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Pathogenicity of *Yersinia enterocolitica* for Mice

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A laboratory infection of *Yersinia enterocolitica* in mice which closely resembles the naturally acquired human infection is described. Intravenous inoculation of mice with small numbers of *Y. enterocolitica* gives rise to a systemic, pyogenic infection involving primarily the spleen, liver, and lungs. Massive neutrophil infiltration of these organs occurs early in the infection, eventually leading to large abscesses and pulmonary consolidation. Mice infected intragastrically show neutrophil infiltration in the Peyer's patches of the distal ileum less than 24 h postinfection. The Peyer's patches are unable to contain the infection which spreads to the mesenteric lymph node, causing large abscesses in the medullary regions. Soon after, the infection becomes systemic with abscesses forming in the liver, spleen, and lungs, and the total peripheral leukocyte count rises dramatically to over 30,000/mm^2. A serological response, in the form of agglutinating antibody, begins to appear 2 weeks after infection. Possible causes of death and the usefulness of this infectious disease model are discussed.

In the last decade, increasing attention has been directed toward *Yersinia enterocolitica* as an etiologic agent in human ileitis, mesenteric lymphadenitis, and septicemia (9, 16, 23) as well as possible sequelae of the enteric infection: arthritis, Reiter's syndrome, erythema nodosum, and others (14). However, the connection between *Y. enterocolitica* and these syndromes has to date been circumstantial, the infection of human volunteers not having been attempted. A serious weakness in the case for a causative relationship to the human disease has been the inability to confirm an early report (21) of the pathogenicity of human isolates for laboratory animals (10, 15). Some attempts to demonstrate virulence in guinea pigs and rabbits (19), as well as in mice (17), involved the administration of extremely high challenge doses given parenterally, with most of the deaths occurring between 24 and 48 h. The very large doses of *Y. enterocolitica* needed to kill the animals and the mean time of death closely resemble endotoxin death and bear no similarity to the human infection. Recently, however, strains which demonstrate marked virulence for laboratory animals have been isolated (3, 18), thus tending to confirm early reports and affording researchers an opportunity to use this organism in the establishment of an animal model for this enteric infection. The report which follows shows the striking similarities between the murine infection induced by *Y. enterocolitica* and the human infection attributed to this agent.

MATERIALS AND METHODS

Animals. Specific pathogen-free male CD-1 mice (Charles River Farms, Wilmington, Mass.) were maintained 10 to a cage under Isocaps (Carworth Lab Cages, New York, N.Y.) on sterilized bedding (San-I-Cel, Paxton, Ill.). The animals were given free access to sterilized food (Charles River Rat/mouse diets, Country Foods, Syracuse, N.Y.) and water.

Mode of infection. *Y. enterocolitica* strain WA (ATCC 27729; NCTC 10938) was used to challenge the mice either intravenously via a tail vein or intragastrically by means of a feeding tube (4). The WA strain was isolated from human blood and subsequently maintained at −70 C (most strains of *Y. enterocolitica* attenuate easily, so isolates obtained from culture collections may require passage through mice prior to use). The organism was subcultured in Trypticase soy broth (BBL, Cockeysville, Md.) and incubated at 25 C for 18 h prior to animal challenge. The intravenous and oral 50% lethal dose (LD_{50}) and the characteristics of the WA strain of *Y. enterocolitica* have been reported elsewhere (3).

Histology. The mice were anesthetized with ether and exsanguinated from the exposed heart before autopsy. Specimens of the thymus, lungs, kidneys, adrenals, liver, spleen, pancreas, mesenteric and peripheral lymph nodes, intestine, and Peyer's patches were fixed immediately in either Bouin's fluid or buffered neutral formalin. The tissues were processed, embedded in paraffin, sectioned, and stained according to standard methods (11).

Hematology and serology. A small portion of heart blood from each animal was taken for total leukocyte determinations and slide smears, whereas serum was collected from the remainder and stored at −20 C. The blood smears were stained with May-

Grünwald-Giemsa and examined. Only mature cells were considered in determining the differential leukocyte counts. A great number of poorly differentiated cells appeared in the blood as the infection progressed and, because of difficulty in the identification of these cells, they were grouped as a special category. The antibody titers of the sera from infected mice were determined using the Microtiter system (Cooke Engineering, Alexandria, Va.). The sera were titered against either merthiolate-killed *Y. enterocolitica* WA (grown at 25 C for 48 to 72 h), autoclaved *Y. enterocolitica* WA (24), or the passive hemagglutination test currently employed at the Ft. Collins Laboratories, Center for Disease Control (CDC), Fort Collins, Colo. (B. W. Hudson, personal communication). The latter test employs sheep erythrocytes sensitized with lipopolysaccharide extracted from cell walls of acetone-killed and autoclaved *Y. enterocolitica* WA.

RESULTS

Intravenous infection. CD-1 mice were challenged intravenously with about 1,000 viable *Y. enterocolitica* WA (5 LD_{50}'s) using a 24-h broth culture grown at 25 C. Three animals were chosen for necropsy at daily intervals after the infection. As early as 24 h postinfection, small areas of hemorrhage were grossly evident in the right lobes of the lung together with small creamy white nodules within the spleens of the infected animals. The number and size of the abscesses in the spleen, liver, and lungs increased, and by day 4 animals began to die; all were moribund by day 6. Histologically, the areas of necrosis in the spleen seen on day 1 contained massive numbers of neutrophils. By day 2, the lesions in the red pulp of the spleen began to show some organization around the periphery, with the center of the lesion consisting primarily of polymorphs together with a great deal of cell debris and amorphous eosinophilic material. The liver showed signs of massive focal infiltration by polymorphs, whereas a similar infiltrate in parts of the lung was of such an extent that the alveolar morphology of the area was destroyed. By day 7, the lesions in the lungs, liver, and spleen were much enlarged and necrotic. Throughout the course of the intravenous infection, no lesions were observed in the kidneys or peripheral lymph nodes, with the exception of one animal which showed infiltration of one of the lumbar nodes, probably due to a subcutaneous infection in the tail during the intravenous challenge. The intestinal tissue, mesenteric lymph nodes, and Peyer's patches were free from any sign of infection in these animals.

Intravenous *Yersinia* infection gave rise to a leukocytosis consisting of neutrophils and monocytes. After an initial depression seen in animals autopsied on day 2, due principally to a decrease in peripheral blood lymphocytes, the total leukocyte count increased over the next few days, but by day 7 it was again depressed in the one surviving mouse. Intravenous inoculation resulted in a shift toward more immature cells in the differential leukocyte counts of animals autopsied after day 3. The serum antibody titer against *Y. enterocolitica* was less than 1:4 in all animals up to day 7, when a titer of 1:8 was detected in the hemagglutination test. Significant titers were noted in a separate set of animals surviving a sublethal infection to day 30 postinfection (Table 1).

Oral infection. To produce an infection more similar in its pathogenesis to the human infection, male CD-1 mice were challenged intragastrically with approximately 10^8 *Y. enterocolitica* WA (0.5 LD_{50}). In the first few days of the infection, the Peyer's patches in the distal ileum showed signs of swelling and hyperemia, whereas all other tissues appeared normal. By day 5, the mesenteric lymph nodes were swollen

TABLE 1. *Serological response to Y. enterocolitica WA infection in CD-1 mice*

Route of infection	Time postinfection (days)	Animal no.	Serum titer	
			CDC PHA test[a]	Merthiolate-killed *Y. enterocolitica* WA
Intravenous	1	1-3	<1:4	<1:4
	2	4-6	<1:4	<1:4
	3	7-9	<1:4	<1:4
	4	10-12	<1:4	<1:4
	7	13	1:8	<1:4
	30	14	1:64	1:128
		15	<1:4	1:512
		16	<1:4	1:128
Intragastric	1	1-3	<1:4	<1:4
	2	4-6	<1:4	<1:4
	5	7-9	<1:4	<1:4
	7	10	<1:4	<1:4
		11	<1:4	<1:4
		12	1:16	<1:4
	9	13-15	<1:4	<1:4
	12	16	1:8	<1:4
		17	<1:4	<1:4
		18	<1:4	<1:4
	14	19	1:32	<1:4
		20	1:4	<1:4
		21	<1:4	<1:4
	16	22	1:32	<1:4
		23	<1:4	1:4
		24	<1:4	1:16

[a] PHA, Passive hemagglutination.

and macroscopic lesions which resembled those seen in the intravenously infected mice were apparent in the spleen. By day 12, large lesions were visible in the liver, spleen, and lungs, the latter being associated with enlargement of the mediastinal lymph nodes. The Peyer's patches became greatly enlarged and suppurative (Fig. 1), and by 2 weeks some of them had ulcerated into the peritoneal cavity with adhesions to the peritoneal wall. The mesenteric lymph node was hemorrhagic and also showed a great deal of suppuration. The thymus at that time was greatly reduced in size. Histologically, evidence of infection could be observed as early as 24 h,

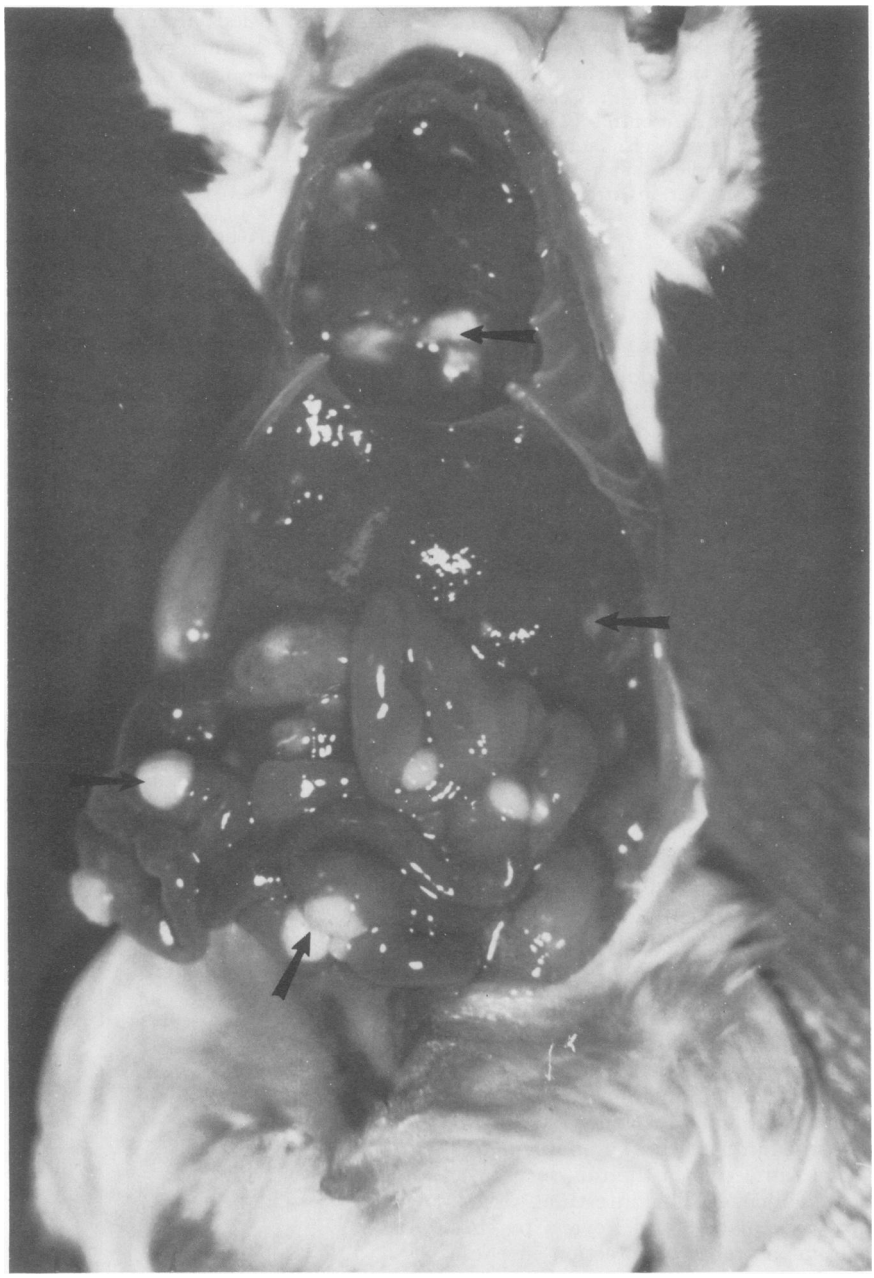

FIG. 1. *Gross pathology of Y. enterocolitica infection in orally infected CD-1 mouse at day 10; the Peyer's patches, liver, spleen, and lungs are abscessed (arrows)* (×2).

with the Peyer's patches in the ileum showing aggregations of neutrophils between the mucosa and the lymphoid nodule (Fig. 2). There was also a marked influx of polymorphs into the lamina propria of the mucosa surrounding the Peyer's patches. The lamina propria in the intestinal mucosa opposite the side of the intestine containing the Peyer's patch was free from polymorphonuclear infiltration, and sections of small intestinal wall not containing Peyer's patches also showed no sign of infection. The Peyer's patches in the duodenum and the upper part of the small intestine generally appeared to be uninfected. However, the cecal Peyer's patch in several of the animals was heavily infected in the same manner as the Peyer's patches in the distal ileum. By day 5, the normal architecture of the Peyer's patches in the ileum had been completely destroyed and, in some instances, abscesses were observed to ulcerate into the lumen of the intestine (Fig. 3). Lesions were also observed at this time in the medullary portion of the mesenteric lymph nodes. The lesions were characterized by an eosinophilic, necrotic center of cell debris and amorphous material. Similar lesions were also observed to occur within the spleen and liver, and, in each case, the infiltrate was almost exclusively neutrophil in nature. The lung pathology appeared as a typical suppurative pneumonia with polymorphonuclear infiltration and exudation into the alveoli and with abscesses frequently occurring. The mediastinal nodes began to undergo a pyroninophilic response, ultimately turning the nodes of some of the animals into sacks of plasma cells. In animals surviving to day 16, the spleen showed hypertrophy but no longer any overt signs of necrosis; however, polymorphonuclear infiltration of the red pulp was still present. This finding may be accounted for by the fact that the sections of spleen were distant from the sites of abscess formation, which at the time was grossly characterized by the presence of only one or two large abscesses in the proximal portion of the organ. The large number of small sites of infiltration grossly visible early in the infection appeared to have disintegrated. The liver likewise was free of necrotic tissue but had many large areas of perivascular infiltration comprised of polymorphs and mononuclear cells. The exudate in the alveoli observed early in the infection cleared; however, the alveolar walls of the lungs were congested with accumulated polymorphs, suggesting either an interstitial or resolving pneumonia. Throughout the infection, the colon remained free of any sign of

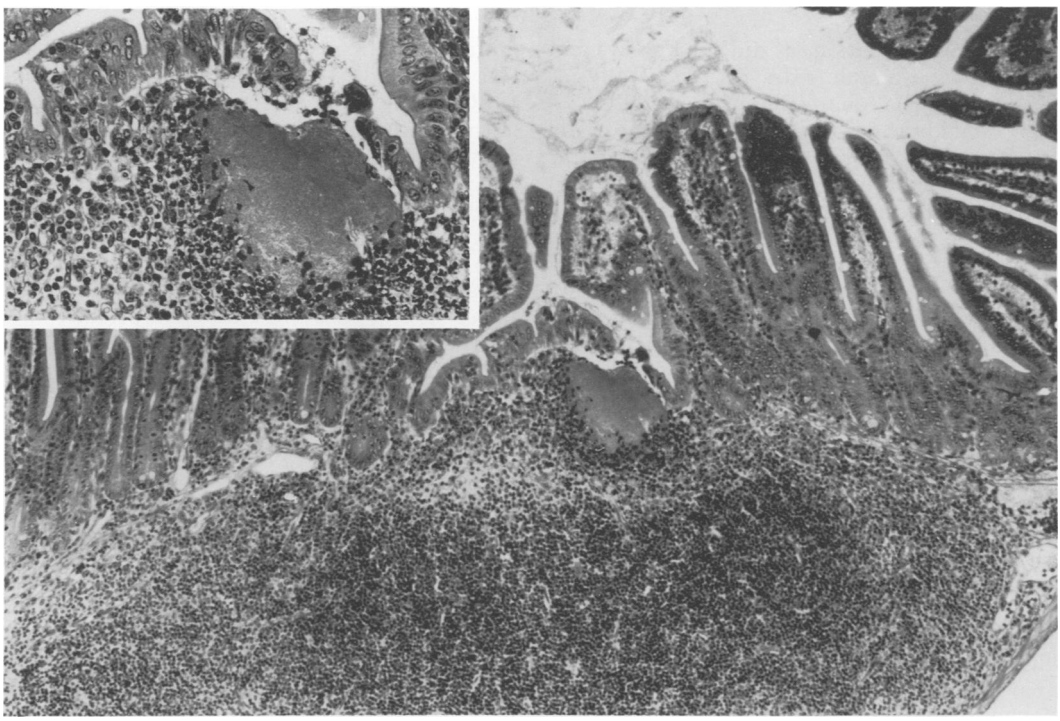

FIG. 2. *Twenty-four-hour lesion in ileal Peyer's patch of orally infected mouse (H & E, ×50). Inset: Higher magnification of lesion; infiltration is polymorphonuclear in nature (H & E, ×100).*

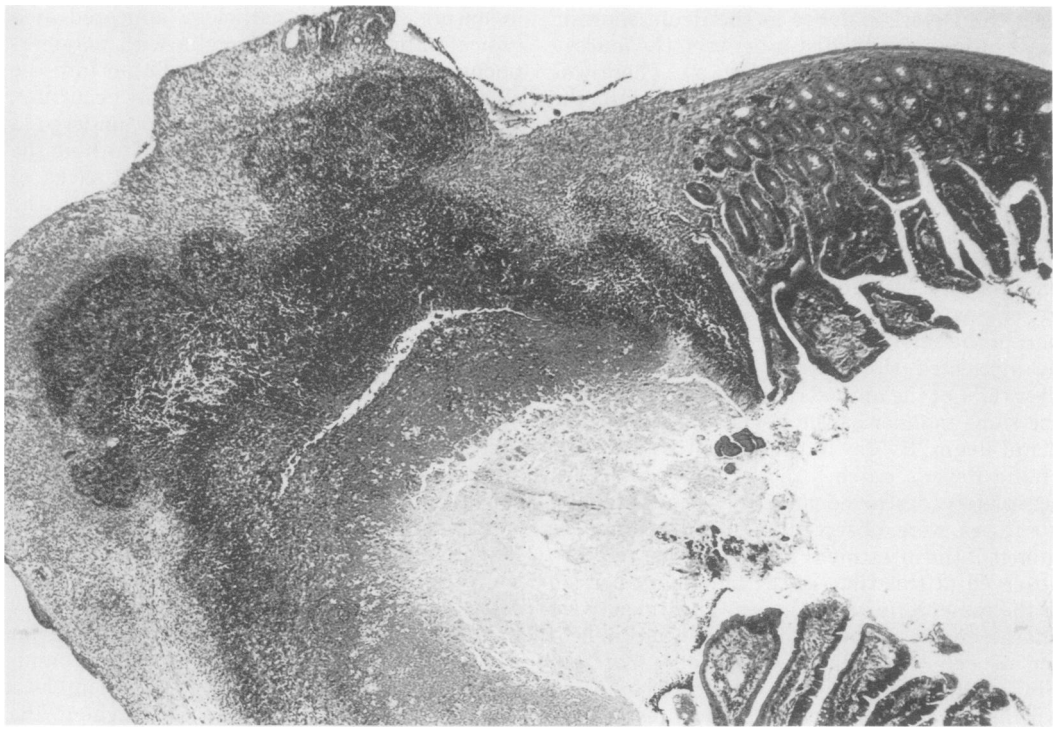

FIG. 3. *Peyer's patch lesion at 14 days after oral infection; the normal architecture of the lymphoid nodule is destroyed, and the abscess has ulcerated into the intestinal lumen (H & E, ×28).*

infection or infiltration, as did the kidneys and the peripheral lymph nodes.

After an initial depression of the total leukocyte counts in the peripheral blood of the orally infected mice, an increase was observed to occur over the succeeding weeks until the count surpassed 35,000/mm^2 in some of the animals. There was also a marked increase in the percentage of neutrophils in the peripheral blood and a significant number of immature cells by day 14 postinfection. Though not quantitated, an increased number of reticulocytes was observed throughout the infection. There was no significant increase in antibody titers in any of the infected animals up to 16 days postinfection. However, sera taken from mice surviving oral infections to day 30 did show significant agglutinin titers, comparable to those observed in intravenously infected mice at day 30.

Cause of death. The cause of death was uncertain, but the possibility that toxin production could be implicated was investigated. For this purpose, a putative toxin was prepared from *Y. enterocolitica* WA cells grown at 25 or 37 C in the manner outlined by Schar and Thal (20) for *Y. pseudotuberculosis* toxin. Twofold dilutions of the material were made so that up to 1 mg was injected intravenously into 5-week-old CD-1 mice. No deaths were observed in any of the groups, and obvious illness only occurred in mice receiving the two highest concentrations of material obtained from those bacteria cultured at 25 C.

Injection of culture filtrates intravenously into CD-1 mice did not produce any illness, and intradermal inoculation of these culture filtrates into guinea pigs did not produce any dermal necrosis.

A crude comparison of the endotoxin content of *Y. enterocolitica* WA and *Salmonella typhi* Ty2 was made by intravenously injecting CD-1 mice with heat-killed (56 C for 2 h) bacteria. The LD_{50}'s for *Y. enterocolitica* WA grown at 25 C and *S. typhi* Ty2 were identical, both equal to 2,200 μg of killed cells or approximately 10^{10} organisms. The LD_{50} of *Y. enterocolitica* WA cultured at 37 C was not reached with doses used but was well over 2,500 μg. Even so, endotoxic shock cannot be discounted and may be the ultimate cause of death in *Y. enterocolitica*-infected mice, since the number of viable bacteria in the organs of moribund mice surpasses 9 logs (2).

DISCUSSION

The results presented demonstrate the exceptional pathological similarity between the naturally acquired infection in man (6) and that induced in mice with the same strain of *Y. enterocolitica*. Observations of gut pathology support the idea that *Y. enterocolitica* alone could be an etiologic agent of regional enteritis and mesenteric lymphadenitis in man, a fact long suspected but still not unequivocally demonstrated for man (8). The disease in mice is a quickly developing and accelerating infection giving rise to well-organized abscesses of neutrophils and cell debris.

The initial site of infection after the oral challenge of CD-1 mice occurs in the Peyer's patches of the distal ileum, the early lesion being a collection of neutrophils within the lymphoid nodules just beneath the mucosal wall. The abscess then enlarges, obliterating the normal architecture of the Peyer's patch, and leads to infection of the abdominal organs and the lungs. The pathology of the infection is almost identical to that seen when systemic infection is brought about by intravenous inoculation. The major difference between the intravenous and intragastric infections seems to be a noninvolvement of the intestinal tract, Peyer's patches, and mesenteric lymph nodes in the former case. The more severe involvement of the liver, spleen, and lungs in the intravenously infected mice may be attributed to the size of the challenge infection used to give a standardized response in all of the mice. Preliminary data on the intravenous infection indicated that death or survival is determined very early in the infection, with half of the animals receiving an LD_{50} dose showing no pathological changes shortly after challenge. When mice are infected intragastrically, however, doses below an LD_{50} regularly give rise to a uniform gut infection, though the extent of the subsequent systemic infection varies considerably.

The pathology observed in the orally infected mouse is very similar to that observed with *Y. pseudotuberculosis* in guinea pigs (13) and in man (1, 5, 7, 12, 22). As in pseudotuberculosis infection, the primary lesions appear as abscesses in the Peyer's patches and caseous necrosis of the mesenteric lymph nodes. Another feature held in common with pseudotuberculosis infection is the absence of granulomas.

The cause of death remains obscure. No toxin production could be detected in *Y. enterocolitica* cultures, and the endotoxin from the organism grown at 37 C seems to be less potent, on a weight for weight basis, than that produced by *S. typhi*. The lower endotoxin content of *Y. enterocolitica* growing at 37 C may still be biologically important when the total number of bacteria present in the tissues of moribund animals is considered (2). Investigators who have used the intraperitoneal route of challenge (17, 19) report that the majority of deaths occurs in 24 to 48 h with abdominal hemorrhage being the predominant pathology, both being characteristic of acute endotoxicosis. However, these observations do not negate the possibility of death due to other causes. Peritonitis, gastrointestinal hemorrhage, liver failure, or pulmonary insufficiency may also contribute to a fatal outcome.

The most useful antigen in titering the mouse sera was the merthiolate-killed *Y. enterocolitica* WA. The CDC passive hemagglutination test, gave rather erratic results with the mouse sera. Autoclaved whole cells gave very poor results and were not included in the data in Table 1.

The essential points of the pathology reported here have been independently confirmed by others (T. J. Quan and B. W. Hudson, personal communication). It therefore appears that some *Y. enterocolitica* strains are indeed capable of infecting laboratory animals and can produce an infection similar to that observed in man. The present experimental animal model has obvious value in the development of diagnostic tests, therapeutic regimens, detailed studies of the pathogenic mechanisms, and the immune response to this increasingly important human disease.

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