capsules in groups of two or three, and eggs were detected in feces of two elk harboring nine or 22 *F. magna*. In the elk where only one fluke was recovered, the fluke was immature and was not encapsulated. Fluke eggs were first detected in feces from elk numbers 2 and 4 on days 201 and 235, respectively (Table 1). Thereafter, fluke eggs were detected in feces from these two elk every collection period until the experiment was terminated. Eggs in feces were not detected in elk number 1 that died or from elk number 3 with one immature fluke recovered.

At necropsy, elk number 1 weighed 68 kg and was in good body and postmortem condition. Approximately 300 ml of dark red fluid containing numerous immature *F. magna* was in the peritoneal cavity. The omasum and mesentery were adhered widely to the abdominal viscera and the abdominal wall. A 10 × 10 × 5 cm mass of inflamed granulation tissue composed of proximal jejunum and mesentery was present. A 4-cm section of jejunum incorporated into the mass, was black, friable and had a 1-cm perforation in the anti-mesenteric border. Hemorrhage and fibrin were associated with the perforation. Numerous subcapsular branching fluke migratory tracts were present on the surface of the liver.

Histologically, liver lesions were similar to those described in the deer. In the small intestine, several 1 to 4 mm diameter necrotic mucosal foci infiltrated with degenerate neutrophils and eosinophils and surrounded by granulation tissue were in the gut wall near the intestinal rupture. The gut wall adjacent to the rupture site was thickened with granulation tissue. Death was attributed to severe acute fibrinous peritonitis.

DISCUSSION

Mature *F. magna* have not been reported from free-ranging mule deer, and Foreyt (1992) noted that *F. magna* was lethal in mule deer infected with 500 metacercariae. Based on those data, mule deer, like domestic sheep and domestic goats were considered unsuitable hosts and unlikely to survive infection with *F. magna*. In the current experiment, fewer metacercariae were used in the inocula to test the hypotheses that mule deer can survive light infections, and that mature parasites release eggs in their feces. Based on the

results of the current experiment, mule deer can survive infection and function as definitive hosts of *F. magna*. In surveys of parasites of mule deer, very few *F. magna* have been reported, and all flukes have been immature (Pybus, 1990). It is likely, therefore, that lack of exposure to metacercariae of *F. magna* is the reason for few reported infections in free-ranging mule deer, rather than fatal infections in mule deer before flukes mature. Different habitat preferences of mule deer compared to white-tailed deer may be one reason for few infected free-ranging mule deer. Mule deer are found predominantly in western North America and frequent semiarid, open forest, brush and shrub lands associated with steep and rough terrain (Mackie et al., 1982), where exposure to metacercariae would be minimal.

As demonstrated in this report, inoculation with 2,000 *F. magna* metacercariae resulted in death of one elk, but ingestion of a single dose of 2,000 metacercariae in free ranging elk may be unlikely. Effects of this magnitude of exposure over a greater time periods is unknown. All three survived the experimental inoculation of 250 metacercariae, and two of these resulted in patent infections. Based on natural and experimental infections in white-tailed deer, the development and prepatent period of *F. magna* in elk appears to be comparable to that in white-tailed deer (Foreyt and Todd, 1976) and the mule deer in this study.

Because of humane concerns, this study was restricted to a limited sample size of four elk and six mule deer. Although infected mule deer and one elk had some signs of infection, humane observers did not consider the animals under undue duress. Lesions were similar to those reported in domestic sheep, domestic goats, and mule deer (Foreyt, 1992), and it is likely that death occurs quickly due to massive hemorrhage resulting from blood vessel rupture caused by migrating flukes.

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