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RATE OF *Listeria monocytogenes* SHEDDING FROM FROGS^{[1][2]}

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Abstract: Leopard frogs (*Rana pipiens*) were orally inoculated with *Listeria monocytogenes*. Most listeriae were shed 3-6 days after inoculation. The evidence indicated that the listeriae did not multiply in frogs and were unable to become established in the gastrointestinal tract.

INTRODUCTION

The isolation of *Listeria monocytogenes* from crustaceans,⁶ fish^{5,7} and frogs^{8,9} is evidence that aquatic animals carry listeriae. Further, the high prevalence of listeriae in leopard frogs (*Rana pipiens*)³ on the Edwin S. George Reserve in Michigan suggested that they might be reservoirs of the bacteria. The objective of this study was to determine the rate and period of time that orally infected leopard frogs shed listeriae.

MATERIALS AND METHODS

Eight male leopard frogs were captured at Whitmore Lake, about 24 km east of the George Reserve in October, 1969, and stored at the Amphibian Facility of The University of Michigan.⁴ At the time of this experiment in February, 1970, the frogs weighed 25-37 g and appeared to be 1-2 years old. During the experiment they were housed in plastic

cages approximately 16x14x10 cm and were fed house crickets (*Acheta domesticus*) at 3-4 day intervals.

The *Listeria* strain used (F28-71) was a Type 4b isolated from a leopard frog on the George Reserve.³ One-tenth ml containing 10^{8.3} washed listeriae was orally inoculated into the stomach of each frog through polyethylene tubing.

All cages were checked for listeriae on the day before inoculation (Day -1), the day of inoculation (Day 0), and the 10 days following inoculation of strain F28-71. Each day the waste water from each frog was collected and measured. Any other solid material in the cage was wiped up with a sterile cotton swab and added to the water sample. Ten ml of fresh sterile tapwater was added to the cage and each frog was replaced. The total listeriae population of each sample was determined by methods described previously.^{1,2} These figures were converted to the percentage of the original inoculum. On the 12th day after inocu-

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lation, the frogs were killed and samples of the heart blood, liver, spleen, testes, and contents of the lower colon were examined for the presence of listeriae.

RESULTS

The results are presented in Table 1. Because several dilutions of the waste water were required to obtain plates with discrete colonies, the percentage figures were sometimes based on plates with five

or less *Listeria* colonies. Two of the eight frogs never shed detectable levels of listeriae. None of the frogs shed a concentration as high as 1% of the original inoculum; the highest level was 0.77% from frog G. Only one frog (E) shed detectable levels with consistency; interestingly, this animal developed an edematous foot shortly after a toe was clipped at the beginning of the experiment. No listeriae were recovered from any of the tissues or animal products sampled on the 12th day.

TABLE 1. Recovery of *Listeria monocytogenes* in the feces of orally infected frogs, as a percentage of the original inoculum.

Frog	Day											
	-1	0	1	2	3	4	5	6	7	8	9	10
F	—	—	—	—	—	—	—	—	—	—	—	—
A	—	—	—	—	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	.04	—	—	—	—
C	—	—	—	—	.04	—	—	—	—	—	—	—
G	—	—	—	—	.77	—	—	—	—	—	.002	—
H	—	—	—	—	.02	.007	—	—	—	—	—	—
B	—	—	—	—	.11	.02	—	—	—	—	—	—
E	—	—	—	—	.10	.02	.02	.02	.02	—	.02	—

DISCUSSION

It is evident that listeriae inoculated into frogs are shed, and this shedding can be monitored. However, the evidence suggests that leopard frogs shed detectable numbers of listeriae irregularly and only for a short time (less than 10 days). There was no evidence of multipli-

cation: the numbers of listeriae observed from each frog were well below the inoculated dose. These data suggest that any listeriae ingested by frogs are passively shed without becoming established in the intestine. There is no evidence that frogs are reservoirs of listeriae on the George Reserve.

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