A SIMPLE ADULT-MOUSE TEST FOR TISSUE INVASIVENESS IN *YERSINIA ENTEROCOLITICA* STRAINS OF LOW EXPERIMENTAL VIRULENCE

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SUMMARY. The virulence of *Yersinia enterocolitica* depends on the presence of a 70-kilobase plasmid, called the Vwa plasmid. This situation is particularly favourable for studies of the mechanism of pathogenicity, but these are hindered by the lack of a suitable animal test to monitor the virulence of the human-pathogenic strains isolated outside the USA which belong to serogroups O:3, O:9 and O:5,27. We observed that, after oral administration to the mouse, the Vwa-positive strains of these serogroups produce a discrete systemic infection while the Vwa-negative strains do not. We present here a simple mouse-virulence test based on this observation.

INTRODUCTION

Virulent strains of *Yersinia enterocolitica* harbour a plasmid of about 70 kilobases (kb) (Gemski *et al.*, 1980; Zink *et al.*, 1980) that belongs to the incompatibility group FI (Bakour *et al.*, 1983). The presence of this plasmid, now referred to as the Vwa plasmid, conditions the virulence of *Y. enterocolitica*, as determined for serogroup-8 strains by invasion of the conjunctival epithelium of guinea-pigs (the Serény test) or by lethality for mice (Gemski *et al.*, 1980).

This 70-kb plasmid encodes calcium dependency at 37°C (Higushi and Smith, 1961; Gemski *et al.*, 1980; Portnoy *et al.*, 1984), autoagglutinability at the same temperature (Laird and Cavanaugh, 1980), and resistance to the bactericidal activity of serum (Pai and DeStephano, 1982). The 70-kb plasmid of serogroup-8 strains also determines the presence in them of the V and W antigens of the plague bacillus (Carter *et al.*, 1980; Perry and Brubaker, 1983) and at least four major outer-membrane proteins (Bolin *et al.*, 1982; Portnoy *et al.*, 1984). However, the invasiveness of *Y. enterocolitica* for HeLa cells (Lee *et al.*, 1977; Une, 1977b) is not dependent on this 70-kb plasmid (Schiemann and Devenish, 1982). Similarly, it does not condition invasiveness of *Y. enterocolitica* for human epithelial-tissue cells (HEp-2) but it codes for the detachment of HEp-2 monolayers (Portnoy *et al.*, 1981) and for the adherence of the bacteria to these cells (Vesikari *et al.*, 1981). A second plasmid (120 kb) is said to be concerned in the

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virulence of strains of serogroups O:8, O:13, 18, O:20 and O:40 (Kay *et al.*, 1982), but will not be considered further in this paper.

A genetic analysis of virulence clearly requires a cheap and easy animal test. Several such tests are available for the aesculin-negative, biotype-1 American strains that belong mainly to serogroup O:8: the Serény test is positive (Feeley *et al.*, 1979; Gemski *et al.*, 1980; Schiemann and Devenish, 1980; Zink *et al.*, 1980) and mice (Carter and Collins, 1974; Quan *et al.*, 1974; Carter, 1975) or gerbils (Wetzler *et al.*, 1968; Quan *et al.*, 1974; Schiemann and Devenish, 1980) die after oral or intraperitoneal inoculation of calcium-dependent cells. However, these tests are inadequate for the human-pathogenic strains isolated outside the USA that belong to serogroups O:3, O:9 and O:5,27, as well as for members of serogroups O:1 and O:2 that are pathogenic for some animals (Wauters, 1970); these strains fail to evoke a keratoconjunctivitis in guinea-pigs (Mollaret and Guillon, 1965; Mors and Pai, 1980; Schiemann *et al.*, 1981; Vesikari *et al.*, 1981) and they do not cause death or clearly identifiable clinical illness in mice (Mollaret and Guillon, 1965; Alonso *et al.*, 1975; Pai and DeStephano, 1982; Aulisio *et al.*, 1983) or gerbils (Schiemann and Devenish, 1980) when given by any route. Nevertheless, in mice, these low-virulence strains are excreted for a long period after oral inoculation (Maruyama *et al.*, 1979; Pearson *et al.*, 1979; Kaneko and Hashimoto, 1983), induce a mild diarrhoea (Laird and Cavanaugh, 1980; Schiemann *et al.*, 1981) and multiply in Peyer's patches (Pai and DeStephano, 1982). The virulence of these strains can be assayed by the intraperitoneal or oral route in nude mice (Alonso *et al.*, 1975), the oral route in axenic mice (Bercovier *et al.*, 1976) or the intraperitoneal route in suckling mice (Aulisio *et al.*, 1983). The addition of iron dextran to an intraperitoneal inoculum leads to some deaths in mice given serogroup-3 or -9 strains, but this is not clearly related to the presence of the 70-kb plasmid (Smith *et al.*, 1981).

We observed that the tissue invasiveness of the "low-virulence" *Y. enterocolitica* strains can be assayed in a simple adult-mouse test: after oral administration to TB (Platteau and Bazin, 1978) or NMRI mice, calcium-dependent strains invade the spleen while calcium-independent isogenic strains do not. This was observed with strains of serogroups O:1, O:2, O:3, O:9 and O:5, 27.

MATERIALS AND METHODS

Bacterial strains. *Y. enterocolitica* strains are listed in table I. All these strains were recloned and tested for calcium dependency, autoagglutinability and presence of the Vwa plasmid. Calcium-independent variants were selected for all the strains that were originally calcium dependent by plating at 37°C on magnesium oxalate (MOX) agar (Higushi and Smith, 1961; Schiemann and Devenish, 1980).

Serogrouping. All *Y. enterocolitica* strains were serotyped by the standard slide-agglutination procedure with type-specific antisera prepared in rabbits (Wauters *et al.*, 1971).

Autoagglutination test. Autoagglutination was detected by comparing overnight cultures grown at 37°C and 28°C in Tryptic Soy Broth (TSB; Difco).

Calcium dependency. This test was performed by plating at 37°C, spots of 20 µl of a 1 in 100 dilution of an overnight culture grown at 28°C, on to Tryptic Soy Agar (TSA) (Difco) and MOX agar as described by Schiemann and Devenish (1980).

Vwa-plasmid detection. This was done essentially as described by Kado and Liu (1981). Lysates were incubated for 45 min at 65°C. Several strains were also analysed by the method described by Cornelis *et al.* (1981). Agarose-gel electrophoresis was performed in vertical 0.8% gels at 30 V for 20 h. Preparations of the Vwa plasmid of strain 439-80, which was fully

TABLE I
Strains of Yersinia studied

Strain no.	Serogroup (O antigens)	Biogroup	Calcium dependency, autoagglutinability, Vwa plasmid present	Origin	Source or reference
<i>Y. enterocolitica</i>					
T1758	1	3	+	Beaver	S. Toma: Toronto, Canada
IP38	2	5	+	Hare	Yersinia Centre, Paris
W774	3	4	+	Human faeces	This laboratory
W783	3	4	+	Human faeces	This laboratory
W804	3	4	+	Human faeces	This laboratory
W835	3	4	+	Human faeces	This laboratory
W845	3	4	+	Human faeces	This laboratory
L11	3	4	+	Pig's tongue	This laboratory
338-80	5	1	—	Human faeces	R. Van Noyen: Belgium
35	5	1	—	Pig's tongue	This laboratory
708-82	5,27	2	+	Human faeces	G. Ghysels: Belgium
605-31	5,27	2	+	Human faeces	J. Goudswaard: Netherlands
25	6	1	—	Pig's tongue	This laboratory
300-82	6,30	1	—	Human faeces	Coninx: Belgium
Y440	7,8	1	—	Human faeces	R. Van Noyen: Belgium
439-80	9	2	+	Human faeces	G. Ghysels: Belgium
W22708	9	2	+	Human faeces	G. Cornelis and C. Colson (1975)
W818	9	2	+	Human faeces	This laboratory
W830	9	2	+	Human faeces	This laboratory
W836	9	2	+	Human faeces	This laboratory
S6	9	2	+	Pig's tongue	This laboratory
301-82	41	1	—	Human faeces	Coninx: Belgium
<i>Y. frederikseni</i>					
S42	3		—	Human faeces	This laboratory
<i>Y. kristensenii</i>					
Y438	12,25		—	Human faeces	R. Van Noyen: Belgium

characterised by Laroche *et al.* (1984), were included in all experiments. Bands corresponding in position with that given by the reference plasmid were taken to indicate the presence of the Vwa plasmid.

Mice. The tests were carried out on adult (2–3-months-old) inbred TB mice (Platteau and Bazin, 1978) and on outbred NMRI mice.

Standard invasiveness test. *Y. enterocolitica* strains were grown at 28°C in TSB with shaking at 150 rpm in conical flasks with four baffles. Bacteria were harvested, washed and resuspended in unsterilised drinking water. The OD₆₆₀ of the suspension was adjusted to 200 Klett units, which corresponds to 10⁹ bacteria/ml.

Batches of three or four NMRI mice in 15 cm × 21 cm × 13 cm cages with 75 g of woodshaving bedding were deprived of water for 24 h (starting at 6 p.m.), and then each batch was allowed to drink *ad libitum* for 48 h from 100 ml of the bacterial suspension. After this, the mice were allowed free access to drinking water for one night. On the next day, the mice were killed with ether and the spleens were removed aseptically. Each spleen was placed in 10 ml of saline and disrupted by a treatment of 15–30 s with an Ultra-turrax TP18 disrupter (instrument also called Takmar in the USA; Janke and Kunkel, W. Germany) equipped with the 18K shaft. The bacterial content of the spleen was concentrated by spinning at 1875 *g* for 30 min, resuspended in 0.3 ml of saline and plated, eventually after dilution, on MacConkey's agar.

Antibody detection. TB mice were deprived of water for 24 h and then allowed to drink a suspension of 10⁹ *Y. enterocolitica*/ml for 24 h. They were then placed in a cold room (4°C) for 6 days and killed on the 7th day after inoculation. The mice were bled from the heart under ether anaesthesia. After coagulation, the serum was used in slide- and tube-agglutination tests. For the latter test, the bacterial suspension was treated with merthiolate (0.2% final concentration) and adjusted to a density of MacFarland No. 2 standard.

RESULTS

Recovery of Vwa-positive strains from the spleen

Preliminary observations (data not shown) revealed that Vwa-positive (Vwa⁺) strain 439-80 did not cause death or illness in TB mice after oral inoculation but invaded deep organs such as the spleen and the liver. Further experiments were performed with this strain to define the optimal conditions for detection of bacteria in the spleen. Some batches of TB mice were placed at 4°C to assess the effect of slight stress on the recovery of *Y. enterocolitica* from the spleen. Mice, 3–4 per cage (as described in *Materials and methods*) were placed for 6 days, without previous acclimatization, at 4°C in a large lit cold-room, after they had drunk (at room temperature) the bacterial suspension for 24 h. The stay at 4°C seemed to increase the number of bacteria recovered from the spleen, and day 7 appeared to be the most appropriate one to kill the mice. Under these conditions, the Vwa⁺ strain 439-80 was always recovered from the spleen while the Vwa-negative (Vwa⁻) variant of the same strain was detected in only two of 17 mice and in much lower number (table II). Under the same conditions, the test was then applied to the 16 strains of *Y. enterocolitica* from serogroups O:1, O:2, O:3, O:5, 27 and O:9. All these strains and their calcium-independent variants were also checked for the presence of the Vwa plasmid by agar-gel electrophoresis. Calcium dependency was in each case associated with the presence of a plasmid of about 70 kb. In addition, strain IP38 (O:2) was found to contain a second plasmid of higher mol. wt. As shown in table II, all the Vwa⁺ strains invaded the spleen (60 out of 62 mice). Counts were generally in the range of 1000 bacteria per spleen but with great variations between individual mice of the same batch. On the contrary, the Vwa⁻ strains could only be recovered from the spleens of 3 out of 61 mice and the counts were extremely low in these exceptional cases (< 10 bacteria per spleen).

Besides these strains of low experimental virulence, six aesculin-positive *Y. enterocolitica* strains from biogroup 1 and two strains recently classified as *Y. frederikseni* and *Y. kristenseni* (Bercovier *et al.*, 1980) were also tested. Such strains have never been found, up to now, to be calcium dependent. None of them harboured a 70-kb plasmid. However, strain 301-82 (O:41) has a plasmid clearly smaller than 70 kb and the *Y. frederikseni* strain S42 has also a plasmid, larger than 70 kb (data not shown). None of these strains was found to invade the spleen of the mice: only one colony was recovered from the spleen of 32 mice (table II).

To determine whether this observation requires the particular TB mouse strain, we repeated the experiment with outbred NMRI mice. The animals were allowed to swallow the bacterial suspension for 48 h and then placed at 4°C for the duration of the experiment. The animals were killed on day 3, 5 or 7 after they started drinking the bacterial suspension. As shown in table III, the invasion of the spleen by Vwa⁺ strains was clearly demonstrated.

For ethical reasons, the need for keeping the animals at 4°C was re-evaluated. Comparative experiments were performed with NMRI mice. After drinking the bacterial suspension for 48 h, some batches of mice were placed in the cold room and some batches were kept at room temperature as described in the standard procedure. The comparative experiment was done with six different strains of *Y. enterocolitica* from four different serogroups (table IV). The test gave satisfactory results when the

TABLE II

Invasion of the spleen by strains of Yersinia bearing the Vwa plasmid (Vwa⁺), and by Vwa-negative variants or strains (Vwa⁻), in TB mice kept at 4°C

Strain no.	Serogroup	Vwa ⁺ form Number of bacteria/spleen of mouse no.				Vwa ⁻ form Number of bacteria/spleen of mouse no.			
		1	2	3	4	1	2	3	4
<i>Y. enterocolitica</i>									
439-80	9	3 × 10 ⁴	5 × 10 ⁴	1 × 10 ⁵	...	0	0	0	...
		1 × 10 ⁵	5 × 10 ⁴	2 × 10 ⁶	3 × 10 ⁵	0	0	0	0
		1 × 10 ⁴	1 × 10 ³	2 × 10 ⁴	...	0	0	0	...
		8 × 10 ³	8 × 10 ³	4 × 10 ³	2 × 10 ⁵	0	0	0	7
		7 × 10 ¹	6 × 10 ¹	2 × 10 ²	...	0	0	1	...
W22708	9	3 × 10 ¹	2 × 10 ³	5 × 10 ³	...	0	0	0	...
W818	9	3 × 10 ²	3 × 10 ²	1 × 10 ³	...	0	0	0	...
W830	9	1 × 10 ⁴	2 × 10 ⁴	1 × 10 ³	...	0	0	0	...
W836	9	1 × 10 ⁵	1 × 10 ³	1 × 10 ⁵	...	0	0	0	...
S6	9	1 × 10 ⁴	1 × 10 ⁴	1 × 10 ³	...	0	0	0	...
W774	3	1 × 10 ⁵	6 × 10 ⁵	3 × 10 ⁵	...	0	0	x	...
W783	3	8	2 × 10 ³	2 × 10 ²	...	0	0	0	...
W804	3	5 × 10 ⁵	6 × 10 ³	5 × 10 ²	...	0	0	0	...
W835	3	6	6	1 × 10 ³	...	0	0	0	...
W845	3	1 × 10 ³	1 × 10 ⁶	7 × 10 ²	...	0	0	8	...
L11	3	1 × 10 ²	1 × 10 ²	1 × 10 ³	...	0	0	0	...
T1758	1	4 × 10 ²	5 × 10 ²	6 × 10 ³	...	0	0	0	...
IP38	2	0	5 × 10 ²	2 × 10 ¹	...	0	0	0	...
708-82	5,27	0	5 × 10 ¹	5 × 10 ²	...	0	0	0	...
605-31	5,27	7 × 10 ²	9 × 10 ¹	3 × 10 ²	...	0	0	0	...
338-80	5	...	...	...	...	0	0	0	0
35	5	...	...	...	...	0	0	0	0
25	6	...	...	...	...	0	0	0	0
300-82	6,39	...	...	...	...	0	0	0	0
Y440	7,8	...	...	...	...	0	0	0	1
301-82	41	...	...	...	...	0	0	0	0
<i>Y. frederikseni</i>									
S42	3	...	...	...	...	0	0	0	0
<i>Y. kristenseni</i>									
Y438	12,25	...	...	...	...	0	0	0	0

x = Mouse died before day 7.

TABLE III

Invasion of the spleen by Vwa⁺ and Vwa⁻ forms of two strains of Y. enterocolitica in NMRI mice kept at 4°C and killed 3, 5 or 7 days after infection

Strain no.	Serogroup	Killed on day	Vwa ⁺ form Number of bacteria/spleen in mouse no.				Vwa ⁻ form Number of bacteria/spleen in mouse no.			
			1	2	3	4	1	2	3	4
439-80	9	3	2 × 10 ⁴	1 × 10 ⁴	3 × 10 ²	0	0	0	0	0
		5	7 × 10 ³	1 × 10 ²	5 × 10 ²	8 × 10 ²	0	0	0	0
		7	1 × 10 ³	5 × 10 ²	3 × 10 ²	5 × 10 ²	0	0	0	6
W774	3	3	6 × 10 ³	9 × 10 ²	2 × 10 ²	3 × 10 ²	0	0	0	0
		5	3 × 10 ³	3 × 10 ³	2 × 10 ⁴	3 × 10 ³	0	0	0	0
		7	1 × 10 ³	1 × 10 ³	1 × 10 ³	1 × 10 ³	0	0	0	0

TABLE IV
Effect of ambient temperature on invasion of the spleen by *Y. enterocolitica* in NMRI mice

Strain no.	Serogroup	Temperature	Vwa ⁺ form								Vwa ⁻ form							
			Number of bacteria/spleen in mouse no.								Number of bacteria/spleen in mouse no.							
			1	2	3	4	5	6	7	8	$\bar{m} \log \pm \sigma^*$	1	2	3	4	5	6	7
439-80	9	4°C RT	184 145	381 30	76 211	111 274	17 645	39 16	53 192	0 151	1.66 ± 0.79 2.1 ± 0.52	0 0	0 0	0 0	0 0	0 0	0 0	0 0
W22708	9	4°C RT	13 20	32 71	60 460	83 15	83 1	327 900	600 900	3 × 10 ³ 0	2.18 ± 0.89 1.61 ± 1.21	0 0	0 0	0 0	0 0	0 0	0 0	0 8
W774	3	4°C RT	1 × 10 ⁴ 1 × 10 ⁴	1 × 10 ³ 3 × 10 ³	1 × 10 ⁴ 7 × 10 ²	5 × 10 ³ 2 × 10 ³	3 × 10 ³ 1 × 10 ³	2 × 10 ³ 4 × 10 ³	1 × 10 ³ 1 × 10 ³	4 × 10 ³ 2 × 10 ³	3.53 ± 0.39 3.19 ± 0.52	0 0	0 0	0 0	0 0	0 0	0 0	0 0
W783	3	4°C RT	900 700	2 × 10 ³ 60	2 × 10 ³ 27	2 × 10 ³ 14	6 × 10 ³ 600	1 × 10 ⁴ 2 × 10 ³	300 2 × 10 ³	1 × 10 ³ 1 × 10 ³	3.32 ± 0.51 2.48 ± 0.9	0 0	0 0	0 0	0 0	0 0	0 0	0 21
605-31	5,27	4°C RT	1 × 10 ³ 300	420 200	300 150	300 82	70 65	42 15	800 1 × 10 ³	23 3 × 10 ³	2.29 ± 0.61 2.28 ± 0.71	0 0	0 0	0 0	0 0	0 0	0 0	0 6
T1758	1	4°C RT	3 12	300 1 × 10 ³	10 0	32 30	1 0	15 0	470 2	4 × 10 ³ 2 × 10 ³	1.61 ± 1.21 1.14 ± 1.35	0 0	0 0	0 0	0 0	0 0	0 0	0 0

RT = room temperature.

* The mean was calculated on the log of the number of bacteria; for this calculation, sterile spleens were given a value of one organism.

animals were kept at room temperature. In this experiment strain T1758 (serogroup 1) gave somewhat lower mean counts than those shown in table I, irrespective of the temperature conditions. This difference may perhaps be attributed to the different mouse strains used.

Pathological examination

The invasion of the spleen was confirmed by histological examination. Three positive spleens of NMRI mice that yielded *c.* 10 000 *Y. enterocolitica* of strain 439-80 per spleen showed focal accumulation of clusters of histiocytic cells in the red pulp, without necrosis (data not shown).

Antibody production

The serum of TB mice infected with Vwa⁺ and Vwa⁻ cultures of *Y. enterocolitica* strain 439-80, kept at 4°C, and killed on day 7, agglutinated the homologous strain. Titres were in the range of 100, and there was no significant difference between mice infected with the Vwa⁺ or Vwa⁻ cultures.

Recovery of Y. enterocolitica from the mesenteric lymph nodes

The detection of anti-*Y. enterocolitica* antibodies in the sera of TB mice infected with the Vwa⁻ strain 439-80 prompted us to look for yersiniae in the mesenteric lymph nodes. In 11 experiments with serogroup O:3 and O:9 strains, mesenteric lymph nodes were collected, ground and inoculated on to plates of MacConkey's agar. Bacteria were recovered from 27 out of 35 lymph nodes from mice infected with Vwa⁺ strains and from 19 of 34 lymph nodes of mice infected with the Vwa⁻ strains.

DISCUSSION

In the conditions described, *Y. enterocolitica* strains of "low experimental virulence" cross the intestinal barrier of the mouse, reach the mesenteric lymph nodes and elicit a weak humoral immune response, independently of the presence of the Vwa plasmid. Vwa⁺ strains in addition produce an asymptomatic systemic infection and can be detected, between days 3 and 7 after inoculation, in the spleen. The histological examination of the spleen revealed focal accumulation of histiocytic cells but no clear abscesses.

To our knowledge, and quite surprisingly, this has not been described before. Mollaret and Guillon (1965) and, later, Alonso *et al.* (1975) examined the spleens of mice infected orally with *Y. enterocolitica* of serotypes O:3 and O:9. These authors observed no morbidity and, unlike us, found that the spleens were sterile; however, their examinations were made at days 30 or 90 instead of days 3-7. Moreover, these studies were conducted before the Vwa plasmid had been described and it is not known whether or not the strains used had the Vwa plasmid. Since the description of this plasmid (Gemski *et al.*, 1980; Zink *et al.*, 1980), a few authors have studied again the experimental pathogenicity of the *Y. enterocolitica* strains of "low experimental virulence" in the adult mouse (Schiemann *et al.*, 1981; Smith *et al.*, 1981; DeStephano,

1982; Schiemann and Devenish, 1982; Aulisio *et al.*, 1983) but they focused on diarrhoea, long-term excretion or lethality.

The present observation thus shows that Vwa⁺ strains of "low experimental virulence", given orally, produce a discrete systemic infection in the mouse. This is in agreement with the observation of Une (1977a) that O:3 and O:9 strains, given intragastrally to the rabbit produced granulomas in the spleen, accompanied by bacterial localisation in this organ.

It is worth noting that the aesculin-positive strains from biogroup 1, and the *Y. frederikseni* and *Y. kristensenii* strains, behaved like the Vwa⁻ variants of the strains of low experimental virulence. This is not unexpected, because these strains are Vwa⁻ and were found to be avirulent in all the previous experimental studies (Une, 1977a, Schiemann *et al.*, 1981). Moreover, prevalence studies in healthy persons and in patients suffering from diarrhoea suggest that these strains are not pathogenic for man (Van Noyen *et al.*, 1981).

Our main purpose in studying experimental infection in the mouse was not the description of the physiopathology of the infected mouse but rather the establishment of an easy test to monitor invasiveness for use in molecular-biology studies of the pathogenicity of *Y. enterocolitica* O:3, O:9 and O:5,27. In particular, we are at present using this test to localise virulence genes on the Vwa plasmids of these strains. In some of the experiments reported in this paper the mice were kept at 4°C. It became clear from our later work that this is not necessary for the detection of invasiveness. We thus strongly recommend performing the test at room temperature as we have described. This test is thus comparable with other mouse tests, such as mouse-diarrhoea (MD) (Laird and Cavanaugh, 1980; Schiemann *et al.*, 1981), the measure of faecal excretion or intestinal colonization (Schiemann *et al.*, 1981; Pai and DeStephano, 1982), the observation of bacterial multiplication in the Peyer's patches (Pai and DeStephano, 1982) and lethality in 1–3-day-old mice (Aulisio *et al.*, 1983). In this respect, the spleen test appears to be particularly easy to perform and to interpret in comparison with some of the other tests. Although the MD test is probably simpler than the spleen test, some authors have found diarrhoea in mice difficult to define (Pai and DeStephano, 1982; Aulisio *et al.*, 1983). The molecular biologist would probably find the spleen easier to excise than the Peyer's patches. Maintaining a regular supply of suckling mice may well complicate the laboratory's mouse-breeding programme. The spleen test should perhaps be coupled with the measure of the ability to colonize the intestine (Ricciardi *et al.*, 1978; Pai and DeStephano, 1982) because these two tests reveal different aspects of the pathogenicity of *Y. enterocolitica* that might depend on different plasmid genes.

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