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Author: BOTZLER, R. G.

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YERSINIAE IN POND WATER AND SNAILS[1]

R. G. BOTZLER,[2] School of Natural Resources, The University of Michigan, Ann Arbor, Michigan 48104, USA

F. T. WETZLER,[3] Department of Environmental and Industrial Health, The University of Michigan

A. B. COWAN, School of Natural Resources, The University of Michigan

T. J. QUAN, Plague Section, Center for Disease Control, Public Health Service, Department of Health, Education & Welfare, P.O. Box 2087, Fort Collins, Colorado 80521, USA

Abstract: A *Yersinia enterocolitica* serotype 9 was isolated from pond water; *Y. enterocolitica*-like bacteria were also isolated from pond water and from three species of snails (*Lymnaea palustris elodes, Helisoma* sp., *Oxyloma retusa*) from the Edwin S. George Reserve in southeastern Michigan. There was evidence for biochemical stability among some of the organisms over a period of years. There also was evidence of transmission of these organisms to snails from the water.

INTRODUCTION

In recent years, a great deal of information has been gathered on *Yersinia pseudotuberculosis* and *Y. enterocolitica* in wild anmials.[9,10,11]

After *Y. enterocolitica* and *Y. enterocolitica*-like bacteria were isolated from deer[11] and from frogs and snails[1] taken from the George Reserve in Michigan, a more detailed study was made to determine which ponds and aquatic species carried these organisms.

MATERIALS AND METHODS

The Edwin S. George Reserve is a 485 ha tract of land in the southwest corner of Livingston County, Michigan, about 40 km northwest of Ann Arbor. About 35% of the reserve is woodlot (predominantly *Quercus-Carya*), about 2% is brushland, and about 40% is grassy upland; the remaining 23% is composed of ponds, swamps, bogs and marshes.

In 1968, aquatic animals were collected between 1 May and 28 August from Crane Pond, Fishhook Marsh, and Southwest Swamp. These included leopard frogs (*Rana pipiens pipiens*), bullfrogs (*Rana catesbeiana*), green frogs (*Rana clamitans melanota*), midland painted turtles (*Chrysemys picta marginata*) and common snapping turtles (*Chelydra serpentina serpentina*).[3] The snails were *Physa sayii, Helisoma* spp., *Lymnaea* (*Stagnicola*) *palustris elodes*, and *Oxyloma retusa*. Limited collections of *O. retusa* and water samples also were made between 2 May and 20 September 1969.

With the exception of *O. retusa*, snails were pooled and each pool was ground with a mortar and pestle after a rinsing in absolute alcohol. The remains of each

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[2] Present address: School of Natural Resources, Humboldt State University, Arcata, California 95521, USA.

[3] Present address: Department of Environmental Health, University of Washington, Seattle, Washington 98101, USA.

pooled sample were added to trypticase soy broth with 0.5% yeast extract and 50 μg/ml of potassium tellurite.

Specimens of *O. retusa* were scrubbed in a solution of 200 ppm Iosan in tap water. After rinsing in sterile distilled water, each pool of snails was ground with a mortar and pestle. Half of the remains were stored in a screw-capped tube containing 10 ml of trypticase soy broth, 0.5% yeast extract, and 25 μg/ml of potassium tellurite (TY-25); the other half was stored in screw-capped tubes containing 10 ml of trypticase soy broth and 0.5% yeast extract (TY-0).

Water samples were thoroughly shaken and filtered through a cellulose triacetate membrane with a pore size of 0.45 μm. The membrane was divided into two parts; one part was stored in a tube of TY-25 and the other was stored in TY-0.

Additional information about the study area, the number of animals sampled, and methods of collecting and handling the specimens has been reported previously.[2]

Bacterial isolations were made on MacConkey Agar[4][5] after the samples were stored at 4 C for approximately one year. The isolates were characterized by standardized methods.[3]

The pathogenicity of the isolates was determined at levels of $10^{6.3}$ and $10^{6.3}$ bacteria, with 18-hr cultures of each isolate grown at 26 C. Three 7-week-old Swiss mice were injected intraperitoneally with each dose of bacteria in 0.1 ml amounts. The mice were observed over a 30-day period.

Representative isolates were sent to the U.S. Public Health Service, Center for Disease Control, Bureau of Laboratories, Fort Collins, Colo., for serotyping tests. Isolates were evaluated by a whole cell bacterial agglutination test with antiserums 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

Nine strains of *Yersinia enterocolitica* and *Y. enterocolitica*-like organisms were isolated. Strains N7-41 and N7-44 (N7 series) were isolated from a pool of two snails (*L. palustris elodes*) collected in the canal between Crane Pond and Fish-Hook Marsh (Map given in earlier report[2]). Strains N19-41 and N19-42 (N19 series) were isolated from a snail (*Helisoma* sp.) collected from Southwest Swamp. Strains N62-41T and N62-42 (N62 series) were isolated from a pool of 46 *O. retusa* collected from Southwest Swamp. Strains W5-42, W9-42, and W10-43 were isolated from three water samples collected from Section b of Crane Pond.

All nine strains were gram-negative, catalase positive rods. They were motile at 26 C, but not at 37 C. All were urease positive, H_2S negative, indol positive, methyl red positive or ±, acetylmethylcarbinol positive, and phenylalanine deaminase negative. All fermented glucose in the O-F test. All were lysine decarboxylase negative and ornithine decarboxylase positive. They grew in KCN broth at 26 C and 37 C. In a purple broth base all nine strains utilized arabinose, xylose, fructose, galactose, mannose, sorbose, lactose, maltose, sucrose, trehalose, glycerol, mannitol, and sorbitol. None of the strains utilized dulcitol or adonitol within 14 days. Variation occurred with citrate, melibiose, salicin and rhamnose (Table 1).

Strains N7-41, N19-41, and N62-42 from snails, and W5-42 and W9-42 from water were inoculated into mice; none of the mice died within 30 days.

Strains N7-44, N19-41, N62-42, W5-42, W9-42, and W10-43 were serotyped. Two strains (5F4-32, 5M4-43) reported earlier[1] also were serotyped. Strain W5-42 from water was identified as *Y. enterocolitica* serotype 9 and strain 5F4-32 from a frog was identified as serotype 8.

[4] Difco Laboratories, Detroit, Michigan.

[5] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

TABLE 1. Biochemical variation among the George Reserve strains of **Yersinia enterocolitica** and **Y. enterocolitica**-like bacteria.

	Y. enterocolitica			*Y. enterocolitica*-like					
	Deer	Frogs	Water	Frogs	Snails			Water	
	M Series	5F4 -32	W5 -42	2F33 & 2F35 Series	5M4 Series	N7 Series	N19 Series	N62 Series	W9 -42	W10 -43
Acetylmethylcarbinol	—	+	+	—	V	+	+	+	+	+
Citrate utilization	V	+	—	+	V	V	V	V	+	+
Ornithine decarboxylase	+	+	+	—	+	+	+	+	+	+
Indol	+	+	+	V	V	+	+	+	+	+
Carbohydrate fermentation (14 days)										
Lactose	—	—	+	+	+	+	+	+	+	+
Rhamnose	ND	—	—	+	+	+	+	+	+	+
Sorbose	ND	+	+	—	+	+	+	+	+	+
Melibiose	—	—	—	V	+	+	ND	+	+	+
Sucrose	V	+	+	—	+	+	+	+	+	+
Salicin	V	+	—	+	+	+	+	+	+	+
Dulcitol	—	—	—	+	—	—	—	—	—	—

+: positive reaction —: negative reaction V: variation among isolates ND: not tested

TABLE 2. Further biochemical variations among selected George Reserve strains of **Yersinia enterocolitica** and **Y. enterocolitica**-like bacteria

	Y. enterocolitica		*Y. enterocolitica*-like				
	5F4 -32	W5 -42	5M4 -43	N7 -44	N62 -42	W9 -42	W10 -43
Lactose O-F (25 C)	O	O	O	O	O	F	O
Aesculin	+	—	+	+	+	+	+
Raffinose	—	ND	+	+	—	+	+
Inositol	+	+	—	—	+	+	—

+: positive reaction —: negaitve reaction F: fermentative O: oxidative ND: not tested

The other strains were not serotypable and are designated as *Y. enterocolitica*-like bacteria.

The variation between the *Y. enterocolitica* and *Y. enterocolitica*-like bacteria isolated from the George Reserve fauna and water is presented in Table 1. Data on strain 5F4-32, the 2F33 and 2F35 series, the 5M4 series and the deer isolates were obtained from previous studies.[1,11]

Strains 5F4-32 and 5M4-43 from frogs, N7-44 and N62-42 from snails, and W5-42, W9-42 and W10-43 from water were further characterized. In addition to reactions previously reported,[1] all reduced nitrates and were ONPG positive; none utilized malonate. They all utilized cellobiose, arabinose, galactose (strain 5F4-32 was not tested), trehalose, sorbose, and maltose. Variations were observed in the lactose O-F Test carried out at 25 C, aesculin hydrolysis, and utilization of raffinose and inositol (Table 2).

DISCUSSION

Of the strains previously identified as *Y. enterocolitica,* the M series was isolated from white-tailed deer (*Odocoileus virginianus*) harvested in the winter of 1965,[11] and strain 5F4-32 was isolated from a pool of three leopard frogs (*Rana pipiens*) collected in the summer of 1966 from Fish-hook Marsh.[1] Of the *Y. enterocolitica*-like bacteria, the 2F33 and 2F35 series were isolated from two green frogs (*Rana clamitans*) collected in the summer of 1966 from George Pond; the 5M4 series were isolated from a snail (*L. palustris elodes*) collected in the summer of 1966 from Fish-hook Marsh.[1] Including the data from the present study, these bacteria were isolated over a 5-year period from deer, two species of frogs, three species of snails, and directly from pond water; the isolates were associated with four or five ponds studied on the reserve.

The strains that could not be serotyped (reported previously[1] as well as in this study) generally follow the biochemical reactions characteristic of *Y. enterocolitica* given by Nilehn.[6] Exceptions are the utilization of citrate, rhamnose, melibiose and raffinose by many of the George Reserve strains.

Strains W9-42 and W10-43, and the N7, N19, and N62 series are virtually indistinguishable from the 5M4 series, and this entire group will be referred to as the snail-water series. The deer isolates, strain 5F4-32, strain W5-42, the 2F33 and 2F35 series, and the snail-water series all appear to be biochemically and serologically distinct. Two possible interpretations are that these organisms are part of one larger population in which different variants are selected under varying circumstances, or that there are a number of stable strains adapted to specific niches.

The isolates of the snail-water series were biochemically very similar, despite being collected from different hosts and ponds over a 2-year interval. Serological and biochemical determinations indicated that strain 5F4-32 from frogs was very similar to a strain of *Y. enterocolitica* reported 30 years earlier from a human in New York.[7] This evidence suggests that at least some of these organisms remain relatively stable and autonomous over a period of years.

The biochemical similarity between the strains in the snail-water series suggests the possibility that *Y. enterocolitica* is transmitted regularly to snails from the water. The apparent transmission of *Y. enterocolitica* to a human from water has been reported recently.[4] The isolation of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria from three of nine water samples from the portion of a pond used frequently by deer would suggest a similar relationship between pond water and the deer. But the consistent biochemical differences between the water isolates and the deer isolates argues against this.

The isolation of *Y. enterocolitica*-like strains from *Oxyloma,* an intermediate host of the deer meningeal worm (*Parelaphostrongylus tenuis*), suggests that these snails could carry these bacteria from infected to uninfected deer. However, other evidence indicates that *Oxyloma* does not play a major role as a carrier of these organisms. Nineteen pooled samples containing 193 snails

were analyzed, but only one pool of 46 snails had *Y. enterocolitica*-like organisms, which indicates that the frequency with which *Oxyloma* carry these bacteria is probably very low. Moreover, the organisms carried by *Oxyloma* were biochemically different from those isolated from the deer.

The isolation of *Y. enterocolitica* or *Y. enterocolitica*-like bacteria from the intestines of the deer, frogs, and snails indicates that food or water is a common source. The food habits of these animals vary, but all need water. In the past, nearly all hypotheses for the perpetuation of yersinioses depended on animal reservoirs. Based on the possible transmission of these bacteria to aquatic snails from the water, and the isolation of yersiniae from water in other studies[4,5,8] we suggest that water or soil also may be a reservoir.

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