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Enterobacteriaceae and *Aeromonas hydrophila* in Minnesota Frogs and Tadpoles (*Rana pipiens*)

DAVID W. HIRD,[1]† STANLEY L. DIESCH,[1] ROBERT G. McKINNELL,[2] EVILLE GORHAM,[3] FRANK B. MARTIN,[4] CRAIG A. MEADOWS,[1] AND MARY GASIOROWSKI[1]

Departments of Large Animal Clinical Sciences,[1] Genetics and Cell Biology,[2] and Ecology and Behavioral Biology[3] and Statistical Center,[4] University of Minnesota, St. Paul, Minnesota 55108

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In 222 *Rana pipiens* frogs and 34 tadpoles captured in and near Minnesota, *Aeromonas hydrophila* and 29 species of *Enterobacteriaceae*, including *Yersinia enterocolitica* and *Salmonella arizonae*, were isolated from intestines. The prevalence of members of the family *Enterobacteriaceae* was lowest in frogs captured in early spring and highest in frogs captured in late summer.

Wild populations of the leopard frog, *Rana pipiens*, have declined dramatically in the upper Midwest and other parts of the United States in the last two decades (1, 9). Bacterial disease is among the several factors suggested to be responsible for the reduced frog populations (1). Redleg disease, attributed to *Aeromonas hydrophila*, is commonly reported in captive frogs in laboratories (8, 10), but its significance in wild populations is not known. Members of the family *Enterobacteriaceae* have been studied in some species of ill or captive frogs (3, 10, 15), but little information is available on their occurrence in wild populations of *R. pipiens*.

The present study was undertaken to provide additional information on the patterns of occurrence of *A. hydrophila* and on the species of *Enterobacteriaceae* occurring in wild populations of *R. pipiens* in and near Minnesota.

Collection. Methods for frog and tadpole collection and sampling were similar to those previously described (13). Frogs were collected from areas of relative abundance from April through October 1980, beginning with emergence from overwintering in lakes (early spring) and later from breeding ponds (spring), foraging sites in grassy habitats adjacent to streams and lakes (summer), and areas of entry to lakes for overwintering (fall). Tadpoles were collected from breeding ponds in late spring and early summer. Sixteen collecting trips were made to 11 sites: Westport Lake, Pope County, Minnesota; Cedar Lake, Scott County, Minnesota; Diamond Lake, Kandiyohi County, Minnesota; farm pond near Becker, Sherburne County, Minnesota; south of Litchfield, Meeker County, Minnesota; near Eagle River, Otter Tail County, Minnesota; Pomme de Terre River, 1 mile (ca. 1.6 km) southeast of Morris, Stevens County, Minnesota; Block Lake, Otter Tail County, Minnesota; northeast of Pine City, Pine County, Minnesota; Sand Hill River near Fertile, Polk County, Minnesota; and unnamed pothole lake in Burke County, North Dakota. Water samples were also collected from some sites.

Sampling and isolation. Heart blood (frogs) and intact 1.5- to 2.0-cm sections of the midgut (frogs and tadpoles) were removed aseptically and inoculated into culture media for *A. hydrophila* isolations as previously described (13). Direct smears were made of midgut contents onto MacConkey agar; the midgut contents were also subjected to enrichment procedures for salmonellae (inoculation into Selenite F broth and incubation at 43°C) and yersiniae (incubation in 1% mannitol at 6°C for 3 weeks). Biochemical tests were performed using the API 20E *Enterobacteriaceae* system (Analytab Products Inc., Plainview, N.Y.) for members of the *Enterobacteriaceae* (16); standard biochemical tests were used for the identification of *A. hydrophila* (6, 7). Isolates identified as *Yersinia enterocolitica* were confirmed and serotyped by Thomas J. Quan.

Findings in frogs. Dead or clinically ill frogs were rarely seen in the field, and nearly all specimens received in the laboratory appeared to be healthy as judged by movements, posture, and skin coloration and luster. A total of 492 enteric isolates was identified from 222 frogs (Table 1). In cases in which several isolations of the same bacterial species were made from an individual frog, those isolates were considered to be a single isolation.

Citrobacter freundii and *A. hydrophila* were the most common isolates; both were cultured

† Present address: Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616.

TABLE 1. Isolation frequency of *A. hydrophila* and of members of the family *Enterobacteriaceae* from intestines of *R. pipiens* frogs, by season and snout vent length

Organism	No. of positive/no. of samples (% positive)				
	Collected April–May		Collected June–October		Total
	≤59[a]	≥60	≤59	≥60	
Aeromonas hydrophila	9/16 (56.3)	10/38 (26.3)	49/97 (50.5)	34/71 (47.9)	102/222 (45.9)
Escherichia coli	0/16 (0.0)	1/38 (2.6)	37/97 (38.1)	28/71 (39.4)	66/222 (29.7)
Edwardsiella tarda	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	0/71 (0.0)	1/222 (0.5)
Citrobacter freundii	4/16 (25.0)	4/38 (10.5)	36/97 (37.1)	59/71 (83.0)	103/222 (46.4)
Citrobacter amalonaticus	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	0/71 (0.0)	1/222 (0.5)
Citrobacter sp.	0/16 (0.0)	0/38 (0.0)	0/97 (0.0)	2/71 (2.8)	2/222 (0.9)
Salmonella arizonae	0/16 (0.0)	0/38 (0.0)	0/97 (0.0)	5/71 (7.0)	5/222 (2.3)
Klebsiella pneumoniae	0/16 (0.0)	0/38 (0.0)	5/97 (5.2)	5/71 (7.0)	10/222 (4.5)
Klebsiella ozaenae	0/16 (0.0)	0/38 (0.0)	2/97 (5.2)	2/71 (2.8)	4/222 (1.8)
Klebsiella oxytoca	0/16 (0.0)	0/38 (0.0)	4/97 (4.1)	11/71 (15.5)	15/222 (6.8)
Enterobacter aerogenes	0/16 (0.0)	0/38 (0.0)	2/97 (2.1)	5/71 (7.0)	7/222 (3.2)
Enterobacter cloacae	1/16 (6.3)	0/38 (0.0)	17/97 (17.5)	1/71 (1.4)	19/222 (8.6)
Enterobacter sakazakii	0/16 (0.0)	0/38 (0.0)	0/97 (0.0)	1/71 (1.4)	1/222 (0.5)
Enterobacter agglomerans	1/16 (6.3)	1/38 (2.6)	5/97 (5.2)	10/71 (14.1)	17/222 (7.7)
Hafnia alvei	1/16 (6.3)	3/38 (7.9)	2/97 (2.1)	40/71 (56.3)	46/222 (20.7)
Serratia marcescens	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	2/71 (2.8)	3/222 (1.4)
Serratia liquefaciens	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	4/71 (5.6)	5/222 (2.3)
Serratia fonticola	1/16 (6.3)	1/38 (2.6)	2/97 (2.1)	14/71 (19.7)	18/222 (8.1)
Serratia odorifera	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	1/71 (1.4)	2/222 (0.9)
Serratia plymuthica	0/16 (0.0)	0/38 (0.0)	1/97 (1.0)	2/71 (2.8)	3/222 (1.4)
Serratia sp.	0/16 (0.0)	0/38 (0.0)	0/97 (0.0)	2/71 (2.8)	2/222 (0.9)
Proteus vulgaris	0/16 (0.0)	0/38 (0.0)	3/97 (3.1)	0/71 (0.0)	3/222 (1.4)
Providencia rettgeri	0/16 (0.0)	0/38 (0.0)	6/97 (6.2)	3/71 (4.2)	9/222 (4.1)
Providencia stuartii	0/16 (0.0)	0/38 (0.0)	2/97 (2.1)	0/71 (0.0)	2/222 (0.9)
Providencia alcalifaciens	0/16 (0.0)	0/38 (0.0)	9/97 (9.3)	4/71 (5.6)	13/222 (5.9)
Morganella morganii	0/16 (0.0)	0/38 (0.0)	14/97 (14.4)	9/71 (12.7)	23/222 (10.4)
Yersinia enterocolitica	1/16 (6.3)	4/38 (10.5)	0/97 (0.0)	2/71 (2.8)	7/222 (3.2)
Yersinia intermedia	0/16 (0.0)	0/38 (0.0)	0/97 (0.0)	1/71 (1.4)	1/222 (0.5)
Yersinia ruckeri	0/16 (0.0)	2/38 (5.3)	0/97 (0.0)	0/71 (0.0)	2/222 (0.9)

[a] Snout vent length in millimeters.

from ca. 46% of the frog intestines sampled. A much greater diversity and prevalence of species of the family *Enterobacteriaceae* were encountered in the intestines of frogs collected in early spring, and *A. hydrophila* isolation rates ranged from 37 to 68% for different collecting sites.

Five isolations of *Salmonella arizonae* (2.3%) were made from the direct midgut smears from individual frogs.

Of the eight *Y. enterocolitica* isolates submitted for serotyping, three were serotype O:Tacoma, three were O:4,33, one was O:6,31, and one could not be typed.

Eleven samples of heart blood yielded the following numbers of isolates: three *A. hydrophila*, three *C. freundii*, two *Hafnia alvei*, one *Klebsiella pneumoniae*, one *Providencia rettgeri*, and one *Enterobacter agglomerans*. Seven of the isolates were from 6 of 18 frogs collected in North Dakota.

Tadpoles. Isolations from 34 tadpole intestines were as follows: *A. hydrophila*, 1 (2.9%); *C. freundii*, 1; *H. alvei*, 11 (32.4%); *Escherichia coli*, 22 (64.7%); *Y. enterocolitica*, 2 (5.9%); *Serratia odorifera*, 1; *E. agglomerans*, 4 (11.8%); *Enterobacter cloacae*, 5 (14.7%); *K. pneumoniae*, 3 (8.8%); and *P. rettgeri*, 1. *A. hydrophila* was not isolated from 28 tadpoles collected from a Sherburne County breeding pond on 13 May and 16 June, but 18 of 24 (75%) froglets collected on 1 July from the same site yielded the organism.

Water. Thirty-eight water samples were collected. From lake overwintering sites (April), *A. hydrophila* was isolated from two of five (40%) samples. From a breeding pond, one of five (20%) samples collected in May and five of nine (56%) collected in June and July yielded *A. hydrophila*. The organism was cultured from 13 of 19 (68%) samples from rivers and lakes near summer foraging sites (July through September).

The methodology used in the present study was not quantitative, and we could not say whether the species isolated were dominant members of the flora or transients. It has been reported that facultative aerobic bacteria represent about 2% of the total bacterial count of the frog gut, the remainder being anaerobes (11).

Anaerobic bacteria are not known to be important frog pathogens and were not considered in this study.

Results of *A. hydrophila* isolation were similar to those of a previous study (13), except that the organism was isolated from few (2.9%) tadpoles in the present study. This may be because collections were made several weeks earlier in the season, and *A. hydrophila* populations in bodies of water appear to increase with warmer water temperatures in the spring and summer (12).

We considered *A. hydrophila*-like isolates that were lysine decarboxylase positive not to be *A. hydrophila*, as stated previously (6). However, others have reported lysine decarboxylase-positive strains of *A. hydrophila* and have demonstrated an association between the presence of lysine decarboxylase activity and cytotoxicity (4, 5).

S. arizonae and *Y. enterocolitica* have been isolated from frogs previously (2, 14), but their pathological significance in frogs is not known.

Most of the frogs from which isolates were obtained from heart blood were collected in North Dakota, the most distant collection site. Frogs captured in Minnesota were almost always dissected within 24 h, but those from North Dakota were dissected 48 to 72 h after capture, suggesting an association between septicemia and transit time to the laboratory. Problems of decreased survival of frogs after shipment have been observed (1).

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