Pelvic inflammatory disease (PID) is the most common serious gynaecological disorder diagnosed in women and can result in debilitating sequelae including infertility, chronic pelvic pain, and ectopic pregnancy. Neisseria gonorrhoeae and Chlamydia trachomatis, two well established PID aetiologies, have declined over the past two decades in most regions and are found less commonly in HIV seropositive women with PID. Other facultative and anaerobic bacteria have been cultured from the endometrium, fallopian tube and pelvic exudate from women with PID, but the role of these organisms as primary PID pathogens or secondary colonisers has not been well established. Nevertheless, in studies of acute salpingitis, no microbial aetiology has been identified in 20%–70% of women. The definitive association of a pathogen with salpingitis is dependent upon its detection in fallopian tube tissue. However, such specimens are difficult to obtain, and thus such studies are not frequently performed.

Mycoplasma genitalium has been detected in the female reproductive tract, but its association with disease has only recently been studied because of the difficulty in culturing this fastidious organism. Thus, although M genitalium was first cultured in 1981 from two of 13 men with non-gonococcal urethritis (NGU), the development and application of specific polymerase chain reaction (PCR) assays a decade later enabled studies to establish its association with NGU. Applying these PCR assays to studies in women, M genitalium has been detected in vaginal, cervical, and endometrial specimens, and has been associated with cervicitis, clinical PID, and histologically diagnosed endometritis in women with acute pelvic pain. While early serological investigations designed to assess an association between PID and M genitalium were inconclusive, M genitalium was shown to cause salpingitis experimentally in several non-human primate species and more recent serological studies established an association between M genitalium and tubal factor infertility. Although most of these studies suggest that M genitalium may be a significant reproductive tract pathogen in women, its ability to ascend to the female upper genital tract and cause tubal disease has not been firmly established.

In the present study M genitalium was assayed using PCR in fallopian tube, endometrial, and cervical samples from women with laparoscopically confirmed acute salpingitis. In addition, laboratory and clinical findings were measured to define factors associated with M genitalium infection in women with salpingitis.

METHODS
A prospective case-control study, defined by HIV serostatus, was utilised to investigate the aetiology of acute salpingitis. Women aged 18–40 presenting with clinically suspected PID at Kenyatta National Hospital (KNH) were screened for study participation between April 2000 and January 2003. Inclusion criteria, identical to those used for our earlier investigation, included a complaint of low abdominal pelvic pain for 2 weeks or less in addition to one or more of the following signs or symptoms: temperature >38°C, dysuria, and complaint of abnormal vaginal discharge. Women who reported pregnancy, abortion, or surgery within the past 6 weeks, or who used any antibiotic within the previous 2 weeks were excluded. Subjects were selected, examined, and underwent laparoscopy within 6 hours of hospital admission.

After giving written informed consent, study participants underwent HIV pretest counselling, a detailed questionnaire, and general and gynaecological examination. A clinical severity score (CSS) was used to assess the clinical severity of PID. During pelvic examination, a single Dacron swab was used to obtain the cervical specimen for M genitalium, N gonorrhoeae, and C trachomatis PCR testing. Blood was obtained for HIV and CD4 lymphocyte counts (HIV seropositive women only).

Abbreviations: CSS, clinical severity score; NGU, non-gonococcal urethritis; PCR, polymerase chain reaction; PID, pelvic inflammatory disease; TOA, tubo-ovarian abscess
To confirm the clinical diagnosis of acute PID and visually stage the severity of acute salpingitis, laparoscopy was performed on all participants by one of two gynaecologists trained in the laparoscopic diagnosis of acute salpingitis. At surgery, the gynaecologist was blinded to laboratory findings, including HIV serostatus. After induction of anaesthesia, and following a full surgical preparation that included application of Betadine three times to the cervix, vagina, and vulva an endometrial biopsy was obtained with a pipelle suction curette (Unimar, Inc, Wilton, CT, USA) for *M. genitalium*, *N. gonorrhoeae*, and *C. trachomatis* testing. The severity of acute salpingitis was graded as (1) mild (tubal erythema or oedema, mobile tubes, with or without spontaneous exudate), (2) moderate (marked tubal erythema and oedema, limited tubal mobility, questionable or no tubal patency, and gross exudate), and (3) severe (pyosalpinx or tubo-ovarian abscess (TOA)). Pus obtained from a pyosalpinx-TOA aspirate or collected from the peritoneal cavity, or tubal ostia biopsy if pus was not present, was tested for *M. genitalium, N. gonorrhoeae*, and *C. trachomatis* by PCR. For analysis, these specimens were treated collectively as fallopian tube specimens.

Subjects with laparoscopic evidence of acute salpingitis received treatment with cefotetan 2 g intravenously every 12 hours and doxycycline 100 mg orally every 12 hours until the CSS was reduced by 75% compared to baseline findings. If the CSS did not improve within 72 hours of antibiotic treatment, intravenous clindamycin 500 mg every 6 hours was added. At discharge, all patients were treated with an additional week of metronidazole 500 mg every 8 hours and doxycycline 100 mg every 12 hours. Women received HIV post-test counselling at time of hospital discharge. Participants were scheduled for re-evaluation at 1, 2, and 4 weeks after discharge.

All samples from the cervix, endometrium, and fallopian tubes, (including abscesses) were collected in a dry tube and 800–1000 µl of 2-SP buffer (0.2 M sucrose in phosphate buffer, pH 7.5) were added, and frozen at −20°C until tested for *N. gonorrhoeae* and *C. trachomatis* by PCR (Ampli-) Roche Diagnostic Systems, Branchburg, NJ, USA) in Kenya. Subsequently, the frozen specimens were shipped to Seattle and analysed for *M. genitalium* using the MgPA-IMW PCR assay after purification of DNA using the MasterPure DNA purification kit (Epicentre, Madison, WI, USA). All PCR assays were performed with an equivalent amount of preprocessed sample (12.5 µl). Among the specimens tested, endometrial (33), and to a lesser extent, fallopian tube (16) specimens were more often inhibited than cervical specimens (three). However, inhibition was always eliminated by a 1:5 or 1:10 dilution of the original sample. We repeated the MgPa-IMW PCR analysis on all initially positive specimens to confirm that they were true positives and not PCR contaminants. Among one, six, and 10 specimens initially positive from the fallopian tube, endometrium, and cervix, respectively, none, one, and four did not repeat as positive and therefore were given a negative final result.

Serum was tested for HIV antibodies by ELISA (Detect HIV, BioChem ImmunoSystems, Montreal, Canada), with positive results confirmed by a second ELISA (Recombinden, Cambridge Biotech, Ireland). CD4 cells from peripheral blood were enumerated using a Facscan (Beckton-Dickinson, Baltimore, MD, USA).

Data were analysed using SPSS for Windows 11.5 (SPSS Inc, Chicago, IL, USA). Univariate analyses included Pearson’s *χ*² and Fisher’s exact tests for categorical data; Mann-Whitney test and Student’s *t* test for continuous variables.

## RESULTS

Salpingitis was laparoscopically confirmed in 142 (90%) of the 158 women enrolled with a clinical diagnosis of acute PID; 16 (10%) women had no evidence of salpingitis and were excluded from the study. Sixteen (11%) of the 142 women with salpingitis did not have specimens sufficient for *M. genitalium* PCR. Of the remaining 123 cases with specimens available for *M. genitalium* testing, 53 (43%), 35 (28%), and 36 (29%), had mild, moderate, and severe salpingitis, respectively, and 50 (40%) were diagnosed with HIV infection. Age, marital status, sexual history and microbiological findings of women who did and did not have specimens tested for *M. genitalium* did not differ significantly (data not shown). However, women who did not have specimens tested for *M. genitalium* tended to have more mild salpingitis (12 (75%) v 56 (44%), *p* = 0.06) and were less likely to be HIV infected (two (13%) v 49 (40%), *p* = 0.05) than women with specimens available.

Table 1 depicts the prevalence of *M. genitalium*, *C. trachomatis*, and *N. gonorrhoeae* detected in cervical, endometrial, and tubal specimens stratified by the severity of salpingitis. *M. genitalium* was detected in the fallopian tube specimen from
found in cervical and endometrial specimens, was rarely detected in fallopian tube samples of women with confirmed salpingitis in our sample set: (1) M genitalium may be associated with milder forms of PID, and therefore would rarely infect our study population, most of whom were hospitalised with more severe disease; (2) cervical and/or endometrial M genitalium infection or colonisation may be associated with salpingitis caused by as yet unidentified pathogens rather than ascending into the fallopian tubes itself; (3) M genitalium, similar to Ureaplasma urealyticum may not cause salpingitis, but rather may colonise the cervix and possibly the endometrium without causing significant tubal pathology, a possibility that cannot be excluded since our study did not contain a true control group for comparison; and (4) M genitalium may infect the fallopian tube in amounts that were undetectable by our PCR assay. Although the sensitivity of the M genitalium assay is similar to that of the Amplicor C trachomatis and N gonorrhoeae assays, it is possible that several freeze-thaw cycles, by the time the samples were shipped to Seattle and tested for M genitalium, contributed to decreased sensitivity.

In our previous investigation of women with outpatient PID, all 10 M genitalium infected women with histologically diagnosed endometritis complained of mild abdominal pain in comparison to 68% of those with endometritis who did not have M genitalium detected (p = 0.06). In the current study, M genitalium was not associated with severity of disease by either clinical criteria (CSS) or the well established laparoscopic scoring system. In fact, M genitalium was evenly distributed among women with mild and moderate salpingitis and was found in two women with severe disease. Nevertheless, the prevalence of M genitalium among a similar population of Kenyan women diagnosed with outpatient PID was slightly higher (16%) than we observed in the present study of hospitalised women (7%), consistent with the hypothesis that M genitalium causes less severe symptomatology.

The increased prevalence of M genitalium in HIV seropositive women in comparison with HIV seronegative women in our study was remarkably similar to that demonstrated by

### Table 2 Comparison of sociodemographic, clinical, and laboratory findings for 126 women diagnosed with acute salpingitis with and without M genitalium infection

<table>
<thead>
<tr>
<th>Sociodemographics</th>
<th>M genitalium positive (n=9)</th>
<th>M genitalium negative (n=114)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean (SD))</td>
<td>26.9 (4.9)</td>
<td>28.1 (5.5)</td>
<td>0.52*</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>3 (33%)</td>
<td>68 (60%)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>3 (33%)</td>
<td>23 (20%)</td>
<td></td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>3 (33%)</td>
<td>18 (16%)</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>0</td>
<td>4 (4%)</td>
<td>0.32†</td>
</tr>
<tr>
<td>Number of sex partners (median, range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last 3 months</td>
<td>1 (0–1)</td>
<td>1 (0–5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Last year</td>
<td>1 (0–2)</td>
<td>1 (0–10)</td>
<td>0.70†</td>
</tr>
<tr>
<td>Lifetime</td>
<td>4 (1–97)</td>
<td>3 (1–97)</td>
<td>0.39</td>
</tr>
<tr>
<td>Clinical findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical severity score (mean (SD))</td>
<td>15.6 (7.1)</td>
<td>15.6 (7.6)</td>
<td>0.88*</td>
</tr>
<tr>
<td>Laparoscopic severity of salpingitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>3 (33%)</td>
<td>50 (44%)</td>
<td>1.0*</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (44%)</td>
<td>31 (27%)</td>
<td>0.47*</td>
</tr>
<tr>
<td>Severe</td>
<td>2 (22%)</td>
<td>34 (30%)</td>
<td>1.0*</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV seropositive</td>
<td>7 (78%)</td>
<td>42 (37%)</td>
<td>0.03*</td>
</tr>
<tr>
<td>CD4 count x10^3/l (mean (SD))</td>
<td>230 (151)</td>
<td>342 (229)</td>
<td>0.23*</td>
</tr>
<tr>
<td>C trachomatis (any site)</td>
<td>1 (11%)</td>
<td>7 (6%)</td>
<td>0.48*</td>
</tr>
<tr>
<td>N gonorrhoeae (any site)</td>
<td>0</td>
<td>21 (19%)</td>
<td>0.36*</td>
</tr>
</tbody>
</table>

*Student’s t test.†Pearson’s r test. ¤Mann-Whitney test. ¶Fisher’s exact test. 
For HIV seropositive subjects only.
Key messages

(1) Mycoplasma genitalium was detected in cervical and endometrial specimens from women with laparoscopically confirmed acute salpingitis.

(2) This study represents the first detection of M genitalium from the fallopian tube.

(3) The prevalence of M genitalium in the fallopian tube among women with salpingitis was low (1% overall and 11% of all M genitalium genital tract infections), similar to that of Chlamydia trachomatis (6% overall and 38% of all C trachomatis infections), but statistically different from that of Neisseria gonorrhoeae (17% overall and 71% of N gonorrhoeae infections).

(4) M genitalium detection was positively correlated with HIV infection in women with acute salpingitis.

Irwin et al19 in a US population, and in our earlier investigation.20 In Nairobi women with outpatient PID, N gonorrhoeae and C trachomatis were more often detected in HIV seronegative women, whereas bacterial vaginosis was more often associated with PID in HIV seropositive women.21 In addition, Enterobacteriaceae and anaerobic Gram negative rods isolated from the endometrium were positively correlated with HIV infection in women with salpingitis.22 Whether HIV infection affects susceptibility to M genitalium infection and disease, or serves as a marker for high risk exposure will require further investigation.

Most evidence to date suggests that M genitalium has a role in PID and its sequelae.11–12 Based on this and our earlier investigation demonstrating an association between M genitalium and endometritis, we expected to detect this organism in fallopian tube specimens from a higher proportion of women with salpingitis. Almost 25 years ago European and American researchers were involved in similar controversy regarding C trachomatis as a cause of salpingitis.23 As a consequence, rather than thwart enthusiasm for M genitalium as a potential cause of female upper genital tract infection, these results should encourage further investigation to determine the role of M genitalium in PID, including salpingitis.

CONTRIBUTORS
CRC designed the study, supervised the collection of data and analysis, and wrote the paper; NRM supervised the field site, collection of data, and assisted with writing of the paper; SGA supervised the M genitalium assay, helped oversee the laboratory procedures in Nairobi, and assisted with writing the paper; RO performed many of the laboratory assays in Kenya, helped with quality control of the M genitalium assay, and assisted with writing of the paper; LEM helped design the protocol and assisted with writing of the paper; JAK helped supervise the field site in Nairobi, oversaw local laboratory procedures, and helped write the paper; WES helped design the protocol, and assisted with writing the paper; PGW helped supervise the Kenyan laboratory and assisted with writing the paper; PAT designed the M genitalium component of the study, helped analyse and interpret results, and co-wrote the paper.

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This study was funded by the Sexually Transmitted Disease Clinical Trial Unit (A1753329) to CRC, RO1 A148634 to PAT, and training grants T22 TW00001 for NRM and T32 AI107140 for LEM.

This study was presented at the International Society of Sexually Transmitted Disease and Research, 27–31 July 2003, Ottawa, Canada. The study protocol was approved by the institutional review board for human subjects at the University of Washington, and by the ethics review committee at Kenyatta National Hospital and the Kenya Medical Research Institute, Nairobi, Kenya. Procedures followed were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki of 1975, revised in 1983. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organisations imply endorsement by the US Government. No author on this manuscript had any conflict of interest, either financial or personal, that may have biased his or her actions.

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Detection of *Mycoplasma genitalium* in women with laparoscopically diagnosed acute salpingitis

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*Sex Transm Infect* 2005 81: 463-466
doi: 10.1136/sti.2005.015701