Factors associated with the acquisition and severity of Gestational Listeriosis

M. M. Suyemoto
P. A. Spears
T. S. Hamrick
J. A. Barnes
E. A. Havell

See next page for additional authors

Follow this and additional works at: https://cufind.campbell.edu/medicine_school

Part of the Medical Immunology Commons
Authors
Factors Associated with the Acquisition and Severity of Gestational Listeriosis

M. Mitsu Suyemoto1, Patricia A. Spears1, Terri S. Hamrick2, Jill A. Barnes3, Edward A. Havell1, Paul E. Orndorff1*

1 Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States of America, 2 Department of Pharmaceutical Sciences, School of Pharmacy, Campbell University Buies Creek, Buies Creek, North Carolina, United States of America, 3 Department of Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States of America

Abstract
Gravid mammals are more prone to listeriosis than their nongravid counterparts. However, many features of the disease in gravid animals are not well defined. We determined, in mice, that increased susceptibility to lethal infection following oral inoculation begins surprisingly early in pregnancy and extends through embryonic development. Pregnancy did not demonstrably increase the spread of listeriae from the intestine to the liver and spleen in the initial 96 h period post inoculation. Consequently, it appeared that gravid animals were competent to contain an enteric infection, but in those instances where escape did occur, a lethal outcome was more likely. Interestingly, colonic colonization level and prevalence, measured 96 h post inoculation, was significantly higher in gravid individuals. In terms of human risk factors for listeriosis, our results suggest that the window of listeriosis susceptibility afforded by pregnancy may be open longer than previously appreciated. Our results also suggest that while gravid animals are competent to contain an enteric infection, enteric carriage rate may be more of a factor in defining disease incidence than previously considered.

Introduction
Common features of listeriosis are (i) ingestion of contaminated food (ii) colonization of the intestine (iii) intestinal translocation (iv) replication in the liver and spleen, and (v) either the resolution of infection or the hematogenous spread to other organs resulting in a systemic infection [1]. Pregnancy greatly increases the risk of listeriosis in virtually all mammalian species [2,3]. Approximately thirty-three percent of clinically documented cases of human listeriosis are in pregnant women [4] and pregnant women constitute approximately 60% of all cases (male and female) aged 10 to 40 years [5].

Despite the well-known association of listeriosis with pregnancy, there is incomplete agreement on how pregnancy increases disease risk. Case studies suggest that the gravid host is most susceptible to listeriosis during the later stage of pregnancy. Indeed, with isolated exceptions [6], disease presentation correlates well with a depression in the host’s cell-mediated immunity late in gestation [7,8]. However, early pregnancy terminations (i.e., spontaneous abortions at the embryonic stage rather than at the later fetal stage) are typically not analyzed microbiologically. The gravid host may also display fewer symptoms of listeriosis in early pregnancy or attribute mild symptoms to more routine discomforts (e.g., morning sickness). Experimental studies in mice, in which the timing of impregnation and initiation of listeriosis by oral inoculation can be tightly controlled [9], indicate that gravid animals are prone to disease earlier than the fetal development stage [10,11]. However, a systematic study to identify the gestational stage at which increased susceptibility first manifests itself has not been done.

In this communication, we report that increased maternal susceptibility to lethal listeriosis begins very early in gestation and extends through embryonic development. Mice at the fetal stage of pregnancy recovered some resistance. Pregnancy did not increase liver and spleen infectivity (measured soon after oral inoculation). However, gestation did increase listerial prevalence and load in the colon throughout gestation.

Results
LD50 determination at progressive gestational stages
The LD50 values determined from orally inoculated nongravid and gravid mice (Fig. 1) revealed that the LD50 was significantly lower at 0.5 gestational days (gd) and reached its apparent nadir (ca., 100-fold less than nongravid) between 3.5 and 6.5 gd. The LD50 increased at 9.5 gd to a value indistinguishable from the nongravid LD50 before decreasing at 14.5 gd at which time the LD50 was ca. 10-fold lower than that for nongravid mice.

Listerial infectivity at progressive gestational stages
The ability of gravid and nongravid mice to limit the translocation of listeriae from the intestine to the liver and spleen was assessed 96 h post inoculation at progressive stages of gestation by examining the cfu in the colon contents and in the
liver and spleen. We found that gestational stage had no demonstrable effect upon the average level of liver and spleen colonization (Fig. 2A). However, there was a significant and dramatic increase in the proportion of gestating mice carrying listeriae in the colon soon after impregnation (Fig. 2B). This same increase in listerial prevalence was registered as statistically significant if colonic listerial cfu per mouse (Fig. 2B) were averaged within groups and compared.

**Discussion**

Despite the well known susceptibility to listeriosis during pregnancy, there is incomplete agreement on how pregnancy increases listeriosis risk. The generalized suppression of immunity in gravid animals late in gestation is often cited as a factor in increased disease susceptibility [10,12]. Consequently, most experimental studies that examine the effect of pregnancy on listerial pathogenesis have employed mice at a late stage in their 18.5 gestational period, typically 14.5 gd. However, a number of reports indicate that mice become more prone to infection well in advance of that gestational stage (reviewed in Orndorff et al.[1]). Most recently, Hamrick et al. [11] showed a dramatic increase in susceptibility to infection at 7.5 gd. In the present report, we orally inoculated mice at different stages of pregnancy and determined susceptibility increased, seemed to eliminate generalized immuno-suppression as the cause of the increased lethality.

Our results indicate that susceptibility to lethal infection occurs very early in gestation. Even animals inoculated at 0.5 gestational days showed a significant increase in susceptibility. Lethality peaked in animals inoculated at 3.5 and 6.5 gd when embryonic development is occurring rapidly. By the time embryonic development ceased (ca. 9.5 gd) and fetal maturation commenced, lethality decreased somewhat.

The strikingly early pregnancy stage (0.5 gd) at which susceptibility increased, seemed to eliminate generalized immunological suppression as the cause of the increased lethality. However, to examine this assumption we determined the ability of gravid animals to initially respond to listerial infection by measuring the level of liver and spleen colonization soon after inoculation of a low listerial dose. The short time interval between inoculation and organ harvest and the low inoculating dose employed largely eliminated complicating intrauterine infections in gravid individuals. Only a small subset of gravid individuals with liver and spleen colonization develop intrauterine infections [11,13,14]. Nevertheless, such infections can serve as a source for reseeding the liver and spleen [15]. Consequently, intrauterine cfu were monitored. We found that comparatively few mice (4 of the 29 gravid animals examined) had uterine infections at 96 h post inoculation (10^5 to 10^6 cfu per organ) and their small representation did not affect the conclusions derived from statistical analysis of liver and spleen cfu: Listerial burdens in the liver and spleen, in gravid and nongravid mice were indistinguishable. This result indicated that the initial immune response of a gravid mouse was similar to that of a nongravid mouse. There was, however, a pronounced increase in the persistence of listeriae in the gravid colon. This latter observation, made possible through the use of the natural oral inoculation route, may warrant further study since, in women, colonic colonization could serve as a valuable predictor of an at-risk pregnancy.
A principal reason for the increased lethality in gravid mice could be the ability of the implanted embryo to serve as a privileged site. Bacterial replication would require sufficient numbers of circulating bacteria to be present to effect colonization [11]. Mouse blastocyst implantation occurs at ca. 3.5 gd [9]. Consequently, circulating listeriae could find a protective environment available at approximately this time. Our observation that susceptibility to lethal infection was increased even at 0.5 gd is consistent with this view because listeriae were present in the livers and splens for periods that overlapped with blastocyst implantation.

The reason that listeriosis lethality decreased later in gestation is not known, but may relate to the presence of a more fully developed placental barrier to infection. Another plausible explanation is that some of these infected individuals gave birth (i.e. expelled potentially infective nidí). These factors, and other perinatal events, introduce variables into the interpretation of results at the later gestational stages but do not impact our finding that gravid animals become more prone to lethal infection at a previously unidentified early stage. This finding clearly casts doubt on the role of generalized immune modulation as an explanation for pregnancy’s effect on listeriosis susceptibility, at least in mice.

Mice, other rodents [16], and humans have a hemochorial placenta and a similar early placental development [17]. Although several rodent species other than mice have been argued to be more representative of humans [18,19], all gravid mammalian hosts develop a strikingly high listeriosis susceptibility, and mice are no exception to this rule. Consequently, the magnitude and implication of observations regarding the onset of susceptibility in any model host should be regarded as compelling.

In summary, our findings suggest that listeriosis could be a factor in early pregnancy loss and support the adoption of routine microbiological evaluation of human concepts spontaneously aborted in early pregnancy. Our results also point out that colonic listerial carriage in pregnant women could be an especially sensitive indicator of exposure and deserving of subsequent careful monitoring.

**Materials and Methods**

**Ethics Statement**

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study was approved by the North Carolina State University Institutional Animal Care and Use Committee (Assurance Number: A3331-01).

**Bacterial culture**

The mouse oral virulent *L. monocytogenes* serotype 4momb strains F6214-1 and PAS351 were used in this study [11]. The strains are equally virulent and isogenic except for a Tn917 insertion in PAS351 that confers lincomycin and erythromycin resistance [11]. The strains were used in all cases. Mice were sacrificed 96 h post inoculation and subjected to Probit analysis for mean differences using the Minitab 14 statistical analysis program. Median differences involving liver and spleen colonization levels were analyzed by the Mann-Whitney rank sum test using the Minitab 14 statistical analysis program. Median differences involving liver infectivity experiments were performed essentially as for the LD<sub>50</sub> experiments except a single dose (averaging 4×10<sup>8</sup> cfu) was used in all cases. Mice were sacrificed 96 h post inoculation and the cfu in the liver, spleen, uterus and colonic contents were enumerated as previously described [11].

**Statistical treatment**

LD<sub>50</sub> values were calculated as previously described [11] and subjected to Probit analysis for mean differences using the Minitab 14 statistical analysis program. Median differences involving liver and spleen colonization levels were analyzed by the Mann-Whitney rank sum test using the Minitab 14 statistical analysis program. Binary outcome data in listericol placental carriage were analyzed for mean differences using Fisher’s exact test offered on Vassar Stats. In all cases significance was defined as P<0.05.

**Acknowledgments**

We thank John Horton, and Rebecca Wolf for a critical reading of the manuscript and helpful advice.

**Author Contributions**

Conceived and designed the experiments: TSH EAH PEO. Performed the experiments: MMS PAS TSH JAB EAH. Analyzed the data: MMS PAS EAH PEO. Contributed reagents/materials/analysis tools: PAS JAB. Wrote the paper: MMS PEO.


