HHS Public Access

Author manuscript

AIDS. Author manuscript; available in PMC 2020 November 15.

Published in final edited form as:

AIDS. 2019 November 15; 33(14): 2211–2217. doi:10.1097/QAD.0000000000002335.

Mycoplasma genitalium infection among HIV-infected pregnant African women and implications for mother-to-child transmission of HIV

Alison C. Roxby, MD, MSc[1,2,3], **Krista Yuhas, MS**[2,8], **Carey Farquhar, MD, MPH**[1,2,3], **Rose Bosire, MBChB, MPH**[6], **Dorothy Mbori-Ngacha, MBChB, MMed, MPH**[7], **Barbra A. Richardson, PhD**[2,5,8], **Patricia A. Totten, PhD**[1,2], **Grace John-Stewart, MD, PhD**[1,2,3,4]

[1]Department of Medicine, University of Washington, Seattle, Washington, USA

[2]Department of Global Health, University of Washington, Seattle, Washington, USA

[3]Department of Epidemiology, University of Washington, Seattle, Washington, USA

[4]Department of Pediatrics, University of Washington, Seattle, Washington, USA

[5]Department of Biostatistics, University of Washington, Seattle, Washington, USA

[6]Centre for Public Health Research, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya

[7]UNICEF, New York, NY

[8]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA

Abstract

Objective: Many sexually transmitted infections (STIs) increase risk of mother-to-child transmission (MTCT) of HIV, but the effect of *Mycoplasma genitalium* (MG) is not known. We hypothesized that MG infection would be common among HIV-infected pregnant women and could be associated with *in utero* and intrapartum MTCT.

Design: Observational case-cohort study

Methods: This study used specimens from a Kenyan perinatal MTCT cohort (1999-2005) involving HIV-infected women and their infants, who received short-course zidovudine for prevention of MTCT. Vaginal swabs collected at 32 weeks gestation were tested for MG using a transcription-mediated amplification assay. Infant perinatal HIV infection was determined at birth and 4 weeks of age by DNA PCR. Using a case-cohort design, a random sample was generated with 3:1 control: case ratio; prevalence and correlates of MG were assessed with Chi-squared and t-tests; predictors of infant outcomes were analyzed using logistic regression.

Results: Among 220 HIV-infected pregnant women evaluated, 47 women (21.4%) had MG. Antenatal MG infection was associated with higher HIV RNA in plasma (5.0 vs. 4.6 $\log_{10}$

[*]**Corresponding Author: Alison Roxby MD, MSc**, Departments of Medicine and Global Health, University of Washington, PO Box 359909, Seattle, WA 98104. Tel: 206-543-4278 Fax: 206-543-4818 aroxby@uw.edu.

Competing Interests: Hologic (Gen-Probe) donated materials for *M. genitalium* laboratory testing, but was not involved in any other aspects of the research. P.A.T. has received remuneration for contract work, consulting, and speakers fees from Hologic and SpeeDx. No other co-authors report any competing interests to disclose.

copies/ml in MG-positive vs. MG-negative women, p=0.02) at 32 weeks. Women with MG were less likely to report prior STIs and genital ulcers (both p=0.05). There was no association found between exposure to MG and perinatal MTCT (OR=0.72, 95% CI 0.35, 1.51, p=0.39).

Conclusions: Vaginal MG infection was frequently detected among Kenyan HIV-infected pregnant women and was associated with higher plasma HIV levels, but was not associated with perinatal transmission of HIV.

Keywords

Mycoplasma genitalium; HIV/AIDS; mother-to-child transmission of HIV; perinatal HIV

Introduction:

Many sexually transmitted infections (STIs) increase risk of mother-to-child transmission (MTCT) of HIV. Despite rapid scale-up of interventions to eliminate MTCT, approximately 180,000 children acquired HIV in 2017.[1] STIs may facilitate perinatal transmission of HIV by increasing genital inflammation and HIV viral shedding; syphilis, gonorrhea (GC), and herpes simplex virus 2 (HSV-2) have all been implicated in MTCT of HIV.[2–5] *Mycoplasma genitalium* (MG), a pathogen of the genital tract of both men and women, is an emerging STI.[6] To our knowledge, the potential influence of MG in MTCT of HIV has not been investigated. MG infection is associated with increased vaginal cytokine levels and inflammation,[7] and women with a high burden of MG have been observed to have increased cervical HIV shedding,[8] providing a potential mechanism by which MG infection could lead to increased *in utero* and/or intrapartum MTCT of HIV.

MG has a wide range of presentations, from asymptomatic to severe, and if untreated, can persist for years. MG has been detected in 5%-20% of sexually active African adults[9], and is more common among adults with HIV infection.[10] Further, MG is linked to clinical illness including acute and chronic non-gonococcal urethritis in men,[11] and urethritis,[12] cervicitis,[13] tubal infertility,[14, 15] endometritis,[16] pelvic inflammatory disease[17] and salpingitis[15, 18] in women. Longitudinal data link MG to female acquisition of HIV.[19, 20] Despite the spectrum of disease caused by MG, and, in select settings, a higher prevalence of MG than GC or chlamydia (CT),[9, 21, 22] MG is not routinely screened for in Africa, nor is it effectively treated by World Health Organization (WHO) syndromic management guidelines for STIs.[23] Antenatal screening for MG is not currently recommended, and there are no FDA approved commercial tests or point-of-care tests available.

Further evidence linking MG to MTCT could provide additional impetus for screening and treatment recommendations, as well as stimulating development of novel and inexpensive diagnostics and more effective treatments. We examined a historical perinatal HIV cohort of Kenyan women to determine the prevalence and correlates of MG infection, and to determine whether antenatal MG infection is associated with increased risk of MTCT.

Methods:

Population:

This study utilized specimens collected from a cohort of HIV-infected pregnant women enrolled and followed in Nairobi, Kenya from 1999-2003 to determine immunologic correlates of MTCT. [24, 25] Pregnant women were eligible for enrollment if HIV-seropositive, less than 28 weeks gestation, and willing to participate in longitudinal follow-up for 1-2 years.

Ethics:

All women gave written informed consent in English or Kiswahili, and the study was approved by the University of Washington Institutional Review Board and the University of Nairobi/Kenyatta National Hospital Ethical Review Committee.

Clinical procedures:

Enrolled women had a pelvic exam at 32 weeks gestation; vaginal Dacron swab specimens were collected and preserved in 1 mL of freezing medium (70% RPMI 1640 with L-glutamine, 25 mM HEPES, 20% fetal bovine serum, 10% DMSO) and cryopreserved at −80°C. Women were provided with antenatal short-course zidovudine (ZDV) for prevention of MTCT, the standard of care at the time. Women were asked to deliver in a hospital facility, where infant blood was collected for HIV testing at birth; infants were seen and followed again at 4 weeks of age for HIV testing.

Laboratory methods:

One hundred microliters of the thawed cryopreserved vaginal swab specimens were subjected to testing for MG using the Aptima research-only transcription-mediated amplification (TMA) assay using primers targeting MG rRNA according to the manufacturer's directions (Hologic).[26] Since rRNA is present in multiple copies per cell, this potentially increases the sensitivity of MG detection over standard PCR techniques. Signals correspond to the hybridized target and express as relative light units (RLU), with RLU≥50,000 considered positive, RLU <5000 considered negative, and the remainder intermediate. Intermediate samples were repeated twice, if repeats remained intermediate, the specimen was reported as "indeterminate."

CD4 counts were measured in Nairobi; in Seattle, HIV RNA levels were measured on serum and genital tract specimens using methods validated for HIV subtypes prevalent in Kenya. [27] Infant HIV status was determined by HIV DNA testing, confirmed by HIV RNA testing, using a prototype HIV-1 viral load assay (Gen-Probe/Hologic).[28] STI testing included: HSV-2 IgG antibody testing by Focus ELISA, confirmed by Western blot; syphilis testing by rapid plasma reagin (BectonDickinson), confirmed by *Treponema pallidum* hemagglutination assay (Randox); *Trichomonas vaginalis* from vaginal swabs by in-pouch culture testing using the APTIMA platform (Gen-Probe/Hologic); CT and NG testing of cervical swabs with the PCR Amplicor CT/NG test (Roche); and bacterial vaginosis (BV) by Gram stain using the scoring method described by Nugent and Hillier[29].

Study Design:

The study used a case-cohort design, which creates a synthetic case-control study by randomly choosing specimens from a larger cohort, enabling cost-effective use of expensive laboratory assays.[30, 31] The primary analysis defined cases as mothers whose infants acquired HIV (with HIV DNA and RNA detected) within the first 4 weeks of life. The exposure of interest was maternal MG detected at 32 weeks gestation. Mother/infant pairs were excluded *a priori* if the gestation included twins, no infant HIV test results available, elective Caesarean section was performed, or vaginal swabs were unavailable. A random number generator was used to select eligible women from the parent cohort for MG testing. The size of the random sample was determined by multiplying the number of cases of MTCT in the cohort by 3, and adding an additional 10% in case of failed assays.[32] MG testing was also conducted on specimens from all women who transmitted HIV to infants perinatally if they were not already included in the random sample.

Statistical Methods:

In the randomly-selected cohort, prevalence and sociodemographic and immunological correlates of MG infection were analyzed among women using univariate tests including Chi-squared or Fisher's exact test for categorical variables or Student t-tests for continuous variables, and correlates were confirmed with a logistic regression model. All other MTCT cases were then added to the random cohort and logistic regression was used to model the association between MG (exposure) and infant HIV (outcome). A multivariate logistic regression model was constructed, adjusting for known cofactors of maternal HIV transmission (maternal age, plasma HIV RNA level, CD4 count, trichomoniasis, BV, syphilis, CT) and any other correlates of MG infection identified in our random cohort . Each cofactor was added stepwise to the model and included in the final model if significantly associated ($p<0.1$).

Sample size and power calculations:

The number of cases of infant HIV acquisition was fixed. Assuming 20% prevalence of MG infection among women, this study had 80% power to detect at least a 2.5-fold increased odds of MTCT among mothers with exposure to MG (2-sided alpha=0.05).

Results:

Case-cohort selection

From the original cohort of 510 women, we identified 408 mother-infant pairs eligible for the primary analysis, which included 67 early infant HIV infections. We then randomly selected 222 samples from the parent cohort. This random sample included 33 women who transmitted HIV to their infants. The remaining 34 cases of MTCT were added to the random cohort for MG testing. In total, 256 swabs were tested for MG, with 254 results.

Demographic and health data of women and infants in the cohort

MG results were obtained from 220 of the 222 randomly-selected cohort (two specimens failed the assay); baseline characteristics are detailed in Table 1. Prevalence of MG infection was high; 47 (21.4%) women had MG detected at 32 weeks gestation. The remainder tested negative; there were no intermediate results. Women had a median age of 25 years and a median of 8 years of education. The median CD4 count of women was 465 cells/mm^3 (interquartile range (IQR)=310-643 cells/mm^3) and mean plasma HIV RNA level was 4.68 log_{10} copies/ml (standard deviation (SD) = 0.89). Prior STIs were reported by 28 women (12.7%) and prior genital ulcer disease (GUD) was reported by 13 women (6%). STI testing in pregnancy revealed 7 women (3.2%) had syphilis; HSV-2 results were not available for all women, but of 137 women tested, 121 were seropositive (88%). Trichomoniasis was diagnosed in 41 women (18.6%), CT in 10 women (4.6%), GC in 2 women (<1%), and 80 women (38%) had BV on Gram stain. Among 220 randomly-selected mothers, there were 33 total perinatal infant HIV infections (15%), 16 (7.3%) detected at birth, reflecting *in utero* transmission, and 17 infants (7.7%) with no HIV detection at birth and HIV detection at 4 weeks of life, indicating intrapartum transmission.

Correlates of maternal MG infection

Correlates of maternal MG infection are summarized in Table 2. Women with MG had similar demographic characteristics (age, education, parity, sex partners) as women without MG infection, but were less likely to report a history of STIs, including GUD (p=0.05 for both). Mean plasma HIV RNA levels at 32 weeks gestation were significantly higher among MG-infected mothers, (5.0 log_{10} copies/ml for mothers with MG v. 4.6 log_{10} copies/ml for mothers without MG, p=0.02). Mothers with MG were more likely to be diagnosed with trichomoniasis while pregnant (28% vs. 16%, p=0.07). Multivariate modeling did not result in different associations.

Case-cohort analysis of MG and MTCT:

The case-cohort analysis included the random sample and all cases of infant HIV (n=254 mother-baby pairs). No association between maternal MG infection and infant HIV was seen in an unadjusted model (odds ratio (OR)=0.72 (95% confidence interval (CI) 0.36, 1.51, p=0.39) (Table 3). Similarly, in an analysis adjusting for plasma HIV RNA level and BV status, known predictors of infant HIV acquisition, MG was not associated with increased odds of infant HIV acquisition (adjusted OR 0.55 (95% CI 0.25, 1.22, p=0.14) and the model showed that maternal plasma HIV RNA level and maternal BV infection remained dominant associations with infant HIV acquisition. We also constructed unadjusted and adjusted models examining maternal MG and different timing of infant HIV acquisition, and did not observe any different associations of MG and *in utero* or intrapartum transmission. Additional multivariate models were run adjusting for significant correlates of MG infection identified in Table 2, including history of STI, history of genital ulcer disease, trichomoniasis in pregnancy, and marital status. Adjusting for these variables did not affect the odds ratios, and so these variables were not included in the final adjusted models.

Discussion

We detected MG in more than 20% of pregnant women with HIV infection in this Kenyan cohort. MG was more commonly detected than GC, CT, and trichomoniasis; only HSV-2 and BV were more prevalent in this population. The prevalence of MG was higher than most previously reported cohorts, with the exception of cohorts of African women at high risk of HIV,[9] and represents an underappreciated burden of this infection in pregnant African women with HIV. Interestingly, MG was detected more often among women who did not self-report a history of bacterial STIs or prior treatment of STIs. This is in contrast to HSV-2 and other STIs which we typically detect more often in women reporting some STI history. Women with MG were also more likely to be diagnosed with trichomoniasis while pregnant (borderline significant p=0.07). Other than trichomoniasis, women with MG had similar proportions of all other STIs detected in pregnancy compared to women without MG, which does not align with our expectation that behaviors that predisposed to MG acquisition would result in positive associations between MG and other STIs.

We did not observe an association between MG infection and early infant HIV infection, including both *in utero* or intrapartum HIV transmission. Although the study had sufficient power to detect a fairly large association (OR >2.4) between MG infection and MTCT, it lacked power to detect a smaller association. The direction of the association identified, however, with OR<1, indicates that it is unlikely that MG increases MTCT. Our failure to find a strong relationship between MG infection and MTCT is, however, reassuring, as numerous barriers exist to antenatal screening and treatment of MG. There are currently no readily available, inexpensive screening methods to detect MG in antenatal or pre-pregnancy settings.[33] We also lack cheap and effective antibiotic therapy to treat MG, especially as macrolide antibiotics are generally avoided during pregnancy.[34] Further, treatment of MG is not always successful, antibiotic resistance to both azithromycin and fluoroquinolones is becoming more prevalent,[35] and relapse rates are common.[36] It is not known whether concurrent treatment of sexual partners is needed to eradicate MG, although best practices for STIs would presume that partner treatment would be recommended.

MG is linked to acquisition of HIV in women in sub-Saharan Africa[19, 20], but it is not known whether women with MG transmit HIV more easily to male sexual partners. Our finding of high prevalence of MG in this cohort, and higher HIV plasma levels in women with MG infection, may be important for targeting efforts to prevent heterosexual HIV transmission.[37, 38] MG infection may continue to have public health significance in the fight against HIV. If, as we have shown here, women with MG have higher plasma HIV RNA levels, then it is biologically plausible that MG-infected women may be more likely to transmit HIV to partners, which would be another indication for improved diagnostic and treatment options for persons with MG. We do not have an explanation for our observation of increased HIV RNA in plasma but not cervicovaginal secretions. We expected the converse given that MG is a genital infection. However, our findings are from a small sample of women and should be evaluated in larger studies.

Our study has some limitations. The vaginal swab specimens used in this study were collected 15 years prior to MG testing, possibly leading to TMA target degradation over

time and underestimation of MG in our population. However, such degradation should not change the direction of an association. We used accepted definitions of timing of infant HIV transmission used in prior studies, however, some infants classified as intrapartum transmission may have acquired HIV from early breastfeeding in the first days of life, which would be unrelated to MG exposure. This is a known challenge in MTCT research and is not unique to this cohort. Cohort studies can suffer from longitudinal loss to follow-up, but we observed very low attrition in the first weeks of the cohort (2% by one month of life). MG status was ascertained at 28 weeks gestation, and some women may have acquired MG, or cleared MG, prior to delivery.[39] We included only HIV-seropositive women, which limits the generalizability of the findings.

Another limitation is that we lacked statistical power to detect low odds of increased MG exposure among mothers transmitting HIV perinatally. The cohort was studied when short-course ZDV monotherapy in late pregnancy was the standard of care and therefore had rates of HIV transmission to infants that are much higher than current perinatal trends; within this cohort, we have previously demonstrated statistically significant associations with MTCT and both HSV-2 and BV[5, 40]. We therefore present evidence that a relationship between MG and MTCT is weaker than the relationship seen with these known cofactors, and the relationship seen was in the direction of no risk (OR <1). While our lack of power is a limitation of this data, it is unlikely that prospective studies done in the current era of low MTCT rates could uncover a more precise or smaller association without requiring a prohibitively large cohort.

In conclusion, we detected a high prevalence of MG in pregnant HIV-infected Kenyan women and did not detect an association between MG and MTCT. A recent technical consultation convened to study MG outlined research gaps in our understanding of the public health implications of MG infection in pregnancy. Considering the high prevalence of MG in our population of pregnant women, we concur with this expert panel that additional research to determine the long term effects of maternal MG and the best treatment options in pregnancy should be a research priority.[41]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors gratefully acknowledge the participation of the women and children in the study.

Study design: ACR, KY, BAR, GJS

Parent cohort data collection: CF, RB, DMN, BAR, GJS

Laboratory testing: PAT

Data analysis: ACR, KY, BAR, GJS

Manuscript writing: ACR, KY, GJS

Manuscript review and revisions: ACR, KY, CF, RB, DMN, BAR, PAT, GJS

Funding: This research was funded by a University of Washington Institute for Translational Health Sciences Small Pilot Award (UL1 TR002319 and UL1TR000423) to A.C.R., supported by the National Center for Advancing Translational Sciences of the National Institutes of Health (NIH), and the following NIH research grants: K23 HD071788-01A1 to A.C.R., K24 AI087399 to C.F., R01 HD 23412 and K24 HD 054314 to G.J.S., and the University of Washington/Fred Hutch Center for AIDS Research, an NIH-funded program (AI027757) supported by the following NIH Institutes and Centers: NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, NIDDK. The funders had no role in study design, data collection and analysis, or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. UNAIDS. UNAIDS Data 2017. In. Geneva; 2017.
2. Cowan FM, Humphrey JH, Ntozini R, Mutasa K, Morrow R, Iliff P. Maternal Herpes simplex virus type 2 infection, syphilis and risk of intra-partum transmission of HIV-1: results of a case control study. AIDS 2008; 22(2):193–201. [PubMed: 18097221]
3. Bollen LJ, Whitehead SJ, Mock PA, Leelawiwat W, Asavapiriyanont S, Chalermchockchareonkit A, et al. Maternal herpes simplex virus type 2 coinfection increases the risk of perinatal HIV transmission: possibility to further decrease transmission? AIDS 2008; 22(10):1169–1176. [PubMed: 18525263]
4. Fawzi W, Msamanga G, Renjifo B, Spiegelman D, Urassa E, Hashemi L, et al. Predictors of intrauterine and intrapartum transmission of HIV-1 among Tanzanian women. AIDS 2001; 15(9): 1157–1165. [PubMed: 11416718]
5. Drake AL, John-Stewart GC, Wald A, Mbori-Ngacha DA, Bosire R, Wamalwa DC, et al. Herpes simplex virus type 2 and risk of intrapartum human immunodeficiency virus transmission. Obstet Gynecol 2007; 109(2 Pt 1):403–409. [PubMed: 17267842]
6. Anagrius C, Lore B, Jensen JS. Mycoplasma genitalium: prevalence, clinical significance, and transmission. Sex Transm Infect 2005; 81(6):458–462. [PubMed: 16326846]
7. McGowin CL, Annan RS, Quayle AJ, Greene SJ, Ma L, Mancuso MM, et al. Persistent Mycoplasma genitalium infection of human endocervical epithelial cells elicits chronic inflammatory cytokine secretion. Infect Immun 2012; 80(11):3842–3849. [PubMed: 22907815]
8. Manhart LE, Mostad SB, Baeten JM, Astete SG, Mandaliya K, Totten PA. High Mycoplasma genitalium organism burden is associated with shedding of HIV-1 DNA from the cervix. J Infect Dis 2008; 197(5):733–736. [PubMed: 18266605]
9. Baumann L, Cina M, Egli-Gany D, Goutaki M, Halbeisen FS, Lohrer GR, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review andmeta-analysis. Sex Transm Infect 2018; 94(4):255–262. [PubMed: 29440466]
10. Cohen CR, Nosek M, Meier A, Astete SG, Iverson-Cabral S, Mugo NR, et al. Mycoplasma genitalium infection and persistence in a cohort of female sex workers in Nairobi, Kenya. Sex Transm Dis 2007; 34(5):274–279. [PubMed: 16940898]
11. Jensen JS. Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases. J Eur Acad Dermatol Venereol 2004; 18(1):1–11.
12. Manhart LE, Holmes KK, Hughes JP, Houston LS, Totten PA. Mycoplasma genitalium among young adults in the United States: an emerging sexually transmitted infection. Am J Public Health 2007; 97(6):1118–1125. [PubMed: 17463380]
13. Manhart LE, Critchlow CW, Holmes KK, Dutro SM, Eschenbach DA, Stevens CE, et al. Mucopurulent cervicitis and Mycoplasma genitalium. J Infect Dis 2003; 187(4):650–657. [PubMed: 12599082]
14. Simms I, Eastick K, Mallinson H, Thomas K, Gokhale R, Hay P, et al. Associations between Mycoplasma genitalium, Chlamydia trachomatis and pelvic inflammatory disease. J Clin Pathol 2003; 56(8):616–618. [PubMed: 12890814]
15. Clausen HF, Fedder J, Drasbek M, Nielsen PK, Toft B, Ingerslev HJ, et al. Serological investigation of Mycoplasma genitalium in infertile women. Hum Reprod 2001; 16(9):1866–1874. [PubMed: 11527890]

16. Cohen CR, Manhart LE, Bukusi EA, Astete S, Brunham RC, Holmes KK, et al. Association between Mycoplasma genitalium and acute endometritis. Lancet 2002; 359(9308):765–766. [PubMed: 11888591]
17. Haggerty CL, Totten PA, Astete SG, Ness RB. Mycoplasma genitalium among women with nongonococcal, nonchlamydial pelvic inflammatory disease. Infect Dis Obstet Gynecol 2006; 2006:30184. [PubMed: 17485798]
18. Cohen CR, Mugo NR, Astete SG, Odondo R, Manhart LE, Kiehlbauch JA, et al. Detection of Mycoplasma genitalium in women with laparoscopically diagnosed acute salpingitis. Sex Transm Infect 2005; 81(6):463–466. [PubMed: 16326847]
19. Mavedzenge SN, Van Der Pol B, Weiss HA, Kwok C, Mambo F, Chipato T, et al. The association between Mycoplasma genitalium and HIV-1 acquisition in African women. AIDS 2012; 26(5): 617–624. [PubMed: 22210630]
20. Vandepitte J, Weiss HA, Bukenya J, Kyakuwa N, Muller E, Buve A, et al. Association between Mycoplasma genitalium infection and HIV acquisition among female sex workers in Uganda: evidence from a nested case-control study. Sex Transm Infect 2014; 90(7):545–549. [PubMed: 24687129]
21. Balkus JE, Manhart LE, Jensen JS, Anzala O, Kimani J, Schwebke J, et al. Mycoplasma genitalium infection in Kenyan and US women. Sex Transm Dis 2018.
22. Birger R, Saunders J, Estcourt C, Sutton AJ, Mercer CH, Roberts T, et al. Should we screen for the sexually-transmitted infection Mycoplasma genitalium? Evidence synthesis using a transmission-dynamic model. Sci Rep 2017; 7(1):16162. [PubMed: 29170443]
23. World Health Organization. Guidelines for the Treatment of Sexually Transmitted Infections. In; 2003.
24. Farquhar C, VanCott TC, Mbori-Ngacha DA, Horani L, Bosire RK, Kreiss JK, et al. Salivary secretory leukocyte protease inhibitor is associated with reduced transmission of human immunodeficiency virus type 1 through breast milk. J Infect Dis 2002; 186(8):1173–1176. [PubMed: 12355371]
25. Farquhar C, Rowland-Jones S, Mbori-Ngacha D, Redman M, Lohman B, Slyker J, et al. Human leukocyte antigen (HLA) B*18 and protection against mother-to-child HIV type 1 transmission. AIDS Res Hum Retroviruses 2004; 20(7):692–697. [PubMed: 15307911]
26. Wroblewski JK, Manhart LE, Dickey KA, Hudspeth MK, Totten PA. Comparison of transcription-mediated amplification and PCR assay results for various genital specimen types for detection of Mycoplasma genitalium. J Clin Microbiol 2006; 44(9):3306–3312. [PubMed: 16954265]
27. Panteleeff D, Emery S, Richardson B, Rousseau C, Benki S, Bodrug S, et al. Validation of Performance of the Gen-Probe Human Immunodeficiency Virus Type 1 Viral Load Assay with Genital Swabs and Breast Milk Samples. Journal of Clinical Microbiology 2002; 40(11):3929–3937. [PubMed: 12409354]
28. Panteleeff DD, John G, Nduati R, Mbori-Ngacha D, Richardson B, Kreiss J, et al. Rapid method for screening dried blood samples on filter paper for human immunodeficiency virus type 1 DNA. J Clin Microbiol 1999; 37(2):350–353. [PubMed: 9889216]
29. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991; 29(2):297–301. [PubMed: 1706728]
30. Sharp SJ, Poulaliou M, Thompson SG, White IR, Wood AM. A review of published analyses of case-cohort studies and recommendations for future reporting. PLoS One 2014; 9(6):e101176. [PubMed: 24972092]
31. Prentice RL. A Case-Cohort Design for Epidemiologic Cohort Studies and Disease Prevention Trials. Biometrika 1986; 73(1):1–11.
32. Cai J, Zeng D. Sample size/power calculation for case-cohort studies. Biometrics 2004; 60(4): 1015–1024. [PubMed: 15606422]
33. Wiesenfeld HC, Manhart LE. Mycoplasma genitalium in Women: Current Knowledge and Research Priorities for This Recently Emerged Pathogen. J Infect Dis 2017; 216(suppl_2):S389–S395. [PubMed: 28838078]

34. Bissessor M, Tabrizi SN, Twin J, Abdo H, Fairley CK, Chen MY, et al. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. Clin Infect Dis 2015; 60(8):1228–1236. [PubMed: 25537875]

35. Murray GL, Bradshaw CS, Bissessor M, Danielewski J, Garland SM, Jensen JS, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerg Infect Dis 2017; 23(5):809–812. [PubMed: 28418319]

36. Hay B, Dubbink JH, Ouburg S, Le Roy C, Pereyre S, van der Eem L, et al. Prevalence and macrolide resistance of Mycoplasma genitalium in South African women. Sex Transm Dis 2015; 42(3):140–142. [PubMed: 25668646]

37. Napierala Mavedzenge S, Muller EE, Lewis DA, Chipato T, Morrison CS, Weiss HA. Mycoplasma genitalium is associated with increased genital HIV type 1 RNA in Zimbabwean women. J Infect Dis 2015; 211(9):1388–1398. [PubMed: 25404521]

38. Gatski M, Martin DH, Theall K, Amedee A, Clark RA, Dumestre J, et al. Mycoplasma genitalium infection among HIV-positive women: prevalence, risk factors and association with vaginal shedding. Int J STD AIDS 2011; 22(3):155–159. [PubMed: 21464453]

39. Cina M, Baumann L, Egli-Gany D, Halbeisen FS, Ali H, Scott P, et al. Mycoplasma genitalium incidence, persistence, concordance between partners and progression: systematic review and meta-analysis. Sex Transm Infect 2019.

40. Farquhar C, Mbori-Ngacha D, Overbaugh J, Wamalwa D, Harris J, Bosire R, et al. Illness during pregnancy and bacterial vaginosis are associated with in-utero HIV-1 transmission. AIDS 2010; 24(1):153–155. [PubMed: 19952542]

41. Martin DH, Manhart LE, Workowski KA. Mycoplasma genitalium From Basic Science to Public Health: Summary of the Results From a National Institute of Allergy and Infectious Disesases Technical Consultation and Consensus Recommendations for Future Research Priorities. J Infect Dis 2017; 216(suppl_2):S427–S430. [PubMed: 28838075]

Table 1:

Characteristics of a random sample of pregnant, HIV-seropositive women in Nairobi, Kenya, 1999-2005.

Characteristic (n=220)	N	Median (IQR) or N (%)
Baseline (32 weeks gestation)		
Age (years)	220	24.5 (22-27)
Education (years)	218	8 (7-12)
Single	220	12 (5.5%)
Employed	220	160 (72.7%)
Age at first intercourse (years)	220	17 (16-19)
Number of lifetime sex partners	220	3 (2-4)
Parity	217	1 (1-2)
History of tuberculosis	220	16 (17.3%)
History of syphilis	220	20 (9.1%)
History of STIs	220	28 (12.7%)
History of GUD	220	13 (5.9)
HSV-2 positive	137	121 (88.3)
CD4 count (cells/mm^3)	216	465 (310-643)
HIV RNA (log_{10} copies/ml)	210	4.68 (0.89)*
Pregnancy		
Diagnosed with tuberculosis	219	2 (0.9%)
Diagnosed with syphilis	217	7 (3.2%)
Diagnosed with genital ulcer disease	219	9 (4.1%)
Diagnosed with bacterial vaginosis	209	80 (38.3%)
Diagnosed with trichomoniasis	220	41 (18.6%)
Diagnosed with gonorrhea	219	2 (0.9%)
Diagnosed with *Chlamydia*	219	10 (4.6%)
Delivery vaginal HIV RNA (log_{10} copies/ml)	150	2.11 (1.70 – 3.13)
Delivery plasma HIV RNA (log_{10} copies/ml)	169	4.15 (3.52-4.74)
M. genitalium detected at antenatal visit	220	47 (21.4%)
Infant characteristics		
All perinatal HIV: HIV detected at or before 4 weeks	220	33 (15%)
In utero transmission: HIV detected at delivery	220	16 (7.3%)
Intrapartum transmission: HIV not detected at birth, but detected at 4 weeks	220	17 (7.7%)

IQR = inter-quartile range; STI = sexually transmitted infections; GUD = genital ulcer disease; HSV-2 = herpes simplex virus 2.

Table 2:

Correlates of *Mycoplasma genitalium* infection from random sample (n=220) of HIV-seropositive pregnant women.

Characteristic	N	Women without *M. genitalium* infection (N=173) Mean (SD) or N (%)	Women with *M. genitalium* infection (N=47) Mean (SD) or N (%)	p-value*
Baseline (32 weeks gestation)				
Age (years)	220	25 (4.4)	25 (3.7)	0.70
Education (years)	218	8.8 (2.7)	8.9 (2.9)	0.86
Single	220	7 (4.1%)	5 (10.6%)	0.08
Employed	220	127 (73.4%)	33 (70.2%)	0.66
Age at first intercourse (years)	220	17.4 (2.4)	17.3 (2.5)	0.81
Lifetime sexual partners	220	3 (1.6)	3 (1.5)	0.86
Parity	217	1.5 (1.3)	1.6 (1.3)	0.69
History of tuberculosis	220	15 (8.7%)	1 (2.1%)	0.13
History of syphilis	220	18 (10.4%)	2 (4.3%)	0.19
History of any sexually transmitted infection (STI)	220	26 (15%)	2 (4.3%)	0.05
History of genital ulcer disease	220	13 (7.5%)	0	0.05
HSV-2 positive	137	91 (87.5%)	30 (91%)	0.60
CD4 count (cells/mm^3)	216	479 (236)	522 (258)	0.29
Plasma HIV RNA (log_{10} copies/ml)	210	4.6 (0.92)	5.0 (0.73)	0.02
Pregnancy				
Diagnosed with tuberculosis	219	2 (1.2%)	0	0.46
Diagnosed with syphilis	217	6 (3.5%)	1 (2.1%)	0.63
Diagnosed with genital ulcers	219	7 (4.1%)	2 (4.3%)	0.96
Diagnosed with BV	209	61 (37%)	19 (43.2%)	0.45
Diagnosed with trichomoniasis	220	28 (16.2%)	13 (27.7%)	0.07
Diagnosed with gonorrhea	219	2 (1.2%)	0	0.46
Diagnosed with chlamydia	219	8 (4.7%)	2 (4.3%)	0.91
Delivery				
Plasma HIV RNA (log_{10} copies/ml)	169	4.0 (1.0)	4.3 (0.78)	0.16
Vaginal HIV RNA (log_{10} copies/ml)	150	2.4 (1.1)	2.6 (1)	0.45

Chi squared test or Fisher's exact test were used to compare categorical variables; t-tests were used to compare continuous variables. HIV RNA testing of plasma (n=169) and vaginal swabs (n=150) was not available at delivery for all mothers; similarly HSV-2 testing was not available for all mothers (n=137).

Table 3:

Mycoplasma genitalium and odds of perinatal, *in utero*, and intrapartum mother to child transmission of HIV

Type of transmission	Characteristic	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
All perinatal HIV transmission	Maternal MG infection	0.72 (0.36 – 1.51)	0.55 (0.25 – 1.22)
	Plasma HIV RNA, $\log_{10}$ copies/ml	2.27 (1.47 – 3.50)	2.57 (1.62 – 4.06)
	Maternal BV	2.14 (1.20 – 3.81)	2.33 (1.26 – 4.34)
	Maternal CD4, cells/mm^3	0.99 (0.99 – 1.00)	-
In utero HIV transmission	Maternal MG infection	0.63 (0.20 – 1.92)	0.49 (0.15 – 1.57)
	Plasma HIV RNA, $\log_{10}$ copies/ml	1.91 (1.07 – 3.42)	2.07 (1.13 – 3.80)
	Maternal BV	3.05 (1.33 – 6.98)	3.24 (1.38 – 7.58)
	Maternal CD4, cells/mm^3	1.00 (0.99 – 1.00)	-
Intrapartum HIV transmission	Maternal MG infection	0.80 (0.33 – 1.96)	0.63 (0.26 – 1.60)
	Plasma HIV RNA, $\log_{10}$ copies/ml	2.21 (1.31 – 3.72)	2.29 (1.35 – 3.86)
	Maternal BV	1.58 (0.78 – 3.21)	1.68 (0.80 – 3.56)
	Maternal CD4, cells/mm^3	0.99 (0.99 –1.00)	-

MG = *Mycoplasma genitalium* infection; BV = bacterial vaginosis, defined as Nugent score ≥ 7; OR = odds ratio; Perinatal HIV transmission is defined as any infant with HIV detected by 4 weeks of life; *In utero* HIV transmission is defined as HIV detected at birth in infants; intrapartum HIV transmission is defined as HIV not detected at birth, and detected at 4 weeks postpartum.

HIV plasma RNA level in $\log_{10}$ copies/ml was measured at 28 weeks gestation.

Infants in whom *in utero* HIV transmission was detected at birth were excluded from the intrapartum HIV analysis.

Adjusted models include the following numbers of infants: Perinatal adjusted model, N=232, *in utero* adjusted model, N=232, intrapartum adjusted model, N=201.