Listeria placental infection

Citation for published version:
Vazquez-Boland, J, Krypotou, E & Scortti, M 2017, 'Listeria placental infection' mBio. DOI: 10.1128/mBio.00949-17

Digital Object Identifier (DOI):
10.1128/mBio.00949-17

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
mBio

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Listeria Placental Infection

José A. Vázquez-Boland, Emilia Krypotou, Mariela Scortti
Division of Infection and Immunity, The Roslin Institute and Edinburgh Medical School (Biomedical Sciences), University of Edinburgh, Edinburgh, United Kingdom

ABSTRACT  The Gram-positive facultative intracellular bacterium Listeria monocytogenes is the causative agent of listeriosis, a severe food-borne infection. Pregnant women are at risk of contracting listeriosis, which can potentially lead to miscarriage, stillbirth, preterm birth, and congenital neonatal infections. While other systemic bacterial infections may result in adverse pregnancy outcomes at comparable frequencies, L. monocytogenes has particular notoriety because fetal complications largely occur in the absence of overt illness in the mother, delaying medical intervention. Here, we briefly review the pathophysiology and mechanisms of maternofetal listeriosis, discussed in light of a recent mBio report on Listeria transplacental infection in a nonhuman primate model.

KEYWORDS  Listeria miscarriage, Listeria monocytogenes, maternofetal listeriosis, placental infection

Although many human systemic bacterial infections may result in miscarriage or stillbirth, unlike Listeria monocytogenes, the causative agents are not considered primarily abortifacient. For example, recent data from the United Kingdom show that of a cohort of 75 pregnant women with invasive Haemophilus influenzae infection, 63% had fetal loss (1). In comparison, with a similar incidence, only 40% of pregnancy-associated listeriosis cases resulted in spontaneous abortion, stillbirth, or neonatal death in the same geographic area (2). Other examples include Brucella, Campylobacter, Coxiella, or Salmonella, to name a few; while not recognized as part of the TORCH (toxoplasmosis, other infections, rubella, cytomegalovirus, and herpes simplex virus) group, all these pathogens may lead to adverse fetal outcome in pregnant women (3–6) and are also well-known causes of septic abortion in farm animals (7). It has been suggested that essentially any invasive bacteria with the ability to survive within host cells can potentially colonize the placenta and fetus via the hematogenous route (8). What then makes L. monocytogenes stand out as a notorious miscarriage-associated microbe? One could argue that this is largely due to perception, owing to the inapparent or mild clinical course of maternal infection before the onset of obstetric signs. This is in contrast to other systemic infections, where fetal complications are mainly regarded as secondary to the mother’s illness.

The absence of obvious outward manifestations preceding listerial miscarriage has been experimentally observed in a study with intragastrically (i.g.) infected cynomolgus macaques recently published by Wolfe et al. in mBio (9). Similar findings were previously reported in rhesus macaques (10, 11), confirming that the lack of specific prodromal signs appears to be characteristic of maternofetal listeriosis. With a similar hemochorial, villous, discoidal placenta and reproductive cycle, nonhuman primates offer a suitable model to study human reproduction and related disorders (12, 13). Macaques are naturally susceptible to L. monocytogenes infection, with clinical outcomes in pregnant monkeys mirroring those in human maternofetal listeriosis. This includes miscarriage or stillbirth in late pregnancy (14) (in pregnant women mostly during late second/third trimester [2, 15]).

Timing of listerial miscarriage. The study by Wolfe et al. (9) is interesting in that...
the monkeys were inoculated in early gestation (single i.g. dose of $10^7$ CFU between gestation days [GD] 36 to 46, term is GD 165), and all four exposed animals miscarried. In contrast, in an earlier study, Smith et al. (11) observed only six stillbirths among 23 late pregnancies following i.g. exposure of macaques at around GD 110. Wolfe et al. concluded that the macaque’s maternal-fetal interface may be particularly sensitive to Listeria infection in early pregnancy and that there might be a greater, unrecognized risk of listerial miscarriage in the first trimester of gestation (9).

However, careful analysis of the existing data for macaques indicates that the suggested increased susceptibility in early pregnancy (9) may be more apparent than real, due to the high experimental infection dose used. In late pregnancy monkeys, Smith et al. (11) observed 16.6% stillbirths after i.g. exposure to $10^3$ to $10^4$ L. monocytogenes CFU, 28.6% stillbirths when using $10^5$ to $10^6$ CFU, whereas the only monkey administered $10^7$ CFU, i.e., the same dose as Wolfe et al. (9) used, delivered a stillborn fetus. No adverse fetal outcomes were observed with a $10^2$ dose. For a given single-dose L. monocytogenes inoculum, a higher rate of blood-borne colonization per tissue mass is to be expected for a smaller uteroplacental unit than for a significantly more-developed one. An overwhelming placental infection in Wolfe et al. is supported by the acute course and short incubation period before fetal demise, only 7 to 13 days postinoculation (9) compared to an average of 47 days until stillbirth in the Smith et al. study (11). The latter is more in keeping with the typical incubation period of maternofetal listeriosis in humans (16), generally associated with lower levels of exposure to L. monocytogenes according to outbreak data ($5 \times 10^4$ CFU/g in the incriminated food (17); $1.9 \times 10^6$ estimated dose for 50% listerial perinatal morbidity (18)).

A number of factors may explain the predominant presentation of maternofetal listeriosis in late pregnancy. For example, the human placenta is thought to become truly hemochorial only in the second trimester (19). This late shift in the nature of the maternofetal interface appears to be unique to great apes. In the macaque, intervillous maternal circulation is established as early as 3 weeks after conception (12, 13), which together with an earlier invasion of spiral arteries by the extravillous trophoblast (EVT) (12) (the primary placental cellular target of L. monocytogenes; see below) (Fig. 1) may contribute to explain the exquisite susceptibility noted by Wolfe et al. (9). The importance of the placental configuration is unclear, however, because listerial abortion in ruminants is also predominantly observed in late pregnancy (7, 20) despite having a different type of placentation (epitheliochorial, with the maternal and fetal circulations physically separated by six tissue layers) (13).

Other possible explanations include the following: (i) predominance of low-level infections requiring a protracted incubation period, where the placenta itself may become a reservoir for maternal reinfection and amplification of the bacterial load (21); (ii) progressive increase in uterine blood flow (and hence exposure of the placenta to blood-borne listeriae) (Fig. 2) relative to the overall cardiac output; (iii) possible exacerbation of critical immune tolerance mechanisms at the maternal-fetal interface in late gestation; (iv) physiological burden of advanced pregnancy, as suggested by the observation that pregnant women carrying multiple fetuses are at greater risk of listeriosis (22); and (v) fetal death during early gestation being more likely to go unreported or remaining etiologically undiagnosed.

**Mechanism of placental colonization and dissemination to the fetus.** The high-dose infection used by Wolfe et al. caused an extensive neutrophilic inflammatory response with disruption of the maternal-fetal barrier of the macaques (9). Vasculitis, thrombosis, and necrosis of the decidual spiral arteries and the presence of bacteria in the intervillous maternal circulation, villous capillaries, and umbilical cord were consistently observed (Fig. 18). While inflammation-mediated hematogenous transmission to the fetus may prevail in an acute or advanced placental infection, the importance of this dissemination pathway at the initial stages of the process or in cases of low-level infections is less clear. L. monocytogenes is an actively invasive pathogen endowed with a stealthy actin-based cell-to-cell spreading mechanism that bypasses the dependency on cell/tissue damage for successful dissemination while allowing escape from immune
control (23) (Fig. 1A). The key importance of this cell-to-cell spread mechanism in transplacental colonization has been demonstrated in vivo in nonprimate animal models using *L. monocytogenes* mutants lacking the virulence factor responsible for it, the actin-polymerizing protein ActA (24, 25).

The primary hematogenous entry route to the placenta (Fig. 2) is more controversial. Studies of guinea pigs, gerbils, and mice show a contributing yet dispensable role for the listerial invasins InlA and InlB (required for internalization into nonphagocytic host cells but not for uptake by professional phagocytes) in placental and fetal colonization (24, 26, 27). Thus, direct invasion by extracellular bacteria seems less likely to be the main mechanism than cell-to-cell spread from infected maternal phagocytes trafficking to the placenta (21) (Fig. 1A). The predominance of one mechanism over the other may critically depend on the infectious dose and degree of infection of the primary target organs (liver and spleen) after listerial intestinal translocation (Fig. 2).

**Placental tropism?** Wolfe et al. (9) observed substantially larger bacterial loads in the decidua and placenta, umbilical cord, amniotic fluid, and fetal tissues (>10⁷ to 10⁸ CFU) compared to the maternal nonreproductive tissue (liver, spleen, and lymph node; <10⁵ CFU). This was interpreted as a demonstration of the tropism of *L. mono-**
cytogenes for the gravid uterus (9). Alternatively, it may simply reflect the relative permissiveness of the placenta to listerial infection compared to other (immunologically) more restrictive maternal tissues and organs, i.e., liver and spleen. There they establish infectious foci that in an immunocompetent individual are efficiently cleared by cell-mediated immunity. In adult people with no predisposing conditions, the process is largely subclinical. In this population, exposure to larger infective doses may cause febrile gastroenteritis and, in rare cases, invasive disease. In immunocompromised adults and elderly people who are unable to mount an efficient T-cell-mediated immune response, the primary infectious foci are inadequately resolved and Listeria bacteria may be released to the bloodstream. This results in febrile bacteremia and, eventually, invasive infection of the brain. In pregnant women, L. monocytogenes colonizes the uterus in addition to the liver and spleen. While the infection is controlled in the latter organs, the placental immune tolerance mechanisms provide a permissive niche for the proliferation of L. monocytogenes. Bacteria from the placental reservoir released to the bloodstream may reinfect the mother’s liver and spleen, contributing to infection maintenance and amplification (21). Transplacental dissemination to the fetus results in abortion, stillbirth, or neonatal sepsis. A late-onset congenital form is also observed in neonates, often accompanied by meningitis. Based on an original depiction in reference 38.

FIG 2 Pathophysiology of food-borne listeriosis. L. monocytogenes bacteria cross the epithelial barrier of the intestine, translocate to the mesenteric lymph nodes, and reach their primary target organs, i.e., liver and spleen. There they establish infectious foci that in an immunocompetent individual are efficiently cleared by cell-mediated immunity. In adult people with no predisposing conditions, the process is largely subclinical. In this population, exposure to larger infective doses may cause febrile gastroenteritis and, in rare cases, invasive disease. In immunocompromised adults and elderly people who are unable to mount an efficient T-cell-mediated immune response, the primary infectious foci are inadequately resolved and Listeria bacteria may be released to the bloodstream. This results in febrile bacteremia and, eventually, invasive infection of the brain. In pregnant women, L. monocytogenes colonizes the uterus in addition to the liver and spleen. While the infection is controlled in the latter organs, the placental immune tolerance mechanisms provide a permissive niche for the proliferation of L. monocytogenes. Bacteria from the placental reservoir released to the bloodstream may reinf ect the mother’s liver and spleen, contributing to infection maintenance and amplification (21). Transplacental dissemination to the fetus results in abortion, stillbirth, or neonatal sepsis. A late-onset congenital form is also observed in neonates, often accompanied by meningitis. Based on an original depiction in reference 38.
targeting mechanisms (unless involving a conserved, promiscuous host receptor); it suggests rather that placental invasion depends on general intrinsic features of the *Listeria* host-pathogen interaction (such as, e.g., cell-to-cell spread in an immunologically relatively permissive environment).

The above does not exclude the existence of *L. monocytogenes* determinants facilitating establishment and proliferation in the maternal reproductive tract and placenta. According to recent data in guinea pigs, a listerial protein of the internalin family, InIP, which is generally conserved in *L. monocytogenes*, appears to specifically aid placental colonization (34). Virulence heterogeneity among *L. monocytogenes* isolates is well documented, and specific “hypervirulent” clonal complexes have been epidemiologically and experimentally associated with invasive (placental and CNS indistinctly) listeriosis (35). Anecdotal evidence hints at the possibility that particular strains might be more prone to cause maternofetal infections versus other clinical presentations. Thus, some listeriosis outbreaks, associated with a specific *L. monocytogenes* strain, have an unusually high frequency of maternal-perinatal cases (36, 37). In ruminants, maternofetal and CNS forms of the disease seldom occur simultaneously in the same herd outbreak (20, 38). Interestingly, considered together, the studies by Wolfe et al. (9) and Smith et al. (10, 11) show that two strains associated with maternofetal listeriosis cases in humans or primates consistently caused miscarriage in experimentally infected macaques with an i.g. dose of 10^7, while two other strains involved in human outbreaks with a low frequency of maternofetal cases did not. Specifically, a *L. monocytogenes* strain (ScottA) from a listeriosis outbreak with few pregnancy-related cases (7 of 42) (39) caused stillbirth at a dose of 10^10 (1/1) but not 10^8 (0/1) unless as part of a mix with isolates associated with maternofetal infections (2/2) (10). Although based on very limited evidence and far from conclusive, these data show a trend that warrants further investigation.

As briefly outlined here, many areas still remain obscure in our understanding of maternofetal listeriosis. Key points for attention include the impact of the bacterial dose on placental infection dynamics, the pathophysiology and determinants of transplacental invasion, and the potential involvement of tropism factors. Also requiring clarification is the role of early immune signaling events prior to transplacental colonization as a precipitating factor in fetal demise (40). Experiments in relevant animal models, including macaques closely replicating the human system, should prove invaluable for illuminating the detailed mechanisms of placental listeriosis and other aspects of *Listeria* pathogenesis.

**ACKNOWLEDGMENTS**

We thank the Wellcome Trust (WT074020MA) and BBSRC core funding to The Roslin Institute (BB/J004227/1) for supporting *Listeria* work in our laboratory.

**REFERENCES**

1. Collins S, Ramsay M, Slack MP, Campbell H, Flynn S, Litt D, Ladhani SN. 2014. Risk of invasive *Haemophilus influenzae* infection during pregnancy and association with adverse fetal outcomes. JAMA 311:1125–1132. https://doi.org/10.1001/jama.2014.1878.
2. Awofisayo A, Amar C, Ruggles R, Elson R, Adak GK, Mook P, Grant KA. 2015. Pregnancy-associated listeriosis in England and Wales. Epidemiol Infect 143:249–256. https://doi.org/10.1017/S0950268814000594.
3. Coughlin LB, Muiguan J, Haddad NG, Mannion P. 2003. *Salmonella* sepsis and miscarriage. Clin Microbiol Infect 9:866–868. https://doi.org/10.1046/j.1469-0691.2003.00605.x.
4. Smith JL. 2002. *Campylobacter jejuni* infection during pregnancy: long-term consequences of associated bacteremia, Guillain-Barre syndrome, and reactive arthritis. J Food Prot 65:696–708. https://doi.org/10.4315/0362-028X-65.4.696.
5. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A. 2009. Q fever during pregnancy: a cause of poor fetal and maternal outcome. Ann Y Acad Sci 1166:79–89. https://doi.org/10.1111/j.1749-6632.2009.04519.x.
6. Khan MY, Mah MW, Memish ZA. 2001. Brucellosis in pregnant women. Clin Infect Dis 32:1172–1177. https://doi.org/10.1086/319758.
7. Givens MD, Marley MS. 2008. Infectious causes of embryonic and fetal mortality. Theriogenology 70:270–285. https://doi.org/10.1016/j.theriogenology.2008.04.018.
8. Vigiliani MB, Bakardjiev AI. 2013. First trimester typhoid fever with vertical transmission of *Salmonella typhi*, an intracellular organism. Case Rep Med 2013:973297. https://doi.org/10.1155/2013/973297.
9. Wolfe B, Wiezp GJ, Schotzko M, Bondarenko GI, Durning M, Simmons HA, Mejia A, Faith NG, Sampene E, Suresh M, Kathariou S, Czuprynski CJ, Golos TG. 2017. Acute fetal demise with first trimester maternal infection resulting from *Listeria monocytogenes* in a nonhuman primate model. mBio 8:e01938-16. https://doi.org/10.1128/mBio.01938-16.
10. Smith MA, Takeuchi K, Brackett RE, McClure HM, Raybourne RB, Williams KM, Babu US, Ware GQ, Broderson JR, Doyle MP. 2003. Nonhuman primate model for *Listeria monocytogenes*-induced stillbirths. Infect Immun 71:1574–1579. https://doi.org/10.1128/IAI.71.3.1574-1579.2003.
11. Smith MA, Takeuchi K, Anderson G, Ware GQ, McClure HM, Raybourne...
RB, Mynte N, Doyle MP. 2008. Dose-response model for Listeria monocytogenes-induced stillbirths in nonhuman primates. Infect Immun 76:726–731. https://doi.org/10.1128/IAI.01366-06.

12. de Rijk EPCT, Van Esch E. 2008. The macaque placenta—a mini-review. Toxicol Pathol 36:1085–1185. https://doi.org/10.1080/0192623308326095.

13. Carter AM. 2007. Animal models of human placentation—a review. Placenta 28(Suppl A):S41–S47. https://doi.org/10.1016/j.placenta.2006.11.002.

14. Egal ES, Ardeshrin A, Mariano FV, Gonzak RO, Montali VA, dos Santos HT, Canfield DR, Yee J, Lemoy MJ, Lerche NW, Tarara RP. 2015. Contribution of endemic listeriosis to spontaneous abortion and stillbirth in a large outdoor-housed colony of rhesus macaques (Macaca mulatta). J Am Assoc Lab Anim Sci 54:399–404.

15. Girard D, Leclercq A, Laurent E, Lecuit M, de Valk H, Goulet V. 2014. Pregnancy-related listeriosis in France. 1984 to 2011, with a focus on 606 cases from 1999 to 2011. Euro Surveill 19(38):pii=20909. doihttps://doi.org/10.2807/1560-7917.EES2014.19.38.20909.

16. Goulet V, King LA, Vaillant V, de Valk H. 2013. What is the incubation period for listeriosis? BMC Infect Dis 13:11. https://doi.org/10.1186/1471-2334-13-11.

17. Pérez-Atienza E, Zigorraga C, Artieda J, Allerka M, Marimon JM. 2014. Two outbreaks of Listeria monocytogenes infection, Northern Spain. Emerg Infect Dis 20:2155–2157. https://doi.org/10.3201/eid2012.140993.

18. FAO/WHO. 2004. Risk assessment of Listeria monocytogenes in ready-to-eat foods. Microbiological risk assessment series 5, technical report. Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy. http://www.who.int/foodsafety/publications/mra_listeria/en/.

19. Benirschke K, Burton GJ, Baergen RN. 2012. Placental types, p 27–39. In Pathology of the human placenta, 6th ed. Springer-Verlag, Berlin, Germany.

20. Low JC, Donachie W. 1997. A review of Listeria monocytogenes and listeriosis. Vet J 157:39–29. https://doi.org/10.1016/S1090-0233(97)80005-6.

21. Bakardjiev AI, Theriot JA, Portnoy DA. 2006. Listeria monocytogenes traffics from maternal organs to the placenta and back. PLoS Pathog 2:e66. https://doi.org/10.1371/journal.ppat.0020066.

22. Mascola L, Ewert DP, Eller A. 1994. Listeriosis: a previously unreported medical complication in women with multiple gestations. Am J Obstet Gynecol 170:1328–1332.

23. Portnoy DA, Auerbach V, de Valk H. 2013. Fetomaternal immune cross-talk and its consequences for maternal and offspring’s health. Nat Med 19:548–556. https://doi.org/10.1038/nm.3160.

24. Warning JC, McCracken SA, Morris JM. 2011. A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. Reproduction 141:715–724. https://doi.org/10.1530/REP-10-0360.

25. Pamer EG. 2004. Immune responses to Listeria monocytogenes. Nat Rev Immunol 4:812–823. https://doi.org/10.1038/nri1461.

26. Faralla C, Rizzuto GA, Lowe DE, Kim B, Cooke C, Shioy LR, Bakardjiev AL. 2016. iNIP, a new virulence factor with strong placental tropism. Infect Immun 84:3584–3596. https://doi.org/10.1128/IAI.00625-16.

27. Maury MV, Tsai YH, Charlier C, Touchon M, Chenal-Franciscus V, Leclercq A, Crisculo A, Gaultier C, Roussel S, Brissais A, Disson O, Rocha EP, Brisse S, Leclut M. 2016. Uncovering Listeria monocytogenes hypervirulence by harnessing its biodiversity. Nat Genet 48:308–313. https://doi.org/10.1038/ng.3501.

28. Linnan MJ, Mascola L, Lou XD, Goulet V, May S, Salminen C, Hird DW, Yonekura ML, Hayes P, Weaver R, Audurier A, Pikayckis BD, Fannin SL, Kleks A, Broome CV. 1988. Epidemic listeriosis associated with Mexican-style cheese. N Engl J Med 319:823–828. https://doi.org/10.1056/NEJM198809293191303.

29. MacDonald PD, Whitwam RE, Boggis JD, MacCormack JN, Anderson KL, Reardon JW, Saah JR, Graves LM, Hunter SB, Sobel J. 2005. Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese. Clin Infect Dis 40:677–682. https://doi.org/10.1086/427803.

30. Vázquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, González-Zorn B, Welhahn J, Kreft J. 2001. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev 14:584–640. https://doi.org/10.1128/CMR.14.3.584-640.2001.

31. Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Pikayctis BD, Holmes MB, Audurier A, Broome CV, Reingold AL. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med 312:404–407. https://doi.org/10.1056/NEJM198502143120704.

32. Rowe JH, Ertelt JM, Xin L, Way SS. 2012. Listeria monocytogenes cytoplasmic entry induces fetal wastage by disrupting maternal Foxp3+ regulatory T cell–sustained fetal tolerance. PLoS Pathog 8:e1002873. https://doi.org/10.1371/journal.ppat.1002873.