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Source: Journal of Wildlife Diseases, 45(4) : 1217-1220

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-45.4.1217>

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Experimental Infection of Bighorn Sheep with Liver Flukes (*Fasciola hepatica*)

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ABSTRACT: Nine Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) were each inoculated orally with 250 metacercariae of *Fasciola hepatica*. Blood and fecal samples were collected at the time of inoculation and at 5, 10, 14, and 20 wk after inoculation. Numbers of fluke eggs in feces, hemoglobin, packed cell volume, and eosinophil values were determined. Five of the bighorn sheep were treated with triclabendazole at 40 mg/kg of body weight 14 wk after inoculation. Based on fecal evaluations, all bighorns developed patent infections. Six weeks after treatment, fluke eggs were not detected in feces from the five treated animals but were present in two of four untreated animals. One untreated bighorn sheep was euthanized 20 wk after inoculation, and 57 adult *F. hepatica* were recovered from the liver. Results from this experiment indicated that bighorn sheep are efficient hosts for *F. hepatica*. Triclabendazole at 40 mg/kg of body weight was safe and, based on fluke egg recovery in feces, apparently an effective treatment. To my knowledge, this is the first published report of *F. hepatica* in bighorn sheep.

Key words: Bighorn sheep, experimental infection, *Fasciola hepatica*, *Ovis canadensis canadensis*.

Fasciola hepatica is a liver fluke that is found commonly in sheep, goats, cattle, and a variety of animals throughout much of the world (Pybus, 2001). Adult flukes inhabit the bile ducts of the liver, and eggs pass with bile into the feces. Intermediate stages of the fluke develop in suitable snail hosts, and final hosts become infected by ingesting the metacercarial stage with vegetation. Immature flukes migrate through hepatic parenchyma before entering and maturing in bile ducts. Clinical signs of infection are often dependent on the numbers of flukes present and range between subclinical infection and death

(Roberts, 1968). Anemia is one of the usual clinical signs of infection, and domestic sheep and goats are particularly susceptible to the deleterious effects of infection (Roberts, 1968; Leathers et al., 1982). Bighorn sheep are hosts of a wide variety of internal and external parasites, but to my knowledge, infection with *F. hepatica* has not been reported. The purpose of this experiment was to document the susceptibility of bighorn sheep to infection.

Nine captive Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*), which ranged from in age from 1 to 7 yr and consisted of six males and three females, were used in this experiment, conducted between 28 March and 27 August 2002. The bighorn sheep were born in captivity and maintained on a 0.4-ha pasture at Washington State University, Pullman, Washington, USA. Feed consisted of natural grasses supplemented with alfalfa hay and an alfalfa-grain pelleted ration. Mineralized salt, water, and shelter were available at all times. Animals were observed on a daily basis.

On day 0 of the experiment, all nine bighorns were each inoculated with 250 metacercariae of *F. hepatica*. 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 the dissecting microscope (30×), transferred in water to a gelatin capsule, and given orally using a balling gun (Nasco West, Modesto, California, USA). Sheep were captured at the time of inoculation and 5,

TABLE 1. Numbers of fluke eggs per gram of feces recovered from bighorn sheep inoculated with 250 metacercariae of *Fasciola hepatica* on day 0.

Animal No.	Age	Sex	Day 0	Wk 5	Wk 10	Wk 14	Wk 20
W23 ^a	4	F	0	0	7	35	0
O47 ^a	7	F	0	0	14	46	0
O11 ^a	2	M	0	0	8	24	0
W27 ^a	1	F	0	0	7	10	0
W30 ^a	1	M	0	0	3	0	0
W2 ^b	5	M	0	0	6	10	25
W31 ^b	2	M	0	0	0	6	0
B11 ^{b,c}	3	M	0	0	24	92	42
W49 ^b	1	F	0	0	7	0	0

^a Treated with triclabendazole at 40 mg/kg of body weight on wk 14.

^b Untreated animals.

^c Euthanized on wk 20—57 mature *F. hepatica* were recovered at necropsy.

10, 14, and 20 wk after inoculation using a drive net, after which they were physically restrained. Blood from the jugular vein and fecal samples from the rectum were collected from all bighorns each time the bighorns were captured. Blood anticoagulated with EDTA was evaluated for hemoglobin concentration (Hb), packed cell volume (PCV), and eosinophil count by standard techniques. Mean differences between the hematology values were compared between the day of inoculation (day 0) and each other sampling date using the paired Student's *t*-test calculated in SAS/PC (Cary, North Carolina, USA). Fecal samples were evaluated for fluke eggs using a modified sedimentation test and for other internal parasites using a sugar flotation (specific gravity=1.27) technique as described by Foreyt (2001). Fourteen weeks after inoculation, five of the bighorns were chosen randomly and treated orally with a 5% triclabendazole suspension (Novartis, Basel, Switzerland) at 40 mg/kg of estimated body weight using a drenching syringe. The remaining four untreated bighorns were drenched with a water placebo. Twenty weeks after inoculation, one untreated bighorn that was passing fluke eggs in feces was euthanized with pentobarbital given intravenously. At necropsy, the bile ducts were dissected, and all flukes were removed and

counted. The liver was then sectioned at 1-cm intervals, soaked in warm water for 30 min, and the liver slices and sediment were examined grossly for additional flukes. The remaining eight bighorns were not euthanized.

All bighorns survived the experiment and remained clinically healthy. None of the bighorns was passing fluke eggs at the beginning of the experiment or at 5 wk after inoculation (Table 1). Fluke eggs (3–24 eggs per gram of feces) were initially detected in eight of nine bighorns 10 wk after inoculation, and eggs were detected in all treated sheep at the time of treatment on wk 14 (Table 1). Hemoglobin and PCV values were significantly lower ($P<0.05$) for all time periods after inoculation when compared with values on day 0. Eosinophil values were significantly higher ($P<0.05$) for all time periods except for the last collection (wk 20) after inoculation (Table 2). Gross lesions in the bighorn that was euthanized at the end of the experiment were typical of mature *F. hepatica* infections and were primarily characterized by thickened and fibrotic bile ducts. Fifty-seven mature *F. hepatica* was recovered from the bile ducts. Representative samples of *F. hepatica* (USNPC no. 101508.00) were deposited in the US National Parasite Collection (USNPC, Agricultural Research Service,

TABLE 2. Mean hemoglobin, packed cell volume (PCV), and eosinophil values ($\pm$ SD) in nine bighorn sheep experimentally inoculated with 250 metacercariae of *Fasciola hepatica*.

Blood parameter	Published values ^a	Day 0	Wk 5	Wk 10	Wk 14 ^b	Wk 20
Hemoglobin (g/dl)	18.1 $\pm$ 3.3	20.3 $\pm$ 0.4	19.1 $\pm$ 1.4 ^c	15.3 $\pm$ 2.4 ^c	14.4 $\pm$ 1.7 ^c	15.9 $\pm$ 1.7 ^c
Packed cell volume (%)	45.2 $\pm$ 6.6	63 $\pm$ 1	58 $\pm$ 5 ^c	47 $\pm$ 8 ^c	40 $\pm$ 8 ^c	52 $\pm$ 5 ^c
Eosinophils (/ μ l)	404	293 $\pm$ 443	1171 $\pm$ 851 ^c	4574 $\pm$ 2089 ^c	1471 $\pm$ 1245 ^c	697 $\pm$ 909

^a Franzmann (1971, 1972); Borjesson et al. (2000).

^b Five bighorns were treated with triclabendazole at 40 mg/kg of body weight.

^c Means are significantly different ($P < 0.05$) from means on day 0.

US Department of Agriculture, Beltsville, Maryland, USA). Immature flukes were not recovered. Other parasite eggs and oocysts detected in fecal samples included low numbers of *Eimeria* spp. and gastrointestinal strongyles in three of the bighorns.

On the basis of laboratory and necropsy results from this experimental study, bighorn sheep are susceptible to infections with *F. hepatica* and are effective definitive hosts. Fluke eggs were recovered from eight of nine bighorns 10 wk after inoculation, which is in agreement with the 8–10-wk prepatent reported in a majority of the literature for domestic ruminants (Pybus, 2001). Fluke eggs were also detected in the ninth bighorn only on wk 14 (Table 1). Although there were no uninoculated controls in this experiment, Hb, PCV, and eosinophil values were compared to mean values on the day of inoculation (day 0). The lower Hb and PCV values and higher eosinophil numbers are consistent with *F. hepatica* infections in other ruminants (Berry and Dargie, 1978). Anemia is one of the hallmarks of infection in ruminants. In acute fascioliasis, anemia is attributed to intrahepatic hemorrhage from parenchymal damage by migrating immature flukes, whereas the anemia of chronic infection is primarily the result of blood loss from the damaged biliary ducts and the ingestion of blood by mature flukes (Sewell et al., 1968; Berry and Dargie, 1978). The daily host blood loss has been estimated at 0.3 to 0.5 ml per fluke (Holmes and McLean,

1971), which would account for the lower Hb and PCV values observed in this study. It is therefore possible that bighorn sheep infected with large numbers of immature or mature flukes would likely be severely anemic and may die. The Hb and PCV values for the nine bighorn sheep at the beginning of the experiment were higher than normal values reported by Franzmann (1971, 1972). This was likely due to the excellent physical condition of the captive bighorns, and it may have been responsible for the lack of clinical disease in the infected bighorns.

Bighorn sheep seem to thrive where grasslands or grass/shrub habitats are found adjacent to or intermixed with precipitous terrain characterized by rocky slopes, ridges, and cliffs or rugged canyons (Johnson, 1983). *Fasciola hepatica* has not been reported from bighorn sheep previously, likely because these habitats are usually not suitable for intermediate snail hosts or infected definitive hosts such as cattle, sheep, and goats. In areas where bighorn sheep habitat is shared by infected definitive hosts, infection is more likely to occur. Additional studies utilizing fecal and necropsy evaluations will likely document the presence and pathogenicity of *F. hepatica* in free-ranging bighorn sheep.

I thank the numerous veterinary students at Washington State University who assisted with the captures and data collection.

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Received for publication 5 November 2008.