Listeria monocytogenes infection in pregnant guinea pigs is associated with maternal liver necrosis, a decrease in maternal serum TNF-α concentrations, and an increase in placental apoptosis

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\textbf{A B S T R A C T}

Stillbirths and spontaneous abortions can result when pregnant women are exposed to the food borne pathogen, Listeria monocytogenes. Fetuses and neonates account for one-third of the 2500 cases annually. The objectives were to determine the dose dependent trends of immunological and pathological effects in pregnant guinea pigs after infection with L monocytogenes. Timed pregnant guinea pigs were treated on gestation day (gd) 35 with doses of 10^4 to 10^8 colony forming units (CFUs) and sacrificed on gd 56. Hepatic lesions were found in dams treated with ≥ 10^5 CFUs. Apoptosis was detected in significantly more placentas from dams treated with ≥ 10^6 CFUs compared to controls. Maternal serum TNF-α concentrations were significantly decreased in all dose groups compared to controls. In conclusion, increases in premature delivery, maternal hepatic effects and placental apoptosis along with a decrease in TNF-α concentrations were associated with L monocytogenes infection in pregnant guinea pigs.

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1. Introduction

Listeriosis is the clinical manifestation of Listeria monocytogenes (L. monocytogenes) infection. In the United States, it is estimated that 500 deaths occur each year due to listeriosis [1]. The primary route of exposure in humans is through the consumption of contaminated foods. Ingestion of L monocytogenes in pregnant women carries the risk of feto-placental transmission [2]. The mother may be asymptomatic or may experience a slight fever while the fetus suffers more serious consequences, such as stillbirth or septicemia of the neonate [3,4].

To gain entry into the systemic circulation after oral ingestion, L monocytogenes must cross the intestinal barrier. A specific protein receptor, E-cadherin, is used to mediate gastrointestinal invasion and transmission of L monocytogenes across the intestinal barrier [5]. The amino acid sequence in the active site of E-cadherin is identical in humans and guinea pigs [6]. After exposure to 10^6 or greater colony forming units (CFUs) L monocytogenes, pregnant guinea pigs have the same etiology and pregnancy outcome [7] as humans [4].

In pregnant guinea pigs, maternal liver invasion appears to occur prior to fetal infection after oral treatment with L monocytogenes [7]. Because maternal exposure to L monocytogenes and subsequent liver invasion can lead to fetal infection, placental transmigration of L monocytogenes is an essential step in L monocytogenes-induced stillbirths. Humans have a hemochorial placenta, and guinea pigs are the rodent species with the most similarity to human placentas [8]. Because of the importance of the maternal–fetal interface and placental transmission of L monocytogenes, the guinea pig’s similarity to the human placenta is very important. In addition, placental invasion and degradation may be an important aspect of infection-induced stillbirths. Toxoplasma gondii, an intracellular parasite, has been shown to cause apoptosis in placental trophoblasts after infection [9]. The induction of apoptosis within the placenta following infection could result in increased pathogen transmission to the fetus.

After pathogen exposure, the immune system's ability to recognize and target the pathogen is necessary to prevent systemic infection. The immune system is altered during pregnancy, likely...
to prevent allograft rejection. It has been suggested that after exposure to an infectious pathogen during pregnancy, a B cell antibody response is elicited instead of an inflammatory response, which occurs in non-pregnant individuals [10]. Two subsets of cytokines elicit different responses after activation. Th helper type-1 (Th1) cytokines induce an inflammatory response which can be harmful to the developing fetus. Specific Th1 cell products have been characterized as inflammatory mediator, including interferon-γ (IFN-γ), and tumor necrosis factor (TNF)-α. In contrast, Th helper type-2 (Th2) cytokine activation results in an anti-inflammatory response. Th2 cytokine's anti-inflammatory response is elicited via B cell antibody production [11]. IL-4, IL-5, IL-6, and IL-10 are Th2 cytokines that are present throughout pregnancy [12,13].

The purpose of this study was to investigate the maternal and fetal responses after oral exposure to L. monocytogenes using timed pregnant guinea pigs. Previously, pregnant guinea pigs and primes have been used to develop a dose–response model for L. monocytogenes–induced stillbirths. In both animal models, the estimated LD50 for fetal mortality was approximately 10^2 CFUs L. monocytogenes [7,14], which is similar to an estimated LD50 for fetal mortality of 1.9 × 10^4 CFUs from a human outbreak of listeriosis [15]. However, no studies using pregnant guinea pigs have been done to determine the effects of infection on the maternal immune system and the possible consequences to the fetus. Our objectives were to [1] determine the effects of infection on the maternal liver [2]; characterize the effects of infection on the maternal immune system; and [3] determine the effects of infection on apoptosis in the placenta.

2. Materials and methods

2.1. Animals

Forty timed pregnant guinea pigs were purchased from Elmhill Labs (Chelmsford, MA) on gestation day (gd) 28 and were housed at the University of Georgia (UGA) animal facility. For details on animal husbandry and treatment, see Williams et al. [7]. Briefly, animals were housed individually with air filters fitted on each cage, and control animals were housed in a separate room from treated animals. The animals were provided sterilized water and chow and were maintained on a 12 h light/12 h dark cycle. Guinea pigs were allowed a 1-week acclimation period before treatment. After treatment, the animals were observed daily for any overt changes in food consumption, fecal output, and behavior, and the animals were weighed once a week. Pregnancy was allowed to continue normally until sacrifice on gd 56 for 2500 g for 24 h in anesthetized guinea pigs. Previously, pregnant guinea pigs and primates have been used to develop a dose–response model for L. monocytogenes–induced stillbirths. In both animal models, the estimated LD50 for fetal mortality was approximately 10^2 CFUs L. monocytogenes [7,14], which is similar to an estimated LD50 for fetal mortality of 1.9 × 10^4 CFUs from a human outbreak of listeriosis [15]. However, no studies using pregnant guinea pigs have been done to determine the effects of infection on the maternal immune system and the possible consequences to the fetus. Our objectives were to [1] determine the effects of infection on the maternal liver [2]; characterize the effects of infection on the maternal immune system; and [3] determine the effects of infection on apoptosis in the placenta.

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2.9. Determination of placental apoptosis

Apoptosis in the placenta was determined by PCR. DNA was extracted using a DNeasy mini tissue kit according to the manufacturer's protocol (QIAGEN Inc., Valencia, CA). Briefly, the 25 ± 1 mg section of placenta was placed in a sterile, DNA-free centrifuge tube and subjected to mechanical grinding. Lysis buffer and protease K were added and mixed by vortexing. The sample was incubated at 55 °C for a minimum of 3 h in a shaking water bath. A DNA binding buffer was added to the sample and mixed thoroughly. The sample was incubated at 70 °C for 10 min. DNA was collected through a series of three washes and a final elution and centrifugation. The optical density was measured using a spectrophotometer (BioPhotometer, Eppendorf, Hamburg, Germany) to determine the amount of genomic DNA present.

Apoptosis was analyzed using a commercially available apoptosis-specific PCR kit, DNA Ladder Assay (Maxim Biotech Inc., Rockville, MD). The ligation mix was added to 1 μg genomic DNA and cooled from 55 to 10 °C over 1 h. The reaction was incubated at 16 °C overnight. Ligated DNA was added to the PCR mixture, a Hot start PCR method was used and Taq Polymerase was added. The recommended temperature profile for PCR reaction (72 °C for 10 min, 94 °C for 1 min, a 2-step cycle of 94 °C for 1 min and 70 °C for 2 min was run for 40 cycles, then 72 °C for 10 min, and 20 °C for a minimum of 30 min) was completed using a Mastercycler EP (Eppendorf, Hamburg, Germany).

The completed reaction solution was loaded on a 1.75% agarose gel. The gel was run at 100 mV for approximately 1 h. The gel was viewed under an ultraviolet hood after staining with ethidium bromide, and pictures were taken using a Nikon camera with a 590 lens.

2.10. Statistical analysis

Data were analyzed using SAS Version 8.2 (Cary, NC). For all statistical analysis, the dam was used as an N = 1. In all dose groups except 10⁷ CFUs L. monocytogenes, all placentas from all dams were analyzed and the numbers ranged from 18 to 62 placentas per dose group. For the highest dose group, 10⁶ CFUs L. monocytogenes, placentas from some premature deliveries were not available. However, at least three placentas from each dam were analyzed for a total of 14 placentas. Statistical significance between control groups and treated groups for maternal antibody response, cytokine concentrations, and placental apoptosis was calculated using an analysis of variance test (ANOVA, p ≤ 0.05). Differences between groups were determined by using a Student–Newman–Keuls (SNK) test (p ≤ 0.05). Comparisons between the number and day of premature delivery were analyzed using a Chi-square test (p ≤ 0.05).

3. Results

Of the forty guinea pigs treated with L. monocytogenes (doses ranging from 10⁴ to 10⁸ CFUs), 10 animals had adverse pregnancy outcomes. Microbiology analyses of these guinea pigs are reported in Williams et al. [7]. When premature labor occurred, all fetuses delivered were stillborn, and L. monocytogenes was recovered from all placentas and fetuses [7]. In addition, whenever L. monocytogenes was recovered from viable fetuses, the corresponding placenta was also infected [7]. No premature deliveries occurred in guinea pigs treated with ≤10⁵ CFUs L. monocytogenes (Table 1). However, two dams treated with 10⁶ CFUs L. monocytogenes each had one nonviable fetus with viable littermates at the time of sacrifice (gd 56), but L. monocytogenes was not recovered from the fetuses [7]. One dam treated with 10⁸ CFUs L. monocytogenes had one nonviable fetus at the time of sacrifice with five viable littermates (data not shown).

Premature deliveries resulting in stillbirths occurred at doses of >10⁶ CFUs L. monocytogenes (Table 1). The number of days until a guinea pig prematurely delivered a stillbirth was dose-dependent. One dam treated with 10⁶ CFUs L. monocytogenes delivered stillbirths on gd 55 (average full term for guinea pigs is 65 days). However, three dams treated with 10⁷ CFUs L. monocytogenes had stillbirths at an average of gd 48 (range gd 44–54). At the highest dose tested, 10⁸ CFUs L. monocytogenes, three dams had stillbirths occurring at an average of gd 45, which was significantly earlier than the animals whose pregnancy continued until the time of sacrifice (gd 56) (p < 0.05). L. monocytogenes was recovered from the placentas and fetuses of all stillbirths [7].

At necropsy, maternal livers were observed for gross hepatic lesions. Dams treated with 10⁴ CFUs L. monocytogenes had no visible hepatic lesions. Focal hepatic lesions (Fig. 1) were visible at higher doses, and the number of lesions appeared to increase as the dose increased. Microscopic examination of the liver revealed two main findings. There were multiple, randomly distributed round foci of hepatocellular necrosis. The foci were surrounded by mixed acute, chronic inflammation and fibrosis. An increase in the extent of necrosis and chronic inflammation was noted as the dose increased (Table 2; Fig. 2). Secondly, there appeared to be a dose-related increase in the numbers of mononuclear cells scattered throughout the sections (Table 2; Fig. 2). Identifying their origin is beyond the scope of this study, but their appearance (round to slightly oval shape, eosinophilic cytoplasm, darkly stained variably shaped nuclei) indicate that they are likely histiocytes or macrophages of monocyte origin.

Maternal serum samples were collected at the time of sacrifice to determine immunological changes including changes in antibody levels. Maternal Listeria antisera titer levels were significantly increased (p < 0.05) from the control levels in dams treated with 10⁶ CFUs L. monocytogenes. However, Listeria antisera titer levels in dams exposed to ≤10⁷ CFUs L. monocytogenes were not significantly altered (Fig. 3).

In addition to maternal Listeria-induced antibody changes, maternal cytokine production was analyzed. An ELISA assay was used to analyze maternal serum for selected cytokines: tumor necrosis factor (TNF)-α, interleukin (IL)-4, and IL-10. TNF-α was significantly decreased (p < 0.05) in all dose groups. However, other cytokine levels were not significantly altered in dams treated

### Table 1

<table>
<thead>
<tr>
<th>Dose</th>
<th>Number of dams</th>
<th>Number of premature deliveries</th>
<th>Average post-treatment day of premature deliveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>10⁴ CFUs</td>
<td>4</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>10⁵ CFUs</td>
<td>11</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>10⁶ CFUs</td>
<td>9</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>10⁷ CFUs</td>
<td>10</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>10⁸ CFUs</td>
<td>4</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

*Statistically significant when compared to control group (p < 0.05).*

### Table 2

<table>
<thead>
<tr>
<th>Dose</th>
<th>Number of dams</th>
<th>Number of dams with ≥1 lesion</th>
<th>Necrosis scorea (average ± S.D.)</th>
<th>Mononuclear cellsb (average ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>0</td>
<td>0.5 ± 0.58</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>10⁴ CFUs</td>
<td>4</td>
<td>0</td>
<td>0.75 ± 0.96</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>10⁵ CFUs</td>
<td>4</td>
<td>2</td>
<td>1.67 ± 1.53</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>10⁶ CFUs</td>
<td>4</td>
<td>3</td>
<td>1.33 ± 0.58</td>
<td>1.67 ± 0.58</td>
</tr>
<tr>
<td>10⁷ CFUs</td>
<td>4</td>
<td>2</td>
<td>2.5 ± 1.4</td>
<td>2.0 ± 1.4</td>
</tr>
</tbody>
</table>

a Necrosis subjectively scored on a 0–3 scale averaged per dose group; 0 = no necrosis present; 1 = few small foci of necrosis with little inflammation; 2 = well-developed focal area of necrosis with increased inflammation; 3 = more extensive lesion as described in score 2.

b Value indicates subjective scoring for the number of mononuclear cells (see text) noted in the sections: 0 = within normal limits; 1 = a minimal to slight increase in the number of cells not involving all fields; 2 = a further increase in number with multiple cells present in most fields; 3 = a noticeably larger numbers of cells present in all fields.
Table 3
Maternal serum cytokine concentrations (pg/ml) in pregnant guinea pigs 21 days after treatment with *L. monocytogenes*

<table>
<thead>
<tr>
<th>Dose</th>
<th>GM-CSF (± S.D.)</th>
<th>MIP-1β (± S.D.)</th>
<th>IFN-λ (± S.D.)</th>
<th>IL-12p70 (± S.D.)</th>
<th>IL-9 (± S.D.)</th>
<th>TNF-α (± S.D.)</th>
<th>IL-4 (± S.D.)</th>
<th>IL-10 (± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.59 (± 22.4)</td>
<td>41.9 (± 39.5)</td>
<td>1.76 (± 0.9)</td>
<td>3.6 (± 0.3)</td>
<td>101.4 (± 16.5)</td>
<td>14.01 (± 16.7)</td>
<td>8.79 (± 7.3)</td>
<td>≤ 2.0d</td>
</tr>
<tr>
<td>10⁴ CFUs</td>
<td>20.3 (± 33.4)</td>
<td>≤ 7.7d</td>
<td>1.45 (± 1.6)</td>
<td>10.6 (± 9.9)</td>
<td>137.3 (± 74.9)</td>
<td>5.12 d, e (± 6.9)</td>
<td>2.55 (± 2.4)</td>
<td>19.8 (± 18.1)</td>
</tr>
<tr>
<td>10⁵ CFUs</td>
<td>5.44 (± 6.3)</td>
<td>34.6 (± 14.6)</td>
<td>0.75 (± 0.6)</td>
<td>24.75 (± 38.2)</td>
<td>42.33 (± 54.5)</td>
<td>≤ 2.5d, e</td>
<td>NDf</td>
<td>NDf</td>
</tr>
<tr>
<td>10⁶ CFUs</td>
<td>4.43 (± 27.8)</td>
<td>40.28 (± 22.5)</td>
<td>4.22 (± 3.0)</td>
<td>8.3 (± 8.2)</td>
<td>71.48 (± 28.1)</td>
<td>3.41 d, e (± 1.8)</td>
<td>6.89 (± 5.6)</td>
<td>37.63 (± 52.3)</td>
</tr>
<tr>
<td>10⁷ CFUs</td>
<td>≤ 1.0d</td>
<td>≤ 7.7d</td>
<td>3.13 (± 1.2)</td>
<td>3.6 (± 0.3)</td>
<td>24.2 (± 28.8)</td>
<td>≤ 2.5 d, e</td>
<td>16.97 (± 8.0)</td>
<td>2.67 (± 1.2)</td>
</tr>
</tbody>
</table>

a Each sample was analyzed in duplicate and serum from ≥ 3 dams was analyzed per dose group; all samples recorded in pg/ml.
b Cytokine levels were analyzed by Lincoplex (see Section 2 for details).
c Cytokine levels were analyzed by ELISA (see Section 2 for details).
d Cytokines were measured at or below the detection limit; the detection limit was used for statistical comparisons when all samples were less than the detection limit.
e Statistically significant (p < 0.05) when compared to control.
f Not done.

with *L. monocytogenes* (Table 3). In addition, a wide range of pro-inflammatory and anti-inflammatory cytokines were analyzed using the Lincoplex kit. No significant differences were seen in the maternal serum cytokines analyzed using the Lincoplex kit (Table 3). The lack of significant changes in cytokine concentrations after maternal treatment with *L. monocytogenes* is likely due to large variation between individual animals or the length of time between infection and sample collection.

Placentas from all dose groups were analyzed to determine whether maternal treatment with *L. monocytogenes* affected placental apoptosis. Some placental apoptosis is normal for late gestation; however, the number of placentas with a detectable amount of apoptosis showed a significant increase in dams treated with ≥ 10⁶ CFUs (Fig. 4). Interestingly, in dams treated with 10⁸ CFUs *L. monocytogenes*, all placentas had a detectable amount of apoptosis. In addition, when amplified DNA was viewed under UV lighting comparing placentas from treated dams with nonviable fetuses to those with viable fetuses, there was greater intensity staining of the bands in placentas from treated dams with nonviable fetuses (Fig. 5).
human disease using common routes of exposure. Previously, primates and mice have been used to study the effects of infection with *L. monocytogenes* on pregnancy. In humans, the bacteria use E-cadherin, an internalin A receptor, to transmigrate the gastrointestinal tract which allows for systemic invasion. Non-human primates appear to be a good model for human listeriosis during pregnancy although the E-cadherin amino acid sequence has not been determined [16]. However, nonhuman primates are expensive and invasive studies are not easily done. Mice are less susceptible to *L. monocytogenes*-induced stillbirth after oral exposure, due to a difference in E-cadherin, more specifically a substitution of a glutamic acid for proline at site 16. Interestingly, using anesthesia during inoculation of mice resulted in mice that were more susceptible to infection with *L. monocytogenes* than non-anesthetized mice [19]. In a previous study, we developed a pregnant animal model for oral exposure to *L. monocytogenes* using timed pregnant guinea pigs [7]. In our current study, we investigated the effects of maternal exposure to *L. monocytogenes* on maternal liver, the maternal immune system, and placenta using timed pregnant guinea pigs.

In our study, stillbirth occurred at doses of \( \geq 10^6 \) CFUs *L. monocytogenes*. At the highest dose tested (\( 10^8 \) CFUs), dams went into premature labor significantly earlier, an average of gd 45 (range gd 42 to gd 50) compared to sacrifice at gd 56 (post-treatment day 21). This is in agreement with a previous primate study that allowed animals to go to full term after treatment with *L. monocytogenes* [14,16]. The primates with adverse pregnancy outcomes on average delivered earlier than the animals with normal pregnancy outcomes [14,16].

After human exposure, the mother will often be asymptomatic until the time of fetal distress or may experience slight nausea or flu-like symptoms [3,4]. In our study, the dams were asymptomatic until the time of premature delivery, and this is similar with that seen in our previous study in primates [14,16]. Grossly visible lesions, confirmed by microscopic examination to be necrotic foci, were seen on the maternal liver at doses of \( \geq 10^5 \) CFUs *L. monocytogenes*. Listeria-induced liver damage has been shown in IV challenged mouse studies and after human exposure [20,21]. Severe necrotizing hepatitis with multiple hepatic lesions resulted from IV infection of *L. monocytogenes* in pregnant mice [20]. In cases of human exposure to *L. monocytogenes*, solitary liver abscesses have been noted [21]. Guinea pig dams with multiple necrotic foci did not appear to be sick and did not have an increase in serum alanine aminotransferase (ALT) levels [7]. In human listeriosis, solitary and multiple liver abscesses have been present in patients with an underlying condition, such as diabetes mellitus [22]. In these cases of listeriosis, serum ALT levels and aspartate aminotransferase (AST) levels were not helpful in diagnosis [22]. However, one study suggests that alkaline phosphatase levels might reveal any necrotic lesions [26]. Similar to our study in guinea pigs, in neonatal listeriosis in humans, granulomas and microabscesses have been reported at autopsy (granulomatosis infantisepctic) [23–25]; this is similar to what was seen in the guinea pig maternal liver. In pregnant guinea pigs, placenta histology did not reveal any necrotic lesions [26]. Similar to our study in guinea pigs, *L. monocytogenes* is commonly isolated from the liver and spleen in humans, although the specific cause of death is unknown. In general, death is attributed to septicemia and the subsequent sequelae.

We measured antibody production against the LLO protein in the circulating maternal serum. At the highest dose of \( 10^6 \) CFUs *L. monocytogenes*, maternal antibody titer levels were significantly higher (4-fold) when compared to the control group. Previously, rhesus monkeys showed an 8- to 32-fold increase in antibody titer levels from dams with a stillbirth when compared to levels from mothers with a normal birth [16]. The lack of response

### 4. Discussion

Exposure to *L. monocytogenes* can result in spontaneous abortions and stillbirths in humans and other mammals. To develop drug therapies and diagnostic tools for early intervention for human listeriosis during pregnancy, animal models are needed that mimic

![Graph](image1)

**Fig. 3.** Antibody titers against listeriolysin-O antigen in sera from guinea pigs 21 days after treatment with *L. monocytogenes*. At the highest dose of \( 10^6 \) CFUs *L. monocytogenes*, the titer level was significantly increased \((p \leq 0.05, n \geq 3)\). *Statistically significant.*

![Graph](image2)

**Fig. 4.** Placental tissue samples analyzed for apoptosis by PCR. All samples were adjusted to \( 1 \mu g \) DNA and subjected to 40 thermocycles. An average of the number of placenta with a detectable amount of apoptosis was determined for each dam, and the mean per treatment group was calculated. A significant increase in the average number of placentas positive for apoptosis was seen in treatment groups \( 10^6, 10^7, \) and \( 10^8 \) CFUs *L. monocytogenes* compared to the control group \((p \leq 0.05, n \geq 3)\). *Statistically significant.*

![Image](image3)

**Fig. 5.** Comparison of apoptosis in placentas of viable and nonviable fetuses from dams treated with \( 10^6 \) CFUs *L. monocytogenes*. Fetuses A, B, and C were littersmates. An increase in banding intensity is seen in lanes 1–3 (nonviable fetuses) compared to lane 4 (viable fetus). A placenta from a control fetus in lane 5 shows no banding.
seen in lower dose groups probably results from the limited contribution of humoral immunity to listeric infection. However, at the time of stillbirth or sacrifice, high antibody titer levels were seen both in primates and guinea pigs indicating that the maternal immune system is capable of mounting an immune response [16]. Interestingly, primate antibody titer levels were higher in animals that had a stillbirth than treated animals with normal deliveries [16].

Cell mediated immunity is largely involved in the destruction of intracellular pathogens, including *L. monocytogenes* [27]. Th1 cytokine production plays a large role in the cell mediated inflammatory immune response. However, Th1 cytokine production, including TNF-α, IL-2, and IFN-γ, is reduced during pregnancy leading to a shift in the Th1/Th2 cytokine ratio [28–30]. In our study, maternal levels of circulating serum cytokines were generally below the detection limit or not significantly altered upon infection with *L. monocytogenes*, with the exception of TNF-α concentrations which were significantly decreased after maternal treatment with *L. monocytogenes* (Table 3).

For the cytokines analyzed in our study, no guinea pig specific ELISA assays were available, and mouse specific ELISA assays were used. Mouse specific ELISA assays have previously been used to analyze guinea pig serum cytokine levels [17,18]. A guinea pig model of chorioamnionitis induced via cervical inoculation with *Escherichia coli* analyzed maternal serum cytokine concentrations, and similar to our results many samples tested below the minimal detectable limit [17]. A guinea pig model of lipopolysaccharide-induced fetal brain injury showed significant increases of maternal TNF-α concentrations using a mouse specific ELISA assay [18]. Our study was designed to analyze only one time point, 21 days post-treatment. We are currently examining cytokine levels at earlier post-infection time points to determine temporal changes in cytokine concentrations and analyzing cytokines within the placenta.

Guinea pigs are the rodent species with a placenta most similar to humans, a hemochorial placenta [8]. Bakardjiev et al. [31] found that the placenta was targeted for proliferation more than other organs after intravenous exposure to the bacteria. In our study, the number of placentas positive for apoptosis increases as the dose increases with the three highest doses having a significant increase in the number of placentas positive for apoptosis when compared to the control animals. In our previous study by Williams et al. [7], the number of placentas from which *L. monocytogenes* is isolated is directly correlated with increasing dose. The increase in apoptosis could be due to significant infection within the placenta, which could lead to placental malfunction and adverse fetal effects. Unregulated apoptosis can result in decreased clearance of apoptotic bodies which can in turn lead to increased degradation.

In conclusion, our study provides additional information on the pregnant guinea pig as a surrogate model for human listeriosis. With exposures to increasing doses of *L. monocytogenes*, the severity of maternal liver damage increases, the number of placentas with detectable levels of apoptosis increases, and the percent of nonviable fetuses increases. Maternal serum TNF-α concentrations were significantly decreased by infection with *L. monocytogenes*, however, all other cytokine levels are not significantly altered at 21 days post-treatment.

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**References**


