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Long-Term Fecal Excretion and Resistance Induced in Mice Infected with *Yersinia enterocolitica*

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European isolates of *Yersinia enterocolitica* serotypes O3 and O9 were shown to infect but not kill mice, which became fecal excreters for up to 135 days. The mice challenged with 500 50% lethal doses of the virulent WA strain of *Y. enterocolitica* serotype O8 survived, and some excreted the virulent strain. This rodent model may be of value in assessing the ecological significance of rodents in the maintenance of *Y. enterocolitica*.

Rodents act as reservoirs for *Yersinia pestis* and *Yersinia pseudotuberculosis*, although epizootics with high mortality may occur. *Yersinia enterocolitica* has been isolated from rodents (1, 7, 8), but its pathogenic effects and survival within murine populations have not been determined. Thus, the importance of rodents as a reservoir is unknown, as is the significance of infected rodents as a source of human disease. However, the high isolation rates reported from Scandinavia and Czechoslovakia (1, 7, 8) would suggest that rodents are chronic excreters since acute lethal infections were associated with low isolation rates. Another interesting finding from these investigations was the variety of serotypes, untypable strains, and aberrant "*Yersinia*-like organisms."

Several workers (2, 9) have attempted to infect laboratory rodents with human strains and have reported their findings in terms of avirulence or inability of most strains to cause infection in mice. The first report of a virulent strain of *Y. enterocolitica* producing high mortality came from the United States, where Carter et al. (4) established the 50% lethal dose (LD_{50}), by the intravenous route, in a hybrid mouse as being 7.0×10^4. European strains of serotypes O3 and O9 were shown subsequently to have enhanced virulence for laboratory mice after repeated passage (J. M. Alonso, personal communication).

These divergent findings led us to investigate and compare the effects of two European-derived strains of serotypes O3 and O9 with that of the virulent WA strain of serotype O8 studied by Carter et al. (4). We found the LD_{50} for *Y. enterocolitica* WA serotype O8 (NCTC no. 10938) in the Porton white mouse to be 8.8×10^1, whereas the European strains O3 and O9 were shown to infect but not kill when given by the intraperitoneal route (Pearson et al., *Proceedings of the Third International Symposium on Yersinia*, vol. 2, in press). The experiments reported here were aimed at establishing the duration of fecal excretion of the introduced strains, and the resistance of the mice to subsequent challenge with a lethal dose of virulent *Y. enterocolitica*.

Six groups of 16 male mice (Porton white strain) were challenged with avirulent European strains of *Y. enterocolitica*; three groups were inoculated with a strain of serotype O3 (Institute Pasteur [IP] strain 134) and three groups with a strain of serotype O9 (IP strain 383). Each of the six groups received a single intraperitoneal dose containing 10^7, 10^5, or 10^3 viable cells. To assess the reported variation in pathogenicity induced by different growing temperatures (3), the experiment was done with cultures grown at 25 and 37°C. Thirty-five days after the initial infection, all mice were challenged with 5×10^4 live organisms of the virulent WA strain (500 LD_{50}s). A control group of 10 normal mice was challenged with the same dose by the intraperitoneal route. Fecal specimens (0.1 g) were collected from cages for the 135 days of the investigation, animals being transferred to clean, autoclaved cages after each sampling. Material from each cage was suspended in 1 ml of phosphate-buffered saline (pH 7.3) and stored at 4°C for 3 days (5) before plating with a standard loop on lactose-sucrose-urea selective agar (6). Plates were examined for *Yersinia* colonies after 24 and 48 h of incubation at 25°C. If negative, a further sample from material stored at 4°C was reexamined at 4 and 6 weeks. Colonies resembling *Y. enterocolitica* were identified by slide agglutination and were subcultured and stored in liquid nitrogen for biochemical testing and checking of the patterns of antibiotic sensitivity.

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A total of 64 isolates were recovered from fecal samples.

Mice given the European isolates O3 and O9 did not die and were found to excrete the organism for several months from the gastrointestinal tract (Table 1). The excretion pattern was most consistent in mice given cultures of serotype O9, particularly those infected with 10^7 organisms. In this latter group, fecal excretion continued for the 135 days of the experiment. There was no difference in the behavior of the mice given organisms grown at 25 and 37°C, of either strain O3 or O9, with any of the three inoculating doses. The challenge with 500 LD_{50}s of the virulent WA strain, given at day 35 of the experiment, killed all control mice within 15 days; 80% of the deaths occurred by day 8.

TABLE 1. *Fecal excretion from mice given Y. enterocolitica by the intraperitoneal route*[a]

Serotype	Days after initial challenge	Dose		
		10^3	10^5	10^7
O3	8	NT	NT	−
	11	NT	NT	−
	12	NT	NT	+
	15	−	−	+
	20	−	−	+
	28	−	−	−
	35[b]	−	−	−
	39	WA	−	WA
	42	WA	WA	WA
	46	−	WA	WA
	49	−	−	WA
	55	−	WA	WA
	71	WA	WA	WA
	83	−	−	−
	89	−	−	−
	109	−	−	−
	135	−	−	−
O9	8	NT	NT	+
	11	NT	NT	+
	12	NT	NT	+
	15	−	+	+
	20	−	−	+
	28	−	−	+
	35[b]	+	+	+
	39	WA	WA/+	+
	42	WA	+	+
	46	WA	WA/+	+
	49	WA	WA	WA/+
	55	WA	−	+
	71	WA	−	+
	83	−	−	+
	89	−	−	+
	109	−	−	+
	135	−	−	+

[a] +, Initial strain isolated; WA, WA strain isolated; NT, not tested; −, no isolation; WA/+, both strains isolated.

[b] WA challenge (5×10^4) given at this time.

However, mice previously given a dose of 10^7 organisms of serotype O3 showed a significant degree of protection, as did mice given either 10^7 or 10^5 cells of serotype O9 (Fig. 1, Komolgorov-Smirnov test, $\alpha = 0.05$). This graph shows that mice with a prior *Y. enterocolitica* infection had a 70% survival rate on day 8 after challenge as compared to an 80% mortality in the controls given the virulent strain. The mice that survived the challenge with the virulent WA strain consistently excreted this strain for up to 36 days, with the possible exception of those previously given 10^7 cells of strain O9. In the latter group, strain WA was only recovered in one sampling period. An attempt was made to assess any variation in properties of the 64 isolates that were recovered during the 135 days of the experiment by means of comparing 26 biochemical tests and 14 antibiotic sensitivities with the properties of the starting cultures. No evidence of variation was detected.

Our findings establish that an outbred laboratory mouse strain is susceptible to both the virulent American WA strain and the relatively avirulent European strains, all of which have been incriminated in human infections. The results are of special interest regarding the duration of fecal excretion, which at 135 days represents a significant part of the life span of a mouse. We attempted to see whether there was any variation to explain the large proportion of untypable and aberrant strains isolated from wild rodents (8), but in the limited series of tests we undertook, no evidence was obtained to support this hypothesis.

Chronic fecal excretion and the reported 10 to 27% isolation rate of *Y. enterocolitica* from wild rodents (1, 7) and adjacent freshwater (8) are evidence that mice are chronic carriers and ex-

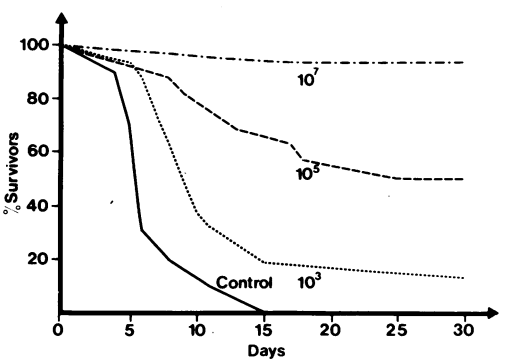

FIG. 1. *Survival of mice challenged intraperitoneally with 500 LD_{50}s of virulent Y. enterocolitica serotype O8 strain WA. Controls, 10^3, 10^5, and 10^7 curves, 5×10^4 virulent WA cells; stated doses of avirulent serotype O9 cells given 35 days before challenge with 5×10^4 virulent WA cells.*

creters which may disseminate organisms to the environment. Both infected water and the coprophilic habit of rodents would serve to perpetuate the infection in rodent burrows. The excretion of highly virulent strains after prior infection with another serotype is of potential ecological significance since strains of low virulence would not affect the dynamics of the rodent population but would contribute to the maintenance and spread of virulent strains. Laboratory mice appear, therefore, to offer an interesting system in which to examine the host response, excretion of the organism, as well as the possibility of creating a model which may reflect certain aspects of the maintenance of *Y. enterocolitica* in aquatic and terrestrial biocycles.

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