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## YERSINAE ISOLATED FROM WAPITI (*Cervus canadensis roosevelti*)

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**Abstract:** *Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria were isolated from 22 of 90 fecal samples taken from five herds of wapiti studied in northwestern California. The serotypes included: (5), (6), (11), (16), (2,19), (4,16) and (6,15). In one herd, all of the organisms were isolated from within a one hectare area. A significantly higher ( $p < .02$ ) prevalence of isolations was obtained during April and May.

### INTRODUCTION

*Yersinia enterocolitica* is a gram negative, rod-shaped bacterium isolated from several birds and mammals.<sup>2,7,8</sup> It is found mainly in the northern hemisphere and generally north of the 20th parallel.<sup>7</sup>

This study was conducted in Prairie Creek State Park, Orick, California, where a wapiti (*Cervus canadensis roosevelti*) population has been protected from predation and hunting. The purpose of this study was to survey the wapiti for yersiniae and to determine the effects of rainfall, habitat, and movements on the frequency of isolation from the different herds.

### STUDY AREA

Prairie Creek State Park is a 4950 ha portion of land in the northwest corner of Humboldt County, California, along the Pacific Ocean. A large percentage of the park is composed of old-growth redwood forest with the majority of the surrounding area consisting of cut-over redwood forest. More detailed descriptions of the study area have been reported previously.<sup>3,5</sup> The wapiti were distributed among five herds: North Beach, South Beach, Gold Bluffs Beach, West Ridge, and Boyes Prairie.

### MATERIALS AND METHODS

Wapiti were observed until they defecated. A sample of feces from the center of the pile was aseptically collected and stored at 4 C in 10 ml of Trypticase Soy Broth<sup>2</sup> with 0.5% yeast extract. The date, herd, sex, age, and identification of the wapiti sample were noted. A more extensive explanation of the sampling methods was reported previously.<sup>5</sup>

Approximately 0.5 ml of the original fecal suspension was incubated at 37 C for 3 hr in 5 ml of Trypticase Soy Broth<sup>2</sup> with 0.5% yeast extract. MacConkey Agar,<sup>3</sup> Trypticase Soy Agar,<sup>2</sup> and SS Agar<sup>3</sup> were then used for initial isolations. The colony types present on all of the media were sampled after 24 hr and 48 hr incubation at 37 C; all isolates were characterized by techniques outlined in Cowan and Steel.<sup>1</sup> Generally, 1% concentrations of filter-sterilized carbohydrates in Purple Broth Base<sup>3</sup> were used, except that a 0.5% concentration of soluble starch autoclaved 10 min at 116 C was used.

A selection of the potential *Yersinia* sp. was sent to Drs. Thomas Quan and Bruce Hudson, U.S. Public Health Service, Center for Disease Control, Bureau of Laboratories, Fort Collins, Colorado

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for serotyping tests. Isolates were evaluated with antisera to *Y. enterocolitica* serotypes 1 to 21, 24, 32, and the new, undesignated types Arizona and Tacoma, as well as with antisera to *Y. pseudotuberculosis* serotypes I through VI.

#### RESULTS

Ninety samples were collected between April and November, 1972: twenty-four were collected from the Gold Bluffs Beach herd, 27 from the Boyes Prairie herd, and 13 from each of the other three

herds. *Y. enterocolitica* and *Y. enterocolitica*-like strains were isolated from 22 (24%) of the 90 fecal samples taken. All of the isolates were gram negative rods motile at 21 C but not at 37 C. All were urease positive, catalase positive, cytochrome oxidase negative, and methyl-red positive. All isolates reduced nitrates to nitrates, but none produced H<sub>2</sub>S on Kligler Iron Agar,<sup>3</sup> or possessed phenylalanine deaminase. Reactions varied with the acetylmethylcarbinol and indole tests (Table 1).

TABLE 1. Variation in *Yersinia* isolates.

Samples	Serotypes	Herd	Lactose	Rhamnose	Sucrose	Acetylmethylcarbinol	Indole	Soluble Starch
15,63, 69	(5) (5)	P	—	—	+	—	+	+
58, 72	(5) (6,15)	SB	—	—	+	—	+	+
3		P	—	—	+	+	+	+
9		B	—	—	+	+	+	+
35		NB	—	—	+	+	+	+
26,33	(2,19) (6)	WR	—	—	+	—	—	+
15,16		P	—	—	+	—	—	+
69	(6)	P	—	—	+	—	+	—
4,6,63	(ns)	P	+	—	+	+	+	+
30	(ns)	B	+	—	+	+	+	+
16,69	(6)	P	+	—	+	—	+	+
44	(6)	NB	+	—	+	—	+	+
72		SB	+	—	+	—	—	+
19,28	(16) (11)	SB	+	+	+	+	+	+
23,24		NB	+	+	+	+	+	+
63		P	+	+	+	—	+	—
45,73	(4,16)	NB	+	+	+	—	+	+
7		B	—	+	+	—	+	+
28		SB	+	+	—	—	+	+

P—Boyes Prairie Herd  
B—Gold Bluffs Beach Herd  
NB—North Beach Herd  
— —Negative Reaction  
( )—Serotype  
(ns)—Non-serotypable

SB—South Beach Herd  
WR—West Ridge Herd  
+ —Positive Reaction  
— —Sample for which Serotype was obtained

Based on the Glucose O-F test, all isolates were fermenters. They all acidified glucose (without gas), sorbitol, mannitol, and glycerol in 14 days. None of the isolates acidified inulin in 14 days. The organisms varied in their ability to break down lactose, rhamnose, sucrose, and soluble starch (Table 1).

Four isolates were serotype 6, three isolates were serotype 5, one was serotype 11, and one was serotype 16 (Table 1). One isolate was serotype 6,15; one was serotype 2,19 and one was 4,16. Two isolates were not serotypable; these are designated as *Y. enterocolitica*-like. None of the isolates were checked for pathogenicity.

In one instance yersiniae were isolated from the same wapiti twice. This was an 8 year-old cow from the Boyes Prairie herd. A non-serotypable strain was isolated in April (sample 4) and a serotype 5 strain was isolated in August (sample 63). These strains were also biochemically different (Table 1).

In the Boyes Prairie herd, 7 of the 16 samples taken from a one ha area at the north end of the prairie had yersiniae. This area was adjacent to the park entrance and headquarters and, in general, had the most exposure to humans. No yersiniae were isolated from 11 samples taken from the other 70 ha of Boyes Prairie. Using a chi-square test, this difference was significant ( $p < .01$ ).

Yersiniae were recovered from samples taken during all months of the study. However, a significantly ( $p < .02$ ) higher prevalence of isolations occurred in April and May; yersiniae were isolated from 11 of 25 samples taken in April and May, but only from 11 of 65 samples taken during the remainder of the study.

There were no significant differences in

yersiniae isolation rates among the five herds. The rates of isolation were: Boyes Prairie—26%, Gold Bluffs Beach—13%, North Beach—38%, South Beach—31%, and West Ridge—23%. These data suggest that yersiniae are present throughout the park area and are available to all of the wapiti present.

#### DISCUSSION

Yersiniae were isolated from 24% of the samples taken. In only one instance were they isolated from the same wapiti twice. In this instance, the isolates were biochemically and serologically different. Thus, the current evidence suggests that while wapiti may regularly ingest these bacteria, they are not reservoirs of yersiniae. However, the occurrence of yersiniae in the gastro-intestinal tract of wapiti suggests that food or water is the source.

During the study, rainfall in excess of 10 cm occurred only in April (13.7 cm) and November (19.0 cm). Most yersiniae were isolated in April or May. The higher rate of yersiniae isolations at this time may have resulted from the presence of the bacteria in the water following the rains. The isolation of yersiniae directly from water in other studies<sup>4,6</sup> suggests that water or soil may be a reservoir. No wapiti were sampled after the rains began in November to further test this hypothesis.

In animals a *Y. enterocolitica* infection may progress rapidly, involving several of the internal organs and resulting in death.<sup>7</sup> However, in this study all of the animals from which yersiniae were isolated appeared healthy. At this time there is no evidence that *Y. enterocolitica* has a significant effect on the wapiti population at Prairie Creek State Park.

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