Prevalence and persistence of asymptomatic *Chlamydia trachomatis* infections in urine specimens from Danish male military recruits

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**Summary:** Danish male military recruits (n = 388) were included in a follow-up study to investigate the prevalence and persistence of asymptomatic *Chlamydia trachomatis* infections. Urine specimens were collected at enrolment and after approximately six months. *C. trachomatis* was detected by polymerase chain reaction (Amplicor, Roche). Questionnaires were filled out concerning sexual behaviour and clinical symptoms.

The prevalence of asymptomatic *C. trachomatis* in Danish male military recruits was 4.6% (18 out of 388). From five *C. trachomatis*-positive men no follow-up sample was obtained. From the remaining 13 *C. trachomatis*-positive men four (31%) were treated for *C. trachomatis* between the two visits (outside the study protocol). Of the remaining nine men, one cleared the infection and eight men (89%) had a persistent infection. The number of lifetime sexual partners was associated with the presence of *C. trachomatis* at enrolment. Although based on small numbers, this follow-up study shows, in contrast to women with asymptomatic *C. trachomatis* infections, a high percentage of *C. trachomatis* persistence in asymptotically infected males.

**Keywords:** prevalence, persistence, *C. trachomatis* infection, clearance

**INTRODUCTION**

*Chlamydia trachomatis* is the most prevalent sexually transmitted agent in the USA and Europe and has an aetiologic role in non-gonococcal urethritis in men. The complications due to *C. trachomatis* infections in men are less frequent and less severe than in women. In women, 70–80% of *C. trachomatis* infections are asymptomatic¹, whereas only 25–30% of the infections in men are asymptomatic. However, more recent studies using sensitive DNA amplification techniques show that up to 50% of *C. trachomatis* infections are asymptomatic in men¹.

The most frequent complications of an untreated *C. trachomatis*-urethritis, epididymitis and prostatitis, are reported in less than 4% of *C. trachomatis*-infected men². Eggert et al.³ found a correlation between the presence of *C. trachomatis* antibodies in males and tubal factor infertility in their female partners. The infections among men will mostly affect their female partners, leading to severe consequences such as pelvic inflammatory disease, ectopic pregnancy and tubal infertility in 8–20% of the infected women⁴. Despite the obvious importance of this microorganism, little is known about the natural course of asymptomatic *C. trachomatis* infections. This is one major issue raised by Wilson and Jungner⁵ in their 10 prerequisites for screening. Elucidation of the duration of infection is essential for disease modelling, as recently stated by Golden et al.⁶ Among women, clearance of asymptomatic *C. trachomatis* infections has been reported, but the number of women who cleared the infection differs depending on the detection techniques, such as cell culture and enzyme immunoassay. No such studies have yet been reported in men.

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Nucleic acid amplification techniques have increased the number of detected asymptomatic C. trachomatis infections by 35%. However, the clinical relevance of the additional detection of asymptomatic C. trachomatis infections is largely unknown. The use of urine specimens instead of inconvenient urethral swabs has greatly increased the participation rates in studies of asymptomatic C. trachomatis infections among men. In a follow-up study, we have investigated the prevalence and persistence of asymptomatic C. trachomatis infection in young men in relation to sexual behaviour and clinical symptoms.

MATERIALS AND METHODS

Study population and clinical specimen collection

Between September and October 1998, 388 male Danish military recruits, aged 18–29 years (93% between 19 and 22 years) were included in a follow-up study of 5–8 months. During the medical check-up at enrolment in the army, participants were requested to collect a first void urine sample. After approximately six months, a second urine specimen was collected (March–April 1999). At both examinations a self-administered questionnaire was completed providing data about age, life-time number of sexual partners, history of C. trachomatis infection, treatment for C. trachomatis in the last six months prior to inclusion, C. trachomatis treatment during follow-up, C. trachomatis treatment of partner during follow-up, and clinical symptoms or a urogenital infection (dysuria, frequent urination, urethral discharge).

All urine specimens were transported by plane at room temperature to a laboratory in The Netherlands (University Hospital Vrije Universiteit, Amsterdam) for C. trachomatis testing by polymerase chain reaction (PCR) (Amplicor, Roche). An internal control to monitor inhibition for amplification was introduced into each amplification reaction. To assess the true C. trachomatis-negative status of urine samples, those with either inhibition in the Amplicor assay, or samples that were C. trachomatis-negative while the proceeding sample was C. trachomatis-positive, were subjected to an in-house PCR using purified DNA from the original urine specimens. The in-house PCR and the DNA isolation were performed as described previously.

Statistical analyses

For statistical testing the Chi-square test or two-tailed Fisher’s exact test were used where appropriate. A P-value of P < 0.05 or less was considered statistically significant.

RESULTS

At enrolment, 18 (4.6%) out of 388 male military recruits had an asymptomatic C. trachomatis infection. After approximately six months, 322 men were included in the follow-up visit. No statistically significant difference was found between the C. trachomatis-negative men and C. trachomatis-positive men with regard to the percentage of non-responders. In five of the 18 men who were positive at enrolment no follow-up sample was obtained (Table 1). Four of the men who had cleared the infection reported that they (and their partners) had been treated for C. trachomatis outside the study protocol during the follow-up period. Of the nine remaining men who were C. trachomatis-positive at first examination, eight (89%) also had a C. trachomatis infection at the second examination, whereas only one man had cleared the infection. The incidence of chlamydial infection among 309 C. trachomatis-negative men included in the follow-up was low as only one man became C. trachomatis-positive (data not shown in Table 1).

Table 2 gives selected baseline characteristics among the 388 male military recruits. No difference according to C. trachomatis status at enrolment was found for C. trachomatis treatment in the six months prior to inclusion. The C. trachomatis-positive men reported a higher lifetime number of partners than C. trachomatis-negative men: among those testing positive, 67% reported to have had more than nine partners, whereas this only applied to 22% of the men with an asymptomatic C. trachomatis infection. The use of urine specimens instead of inconvenient urethral swabs has greatly increased the participation rates in studies of asymptomatic C. trachomatis infections among men.

C. trachomatis detection

All urine specimens were tested for C. trachomatis by PCR according to the instructions of the manufacturer (Amplicor, Roche Diagnostic Systems, Basel, Switzerland). An internal control to monitor inhibition for amplification was introduced into each amplification reaction. To assess the true C. trachomatis-negative status of urine samples, those with either inhibition in the pre-amplification reaction were subjected to an in-house PCR using purified DNA from the original urine specimens.
**DISCUSSION**

In a 5–8 months follow-up study among a low *C. trachomatis* prevalence population of male Danish military recruits we showed that a high percentage of asymptomatic *C. trachomatis* infections in men persisted. These data concerning the duration of chlamydial infections are essential for disease modelling, especially since the clinical relevance of the additionally detected asymptomatic *C. trachomatis* infections, as assessed by DNA amplification assays, is still unknown.

In this male population, 4.6% had an asymptomatic infection based on *C. trachomatis*-DNA detection by PCR in mailed urine specimens. The prevalence in the investigated population is comparable to the prevalence of 4.1% found recently by Stary *et al.*12 using PCR in asymptomatic male military recruits, although the mean age in that particular group was slightly higher (20–37 years old), and urine specimens were used directly without prior mailing to the laboratory. However, the approach of using mailed urine specimens has recently been shown by our group to be reliable8.

To date, there is no information concerning the natural course of asymptomatic *C. trachomatis* infections in men. Symptomatic *C. trachomatis* infection in men has been studied up to 56 days with a clearance rate of 15%.13 Our study is the first to examine persistence and clearance of asymptomatic *C. trachomatis* infections in males with a follow-up of approximately six months. We showed that eight out of nine (89%) of the men had a persistent *C. trachomatis* infection. In five of the men who were *C. trachomatis*-positive at enrolment no follow-up samples were obtained. Even if we hypothesize that these men all became *C. trachomatis*-negative at follow-up, the persistence rate would still be high (8/17 = 57%).

A study focusing on the detection of *C. trachomatis* in semen showed that part of the *C. trachomatis*-positive semen samples remained positive over a period of two years, suggesting persistence of *C. trachomatis* in men for long periods of time14, although reinfection in that study could not be ruled out. McCormack *et al.*15 showed in 1979 for the first time, using culture and serology, that *C. trachomatis* infections could clear spontaneously in women. They reported a clearance rate of 50% after 16–17 months.

To study the factors associated with a *C. trachomatis* infection, questionnaire information was obtained at enrolment and after approximately six months of follow-up. The lifetime number of sexual partners was associated with *C. trachomatis* positivity at enrolment. This association has also been found for asymptomatically infected Danish women.10 Neither non-specific urogenital complaints (self-reported) nor specific urogenital complaints were associated with having a *C. trachomatis* infection. Thus, these self-reported complaints were not useful for selective screening purposes and identification of potential high-risk groups.

Four men (31%), initially *C. trachomatis*-positive, reported to have received anti-chlamydial antibiotic treatment during follow-up. Several explanations for receiving chlamydial specific antibiotics are possible: 1) the men became symptomatic and contacted a physician for treatment, 2) their partners became symptomatic and the men were treated together with the women, 3) the women were asymptptomatically infected and were found to be positive during a routine medical check-up (pregnancy, insertion of an intrauterine device) and were treated together with their men. Since in men more symptomatic *C. trachomatis* infections are reported, these four cases most likely converted to a symptomatic infection for which a physician was contacted.

In conclusion, although based on small numbers, this study showed a high rate of persistence of asymptomatic *C. trachomatis* infections in men in contrast to asymptomatic *C. trachomatis* infections in women (see Morré *et al*, this supplement, pp. 12–18). Nearly a third of the initially asymptomatic *C. trachomatis* infections were treated during follow-up. However, large-scale studies are needed to provide more accurate estimates of the persistence rates of asymptomatic *C. trachomatis* infections in men.

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**Table 2. Baseline characteristics among 388 male military recruits in Denmark according to Chlamydia trachomatis (CT) status at enrolment**

<table>
<thead>
<tr>
<th>Self-reported treatment for CT within six months prior to enrolment</th>
<th>CT positive (n = 18)</th>
<th>CT negative (n = 370)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>0 (0)</td>
<td>2 (0.5)</td>
<td></td>
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**Life-time number of sexual partners**

| 0–4 | 0 (0) | 191* (52) |
| 5–9 | 6 (33) | 99 (27) |
| 10–14 | 8 (44) | 40 (11) |
| 15–19 | 3 (17) | 18 (5) |
| 20+ | 1 (6) | 22 (6) |

**Men with unspecific urological or urogenital complaints**

| Dysuria | 2 (11) | 9 (2.4) |
| Urethral discharge | – | 3 (0.8) |
| Dysuria and frequent urination | – | 2 (0.5) |

*19 persons had never had sexual intercourse
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