Dose-Response of *Listeria monocytogenes* after Oral Exposure in Pregnant Guinea Pigs

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**ABSTRACT**

Listeriosis, a severe disease that results from exposure to the foodborne pathogen *Listeria monocytogenes*, is responsible for ~2,500 illnesses and 500 deaths in the United States each year. Pregnant women are 20 times more likely to develop listeriosis than the general population, with adverse pregnancy outcomes that include spontaneous abortions, stillbirths, and neonatal meningitis. The objective of this study was to determine an infective dose that resulted in stillbirths and infectivity of selected tissues in pregnant guinea pigs. Pregnant guinea pigs were exposed orally on gestation day 35 to 10⁴ to 10⁸ *L. monocytogenes* CFU in sterile whipping cream. *L. monocytogenes* was recovered at 64, 73, 90, and 100% from the livers of animals infected with 10⁵, 10⁶, 10⁷, and 10⁸ CFU, respectively. In dams exposed to ≥10⁸ CFU, *L. monocytogenes* was cultured from 50% of the spleen samples and 33% of the gallbladder samples. Eleven of 34 dams infected with ≥10⁶ CFU delivered stillborn pups. *L. monocytogenes* was cultured from the placenta, liver, and brain tissue of all stillbirths. Dams that delivered nonviable fetuses after treatment with ≥10⁷ *L. monocytogenes* CFU had fecal samples positive for *L. monocytogenes* at every collection posttreatment. On the basis of a log-logistic model, the dose that adversely affected 50% of the pregnancies was approximately 10⁷ *L. monocytogenes* CFU compared with that estimated from a human outbreak of 10⁷ CFU. Listeriosis in pregnant guinea pigs can result in stillbirths, and the overall disease is similar to that described in nonhuman primates and in humans.

Listeriosis results from exposure to *Listeria monocytogenes*, a gram-positive bacterium found in the environment that is capable of contaminating various foods. Almost one third of the listeriosis cases occur in pregnant women and carry the risk of fetoplacental infection that can result in stillbirths, the occurrence of preterm delivery, or severe health effects, such as septicemia, pneumonia, or meningitis (21, 25). Clinical manifestations in the fetus appear to be dependent on the point of gestation when exposure occurs. Second- and third-trimester infections can result in premature delivery that is followed by neonatal illness or preterm delivery of a stillborn (9, 11). Listeriosis most commonly occurs during the third trimester.

A pregnant animal model that expresses similar clinical manifestations and mimics the primary human exposure route is essential to accurately assess the risk of maternal exposure to *L. monocytogenes* to the fetus or neonate. The risk assessment by the U.S. Food and Drug Administration–U.S. Department of Agriculture–Centers for Disease Control and Prevention (FDA-USDA-CDC) (26) that evaluates *L. monocytogenes* in ready-to-eat foods relied on a dose-response curve obtained from mouse data. Because the only animal data for which there was sufficient information to obtain a dose-response curve was from a mouse study, the FDA-USDA-CDC applied an adjustment factor to mouse data for estimating human risk (26). However, mice may not be an appropriate surrogate for human listeriosis. Mice are not susceptible to stillbirths after oral exposure to *L. monocytogenes*, and the dose-response data used by the FDA-USDA-CDC were based on death rates in adult mice (12). This difference in susceptibility may be due to the E-cadherin receptor, which has been proposed as the receptor used by *L. monocytogenes* to gain entry into intestinal epithelial cells.

A single amino acid substitution located at position 16 in murine E-cadherin (proline to glutamic acid) results in a loss of the ability of *L. monocytogenes* and the receptor to interact efficiently (17). A transgenic mouse that expresses human E-cadherin demonstrates the interactions of *L. monocytogenes* internalin and E-cadherin in vivo (18); however, these receptors are localized within the intestine and may not be associated with other tissues, specifically, the placenta.

Previous studies show that pregnant nonhuman primates are susceptible to listeriosis both naturally and experimentally (1, 20, 24). Furthermore, fetal infections in both humans and nonhuman primates can lead to similar conditions, such as abortions, stillbirths, and neonatal deaths (5, 20). For humans and nonhuman primates, the pathogenesis and morphological findings associated with stillbirths due to *L. monocytogenes* are essentially the same (1, 5). Yet the number of primates needed to thoroughly

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examine the low-dose region of the dose-response curve and to conduct mechanistic studies is prohibitive, and presently, the sequence of their E-cadherin protein has not yet been published.

Studies conducted in the 1970s used nonpregnant guinea pigs to investigate the interaction between Listeria and host cells. With electron microscopy, the interaction between L. monocytogenes and intestinal epithelial cells was investigated after guinea pigs had been infected with \(10^9\) CFU through a stomach tube (23). These studies showed that L. monocytogenes entered the epithelial cells of the small intestine and multiplied before being phagocytosed by macrophages (23). Pregnant guinea pigs have also been used to characterize Listeria isolates (8). Interestingly, guinea pig E-cadherin binds to internalin, and it has the same amino acid sequence as human E-cadherin (17). Despite the preponderance of studies in mice, guinea pigs have been identified as the small animal of choice for L. monocytogenes studies because of the E-cadherin molecule (17).

The objectives of this study were to use pregnant guinea pigs that were orally exposed to L. monocytogenes to (i) examine the pregnant guinea pig as a surrogate model for human listeriosis; (ii) determine the maternal dose that results in the placental transmigration leading to fetal tissue infectivity, stillbirths, or both; and (iii) relate adverse fetal outcomes to maternal fecal shedding and tissue infectivity.

**MATERIALS AND METHODS**

**Inoculum preparation.** The L. monocytogenes strain used in this study was isolated from a Listeria-induced stillbirth from a rhesus monkey and was subsequently used to induce stillbirths in a primate study by Smith et al. (24). Strain 12443 (serotype 1/2a) is currently stored on cryo-beads (Prolab Diagnostics, Austin, Tex.) and maintained at -80°C. L. monocytogenes cells were grown during three successive overnight transfers in 10-ml aliquots of tryptic soy broth (BD Diagnostics, Sparks, Md.) and incubated at 35°C. Cultures were then harvested by centrifugation (9,000 × g at 4°C for 30 min), washed twice, and resuspended in sterile phosphate-buffered saline (PBS, pH 7.2). Cultures were diluted to give final concentrations from \(10^8\) to \(10^4\) CFU/ml. The appropriate number of L. monocytogenes cells was added to commercial heavy whipping cream that contained 36% milk fat, and the inoculum was sweetened with 0.5 g of Splenda. Whipping cream was chosen as the vehicle, because previous studies completed by our laboratory have indicated that whipping cream is an effective method of delivering L. monocytogenes and producing stillbirths in nonhuman primates (24). Also, the fat content of the vehicle used to deliver L. monocytogenes inoculum does not affect the fecal shedding or infectivity of L. monocytogenes in mice (22). Whipping cream was sterilized by autoclaving at 121°C for 13 min. Sterile PBS (1.0 ml) was added to the whipping cream and administered to the control animals.

The number of L. monocytogenes cells in the inoculated sample was confirmed by serially diluting the cell suspension in PBS (0.01 M) and plating onto Listeria selective agar (Oxoid, Ogdensburg, N.Y.). The plates were incubated at 35°C for 24 h before colony enumeration.

**Animals and treatment.** Timed-pregnant guinea pigs were obtained from Elm Hill Breeding Laboratories (Chelmsford, Mass.) on gestation day 29, housed in cages containing air filters, and maintained on a 12-h light:dark cycle. The temperature and humidity were 21 ± 1°C and 55% ± 15%, respectively. The animals were provided sterilized water and Purina autoclavable guinea pig diet (PMI Nutrition International, St. Louis, Mo.) ad libitum. A 22.1-g encapsulated vitamin C supplement (Prima-Treats F0308, Bio-Serv, Frenchtown, N.J.) was supplied once per week. Animals were trained to orally ingest the vehicle 2 days prior to treatment by providing controlled delivery with a transfer pipette. By the day of treatment, guinea pigs freely drank the sweetened whipping cream.

The normal gestational period for guinea pigs is approximately 65 days. On the basis of the time it took for a stillbirth to manifest in our primate study (24), we allowed 21 gestation days before sacrifice to allow L. monocytogenes colonization and invasion of the placenta and fetus.

Inoculum doses ranged from \(10^6\) to \(10^4\) L. monocytogenes CFU/5 ml of sterile whipping cream. Immediately before administering the inoculum, control animals were transferred to and maintained in a separate room. The animals were observed daily for changes in the amount of fecal output or appearance, changes in the degree of activity or appearance, and weight loss. Guinea pigs were sacrificed on gestation day 56, except for five animals, which delivered stillbirths prematurely.

**Tissue collection and L. monocytogenes confirmation.** Following necropsy, maternal and fetal tissues were collected and weighed, transferred to a primary enrichment broth, and used for further analysis. The tissues analyzed included the following: the maternal serum, gallbladder, liver, and spleen, along with the placenta, fetal liver, and brain. Tissues and fecal samples were confirmed positive for L. monocytogenes according to the methodology set forth by the USDA, as described by Cook (6), which includes both qualitative and quantitative detection methods. The quantitative method uses direct plating on Listeria selective agar (Remel, Lenexa, Kans.). The qualitative method includes enrichment in both nonselective and selective media and then plating onto Listeria selective agar. Immediately following the necropsy, tissue samples were placed in 100 ml of nonselective enrichment medium, modified University of Vermont broth (BD Diagnostics). After 24 h, 0.1 ml of the modified University of Vermont–enriched sample was transferred to 9.9 ml of the secondary enrichment, Fraser broth (Remel). Positive Fraser samples were streaked onto Listeria selective agar and incubated for 24 h at 35°C. Selected isolated colonies were streaked onto Rapid L’mono plates (Bio-Rad, Hercules, Calif.) for confirmation as L. monocytogenes.

**Fetal viability.** Fetuses were examined in utero and classified as normal or nonviable. A stillbirth was defined as a nonviable fetus that delivered prematurely. Fetal viability at the time of sacrifice was based on several factors: physical characteristics, including eyelid skin formation and eyelid opening; skin color and formation; hair growth; pinna formation; appearance of placenta and cord blood; appearance of cyanosis; and notably smaller size for gestational age if viable littermates were present.

**Statistical analysis.** Differences in fetal weights and lengths were analyzed by a one-way analysis of variance and least significant differences for mean separation, \(P \leq 0.05\) (version 8.2, SAS, Cary, N.C.). The remaining data were analyzed by the Kruskal-Wallis test for multiple comparisons and Dunnett’s method for comparison with the control (version 3.1, SigmaStat, Sioux City, S.D.). To calculate the 50% infectivity dose (ID\(_{50}\)) of tissues, a log-logistic model (version 8.0, PSI Plot, Pearl River, N.Y.) was fitted to the raw data for the maternal liver, spleen, or gallbladder.

The equation was as follows: \(f = a[(1 + \exp[b(x - c))] + d\), and the parameters were as follows: \(a = 334159\), \(b = 0.813\),
TABLE 1. Isolation of L. monocytogenes from maternal and fetal tissues after oral challenge during pregnancy

<table>
<thead>
<tr>
<th>Maternal dose</th>
<th>Liver&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Spleen&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gallbladder&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of dams with infected fetuses (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Placenta&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fetal liver&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fetal brain&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/9 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/9 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/9 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/9 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/33 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/33 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/33 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;8&lt;/sup&gt; CFU</td>
<td>1/4 (25) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/4 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/4 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/4 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/18 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/18 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/18 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;5&lt;/sup&gt; CFU</td>
<td>7/11 (64) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/11 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/11 (9) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/11 (18) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/62 (3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/62 (3) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/62 (0) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;6&lt;/sup&gt; CFU</td>
<td>8/9 (89) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>2/9 (22) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/9 (11) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/9 (22) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/41 (7) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>6/41 (15) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/41 (12) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;7&lt;/sup&gt; CFU</td>
<td>8/9 (89) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/8 (38) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/8 (25) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/9 (33) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>13/31 (42) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>12/31 (39) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>11/31 (35) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;8&lt;/sup&gt; CFU</td>
<td>4/4 (100) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/4 (75) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>1/3 (33) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/4 (75) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>9/14 (64) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>12/17 (71) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>12/17 (71) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of positive tissues/total number exposed (% positive).
<sup>b</sup> Dams with at least one infected fetus.
<sup>c</sup> Groups with different letters are significantly different (P < 0.05).

RESULTS

There was a dose-dependent relationship in the colonization of L. monocytogenes within the maternal liver, spleen, and gallbladder tissue samples (Table 1). However, at doses that were <10<sup>6</sup> CFU, L. monocytogenes was not isolated from any maternal spleens and from only 1 of 15 gallbladders. In contrast, L. monocytogenes was isolated from liver tissue at the lowest dose tested (10<sup>4</sup> CFU) in one of four animals. Dams that received ≥10<sup>5</sup> L. monocytogenes CFU had a significantly higher percentage of L. monocytogenes–infected livers than did controls and dams exposed to 10<sup>4</sup> CFU. Dams exposed to 10<sup>6</sup> L. monocytogenes CFU had a significantly greater percentage of positive spleen samples than did controls or dams exposed to <10<sup>8</sup> L. monocytogenes CFU. Although there were no statistical differences in the isolation of L. monocytogenes from gallbladder samples among different treatment groups, L. monocytogenes was isolated from at least one gallbladder from groups infected with ≥10<sup>5</sup> L. monocytogenes CFU. The control samples were negative for L. monocytogenes throughout the experiment (Table 1).

Three of the 40 exposed guinea pigs were not pregnant; however, the liver, spleen, and gallbladder samples were analyzed for culturable L. monocytogenes. Two of the guinea pigs were infected with 10<sup>6</sup> L. monocytogenes CFU, and one was infected with 10<sup>8</sup> L. monocytogenes CFU. Numbers of L. monocytogenes CFU were cultured from all of the nonpregnant animals’ livers. One guinea pig that was infected with 10<sup>6</sup> L. monocytogenes CFU had an infected spleen. None of the nonpregnant guinea pigs had infected gallbladders at the time of sacrifice (data not shown).

Of the pregnancies that resulted in fetal mortality, 90, 70, and 50% of the dams’ livers, spleens, and gallbladders were infected with L. monocytogenes, respectively (data not shown). Interestingly, in dams that delivered preterm stillbirths, L. monocytogenes was cultured from all maternal livers and spleens. When comparing infectivity in dams, the liver had the lowest calculated ID<sub>50</sub> compared with the spleen and gallbladder (Table 2), but invasion of the liver was not a predictor of fetal mortality, because animals with normal fetuses had ID<sub>50</sub> values similar to those with nonviable fetuses (Table 2).

Visible hepatic lesions from infected dams suggested liver damage (14); thus, we investigated serum alanine aminotransferase (ALT) levels as an indicator of damage. Because of the unavailability of guinea pig reagents, a human kit was utilized during these experiments (13). There were no significant differences in serum ALT activity at any dose tested (data not shown). However, these results did not include animals that prematurely delivered stillbirths.

Following the maternal ingestion of ≥10<sup>5</sup> CFU, L. monocytogenes was isolated from the placenta and fetus (Table 1). In general, if L. monocytogenes infected a placenta, there was a high likelihood that the fetal liver and brain would also be infected. When the fetal brain was infected, the fetal liver for that animal was always also infected with L. monocytogenes.
TABLE 3. Average weights and lengths of gestational day-
matched fetuses from dams orally challenged with L. mono-
cytogenes$\textsuperscript{a}$

<table>
<thead>
<tr>
<th>Maternal dose</th>
<th>Mean weight ± SD (g)</th>
<th>Mean length ± SD (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.2 ± 6.2</td>
<td>7.65 ± 1.04</td>
</tr>
<tr>
<td>$10^4$ CFU</td>
<td>75.1 ± 19.2$^b$</td>
<td>7.65 ± 0.18</td>
</tr>
<tr>
<td>$10^5$ CFU</td>
<td>60.1 ± 5.1</td>
<td>7.09 ± 0.51</td>
</tr>
<tr>
<td>$10^6$ CFU</td>
<td>61.7 ± 8.2</td>
<td>7.87 ± 1.19</td>
</tr>
<tr>
<td>$10^7$ CFU</td>
<td>56.8 ± 10.1</td>
<td>7.19 ± 0.79</td>
</tr>
<tr>
<td>$10^8$ CFU</td>
<td>54.3$^c$</td>
<td>6.65$^c$</td>
</tr>
</tbody>
</table>

$^a$ Excludes premature deliveries and stillbirths not age matched.
$^b$ Denotes samples that are statistically different from control samples.
$^c$ n = 1 dam with 5 fetuses; all other dams infected with $10^8$ CFU had prematurely delivered stillbirths.

When compared with controls, there was a significant increase of fetal weights in dams exposed with $10^4$ CFU (Table 3), but there was no significant change in the other treatment groups. Note that the weights and lengths of fetuses for the $10^8$ treatment group are based on one litter, because the remaining three litters resulted in preterm stillbirths and were therefore not at the appropriate gestational age for comparisons.

$L. monocytogenes$ was isolated from all fetal liver and fetal brain tissues examined from each stillbirth, suggesting that a high degree of infectivity was incompatible with life and resulted in premature delivery (Fig. 1). None of the placental or fetal brain samples from infected dams with normal fetuses were positive for $L. monocytogenes$. However, some normal fetuses of exposed dams had infected livers (Fig. 1). All control fetuses were negative for $L. monocytogenes$ for all tissues examined.

$L. monocytogenes$ was cultured from the livers of all dams with ≥1 infected fetal brain (Table 4). When the fetal brain was infected, there seemed to be a high correlation among fetal liver, placenta, and maternal liver infectivity.

Exposed dams with viable fetuses showed a dose-dependent increase in fecal shedding (Fig. 2). Infected dams with nonviable fetuses or stillbirths shed $L. monocytogenes$ for longer time periods than those with normal fetuses (data not shown). Guinea pigs that were exposed to ≥$10^6$ $L. monocytogenes$ CFU had an increase in the number of livers (Fig. 1).

A dose-response curve based on fetal mortality was constructed for the guinea pig fetal mortality data. For the logistic regression model, the mortality rate is given by the following formula:

$$P = \frac{1}{1 + \exp(-\beta(x - \mu))}$$

where $\mu$ denotes the 50% lethal dose (LD$_{50}$), and $\beta$ governs the rate at which mortality increased with increase log dose $x$ of $L. monocytogenes$. Under this model, that rate is maximized at the LD$_{50}$. In addition, the first derivative of this risk function with respect to dose is symmetric about the LD$_{50}$. Note that the logistic regression model is a special case in which $\gamma = 0$. Under this model, the LD$_{50}$ is given by the following formula:

$$\ln LD_{50} = \mu + \frac{1}{\beta} \log \frac{0.5^{1/\gamma}}{1 - 0.5^{1/\gamma}}$$

The parameter estimates for the model are as follows: $\beta = 1.4776$ and $\mu = 7.3008$. The estimated $\ln LD_{50}$ is 7.3008, and the estimated LD$_{50}$ is $1.999 \times 10^7$ (95% confidence limits of $0.352 \times 10^7$ and $11.340 \times 10^7$). The complimentary log-log and power logistic were also examined for their fit to the dose-response data. There is little to distinguish between the goodness-of-fit characteristics of the three models, as indicated by the similarity of their log likelihoods. The complimentary log-log function fit the data most poorly, with a log likelihood of −13.579, compared with a log-likelihood value for the logistic model of

FIGURE 1. Infection of fetal tissues after dams were fed $L. monocytogenes$. Fetuses were classified as stillbirths when they were delivered prematurely and were not viable. All other fetuses were examined in utero at the time of sacrifice and classified as normal or nonviable. Fetal tissues were categorized by birth outcome (normal, nonviable, and stillborn fetuses), and the bars represent the percentage of tissues positive for $L. monocytogenes$ in each group. n, number of dams.
FIGURE 2. Fecal shedding of L. monocytogenes in dams fed L. monocytogenes. Two samples were collected pretreatment to ensure that the animals were not shedding L. monocytogenes prior to treatment; three samples per week were collected posttreatment. Maternal fecal samples were categorized by dose group and birth outcome.

FIGURE 3. Dose-response of L. monocytogenes–induced stillbirths in guinea pigs. A log-logistic model was used to fit the dose-response data (solid line) on the basis of the dose resulting in fetal deaths. The estimated LD50 was $1.999 \times 10^7$ L. monocytogenes CFU. Solid dots represent the average mortality for each dose group. Dashed lines represent 95% confidence limits to fitted data.

DISCUSSION

We add to the growing literature that pregnant guinea pigs can be used as a model for human listeriosis by orally challenging dams with L. monocytogenes and monitoring the following endpoints as indicators of infection: infectivity of maternal and fetal tissue, maternal fecal shedding, and birth outcomes. Research has shown that following exposure to L. monocytogenes, pregnant guinea pigs exhibit clinical manifestations similar to humans, including fetal abortions (8) as well as gastrointestinal tract disturbances (16). Intraperitoneal injection of L. monocytogenes also resulted in abortions in guinea pigs when the animals were treated with infected tissues (8).

Our studies support the use of the pregnant guinea pig as a surrogate for oral exposure in humans on the basis of similarities in birth outcome and tissue infectivity. The similarities probably relate to the mechanism of how L. monocytogenes gains entry into the body. Recently, it was shown that E-cadherin, which mediates the transmigration of L. monocytogenes into mammalian epithelial cells, has the same sequence in both humans and guinea pigs (17). E-cadherin protein is important in the binding of L. monocytogenes to epithelial cells. A single amino acid transition can alter the active site’s configuration, as in the mouse, resulting in the inability of L. monocytogenes to bind to the receptor (17).

Early diagnosis and antimicrobial treatment of listeriosis during pregnancy can result in the birth of a healthy infant (15). However, the lack of biomarkers or early diagnostic tests can result in fetal deaths. Because liver lesions were observed in treated guinea pigs (14), we examined serum ALT levels as a potential biomarker of L. monocytogenes infection. We did not see dose-related changes in serum ALT levels; however, these results did not include animals that prematurely delivered stillbirths, which may have experienced more severe liver damage. The lack of a serum ALT response is also consistent with a listeriosis human case study that analyzed liver abscesses in eight elderly individuals with diabetes (4). Laboratory

<table>
<thead>
<tr>
<th>Maternal dose</th>
<th>No. of litters</th>
<th>% litters with ≥1 fetal death</th>
<th>Total no. of fetuses</th>
<th>% fetal mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>0</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>$10^4$ CFU</td>
<td>4</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>$10^5$ CFU</td>
<td>11</td>
<td>0</td>
<td>64</td>
<td>0</td>
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<tr>
<td>$10^6$ CFU</td>
<td>9</td>
<td>22</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>$10^7$ CFU</td>
<td>9</td>
<td>33</td>
<td>42</td>
<td>43</td>
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<tr>
<td>$10^8$ CFU</td>
<td>4</td>
<td>75</td>
<td>21</td>
<td>95</td>
</tr>
</tbody>
</table>
tests showed that transaminase levels were atypical in only two of the eight individuals. Interestingly, alkaline phosphatase levels were elevated in seven of the eight individuals. Thus, alkaline phosphatase may be a more appropriate biomarker for *L. monocytogenes* damage to the liver than serum ALT.

Fecal shedding appears to be a better biomarker of *L. monocytogenes* infection than serum ALT levels. Interestingly, our fecal shedding results (Fig. 2) are similar to a previous primate study in which pregnant rhesus monkeys that delivered stillborn infants shed *L. monocytogenes* at a higher rate and for a longer period than in normal pregnancies (24). As seen in Figure 2, dams that received higher doses of *L. monocytogenes* had more positive fecal samples than did dams that received lower doses. Although fecal shedding is not an absolute predictor of stillbirth, animals with greater than 60% positive fecal samples had pregnancies that ended in stillbirths.

To our knowledge, no previously published dose-response studies of *L. monocytogenes* have used oral exposure in guinea pigs, although previous studies have used other routes of exposure, including intravenous, intraperitoneal, and intracardial injections, to expose guinea pigs to *L. monocytogenes* (2, 3, 7). Because humans are exposed to *L. monocytogenes* through ingestion, experimentally bypassing the gastrointestinal tract may lead to differences in virulence and infective dose estimates for the pathogen. By means of an exposure regimen consistent with human exposures, better dose estimates of maternal and fetal adverse outcomes can be obtained. Our research shows that pregnant guinea pigs orally exposed to *L. monocytogenes* had an increased, dose-dependent risk of delivering stillborn infants. Also, *L. monocytogenes* isolates could be cultured from maternal tissues and fetal materials, as well as from fetal tissues.

Three dose-response models were examined for their fit to our data. There was no significant difference between the two models with the best fit (logistic and power logistic). In addition, the estimated LD$_{50}$ value was not sensitive to the model used to fit these data. On the basis of a logistic fit to the dose-response data, the dose adversely affecting 50% of the pregnancies was $1.999 \times 10^7$ (95% confidence limits from $3.52 \times 10^6$ to $1.1 \times 10^8$) *L. monocytogenes* CFU. This can be compared with that estimated in the Food and Agriculture Organization, World Health Organization risk assessment (10) of $1.9 \times 10^6$ for perinates or neonates after a population of pregnant women was exposed. This ID$_{50}$ value was calculated from an exponential dose-response curve based on attack rates from a Mexican-style cheese outbreak (19). Thus, calculations based on two different species (humans and guinea pigs) and two different dose-response models (exponential and logistic) estimated the 50% affected rate at about an order of magnitude difference. These results add to our accumulating knowledge, suggesting that guinea pigs are appropriate surrogate models for studying the fetoplacental transmission of *L. monocytogenes* in humans and that dose-response information from these animals can be used in human risk assessment.

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