Effect of Fat Content on Infection by *Listeria monocytogenes* in a Mouse Model

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**ABSTRACT**

An estimated 2,500 cases of listeriosis occur annually in the United States. Listeriosis is particularly severe among pregnant women and immunocompromised individuals. Little is known regarding the effect of the food matrix on the ability of *L. monocytogenes* to survive in the gastrointestinal tract and cause systemic infection. Mice were inoculated with various doses of *L. monocytogenes* in skim milk, Half & Half, or whipping cream to determine whether differences in milk fat content influence the ability of *L. monocytogenes* to survive passage through the gut and infect the liver or spleen. The number of fecal samples positive for *L. monocytogenes* increased with increasing doses of *L. monocytogenes* for all three vehicles. The number of *L. monocytogenes* cells isolated from liver or spleen of mice dosed with *L. monocytogenes* was not significantly different among treatment vehicles. Dose-response models revealed that as the dosage of *L. monocytogenes* was increased in different milk vehicles, the number of *L. monocytogenes* cells in liver or spleen also increased. Although fat content of food had no dose-dependent effect on *L. monocytogenes* infection in the murine gastrointestinal tract, we cannot discount the possibility that it may be a factor in *L. monocytogenes* infections of humans because of differences in the physiology of gastrointestinal tracts of mice and humans.

*L. monocytogenes* is a foodborne pathogen that causes listeriosis and can lead to gastrointestinal infection, meningitis, spontaneous abortions, septicemia, primary bacteremia, and endocarditis (9). Listeriosis may occur sporadically or as part of an outbreak. Consumption of highly contaminated foods is considered to be the principal route of *L. monocytogenes* infection. Pasteurized milk (11), soft cheese (23), Mexican-style cheese (2), and deli meats (22, 25) have been associated with outbreaks of listeriosis. In 2000, the Centers for Disease Control and Prevention (CDC) (3) reported that of all the foodborne pathogens tracked by them, *L. monocytogenes* was the causative agent associated with the second highest fatality rate (21%) and the highest hospitalization rate (90.5%). Immunocompromised persons and pregnant women are particularly susceptible to listeriosis (1). The pathogen may translocate from the gastrointestinal (GI) tract to the liver, spleen, central nervous system, placenta, or fetus.

*L. monocytogenes* is frequently isolated from a wide variety of foods (10, 23). Although outbreaks have been associated with many different types of food, little is known regarding the effect of the food matrix on the ability of *L. monocytogenes* to survive in and colonize the GI tract. Although food matrix effects may influence dose-response relationships associated with *L. monocytogenes*, data are insufficient to consider food matrix as a variable within the hazard characterization of risk assessment (14, 26). A better understanding of the effect of fat content of foods on the probability of developing listeriosis may be relevant to determining dose-response relationships for *L. monocytogenes*, and research is needed in this area.

To our knowledge, no studies in animal surrogates have assessed the effects of food matrix on dose response (29); hence, this study was carried out to determine the effect of fat content of dairy products on *L. monocytogenes* colonization and infection in a mouse model. The specific objectives of this study were to (i) compare the extent of colonization and dissemination of *L. monocytogenes* in the GI tract, liver, and spleen of mice after peroral administration in milk products of different fat contents (skim milk, 0.25% milk fat; Half and Half, 11% milk fat; and whipping cream, 30% milk fat) and (ii) develop dose-response curves for *L. monocytogenes* infection as influenced by food vehicles of different fat content.

**MATERIALS AND METHODS**

Adult female mice (BALB/c, Charles Rivers Labs, Raleigh, N.C.) were held under specific-pathogen-free conditions (six animals per group, experiment was conducted three times), with free access to sterile food and water, for 24 h before the beginning of the study and throughout the study period. All animals used in the study were handled in accordance with National Institutes of Health guidelines, and the study was approved by the University’s Institutional Animal Use and Care Committee.

The *L. monocytogenes* strain 12443 (serotype 1/2a) used in the study was isolated from a monkey clinical specimen obtained from a spontaneously aborted fetus from Yerkes National Primate Center (Emory University, Atlanta, Ga.) (28). The culture was stored on latex beads at −80°C. Cultures were grown by overnight incubation at 37°C in tryptic soy broth (Fisher Scientific Inter-
FIGURE 1. Percentage of mice confirmed positive for L. monocytogenes in feces after ingesting L. monocytogenes in skim milk (SM), Half & Half (HH), or whipping cream (WC). Mice were euthanized on day 4 after oral administration of approximately 10^6 to 10^9 CFU of L. monocytogenes (n = 18 mice per dose per treatment). *Significantly different (P < 0.05).

was performed according to the methods described by the CDC (16).

Results were analyzed statistically, and dose-response data were fitted into a four-parameter logistic sigmoidal model based on the Hill equation with Graphpad Prism 4.0 software (24). This model was selected because its equation describes a typical dose-response relationship (4, 5). The general equation is

\[ Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + 10^{\log EC_{50} - X} \text{Hill Slope}} \]  

where X is the logarithm of L. monocytogenes dose, Y is the log value of L. monocytogenes concentration (CFU per gram), the variables Max and Min are the log values of the maximum and minimum L. monocytogenes concentration (CFU per gram), respectively, and log EC_{50} is the log value of the logarithm of L. monocytogenes dose when L. monocytogenes counts are midway between maximum and minimum plateaus of the L. monocytogenes infection. HillSlope describes the steepness (slope) of the curve at its midpoint.

The significance of differences in results for milk fat vehicles at the same dose were determined with Student’s t test. A P value of <0.05 was used to determine significance. All calculations were performed with GraphPad Prism. To compare the dose-response models for the three milk fat vehicles, the individual data sets were combined, and fitted curves were compared with GraphPad Prism.

RESULTS

Mice were given different oral doses of L. monocytogenes in skim milk, Half & Half, or whipping cream to determine whether fat content of a food influences the ability of L. monocytogenes to survive passage through the gut and infect the liver or spleen. The number of fecal samples positive for L. monocytogenes increased with higher doses of L. monocytogenes for all three milk vehicles. At high doses (approximately 10^9 CFU), all animals shed L. monocytogenes in their feces regardless of the fat content of the vehicle used for feeding. However, at doses of 10^6 to 10^4 CFU, the animals receiving L. monocytogenes in whipping cream consistently had fewer fecal samples that were positive for L. monocytogenes. At 1.41 × 10^6 CFU, L. monocytogenes shedding from animals receiving doses in whip-

Fecal samples were collected before dosing, on the day after dosing, and on the day the animals were euthanized and suspended in UVM enrichment broth (Fisher) at 1 g/10 ml. After 2 min of aseptic maceration in a stomacher (Stomacher 400 laboratory blender, Seward, Worthington, UK), the samples were plated on modified Oxford agar (MOX; Oxoid, Basingstoke, UK). The suspension was then incubated at 37°C for 24 h before enrichment in Fraser broth (Fisher) for confirmation of identification. The minimum sensitivity of the enumeration assay was 10^2 CFU/g. Four days after treatment, animals were euthanized by CO2 asphyxiation, and liver, spleen, and cecum were removed aseptically. Each tissue was weighed and macerated in PBS at a ratio of 1:10 (wt/vol). The tissue suspensions were serially diluted (1:10 in PBS) and surface plated on MOX and TSA in duplicate. MOX plates were incubated at 37°C for 48 h, and TSA plates were incubated at 37°C for 24 h to determine bacterial counts in each organ. Colonies were examined with Henry illumination for typical Listeria appearance. Selected colonies were confirmed as L. monocytogenes by Gram stain and API Listeria tests (bioMérieux, Hazelwood, Mo.).

The pulsed-field gel electrophoresis (PFGE) patterns of liver and spleen tissue isolates were compared with those of the original L. monocytogenes strain to verify that they were the same. PFGE was performed according to the methods described by the CDC (16).

Bovine milk–based carriers containing different concentrations of fat (skim milk, 0.25% milk fat; Half & Half, 11% milk fat; and whipping cream, 30% milk fat) were autoclaved at 121°C for 15 min. To prepare the treatment inoculum, the L. monocytogenes pellet was serially diluted in the appropriate autoclaved milk-based carrier to yield cell suspensions of approximately 10^2, 10^4, 10^6, and 10^8 CFU. Mice were challenged by gavage with 0.3 ml of inoculum. Six animals were evaluated per target dose (0, 10^2, 10^4, 10^6, 10^8, 10^9 CFU) per treatment (skim milk, Half & Half, and whipping cream). The study was conducted three times (total of 18 mice per dose per treatment). The actual mean doses were 3.63 × 10^2, 6.60 × 10^4, 1.41 × 10^6, 3.80 × 10^8, and 1.62 × 10^9 CFU, respectively.

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Percentage of mice confirmed positive for *L. monocytogenes* in livers (a) or spleens (b) after ingestion of *L. monocytogenes* in skim milk (SM), Half & Half (HH), or whipping cream (WC). Mice were euthanized on day 4 after oral administration of approximately $10^2$ to $10^9$ CFU of *L. monocytogenes* ($n=18$ mice per dose per treatment). There are no significant differences among groups given different milk-based vehicles when comparing the percentage of mice confirmed positive for *L. monocytogenes* based on samples from either liver or spleen.

Dosing cream was significantly lower than that for animals receiving other vehicles ($P < 0.05$; Fig. 1). After treatment with $6.60 \times 10^4$ CFU, fecal shedding of *L. monocytogenes* was highly variable and vehicle effects were not significant ($P < 0.05$).

The percentage of mouse livers and spleens that were positive for *L. monocytogenes* in each dose group was not significantly different when *L. monocytogenes* was administered in skim milk, Half & Half, or whipping cream (Fig. 2a and 2b). However, there were similar trends in both liver and spleen that were dose dependent. After treatment with approximately $10^6$ CFU, *L. monocytogenes* was isolated from liver and spleen of all mice regardless of vehicle (Fig. 2a and 2b). There was no significant difference in recovery of *L. monocytogenes* within dose groups ($P < 0.05$).

Selected colonies from fecal and tissue samples obtained from each treated group of mice were assayed by PFGE. PFGE banding patterns for these colonies were identical to those of the treatment strain in all cases (data not shown), indicating that the treatment strain was responsible for infection, and no contaminating or indigenous *L. monocytogenes* were detected.

Dose-response plots of *L. monocytogenes* (CFU per gram of organ) isolated from mouse livers or spleens receiving different doses of *L. monocytogenes* in skim milk, Half & Half, or whipping cream are shown in Figures 3a and 3b. *L. monocytogenes* was not detected by direct counting in livers or spleens 4 days after oral exposure to approximately $10^2$ CFU administered through any of the three vehicles; however, both livers and spleens were infected in mice receiving doses of $\geq 10^4$ CFU in any of the three vehicles. The degree of infection based on *L. monocytogenes* counts from livers and spleens of animals receiving doses of $10^4$ to $10^9$ CFU was similar for each vehicle of administration (Fig. 3a and 3b). At doses of $10^9$ CFU and above, organ burden plateaued, and there was no significant difference based on vehicle in the number of *L. monocytogenes* isolated from liver or spleen.

The doses necessary for eliciting 50% infection (EC$_{50}$), as estimated assuming a four parameter logistic sigmoidal dose-response relationship, were $3.6 \times 10^4$, $2.0 \times 10^4$, and $1.3 \times 10^4$ CFU when administered in whipping cream, Half & Half, and skim milk, respectively. These EC$_{50}$s were not significantly different. Hence, infectivity in mice 4 days after oral exposure to *L. monocytogenes* does not appear to be influenced by milk fat content of the delivery vehicle at any dose level within a very broad range. The estimated EC$_{50}$s based on the dose-response model and maximum
FIGURE 4. Global curve fit for sigmoidal dose response of L. monocytogenes counts in livers (a) or spleens (b) of mice fed L. monocytogenes in vehicles with different fat contents: skim milk (SM), Half & Half (HH), or whipping cream (WC) (n = 18 mice per dose per treatment). The minimum dose (Min), maximum dose (Max), EC$_{50}$, and log EC$_{50}$ represent the midpoint dose between the Min and Max doses, the HillSlope is the calculated slope of the dose-response curve, and R$^2$ represents the correlation coefficient of the fit of the curve to the data points.

level of infection at 4 days after exposure were each similar for all three test vehicles.

To evaluate differences among the dose-response curves for the three vehicles, a global curve was fitted to the combined data set for all fat-containing vehicles for L. monocytogenes counts in spleens or livers separately (Fig. 4a and 4b). The global curve was compared with each of the respective curves of the three milk fat vehicles using a modeled curve comparison procedure in Graphpad Prism (24). The analysis revealed that the global fit curve was not significantly different (P < 0.05) for L. monocytogenes counts in spleens or livers from the three individual curves for each milk fat vehicle.

DISCUSSION

Outbreaks of listeriosis associated with low concentrations of L. monocytogenes are frequently reported in foods with high fat content, such as Queso blanco or fresco cheese (20) and butter (21). These high-fat foods are associated with outbreaks where the estimated ingested dose was relatively low (<10$^4$ CFU). Our studies in pregnant primates suggest that infectivity increases in high-fat vehicles (28). No reports have been published concerning direct comparison of the effect of fat content of a food on L. monocytogenes colonization of the GI tract and infection of the liver or spleen of mice. The purpose of our study was to determine the effect of vehicle fat content on L. monocytogenes 1/2a intestinal colonization and infection of liver and spleen in mice.

Intragastric exposure to L. monocytogenes results in systemic infection in selected strains of mice (8, 15, 17, 18). In the mice in our study that were exposed to L. monocytogenes at a dose of approximately 10$^4$ CFU, intestinal colonization was highly variable, and significant differences between groups treated with different vehicles could not be demonstrated. At high exposures (10$^8$ or 10$^9$ CFU), the incidence of fecal shedding was very high (≥83% of exposed animals shed L. monocytogenes) and independent of the fat content of the vehicle. At approximately 10$^6$ CFU, incidence of shedding appeared to be less when whipping cream (high fat) was used as the vehicle of administration than when Half & Half or skim milk were used. Thus, in the mouse model the effects of vehicle fat content on L. monocytogenes shedding were subtle and occurred only at selected doses.

Systemic infection was seen with each vehicle at all dosages. Both the incidence and level of infection were dose dependent, but this relationship was most apparent for incidence of infection (Fig. 3a and 3b). Organ burdens were near or at plateau levels over the dose range of 10$^4$ to 10$^6$ CFU. The modest reduction in susceptibility to Listeria in a high-fat vehicle (i.e., reduced fecal shedding at a dose of 10$^6$ CFU) may reflect the physiological response to ingestion of fat (29, 30). This mouse model did not exhibit an increase in susceptibility to systemic infection when L. monocytogenes was administered in a high-fat vehicle. However, host-dependent differences in susceptibility to listeriosis has been reported. The E-cadherin molecule in human and guinea pig gut epithelial cells contains a proline at position 16 to which internalin on the bacterial surface is able to bind (6, 13, 19). Lecluit et al. (19) confirmed that a single amino acid difference in murine E-cadherin, a glumatic acid at position 16, does not allow binding of the L. monocytogenes internalin A protein, which is critical for invasion of intestinal epithelial cells. This amino acid difference also explains why mice are less susceptible to Listeria infection via oral inoculation, even though oral transmission is the usual route in humans. Another difference between mice and humans is in gastric pH, which is about 4.0 in mice and about 2.0 to 2.5 in humans (7, 12).

The effects of dietary intake of fat must be separated from the effects of the food matrix. In studies in which L. monocytogenes was administered to rats in saline, Sprong et al. (29) reported that fecal excretion of Listeria was significantly reduced for rats fed high-milk-fat diets compared with that for rats fed low-fat diets. Shinomiya et al. (27) found that in mice fed a high-fat diet and given a sublethal dose of L. monocytogenes, pathogen numbers increased during the first 3 days of infection, whereas pathogen numbers in control mice declined during the same period. The physiological responses to long-term dietary fat ingestion.
are distinct from those to acute ingestion of contaminated high-fat foods.

Studies with a small number of pregnant primates given \(L.\) monocytogenes in skim milk, Half & Half, or whipping cream suggested that stillbirths occurred at lower concentrations among animals receiving \(L.\) monocytogenes in whipping cream than among animals that received \(L.\) monocytogenes in skim milk or Half & Half (28). Analysis of results of fecal shedding of \(L.\) monocytogenes according to the vehicle of administration revealed that animals receiving \(L.\) monocytogenes in whipping cream shed up to 18 times more \(L.\) monocytogenes per gram of feces than those receiving the pathogen in Half & Half; however, because of the small number of animals treated with different vehicles, a statistical analysis could not be performed (28).

The results of these studies reveal various responses to \(L.\) monocytogenes administered in high-fat foods or diets. Fecal shedding of \(L.\) monocytogenes in mice was higher when the pathogen was given in skim milk than when it was given in whipping cream, although this difference was significant only at one dose. The number of fecal samples positive for \(L.\) monocytogenes and the incidence of infection in liver and spleen increased with increasing dose, regardless of fat content of the delivery vehicle.

Intestinal colonization by \(L.\) monocytogenes may depend on fat content of diets or vehicles of administration, presence of E-cadherin, gastric pH, and several other factors such as bile salts, fatty acids, and minerals. The effects of such factors may help explain differences among studies conducted with different animal species. Although fat content of the food had no significant effect on \(L.\) monocytogenes colonization of the murine GI tract, the lack of significant differences among groups treated with different vehicles suggests that high numbers of \(L.\) monocytogenes in food overcome any effects of food matrix. However, because of differences in the physiology of GI tracts of mice and humans, we cannot discount the possibility that fat content may be a factor in \(L.\) monocytogenes infections in humans.

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REFERENCES