Journal of Food Protection, Vol. 69, No. 4, 2006, Pages 842–846

Comparison of *Listeria monocytogenes* Virulence in a Mouse Model

KAZUE TAKEUCHI,[1] NUTAN MYTLE,[1] SONYA LAMBERT,[1,2] MARGARET COLEMAN,[3]† MICHAEL P. DOYLE,[1] AND MARY ALICE SMITH[1,2]*

[1]*Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223;* [2]*Department of Environmental Health Science, University of Georgia, 206 Environmental Health Science Building, Athens, Georgia 30602; and* [3]*U.S. Department of Agriculture, Agricultural Research Service, Princess Anne, Maryland 21853, USA*

MS 05-245: Received 13 May 2005/Accepted 10 November 2005

ABSTRACT

Listeriosis results from exposure to the foodborne pathogen *Listeria monocytogenes.* Although many different strains of *L. monocytogenes* are isolated from food, no definitive tests currently predict which isolates are most virulent. The objectives of this study were to address two major data gaps for risk assessors, variability among *L. monocytogenes* strains in pathogenicity and virulence. Strains used in our monkey clinical trial or additional food isolates were evaluated for their virulence and infectivity in mice. All strains were equally pathogenic to immunocompromised mice, causing deaths to 50% of the population 3 days after exposure to doses ranging from 2 to 3 log CFU. Doses resulting in 50% deaths on the fifth day after administration were 1 to 2 log lower than those on the third day, indicating that the full course of pathogenicity exceeds the 3-day endpoint in immunocompromised mice. Three strains were chosen for further testing for their virulence and infectivity in liver and spleen in normal (immunocompetent) mice. Virulence was not significantly different ($P > 0.05$) among the three strains, all resulting in deaths to 50% of mice at 5 to 7 log CFU by 5 days after administration. All strains were equally infective in liver or spleen, with higher numbers of *L. monocytogenes* directly correlated with higher doses of administration. In addition, there was no preference of organs by any strains. The lack of strain differences may reflect the limitation of the mouse model and suggests the importance of using various models to evaluate the pathogenicity and virulence of *L. monocytogenes* strains.

The Centers for Disease Control and Prevention, based on active surveillance from 2000, estimates that approximately 2,500 people in the United States become ill annually from foodborne *Listeria monocytogenes*, and approximately 500 die *(6).* The consumption of contaminated ready-to-eat foods is considered to be the principal route of *L. monocytogenes* infection. Soft cheese *(3, 7, 19),* pasteurized milk *(4),* and deli meats *(5)* have been associated with outbreaks of listeriosis. Four serotypes (4b, 1/2a, 1/2b, and 1/2c) among 13 known serotypes have been isolated from a wide range of foods *(12).* Three of these serotypes (4b, 1/2a and 1/2b) are associated with the majority of human listeriosis *(12).*

Risk factors for infection are underlying illnesses, impaired immune system, extreme age, and pregnancy *(12, 20).* In healthy individuals, infections are generally rare, sporadic, and mild, with symptoms characterized as flu-like *(12, 19, 20).* However, the outcome of listeriosis is serious in susceptible individuals, potentially leading to septicemia and meningitis *(12, 19, 20).* In pregnant women, listeriosis primarily affects the fetus or neonate and can result in spontaneous abortion or stillbirth. Studies have linked listeriosis to 1 to 6% of spontaneous abortions in western European countries *(10).*

Because human trials for *L. monocytogenes* are unethical because of the potential fatal outcome for susceptible individuals, animal surrogate studies must be developed to describe dose-response relationships for various adverse endpoints and for extrapolation to human dose-response models. Nonhuman primates have been used for dose-response studies *(11, 28),* but the cost and limited numbers of primates per dose group make the use of nonhuman primates for *L. monocytogenes* strain comparisons prohibitive. The objective of our study was to screen a panel of *L. monocytogenes* strains for pathogenicity and invasion of the liver and spleen in mice. The panel included strains used in a primate study *(28)* and additional strains isolated from food linked to human illness *(5, 13, 27).*

The mouse is the most commonly used species for the study of *L. monocytogenes,* and there are numerous publications on the pathogenicity, virulence, infectivity, genetic factors, and other characteristics of different *L. monocytogenes* strains. However, many studies use three or fewer strains and cannot be directly compared to other studies because of differences in strains used, animal models used for testing, or interlaboratory methods. Results obtained in this study provide information on virulence that can aid in direct comparisons of *L. monocytogenes* strains that are known human or primate pathogens and three food isolates. Additionally, we knew little about the virulence of the nonhuman primate clinical isolate, strain 12443, which we used in our pregnant nonhuman primate model *(28),* and this

* Author for correspondence. Tel: 706-542-1599; Fax: 706-542-7472; E-mail: masmith@uga.edu.

† Present address: Syracuse Research Corporation, Environmental Science Center, 301 Plainfield Road, Suite 350, Syracuse, NY 13212, USA.

TABLE 1. *Isolation source and serotype of* Listeria monocytogenes *strains*

Clinical isolate	Serotype	Source of isolation
Scott A	4b	Human clinical isolate
G3982	4b	Human clinical isolate associated with outbreak linked to Mexican-style cheese
G3990	4b	Human clinical isolate associated with outbreak linked to hot dog
H7550	4b	Human clinical isolate associated with outbreak linked to hot dog
H9666	1/2c	Human clinical isolate from blood
12443	1/2a	Monkey clinical isolate
12375	4b	Monkey clinical isolate
101M	4b	Beef pork sausage
F6854	4b	Turkey frankfurters
H7776	4b	Frankfurters

study helps provide information on this strain compared with known human pathogens.

MATERIALS AND METHODS

Mice. Female ICR mice, 18 to 20 g, were obtained from Harlan Sprague Dawley (Indianapolis, Ind.). Mice were housed in groups of five in cages covered by wire lids and filters with free access to sterile food and water. The mice were held under these conditions for 24 h before the study began and throughout the study period. All animals used in this study were handled in accordance with National Institutes of Health guidelines, and their use was approved by the University of Georgia Institutional Animal Use and Care Committee.

Bacterial strains and preparation of inocula. The *L. monocytogenes* strains used in this study included several from our nonhuman primate study (human clinical isolates Scott A, G3982, G3990, and H7550 and monkey clinical isolates 12443 and 12375) *(28)* in addition to three foodborne isolates (101M, beef-pork sausage *(13)*; F6854, turkey frankfurter *(27)*; and H7776, frankfurter *(5)*) provided by the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), Wyndmoor, Pa. (Table 1). Cultures were stored on latex beads at −80°C. Cultures were grown during three successive overnight transfers at 37°C in tryptic soy broth (Difco, Becton Dickinson, Sparks, Md.). Cells were washed twice with 0.01 M potassium phosphate-buffered saline (PBS; pH 7.2) by centrifugation (3,500 × *g*) for 10 min at room temperature, sedimented in a final wash, and then resuspended in PBS. Cell numbers in suspensions were determined by surface plating serial dilutions (1:10) on tryptic soy agar (Difco, Becton Dickinson) in duplicate. Colonies were enumerated after 24 h of incubation at 37°C. Inocula were prepared by diluting cell suspensions serially (1:10) in PBS.

Assessment of mouse pathogenicity. The pathogenicity of *L. monocytogenes* isolates was determined in immunocompromised female ICR mice by intraperitoneal (i.p.) injection of test strains. Mice were immunocompromised by i.p. injection of 200 mg/kg of body weight of carrageenan (Sigma type II, Sigma Chemical Co., St. Louis, Mo.) 24 h before inoculation with *Listeria (16, 30).* Five mice were injected with each strain in 0.1 ml PBS at approximately 4 log CFU. *L. monocytogenes* H9666 and *Listeria innocua* were used as positive and negative controls, respectively. Strain H9666 was previously tested in our laboratory and found to infect both immunocompromised and normal mice *(30).* Control mice were administered 0.1 ml of sterile PBS i.p. Mice were observed for death three times daily for 5 days. Any signs of illnesses, such as ruffled fur and lethargy (lack of movement), were also recorded. Isolates that caused at least one death within 5 days were designated as pathogenic *(16).* Strains that did not cause death within 5 days were designated as nonpathogenic.

Evaluation of dose response. The effect of different doses on mice was determined for *L. monocytogenes* strains that were pathogenic to immunocompromised mice. Mice were immunocompromised as previously described. Five concentrations of each *L. monocytogenes* strain were prepared as described above and inoculated in mice (five mice per concentration). Concentrations administered were based on the outcome of the pathogenicity screening test. Control mice were given 0.1 ml of sterile 0.01 M potassium PBS (pH 7.2). *L. monocytogenes* H9666 and *L. innocua* were inoculated in mice to serve as positive (virulent) and negative (nonvirulent) controls, respectively. Mice were observed for death three times a day for 5 days. The method of Reed and Muench *(26)* was used to estimate doses that resulted in the death of 50% of mice. When 50% of deaths occurred outside the range of the dose tested, the 50% lethal dose (LD_{50}) was determined by extrapolation.

Infectivity determination. *L. monocytogenes* inocula of pathogenic strains were prepared as described above. Based on results of the pathogenicity experiment, one concentration that resulted in consistent death of the mice was selected for the evaluation of infection of liver and spleen. Three *L. monocytogenes* strains were inoculated i.p. in normal (immunocompetent) mice. A known virulent strain of *L. monocytogenes* (H9666) and a nonvirulent strain (*L. innocua)* were used as positive and negative controls, respectively. Control mice were injected with PBS. Based on preliminary experiments and previous work in our laboratory *(30),* necropsy dates were selected. Mice were euthanized by carbon dioxide asphyxiation, and necropsy was performed. Livers and spleens were aseptically removed from mice, weighed, and macerated in PBS. The tissue suspensions were serially diluted in PBS and surface plated on tryptic soy agar in duplicate to determine the bacterial counts in each organ. Plates were incubated at 37°C for 24 h prior to enumeration. Colonies were examined for typical *Listeria* appearance by Henry illumination. Select colonies were confirmed as *L. monocytogenes* by standard tests *(12, 16).* In addition, pulsed-field gel electrophoresis (PFGE) patterns of isolates from tissue were compared with the strains used for inoculation to determine the stability of the original strains during passage through the mouse. PFGE *(15)* was performed using AscI as a restriction enzyme and *L. monocytogenes* strain H2446, which produces 14 bands with AscI, as a standard.

Statistical analysis. Data were analyzed statistically by analysis of variance and Duncan's multiple range test using Statistical Analysis Software (version 6.12, SAS, Cary, N.C.) at 5% level of significance.

RESULTS

Assessment of mouse pathogenicity and lethality dose determination. All strains except for *L. innocua* (nonpathogenic control) were pathogenic to immunocompromised mice. PBS injected i.p. did not kill any mice, either immunocompromised or normal. Inoculation with *L. monocytogenes* caused mouse coats to appear rough 2 to 3 days after inoculation. If the illness progressed, mice developed

TABLE 2. *Dose of* Listeria monocytogenes *strains required to result in deaths to 50% of immunocompromised mice*

Strain	Dose ± SD (log CFU/mouse) resulting in death on[a]: Day 3	Day 5
L. innocua	No death	No death
H9666	4.4 ± 0.5	2.5 ± 0.5
G3982	3.6 ± 1.4	2.4 ± 0.4
12443	5.7 ± 1.2	3.3 ± 0.9
Scott A	3.8 ± 1.4	2.5 ± 0.6
H7550	4.4 ± 1.5	2.9 ± 1.3
12375	4.0 ± 1.6	3.0 ± 1.4
G3990	4.8 ± 0.9	3.2 ± 1.0
F6854	4.2 ± 1.3	2.5 ± 0.8
H7776	5.3 ± 1.0	3.2 ± 1.7
101M	4.7 ± 0.1	2.7 ± 0.1

[a] There were no significant differences among strains. LD_{50} values were significantly higher on day 3 compared to day 5 for all *L. monocytogenes* strains tested.

diarrhea and rapid and shallow breathing, and then died or were humanely euthanized. Most deaths occurred by the fourth day after inoculation with *L. monocytogenes* strains. Some mice that developed roughness on their coats never progressed into a lethargic state and recovered from the infection. The doses resulting in deaths in 50% of inoculated immunocompromised mice 3 and 5 days after administration are shown in Table 2. Test strains did not significantly differ in LD_{50} values ($P > 0.05$) on either the third or fifth day postadministration. On posttreatment day 3, LD_{50} doses for immunocompromised mice ranged from approximately 4 to 6 log CFU per mouse. On posttreatment day 5, the estimates of LD_{50} were reduced by 1 to 2 log CFU.

The *L. monocytogenes* strains having the highest and lowest LD_{50} in immunocompromised mice (12443 and G3982, respectively), strain H9666 (pathogenic control), and *L. innocua* (nonpathogenic control) were further tested in normal (immunocompetent) mice. Although all *L. monocytogenes* strains, except the nonpathogenic control *L. innocua*, resulted in death in normal mice, there were no significant differences ($P > 0.05$) in virulence (based on LD_{50}) among *L. monocytogenes* strains for the experimental conditions tested. However, there was a significant difference ($P < 0.05$) when comparing the third and fifth day LD_{50} in normal mice for all three strains. H9666 (pathogenic control), G3982, and 12443 required approximately 7 log CFU for death to result in 50% of mice on the third day and 4 to 5 log CFU on the fifth day (Table 3). For all strains tested, a higher dose of *L. monocytogenes* was consistently required to cause deaths in normal mice compared with immunocompromised mice; however, statistical comparisons of doses between the two groups were not done.

The invasion and persistence of each strain in the liver and spleen was evaluated in normal mice for two *L. monocytogenes* test strains, 12443 and G3982, and pathogenic and nonpathogenic controls, *L. monocytogenes* H9666 and *L. innocua* (Figs. 1 and 2). There were no significant differences ($P > 0.05$) among strains in their persistence in liver or spleen. *L. monocytogenes* strains (CFU) were equally recovered from both organs of all treated mice. An increase in recovery was observed at higher doses for all strains. For mice treated with *L. innocua*, 60% had livers and spleens positive for *L. innocua*. There were no incidences where *L. innocua* was recovered from only one of the two organs without recovery from the other.

TABLE 3. *Dose of* Listeria monocytogenes *strains required to result in deaths to 50% of normal (immunocompetent) mice*[a]

Strain	Dose ± SD (log CFU/mouse) resulting in death on[b]: Day 3	Day 5
L. innocua	No death	No death
H9666	6.8 ± 0.1	5.6 ± 1.5
G3982	6.7 ± 1.6	5.7 ± 1.6
12443	6.7 ± 2.5	4.2 ± 1.3

[a] LD_{50} was outside the test dose range in one of the three experiments for each strain and was estimated by extrapolation.

[b] There were no significant differences among strains. LD_{50} values were significantly higher on day 3 compared to day 5 for all *L. monocytogenes* strains tested.

Selected colonies from each treated group of mice were tested by PFGE analysis for quality control. PFGE patterns were identical to the treatment strains in all cases. This result provides evidence of the stability of the genetic structure, at the level of PFGE sensitivity, of the strains during the course of infection and verifies that the recovered strain was the same as the treatment strain and not from a contaminating source.

DISCUSSION

All *L. monocytogenes* strains tested in our study, except the negative control strain *L. innocua*, were pathogenic to

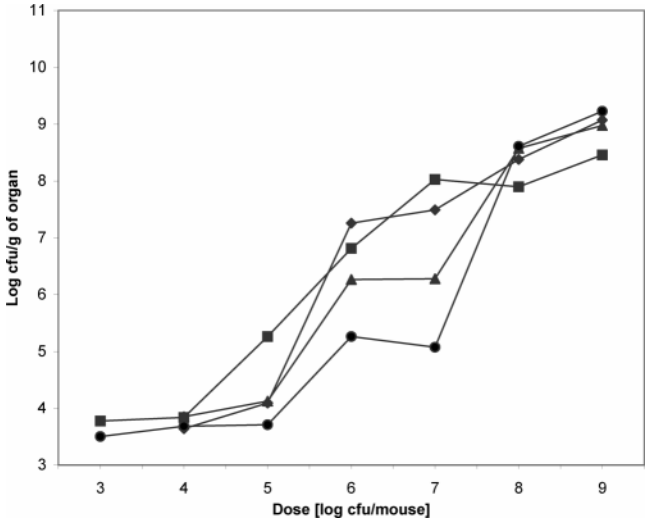

FIGURE 1. *Comparison of the infectivity of different* Listeria monocytogenes *strains in liver of normal mice.* ■, L. monocytogenes *strain 12443;* ▲, L. monocytogenes *strain G3982;* ♦, L. monocytogenes *strain H9666;* ●, L. innocua. L. monocytogenes *strain H9666 and* L. innocua *were tested as pathogenic and nonpathogenic control, respectively.*

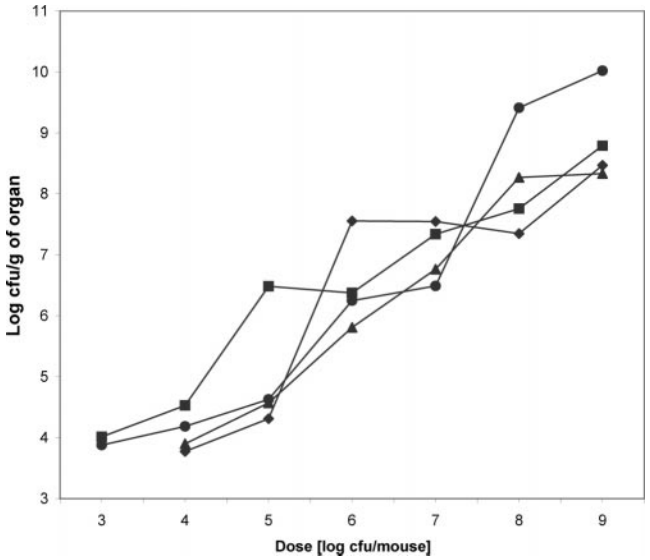

FIGURE 2. *Comparison of the infectivity of different* Listeria monocytogenes *strains in spleen of normal mice.* ■, L. monocytogenes *strain 12443;* ▲, L. monocytogenes *strain G3982;* ◆, L. monocytogenes *strain H9666;* ●, L. innocua. L. monocytogenes *strain H9666 and* L. innocua *were tested as pathogenic and nonpathogenic control, respectively.*

immunocompromised mice regardless of the source. The LD_{50} doses determined in the study were comparable to those observed in other studies using i.p. injection *(1, 8, 21, 25).* However, the differences in virulence among strains reported by other researchers were not observed in our study *(21, 25, 29).* Barbour et al. *(2)* has shown that intravenous injections (i.v.) result in lower LD_{50} estimates in mice than intragastric exposures. This result is expected because i.v. injection bypasses host defense mechanisms that normally constrain progression of disease from oral exposure routes. For the same reasons, differences may also be expected for LD_{50} estimates from i.p. injections compared with oral infection *(17).* The increased dose of *L. monocytogenes* strains required to cause deaths in 50% of normal (immunocompetent) mice as compared with immunocompromised mice confirms that impaired immunity increases the risk of listeriosis *(29).* However, in our study, pathogenesis in both normal and immunocompromised mice appears to require more than 3 days for full development based on the significantly lower ($P < 0.05$) LD_{50} at five days posttreatment compared with 3 days posttreatment (Tables 2 and 3).

In a normal course of infection by *L. monocytogenes* from foods, the organism will enter the host through epithelial cells in the intestinal tract *(9, 22).* Once inside the host cell, *L. monocytogenes* can evade cell-mediated immunity, multiply in the cytoplasm, and migrate toward the cell periphery to invade macrophages and enter the lymphatics and lymph nodes. At this point, *L. monocytogenes* can enter the bloodstream and further disseminate to tissues such as liver and spleen. The extent of invasion was previously related to the virulence of strains *(2, 23).* In our study, we recovered similar levels (CFU) of all three strains from liver and spleen of normal mice. These results are not surprising because there was no significant difference ($P > 0.05$) in doses required to cause deaths in 50% of mice. However, higher rates of invasion observed at higher levels of inoculum provide evidence of dose dependency for severity of listeriosis regardless of strain. Such a mechanism suggests that a high dose of pathogens may overwhelm the host defenses, resulting in an increased likelihood that a fraction of the pathogen dose successfully evades the host immune defenses, survives to invade host tissues, and replicates in host tissues to the magnitude necessary to cause severe damage. In contrast, this mechanism of density-dependent severity suggests that low dose challenges may be effectively eliminated by host defenses prior to significant tissue damage.

In our study, as in others, invasion of liver and spleen was also observed with nonpathogenic *L. innocua.* Menudier et al. *(23)* observed infection of spleen and liver in 5 and 10% of mice at 0.13 and 0.24 log CFU/g of organ, respectively, after exposure to 10^6 CFU *L. innocua* by the i.p. route of exposure. von Koenig et al. *(31)* has also demonstrated that *L. innocua* can enter the spleen at approximately 4 log CFU per spleen when mice are i.p. infected at 9 log CFU, although *L. innocua* was incapable of growing in the spleen and steadily decreased in numbers with time. After 3 days postinoculation, the numbers decreased to approximately 2 log CFU per spleen and became undetectable after 6 days *(31).* Our observed rates and levels of infection of liver and spleen by *L. innocua* were higher than these observations. Differences in susceptibility to listeriosis among different mouse strains has been demonstrated *(14, 21)* and may account for the differences in our results from previous studies.

Golnazarian et al. *(14)* used hydrocortisone acetate or cimetidine to immunocompromise mice and subsequently i.p. injected mice with *L. monocytogenes* strains in doses ranging from 1.0 to 7.0 log CFU/ml. In our study, we used carrageenan to immunocompromise mice and 24 h later injected *L. monocytogenes* in doses ranging from 1 to 7 log CFU/ml. Carrageenan was selected as the macrophage-suppressing agent because of its ability to cause an eightfold decrease in the number of macrophages in mouse peripheral blood within 2 days and because of its long-lasting effect, with full recovery requiring 15 days *(29).* Cortisone acetate has also been shown to suppress cellular immunity and to increase the susceptibility of mice to *Listeria* infection *(14, 24).* However, cortisone acetate's effect wanes rapidly after 24 h *(24),* indicating that it would probably not be effective for the duration of the infection. Golnazarian et al. *(14)* reported LD_{50} results from i.p. injected mice ranging from 3 to 6 log CFU/ml. Other reported values for the LD_{50} of *L. monocytogenes* in mice range from 3 to 10 log CFU, depending on the strain of *Listeria,* the strain of mouse, and the route of inoculation *(1, 14).* In order for valid inferences to be drawn from the diverse reports of LD_{50} from various mouse models to predict likelihood and severity of adverse effects in humans, a conceptual model is needed that elucidates the controlling events of pathogenesis for scaling within and between host species.

The difficulty of using animal models to study the

mechanisms of pathogenicity and virulence of *L. monocytogenes* to understand human listeriosis has been recognized by several researchers. One example is recent evidence for the role of internalin for *L. monocytogenes* invasion of human epithelial cells. Mouse E-cadherin is not a receptor for internalin as it is in humans *(18).* Other factors may also be different between mouse and human listeriosis. In addition, different methods of administration of *L. monocytogenes* may result in different outcomes due to changes in the course of infection. Our study demonstrates the importance of evaluating the pathogenicity and virulence of *L. monocytogenes* strains in multiple animal models to expand knowledge of the mechanisms of listeriosis and enable more rigorous scientific inferences to build defensible dose-response models for human listeriosis.

ACKNOWLEDGMENTS

This work was supported by the National Alliance for Food Safety & Security through USDA-ARS specific cooperative agreement 58-1935-1-129 and Hatch funds to M. A. Smith. We are grateful to the Centers for Disease Control and Prevention's Listeria Reference Laboratory, Atlanta, Ga., for providing human clinical samples and the USDA-ARS Microbial Food Safety Research Unit, Wyndmoor, Pa., for supplying *L. monocytogenes* food isolates.

REFERENCES

1. Audurier, A., P. Pardon, J. Marly, and F. Lantier. 1980. Experimental infection of mice with *Listeria monocytogenes* and *L. innocua. Ann. Microbiol.* 131**:**47–57.
2. Barbour, A. H., A. Rampling, and C. E. Hormaeche. 1996. Comparison of the infectivity of isolates of *Listeria monocytogenes* following intragastric and intravenous inoculation in mice. *Microbiol. Pathogen* 20**:**247–253.
3. Centers for Disease Control. 1985. Listeriosis outbreak associated with Mexican-style cheese—California. *Morb. Mortal. Wkly. Rep.* 34**:**357.
4. Centers for Disease Control and Prevention. 1998. Epidemiologic notes and reports update—listeriosis and pasteurized milk. *Morb. Mortal. Wkly. Rep.* 37**:**764–766.
5. Centers for Disease Control and Prevention. 1998. Multistate outbreak of listeriosis—United States, 1998. *Morb. Mortal. Wkly. Rep.* 47**:**1085–1086.
6. Centers for Disease Control and Prevention. 2000. Multistate outbreak of listeriosis—United States, 2000. *Morb. Mortal. Wkly. Rep.* 49**:**1129–1130.
7. Centers for Disease Control and Prevention. 2001. Outbreak of listeriosis associated with homemade Mexican-style cheese—North Carolina, October 2000–January 2001. *Morb. Mortal. Wkly. Rep.* 50: 560–562.
8. Conner, D. E., V. N. Scott, S. S. Sumner, and D. Bernard. 1989. Pathogenicity of foodborne, environmental and clinical isolates of *Listeria monocytogenes* in mice. *J. Food Sci.* 54:1553–1556.
9. Czuprynski, C. J. 1994. Host defense against *Listeria monocytogenes*: implications for food safety. *Food Microbiol.* 11:131–147.
10. Enocksson, E., B. Wretlind, G. Sterner, and B. Anzen. 1992. Listeriosis during pregnancy and in neonates. *Scand. J. Infect. Dis. Suppl.* 71:89–94.11.
11. Farber, J. M., E. Daley, F. Coates, N. Beausoleil, and J. Fournier. 1991. Feeding trials of *Listeria monocytogenes* with a nonhuman primate model. *J. Clin. Microbiol.* 29:2606–2608.
12. Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol. Rev.* 55:476–511.
13. Glass, K. A., and M. P. Doyle. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol.* 55:1565–1569.
14. Golnazarian, C. A., C. W. Donnelly, S. J. Pintauro, and D. B. Howard. 1989. Comparison of infectious dose of *Listeria monocytogenes* F6817 as determined for normal versus compromised C57B1/6J mice. *J. Food Prot.* 52:696–701.
15. Graves, L. M., and B. Swaminathan. 2001 PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulse-field gel electrophoresis. *Int. J. Food Microbiol.* 65:55–62.
16. Hitchins, A.D. 1998. Listeria monocytogenes, chap. 10. *In* U.S. Food and Drug Administration, Bacteriological analysis manual, 8th ed. Available at: http://www.cfsan.fda.gov/~ebam/bam-10.html#authors. Accessed 19 June 2000.
17. Lammerding, A. M., K. A. Glass, A. Gendron-Fitzpatrick, and M. P. Doyle. 1992. Determination of virulence of different strains of *Listeria monocytogenes* and *Listeria innocua* by oral inoculation of pregnant mice. *Appl. Environ. Microbiol.* 58:3991–4000.
18. Lecuit, M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner, and P. Cossart. 1999. A single amino acid in E-cadherin responsible for host specificity toward the human pathogen *Listeria monocytogenes. EMBO J.* 18:3956–3963.
19. Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome. 1988. Epidemic listeriosis associated with Mexican-style cheese. *N. Engl. J. Med.* 319:823–828.
20. Low, J. C., and W. Donachie. 1997. A review of *Listeria monocytogenes* and listeriosis. *Vet. J.* 153:9–29.
21. Mainou-Fowler, T., A. P. MacGowan, and R. Postlethwaite. 1988. Virulence of *Listeria* spp: course of infection in resistant and susceptible mice. *Int. Med. Microbiol.* 27:131–140.
22. McLauchlin, J. 1997. The pathogenicity of *Listeria monocytogenes*: a public health perspective. *Rev. Med. Microbiol.* 8**:**1–14.
23. Menudier, A., C. Bosgiraud, and J. A. Nicolas. 1991. Virulence of *Listeria monocytogenes* serovars and *Listeria* spp. in experimental infection of mice. *J. Food Prot.* 54:917–921.
24. North, R. J. 1971. The action of cortisone acetate on cell-mediated immunity to infection. *J. Exp. Med.* 134:1485–1500.
25. Pine, L., G. B. Malcolm, and B. D. Plikaytis. 1990. *Listeria monocytogenes* intragastric and intraperitoneal approximate 50% lethal doses for mice are comparable, but death occurs earlier by intragastric feeding. *Infect. Immun.* 58:2940–2945.
26. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. *Am. J. Hyg.* 27:493.
27. Schwartz, B., C. A. Ciesielski, C. V. Broome, S. Gaventa, G. R. Brown, B. G. Gellin, A. W. Hightower, and L. Mascola. 1988. Association of sporadic listeriosis with consumption of uncooked hot dogs and undercooked chicken. *Lancet 2:*779–782.
28. Smith, M. A., K. Takeuchi, R. E. Brackett, H. M. McClure, R. B. Raybourne, K. M. Williams, U. S. Babu, G. O. Ware, J. R. Broderson, and M. P. Doyle. 2003. Nonhuman primate model for *Listeria monocytogenes* induced stillbirths. *Infect. Immun.* 71:1574–1579.
29. Stelma, G. N., A. L. Reyes, J. T. Peeler, D. W. Francis, J. M. Hunt, P. L. Spaulding, C. H. Johnson, and J. Lovett. 1987. Pathogenicity test for *Listeria monocytogenes* using immunocompromised mice. *J. Clin. Microbiol.* 25:2085–2089.
30. Takeuchi, K., M. A. Smith, and M. P. Doyle. 2003. Pathogenicity of food and clinical *Listeria monocytogenes* isolates in a mouse bioassay. *J. Food Prot.* 66:2362–2366.
31. von Koenig C. H. W., B. Heymer, H. Hof, and H. Finger. 1983. Course of infection and development of immunity in experimental infection of mice with *Listeria* serotypes. *Infect. Immun.* 40:1170–1177.