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## SUSCEPTIBILITY OF ELK TO LUNGWORMS FROM CATTLE

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**ABSTRACT:** Two studies were conducted to determine the infectivity of the lungworm, (*Dictyocaulus viviparus*) of cattle origin, in Rocky Mountain elk (*Cervus elaphus nelsoni*) or wapiti. In the first study, each of three 9-mo-old elk was administered 3,000 *D. viviparus* larvae from cattle using a nasogastric tube. In the second study, four 16-mo-old elk were each inoculated with 2,000 *D. viviparus* from cattle using a nasogastric tube. Elk were observed daily for signs of respiratory disease, and fecal samples were collected during the studies and evaluated for lungworm larvae using a modified Baermann technique. One elk was euthanatized during the patent period for recovery of adult lungworms, and three elk were euthanatized after larvae were no longer detected in feces. Lungworm larvae were not detected before inoculation in any of the 16-mo-old elk, but were detected 22 days after inoculation in one elk, 23 days after inoculation in two elk and 24 days after inoculation in all four elk. The prepatent period of this cattle isolate of *D. viviparus* in elk is therefore 22 to 24 days. The precise prepatent period was not determined in the three 9-mo-old elk, but larvae were detected in all three elk 25 days after inoculation. Numbers of larvae ranged from 1/ to 101/g feces with peak larval detection occurring 32 to 50 days after inoculation. Elk shed larvae from 22 to 83 days after inoculation, and patent periods of the parasite ranged from 24 to 62 days. Clinical signs of respiratory disease, with the exception of mild coughing after exercise, were not observed during the infections. Results from this experiment indicated that *D. viviparus* larvae of cattle origin can mature in elk and larvae can be passed in large numbers in feces, but this cattle isolate of *D. viviparus* was not highly pathogenic in elk.

**Key words:** Cattle, *Cervus elaphus nelsoni*, *Dictyocaulus viviparus*, elk, experimental infection, lungworm, susceptibility.

### INTRODUCTION

The lungworm (*Dictyocaulus viviparus*) is a relatively common parasite in free-ranging elk (*Cervus elaphus*), also called wapiti in the northwestern USA. In one study of 298 elk in Montana, necropsy results indicated 44% of the adult elk and 35% of the elk calves were infected (Worley et al., 1969). In Wyoming, evaluation of feces for larvae indicated up to 70% of the Rocky Mountain elk were infected (Bergstrom, 1975). In Washington, 66 of 381 (17%) fecal samples tested from free-ranging Rocky Mountain elk from different areas of the state were positive for *D. viviparus* larvae between 1992 and 1999 (W. J. Foreyt, unpubl. data). *Dictyocaulus viviparus* has been implicated as a parasite that contributes to mortality of free-ranging elk, especially in herds subjected to se-

vere weather, poor nutrition and heavy tick infestations (Worley, 1979). However, the significance of *D. viviparus* infections in free-ranging Rocky Mountain elk has not been well documented. In two studies, we experimentally infected three 9-mo-old elk and four 16-mo-old elk with 2,000 or 3,000 *D. viviparus* larvae of cattle origin to determine the infectivity and pathogenicity of this cattle isolate in elk.

### MATERIALS AND METHODS

In the first study, three 9-mo-old Rocky Mountain elk (*C. elaphus nelsoni*), including two females and one male, were used. Elk were born in captivity at the Idaho Department of Fish and Wildlife Health Laboratory (near Caldwell, Idaho; 43°60'N, 116°50'W), maintained in a 0.5 ha outdoor pen, and fed a diet of alfalfa hay and grain. Elk had access to fresh water and trace mineral salt at all times. On 27 February 1997 each elk was restrained in a

squeeze chute and inoculated orally into the rumen with 3,000 larvae of *D. viviparus* in 50 ml of water using a nasogastric tube, and then flushing the tube with 300 ml water. Third stage *D. viviparus* larvae were obtained from L. Smith (Lodi, Wisconsin, USA), who cultured them from the feces of cattle that had been experimentally infected with cattle-strain *D. viviparus*. Before inoculation, larvae were held at 4 C for approximately 3 days and then warmed to 20 C for 12 hr before inoculation. Viability of larvae was determined by movement of the larvae. On the day of inoculation, a fecal sample was collected from the rectum of each elk and evaluated for parasite eggs and oocysts (1 g feces) using a modified fecal flotation test (sugar, sp. gr. 1.27), and for larvae (15 g feces) with a modified Baermann test (Foreyt, 1997). Additional fecal samples were collected from each elk on post-inoculation days 25, 28, 32, 35, 39, 42, 46, 49, 83, and 111 by observing each elk defecate and then collecting the fresh feces from the ground. Fecal samples were evaluated for lungworm larvae using a binocular microscope at a magnification of 200 $\times$ . First stage larvae of *D. viviparus* were identified based on morphology, including the distinct intestinal cells (Foreyt, 1997). Fifteen larvae, five from each elk, were measured to the nearest  $\mu$ m. Elk were observed daily for signs of respiratory involvement such as coughing or increased respiratory rates. Non-infected control elk were not used in the first study because *D. viviparus* has never been isolated from animals in this facility, the pens were essentially devoid of vegetation, the elk were born in captivity, and the elk did not have larvae in feces on the day of inoculation.

In the second study, four male elk were obtained as 10-mo-old weaned calves from the Starkey Experimental Forest and Range in northeastern Oregon (45°29'N, 118°93'W), transported to Washington State University, Pullman, Washington (46°43'N, 117°11'W) in April, 1998, and maintained in a 0.4 ha outdoor pen for six months before the study began. The elk had access to pasture grasses, supplemental alfalfa hay, mineralized salt and water. Fecal samples were collected weekly during the six-month acclimation period and examined for parasite eggs, oocysts, and larvae as described previously. Infective third-stage larvae of *D. viviparus* of cattle origin were obtained and handled as described for the first study.

On 29 September 1998 each 16-mo-old elk was captured in a drive net, restrained physically, and inoculated with 2,000 lungworm larvae as described for the first study. After inoculation, fecal samples were collected from each elk daily for 29 days, and then three times

weekly until larvae were no longer detected in feces. All fecal samples were collected by observing each elk defecate and then picking up numerous pellets (>16 g) from the ground after defecation. One elk was euthanatized 37 days after inoculation for the purpose of recovering adult lungworms for identification. That elk was immobilized with 400 mg of xylazine (Moby Corporation, Shawnee Mission, Kansas, USA) and then euthanatized with a .25 caliber captive bolt (Cash Special, Accles and Shelvoke LTD, Birmingham, UK.) to the head. Standard necropsy procedures were performed on the elk with emphasis on parasite recovery from the lungs. Lungs were removed intact, filled with water and the water was then decanted into shallow containers to recover parasites. The procedure was repeated four times. Trachea, bronchi and all major air passages were opened with a scissors, examined grossly for parasites and washed several times in shallow containers. All visible parasites were removed from lungs and washings with a forceps and placed in 10% formalin. After decanting excess water from the lung washings, all sediment was examined under a dissecting microscope at 30 $\times$ , and all parasites were placed in 10% formalin. Parasites were identified by descriptions published by Soulsby (1965). Representative samples of lung, mediastinal lymph nodes, brain, liver, kidney, spleen, cardiac and skeletal muscle, and adrenal glands, were preserved in 10% formalin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin for histopathological evaluation. On day 85 after inoculation, when larvae were no longer detected in feces, the remaining three yearling elk were euthanatized and evaluated grossly and histopathologically as indicated previously.

## RESULTS

Larvae of *D. viviparus* were not detected in the feces of the three 9-mo-old elk in the first study on the day of inoculation (day 0), or on postinoculation days 83 and 111. Larvae were detected in feces of each elk between postinoculation days 25 and 49 (Table 1). Numbers of larvae per gram of feces (lpg) ranged from 3/ to 101/g of feces (Table 1). Mean ( $\pm$ SE) size of the larvae ( $n$  = 15 larvae, 5 larvae per elk) was 377 ( $\pm$ 6.8)  $\mu$ m long  $\times$  20 ( $\pm$ 0)  $\mu$ m wide. Other parasite eggs and oocysts detected in low numbers in the feces were *Capillaria* sp. ( $n$  = 3 elk), *Trichuris* sp. ( $n$  = 2 elk), *Nematodirus* sp. ( $n$  = 3 elk), and *Ei-*

TABLE 1. Summary of results from seven elk inoculated with *Dictyocaulus viviparus* from cattle.

Animal number	Age in months	Sex	Number of larvae given	Days after inoculation that larvae were detected	Day of peak larval output	Numbers of larvae <sup>a</sup> range (mean)
330	9	F	3,000	25–49	32	5–74 (35.1)
331	9	F	3,000	25–49	32	3–101 (27.5)
332	9	M	3,000	25–49	32	3–29 (14.0)
Y1	16	M	2,000	24–66	50	<1–12 (3.1)
Y2	16	M	2,000	23–66	36	<1–48 (18.0)
Y16 <sup>b</sup>	16	M	2,000	24–37 <sup>b</sup>	34 <sup>b</sup>	<1–6 (2.5) <sup>b</sup>
Y64	16	M	2,000	22–83	34	<1–15 (4.2)

<sup>a</sup> Numbers represent larvae per gram of feces.<sup>b</sup> Euthanatized on postinoculation day 37.

meria spp. ( $n = 1$  elk). Signs of respiratory disease were not observed in any elk during this experiment.

In the second study, lungworm larvae were not detected in feces of any yearling elk during the 6 mo acclimation period or daily for 21 days after inoculation. First stage larvae of *D. viviparus* were detected in the feces of one elk 22 days after inoculation, in two elk 23 days after inoculation, and in all four elk 24 days after inoculation. Therefore, the prepatent period of this cattle strain of *D. viviparus* in elk is 22 to 24 days. Larvae were consistently detected for 43, 44, and 62 days, respectively, after larval shedding began in three elk indicating a patent period of approximately 43 to 62 days. Numbers of larvae in feces ranged from 1 to 32 larvae per gram of feces with peak shedding occurring between day 32 and 50 days after inoculation (Table 1). Fifteen adult *D. viviparus* (USNPC accession no. 089002) were recovered from the lungs of elk Y16 that was euthanatized 37 days after inoculation. No lungworms were recovered from the three elk euthanatized 85 days after inoculation. Additional eggs and oocysts detected in feces throughout the study included low numbers of *Trichuris* sp., *Capillaria* sp., *Nematodirus* sp., *Eimeria* spp., and unidentified strongyle eggs.

Moderate coughing was noticed in all four yearling elk between 18 and 29 days

after inoculation, especially after running, but none of the elk were depressed, lacked energy, or had noticeably increased or strained respiration rates when resting. Based on our observations, *D. viviparus* in elk at the exposure levels used did not appear to adversely affect their health.

At necropsy, all four elk were in excellent body condition and gross lesions were not observed in any elk. Histopathologic lesions were similar in four lung sections from the one elk euthanatized during the patent period of *D. viviparus*, and indicated mild, multifocal eosinophilic and lymphocytic bronchiolitis with multifocal epithelial hyperplasia. Pulmonary septa were only mildly hypercellular due to occasional lymphocytes and eosinophils. Interlobular septa were occasionally widened by finely granular, intraalveolar hemorrhage and smaller amounts of accompanying edema. Scattered bronchioles had convoluted, hyperplastic epithelium extending into the lumens. Adult parasites, larvae, or embryonated eggs were not observed in the tissues. Lung sections from the three other elk had changes consistent with parasitism and were characterized by a moderate multifocal lymphocytic and eosinophilic peribronchitis. Adult parasites, larvae or eggs were not observed in histological sections. Mediastinal lymph nodes from all elk had increased numbers of follicles, and increased numbers of eosinophils were present in the medullary sinuses. In heart

and skeletal muscle sections, *Sarcocystis* sp. was present in all animals. Other tissues including brain, liver, kidney, spleen, and adrenal gland were considered histologically normal.

### DISCUSSION

Results from these studies indicated that *D. viviparus* of cattle origin matured in elk, passed larvae initially 22 to 24 days after inoculation and produced larvae consistently for a maximum of 62 days. The inoculum of 2,000 or 3,000 larvae per elk did not cause significant respiratory disease in any of the elk, although moderate coughing after exercise was evident in the four yearling elk between 18 and 29 days after inoculation. In a previous study, Presidente et al. (1972) inoculated one elk calf with 24,000 *D. viviparus* larvae of cattle origin. Although the prepatent period was not determined in that study, the infection became patent and the elk passed <7 lpg of feces between days 25 and 59 after inoculation. No clinical signs of infection were observed in that study. In our studies, 2,000 or 3,000 larvae per elk resulted in up to 101 lpg of feces, but <30 lpg was most common (Table 1). Based on our data, we conclude that *D. viviparus* of cattle origin can mature in elk and produce large numbers of larvae during the patent period. Free-ranging or captive elk that share pastures with lungworm-infected cattle likely can develop infections of *D. viviparus* from cattle. However, because of the high prevalence of *D. viviparus* observed in elk in the northwestern USA, and the isolation of many elk herds from infected cattle and other potential reservoir hosts of *D. viviparus*, it is also likely that infections in elk can result from ingestion of infective larvae of elk origin. Strain differences of *D. viviparus* originating from wild cervids or domestic livestock may also account for differences in pathogenicity. Using experimentally infected cattle, Corrigall et al. (1988) demonstrated that cattle infected with the red deer strain

of *D. viviparus* had milder infections than cattle infected with the cattle strain.

Although respiratory disease was not observed in the elk in this experiment, the pathogenicity of *D. viviparus* infection in free-ranging elk has not been well documented. In farmed elk, light to moderate infections (<20 adult parasites) are reported to have little effect on host health, but severe infections may be sufficient to block airways and cause death through asphyxiation (Haigh and Hudson, 1993). In cattle, the pathogenesis of *D. viviparus* is well documented, especially in cattle less than 1-yr-old, and includes coughing, increased respiratory rates, bronchitis, bronchiolitis, consolidation and collapse of lung lobes, secondary bacterial pneumonia and death (Breeze, 1985). Dictyocauliasis in farmed red deer (*C. elaphus elaphus*) is a relatively common infection. In New Zealand, recent surveys in weaner red deer have indicated a prevalence of 8% (Audige et al., 1998). Numbers of *D. viviparus* larvae in feces were reduced with age in red deer, suggesting a resistance to lungworm disease during the first year of life (Audige et al., 1998). Our results which indicated limited pathogenicity following administration of 2,000 or 3,000 larvae in the seven elk agree with the one elk inoculated with 24,000 larvae of bovine origin (Presidente et al., 1972), and with reports regarding experimental infections in red deer. Studies in three 6-mo-old red deer inoculated with 500 *D. viviparus* larvae per kg of body weight (no red deer weights given) for 17 consecutive days indicated that infected red deer had reduced food intakes and weight gains compared to one noninfected red deer, but that the infection was mild (Corrigall et al., 1982). The pathology in the infected red deer was different from the pathology observed in cattle in that alveolar epithelialisation was limited and hyaline membrane formation and interstitial emphysema were not observed. Their conclusions that young red deer readily acquire infection and are tolerant of infection support our findings in the present ex-



periment. However, additional reports of *D. viviparus* infections in red deer in New Zealand indicate that *D. viviparus* can cause high mortality in farmed red deer (Charleston, 1980). Therefore, additional experimental work using elk of different ages, nutritional status, numbers of larvae administered, and strains of *D. viviparus* will clarify whether lungworm burdens in free-ranging and farmed elk can potentially result in parasitic bronchitis, pneumonia and death. However, based on field and experimental data, *D. viviparus* alone does not generally seem to be an important pathogen in free-ranging or captive elk unless other predisposing factors are present.

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