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Susceptibility of Bighorn Sheep (*Ovis canadensis*) to Experimentally-Induced *Fascioloides magna* Infections

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ABSTRACT: Three captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*), consisting of a female lamb, a yearling ram, and a 2½-yr-old castrated ram were inoculated orally with 50 (n = 1) or 100 (n = 2) metacercariae of *Fascioloides magna* in November 1991. All three sheep died from fluke infection on postinoculation days 104, 140, and 197, respectively. Numbers of *F. magna* recovered were 3 (3%), 18 (36%), and 21 (21%). All flukes were immature and were recovered from liver (n = 36), lungs (n = 2), or peritoneal spaces (n = 4). Two white-tailed deer (*Odocoileus virginianus*), each were inoculated orally with 100 metacercariae at the same time as the bighorn sheep. Eggs of *F. magna* were detected in the feces of the deer on postinoculation days 199 and 211, respectively. Both deer remained healthy for the year-long experiment. Thus, bighorn sheep are susceptible to infection with *F. magna* and are likely to die within approximately 6 months of exposure.

Key words: *Fascioloides magna*, bighorn sheep, *Ovis canadensis*, liver flukes, experimental infection.

White-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) are definitive hosts of the large American liver fluke *Fascioloides magna* and usually are not affected clinically by the parasite (Foreyt, 1981). In some hosts such as bison (*Bison bison*), cattle, moose (*Alces alces*), and llamas (*Lama glama*), *F. magna* survives and often matures in the liver, but does not pass eggs in feces (Lankester, 1974; Foreyt and Todd, 1976; Foreyt and Parish, 1990). In other hosts, such as domestic sheep, domestic goats, and mule deer (*Odocoileus hemionus hemionus*), the infection usually is lethal before the parasite matures in the liver (Foreyt and Todd, 1976; Foreyt and Leathers, 1980; Foreyt, 1992). Bighorn sheep (*Ovis canadensis*) are susceptible to a variety of infectious diseases (Spraker and Adrian, 1990), but the importance of many of the diseases have not been investigated. Although the liver fluke *F. magna* has not been reported from bighorn sheep, the potential for infection is present in areas where bighorn sheep share common habitat with infected white-tailed deer or elk. My objective was to determine the susceptibility of bighorn sheep to experimentally induced infections of *F. magna*.

Three captive Rocky Mountain bighorn sheep (*O. canadensis canadensis*), consisting of a female lamb, a yearling ram, and a 2½-yr-old castrated ram were inoculated orally with either 50 (n = 1) or 100 (n = 2) viable metacercariae of *F. magna* (Table 1). All sheep had been in captivity at Washington State University (WSU), Pullman, Washington (USA) for at least 1 yr and were not related. Metacercariae were obtained from Baldwin Enterprises (Monmouth, Oregon, USA). Viability of the metacercariae was determined by movement of the flame cells, and metacercariae were counted under a dissecting microscope (30×), transferred in water to a gelatin capsule, and given orally using a balling gun (Nasco West, Modesto, California, USA). Bighorn sheep were maintained together with two additional uninoculated 7-mo-old bighorn ram lambs on a 0.4-ha pasture at WSU for the entire experiment. Forage consisted of natural grasses and supplemental alfalfa pellets; mineralized salt and fresh water were available at all times. Two 6-mo-old male white-tailed deer each were inoculated also with 100 metacercariae as described previously on the same day as the three bighorn sheep, and were maintained in the same pen with the five bighorn sheep.

Fecal samples were collected monthly from each sheep and deer by observing each animal defecate and then collecting

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TABLE 1. Recovery of *Fascioloides magna* from bighorn sheep experimentally infected in November 1991.

Animal number	Sex	Age (years)	Postinoculation day of death	Metacercariae given	*Fascioloides magna* recovered	
					Number	Mean size in mm (range)
Bighorn 1	M[a]	1 ½	104	100	3[b]	17 (5–24)
Bighorn 2	M	2 ½	197	100	21[c]	26[d] (17–39)
Bighorn 3	F	½	140	50	18[b]	26[e] (15–31)

[a] M, male; F, female.
[b] Includes 1 fluke in the lungs and 1 in the peritoneal spaces.
[c] Includes 1 fluke in the lungs and 3 in the peritoneal spaces.
[d] 17 flukes were measured.
[e] 16 flukes were measured.

the corresponding fecal sample from the ground. The Flukefinder sedimentation technique (Visual Difference, Moscow, Idaho, USA) was used to isolate fluke eggs in feces. Eggs were detected under a dissecting microscope (30×). Sheep that died were submitted to the Washington Animal Disease Diagnostic Laboratory (Pullman, Washington) for necropsy evaluation. At necropsy, all organs were examined grossly, and pieces of liver and lung were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and evaluated histologically for microscopic lesions. Livers and lungs were sliced at 1 cm intervals, and intact flukes were measured. Only anterior ends of flukes were counted when pieces of flukes were recovered. Liver and lung slices were washed thoroughly, and the sediment was examined under a dissecting microscope (30×) for trematode ova.

All three inoculated bighorn sheep died from fluke infection between 104 and 197 days after inoculation (Table 1). Within 2 wk before death, the bighorn sheep had some clinical signs including depression, weight loss, weakness, or loss of appetite. At necropsy, three to 21 *F. magna* were recovered from each sheep, and fluke recovery based on metacercariae given was between 3% and 36% (Table 1). All flukes recovered were immature, and were recovered from liver (n = 36), lungs (n = 2), and peritoneal spaces (n = 4).

At necropsy, significant lesions occurred in liver and lungs. Infection was similar in all sheep and was characterized by multifocal pyogranulomatous hepatitis, necrotizing hemorrhagic pneumonia, and hematin pigment accumulations in liver and other internal organs. Between 50 and 300 ml of serosanguinous fluid were present in thoracic cavities of the three sheep. Fibrin strands and tags were attached to visceral pleura, and occasional black necrotic and hemorrhagic fluke migration tracts were present in lungs. Fibrinous tags covered most abdominal serosa, and some organ surfaces were adhered. In two sheep, lungs adhered to the parietal pleura. Liver was the most severely affected organ, being swollen to approximately twice normal size in all sheep. Coagulative hepatic necrosis was common, and friable parenchyma was infiltrated by numerous tortuous tunnels filled with blood, black pigment, and immature flukes. The following organs and tissues were considered normal: brain, thyroid glands, heart, thymus, pancreas, mesenteric lymph nodes, spleen, adrenal glands, kidneys, urinary and reproductive tracts, muscles, appendicular joints, and gastrointestinal tract.

Histologically, 50% to 75% of each sheep liver was replaced by anastomosing cords of collagen, macrophages, lympho-

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cytes, 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.

Both inoculated white-tailed deer remained clinically healthy during the 365-day experiment, and were not euthanized. Eggs of *F. magna* were detected in feces of the deer on postinoculation days 199 and 211, respectively, and at each sampling period thereafter. The two uninoculated bighorn sheep remained clinically healthy during the experiment, and eggs of *F. magna* were not detected in the feces. These two sheep later were euthanized with an intravenous injection of 30 g of pentobarbetol (Anthony Products Company, Arcadia, California) as part of another study, and no *F. magna* were detected at necropsy.

Because of humane concerns, only three bighorn sheep were inoculated in this experiment. Although infected sheep demonstrated 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 occured quickly due to massive hemorrhage resulting from blood vessel rupture by migrating flukes.

Fascioloides magna was lethal to bighorn sheep at the levels of metacercariae administered. Because one sheep died with only three flukes present at necropsy, it is likely that few or possibly even one fluke may be lethal in bighorn sheep. These results are similar to those of *F. magna* infections in domestic sheep and domestic goats in which infected animals die with very few flukes within 6 mo after infection (Foreyt and Todd, 1976; Foreyt and Leathers, 1980). Therefore, bighorn sheep survival may be impaired in areas where *F. magna* is enzootic. Typical bighorn sheep habitat is often characterized by grasslands, or grass and shrub areas adjacent to or intermixed with precipitous rocky terrain (Johnson, 1983); but in states such as Washington, bighorn sheep occupy or cross habitats that are aquatic habitats for potential intermediate snail hosts for *F. magna*. In those areas, the potential for infection may be important for bighorn sheep survival. The presence of *F. magna* in deer or elk should be considered when translocating bighorn sheep into new areas.

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