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New Strain of *Yersinia enterocolitica* Pathogenic for Rodents

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A strain of *Yersinia enterocolitica*, biotype 2, serotype 8, isolated from the blood of a human patient has been found to be highly pathogenic for rodents.

Human infection with *Yersinia enterocolitica* is thought to have been first reported in New York State. From 1923 through 1940, 11 strains of an unidentified organism, later called *Bacterium enterocoliticum*, were isolated from patients in rural areas of New York, primarily children with enteric disease (5). Most of the isolates were reported to be pathogenic for laboratory animals on initial isolation, but attenuated quickly after subculture on artificial media (14). However, many attempts by European workers to demonstrate experimental pathology with strains of *Y. enterocolitica* have met with failure (2-4, 6, 8, 10). Mollaret and Guillon (10) examined 106 strains of *Y. enterocolitica* for animal pathogenicity by using different routes of inoculation in several species of animals and adjuvants or pathogenic agents to potentiate virulence, but all gave negative results. This failure to produce disease in laboratory animals was not considered due to attenuation because a number of their attempts were made with strains isolated only a few days previously.

A request for pathogenic strains of *Y. enterocolitica* from the New York State Department of Health, Albany, yielded two isolates, both of which proved to be nonpathogenic for mice (Table 1), having either been attenuated or never been pathogenic in the first place. In view of the many failures by European workers to demonstrate pathogenicity with fresh isolates of *Y. enterocolitica*, it would appear that either the New York strains are peculiar among the *Y. enterocolitica* group as regards pathogenicity for laboratory animals, or factors not directly associated with the isolates used by the early workers were responsible for the mortalities observed. With the results reported here, however, we are able to confirm the early work showing that some strains of *Y. enterocolitica* are highly pathogenic for rodents, particularly mice.

On 7 November 1972, *Y. enterocolitica* was isolated in pure culture from the blood of a human patient (W.A.) (E. E. Keet, manuscript submitted for publication). This isolate proved to be highly pathogenic for mice. As shown in Table 1, *Y. enetrocolitica* strains B 1221 and C 5819 (both from Albany) are nonpathogenic by the intravenous (i.v.) route for both the B6D2 (C_{57}B1/6 $\times$ DBA F_1) and the CD-1 strains of mice. Strain *Y. enterocolitica* WA, on the other hand, is extremely virulent for both strains of mice by the i.v. and oral routes of infection. *Y. enterocolitica* WA has also been shown to be pathogenic for rats by the i.v. and subcutaneous routes (D. D. McGregor, unpublished data). Unlike *Y. pseudotuberculosis*, *Y. enterocolitica* WA is very much less pathogenic for guinea pigs, the oral mean lethal dose (LD_{50}) being greater than 5×10^9 organisms. The LD_{50} in all cases was determined by the introduction, by either the i.v. or oral routes, of 37 C log phase cultures diluted in physiological saline into 6-week-old mice or 500-g guinea pigs. Deaths were monitored over a minimum of 30 days.

The biochemical and seriological characteristics of the WA strain of *Y. enterocolitica* are given in Table 2 and are consistent with those published by Nilehn (12) and Mollaret and Chevalier (9) for the *Y. enterocolitica* group. These would place the WA strain into biotype 2 (12). An interesting variation from other biotype 2 isolates regards its metabolism of lactose. On intial isolation from the blood, the organism produced acid from lactose, but upon subculture it has remained lactose negative.

The growth versus temperature kinetics for *Y. enterocolitica* WA were determined by placing identical broth cultures of the organism in a temperature-gradient block at increments of 2 C [see Wayne (15) for block specifications]. Turbidimetric determinations and viable counts were done at frequent intervals up to 72 h. The optimum temperature for growth is 25 to

TABLE 1. *LD_{50} of Y. enterocolitica strains*

Strains	B6D2		CD-1	
	i.v. LD_{50}	Oral LD_{50}	i.v. LD_{50}	Oral LD_{50}
1221	$>10^8$		$>10^8$	
5819[a]	$>10^8$		$>10^8$	
WA[a]	7×10^4	3×10^8	2.4×10^2	2×10^8

[a] Indole-positive strains.

TABLE 2. *Characteristics of Y. enterocolitica strain WA*

Characteristics	Reaction
Carbohydrate (at 37 C)	
Citrate[a]	–[b]
Dulcitol	–
Glucose	A
Inositol	A
Lactose	–
Maltose	A
Mannose	A
Rhamnose	–
Salicin	–
Sorbitol	A
Sucrose	A
Trehalose	A
Xylose	A
Biochemical (general)	
Esculin hydrolysis	–
Arginine dihydrolase	–
β-D-galactosidase	+
Catalase	+
Gelatin	–
Indole	+
Lysine decarboxylase	–
Methyl red	+
Nitrate reduction	+
Ornithine decarboxylase	+
Oxidase	–
Urease[c]	+
Voges-Proskauer (25 C)	+
Voges-Proskauer (37 C)	–
Other	
Motility (25 C)	+
Motility (37 C)	–
Lysogenic phage	–
Biotype	2
Serotype	8

[a] Simmons citrate agar, BBL, Cockeysville, Md.

[b] Key: –, no reaction or negative reaction; +, positive reaction; A, acid formed.

[c] Bacto-urea base concentrate, Difco Laboratories, Detroit, Mich.

26 C, but no real difference was observed over a range of 20 to 39 C. There was a drop in the viable count of tubes placed at temperatures above 43 C, but good growth was observed at temperatures as low as 4 C. Thus the temperature requirements for growth are very similar to those of other strains of *Y. enterocolitica* (9).

Attempts at induction of a lysogenic phage, by using the chloroform technique of Kjems (7) or ultraviolet light, were negative. The WA strain, used as the releasing strain, was grown in broth at both 25 C and at 37 C, spread on agar plates (1), and exposed to chloroform or ultraviolet light. The plates were overlayed with agar suspensions of strains WA, 5819, or 1221 which had been grown at both 25 C and 37 C. In none of the cases were zones of lysis observed. These negative findings are consistent with those of Nilehn and Ericson (13) for indole-positive strains. With the use of the lysotype grouping proposed by Nicolle et al. (11), *Y. enterocolitica* WA has been considered lysogenic type 10_0 (Mollaret, personal communication).

On the basis of studies thus far performed on strain WA, the factors responsible for the virulence of the organism remain obscure. The colonial morphology, biotype, serotype, and lysogenic grouping are the same for both WA and 5819. If strain 5819 has lost its virulence through attenuation, it has not done so by converting from a smooth to a rough colony type. Its colonial morphology is identical to that of strain WA, both being small (1.0 mm) and smooth after 48 h of incubation at either 25 C or 37 C. The virulence of strain WA cannot be attributed to its passage through a human host because a strain of *Y. enterocolitica* of the same serotype and lysogenic group isolated from the water which infected the human patient (Keet, submitted for publication) also demonstrates virulence for CD-1 mice by the intravenous route.

Because it is necessary that a strain of *Y. enterocolitica*, pathogenic for laboratory animals, be available for studies of the pathogenesis of *Yersinia* enteritis and the immune responses involved, the WA strain has been deposited at the American Type Culture Collection (ATCC 27729), the National Collection of Type Cultures (NCTC 10938), and the *Yersinia* collection of the Pasteur Institute, Paris (Ye 2705). Precautions have been taken to insure that the isolates deposited are indeed virulent. In this laboratory, the best method for preservation of viability and virulence is storage at –70 C. In view of early reports of attenuation (14), it may not be wise to transfer the WA strain on artificial media for long periods of time without animal passage, though in our hands, attenuation has not been observed after 8 months on artificial media.

We are indebted to the New York State Department of Health, Albany, for isolates of *Y. enterocolitica* and for confirmation of the biochemical tests on the WA strain and likewise to A. C. Sonnenwirth, St. Louis, for independent confirmation of the classification of the WA strain. We are particularly grateful to H. H. Mollaret, Paris, for determining the serotype and lysogenic group of both the blood and water *Y. enterocolitica* isolates. We also acknowledge the expert technical assistance of Fred J. Jarnot and Lorraine Schuler.

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