Chlamydia trachomatis infection in fertile and subfertile women in Rwanda: prevalence and diagnostic significance of IgG and IgA antibodies testing

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BACKGROUND: In many developing countries, little is known about the prevalence of genital Chlamydia trachomatis infections and complications, such as infertility, thus preventing any policy from being formulated regarding screening for C. trachomatis of patients at risk for infertility. The objective of the present study was to determine the prevalence of C. trachomatis and evaluate the diagnostic utility of serological markers namely anti-C. trachomatis IgG and IgA antibodies in women attending an infertility clinic.

METHODS: Serum and vaginal swab specimens of 303 women presenting with infertility to the infertility clinic of the Kigali University Teaching Hospital and 312 fertile controls who recently delivered were investigated. Two commercial species-specific ELISA were used to determine serum IgG and IgA antibodies to C. trachomatis and vaginal swabs specimens were tested by PCR. Hysterosalpingography (HSG) was performed in subfertile women.

RESULTS: The PCR prevalence of C. trachomatis infection was relatively low and did not differ significantly among subfertile and fertile women (3.3 versus 3.8%). Similarly, no significant differences in overall prevalence rates of C. trachomatis IgG and IgA among both groups were observed. The only factor associated with C. trachomatis infection in our study population was age, 25 years. The seroprevalence of IgG in both assays (86.4% for ANILabsystems and 90.9% for Virancell) was significantly higher in the group of PCR C. trachomatis-positive women compared with that of PCR-negative women. Evidence of tubal pathology identified by HSG was found in 185 patients in the subfertile group (67.8%). All the serological markers measured in this study had very low sensitivities and negative predictive values in predicting tubal pathology. The specificities for ANILabsystems IgG, Virancell IgG, Anilabsystem IgA and positive C. trachomatis DNA to predict tubal pathology were 84, 86, 95 and 98%, respectively, whereas their respective positive predictive values were 73, 76, 81 and 80%.

CONCLUSIONS: The prevalence of C. trachomatis in our study population in Rwanda appears to be low and women aged <25 years are more likely to have genital infection with C. trachomatis. Since serological testing for Chlamydia shows an excellent negative predictive value for lower genital tract infection, specific peptide-based serological assays may be of use for screening in low prevalence settings. Our data suggest that C. trachomatis is not the primary pathogen responsible for tubal pathology in Rwandan women.

Key words: chlamydia infection / prevalence / diagnostic tests / serology / subfertility

Introduction

Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium and is one of the most common bacterial sexually transmitted infections (STIs) throughout the world (Manavi, 2006). It is estimated that every year ~92 million new cases of C. trachomatis infection occur worldwide. More than two-thirds of these cases occur in the developing world, where diagnostic and treatment facilities are almost...
non-existent (Gaydos et al., 2004). The majority of urogenital infections due to C. trachomatis infections are asymptomatic (Peipert, 2003). Thus, most infected people remain undetected and untreated as they do not seek medical attention. In women, untreated infection in the lower genital tract may ascend to upper genital tract and can cause pelvic inflammatory disease (PID) with the risk of serious reproductive complications, such as tubal factor infertility and ectopic pregnancy (Peipert, 2003). An earlier study observed that risk of tubal infertility increased in direct proportion to the number of past pelvic infections: from 11% after one episode of PID, rising to 23% and 54% after two and three episodes, respectively (Westrom et al., 1999; Lepine et al., 1998; Andersen et al., 2011).

High prevalence rates of C. trachomatis and the asymptomatic course of infection have led some industrialized countries to implement screening programmes in order to reduce the rate of PID and prevent the development of reproductive sequelae (Scholtes et al., 1996; Low and Hocking, 2010). Currently, various diagnostic methods, such as isolation in cell culture, antigen detection methods and nucleic acid amplification tests (NAATs) for direct detection of C. trachomatis, are available. Of these methods, NAAT is the most attractive diagnostic tool for screening of asymptomatic individuals because of its sensitivity and specificity and its use on non-invasively collected specimens such as vaginal swabs (Johnson et al., 2002). However, NAAT requires specialized equipment and infrastructure. Thus, the diagnostic capability for direct detection of C. trachomatis in developing countries such as Rwanda is limited to a few research laboratories. Although serology cannot replace methods aiming at the direct detection of C. trachomatis, there are clinical settings in which reliable serological tests can be helpful. For instance, in women with infection of their upper genital tract in whom the pathogens are no longer detectable locally, a positive serological test may be the only indication of chlamydial involvement (Bas et al., 2001). Additionally, determination of antibodies to C. trachomatis may be useful in establishing whether a patient has had previous infection.

Chlamydia immunoglobulin (Ig) G antibodies are thought to persist for years and have been used as markers of a previous C. trachomatis infection (Ngew, 1996). Elevated levels of Ig A antibodies are considered to be markers of chronic infection and have been studied previously in the relationship between chronic Chlamydia pneumoniae infections and respiratory disease (Falck et al., 2002).

In many developing countries, little is known about the prevalence of genital C. trachomatis infections and its complications, such as infertility, thus preventing any policy from being formulated regarding screening of patients at risk for infertility. Screening tests are useful in establishing the risk for tubal pathology in an individual patient. For preventive as well as therapeutic measures, it is highly desirable to have sensitive screening markers for chlamydial infection. Therefore, it is important to understand the diagnostic value of serological testing in different clinical conditions.

Chlamydia antibody testing (CAT) has been introduced as a non-invasive screening method in fertility work-up on a large scale (Mol et al., 1997; Akande et al., 2003) after it had become evident that an association exists between Chlamydia IgG antibodies in serum and tubal pathology (Punnonen et al., 1979). Different serological assays have been developed for the detection of antibodies to C. trachomatis, including the complement fixation test, microimmunofluorescence (MIF) assay, enzyme immunoassays (EIAs) and immunoblotting tests. Although the MIF test is generally considered to be the ‘gold standard’ for serological diagnosis of chlamydial infections, interpretation of the results is problematic because this assay lacks standardization, is subjective and its specificity is questionable because of cross-reactivity with other chlamydial species (Moss et al., 1993; Gijsen et al., 2001). A variety of EIA tests have commercially become available, including several based on recombinant peptides of the C. trachomatis major outer membrane protein, which are more species specific. Moreover, the results can be objectively analysed by computer (Caldwell et al., 1981). The availability of a well-characterized and technically accessible EIA test holds promise for initial screening and large epidemiological studies of C. trachomatis infections in a developing country such as Rwanda.

The objective of the present study was to determine the prevalence of C. trachomatis and evaluate the diagnostic utility of anti-C. trachomatis IgG and IgA antibodies in women attending an infertility clinic in a developing country. Among all available EIAIs, the ANILabsystems (ANILabsystems Ltd., Vantaa, Finland) and Vircell (Vircell, Santa Fe, Spain) assays were selected because of better concordance with MIF serology in our previous study (unpublished data).

Materials and Methods

Study population and clinical procedures

The study population consisted of women seen at an infertility research clinic at the Kigali Teaching Hospital in Rwanda (the largest public hospital in Rwanda) between November 2007 and March 2010, who were originally recruited for a case–control study investigating the aetiology and risk factors of infertility and its link with HIV/STIs. Characteristics of all included women, their male partners and the materials and methods used in this study have been published previously (Dhont et al., 2010).

In brief, the National Ethics Committee of Rwanda and the Ethics Committee of the Ghent University Hospital approved the study before initiation, and all participants gave informed consent. At total of 312 female cases and 312 female controls were recruited. All women invited their male partners with a written note to participate on separate occasion. Male partners of 81% of the infertile women (254 partners) and 61% of the fertile women (189 partners) agreed to participate. Infertility was defined as inability to conceive for 1 year or more despite having regular unprotected intercourse, and included both primary and secondary infertility. Fertile controls, recruited at the level of the community, were defined as non-pregnant women who had delivered between 6 and 18 months ago.

A trained nurse interviewer administered a structured questionnaire gathering socio-demographic characteristics and information on previous gynaecological problems, including whether they ever had physician-diagnosed genital infections or specific symptoms associated with both upper and lower genital tract infections. The questionnaire also included detailed questions on acute genital tract symptoms such as vaginal discharge, pruritus, abdominal pain and dysuria. All participants received a clinical gynaecological examination by the same trained nurse and supervisor by a gynaecologist. A single venous blood sample for C. trachomatis serological testing and a swab of upper vaginal secretions for C. trachomatis PCR testing were collected from all participating women. Aliquots of serum and vaginal specimen were stored at −80°C until shipment on dry ice to the laboratory of the Ghent University Hospital.

Women in an infertile relationship also received a tubal patency test using hysterosalpingography (HSG) in most cases and a laparoscopy in...
some cases. Tubal pathology (uni or bilateral) was defined as the presence of adhesions or occlusion involving the fallopian tube and ovary and hydro-
salpinx in the absence of endometriosis.

**Laboratory methods**

Serological testing for *C. trachomatis* and PCR testing of vaginal specimens were performed in the laboratory of Ghent University Hospital, Belgium.

Frozen samples were shipped on dry ice, and after they had thawed, serum samples were tested for IgG and IgA antibodies specific to *C. trachomatis* by using two commercially available peptide-based ELISA, ANILabsystems (ANILabsystems Ltd., Vantaa, Finland) and Vircell (Vircell, Santa Fe, Spain). Both ELISAs were performed manually according to the manufacturer’s instructions. Briefly, sera were diluted 1:100 in PBS and tested in 96-well plates coated with *C. trachomatis* antigens. The plates were incubated for 30 min at 37°C in a humid chamber. The plates were washed five times with PBS. Horseradish peroxidase-
conjugated anti-IgG or IgA was then added to the wells and incubated for 30 min at 37°C. The plates were washed again five times with PBS. To each well, tetramethylbenzidine substrate was added and the plates were incubated for 15 min at room temperature. Finally, sulphuric acid was added to stop the colour reaction and optical density was read immediately at 450 nm using the BEP III Behring ELISA Processor (Siemens AG, Erlangen, Germany) spectrophotometer. The intensity of the colour is proportional to the concentration of the specific antibody in the sample. The signal-to-cut-off indices were categorized according to the manufacturer’s instructions.

Vaginal specimens were tested using the Abbott RealTime CT/NG PCR assays (Abbott, Des Plaines, IL, USA), a PCR technology with homogenous real-time fluorescence which targets the cryptic plasmid of *C. trachomatis* and the opacity gene of *Neisseria gonorrhoeae* (Marshall et al., 2007). Specimens were processed and stored in the laboratory as per the manufacturer’s instructions (Abbott).

**Statistical analysis**

Data collected during interviews and laboratory investigations were single entered and rigorously verified and cleaned using MS Access 2000 (Microsoft, Seattle, USA). SPSS version 16 for Windows programme (SPSS, Inc., Chicago, USA) was used for statistical analysis of the data.

For comparison of the EIAs to the group of *C. trachomatis*-positive patients and to detect tubal pathology, two-by-two tables were used to calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Statistical comparison was carried out by the χ² test, Fisher’s exact test and McNemar test, where appropriate. A P-value of <0.05 was considered significant.

**Results**

Of a total of 339 potentially infertile women who presented to the clinic, 312 were confirmed eligible and enrolled. Of 407 fertile women identified in the community, 352 (86%) came to the clinic and 312 were confirmed eligible and enrolled. Educational level and employment did not differ between responders and non-responders. Vaginal swabs of 312 controls and 303 infertile women were investigated. IgG results were available for 303 infertile and 312 fertile women and IgA results for 303 infertile and 122 fertile women. Women found positive for *C. trachomatis* by PCR assay (*n* = 22) represented in the infertile (*n* = 10) or fertile (*n* = 12) group. Of the 312 subfertile women enrolled, 273 had HSG examination performed. The analysis for this work will focus on the above described group of women whose ages range between 21 and 45 years old (median age of 29 years).

**Prevalence and seroprevalence rates of *Chlamydia trachomatis***

The rates of *C. trachomatis* infection as detected by PCR did not differ significantly between the fertile and infertile groups (10/308, 3.8%; 12/312, 3.3%, respectively; *P* = 0.5) (Table I). The patients with *C. trachomatis* infection were more likely to be aged 21–25 years (*P* = 0.012) and to have pruritis (*P* = 0.05). The prevalence of *C. trachomatis* infection was higher in the women with vaginal discharge (9.1%) compared with those with other symptoms (3.3%), but this did not reach statistically significance (*P* = 0.08).

No significant differences in overall prevalence rates of *C. trachomatis* IgG antibodies were found in the two assays (Table II). The overall prevalence of IgG antibodies was 18.5%...
with ANILabsystems and 18.7% (115/615) with Vircell; and no significant statistical difference between the two groups was observed. IgG was positive by both assays in 13.7% (84/615) with a good agreement by kappa value ($\kappa = 0.67$). The prevalence of *C. trachomatis* IgA antibodies was very low in the two groups. Significantly higher prevalence of *C. trachomatis* IgG ($P, 0.001$) and, to a lesser extent, IgA antibodies ($P = 0.246$) were found by the two ELISAs in the group of PCR *C. trachomatis*-positive patients compared with PCR-negative patients. Ig G antibodies were detected in 19 (86.4%) cases by ANILabsystems and in 20 (90.9%) cases by Vircell.

### Table II Prevalence of *C. trachomatis* antibodies (IgG and IgA) in the studied population of infertile and fertile women by ANILabsystems and Vircell assays.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Prevalence</th>
<th>ANILabsystems</th>
<th>%</th>
<th>Vircell</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfertility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>303</td>
<td>57</td>
<td>18.8</td>
<td>53</td>
<td>17.3</td>
</tr>
<tr>
<td>IgA</td>
<td>303</td>
<td>24</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>312</td>
<td>57</td>
<td>18.3</td>
<td>62</td>
<td>19.9</td>
</tr>
<tr>
<td>IgA</td>
<td>122</td>
<td>5</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em> DNA positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>22</td>
<td>19</td>
<td>86.4</td>
<td>20</td>
<td>90.9</td>
</tr>
<tr>
<td>IgA</td>
<td>14</td>
<td>2</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(114/615) with ANILabsystems and 18.7% (115/615) with Vircell; and no significant statistical difference between the two groups was observed. IgG was positive by both assays in 13.7% (84/615) with a good agreement by kappa value ($\kappa = 0.67$). The prevalence of *C. trachomatis* IgA antibodies was very low in the two groups. Significantly higher prevalence of *C. trachomatis* IgG ($P < 0.001$) and, to a lesser extent, IgA antibodies ($P = 0.246$) were found by the two ELISAs in the group of PCR *C. trachomatis*-positive patients compared with PCR-negative patients. Ig G antibodies were detected in 19 (86.4%) cases by ANILabsystems and in 20 (90.9%) cases by Vircell.

### Table III Comparison between the real-time PCR and IgG ELISA assays.

<table>
<thead>
<tr>
<th>Assays</th>
<th>Symptomatic</th>
<th></th>
<th></th>
<th>Asymptomatic</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCR Positive</td>
<td>Negative</td>
<td>McNemar P-value</td>
<td>$\kappa$-value</td>
<td>PCR Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>ANILabsystems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>13</td>
<td>0.02</td>
<td>0.35</td>
<td>14</td>
<td>82</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>65</td>
<td></td>
<td></td>
<td>2</td>
<td>433</td>
</tr>
<tr>
<td>Vircell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>12</td>
<td>&lt;0.001</td>
<td>0.44</td>
<td>14</td>
<td>83</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>66</td>
<td></td>
<td></td>
<td>2</td>
<td>432</td>
</tr>
</tbody>
</table>

**Correlation between the presence of *Chlamydia trachomatis* DNA and the presence of chlamydial antibodies in serum**

The degree of agreement is based on interpretation of values of the Kappa statistic as described previously in the literature, in which $0.21 \leq \kappa < 0.40$ is ‘fair’ agreement and $0.41 \leq \kappa < 0.60$ ‘moderate’ agreement. Statistical analysis by Kappa test revealed moderate agreement between the Vircell and the PCR ($\kappa = 0.44$) in symptomatic patients while both assays showed fair agreement with the PCR ($\kappa = 0.21$) in asymptomatic patients (Table III). For the results of both ELISA IgG assays and PCR for *C. trachomatis* detection, a statistically significant difference was found by McNemar test ($P < 0.05$).

In comparison to PCR as the gold standard, we determined the sensitivity, specificity, positive and negative predictive values of the serological tests for current genital infection with *C. trachomatis* (Table IV). The sensitivities of the IgG ANILabsystems and Vircell ELISA were 86 and 91%, respectively, while the specificities of both assays were 84%. While PPVs for serological assays were very low (17%), excellent negative predictive values were observed for both assays (99%). Similarly, IgA serum antibodies exhibited high specificity (93%) and negative predictive value (97%).

### Validity of serological markers in detecting tubal pathology identified by HSG

Evidence of tubal pathology identified by HSG was found in 185 patients (67.8%). For ANILabsystems IgG assay, of the 52 women with positive IgG serum antibodies, 38 had tubal pathology, whereas 147 of 221 women with negative CAT had tubal pathology. No statistically significant differences were found between the two IgG assays. All the serological markers had very low sensitivities and negative predictive values in predicting tubal pathology. Similarly, the presence of *C. trachomatis* DNA in the lower genital tract exhibited poor...
Discussion

*Chlamydia trachomatis* is one of the most prevalent sexually transmitted pathogens, and while most urogenital *C. trachomatis* infections are initially asymptomatic, they may subsequently cause considerable long-term morbidity. Chlamydial infections are primarily of great concern in women since the manifestations and consequences are more damaging to the reproductive health in women than in men (Jeffrey, 2003). Understanding the burden of *C. trachomatis* in a population is based primarily on studying the prevalence of current and past infections, using sensitive and specific laboratory techniques. It is thus important to determine its prevalence in asymptomatic groups of women with different clinical conditions.

To our knowledge, this is the first study using both the EIA and PCR assays for the detection of *C. trachomatis* in serum and genital swab specimens, respectively, in Rwanda and East Africa.

In our study population, the overall prevalence of *C. trachomatis* as determined by direct PCR examination was 3.6%. In Rwanda, no information about the prevalence is available. Our results show a relatively low prevalence of *C. trachomatis* among healthy fertile women (3.8%) as well as among infertile women (3.2%), but these values are comparable to other studies from sub-Saharan Africa (Buve et al., 2001; Nagot et al., 2004; Siemer et al., 2008). In our previous published paper on the same population (Dhont et al., 2011), secondary infertile women exhibited a higher risk sexual behaviour profile in their lifetime than primary infertile and fertile women. Surprisingly, we did not find a higher prevalence of infection among infertile couples than among fertile controls despite the higher level of reporting recent high-risk sexual behaviour. This might in part be due to the more vigorous treatment-seeking behaviour among women in infertile relationships when facing genital symptoms (Dhont et al., 2011).

PCR detection rates in our study surprisingly correlate well with those reported in asymptomatic subfertile and pregnant women in Europe and North America (Garland et al., 2000; de Barbeyrac et al., 2006). However, high detection rates of *C. trachomatis* have been reported in some other studies carried out in developing countries (Jenab et al., 2010; Santos et al., 2003; El Qouqa et al., 2009).

The performance in terms of sensitivity and negative predictive value. The specificities for ANILabsystems IgG, Vircell IgG, ANILabsystems IgA and positive *C. trachomatis* DNA to predict tubal pathology were 84, 86, 95 and 98%, respectively whereas their respective PPVs were 73, 76, 81 and 80%.

**Table IV**: Sensitivities, specificities, PPV and NPV of the two serological assays in relation to the current genital infection with of *C. trachomatis* by PCR.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>No. of samples</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANILabsystems IgG</td>
<td>Positive</td>
<td>19</td>
<td>86 (64–96)</td>
<td>84 (81–87)</td>
<td>17 (10–25)</td>
<td>99 (98–100)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3</td>
<td>91 (69–98)</td>
<td>84 (81–87)</td>
<td>17 (11–26)</td>
<td>99 (98–100)</td>
</tr>
<tr>
<td>Vircell IgG</td>
<td>Positive</td>
<td>20</td>
<td>94 (76–97)</td>
<td>84 (81–87)</td>
<td>17 (11–26)</td>
<td>99 (98–100)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>91 (69–98)</td>
<td>84 (81–87)</td>
<td>17 (11–26)</td>
<td>99 (98–100)</td>
</tr>
<tr>
<td>ANILabsystems IgA</td>
<td>Positive</td>
<td>2</td>
<td>91 (83–97)</td>
<td>93 (91–95)</td>
<td>6 (1–23)</td>
<td>97 (94–98)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>94 (76–97)</td>
<td>95 (93–97)</td>
<td>6 (1–23)</td>
<td>97 (94–98)</td>
</tr>
</tbody>
</table>

Education level, vaginal discharge, dysuria, low acute abdominal pain and infertility were not associated with genital *C. trachomatis* infection in our study. Consistent with established knowledge on *C. trachomatis* infection epidemiology (Paukku et al., 2003; Peipert, 2003), age <25 years was the single most important factor associated with *C. trachomatis* in our study population.

Chlamydia antibodies may be the only indication of past Chlamydia involvement. Our findings of an IgG seroprevalence ranging between 17.3 and 18.8% and IgG seroprevalence rates of 7.4% in subfertile women are in agreement with that reported in other populations of infertile patients (Eggert-Kruse et al., 1997; Morre et al., 2002; de Barbeyrac et al., 2006). The seroprevalence rates for both IgG assays were significantly higher in the group of PCR *C. trachomatis*-positive women. The seroprevalence rates for women confirmed to be positive for *C. trachomatis* by PCR in our study (IgG seroprevalence rate range, 86.4–90.9%, IgA seroprevalence rate of 14.3%) were similar to those from previous published studies for women confirmed to be positive for *C. trachomatis* by PCR (Clad et al., 1994; Morre et al., 2002; Bax et al., 2003). Consistent with our report, Narvanen et al. (1997) using the ANILabsystems ELISA, also found that among culture-positive women, IgG antibodies could be detected in 84.2%. In patients with a current *C. trachomatis* infection, low prevalence rates of IgA antibodies are found (Table II). Therefore, IgA antibodies do not indicate current *C. trachomatis* infection. The role of IgA antibodies in the serological diagnosis of *C. trachomatis* with the currently available assays remains negligible (Clad et al., 2000).

Correlation between the ELISA assays and the PCR assay revealed a fair-to-moderate agreement as determined by the kappa statistics and the difference was statistically significant (Table III). The relatively balanced sensitivities and specificities ranges, 86–91% and 84%, respectively, for both IgG assays in our study are inconsistent with those of other studies that have shown a poor correlation between ELISA assay and PCR assay (Rabenau et al., 2000; Joyee et al., 2007). While chlamydial serology cannot replace methods for agent detection in diagnosing these infections, the negative predictive values of both ELISA IgG assays were extremely high. Because of the high negative predictive value, the presence of *C. trachomatis* DNA in women with negative serum IgG antibodies is unlikely. In consequence, negative serological testing for Chlamydia excludes an active infection of the lower female genital tract in comparison with PCR with a high probability in our population (Table IV). However, the two ELISA assays had low PPV when compared with current infection with *C. trachomatis*, as has also been reported by others (Rabenau et al., 2000).
et al., 2000). The fact that some women (two for Vircell ELISA and three for ANILabsystems ELISA) with positive PCR had no Chlamydia IgG antibodies could be due to a very low IgG response (below the detection level) or to differences in the host ability to develop an immune response to a given antigen; it may be related to HLA class II alleles, since Zhong and Brunham have reported that the antibody responses to some C. trachomatis markers such as hsp60 and hsp70 are major histocompatibility complex linked in mice (Zhong and Brunham, 1992).

In the present study, we also tried to find whether CAT has any predictive value for tubal disease. This would be very important for early selection of women for tubal testing either by HSG or laparoscopy. Infertile women with tubal damage were more likely to be older than fertile women and women without tubal damage (Dhont et al., 2010). We observed that IgG C. trachomatis antibody positivity in subfertile women with tubal damage on HSG did not differ significantly from that of subfertile women without tubal damage (Table V). Both assays achieved a low sensitivity (20%) and a relatively good specificity (range 84–86%) for detecting serum IgG antibodies. These were within the range in a meta-analysis (Mol et al., 1997), which showed that the sensitivities and specificities of CAT varied between 21 and 90% and between 29 and 100%, respectively. There is controversy regarding the value of CAT in predicting tubal disease, with some authors suggesting that CAT can accurately predict women’s probability of tubal pathology and others believing that is of little use in the infertility work-up (den Hartog et al., 2004; Siemer et al., 2008). The differences in CAT tests, threshold levels for a positive test, reference standard and definition of tubal pathology used make comparison between different studies difficult (Land et al., 1998, 2003). In our study the limited value of CAT in predicting tubal pathology could be due to the low IgG seroprevalence in our population as compared with several other studies that found prevalences ranging from 40 to 65% among infertile women (Thomas et al., 2000; Morre et al., 2002). It appears that the C. trachomatis is not the primary pathogen responsible for tubal pathology in our population. Tubal factor infertility caused by other pathogens than C. trachomatis is found in 25–50% of cases, which limits the use of CAT on its own (Rice and Schachter, 1991).

One limitation of our study is that we used HSG to verify tubal pathology, which is not the gold standard test and suffers from possible false positive and false negative results in comparison to laparoscopy.

In this study, serum IgA antibodies exhibited slightly higher performance in terms of specificity and PPV as compared with IgG. The PPV of C. trachomatis IgA is also higher than reported PPV of C. trachomatis IgG, which ranges from 30 to 65% (Eggett-Kruse et al., 1997; Veeneman and van der Linden, 2002; Ackande et al., 2003; Land et al., 2003; Logan et al., 2003). Specific IgA antibodies have been associated with chronic inflammation and infection (Falck et al., 2002), and it has been suggested that serum IgA antibodies may be more reliable markers of persistent chlamydial infections (Saikku, 1999).

In conclusion, the prevalence of C. trachomatis in Rwanda appears to be low, and is surprisingly comparable to studies from developed countries, but it differs from reports from some developing countries where high rates prevail. Women aged <25 years are more likely to have genital infection with C. trachomatis. Thus, if it were decided in Rwanda to introduce screening for genital infections in asymptomatic women, selective screening of younger women should be considered. Since serological testing for Chlamydia shows an excellent negative predictive value for lower genital tract infection, specific peptide-based serological assays may be of use for screening in low prevalence settings. PCR could be used as a second line test for the diagnostic purposes for individuals with lower genital tract infection. In high-risk settings, screening with PCR is more efficient since infectious carriers are directly identified and a high percentage of antibody-positive individuals are not suffering from active infection. Findings of the present study are based on specimens from asymptptomatically infected women; studies addressing symptomatically infected women and women with late complications will further accurately evaluate the diagnostic utility of these peptide-based assays, which could be useful for effective prevention and intervention measures. Finally, further research is required to examine more closely the aetiological role of other STI microorganisms in tubal damage in Rwanda.

### Authors’ roles

The study was conceived and designed by C.M., N.D., M.T., G.C. and E.P. C.M. and N.D. contributed to specimen and data collection. C.M. analysed the data and drafted the article. N.D., R.V. and E.P. assisted with data analysis and interpretation of data. R.V., G.C. and E.P. revised the article critically. G.C. and E.P. provided overall supervision and critical revision of the article.

### Table V Validity of serological markers in detecting tubal pathology identified by HSG (n = 273).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>Tubal pathology</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Present</td>
<td>Absent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>ANILabsystems IgG</td>
<td>Positive</td>
<td>38</td>
<td>21 (15–27)</td>
<td>84 (74–91)</td>
<td>73 (59–84)</td>
<td>33 (27–40)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>147</td>
<td>84 (74–91)</td>
<td>73 (59–84)</td>
<td>33 (27–40)</td>
<td>84 (74–91)</td>
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<tr>
<td>Vircell IgG</td>
<td>Positive</td>
<td>37</td>
<td>20 (15–27)</td>
<td>86 (77–92)</td>
<td>76 (61–86)</td>
<td>34 (28–41)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>148</td>
<td>86 (77–92)</td>
<td>76 (61–86)</td>
<td>34 (28–41)</td>
<td>86 (77–92)</td>
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<tr>
<td>ANILabsystems IgA</td>
<td>Positive</td>
<td>18</td>
<td>9 (6–15)</td>
<td>95 (88–99)</td>
<td>81 (59–94)</td>
<td>33 (28–40)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>167</td>
<td>95 (88–99)</td>
<td>81 (59–94)</td>
<td>33 (28–40)</td>
<td>95 (88–99)</td>
</tr>
<tr>
<td>Chlamydia trachomatis DNA</td>
<td>Positive</td>
<td>8</td>
<td>4 (2–9)</td>
<td>98 (91–100)</td>
<td>80 (44–96)</td>
<td>32 (27–39)</td>
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<tr>
<td></td>
<td>Negative</td>
<td>177</td>
<td>80 (44–96)</td>
<td>32 (27–39)</td>
<td>80 (44–96)</td>
<td>32 (27–39)</td>
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</table>

<table>
<thead>
<tr>
<th>Present Absent % (95% CI)</th>
<th>% (95% CI)</th>
<th>% (95% CI)</th>
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<td>Negative</td>
<td>177</td>
<td>80 (44–96)</td>
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</table>
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