Impact of Waddlia chondrophila infection on pregnancy in the mouse

D. Baud¹, A. Ammerdorffer¹,², Y. Buffe¹, M. Vouga¹, G. Greub³ and M. Stojanov¹
1) Materno-fetal and Obstetrics Research Unit, Department Woman-Mother-Child, Lausanne University Hospital, Switzerland, 2) Department of Medical Microbiology and Infection Control, Laboratory of Immunogenetics, Vrije Universiteit Amsterdam, the Netherlands and 3) Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Switzerland

Abstract

The intracellular bacterium Waddlia chondrophila, which belongs to the Chlamydiales order, was found to be associated with miscarriage in humans. There is little to no knowledge regarding the mode of infection, impact on the neonate and pathophysiology of this emerging bacterium. We have previously shown that W. chondrophila induces a systemic infection, organ pathology and elicits T helper type 1-associated humoral immunity in a murine model of genital infection. In the present study, we took advantage of this model of infection to evaluate the impact of this bacterium on the mouse pregnancy. We used two routes of inoculation, vaginal and intrauterine, to introduce infection before and after mating. Our results show that genital infection by W. chondrophila did not have any significant impact on gestation length and maternal weight gain, nor on the number of offspring and their weight. This observation indicates that the mouse model of infection is not suitable to study the effect of W. chondrophila on pregnancy and alternative models of infection, including in vitro ones, should be used. Moreover, an indirect immunopathological mechanism activated by this bacterium should be further explored.

© 2019 The Author(s). Published by Elsevier Ltd.

Keywords: Adverse pregnancy outcomes, chlamydiales, genital model, mouse, Waddlia chondrophila

Original Submission: 24 April 2019; Revised Submission: 14 November 2019; Accepted: 18 November 2019
Article published online: 23 November 2019

Introduction

Waddlia chondrophila is an obligate intracellular bacterium that has previously been associated with miscarriage in humans and cattle [1,2]. Several studies performed in different geographical regions showed that women with high antibody titres against W. chondrophila were at a higher risk of miscarriage compared with control women [3,4]. Moreover, W. chondrophila was identified by quantitative PCR and immunohistochemistry in the genital tract and placenta of women with miscarriages [5]. Similar findings were identified in cattle [6]. Nevertheless, the exact mechanism of action of W. chondrophila that leads to this increased rate of miscarriage is still unknown.

Wheelhouse et al. have recently tried to imitate W. chondrophila-associated abortion in a bovine model of infection using intravenous inoculation of this bacterium into gravid cows [7]. Although the study did not confirm the role of W. chondrophila as an abortive agent, one animal showed pathogen-associated lesions in the placenta, suggesting a tropism towards reproductive organs. To study the role of W. chondrophila in miscarriages, we have previously developed a genital mouse-model of infection [8]. Intrauterine infection by W. chondrophila was able to induce a systemic infection and organ pathology, while eliciting a T helper type 1-associated humoral immunity, typical of Chlamydia-like bacteria.

Infection with W. chondrophila (later leading to persistent infection) may occur much earlier than conception. Pregnancy is characterized by a relative deficit in T helper type 1 cell-mediated immunity and so may lead to the reactivation of an induced persistent W. chondrophila infection, cell damage and miscarriage [9]. An autoimmune response to an epitope...
Mock controls). Infection was performed by injecting 100 μL mock control or W. chondrophila inoculum (~10^7 bacteria/μL). Two weeks after the introduction of the infection, two females were placed in a cage housing a male mouse. This was chosen according to our previous experiments, which demonstrated a maximum immune response and organ pathology at 2 weeks post-infection [9]. Vaginal plug assessments were completed on a daily basis and once observed, female mice were housed in separate cages to avoid cross-contamination. Blood samples (~50 μL) were collected from the tail before infection, 2 days before mating and at days 7 and 14 post-mating.

### Infection during the pregnancy: vaginal infection procedure

In total, six experiments were performed, including 26 mock controls and 26 W. chondrophila-infected mice. Mating was carried out by placing two females with one male per cage. Vaginal plug assessments to identify sexual activity were completed daily. One week after the appearance of the vaginal plug, mice were infected vaginally with 20 μL mock or W. chondrophila inoculum (~5 × 10^7 bacteria/μL) via manual restraint and infection using a pipette and sterile filter tip. No anaesthesia was used for this procedure. After infection, females were housed separately to prevent cross-contamination. Blood samples (~50 μL) were collected from the tail before infection, 2 days before mating and at days 7 and 14 post-mating.

### Microimmunofluorescence assay

All blood samples collected before and during the infection, as well as at euthanasia, were used to isolate plasma using Microvette 500 μL Lithium-Heparin (Sarstedt, Numbrecht, Germany). Samples were centrifuged for 5 min at 2000 g and plasma was collected in a separate tube and stored at −20°C until further processing. Microimmunofluorescence was used to detect anti-W. chondrophila antibodies using a formalin-inactivated (0.3%) W. chondrophila strain ATCC VR-1470 as an antigen. Serum samples were used at dilutions of 1/32 and 1/64 in phosphate-buffered saline supplemented with 1% bovine serum albumin (Serva, Heidelberg, Germany). Goat anti-mouse IgG secondary antibodies conjugated to Alexa Fluor 488 dye (Thermo Fisher Scientific, Allschwil, Switzerland; diluted 1/1000) were used to detect the presence of IgG. Microimmunofluorescence analyses were evaluated by two independent readers. Weighted Cohen’s κ coefficient was determined for each experiment to judge the agreement of the results and always ranked between ‘good’ and ‘very good’.

### Statistical analysis

GraphPad Prism 7 software (GraphPad Software Inc., San Diego, CA, USA) was used to analyse and present data. The data are expressed as mean (±standard error of the mean) unless otherwise stated.
indicated otherwise. Differences between experimental groups were tested using the Mann–Whitney U test, and a p value of <0.05 was considered statistically significant.

**Results**

**Vaginal versus intrauterine infection with *W. chondrophila***

We have previously used the intrauterine route of infection to evaluate the pathogenicity of *W. chondrophila*. Although this route is useful for pre-mating infection, it is not compatible with infection during gestation, as it would independently induce miscarriages. For this reason, pregnant mice were infected via the vaginal route.

Schematic representations of the pre- and post-mating infection procedures are depicted in Fig. 1(a). After co-habitation with male mice, female mice were evaluated daily for the presence of mating plugs. In our experimental scenario, the pregnancy rate of vaginally infected mice was 92.3% (n = 52), with no significant difference between mock and *W. chondrophila*-infected mice. The pregnancy rate was lower for intrauterine-infected mice, reaching 70.5% (n = 44). The observed difference could be explained by the difference in the invasiveness of the two routes of infection, as the catheter used for the intrauterine inoculation might cause damage to the reproductive organs. This reasoning is supported by the fact that mice failing to become pregnant were equally distributed between the mock-infected (n = 6) and *W. chondrophila*-infected (n = 7) groups.

**Impact of pre- and post-mating infection with *W. chondrophila***

Similar serological results were seen at delivery for the two different routes of inoculation (Fig. 2(a)). We avoided blood sampling during gestation to minimize the stress on the pregnant mice. All mock-infected mice had a negative serology. Exposure to *W. chondrophila* did not affect the duration of the gestation compared with mock controls for both routes of infection (Fig. 2(b)). There was a significant difference in length of gestation between the two routes of infection, which could be again explained by the increased invasiveness of the intrauterine infection procedure, as mentioned above. The weight gain of the pregnant mice was similar for both mock-infected and infected mice infected via the intrauterine route (Fig. 2(c)). Moreover, infection by *W. chondrophila* did not affect the average number of pups per pregnant mouse (Fig. 2(d)), nor the average weight of pups from the same litter (Fig. 2(d)), regardless of the route of infection.

**Discussion**

*Waddlia chondrophila* has been linked to adverse pregnancy outcomes in humans and bovines [2,6]. To study the abortive role of this bacterium, we have previously developed a female mouse model of genital infection in which intrauterine inoculation of the bacterium caused a strong immune response and disseminated infection [8]. The present study aimed at evaluating the impact of *W. chondrophila* infection on reproductive success using the above-mentioned murine model of infection. We used the intrauterine route of inoculation to study the impact of infection before mating and the vaginal route to study the influence of post-mating infection. The latter was used to avoid the potential higher risk of abortion induced by the technical aspects of intrauterine inoculation in already pregnant mice. The intrauterine route of infection was used before mating to increase the number of bacteria reaching the uterine cavity, which we previously demonstrated to induce a
significant immune response and local, as well as distant pathology 2 weeks post-infection [8].

Our results indicate that in our murine model of genital infection, *W. chondrophila* does not result in adverse pregnancy outcomes despite eliciting an immune response. Both routes of inoculation produced a strong immune response that peaked between days 14 and 21 post-infection. The infection did not impact any of the outcomes presented in the study, including the litter size and weight of the pups, duration of the gestation and maternal weight gain.

The fact that *W. chondrophila* was isolated from human and bovine samples, but never in rodents, may indicate that the mouse model of infection is not suitable to study the impact of *W. chondrophila* on gestation. There are some similarities with *Chlamydia trachomatis*, which also does not induce pathologies in a mouse model of genital infection. When inoculated vaginally or into the uterus, the bacterium is quickly cleared by the mouse immune system [13]. Conversely, this infection is closely related to *Chlamydia muridarum*, the murine counterpart of *C. trachomatis*, which causes similar obstetric issues as *C. trachomatis* in humans [14].

Recently, a bovine model of infection has been used to address this issue [7]. Although the infection did not produce a significant increase in miscarriage, in one heifer (out of nine),...
W. chondrophila was successfully re-isolated from the diseased placenta. Although this suggests that there may be a tropism towards the reproductive tract, it also indicates that the bacterium is not systematically associated with adverse pregnancy outcomes.

In conclusion, infection with W. chondrophila was not associated with an increased rate of miscarriage in our mouse model of genital infection. Given the strong serological link in humans and the genital tropism of this bacterium, future research should focus more on immunopathological mechanisms as direct infection by W. chondrophila might not be the main cause of adverse pregnancy outcomes.

**Conflicts of interest**

The authors have no conflicts of interest to declare.

**Author contributions**

MS, AA and DB conceived the experimental design. AA, YB, MV and MS performed the experiments. MS, AA, YB, MV and DB interpreted the data. MS and DB prepared the figures and wrote the manuscript. GG reviewed the manuscript. All authors read and approved the final manuscript.

**Funding**

This work was supported by the Department of Obstetrics and Gynaecology, Lausanne University Hospital, Switzerland and by the Swiss National Science Foundation grant numbers 310030-156169/1, 320030-169853/1 and 320030-169853/2 attributed to David Baud. David Baud is also supported by the Fondation Leenaards through the Bourse pour la relève académique, by the Fondation Divesa and by the Loterie Romande. No conflicts of interest to declare.

**References**

[1] Ammerdorffer A, Stojanov M, Greub G, Baud D. Chlamydia trachomatis and chlamydia-like bacteria: new enemies of human pregnancies. Curr Opin Infect Dis 2017;30:289–96.

[2] Baud D, Greub G. Intracellular bacteria and adverse pregnancy outcomes. Clin Microbiol Infect 2011;17:1312–22.

[3] Baud D, Thomas V, Arafu A, Regan L, Greub G. Waddlia chondrophila, a potential agent of human fetal death. Emerg Infect Dis 2007;13:1239.

[4] Hornung S, Thuong BC, Gyger J, Kebbi-Beghdadi C, Vasilevsky S, Greub G, et al. Role of Chlamydia trachomatis and emerging Chlamydia-related bacteria in ectopic pregnancy in Vietnam. Epidemiol Infect 2015;143:2635–8.

[5] Baud D, Goy G, Osterheld M-C, Croxatto A, Borel N, Vial Y, et al. Role of Waddlia chondrophila placental infection in miscarriage. Emerg Infect Dis 2014;20:460–4.

[6] Dilbeck-Robertson P, McAllister MM, Bradway D, Evermann JF. Results of a new serologic test suggest an association of Waddlia chondrophila with bovine abortion. J Vet Diagn Invest 2003;15:568–9.

[7] Wheelhouse N, Flockhart A, Ritchison K, Livingstone M, Finlayson J, Flachon V, et al. Experimental challenge of pregnant cattle with the putative abortifacient Waddlia chondrophila, Sci Rep 2016;6.

[8] Vasilevsky S, Gyger J, Piersigilli A, Pilloux L, Greub G, Stojanov M, et al. Waddlia chondrophila induces systemic infection, organ pathology, and elicits Th1-associated humoral immunity in a murine model of genital infection. Front Cell Infect Microbiol 2015;5:76.

[9] Baud D, Regan L, Greub G. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. Curr Opin Infect Dis 2008;21:70–6.

[10] Baud D, Vulliemoz N, Ammerdorffer A, Gyger J, Greub G, Castella V, et al. Waddlia chondrophila, a Chlamydia-related bacterium, has a negative impact on human spermatozoa. Hum Reprod 2017;1–8.

[11] Goy G, Croxatto A, Posfay-Barbe KM, Gervaix A, Greub G. Development of a real-time PCR for the specific detection of Waddlia chondrophila in clinical samples. Eur J Clin Microbiol Infect Dis 2009;28:1483–6.

[12] Kaushic C, Ashkar AA, Reid LA, Rosenthal KL. Progesterone increases susceptibility and decreases immune responses to genital herpes infection. J Virol 2003;77:4558–65.

[13] Steurdevant GL, Caldwell HD. Innate immunity is sufficient for the clearance of Chlamydia trachomatis from the female mouse genital tract. Pathogens Dis 2014;72:70–3.

[14] Gondek DC, Olive AJ, Stary G, Starnbach MN. CD4+ T cells are necessary and sufficient to confer protection against Chlamydia trachomatis infection in the murine upper genital tract. J Immunol 2012;189:2441–9.