SHORT REPORT

Mycoplasma genitalium is not associated with adverse outcomes of pregnancy in Guinea-Bissau

A-C Labbé, E Frost, S Deslandes, A P Mendonça, A C Alves, J Pépin

Sex Transm Infect 2002;**78**:289–291

Objective: To evaluate the impact of *Mycoplasma genitalium* on the outcome of pregnancy.
Methods: Cervical samples from women who had previously participated in a case-control study (designed to assess the impact of syphilis and HIV-2 on the outcome of pregnancy in Guinea-Bissau) were processed using a PCR assay to detect the presence of *M genitalium*. Controls were women who had delivered a term neonate with a birth weight over 2500 g. Cases were classified into four groups of mothers according to the outcome of pregnancy: stillbirths, spontaneous abortions, premature deliveries, and small for gestational age (SGA) babies.
Results: Among the 1014 women included in this study, 6.2% were infected with *M genitalium*. *M genitalium* infection was not significantly associated with any of the adverse outcomes of pregnancy studied. Odds ratios (OR) for premature or SGA delivery in the presence of *M genitalium* infection were 1.37 (95% CI 0.69 to 2.60) and 0.44 (95% CI 0.01 to 2.75), respectively. For abortions and stillbirths, OR were respectively 0.61 (95% CI 0.07 to 2.51) and 1.07 (95% CI 0.42 to 2.42).
Conclusion: *M genitalium* appears not to have a deleterious impact on the outcome of pregnancy.

Mycoplasma genitalium has recently been shown to be strongly associated with non-gonococcal urethritis (NGU) in developed and developing countries, and increasing evidence supports the role of this organism in the aetiology of NGU.[1–4] This pathogen has also been detected by polymerase chain reaction (PCR) in the lower genital tract of 7–20% of women attending sexually transmitted diseases (STD) clinics.[1 5] *M genitalium* was found in seven (6.6%) of 106 women with chlamydia negative cervicitis or adnexitis but in none of 80 pregnant asymptomatic women.[6] The involvement of *M genitalium* in pelvic inflammatory disease (PID) remains unclear: it adheres to fallopian tube epithelial cells in culture[1] and produces salpingitis in animal models,[1] but more studies in women are needed.[4]

The role of *M genitalium* in maternal infections and its impact on the outcome of pregnancy has only been sparsely evaluated. The first published study conducted among pregnant women failed to detect this organism by culture and PCR in 232 samples of amniotic fluid collected at the time of caesarean delivery.[7] More recently, *M genitalium* was found in only 5/124 women who delivered prematurely and its presence in the vagina at mid-trimester was not found to be associated with subsequent spontaneous preterm birth.[8]

We have developed a modified version of Jensen's PCR method for the detection of *M genitalium*[5] to study the aetiology of urethral discharge in sub-Saharan Africa.[2 3] To elucidate the potential contribution of *M genitalium* to adverse outcomes of pregnancy, we used the same PCR assay to detect the presence of *M genitalium* in cervical secretions of women who had participated in a study initially designed to assess the impact of syphilis and HIV-2 on the outcome of pregnancy in west Africa.[9]

METHODS

From June 1997 to April 1998, we conducted an unmatched case-control study to assess the impact of syphilis and HIV-2 on pregnancy outcomes in Bissau, Guinea-Bissau. The inclusion and exclusion criteria, the demographic information pertaining to the study participants, and the laboratory methods have been previously described.[9] Briefly, women living in Bissau who gave birth or aborted at the Simao Mendes Hospital obstetrical ward were invited to participate in the study within 24 hours of delivery or abortion. Controls were women who had delivered a term neonate with a birth weight over 2500 g. Cases were classified into four groups of mothers according to the outcome of pregnancy: stillbirths, spontaneous abortions, premature deliveries, and small for gestational age (SGA) babies. Informed consent was obtained, and women were interviewed by midwives for demographic information and sexual, medical, and obstetric histories. They were examined by a physician, blood was collected for syphilis and HIV serology, and a cervical swab was obtained. Seven days later, when the mothers came back to obtain the results of the syphilis serology (and treatment when required), a vaginal swab to identify *Trichomonas vaginalis* (wet preparation) and a second cervical swab were obtained. The cervical swabs were stored at 4°C in Amplicor transport medium (Roche Diagnostic Systems) until transportation to the University of Sherbrooke, Canada, where the first and second swabs were pooled together and submitted for PCR detection of *N gonorrhoeae*.

The same pools of first and second swabs were later (after 24–28 months of storage) used for the detection of *M genitalium*, using a seminested PCR procedure adapted from Jensen *et al*.[5] The details of the protocol used for amplification and the primers' sequences have been described elsewhere.[2 3] Of the 1341 women who participated in the original study, cervical samples for PCR detection of *M genitalium* were available for the first 1014 women enrolled (June-December 1997), as the last specimens obtained were destroyed during the civil war that broke out in Bissau in June 1998. Data were entered on the EPI-INFO 6.0 package and analysed with EPI-INFO and STATA 5.0. Proportions were compared with the χ^2 test or with Fisher's tests if numbers were small. The sample size achieved was sufficient to detect a 2.3-fold increased risk of prematurity among women infected with *M genitalium* ($\beta=0.2$; $\alpha=0.05$).

RESULTS

Among the 1014 women included in this study, 6.2% were infected with *M genitalium*. The prevalence of *M genitalium* according to demographic, behavioural, clinical or laboratory characteristics is presented in table 1 for the entire group of women for whom cervical samples were obtained (controls and all categories of cases). Among women in the control

Table 1 Prevalence of *M genitalium* according to demographic, behavioural, clinical, or laboratory characteristics

	M genitalium present
Age (years)	
14–19 (n=322)	6.5%
20–24 (n=334)	7.8%
⩾25 (n=320)	4.1%
Age of sexual debut (years)	
10–15 (n=312)	8.0%
16–18 (n=399)	5.5%
⩾19 (n=130)	6.2%
Number of sexual partners (last 12 months)	
One (n=948)	6.5%
More than 1 (n=63)	3.4%
Previous pregnancies	
None (n=363)	5.8%
1–2 (n=355)	8.7%
3 or more (n=296)	3.7%
Syphilis serology	
RPR– and/or TPHA– (n=965)	6.0%
RPR+/TPHA+ (n=49)	7.9%
HIV serology	
Negative (n=916)	6.2%
HIV-1 positive (n=33)	12.1%
HIV-2 positive (n=56)	1.8%
HIV-1 and HIV-2 positive (n=6)	16.7%
N gonorrhoeae	
Negative (n=908)	6.2%
Positive (n=78)	7.7%
T vaginalis	
Negative (n=690)	7.0%
Positive (n=194)	3.6%

Total for each characteristic vary because of missing values.
p Value >0.05 for all comparisons.

group (n=600), 6.0% were infected with *M genitalium, and M genitalium* infection tended to be more common in the presence of maternal HIV-1 infection (either single or dual): 20.0% (3/15) of HIV-1 infected women were infected with *M genitalium* as opposed to 5.9% (33/555) of HIV negative women (odds ratio 3.95; 95% confidence interval 0.68–15.61; p=0.06). The prevalence of *M genitalium* infection among controls and each category of cases is shown in table 2. *M genitalium* infection was not significantly associated with any of the adverse outcomes of pregnancy studied.

DISCUSSION

We identified *M genitalium* infection in 6.2% of women who had delivered or aborted at the national reference hospital of Guinea-Bissau. To our knowledge, this study is the first to report on the prevalence of *M genitalium* among pregnant women, using cervical samples. Through our case-control design, we were able to assess the impact of such infections on various outcomes of pregnancy. We did not find a statistically significant association between *M genitalium* infection and stillbirth, abortion, prematurity, and delivery of a SGA baby. Our findings extend on those of Lu *et al* who did not find an increased risk of preterm delivery in women with vaginal *M genitalium* infection at mid-trimester.[8] Given our sample size, we can not rule out *M genitalium* having a weak effect on the outcome of pregnancy, as the upper limits of the confidence intervals for the odds ratios that we measured were between 2.5 and 3.0, but this would have little public health importance since this pathogen is found in only 6.2% of pregnant women in Bissau.

The role of other mycoplasmas (*M hominis* and *Ureaplasma urealyticum*) on the outcome of pregnancy has been studied by others,[10] with inconsistent results owing to differences in study design and failure to identify and adjust for potential confounding factors. Furthermore, the ubiquity of these organisms (*M hominis* and *U urealyticum* colonisation is found in 5–49% and 43–81% of pregnant women, respectively)[11] and the high frequency of coinfection with other STD agents make it challenging to assess their own contribution to such disorders. Overall, there is little evidence implicating *M hominis* as a cause of adverse outcomes of pregnancy[1] but the potential role of *U urealyticum* in adverse pregnancy outcomes continues to be debated.[1 10 12] As expected for most genital pathogens, an animal model of *U urealyticum* infection suggested that if this organism was involved in adverse outcomes of pregnancy, it would probably do so by invasion of the upper reproductive tract of only a subpopulation of those colonised in the lower tract.[10] This phenomenon might occur with *M genitalium* as well and could explain the absence of correlation with adverse outcomes of pregnancy in our study population since chorioamnion infection was not assessed.

We observed a trend for *M genitalium* being more frequent in HIV-1 infected mothers than among their seronegative counterparts in the control group. This association has also been observed in a smaller case-control study using PCR detection of *M genitalium* in urethral specimens of asymptomatic male patients.[13] It deserves additional studies especially since *M genitalium* has been identified recently as a potential cofactor of transmission of HIV among American discordant couples.[4 14]

In conclusion, although *M genitalium* is now considered an aetiological agent of male urethritis rather than an innocent bystander co-transmitted with a true pathogen,[1 2 4] *M genitalium* appears not to have a deleterious impact on the outcome of pregnancy. However, its role in cervicitis and PID, as well as its association with HIV, need further investigations.

ACKNOWLEDGEMENTS

This study received the financial support of the Canadian International Development Agency through its scholarship programme administered by the Canadian Bureau for International Education.

CONTRIBUTORS

ACL, JP, EF, and ACA conceived the study; ACL and APM carried out the field work while ACA was the field supervisor; SD and EF carried out laboratory analyses; ACL and JP performed the statistical analyses; ACL wrote the first draft of the manuscript, and all authors were involved in discussion of the results and assistance in writing the paper; JP supervised all steps of the project.

Table 2 Prevalence of *M genitalium* by PCR according to the outcome of pregnancy

	Positive	Negative	Odds ratio (95% CI)
Controls (n=600)	36 (6.0%)	564	1.00
Premature delivery (n=199)	16 (8.0%)	183	1.37 (0.69 to 2.60)
Stillbirths (n=125)	8 (6.4%)	117	1.07 (0.42 to 2.42)
Abortions* (n=53)	2 (3.8%)	51	0.61 (0.07 to 2.51)
SGA (n=37)	1 (2.7%)	36	0.44 (0.01 to 2.75)

SGA = small for gestational age; CI = confidence interval.
*Median gestational age at abortion was 2.5 months.

Authors' affiliations
A-C Labbé, J Pépin, Centre for International Health, University of Sherbrooke, Canada
E Frost, S Deslandes, J Pépin, Department of Microbiology and Infectious Diseases, University of Sherbrooke, Canada
A P Mendonça, A C Alves, Simao Mendes Hospital, Bissau, Guinea-Bissau

Correspondence to: Dr Annie-Claude Labbé, Département de Microbiologie, Hôpital Maisonneuve-Rosemont, 5415 boulevard l'Assomption, Montréal, Québec, Canada H1T 2M4; labbeac@yahoo.fr

Accepted for publication 11 April 2002

REFERENCES

1 **Taylor-Robinson D**, Furr PM. Update on sexually transmitted mycoplasmas. *Lancet* 1998;**351(Suppl 3)**:12–15.
2 **Pepin J**, Sobela F, Deslandes S, *et al*. Etiology of urethral discharge in West Africa: the role of Mycoplasma genitalium and Trichomonas vaginalis. *Bull World Health Organ* 2001;**79**:118–26.
3 **Morency P**, Dubois MJ, Gresenguet G, *et al*. Aetiology of urethral discharge in Bangui, Central African Republic. *Sex Transm Infect* 2001;**77**:125–9.
4 **Taylor-Robinson D**, Horner PJ. The role of Mycoplasma genitalium in non-gonococcal urethritis. *Sex Transm Infect* 2001;**77**:229–31.
5 **Jensen JS**, Uldum SA, Sondergard-Andersen J, *et al*. Polymerase chain reaction for detection of Mycoplasma genitalium in clinical samples. *J Clin Microbiol* 1991;**29**:46–50.
6 **Uno M**, Deguchi T, Komeda H, *et al*. Mycoplasma genitalium in the cervices of Japanese women. *Sex Transm Dis* 1997;**24**:284–6.
7 **Blanchard A**, Hamrick W, Duffy L, *et al*. Use of the polymerase chain reaction for detection of Mycoplasma fermentans and Mycoplasma genitalium in the urogenital tract and amniotic fluid. *Clin Infect Dis* 1993;**17(Suppl 1)**:S272–9.
8 **Lu GC**, Schwebke JR, Duffy LB, *et al*. Midtrimester vaginal Mycoplasma genitalium in women with subsequent spontaneous preterm birth. *Am J Obstet Gynecol* 2001;**185**:163–5.
9 **Labbé AC**, Mendonca AP, Alves C, *et al*. The impact of syphilis, HIV-1 and HIV-2 on the outcome of pregnancy in Bissau, Guinea-Bissau. *Sex Transm Dis* 2002; **29**:157–67.
10 **Cassell GH**. Ureaplasma Infection. In: Hitchcock PJ, MacKay HT, Wasserheit JN, *et al*, eds. *Sexually transmitted diseases and adverse outcomes of pregnancy*. Washington DC: American Society for Microbiology, 1999.
11 **Luki N**, Lebel P, Boucher M, *et al*. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. *Eur J Clin Microbiol Infect Dis* 1998;**17**:255–63.
12 **Hillier SL**, Martius J, Krohn M, *et al*. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. *N Engl J Med* 1988;**319**:972–8.
13 **Martinelli F**, Garrafa E, Turano A, *et al*. Increased frequency of detection of Ureaplasma urealyticum and Mycoplasma genitalium in AIDS patients without urethral symptoms. *J Clin Microbiol* 1999;**37**:2042–4.
14 **Perez G**, Skurnick JH, Denny TN, *et al*. Herpes simplex type II and Mycoplasma genitalium as risk factors for heterosexual HIV transmission: report from the heterosexual HIV transmission study. *Int J Infect Dis* 1998;**3**:5–11.