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Authors: MARTYNY, J. W., and BOTZLER, R. G.

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## *Listeria monocytogenes* ISOLATED FROM WAPITI (*Cervus canadensis roosevelti*)

J. W. MARTYNY<sup>□</sup> and R. G. BOTZLER, School of Natural Resources,  
Humboldt State University, Arcata, California 95521, U.S.A.

**Abstract:** Serotypes 1 and 4 *Listeria monocytogenes* were isolated from 14 of 72 wapiti among four of five herds studied in northwestern California. More isolations of *Listeria* were made during the summer months than during the other months of the study from one of the herds. The pathogenicity of the strains varied with their ability to ferment xylose and rhamnose.

### INTRODUCTION

*Listeria monocytogenes* is a gram positive rod-shaped bacterium that has been isolated from many species of mammals and birds as well as aquatic animals, soil, silage, and water.<sup>3,5</sup>

The study was conducted in Prairie Creek State Park, Orick, California where a wapiti population has been protected from predation and hunting. The purpose of this study was to survey the wapiti for *L. monocytogenes* 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 4,950 hectare portion of land in the northwest corner of Humboldt County, California, along the Pacific Ocean. The climate of the area consists of wet winters and dry summers. The major vegetation is coastal redwood (*Sequoia sempervirens*), Douglas fir (*Pseudotsuga menziesii*), Sitka spruce (*Picea sitchensis*) and red alder (*Alnus rubra*) with understories of evergreen huckleberry (*Vaccinium ova-tum*), salmonberry (*Rubus spectabilis*),

salal (*Gautheria shallon*) and swordfern (*Polystichum munitum*). A large percentage of the park is composed of mature redwood with the majority of the surrounding area consisting of cut-over redwood.

The wapiti studied were distributed among five herds. The North Beach herd and the Gold Bluffs Beach herd were mainly in grassy areas between the ocean and the redwood forest. The South Beach herd and the West Ridge herd spent most of their time in cut-over redwood habitat. The Boyes Prairie herd occupied a large prairie habitat in the south-central portion of the park. Two of the herds (the Gold Bluffs Beach herd and the Boyes Prairie herd) are semi-tame and can be approached on foot whereas the other wapiti are less accustomed to man. A more complete description of the study area is given by Martyny.<sup>8</sup>

### MATERIALS AND METHODS

#### Field Collections

The wapiti were observed until they defecated. A sample of feces was collected and stored at 4 C for at least 6 months in 10 ml of Trypticase Soy

<sup>□</sup> Current address: Tri-County District Health Dept., 4301 E. 72nd Ave., Commerce City, Colo. 80022, U.S.A.

Broth<sup>2</sup> with 0.5% yeast extract. The date, herd, sex, age and identification of the elk sampled was recorded.

Four samples were taken each month from the Gold Bluffs Beach herd and the Boyes Prairie herd; two wapiti from each herd (a male and a female) were repeatedly sampled throughout the study; the remaining two samples were taken from different animals each month. Two samples per month were taken from the other three herds.

#### Listeria Isolations

One ml of the original fecal suspension was overlaid onto 9 ml of Tryptose Broth<sup>3</sup> with 3.75% potassium thiocyanate and incubated at 21 C. After 48 hours a loopful was streaked for isolation on Tryptose Agar<sup>3</sup> plates. The plates were examined at 24 and 48 hours under a Henry light to detect potential *Listeria* colonies.<sup>4</sup>

#### Biochemical Characteristics

All motile, gram positive, catalase positive, and oxidase negative isolates were characterized by methods outlined in Cowan and Steel.<sup>2</sup> Generally we used 1% concentrations of filter-sterilized carbohydrates in Purple Broth Base,<sup>3</sup> except for a 0.5% concentration of soluble starch autoclaved 10 min at 116 C.

#### Serological Characteristics

The isolates were serotyped by W. C. Eveland at The University of Michigan using a direct fluorescein-labelled antibody method.<sup>7</sup>

#### Pathogenicity to Mice

Pathogenicity was determined by injecting 0.1 ml of an 18 hour culture of 10<sup>8</sup> organisms intraperitoneally into white laboratory mice. Deaths were recorded

over a 10 day period. Attempts were made to isolate *Listeria* from the heart blood of the dead mice.

#### RESULTS

A total of 90 samples were collected from 72 wapiti between April and November, 1972. Twenty-four samples were collected from the Gold Bluffs Beach herd, 27 from the Boyes Prairie herd, and 13 from each of the other three herds.

*Listeria* was isolated from 15 (17%) of the 90 fecal samples taken, and 14 (19%) of the 72 wapiti sampled. All of the isolates were gram positive, oxidase negative, and motile at 21 C. All were methyl red positive, weakly acetymethyl-carbinol positive, and produced acid slants and acid butts on Kilgler's Iron Agar<sup>3</sup> with no production of H<sub>2</sub>S or gas within 24 hours. None of the isolates reduced nitrate to nitrite, utilized citrate, or produced indole or urease.

Based on the O-F test, all of the isolates were fermentative and acidified glucose between 2 to 9 days. All acidified lactose, sucrose, soluble starch, and melezitose within 14 days. None of the isolates acidified inositol within 14 days. Variable results were obtained in xylose, rhamnose, sorbitol, and mannitol (Table 1).

Based on pathogenicity and biochemical reactions, two major groups were found. Among the isolates tested for pathogenicity those that were xylose positive and rhamnose negative (3b, 29a, 61a, 88c) were nonpathogenic to mice. Those isolates that were xylose negative and rhamnose positive (37d, 39a, 48c, 39d) were pathogenic to mice. This pattern was also found in the strains isolated from the George Reserve in Michigan.<sup>1</sup> All of the pathogenic strains killed mice within 48 hours. *Listeria* was consistently recovered from the heart blood of the dead mice.

<sup>2</sup> BBL Laboratories, Baltimore, Maryland.

<sup>3</sup> Difco Laboratories, Detroit, Michigan.

TABLE 1. Variation among *Listeria* isolates.

Isolates	Herd	Serotypes	Xylose	Rhamnose	Sorbitol	Mannitol
3 <i>a,b,c</i>	Prairie	4	+	—	—	—
29 <i>a,b</i>	Beach	1	+	—	—	—
31 <i>b</i>	Beach	ND	+	—	—	—
51 <i>b,c,d</i>	Beach	*	+	—	—	—
61 <i>a,b,c,d</i>	Beach	1,4	+	—	—	—
62 <i>a,b,c,d</i>	Beach	4	+	—	—	—
88 <i>a,b,c</i>	S. Beach	1,1	+	—	—	—
91 <i>b</i>	N. Beach	1	+	—	—	—
91 <i>a,c</i>	N. Beach	1	+	—	—	+
29 <i>c</i>	Beach	ND	+	—	+	—
31 <i>a,c</i>	Beach	1	+	—	+	—
51 <i>a</i>	Beach	1	+	—	+	—
44 <i>a,b,c,d</i>	N. Beach	1,*	+	—	+	—
57 <i>a,b,c</i>	S. Beach	1,1	+	—	+	—
37 <i>a,b,c,d</i>	Prairie	4,4	—	+	+	—
39 <i>a,b,c</i>	Prairie	*	—	+	+	—
83 <i>a,b</i>	Prairie	1	—	+	+	—
48 <i>a,b,c,d</i>	Beach	4	—	+	+	—
86 <i>a,b,c,d</i>	Beach	4	—	+	+	—
39 <i>d</i>	Prairie	ND	—	+	—	—

Prairie — Boyes Prairies Herd

Beach — Gold Bluffs Beach Herd

N. Beach — North Beach Herd

S. Beach — South Beach Herd

+: positive reaction

—: negative reaction

ND: not serotyped

\*: equally strong reaction with type 1 and 4 antisera

italic letters: Isolates serotyped

Serologically the isolates fell into two groups: serotypes 1 and 4. Each serotype was represented in both the pathogenic and the nonpathogenic groups (Table 1).

From one adult cow wapiti from the Gold Bluffs Beach herd, both serotypes were recovered in the August sampling; isolate 61*a* was serotype 1 and isolate 61*c* was serotype 4. Biochemically, no difference was found between these isolates. *Listeria* was also recovered from the same animal in the June sampling (isolates 29*a, b, c*); isolate 29*a* was serotype 1. Isolate 29*c* also differed biochemically from the August isolates in that it

acidified sorbitol. No *Listeria* were isolated from this wapiti during the May or July sampling. This wapiti was the only one of the four repeatedly sampled which yielded *Listeria* more than once.

Two isolates acidified mannitol (Table 1). Isolate 91*a* was serotyped and is serotype 1. To our knowledge, this has been reported from only one other strain of *Listeria*.<sup>6</sup> That strain was isolated from chinchilla feces and differed from our strain in its inability to acidify sucrose, xylose, and melezitose. It seems that the inability to ferment mannitol cannot be held as a key characteristic of *Listeria*.

Listeriae were isolated from four of the five herds sampled. The isolation rates were: Boyes Prairie—15%, Gold Bluffs Beach—29%, North Beach—15%, and South Beach—14% (Table 2). No listeriae were recovered from the West Ridge herd; however, the organisms may have been simply overlooked. None of the herds had a significant difference in the rate of isolation when compared to the other herds sampled. These results suggest that *Listeria* is present and available to all of the wapiti present on the area.

The rate of isolation was correlated to season in the Gold Bluffs Beach herd. Using a chi square test, more *Listeria* were isolated in the summer (June, July, and August) than the other months of the study ( $P < .10$ ). During this time the rainfall was minimal (2.8 cm total for the 3 months) and free water was limited. The temperatures were the highest during these months, ranging from a mean daily midrange of 13 C in June to 16 C in August.

TABLE 2. Isolation rates of listeriae by month from each herd.

Month	# of listeriae isolates/# of samples					Total
	P	B	SB	NB	WR	
April	1/6	0/0	0/0	0/0	0/0	1/6
May	0/4	0/7	0/3	0/3	0/2	0/19
June	2/4	2/4	0/2	0/2	0/2	4/14
July	0/5	2/4	0/2	1/2	0/3	3/16
August	0/3	2/3	1/2	0/2	0/2	3/12
September	0/1	0/1	0/2	0/2	0/2	0/8
October	1/4	0/3	0/0	0/0	0/0	1/7
November	0/0	1/2	1/2	1/2	0/2	3/8
Total	4/27	7/24	2/13	2/13	0/13	15/90

P — Boyes Prairie Herd  
B — Gold Bluffs Beach Herd  
NB — North Beach Herd

SB — South Beach Herd  
WR — West Ridge Herd

## DISCUSSION

There is little evidence that the wapiti are reservoirs for *Listeria* at Prairie Creek State Park. Only one of the four animals sampled repeatedly for *Listeria* was ever positive more than once, and then only on an irregular basis.

The higher isolation rate for the Gold Bluffs Beach herd during the summer months could be due to a reduction in the number of watering areas, resulting in a higher concentration of animals in each area. Botzler et al.<sup>1</sup> found that aquatic habitats frequented by deer had

higher isolation rates than other areas sampled; the presence of *Listeria* in aquatic animals suggests that it can be directly acquired from the water. If this is true, then a reduction in the number of the watering areas could result in the wapiti frequenting fewer areas; the slow runoff would not wash the bacteria out of these areas as fast and result in higher isolation rates in the animals.

All of the animals studied appeared to be healthy. At this time there is no evidence to indicate that *Listeria* has a significant effect on the wapiti population at Prairie Creek State Park.

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