DOSE RESPONSE, INFECTIVITY AND STILLBIRTHS IN PREGNANT GUINEA PIGS INOCULATED WITH *LISTERIA MONOCYTOGENES*

by

DENITA WILLIAMS

(Under the Direction of MARY ALICE SMITH)

ABSTRACT

Listeria monocytogenes is responsible for approximately 2500 cases and 500 deaths of listeriosis each year. Pregnant women are 20 times more likely to develop listeriosis than the general population with adverse pregnancy outcomes including low birth weight, spontaneous abortions, or stillbirths. Objectives of this study were to use pregnant guinea pigs that were orally-exposed to *L. monocytogenes* to (1) determine the maternal dose that results in placental transmigration leading to fetal tissue infectivity and/or the occurrence of stillbirths, and (2) relate adverse fetal outcomes to maternal fecal shedding, tissue infectivity, and/or liver damage in order to establish a biomarker of exposure.

Eleven of 34 dams treated with $\geq 10^5$ CFU delivered stillborn pups. *L. monocytogenes* cells were cultured from placenta, liver, and brain tissue from all stillbirths. The dose adversely affecting 50% of the pregnancies was 10^7 CFU, which is similar to that estimated in humans at 10^6 CFU.

INDEX WORDS: *Listeria monocytogenes*, Listeriosis, Spontaneous Abortions and Stillbirths

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DEDICATION

The thesis entitled DOSE RESPONSE, INFECTIVITY AND STILLBIRTHS IN PREGNANT GUINEA PIGS INOCULATED WITH *LISTERIA MONOCYTOGENES* is dedicated to my sister, RANDI WILLIAMS and my pet, SISSY WILLIAMS. Thanks for giving so graciously of your love and support throughout this process. Randi, although in many cases you used the "tough love" approach, your advice, well wishes and prayers were not in vain. I would also like to recognize Sissy's continuous loyalty throughout my graduate career despite me not being able to return the deed at times. I love both of you!

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CHAPTER 1

INTRODUCTION

Pre-term delivery of a stillborn or neonatal illness can result from exposure of a pregnant woman to *Listeria monocytogenes (L. monocytogenes). L. monocytogenes* is an ubiquitous organism, therefore many humans are likely to be exposed. Yet, certain subpopulations are more susceptible to *L. monocytogenes* infection resulting in adverse health conditions. Almost one-third of the cases involving listeriosis occur in pregnant women and carry the risk of feto-placental infection that results in severe health conditions such as septicemia, pneumonia, or meningitis and in about 25% of cases, the occurrence of pre-term delivery or stillbirth (Mylonakis et al., 2002). Hormonal and immunological changes that take place during pregnancy are thought to influence the susceptibility of pregnant women. In most cases, the pregnant woman is asymptomatic; however, there have been some reports of women experiencing influenza-like illness with fever, headache, and myalgia (Abram et al., 2002). Adverse fetal effects resulting from listeriosis most commonly occur as a result of third trimester maternal exposures. Second- and third-trimester infections can result in premature delivery followed by neonatal illness or pre-term delivery of a stillborn (Farber and Peterkin, 1991; Gellin and Broome, 1989). The effects that are experienced by the fetus appear to be dependent on the point of gestation at time of exposure.

The FDA has identified animal studies that assess effects of *L. monocytogenes* on the maternal and fetal compartments in a dose-dependent relationship as a data gap in their *L. monocytogenes* risk assessment of certain ready-to-eat foods (FDA, 2003). A pregnant animal

model that expresses similar side effects and mimics the primary human exposure route is essential to accurately assess the risk of maternal exposure to *L. monocytogenes* on the resulting fetus and/or neonate.

Research has shown that following exposure to *L. monocytogenes*, pregnant guinea pigs exhibit similar side effects to humans including fetal abortion (Dutta and Malik, 1981) as well as gastrointestinal tract disturbances (Lecuit and Cossart, 2002). Recently, it was discovered that E-cadherin, which mediates transmigration of *L. monocytogenes* into mammalian epithelial cells, has the same sequence in both humans and guinea pigs (Lecuit et al., 1999; Schubert et al., 2002). Preliminary studies conducted by our laboratory (Williams et al., 2004) as well as previous studies (Bakardjiev et al., 2004) support using the pregnant guinea pig as a surrogate for human listeriosis.

The objectives of this study were to use pregnant guinea pigs that were orally-exposed to *L. monocytogenes* to (1) determine the maternal dose that results in placental transmigration leading to fetal tissue infectivity and/or the occurrence of stillbirths, and (2) relate the adverse fetal outcomes to maternal fecal shedding, tissue infectivity, and/or liver damage in order to establish a biomarker of exposure.

CHAPTER 2

LITERATURE REVIEW

Listeriosis and Pregnancy. Almost one-third of the cases involving listeriosis occur in pregnant women and carry the risk of feto-placental infection (Bakardijiev et al., 2004) that could result in severe health effects such as septicemia, pneumonia, or meningitis and in some cases, the occurrence of pre-term delivery or stillbirth (Teberg et al., 1987; Mylonakis et al., 2002). In humans, *L. monocytogenes* has a predilection for the feto-placental unit (Bortolussi et al., 1995; Buchdahl et al., 1990). Listeriosis most commonly occurs during the third trimester (Buchdahl et al., 1990; Gellin et al., 1989) and is probably related to the major decline in cell-mediated immunity that occurs at 26-30 weeks of gestation (Weinberg, 1984). Infections are rare during the second trimester and even rarer during the first trimester (Buchdahl et al., 1990; Fuchs et al, 1994).

In most listeriosis cases, the pregnant woman is asymptomatic; however, there have been some reports of women experiencing influenza-like illness with fever, headache, and myalgia (Abram et al., 2002). The mild flu-like symptoms occur in approximately two-thirds of infected pregnant women and transpire during the period of bacteremia. Severe listeriosis in pregnant women is rare (Sirry et al., 1994), and listeriosis in the pregnant female is usually self-limiting with the delivery of an infected baby. Contrastingly, some animal studies indicate that both the mother and fetus are at risk for serious illness if the mother is infected by *L. monocytogenes* during pregnancy. In a previous study, *L. monocytogenes*-infected pregnant mice did not clear the pathogen from their spleens and livers as efficiently as infected virgin mice, and pregnant

mice had a much higher mortality rate (Schlech et al., 1993; Luft et al., 1982). Also, necropsy of dead pregnant mice revealed that most of the mice had resorbed their fetuses (Decker et al., 1991). Primarily, pregnancy-related listeriosis affects the fetus or neonate. The effect of fetal *Listeria* infection is dependent on the point in gestation time when infection occurs (Smith et al., 2003). First-trimester infection leads to spontaneous abortion, whereas second-and third-trimester infections lead to preterm birth followed by neonatal illness or fetal death with preterm delivery of a stillborn (Cruikshank et al., 1989; Enocksson et al., 1992; Farber and Peterkin, 1991). Schlech et al., (1983) demonstrated this using pregnant rodents. Rodents that were injected with *L. monocytogenes* during the third trimester had more fetal resorptions, stillbirths, or heavily infected pups than animals treated in early or late gestation. These data suggest that late gestational infection had greater adverse effects on fetal outcome as compared to infection during the earlier or middle part of pregnancy.

The changes in hormonal and immunological parameters that take place during pregnancy influence the susceptibility of pregnant women to *L. monocytogenes* infection. There is evidence that pregnancy leads to suppression of cell-mediated immunity and a substantial increase of progesterone production in the mother in order to prevent fetal rejection (Sacks et al., 1999). However cell mediated immunity is necessary for the defense against intracellular pathogens, and therefore suppression of this immune response may predispose the fetus to infection with intracellular pathogens, such as *L. monocytogenes*.

During gestation when cell-mediated immunity is mildly impaired (Weinberg et al., 1984), pregnant women are prone to develop listerial bacteremia. *Listeria monocytogenes* cells may proliferate in the placenta in areas that appear to be unreachable by usual defense mechanisms. For unexplainable reasons, maternal CNS infection (the most commonly

recognized form of listeriosis in other at-risk groups) is extremely rare during pregnancy in the mother when other risk factors are absent (Gellin et al., 1989; Gellin et al., 1991). Acquired immunity to *L. monocytogenes* is entirely cell mediated and largely dependent on cytotoxic CD8+T cells that recognize and lyse infected cells (Zinkernagel et al., 1974).

Increases and decreases of cytokine production are necessary for regulating homeostasis in the body during infection. During pregnancy, cytokine production is tightly regulated to avoid deleterious effects to the fetus. T helper 1-type (Th1) cytokines provoke an inflammatory response when activated, and T helper 2-type (Th2) cytokines have an anti-inflammatory response when activated. Th1 cytokines have been shown to have deleterious effects on the placenta and fetus (Raghupathy et al., 2001). As inflammatory mediators, Th1 cytokines also elicit cell-mediated cytotoxic responses, which can have harmful effects on the fetus. Specific Th1 cell products have been recognized; interleukin (IL)-2, interferon (IFN)-λ, tumor necrosis factor (TNF)-β. Th2 cells are elicited for involvement with an anti-inflammatory response via B cell antibody production (Maksheed et al., 2000). IL-4, IL-5, IL-6 and IL-10 are Th2 cytokines that are thought to be up-regulated during pregnancy. Infection from other pathogens during pregnancy show that the normal relative ratio of Th1 and Th2 cytokines might be disrupted leading to deleterious effects to the fetus (Lin et al., 2003; Yeo et al., 2005).

Guinea Pig as Surrogate for Human Listeriosis. A pregnant animal model that expresses similar side effects as humans is essential to accurately assess the risk of maternal exposure to *L. monocytogenes* on the resulting fetus and/or neonate. In 2003, the FDA conducted a risk assessment for *L. monocytogenes* in ready-to-eat foods relying on a dose response curve obtained from mouse data. Due to the limited quantity and insufficient quality of existing human data, FDA applied an adjustment factor to mouse data for estimating human risk

(FDA, 2003). Mice were the only animal data for which there was sufficient information to obtain a dose-response curve. Based on molecular differences in their receptor protein (E-cadherin) which is essential for transmigration of *L. monocytogenes* across the intestinal barrier and because the adverse health effects are not the same (death in mice opposed to stillbirths/meningitis in humans), mice are not good animal models in estimating dose response for human listeriosis.

A single amino acid shift located at position sixteen in murine E-cadherin (proline to glutamic acid) results in a loss of the ability for *L. monocytogenes* and the receptor to interact (Lecuit et al., 1999). A transgenic mouse that expressed human E-cadherin solely in enterocytes was developed in order to demonstrate the interactions of internalin and E-cadherin *in vivo* (Lecuit et al., 2001); however these receptors are localized within the intestine and may not be associated with other tissues, more specifically, the placenta. Despite this molecular advancement, comparisons between quantitative expression in humans and transgenic mice have not been published.

Previous studies show that pregnant nonhuman primates are susceptible to listeriosis both naturally and experimentally (McClure et al., 1986; Anderson et al., 1993; Smith et al., 2003). Furthermore, fetal infections in both humans and nonhuman primates can lead to similar conditions such as abortions, stillbirths, and/or neonatal deaths (Chalifoux et al., 1981; McClure et al., 1986). For humans and nonhuman primates, the pathogenesis and morphological findings associated with stillbirths due to *L. monocytogenes* are essentially the same (Anderson et al., 1993; Chalifoux et al., 1981). Nonhuman primates exhibit dose responses that are similar to humans. Yet the number of primates needed to thoroughly 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 is not published.

Studies conducted in the 1970s used non-pregnant guinea pigs to investigate the interaction between *Listeria* and host cells. Using electron microscopy, the interaction between *L. monocytogenes* and intestinal epithelial cells was investigated after pre-conditioned guinea pigs had been treated with 10^9 CFU through a stomach tube (Racz et al., 1972). These studies showed that *L. monocytogenes* entered the small intestine epithelial cells and multiplied there before being phagocytosed by macrophages (Racz et al., 1972). Pregnant guinea pigs were one of several animals used to characterize *Listeria* isolates (Dutta and Malik, 1981). Research has shown that following exposure to *L. monocytogenes*, pregnant guinea pigs exhibit similar side effects to humans including fetal abortion (Dutta and Malik, 1981) as well as gastrointestinal tract disturbances (Lecuit and Cossart, 2002). In a previous study, pregnant guinea pigs aborted on the 3rd and 7th day following infection with *L. monocytogenes* by intraperitoneal injection (Dutta and Malik, 1981), but the experimental design did not include the treatment dose.

Recently, it was discovered that E-cadherin, which mediates transmigration of *L. monocytogenes* into mammalian epithelial cells, has the same sequence in both humans and guinea pigs (Lecuit et al., 1999; Schubert et al., 2002). The sequence of the E-cadherin protein is extremely important in the binding of *L. monocytogenes* to epithelial cells and a single amino acid transition can alter the active site's configuration as previously discussed with the mouse model. Preliminary studies conducted by our laboratory (Williams et al., 2004) as well as previous studies (Bakardjiev et al., 2004) support using the pregnant guinea pig as a surrogate for human listeriosis. Bakardjiev et al. (2004) used the pregnant guinea pig to study feto-placental transmission of *L. monocytogenes* following invasion of the maternal system through

intravenous inoculation. Surprisingly, none of the published listeriosis studies that used the guinea pig as an animal model used ingestion as the route of exposure. Humans are exposed to *L. monocytogenes* through ingestion and experimentally by-passing the gastrointestinal tract probably leads to differences in dissemination and disease. By using an exposure regimen that is realistic to human exposures, we will be better equipped to estimate maternal and fetal health conditions following exposures to *L. monocytogenes*.

Pathogenicity of *Listeria monocytogenes*. The genus *Listeria* contains six species: *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeliger*i, and *L. welshimeri*. Of these six, *L. monocytogenes* and *L. ivanovii* are currently the only species to be classified as pathogenic. They cause listeriosis, an opportunistic infection of humans and animals involving severe clinical manifestations such as meningoencephalitis or septicemia and in pregnancy-related cases abortions, stillbirths, or neonatal deaths. The natural habitat is thought to be the surface layer of soil rich in decaying plant matter. From this habitat they gain access to host *via* ingestion of contaminated food sources. *L. monocytogenes* can infect a wide range of animal species, including mammals and birds.

L. monocytogenes possesses some unusual characteristics that make it unique from other food-borne pathogens and contribute to the organism's ubiquitous nature. *L. monocytogenes* replicates at an extremely broad temperature range (1°C to 45°C). Therefore, not only can *L. monocytogenes* survive at the normal refrigeration temperature of 4°C, the cells can also multiply. *L. monocytogenes* can also replicate at high salt concentrations (Wing et al., 2002). The most commonly affected human populations include: neonates, elderly, pregnant, and immunocompromised individuals. Over the years, *L. monocytogenes* has evolved the ability to invade and multiply within eukaryotic cells. Except for vertical transmission from mother to

fetus and rare instances of cross contamination in the delivery suite or newborn nursery (Farber et al., 1991b) human to human infection has not been documented. Infection most likely begins following an oral exposure to the organism. The oral inoculum required to produce clinical infection is unknown: experiments in healthy non-pregnant mammals including cynomolgus monkeys (Farber et al., 1991) and goats (Miettinen et al., 1990) indicate that $\geq 10^9$ organisms are required. Data from a previous study conducted in our laboratory indicate that pregnant rhesus monkeys are susceptible to listeriosis and spontaneous abortions occurred following oral exposure at doses $\geq 10^6$ CFU (Smith et al., 2003). The incubation period for invasive illness is not well established, but evidence from a few cases with exposures by ingestion points to incubation periods ranging from 11 days to 70 days (with mean incubation period, 31 days) (Linnan et al., 1988).

L. monocytogenes is a typical facultative intracellular parasite. It is able to proliferate within macrophages and a variety of normally nonphagocytic cells, such as epithelial and endothelial cells and hepatocytes. Following ingestion, it is advantageous for an intracellular pathogen such as *L. monocytogenes* to invade mammalian cells as efficiently as possible. *L. monocytogenes* crosses the mucosal barrier of the intestine by using its surface protein, internalin, to interact with the host's E-cadherin protein that is located along the intestinal wall. Once in the bloodstream, the organism may disseminate hematogenously to any site; however, *L. monocytogenes* has a particular predilection for the CNS and the placenta (Lorber, 1997).

The mechanisms of how *L. monocytogenes* moves from cell to cell avoiding the maternal immune system has been well documented. Internalin, an 80-kD member of the family of leucine-rich repeat proteins, interacts with E-cadherin resulting in induction of phagocytosis (Cossart et al., 1996; Mengaud et al., 1996). Once phagocytosis has occurred, listeriolysin O, a

major virulence factor along with phospholipases, enables listeriae to escape from phagosomes and avoid intracellular killing (McKay et al., 1991; Portnoy et al., 1992). Once free in the cytoplasm, the bacteria can divide (doubling time, ~1 hour) and by inducing host-cell actin polymerization, propel themselves to the cell membrane (Tilney et al., 1989; Sanger et al., 1992). By pushing against the host cell membrane, the organism subsequently forms elongated pseudopod-like projections that can be ingested by adjacent cells such as macrophages, enterocytes, and hepatocytes. The bacterial oligoproline-containing surface protein, Act A, is necessary for the induction of actin filament assembly and cell to cell spread (Southwick et al., 1996). Through this life-cycle, *L. monocytogenes* can move from cell to cell without being exposed to antibodies, complement, or neutrophils. The entire cycle is completed in approximately 5 hours.

***Listeria monocytogenes* and Food Matrices.** Although diseases caused by *L. monocytogenes* occur at a low rate relative to those caused by other food-borne pathogens, the organism is second only to *Salmonella* spp. in the estimated number of food-related deaths in the United States (Mead et al., 1999). Since 1981, epidemiologic investigations have repeatedly indicated that the consumption of contaminated food is a primary vehicle of transmission of listeriosis. Presently, the Food and Drug Administration has classified the following foods as being at high risk for *Listeria* contamination: ready-to-eat foods, processed foods, deli meats, smoked seafood, soft cheeses and dairy products.

The first confirmed food-borne outbreak of listeriosis occurred in 1981 in Canada. A case-control study implicated locally prepared coleslaw as the vehicle, and the epidemic strain was subsequently isolated from an unopened package of this product (Schlech et al., 1983). The next documented outbreak was in Boston, Massachusetts in 1983 and included 49 cases over a

two month period; a case-control study implicated pasteurized milk as the vehicle (Fleming et al., 1985). In 1985 in California, an outbreak of listeriosis with 142 cases was traced to a Mexican-style cheese (Linnan et al., 1988). Ninety-three cases or 65.5% occurred in pregnant women or their offspring, and of the forty-eight total deaths that occurred, twenty were fetuses and ten were neonates. A *L. monocytogenes*-contaminated soft cheese was responsible for a 4-year (1983-1987) outbreak of 122 cases in Switzerland (Bille et al., 1989) and a contaminated paté caused a 300-case outbreak in the United Kingdom in 1989 to 1990 (Gilbert et al., 1993). In France, contaminated pork tongue in aspic was the principal vehicle of 279 cases of listeriosis in ten months in 1992 (Jacquet et al., 1995), potted pork was associated with 39 cases in 1993 (Goulet, 1995), and soft cheese was the vehicle of 33 cases in 1995 (Loncarevic et al., 1995). The total exposed is not available for any of the above outbreaks.

In 2001, FDA acknowledged that the environment (in this case food matrix) may influence the pathogenic state of *L. monocytogenes* which could ultimately influence the predicted infectious dose for humans (FDA, 2001). However, there were limited studies available that used animal models to assess the effect of food content on the organism's ability to produce illness. Farber et al. (1991) administered sterile whole milk along with Maalox to female cynomolgous monkeys to determine the effect of gastric acidity on infection caused by *L. monocytogenes*. Observations by Farber et al. (1991) were consistent with a study conducted in 1989 by Golnazarian et al. where they administered cimetidine to mice to decrease their gastric acid secretion (Farber et al., 1991; Golnazarian et al., 1989). Golnazarian observed no significant differences between normal and cimetidine-treated mice (Golnazarian et al., 1989). Both studies concluded that acidity did not significantly affect the pathogens ability to produce illness. Several outbreaks of salmonellosis suggest that low infective doses are associated with

high fat content foods, such as chocolate (Craven et al., 1975; D'Aoust et al., 1975; Greenwood et al., 1983) cheese (D'Aoust et al., 1985; Hedberg et al., 1994) and paprika-powdered potato chips (Lehmacher et al., 1995). The *Listeria* outbreaks stated above were predominately associated with foods that are high in fat content suggesting that lipid content may be an important factor in the pathogenic state of *L. monocytogenes*.

In 2001, FDA/CDC/USDA jointly published a risk assessment for *L. monocytogenes* based on a dose response curve from mouse studies. However, mice may not be predictive models for human listeriosis. Although rhesus monkeys are good models, the molecular character of their E-cadherin has not been established and the numbers of animals needed to conduct low dose studies is prohibitive. Our study used pregnant guinea pigs to develop additional dose response information for *L. monocytogenes*-induced stillbirths.

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CHAPTER 3

DOSE RESPONSE OF *LISTERIA MONOCYTOGENES* AFTER ORAL EXPOSURE IN PREGNANT GUINEA PIGS [1]

[1] D. Williams, E.A. Irvin, R.A. Chmielewski, J. Frank, and M.A. Smith. Submitted to *Journal of Food Protection*, 07/25/2006.

Abstract

Listeriosis is a severe disease that results from the foodborne pathogen, *Listeria monocytogenes* and is responsible for ~ 2500 cases and 500 deaths each year. Pregnant women are 20 times more likely to develop listeriosis than the general population with adverse pregnancy outcomes including low birth weight, spontaneous abortions, stillbirths, or neonatal meningitis. The objective of this study was to determine an infective dose, infectivity, and corresponding stillbirths in pregnant guinea pigs. Pregnant guinea pigs (n = 4-11/dose) were treated orally on gestation day (gd) 35 with 10^4 to 10^8 *L. monocytogenes* CFU in sterile whipping cream. *L. monocytogenes* cells were recovered at 64, 73, 90, and 100 percent from liver of animals treated with 10^5, 10^6, 10^7, 10^8 CFU, respectively. In dams dosed with ≥ 10^6 CFU, *L. monocytogenes* cells were cultured from 50% of the spleen and 33% of the gallbladder samples. Eleven of 34 dams treated with ≥ 10^6 CFU delivered stillborn pups. *L. monocytogenes* cells were cultured from placenta, liver, and brain tissue from all stillbirths. Dams with nonviable fetuses shed *L. monocytogenes* for longer periods of time. Fetuses from control dams weighed significantly less than those from dams treated with 10^4 CFU ($p < 0.05$) and were significantly longer than fetuses from dams treated with 10^8 CFU ($p < 0.05$). The dose adversely affecting 50% of the pregnancies was 10^7 CFU which is similar to that estimated in humans at 10^6 CFU.

Listeriosis results from exposure to *Listeria monocytogenes* (*L. monocytogenes*), a gram-positive bacterium that is found in the environment and capable of contaminating various foods. Almost one-third of the listeriosis cases occur in pregnant women and carry the risk of feto-placental infection that can result in stillbirths or the occurrence of preterm delivery, or severe health effects such as septicemia, pneumonia, or meningitis *(18, 21)*. The side effects that are experienced by the fetus appear to be dependent on the point of gestation at time of exposure. Second-and third-trimester infections can result in premature delivery followed by neonatal illness or pre-term delivery of a stillborn (*9, 11*). Listeriosis most commonly occurs during the third trimester.

The FDA has identified animal studies that assess effects of *L. monocytogenes* on the maternal and fetal compartments in a dose-dependent relationship as a data gap in their *L. monocytogenes* risk assessment of certain ready-to-eat foods (*10*). A pregnant animal model that expresses similar side effects and mimics the primary human exposure route is essential to accurately assess the risk of maternal exposure to *L. monocytogenes* on the resulting fetus and/or neonate.

The objectives of this study were to use pregnant guinea pigs that were orally-exposed to *L. monocytogenes* to (1) examine the pregnant guinea pig as a surrogate model for human listeriosis, (2) determine the maternal dose that results in placental transmigration leading to fetal tissue infectivity and/or the occurrence of stillbirths, and (3) relate the adverse fetal outcomes to maternal fecal shedding, tissue infectivity, and/or liver damage in order to establish a biomarker of exposure.

Materials and Methods

Inoculum Preparation. The *L. monocytogenes* strain used in this study was isolated from a *Listeria*-induced stillbirth from a rhesus monkey, which was subsequently, used to induce stillbirths in a primate study by Smith et al. (*20*). Strain 12443 is currently stored on cryo-beads (Prolab Diagnostics, Austin, TX) and maintained at -80°C in our laboratory. Prior to treatment, *L. monocytogenes* cells were activated by three successive transfers in 10 ml aliquots of Tryptic Soy Broth (BD Diagnostics, Sparks, Maryland) and incubated at 35°C for 24 hours. Cultures were then harvested by centrifugation (9,000 x g at 4°C for 30 min), washed twice and resuspended in sterile phosphate buffer saline (PBS). Cultures were diluted to give final concentrations over a range of 10^8 to 10^4 colony forming units (CFU) per ml. The appropriate number of *L. monocytogenes* cells was added to commercial heavy whipping cream which contained 36% milk fat, and the inoculum sweetened with 0.5 grams of Splenda®. Whipping cream was sterilized by autoclaving at 121°C for 13 minutes. Sterile PBS (1.0 ml) was added to the whipping cream and administered to control animals.

The number of *L. monocytogenes* cells in the inoculated sample was determined by serially diluting the cell suspension in PBS (0.01M) and plating onto Listeria Selective Agar (LSA) (Oxoid, Ogdenburg, NY). The plates were incubated at 35°C for 24 h before colony enumeration. The cell populations obtained were used to confirm the dose of *L. monocytogenes* administered to the guinea pigs.

Animals and Treatment. Timed-pregnant (bred on specified date) guinea pigs were obtained from Elm Hill Breeding Laboratories on gestation day (gd) 29, housed in cages containing air-filters, maintained on a 12 hour light/dark cycle, and temperature and humidity were 70ºC ± 2ºC and 55% ± 15%, respectively. The animals were provided sterilized water,

sterilized Purina autoclavable guinea pig diet (PMI Nutrition International, St. Louis, MO), and a 22.1g encapsulated vitamin C supplement (Prima-Treats #F0308, Bio-Serv, Frenchtown, NJ) was given once a week. Following a one-week acclimation period at the UGA animal facility, guinea pigs were orally-treated on gd 35 with *L. monocytogenes* inoculum, and pregnancy allowed to continue normally. Inoculum doses ranged from 10^4 to 10^8 *L. monocytogenes* CFU per 5 mls of sterile whipping cream. Two consecutive days prior to treatment with *L. monocytogenes*, the guinea pigs were hand-fed 5 ml of the treatment vehicle to train the animals to orally ingest the vehicle, and immediately before treatment with the inoculum, control and treated animals were transferred to separate rooms. The animals were observed daily for changes in the amount of fecal output or appearance, change in degree of activity or appearance, or weight loss. Guinea pigs were sacrificed on gd 56 and tissue samples were collected for further analysis.

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. Tissues analyzed include: maternal blood, gallbladder, liver, and spleen, along with placenta and fetal liver and brain. Tissues were confirmed positive for *L. monocytogenes* according to USDA's methodology described by Cook (*7*), which includes both qualitative and quantitative detection methods. Throughout the study, fecal samples were collected and analyzed as previously described. The quantitative method used direct plating on LSA (Remel, Lenexa, KS). The qualitative method includes enrichment in both nonselective and selective media followed by plating onto LSA. Immediately following the necropsy, tissue samples were placed in 100 ml of the nonselective enrichment medium, UVM broth (BD Diagnostics, Sparks, Maryland). After 24 hours, 0.1 ml of UVM-enriched sample was transferred to 9.9 ml of the secondary enrichment, Fraser Broth (Remel, Lenexa, KS). Positive

Fraser samples were streaked onto LSA and incubated for 24 h at 35°C. Selected isolated colonies were streaked onto Rapid L'mono plates (Bio-Rad, Hercules, CA) for confirmation as *L. monocytogenes*.

Serum Alanine Aminotransferase (ALT) Activity. Maternal blood samples were collected at necropsy by cardiac puncture. Serum was collected via centrifugation at 2500 x g for 25 minutes at 4°C. Due to the unavailability of guinea pig reagents, a human kit was used. The protocol and reagents were obtained from Eagle Diagnostics Laboratory (Desoto, TX) and methodology adopted from the International Federation of Clinical Chemistry *(6)*. Briefly, 1.0 ml of ALT reagent was added to 0.1 ml of serum, mixed by swirling, and incubated for two minutes at 37°C. The absorbance was recorded at 2, 3, and 4 minutes at a wavelength of 340 nm. The concentration of ALT was based on the change in absorbance per minute.

Fetal Viability. Fetal viability at the time of sacrifice was based on several characteristics: physical characteristics including eyelid skin formation and eyelid opening, skin color and formation, hair growth, pinna formation and the appearance of placenta and cord blood.

Statistical Analysis. Differences in fetal weights and lengths were analyzed using ANOVA t-test and least significant differences for mean separation, $p \leq 0.05$ (SAS, version 8.2, Cary, N.C.). The remaining data were analyzed using Kruskal-Wallis Test for multiple comparisons and Dunnett's Method for comparison with the control (SigmaStat, version 3.1). The dose response curve for fetal mortality was constructed using a log logistic fit to the data utilizing PSI-Plot software (version 8.0, Pearl River, NY).

Results

There appeared to be a dose dependent relationship in the colonization of *L. monocytogenes* within maternal liver, spleen and gallbladder tissue samples (Table 1). However, at doses < 10^6 CFU, *L. monocytogenes* was not isolated from any maternal spleens and only 1 of 15 gallbladders. In contrast, *L. monocytogenes* was isolated from liver tissue at the lowest dose tested (10^4 CFU) in 1 of 4 animals and there was a significant difference in animals receiving 10^5 CFU or greater compared to control. Although there were no statistical differences in isolation of *L. monocytogenes* from gallbladder samples among differing treatment groups, there appears to be a dose dependent increase in positive samples from dams treated with $\geq 10^5$ *L. monocytogenes* CFU. The control samples were negative for *L. monocytogenes* throughout the experiment (Table 1).

Three of the forty treated guinea pigs were not pregnant; however, liver, spleen, and gallbladder samples were analyzed for culturable *L. monocytogenes* cells. Two of the guinea pigs were treated with 10^6 *L. monocytogenes* CFU and one with 10^8 *L. monocytogenes* CFU. *L. monocytogenes* cells were cultured from all of the nonpregnant animals' livers. One guinea pig that was treated with 10^6 *L. monocytogenes* CFU had an infected spleen. None of the non-pregnant 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. The liver appears to be the most sensitive indicator of *L. monocytogenes* invasion with a lower infectivity dose for 50% (ID50) of the dams with at least

one fetal death, but invasion of liver was not a predictor of fetal mortality because animals with normal fetuses had a similar ID50 as those with nonviable fetuses (Table 2).

Visual inspection of livers from treated dams suggested liver damage (*12*), thus we determined serum ALT levels as an indicator of damage. Figure 1 compares maternal serum alanine aminotransferase levels with increasing doses of *L. monocytogenes*. There were no significant differences in serum ALT activity at any dose tested. These data do seem to contrast with the gross pathology results (*12*). However, these results do not include animals that prematurely delivered stillbirths, which may have more severe liver damage.

Following maternal ingestion, *L. monocytogenes* cells were isolated from the placenta and fetus (Table 3). In general, if *L. monocytogenes* infected a placenta, there was a high likelihood that the fetal liver and brain would also be infected. In all cases where the fetal brain was infected, the fetal liver for that animal was also infected. Both fetal liver and brain tissue were infected by *L. monocytogenes* to about the same extent.

When compared to controls, there was a significant increase of fetal weights in dams treated with 10^4 CFU, but there was a significant decrease in fetal lengths in dams treated with 10^8 CFU (Table 4). Note that weights and lengths of fetuses for the 10^8 treatment group are based on one litter because the remaining four litters resulted in pre-term stillbirths.

Fetuses were examined *in utero* and classified as normal or nonviable. A stillbirth was defined as a nonviable fetus that delivered prematurely. *L. monocytogenes* cells were isolated from tissues examined from each stillbirth suggesting that a high degree of infectivity was incompatible with life and resulted in premature delivery (Figure 2). None of the placental or fetal brain samples from treated dams with normal fetuses were positive for *L. monocytogenes*.

However, some normal fetuses of treated dams had infected livers (Figure 2). 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 6). When fetal brain was infected there seemed to be a high correlation among fetal liver, placenta, and maternal liver infectivity.

All treated dams showed a dose dependent increase in fecal shedding (Figure 3). Also, all treated dams with nonviable fetuses and stillbirths shed *L. monocytogenes* for longer periods of time than those with normal fetuses (data not shown). Our results are similar to a previous primate study where pregnant rhesus monkeys that delivered stillborn infants shed *L. monocytogenes* at a higher rate and for a longer period of time compared to normal pregnancies (*20*).

Guinea pigs that were exposed to $\geq 10^6$ *L. monocytogenes* CFU had an increase in the number of litters with at least 1 fetal death and in the percent of fetal mortality (Table 5). A dose response curve based on doses resulting in fetal mortality was constructed using a log logistic fit to the mortality data (Figure 4). The calculated lethal dose that resulted in 50% fetal deaths is 1.93×10^7 *L. monocytogenes* CFU.

Discussion

A pregnant animal model that is exposed in the same way and expresses similar side effects as humans is essential to accurately assess the risk of maternal exposure to *L. monocytogenes* for the resulting fetus and/or neonate. The FDA/USDA/CDC's *(10)* risk assessment evaluating *L. monocytogenes* in ready-to-eat foods relied on a dose response curve obtained from mouse data. Due to the limited quantity and insufficient quality of existing human data, FDA applied an adjustment factor to mouse data for estimating human risk, as the only

animal data for which there was sufficient information to obtain a dose-response curve was based on mice (*10*). Based on molecular differences in the mouse E-cadherin receptor (used by *L. monocytogenes* to cross the intestinal barrier in humans) and because the adverse health effects are not the same (death in adult mice as opposed to stillbirths/meningitis in humans), mice are not the most appropriate animal model to use in estimating dose response for human listeriosis after oral exposure.

A single amino acid shift located at position sixteen in murine E-cadherin (proline to glutamic acid) results in a loss of the ability for *L. monocytogenes* and the receptor to interact (*14*). A transgenic mouse that expressed human E-cadherin solely in enterocytes was developed in order to demonstrate the interactions of internalin produced by *Listeria* and E-cadherin *in vivo* (*15*); however these receptors are localized within the intestine and may not be associated with other tissues, more specifically, the placenta. Despite this molecular advancement, comparisons between quantitative expression in humans and transgenic mice have not been published.

Previous studies show that pregnant nonhuman primates are susceptible to listeriosis both naturally and experimentally *(1, 17, 20)*. Furthermore, fetal infections in both humans and nonhuman primates can lead to similar conditions such as abortions, stillbirths, and/or neonatal deaths (*5, 17*). For humans and nonhuman primates, the pathogenesis and morphological findings associated with stillbirths due to *L. monocytogenes* are essentially the same (*1, 5*). Nonhuman primates exhibit dose responses that are similar to humans. Yet the number of primates needed to thoroughly 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 is not published.

Studies conducted in the 1970s used non-pregnant guinea pigs to investigate the interaction between *Listeria* and host cells. Using electron microscopy, the interaction between *L. monocytogenes* and intestinal epithelial cells was investigated after pre-conditioned guinea pigs had been treated with 10^9 CFU through a stomach tube (*19*). These studies showed that *L. monocytogenes* entered the small intestine epithelial cells and multiplied there before being phagocytosed by macrophages (*19*). Pregnant guinea pigs were one of several animals used to characterize *Listeria* isolates (*8*). Research has shown that following exposure to *L. monocytogenes*, pregnant guinea pigs exhibit similar side effects to humans including fetal abortion (*8*) as well as gastrointestinal tract disturbances (*16*). In a previous study, pregnant guinea pigs aborted on the 3rd and 7th day following infection with *L. monocytogenes* by intraperitoneal injection (*8*), but the animals were treated with infected tissues and the treatment dose was unknown.

Our studies support using the pregnant guinea pig as a surrogate for oral exposure in humans based on 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 discovered that E-cadherin, which mediates transmigration of *L. monocytogenes* into mammalian epithelial cells, has the same sequence in both humans and guinea pigs (*14*). The sequence of the E-cadherin protein is extremely important in the binding of *L. monocytogenes* to epithelial cells and a single amino acid transition can alter the active site's configuration as previously discussed with the mouse model.

Bakardjiev et al. (*2*) used the pregnant guinea pig to study feto-placental transmission of *L. monocytogenes* following invasion of the maternal system through intravenous inoculation. No previously published listeriosis studies that used the guinea pig as an animal model used

ingestion as the route of exposure. Since humans are exposed to *L. monocytogenes* through ingestion, experimentally by-passing the gastrointestinal tract may lead to differences in virulence and infective dose estimates for the pathogen. By using an exposure regimen consistent with human exposures, we can obtain better estimates of maternal and fetal health conditions following exposure to *L. monocytogenes*.

In this study, we demonstrate that pregnant guinea pigs can be used as surrogates for human listeriosis by orally dosing the dams with *L. monocytogenes* cells and monitoring the following endpoints as indicators of infection: maternal and fetal tissue infectivity, maternal fecal shedding and birth outcomes. In a previous study that tracked the dissemination of *L. monocytogenes* between maternal and fetal tissues, nonpregnant guinea pigs were able to clear *L. monocytogenes* from their spleens (*3*). In contrast, in our study one of three nonpregnant guinea pigs was unable to clear *L. monocytogenes* cells from her spleen (data not shown). The different exposure regimen (injection vs. oral) may be the reason for the contrasting results. In our study, pregnant guinea pigs were also unable to clear *L. monocytogenes* from their livers, which is similar to results seen in a previous study *(3)*. In contrast to a study using injection as the exposure regimen *(3)*, pregnant guinea pigs in our study that were exposed to $\leq 10^5$ CFU had clearance rates of 100% from their spleens. Dams exposed to 10^6, 10^7, and 10^8 CFU had a clearance rate of 78%, 62% and 25%, respectively (Table 1).

In this study serum alanine aminotransferase levels were determined to detect liver injury. The results are consistent with a human case study that analyzed liver abscesses in eight elderly diabetic individuals due to *L. monocytogenes* exposure (*4*). Laboratory tests revealed that transaminase levels were atypical in only two of eight cases. Intriguingly, alkaline phosphatase levels were elevated in seven of the eight cases. Like humans, pregnant guinea pigs may exhibit

elevated phosphatase levels and normal transaminase levels, or the liver damage may be temporary and not result in liver damage in dams.

Early diagnosis and antimicrobial treatment of listeriosis during pregnancy can result in the birth of a healthy infant (*13*). However, the lack of biomarkers or diagnostic tests results in fetal deaths for a treatable disease. Pregnant guinea pigs offer the promise of a surrogate model for human listeriosis that can be used to develop diagnostic procedures and test treatments. Our study shows that pregnant guinea pigs orally exposed to *L. monocytogenes* had an increased risk of delivering stillborn infants that was dose dependent. Isolates could be cultured from maternal fecal matter and tissues as well as fetal tissues. The dose adversely affecting 50% of the pregnancies was 10^7 CFU which is similar to that estimated in humans at 10^6 CFU. These results add to our accumulating knowledge suggesting that guinea pigs are appropriate surrogate models for studying feto-placental transmission of *L. monocytogenes* in humans, and dose response information from these animals could be used in human risk assessment.

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Figure Legends

Figure 1. *Summary of maternal serum alanine aminotransferase levels.* Dams who delivered pre-term stillbirths are not included in the analyses. Fresh and frozen serum was used in the analyses. Error bars indicate standard deviation.

Figure 2. *Infection of fetal tissues from dams treated with* 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 percent of tissues positive for *L. monocytogenes* in each group.

Figure 3. *Fecal shedding of* L. monocytogenes *in dams fed with* L. monocytogenes. Two samples were collected pre-treatment to assure that the animals were not shedding *L. monocytogenes* prior to treatment; Three samples per week were collected post-treatment. Maternal fecal samples were categorized by their birth outcome.

Figure 4. *Dose response of* L. monocytogenes-*induced stillbirths in guinea pigs.* The dose response curve is based on the dose resulting in fetal deaths. The estimated LD 50 is 1.93×10^7 CFU *L. monocytogenes*.

Table 1. Isolation of **L. monocytogenes** *from maternal tissues after oral challenge during pregnancy*

Treatment Dose	Liver # positive/total # treated (% positive)	Spleen # positive/total # treated (% positive)	Gallbladder # positive/total # treated (% positive)
Control	**0/9 (0%)[a]**	**0/9 (0%)**	**0/9 (0%)**
10^4 CFU	**¼ (25%)[a]**	**0/4 (0%)**	**0/4 (0%)**
10^5 CFU	**7/11 (64%)[b]**	**0/11 (0%)**	**1/11 (9%)**
10^6 CFU	**8/9 (89%)[b]**	**2/9 (22%)**	**1/9 (11%)**
10^7 CFU	**8/9 (89%)[b]**	**3/8 (38%)**	**2/8 (25%)**
10^8 CFU	**4/4 (100%)[b]**	**3/4 (75%)**	**1/3 (33%)**

[a,b] Groups with different superscripts are significantly different ($p<0.05$).

Table 2. ***Doses of*** **L. monocytogenes** ***resulting in 50% of maternal tissues infected based on birth outcome***

Maternal Tissues	Group	Birth Outcome	Calculated 50% Infectivity Dose CFU
Liver	Control	Normal Fetuses n=9[a]	--*
	Treated	Normal Fetuses n=29	6×10^4
		Nonviable Fetuses[b] n=8	1×10^5
Spleen	Control	Normal Fetuses n=9	--
	Treated	Normal Fetuses n=29	$>1 \times 10^8$
		Nonviable Fetuses n=7	4×10^6
Gallbladder	Control	Normal Fetuses n=9	--
	Treated	Normal Fetuses n=29	$>1 \times 10^8$
		Nonviable Fetuses n=6	1×10^7

--*= Not Detected

[a] = Number of Dams

[b]= Dams with at least one nonviable fetus per litter

Table 3. Fetal tissues positive for **L. monocytogenes** *after maternal oral-exposure*

Maternal Dose	No. of Dams with Infected Fetus (%)[a]	Placenta (% Positive)	Fetal Liver (% Positive)	Fetal Brain (% Positive)
Control	0/9 (0%)	0/33 (0%)	0/33 (0%)	0/33 (0%)
10^4 CFU	0/4 (0%)	0/18 (0%)	0/18 (0%)	0/18 (0%)
10^5 CFU	2/11 (18%)	2/62 (3%)	2/62 (3%)	0/62 (0%)
10^6 CFU	2/9 (22%)	3/41 (7%)	6/41 (15%)	5/41 (12%)
10^7 CFU	3/9 (33%)	13/31 (42%)	12/31 (39%)	11/31 (35%)
10^8 CFU	3/4 (75%)	9/14 (64%)	12/17 (71%)	12/17 (71%)

[a] Dams with at least one infected fetus.

Table 4. ***Average weights and lengths of gestational day matched fetuses from dams orally-challenged with*** **L. monocytogenes**[a]

Maternal Dose	Mean Weights ± SD[b] (g)	Mean Lengths ± SD (cm)
Control	**54.2 ± 6.2**	**3.01 ± 0.41**
10^4 CFU	**75.16 ±17.80**[c]	**3.01 ± 0.07**
10^5 CFU	**60.1 ± 5.1**	**2.79 ± 0.20**
10^6 CFU	**61.7 ± 8.2**	**3.10 ± 0.46**
10^7 CFU	**56.8 ± 10.1**	**2.83 ± 0.31**
10^8 CFU	**54.3 ± 6.5**	**2.62**[d]

[a] Excludes premature deliveries and stillbirths not age-matched

[b] Denotes standard deviation

[c] Denotes samples that are statistically different from control samples

[d] N=1: All other dams treated with 10^8 CFU had stillbirths

Table 5. ***Dose response of fetal mortality after maternal challenge with*** **L. monocytogenes**

Maternal Dose	Number of Litters	Percent of Litters with ≥ 1 Fetal Death	Total Number of Fetuses	Percent Fetal Mortality
Control	9	0%	38	0%
10^4 CFU	4	0%	18	0%
10^5 CFU	11	0%	64	0%
10^6 CFU	9	22%	41	15%
10^7 CFU	9	33%	42	43%
10^8 CFU	4	75%	21	95%

Table 6. ***Comparison of Tissues from Each Infected Fetus Matched to Its Specific Placenta and Maternal Liver***

Treatment	Number of Dams with ≥ 1 Infected Fetal Brain	Maternal Liver	Placenta No. Positive/Total	Fetal Liver No. Positive/Total	Fetal Brain No. Positive/Total
10^6 CFU	1	+	2/5	5/5	5/5
10^7 CFU	2	+	11/11	10/11	11/11
10^8 CFU	3	+	12/12	10/12	12/12

Figure 1

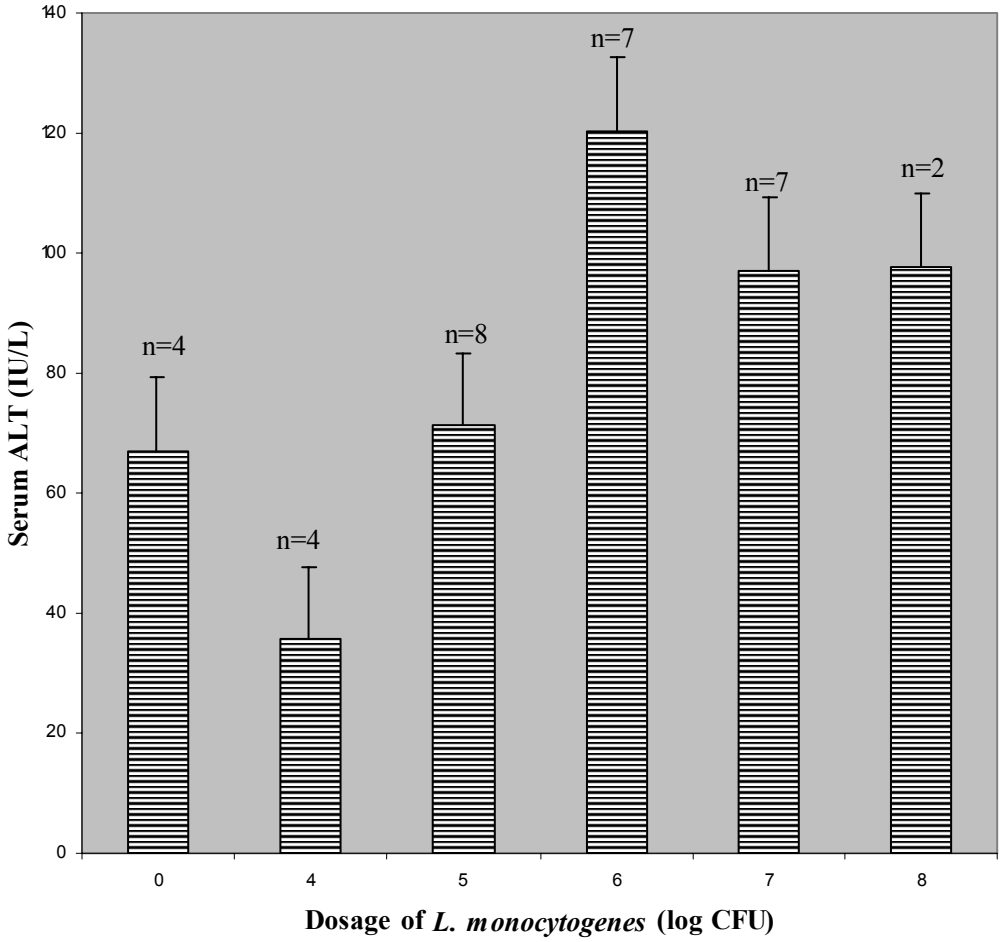

Serum ALT (IU/L)
140
120
100
80
60
40
20
0
n=4
n=4
n=8
n=7
n=7
n=2
0
4
5
6
7
8
Dosage of L. monocytogenes (log CFU)

Figure 2

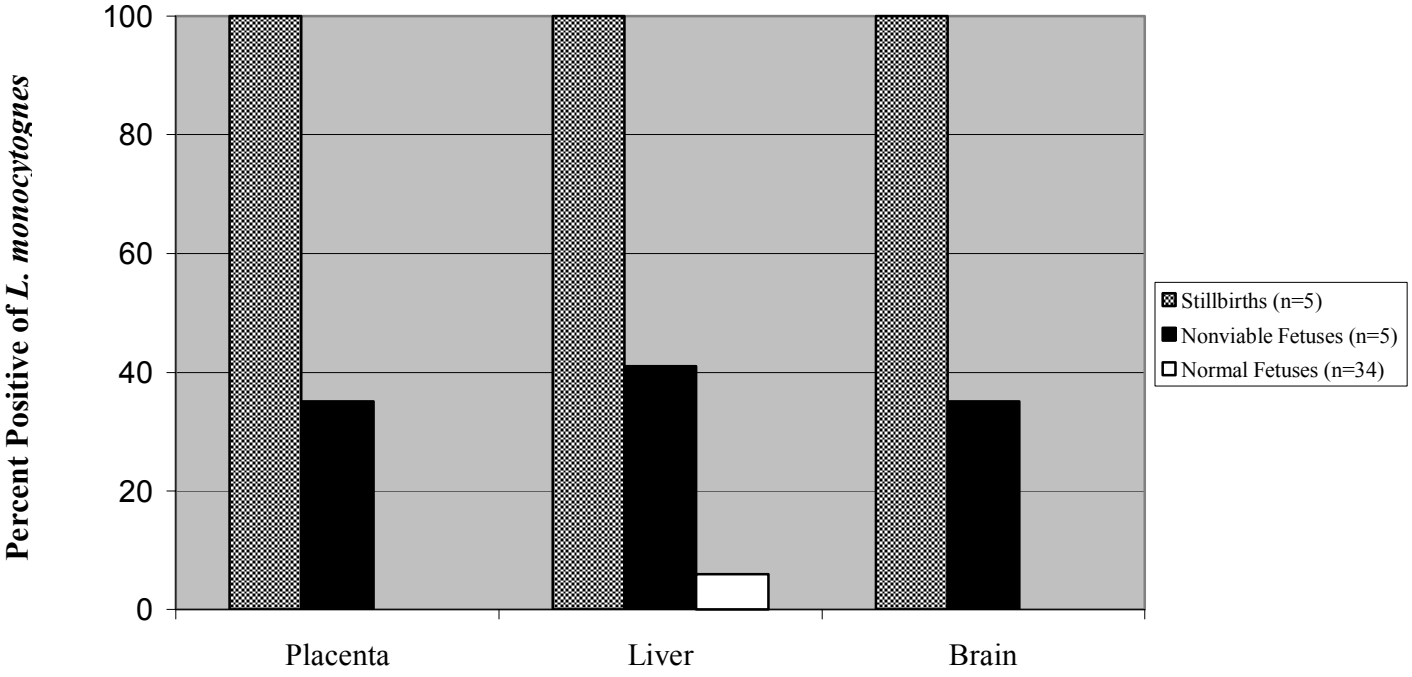

Percent Positive of L. monocytogenes
100
80
60
40
20
0
Placenta
Liver
Brain
Stillbirths (n=5)
Nonviable Fetuses (n=5)
Normal Fetuses (n=34)

Figure 3

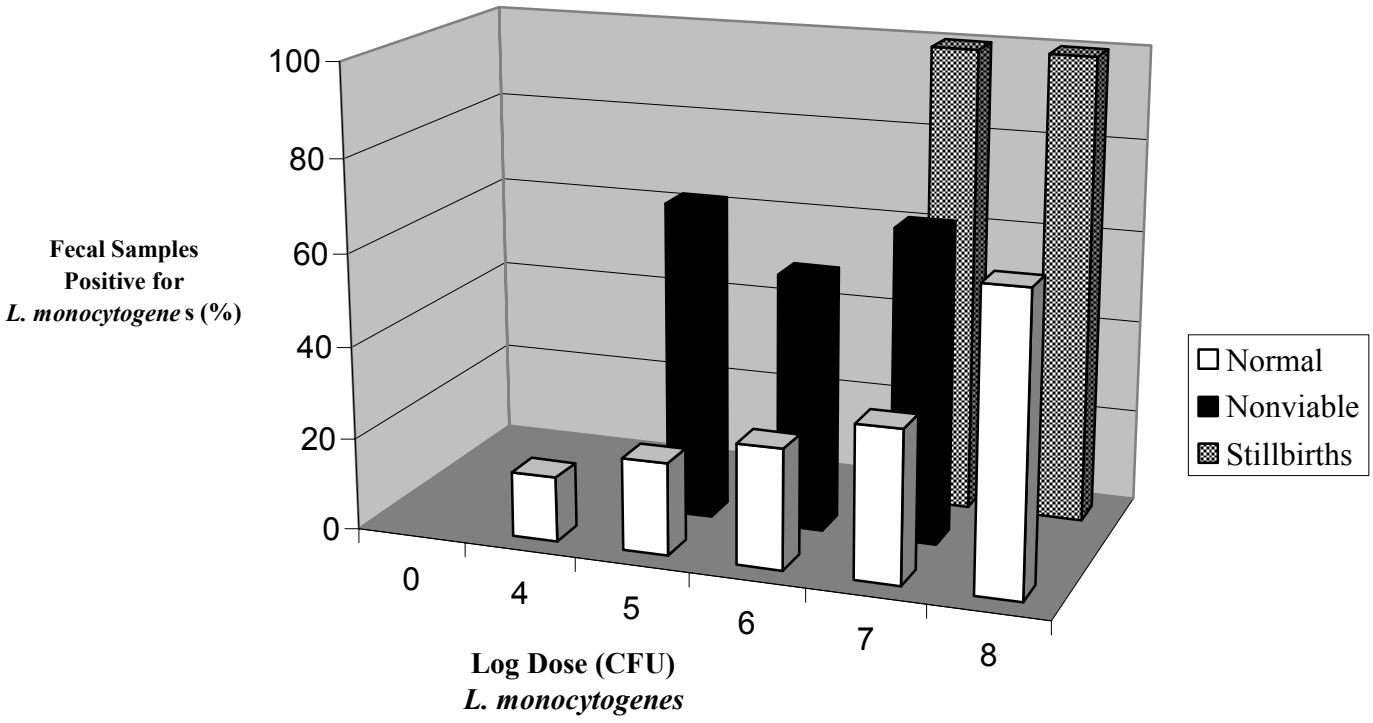
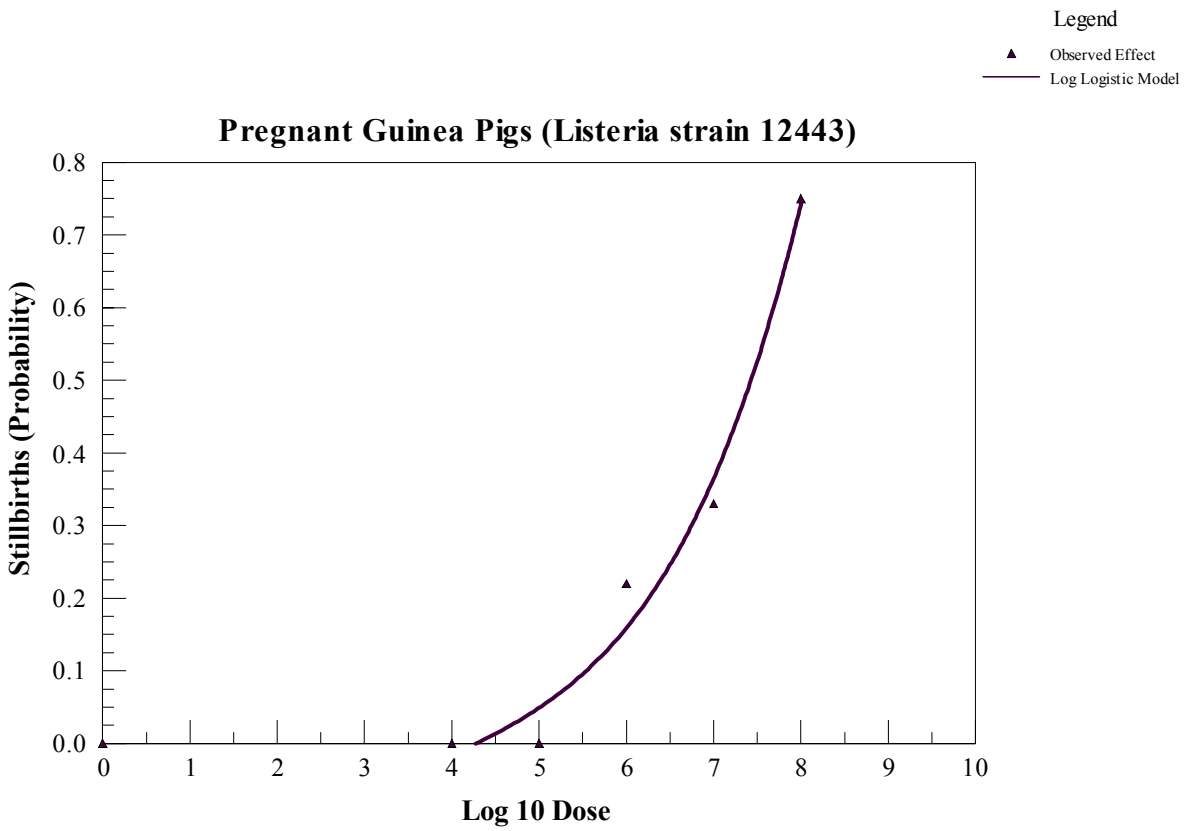

Fecal Samples Positive for L. monocytogenes (%)
100
80
60
40
20
0
0
4
5
6
7
8
Log Dose (CFU) L. monocytogenes
Normal
Nonviable
Stillbirths

Figure 4


Legend
Observed Effect
Log Logistic Model
Pregnant Guinea Pigs (Listeria strain 12443)
Stillbirths (Probability)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
1
2
3
4
5
6
7
8
9
10
Log 10 Dose

CHAPTER 4

SUMMARY AND CONCLUSIONS

The genus *Listeria* contains six species and of these six species, *Listeria monocytogenes* has proven to be the most pathogenic to both animals and humans. *L. monocytogenes* is a gram positive soil organism that has evolved the ability to invade and multiply within eukaryotic cells. *L. monocytogenes* replicates at an extremely broad temperature range (1°C to 45°C). Therefore, not only can *L. monocytogenes* survive at the normal refrigeration temperature of 4°C, the cells can also multiply. *L. monocytogenes* can also replicate at high salt concentrations (Wing et al., 2002). Most of the *Listeria* cases are attributable to food borne *Listeria* because 85 to 95% of listeriosis is food borne (Buzby et al., 1996). The Food and Drug Administration has classified the following foods as being at high risk for *Listeria* contamination: ready to eat foods, processed foods, deli meats, smoked seafood, soft cheeses and dairy products.

L. monocytogenes is a ubiquitous organism; therefore many humans are likely to be exposed. Yet, certain subpopulations are more susceptible to *L. monocytogenes* infection resulting in adverse health conditions. Unfortunately, pregnant women are among this "at-risk" group (Enocksson et al., 1992; Gellin et al., 1991; Hitchins et al., 1996). In human listeriosis, *L. monocytogenes* has a predilection for the feto-placental unit; consequently, maternal exposures pose a risk to fetuses and/or neonates (Buchdahl et al., 1990; Gellin et al., 1989). In many cases, the maternal unit is asymptomatic which results in an infected fetus unexpectedly delivering prematurely with complexities such as meningitis or septicemia or even worse stillborn. The side effects that are experienced by the fetus appear to be dependent on the point of gestation at

time of exposure. Listeriosis most commonly occurs during the third trimester. Second- and third-trimester infections can result in premature delivery followed by neonatal illness or pre-term delivery of a stillborn (Farber and Peterkin et al., 1991; Gellin and Broome, 1989). Listeriosis most commonly occurs during the third trimester.

The objectives of this study were to use pregnant guinea pigs that were orally-exposed to *L. monocytogenes* to (1) examine the pregnant guinea pig as a surrogate model for human listeriosis, (2) determine the maternal dose that results in placental transmigration leading to fetal tissue infectivity and/or the occurrence of stillbirths, and (3) relate the adverse fetal outcomes to maternal fecal shedding, tissue infectivity, and/or liver damage in order to establish a biomarker of exposure.

Our study shows that pregnant guinea pigs orally exposed to *L. monocytogenes* had an increased risk of delivering stillborn infants that was dose dependent. Isolates could be cultured from maternal fecal matter and tissues as well as fetal tissues. The dose adversely affecting 50% of the pregnancies was 10^7 CFU which is similar to that estimated in humans at 10^6 CFU. These results add to our accumulating knowledge suggesting that guinea pigs are appropriate surrogate models for studying feto-placental transmission of *L. monocytogenes* in humans, and dose response information from these animals could be used in human risk assessment.

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