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Listeria monocytogenes Intragastric and Intraperitoneal Approximate 50% Lethal Doses for Mice Are Comparable, but Death Occurs Earlier by Intragastric Feeding

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The intraperitoneal (i.p.) and intragastric (i.g.) mouse approximate 50% lethal dose values (ALD_{50}s) were determined for 15 food and clinical isolates of *Listeria monocytogenes*. Although all strains gave i.g. ALD_{50}s comparable to or less than their i.p. ALD_{50}s, the i.g. feeding of most strains produced more deaths within the first 3 days of the 6-day test than did i.p. injection. ALD_{50}s ranged from 50 to 4.4×10^5 cells with approximately 1-log 95% confidence intervals. Of five strains tested by suspension in milk or by growth in milk, none gave i.g. ALD_{50}s that were lower than those of washed cells. Results with 10- to 21-g mice supported the use of 15-g mice for i.g. testing; 21-g mice were more resistant to i.g. infection. These results indicate that i.g. feeding permits an evaluation of the role of the carrier (such as milk) in the determination of listerial virulence, permits strain characterization by i.p. and i.g. ALD_{50}s, and emphasizes a potentially more rapid infection when the bacterium is introduced i.g.

Between 1981 and 1988, major outbreaks of listeriosis have emphasized the potential of *Listeria monocytogenes* as the causal agent of food-borne epidemics. Of four epidemics, occurring in Nova Scotia (16), Boston (6), Los Angeles (7), and Vaud, Switzerland (1), the last three were directly or retrospectively related to milk or milk products. In addition, as reviewed by Yousef et al. (19), numerous isolations of *L. monocytogenes* have been made from various cheeses.

Recently, we examined the growth of *Listeria* species in laboratory-sterilized pasteurized milk, pasteurized milk, raw milk, and commercially available sterile milk (14). During these experiments, we asked whether growth of *L. monocytogenes* strains in such substrates affected their relative virulence. Consequently, duplicate tubes of these milk cultures were stored at −60°C, and single-step isolates or whole-milk cultures were tested later for mouse virulence, using intraperitoneal (i.p.) inoculation of the isolated culture or intragastric (i.g.) feeding of the milk cultures themselves. Although milk in itself did not affect virulence, the results emphasized the value of direct comparisons of approximate 50% lethal doses (ALD_{50}s) obtained by i.p. and i.g. inoculations. ALD_{50} values by both routes of inoculation were comparable, but deaths occurred earlier with most strains when they were introduced orally. Such comparisons have not been described previously for this food-borne causal organism.

MATERIALS AND METHODS

Bacterial strains and culture media. The strains used and their characteristics are given in Table 1. For the preparation of standard washed-cell inocula, the strains were grown with shaking (70 rpm) at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) for 16 to 18 h. The cells were centrifuged, washed twice with sterile deionized water, and reconstituted in water to the original volume, giving approximately 10^9 CFU/ml. The suspensions were divided into 1.5-ml samples and frozen at −60°C. Standard inocula were used within 2 weeks after preparation unless stated otherwise. For preparation of infective doses, the inocula were rapidly thawed and diluted in 1/10 dilutions in phosphate-buffered saline (0.01 M, pH 7.2). The preparations were kept in ice until administered to the animals.

For preparation of laboratory-sterilized milk, 10-ml samples of 4% pasteurized milk in screw-cap test tubes (20 by 150 mm) were heated to 97 to 98°C for 1 min on 3 consecutive days as described previously (14). Cultures were prepared by inoculation with 10^5 cells per ml and incubation at 5°C for 30 days in a slant position. The cultures were shaken gently several times on alternate days. After the 30-day growth period, these cultures were stored at −60°C for approximately 9 months.

Zero-time CFU of each milk culture or standard inoculum was determined for each experiment by plating the inocula on tryptose blood agar base fortified with 0.1% each glucose and yeast extract.

Mouse inoculations. In general, 21-day-old (9.5- to 12-g), 25- to 26-day-old (13- to 17-g), 27-day-old (16- to 18-g), and 33-day-old (20- to 22-g) white female NCR mice were used. Animals were ordered on the basis of weight, were weighed daily, and were used at the weights indicated for the respective experiments. Inoculations were done at 48 h or later after receipt; this time was believed sufficient to adapt the mice to their surroundings. Mice were inoculated i.p. with 0.25 ml of inoculum, using a 1-ml tuberculin syringe fitted with a no. 22 1-in. (2.54-cm) needle. Four sequential 1/10 dilutions were used, one dilution per lot of either 5 or 10 mice. Similarly, for i.g. feeding, we used a no. 20 1.5-in. (3.81-cm) animal-feeding needle (Popper and Sons, Inc., New Hyde Park, N.Y.). The moistened feeding needle was slipped past the pharynx into the stomach, where the inoculum was injected. The mice were not anesthetized for this procedure. Care was taken not to injure the animals; no animals were used in which there was any indication of injury or inhalation of the inoculum.

Determination of ALD_{50}s. Only 5 to 10 mice were used per

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TABLE 1. Strains of *L. monocytogenes* used

Strain	Source[a]	Serotype	Isolated from:	ALD_{50}[b]
1778+H1b	ATCC 43249	1/2a	Guinea pig	3.0×10^5
1778−H1b	ATCC 43248	1/2a, 4b	Guinea pig	0.7×10^9
F2380	REW		Cheese, California epidemic	2.0×10^4
F2392	REW	4b	Cheese, California epidemic	NDP
F7243	REW	4b	Patient, California epidemic	2.1×10^6
F7245	REW	4b	Patient, California epidemic	1.3×10^6
F7208	REW	3a	Unknown	1.6×10^5
G9599	REW	4(?)	Patient	3.5×10^2
G1032	REW	4(?)	Patient	7.8×10^2
G2228	REW	1/2a	Patient	NDP
G2261	REW	1/2b	Raw filet mignon	NDP
G2618	REW	1/2a	Turkey franks	NDP
G2198	REW	1/2a	Turkey franks	NDP
F5738	BS	1/2a	Patient	NDP
F6646	BS	1/2b	Patient	NDP

[a] Cultures were received from American Type Culture Collection (ATCC) and from Robert E. Weaver (REW) and B. Swaminathan (BS), Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control.

[b] Published LD_{50}s are given in reference 14; NDP, not determined before the experiments reported here. Remaining ALD_{50}s were determined earlier. Three-week-old white female NCR mice (5 or 10 per 10^{-1} dilution) were inoculated i.p. with 0.5 ml of a phosphate-buffered saline suspension of bacterial cells, using a no. 22 1-in. needle. Values were determined on day 6 and were calculated by the Spearman-Karber method (5).

lot; classical LD_{50} values for which higher numbers of animals are used were not determined. The values are presented as ALD_{50}s. ALD_{50}s, variance, and 95% confidence intervals were calculated by the Spearman-Karber method as described elsewhere (5, 8). Calculations were based on a 6-day incubation period; deaths were recorded every 24 h, and cumulative deaths by a specific strain were determined by summing the deaths, on a given day, at all dilutions of inoculum. Curve differences were analyzed by both the Wilcoxon and log rank procedures (10).

RESULTS

Relative ALD_{50}s of *Listeria* strains before and after growth in laboratory-sterilized milk. In an initial experiment, *Listeria* strains were grown for 30 days at 5°C in laboratory-sterilized milk. These strains, isolated on tryptose blood agar base fortified with 0.1% yeast extract and 0.1% glucose, were then compared with the stock cultures for relative virulence by i.p. injection. Within the range of 1 logarithm, the ALD_{50} values were the same for both the stock culture and its milk-grown derivative (Table 2) and were not significantly different at the 0.05 level. The values observed were similar to those observed earlier (Table 1). However, in the case of strain 2392, the ALD_{50} of the milk-grown culture was significantly lower than that obtained with the stock tryptose blood agar-grown culture (Table 2). With strain 1778−H1b, a nonhemolytic avirulent mutant of 1778+H1b (15), no conversion back to virulence was found. Thus, prolonged growth in milk did not select for more virulent *Listeria* populations as tested by i.p. inoculation.

Determination of i.g. ALD_{50}s of washed cells and washed cells suspended in or grown in milk. A direct comparison of the i.g. ALD_{50}s was made of *L. monocytogenes* strains suspended in phosphate-buffered saline, suspended in nonsterilized pasteurized milk, or grown at 5°C for 34 days in the same lot of milk in which the strain was also suspended (Table 3). When five strains were tested by suspension in or growth in milk, none showed ALD_{50} values which were, at the 0.05 level of significance, smaller than those observed for the washed cells themselves. Overall, milk as a carrier of *Listeria* strains or as a selective substrate for growth did not

TABLE 3. Comparative mouse i.g. ALD_{50}s of *L. monocytogenes* strains each introduced i.g. as a culture grown in pasteurized milk, as a suspension in pasteurized milk, or as a washed cell suspension

Strain	Treatment	ALD_{50}	95% confidence interval
1778+H1b	Washed cells	1.48×10^5	4.48×10^4–7.28×10^5
	Added to milk	2.34×10^6	3.32×10^5–1.65×10^7
	Grown in milk	1.26×10^6	2.25×10^5–7.05×10^6
F2380	Washed cells	6.64×10^5	4.09×10^4–1.28×10^6
	Added to milk	3.63×10^5	5.14×10^4–2.56×10^6
	Grown in milk	1.23×10^5	4.90×10^4–3.90×10^5
F7243	Washed cells	1.58×10^5	2.02×10^4–1.24×10^6
	Added to milk	3.98×10^5	9.28×10^4–1.71×10^6
	Grown in milk	2.88×10^5	4.57×10^4–1.82×10^6
F7245	Washed cells	4.36×10^5	6.92×10^4–2.75×10^6
	Added to milk	7.94×10^4	1.85×10^4–3.40×10^6
	Grown in milk	3.72×10^4	7.64×10^3–1.81×10^5
G9599	Washed cells	3.16×10^3	8.60×10^2–1.16×10^4
	Added to milk	7.94×10^3	1.61×10^3–3.92×10^4
	Grown in milk	3.39×10^4	2.96×10^4–4.27×10^4

TABLE 2. Comparison of i.p. ALD^{50}s of *L. monocytogenes* grown on modified tryptose blood agar base and in milk[a]

Strain	Tryptose blood agar base		Milk	
	ALD_{50}[b]	95% confidence interval	ALD_{50}	95% confidence interval
1778+H1b	2.4×10^5	1.1×10^5–5.2×10^5	8.1×10^5	8.8×10^4–1.7×10^6
1778−H1b	$>1.8 \times 10^6$[c]	NA[d]	$>2.7 \times 10^6$[c]	NA
F2380	4.1×10^4	2.6×10^4–$1.7 - 10^5$	8.2×10^4	2.8×10^4–2.4×10^5
F2392	1.2×10^5	6.3×10^4–2.4×10^5	1.8×10^4	6.7×10^3–4.6×10^4
F7208	4.1×10^4	3.0×10^4–5.6×10^4	2.1×10^4	1.5×10^4–2.8×10^4

[a] Stock (mother) cultures were inoculated into laboratory-sterilized pasteurized milk to give 10^6 CFU/ml. The inoculated milk samples were incubated at 5°C for 30 days and were then plated at 10^4 to 10^5 dilutions on tryptose blood agar base foritified with 0.1% glucose and 0.1% yeast extract. Upon verification that the agar plates contained no contaminants, the surface growth was removed, suspended in phosphate-buffered saline, and used for mouse inoculations. Milk was sterilized as described previously (14).

[b] Determined by four sequential 10^{-1} dilutions of inoculum (10 mice per dilution) starting at approximately 3×10^6 CFU per mouse.

[c] No deaths occurred at lowest dilution. See Table 1.

[d] NA, Not applicable.

TABLE 4. Comparative mouse ALD_{50}s of *L. monocytogenes* strains determined by i.p. and i.g. inoculations

Expt[a]	Strain	Inoculation	ALD_{50}	95% confidence interval
1-27-89	1778+H1b	i.p.	1.05×10^5	2.14×10^4–5.20×10^5
		i.g.	2.65×10^5	4.73×10^4–1.48×10^6
	F2380	i.p.	1.48×10^5	2.64×10^4–8.29×10^5
		i.g.	2.34×10^3	5.47×10^2–1.00×10^4
	F7243	i.p.	1.40×10^5	5.56×10^4–3.51×10^5
		i.g.	3.24×10^4	4.59×10^3–2.28×10^5
	G9599	i.p.	2.52×10^2	5.84×10^1–1.08×10^3
		i.g.	4.00×10^3	6.32×10^2–2.52×10^4
	F5738	i.p.	4.69×10^3	1.27×10^3–1.72×10^4
		i.g.	2.96×10^5	3.40×10^4–2.56×10^6
	F6646	i.p.	3.13×10^4	1.25×10^4–7.87×10^4
		i.g.	4.96×10^4	5.66×10^3–1.24×10^5
2-27-89	F2380	i.p.	1.74×10^5	6.03×10^4–5.00×10^5
		i.g.	2.78×10^5	8.60×10^4–8.81×10^5
	F7243	i.p.	7.94×10^5	2.21×10^5–2.85×10^6
		i.g.	2.51×10^5	3.43×10^3–1.84×10^7
	G9599	i.p.	3.77×10^2	1.26×10^2–1.13×10^3
		i.g.	4.75×10^3	1.40×10^3–1.61×10^4

[a] Experiment 1-27-89 used 5 mice per 10^{-1} dilution; experiment 2-27-89 used 10 mice per 10^{-1} dilution. Four dilutions were tested.

influence the virulence of these strains when they were administered by i.g. feeding.

In the above-described experiments, the milk used for cultures (or suspension) was kept frozen at −60°C until it was to be used experimentally. Although previous results have shown that individual listerial milk cultures can vary considerably in the final CFU obtained per milliliter and in physical aspects, as shown by coagulation or separation of the milk (14), the cultures obtained here were not coagulated and were homogeneous. Only one strain, 1778+H1b (Table 3, grown in milk), showed the presence of contaminants in the pasteurized milk culture when plated at the 10^{-5} dilution for determination of the CFU at zero time for inoculation. This contamination apparently had no appreciable effect on the resultant ALD_{50}. The overall results (Table 3) showed little differences between the i.g. ALD_{50}s of washed-cell suspensions, washed-cell suspensions added to milk, and the milk-grown cultures.

Direct comparison of i.p. and i.g. ALD_{50}s of washed cells. The i.g. ALD_{50}s found in the foregoing experiment for washed-cell suspensions were similar to the i.p. ALD_{50}s observed previously (Tables 1 and 2). These results were unexpected in the light of previous publications (2, 11, 12, 20) which suggested that the i.g. ALD_{50}s obtained would be 100 to 1,000 times greater than those obtained by i.p. inoculation. Therefore, we directly compared the ALD_{50}s obtained by i.p. inoculation and i.g. feeding of several *L. monocytogenes* strains (Table 4). Strains 1778+H1b, F7243, G9599, and F6646 gave respective i.g. and i.p. ALD_{50}s that were not different at the 0.05 level of significance; strains F2380 and F5738 showed greater virulence by the i.g. route. In the repeat experiment (Table 4), the i.p. and i.g. values of F2380 and F7243 were essentially equal, although the i.p. ALD_{50} for strain 9599 was smaller than the i.g. value. Although there was some variation between experiments, these results emphasized that all of the strains were strongly virulent when administered i.g.

Subsequent to day 1 of inoculation, it appeared that infection and death occurred more rapidly after i.g. feeding. This was readily seen when the cumulative deaths were plotted per day for each route of inoculation (Fig. 1). In the cases of infection with strains 7243 and 2380, the more rapid death after i.g. inoculation was clearly evident; this result was less marked with strains 1778+H1b and 9599 (Fig. 1). With strain 5738, deaths occurred earlier after i.g. feeding, whereas with strain 6646, deaths occurred at the same time after both i.p. and i.g. inoculations (data not shown).

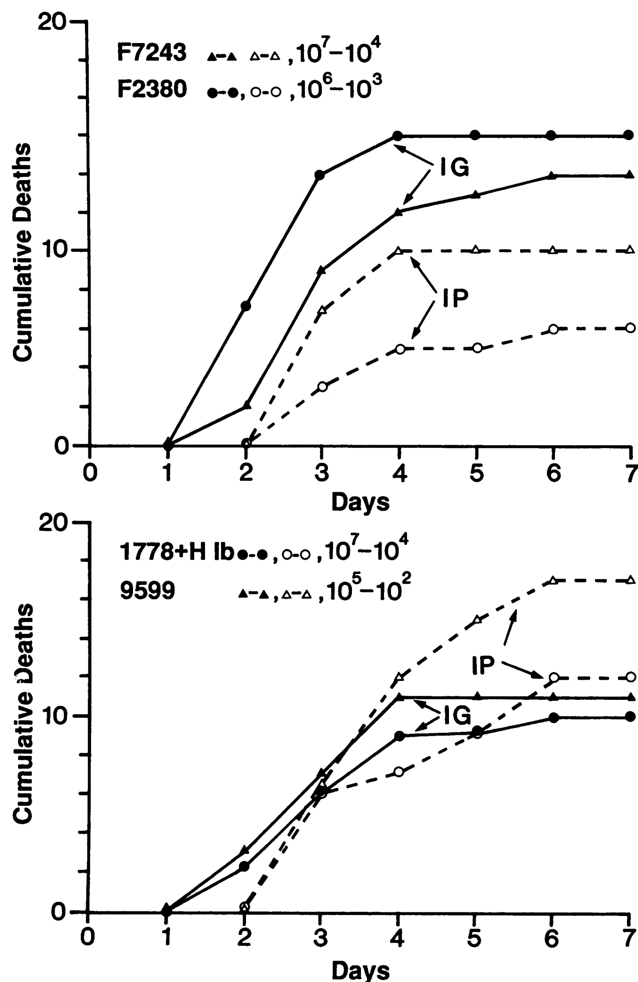

FIG. 1. Comparative rates of cumulative deaths (sum at all dilutions of inoculum) of mice inoculated i.g. or i.p. with *L. monocytogenes* strains (experiment 1-27-89, Table 4).

In a subsequent experiment with additional strains (G1032, F2228, F2618, F2198, and F2261), all strains caused earlier deaths, and one strain (2261) gave a lower ALD_{50} when administered i.g. For the remaining four strains, the i.p. and i.g. ALD_{50}s were not significantly different at the 0.05 level.

Effect of mouse weight on the infection responses after i.g. and i.p. inoculation of *L. monocytogenes* strains. The results of the second experiment (Table 4) gave i.g. ALD_{50} values for the respective strains which were approximately 10 times greater than those observed in the earlier experiment. We attributed this result to what appeared to be a delivery of larger mice in the second experiment, although both were received as 21-day-old mice. The effect of mouse weight on the infection response was therefore tested directly by comparing the rapidity of death by i.p. and i.g. inoculation

TABLE 5. ALD_{50}s of *L. monocytogenes* strains inoculated i.p. or i.g. into mice of different weights

Strain	W^a (g)	i.p.		i.g.	
		ALD_{50}	95% confidence interval	ALD_{50}	95% confidence interval
G1032	10.5	5.0×10^1	1.6×10^1–1.5×10^2	2.0×10^2	3.1×10^1–1.2×10^3
	15.1	1.2×10^2	5.0×10^1–3.1×10^2	5.0×10^1	1.6×10^1–1.5×10^2
	16.2	8.1×10^2	2.5×10^1–6.2×10^2	2.0×10^2	3.2×10^1–1.3×10^3
	21.2	2.0×10^2	4.7×10^1–8.7×10^2	8.1×10^3	1.1×10^3–5.7×10^4
G2618	10.5	4.8×10^3	1.3×10^3–1.8×10^4	4.8×10^3	1.5×10^3–1.5×10^4
	15.1	1.2×10^3	2.1×10^2–6.7×10^3	2.1×10^3	3.8×10^2–1.2×10^4
	16.2	5.6×10^3	1.3×10^3–2.4×10^4	1.4×10^4	2.5×10^3–7.9×10^4
	21.2	3.5×10^2	6.3×10^2–2.0×10^4	3.5×10^4	4.1×10^3–3.1×10^5
G2261	15.1	1.2×10^5	2.7×10^4–4.9×10^5	7.2×10^2	1.7×10^2–3.1×10^3
	16.2	5.9×10^4	1.1×10^4–3.3×10^5	3.7×10^3	4.8×10^2–2.9×10^3
	18.6	1.2×10^4	4.6×10^5–2.0×10^5	ND[b]	
	21.2	5.9×10^4	1.6×10^4–2.2×10^5	5.9×10^4	1.1×10^4–3.3×10^5
G2228	15.1	8.4×10^3	2.3×10^3–3.1×10^4	2.1×10^3	3.8×10^2–1.2×10^4
	16.2	8.4×10^3	1.2×10^3–5.9×10^4	8.4×10^3	9.2×10^2–7.7×10^4
	18.6	1.3×10^4	5.2×10^3–6.6×10^4	ND	
	21.2	8.4×10^4	1.4×10^4–5.3×10^5	8.4×10^4	3.0×10^4–2.4×10^5
G9599	16.2	9.7×10^1	1.9×10^1–4.7×10^2	3.8×10^1	8.4×10^0–1.8×10^2
	21.2	6.4×10^2	1.7×10^2–2.4×10^3	2.4×10^3	2.8×10^2–2.1×10^4

[a] Standard deviations for the respective weights were 10.5 ± 1.0, 15.1 ± 1.7, 16.2 ± 1.3, 18.6 ± 0.8, and 21.2 ± 1.6.
[b] ND, Not done.

and the comparative ALD_{50}s when 10- to 21-g mice were used (Table 5).

Of the ALD_{50}s determined after i.p. inoculation, there was no significant difference for the values determined within the different weights of mice for any given strain tested (Table 5). However, when determined after i.g. feeding, the ALD_{50} values for four of the five strains (G1032, G2618, G2228, and G9599) were significantly higher for the 21-g mice than for the 10- or 15-g mice, suggesting that the smaller mice were more sensitive to i.g. feeding than to i.p. inoculation.

In an experiment using strains G9599, G1032, G2261, G2618, and G2228, a direct comparison was made of the cumulative death curves of 16- and 21-g mice inoculated i.g. or i.p. In general, deaths occurred earlier when i.g. feeding was used for either 16- or 21-g mice, although fewer deaths occurred when 21-g mice were used (Fig. 2). Analyses of the curves of the 16-g mice of strains G9599, G1032, and G2261 inoculated i.g. showed more deaths within the first 3 to 4 days than were observed after i.p. inoculation; as analyzed by the log rank and Wilcoxon tests, the i.g. and i.p. curves were significantly different (Fig. 2, strain G9599). Although the death curves of strains G2618 and G2228 appeared to be distinct, they were not found to be significantly different (Fig. 2, strain G2228). However, when 21-g mice were used for the five strains, both routes of inoculation for four strains gave cumulative death curves that could not be differentiated graphically (data not given); however, i.g. inoculation of strain G9599 appeared to produce more rapid death initially than did i.p. injection (Fig. 2). None of the five strains showed cumulative death curves of 21-g mice in which the respective i.g. and i.p. cumulative deaths were significantly different by the log rank or Wilcoxon test. These results and the results of additional experiments supported the conclusion that mice of 14 to 16 g are more sensitive than 21-g mice to i.g. inoculation. Although i.g. feeding of mice may produce more rapid deaths (dependent on the strain), the resultant ALD_{50}s will not necessarily be smaller than those that would be observed upon i.p. injection.

Intragastric feeding of strains 2380, G9599, 1778+H1b, and F7243 to 19- to 20-g mice at the level of 10^8 CFU per mouse produced 80 to 100% mortality by day 2.

DISCUSSION

The strains chosen for the various experiments were selected for the purposes of observing differences based on phenotypic characters, serotype, association with epidemics, or food and patient relationships. Strain 1778+H1b and its avirulent derivative (1778−H1b) were derived from the strain recommended to be used as the type strain (15). Strains F2380, F2392, F7243, and F7245 were isolated from cheeses or patients of a California epidemic, whereas strains G2228, G2618, and G2198, which were all of the same enzyme type, serotype, and phenotypic characteristics, were isolated from a patient and respective refrigerator

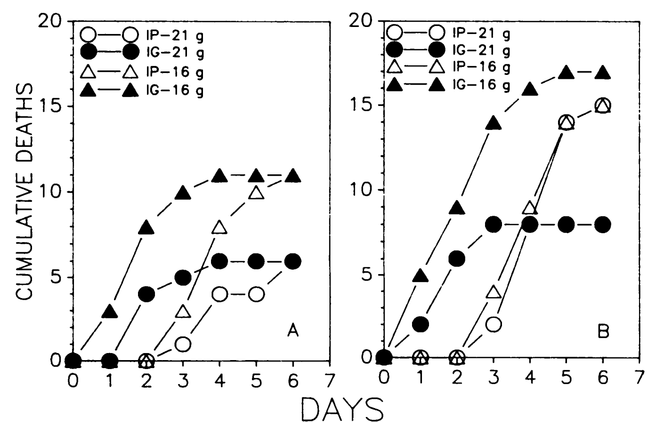

FIG. 2. Effect of mouse weight on the rate of cumulative deaths (sum at all dilutions of inoculum) of mice inoculated i.g. or i.p. with *L. monocytogenes* strains G2228 (A) and G9599 (B).

contents (B. Swaminathan, personal communication). Strains G9599 and G1032, both isolated from patients, were chosen because they have shown consistently high levels of virulence, with ALD_{50}s of the order of 10^2. Among all strains used in the work reported here or elsewhere, the i.p. ALD_{50}s ranged from 10^6 to 10^2, and as reported here, these values have been consistently reflected by the associated i.g. ALD_{50}s. No particular association of relative virulence was made with a given characteristic except for strains G9599 and G1032. These strains showed high virulence and are rhamnose-negative *L. monocytogenes* strains (Robert E. Weaver, personal communication).

Except for strain 2392 (Table 2), there was observed no overt effect of milk for increased virulence, either as a substrate for growth, as a selective environment, or as a virulence factor in itself. Woodbine and collaborators (3, 18) described the increased virulence of a strain of *L. monocytogenes* when cultured at 4°C before inoculation into the air sac of chicken embryos. In the experiments described here, all milk cultures were incubated approximately 30 days at 5 to 8°C before either i.p. injection or i.g. feeding; such inoculations did not differ significantly in virulence from those of washed cell suspensions grown at 37°C. Very recently, Czuprynski et al. (4) reported that growth at 4°C significantly increased the intravenous virulence of four *L. monocytogenes* strains (three clinical strains and one laboratory strain) but had no effect on i.g. virulence. Virulence was determined by bacterial counts within selected organs and tissues over a period of 6 to 8 days after inoculation; the data suggested that all mice, which were 5 to 6 weeks of age, recovered at the infectious dose used.

Our results of relative equality of i.p. and i.g. ALD_{50}s were unexpected in light of published results which suggested that ALD_{50} values obtained by i.g. feeding would be much greater than those obtained by i.p. injection (2, 11, 12, 20). In the review of these papers or of papers in which the mouse model was used for the early investigation of the relative virulence of *L. monocytogenes* in mice, guinea pigs, hamsters, rabbits, monkeys, and lambs (by using respiratory, i.p., intravenous, and intracerebral routes of infection) (9, 17), we were unable to find information in which a direct comparison of ALD_{50} values was made by using the i.g. and i.p. routes of inoculation in mice.

MacDonald and Carter (11), using an i.g. feeding tube, inoculated 0.2 ml of a suspension having 2.5×10^8 CFU into 5- to 8-week-old mice. Such doses consistently gave infection in the Peyer's patches, whereas infection was sporadic with lower numbers of bacteria. No deaths were reported, although in some cases the animals were incubated for as long as 10 days; after 8 days, few animals showed the presence of *L. monocytogenes* in the tissues. However, the mice used were 5 to 8 weeks old. Intragastric inoculations of mice at the levels of 10^7 to 10^8 CFU by Miller and Burns (12) were made into mice weighing 18 to 22 g. The mice were sacrificed on days 4, 7, and 14; no deaths were reported, although 6 of 10 pregnant mice had interruption of pregnancy, with fetal death. Zachar and Savage (20) inoculated both germfree and normal mice, using an i.g. gavage of sterile polyethylene tubing. In normal mice, no deaths occurred when 5×10^7 CFU was administered, although approximately 30% of the germfree mice died between days 5 and 8. Ages and weights of the mice were not given. Audurier et al. (2) inoculated 25- to 30-g female mice orally by the use of infected drinking water. Comparative ALD_{50}s determined intravenously and orally were 2.6×10^5 and 7.0×10^{10}, respectively. Not only did it take high CFU to induce splenic infection, but also the results were not reproducible in repetitive experiments when the oral route of infection was used.

Ziegler (21) has described the relationship of macrophage processing and presentation of listerial antigens to T cells for the development of an effective resistance to *Listeria* strains. In their study of resistance to listerial infection in mice, Ohara et al. (13) studied the development of protective macrophages with increasing age of the mice. In 1- and 2-week-old mice, macrophages did not show killing activity against *L. monocytogenes*, but such activity began when the mice were 3 weeks old and continued to develop as the mice reached adult maturity by 8 weeks. Their results would appear to relate to our observations presented here in which 5-week-old mice no longer showed the i.g. sensitivity to oral infections observed in 10.5- to 16-g mice. Our experience suggests the use of 14- to 15-g mice to differentiate i.p. and i.g. ALD_{50}s, since smaller mice presented problems to us in handling and i.g. feeding.

Recognition that the i.g. route of inoculation for mice reflects the values obtained by i.p. inoculation is of interest from several aspects. First, as we report here for milk, i.g. inoculation permits an evaluation of the role of the carrier (food or infection modulators such as dextran sulfate) in food-borne *Listeria* infections. Second, it permits a characterization of virulence by both i.p. and i.g. ALD_{50}s. Finally, the results appear to emphasize, unexpectedly, the rapidity of the infection in young mice after inoculation by the i.g. route.

Only 0.25-ml volumes were introduced, either i.p. or i.g., and extreme care was taken not to injure the animals during i.g. inoculations; no animals were used in which there was suspicion of injury or inhalation of the inoculum. With exceedingly few exceptions, no deaths were observed within 24 h, and the mice appeared active and healthy during this period after i.g. inoculation. However, i.g. inoculations at high levels of CFU per mouse or with the use of very young mice would have the potential of causing deaths within the first day after inoculation.

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