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Authors: Foreyt, William J., and Lagerquist, John E.

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Experimental Infections of *Eimeria wapiti* and *E. zuernii*-like Oocysts in Rocky Mountain Elk (*Cervus elaphus*) Calves

William J. Foreyt and John E. Lagerquist, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164, USA

ABSTRACT: Four Rocky Mountain elk (*Cervus elaphus*) 10 to 14 wk of age each were inoculated orally with a mixture of 50,000 sporulated oocysts of an *Eimeria zuernii*-like apicomplexan (70%) and *E. wapiti* (30%). Maximum numbers of oocysts per gram of feces (OPG) in each elk ranged from 985 to 15,517, but all calves remained healthy and clinical signs of coccidiosis were not observed. The prepatent period for *E. zuernii* was 8 days and the patent period approximately 37 days, with a maximum mean (±SE) recovery of 6,643 (±3,756) OPG on postinoculation day 8. The prepatent period for *E. wapiti* was 10 to 12 days and the patent period approximately 8 days, with a maximum mean recovery of 4,408 (±2,308) OPG on postinoculation day 12. Based on these data, infections of *E. zuernii* and *E. wapiti* at the numbers given were not pathogenic in healthy elk calves.

Key words: Elk, *Cervus elaphus*, *Eimeria zuernii*, *Eimeria wapiti*, coccidia, experimental study.

Coccidia of the genus *Eimeria* are obligatory protozoan parasites commonly detected in most species of ruminants. Infections of coccidia in domestic livestock can result in morbidity and mortality, particularly in young animals, with the greatest damage occurring in the epithelium of the small intestine. Clinical signs of coccidiosis include diarrhea, dehydration, loss of appetite, weight loss, general weakness and death (Fernando, 1982).

Three species of *Eimeria*, *E. wapiti*, *E. wassilewskii* and *E. zuernii*, have been identified in elk (*Cervus elaphus*) in North America (Haigh and Hudson, 1993). An unidentified eimeriid also was found in the epididymis of a 2-yr-old elk by Hrudka et al. (1983). Previous reports of coccidial infections in elk have been limited primarily to species descriptions and geographic locations of oocyst recoveries (Honess, 1955; Jolley, 1982); controlled experimental infections of elk have not been reported. Our objectives were to determine the prepatent and patent periods, and to evaluate the pathogenicity of *E. wapiti* and *E. zuernii*-like apicomplexan in experimentally infected elk calves.

Seven Rocky Mountain elk calves were used: six males and one female. During June 1993, five male calves 3 to 12 days old were obtained from the U.S. Forest Service Starkey Experimental Research Project near La Grande, Oregon (USA) (45°35′N, 118°10′W), and one 14-day-old female calf was obtained from a zoo in Spokane, Washington (USA) (47°40′N, 117°40′W). In July 1993, a male calf <7-days-old was obtained from the Washington Department of Wildlife near Wenatchee, Washington (47°25′N, 120°35′W). All seven calves were brought to Washington State University, Pullman, Washington, and housed in a facility with a concrete floor and which allowed free access indoors or outdoors. Alfalfa hay and water were provided, and straw for bedding was placed in the indoor area. The calves were bottle-raised on a commercial milk replacer (Merricks Kid Replacer, Middleton, Wisconsin, USA) until released in a 2 ha pasture at 2 mo of age. Alfalfa hay, alfalfa pellets, mineralized salt and water were available at all times.

The experimental coccidia inoculum was prepared from feces obtained from naturally infected captive 8 to 10-wk-old elk calves on an elk ranch near Moscow, Idaho (USA) (46°40′N, 117°W). The calves had no known contact with other ungulates. Oocysts were concentrated by mixing the fecal pellets in a container of tap water and filtering the mixture through two sieves

with openings of 500 and 250 μm, respectively. This sediment was mixed with 2% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$), placed in a 4 l flask, aerated with surgical tubing attached to an air supply valve, and kept at 21 C for 20 days, at which time more than 80% of the oocysts had sporulated as determined by microscopic evaluation. Oocysts were concentrated and the potassium dichromate removed from the inoculum by placing the fecal suspension in 800 ml glass beakers, allowing the oocysts to settle for at least 1 hr, decanting two-thirds of the supernatant fluid and replacing this with fresh tap water. This procedure was repeated six times until the supernatant was clear. Based on direct microscopic observation of the size and morphology of approximately 300 oocysts, the inoculum contained approximately 70% *E. zuernii*-like apicomplexan (hereafter referred to as *E. zuernii senso lato*) and 30% *E. wapiti* sporulated oocysts.

On the day of inoculation, four elk calves (numbers 1 to 4) each received approximately 50,000 sporulated oocysts administered orally with a dosing syringe, and three calves (numbers 5 to 7) were uninoculated controls. All calves were approximately 14-wk-old on experimental day 0, except calf number 4, which was 10-wk-old. Initial fecal samples were collected from the rectum on day 0, and additional fecal samples were collected at varying intervals from 6 to 45 days postinoculation (PI) (Table 1) by observing calves defecate and immediately collecting the feces from the ground. One gram of feces from each fecal sample was examined microscopically for the presence and numbers of coccidial oocysts by a sugar flotation technique (specific gravity = 1.27) (Foreyt, 1986). Oocysts were viewed at 400× and measured with an ocular micrometer (Baxter Healthcare Corporation, Redmond, Washington). Species were identified on the basis of morphology and size (Jolley, 1982). All calves were observed daily for signs of disease, and weighed on days 2 and 58 PI to detect differences in weight gain between inoculated and control groups.

TABLE 1. Mean and range of *Eimeria wapiti* and *E. zuernii*[a] oocysts detected per gram of feces from four elk calves experimentally inoculated with 50,000 sporulated oocysts.

Days post-inoculation	*E. wapiti*		*E. zuernii*[a]	
	$\bar{x}$	Range	$\bar{x}$	Range
0	0	0	2	0–3
6	3	0–11	4	0–10
8	0	0	6,643	174–15,517
10	87	0–271	149	90–214
12	4,406	77–9,533	3,003	643–5,544
13	1,231	706–1,581	212	8–352
14	1,018	256–2,059	216	108–345
15	279	123–607	137	44–287
16	16	0–52	89	18–197
18	1	0–1	12	0–26
20	0	0	29	0–110
22	0	0	18	0–36
24	0	0	7	0–20
27	0	0	8	2–14
29	0	0	17	0–42
31	0	0	24	8–48
38	0	0	11	0–18
45	0	0	3	0–6

[a] *Eimeria zuernii*-like apicomplexan protozoan.

On experimental days 0 and 6, coccidial oocysts were detected in the feces of all seven elk, with <20 oocysts per gram of feces (OPG) in both the inoculated and the uninoculated elk (Table 1). On day 8 PI, markedly increased numbers of *E. zuernii* were recovered from feces of all four inoculated calves ($\bar{x}$ ± SE = 6,643 ± 3,756, range = 174 to 15,517 OPG). Recovery of *E. zuernii* oocysts from feces decreased steadily once peak oocyst production was attained on day 8 for two calves ($\bar{x}$ = 12,871 OPG), and on day 12 for two calves ($\bar{x}$ = 668 OPG), although low numbers of oocysts continued to persist in feces of the inoculated calves through day 38 PI (calves 2 and 3) or day 45 PI (calves 1 and 4), the last day fecal samples were collected. The mean (±SE) size of 50 unsporulated oocysts of *E. zuernii* (Fig. 1) was 19.7 μm ± 0.2 × 14.7 μm ± 0.2. *Eimeria wapiti* oocysts (Fig. 1) were detected in the feces of three calves on day 10 PI ($\bar{x}$ = 116 OPG),

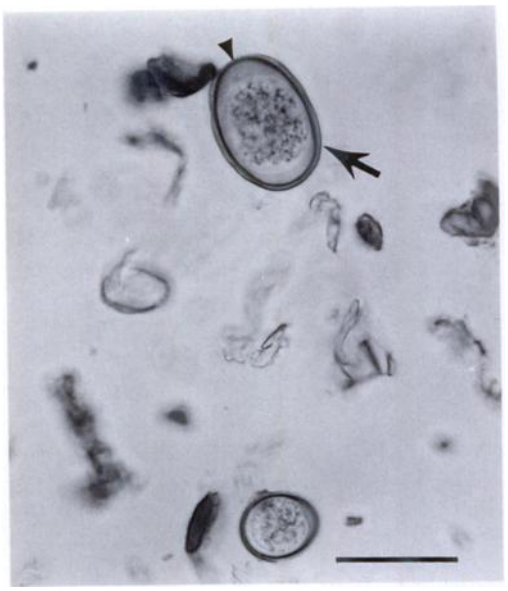

FIGURE 1. Unsporulated oocysts of *Eimeria wapiti* (arrow) and *Eimeria zuernii* from elk (*Cervus elaphus*) in this study. Arrowhead indicates micropyle on *E. wapiti*. Bar = 30 μm.

and calf 3 on day 12 PI (964 OPG). Maximum mean (±SE) number of OPG of *E. wapiti* (4,408 ± 2,308) from inoculated calves occurred on day 12 PI. Presence of *E. wapiti* oocysts in feces decreased rapidly in all inoculated calves; no oocysts were detected beyond day 18 PI. Mean (±SE) size of 50 unsporulated oocysts of *E. wapiti* was 33.4 μm ± 0.2 × 24.0 μm ± 0.2. An 8 day prepatent period for *E. zuernii* was determined with a patent period of approximately 37 days. The prepatent period for *E. wapiti* was approximately 10 to 12 days, and the patent period approximately 8 days. The patent period of *E. wapiti* was short and definitive, in contrast to the prolonged patent period of *E. zuernii* in which low level oocyst production continued for 37 days in two of the inoculated elk.

Clinical signs of disease were not observed in the inoculated calves throughout the experiment. Fecal samples remained firm and pelleted, with no diarrhea or blood detected. Low numbers of coccidial oocysts ($\bar{x}$ ± SE = 8.5 ± 2.4 OPG) which morphologically were the same species as in the inoculum were detected in feces of uninoculated control elk throughout the study. Numbers of oocysts exceeded 20 OPG on only four occasions (30, 34, 48, and 95 OPG). Mean (±SE) weights of the inoculated and uninoculated groups were 76.4 (±11) and 74.5 (±7) kg on PI day 2, and 80.0 (±9) and 80.9 (±11) kg on PI day 58, respectively.

The lack of clinical signs of coccidiosis in this study corresponds with previous observations of coccidia in elk (Jolley, 1982; Haigh and Hudson, 1993), and experimentally infected white-tailed deer (*Odocoileus virginianus*) (Conlogue and Foreyt, 1984). Coccidiosis in cattle is characterized by diarrhea which may contain blood, emaciation, dehydration, weakness, and death in severe cases (Ernst and Benz, 1986), and usually is associated with overcrowding, stress, and fecal contamination of feed or water sources. These conditions are less likely to be encountered by free-ranging elk, and it is unlikely free-ranging elk would become exposed to the high number of oocysts the elk calves received in this experiment. A prepatent period of 16 to 17 days has been reported for *E. zuernii* in cattle (Ernst and Benz, 1986), which greatly exceeds the 8 day prepatent period for *E. zuernii* we observed in this experiment. *Eimeria* spp. exhibit a high degree of host specificity, and it is uncommon for a species of *Eimeria* to infect more than one host genus (Joyner, 1982). It is possible that *E. zuernii* of elk origin is uniquely adapted in elk and therefore may have some different characteristics, such as a shorter prepatent period than in cattle, or the organism only resembles *E. zuernii* morphologically, but is a different species. Detailed morphological study and cross-transmission studies are needed to definitively determine the taxonomic status of this coccidian.

Mean size of unsporulated oocysts of *E. zuernii* in this study was similar to previous descriptions in elk (Jolley, 1982; Levine and Ivens, 1970), but the mean size of 33.4 × 24.0 μm of unsporulated *E. wap-*

iti oocysts we report was somewhat smaller than the 38.2 × 26.3 μm reported by Levine and Ivens (1970). Oocyst size is dependent on the stage of patency, the number of oocysts present within the host, and the individual animal infected (Joyner and Long, 1974). In our study, unsporulated oocysts were measured from fecal samples within 48 hr of collection.

That low numbers of coccidial oocysts were detected in the feces of all seven calves on experimental day 0 or 6 was of no probable consequence to the experiment. Recovery of oocysts from feces of the uninoculated control calves throughout the experiment remained very low or were absent ($\bar{x} \pm SE = 8.5 \pm 2.4$ OPG), compared with the uniform increase and decline of oocyst production among the four inoculated calves. The relatively low recovery of *E. zuernii* oocysts in all four inoculated calves on day 10 PI is unexplained.

We report the experimentally determined prepatent and patent periods for *E. zuernii* and *E. wapiti* in elk. Experimental *E. zuernii* and *E. wapiti* infections in these 10 to 14 wk old elk calves were asymptomatic; clinical signs of coccidiosis were not observed in any of the calves. No differences were observed in fecal consistency or health status between the inoculated and uninoculated calves. The importance of coccidia in wild elk populations has not been determined, but based on our data, we believe that *E. zuernii* and *E. wapiti* at the concentrations given are not pathogenic in healthy elk >10 wk old.

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