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Men and Women Have an Equal Oropharyngeal and Anorectal *Chlamydia trachomatis* Bacterial Load: A Comparison of 3 Anatomic Sites

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Background. The *Chlamydia trachomatis* bacterial load could have impact on transmission and sequelae. This is the first study providing comparison of *C. trachomatis* load at 3 anatomic sites estimated by cycle quantification (Cq) values.

Methods. Data from 7900 *C. trachomatis*-positive samples were included (2012–2018). Cq value was used as an inversely proportional measure for *C. trachomatis* load. Multivariable linear regression analyses assessed differences in mean Cq values.

Results. Vaginal swabs had the lowest Cq values (31.0) followed by urine (32.5), anorectal swabs (34.0), and oropharyngeal swabs (36.8) ($P < .001$). Men and women had similar oropharyngeal (36.4 vs 37.3; $P = .13$) and anorectal (34.2 vs 33.9; $P = .19$) Cq values. Men (32.2) and women (30.7) aged <25 years had lower urogenital Cq values than men (32.8) and women (31.9) aged ≥25 years ($P < .001$). HIV-positive patients had higher urogenital Cq values than HIV-negative patients (33.8 vs 32.6; $P < .03$).

Conclusions. Men and women have a similar *C. trachomatis* load at extragenital locations arguing for similar transmission potential and clinical relevance. Older patients and HIV-coinfected patients had lower *C. trachomatis* load, suggesting exposure to previous *C. trachomatis* infections potentially leading to partial immunity reducing load.

Keywords. *Chlamydia*; bacterial load; urogenital; anorectal; oropharyngeal; extragenital.

Chlamydia trachomatis is the most reported bacterial sexually transmitted infection (STI) worldwide [1]. *C. trachomatis* is associated with reproductive sequelae in women, such as pelvic inflammatory disease, ectopic pregnancy, infertility, and chronic lower abdominal pain [2].

The *C. trachomatis* bacterial load, frequently expressed as the number of bacteria/mL, could potentially affect transmission of the disease and sequelae [3, 4]. Currently, it is not clear what determines a high *C. trachomatis* load in a patient. Although symptoms may be associated with a higher *C. trachomatis* load, studies to date show a weak association [3, 5, 6]. According to a systematic review, only a few nucleic acid amplification test (NAAT)-based studies on associations with *C. trachomatis* load have been conducted [3, 5, 7–10]. Sample sizes of these studies were small and most studies had a small number of cases within categories of independent determinants [3, 5, 7–10]. The assessment of determinants for a high *C. trachomatis* load could be relevant. For example, viral STIs like human immunodeficiency virus (HIV) and herpes simplex virus have shown increased transmission potential with higher viral load [3]. Likely the transmission potential of *C. trachomatis* is also dependent on *C. trachomatis* load [3].

Extragenital infections, mainly anorectal infections, are common among men who have sex with men (MSM) and women [11]. Several studies have assessed the *C. trachomatis* load of different anatomic locations and sample types [3, 5, 7, 12, 13]. However, those studies used different methods to quantify and report *C. trachomatis* load making them less comparable. Therefore, standardization of *C. trachomatis* load measurements is needed [14]. In the current study, we compared the *C. trachomatis* load of all relevant urogenital and extragenital sites using the same methods.

Coinfections with *Neisseria gonorrhoeae* are prevalent among *C. trachomatis*-positive high-risk individuals such as MSM, young people (aged <25 years), and some ethnic groups, and these coinfections might influence *C. trachomatis* load [15–17]. According to a systematic review, only 6 studies to date have assessed the association between coinfections and *C. trachomatis* load [3]. One such study observed a higher anorectal *C. trachomatis* load among *C. trachomatis*-positive men coinfected with anorectal *N. gonorrhoeae* compared to anorectal *N. gonorrhoeae*-negative men [18]. Another study

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suggested a lower urogenital *C. trachomatis* load among women concurrently infected with *C. trachomatis* and *N. gonorrhoeae*; however, this was not statistically significant (P = .06) [19]. Associations between *C. trachomatis* load and coinfections with other STIs like HIV and syphilis were not observed and this is still unclear due to lack of research evidences [3, 12, 18, 20]. Nevertheless, coinfection with other STIs might have impact on acquiring and transmitting *C. trachomatis*.

To our knowledge, this is the first study to date providing a comparison of *C. trachomatis* load of all anatomic sites in both men and women estimated by cycle quantification (Cq) values. Our objectives were to assess whether different sample types have a different *C. trachomatis* load and whether age and coinfection with *N. gonorrhoeae*, HIV, or syphilis were associated.

METHODS

Study Population

Data of *C. trachomatis*-positive patients (aged ≥16 years) from January 2012 to June 2018 were used in this cross-sectional study. Data derived from 7224 consultations (with 1 or more *C. trachomatis*-positive samples available) originating from 6170 *C. trachomatis*-positive patients (from a total of n = 62 306 *C. trachomatis* test consultations; 11.6% *C. trachomatis* positive) were obtained from the laboratory registry of the Medical Microbiology Laboratory of Maastricht University Medical Center. All samples were tested for *C. trachomatis* with the same NAAT for both plasmid and chromosomal DNA (Cobas 4800, Roche Diagnostics), as per the manufacturer's protocol [21]. We used the NAAT-derived Cq value as a proxy for *C. trachomatis* load. The Cq value reflects the number of amplification cycles that occur before a positive *C. trachomatis* signal is detected. Thus Cq values show inverse relationships, that is a low Cq value indicated a high load and vice versa [22–24].

Patients were tested by taking samples from different anatomic locations (urogenital, anorectal, and oropharyngeal) according to testing guidelines and indications [25]. The study included data from all *C. trachomatis*-positive samples for which the Cq value could be retrieved (99.2%; 7900/7965; Figure 1). For men, data from first void urine samples (further referred to as urine samples) and urethral swabs were available. Mean Cq values from urine samples (n = 2601, mean = 32.49 [SD 3.18]) and urethral swabs (n = 10, mean = 33.47 [SD 4.89]) were similar (P = .54), therefore urethral swabs of men were excluded from the analysis. For women, urogenital data were available from urine samples, vaginal swabs, and cervical swabs. The mean Cq values from vaginal swabs (n = 3273, mean = 31.00 [SD 3.72]) and cervical swabs (n = 147, mean = 30.49 [SD 3.94]) were comparable in women (P = .11), therefore values for cervical swabs were excluded from the analysis.

Cycle Quantification Validation for *C. trachomatis* Load

In order to ensure that the Cq value can indeed be used as a proxy for *C. trachomatis* load in the different sample types, we compared the Cq values obtained from the Cobas system with previously determined quantitative in-house polymerase chain reaction (PCR) [12, 21, 26]. Absolute *C. trachomatis* load values (bacteria/mL) were available from a subset of the clinical STI clinic population: 129 urine samples and 69 anorectal swabs from men, and 403 vaginal swabs and 101 anorectal

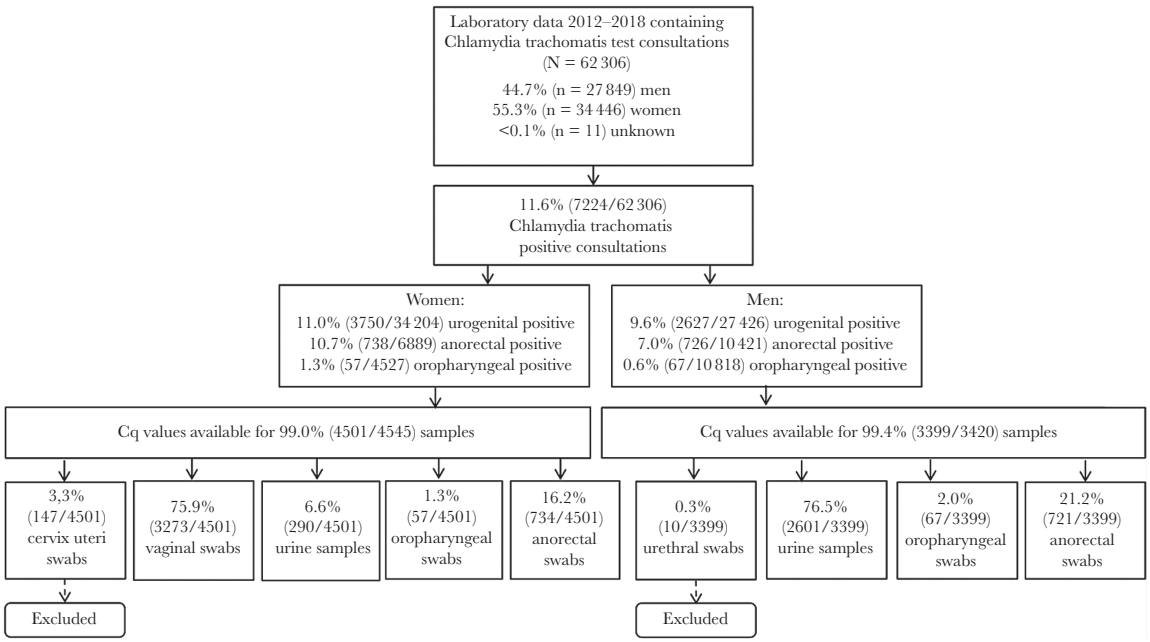

Figure 1. Flowchart, including urogenital and extragenital *Chlamydia trachomatis* samples from men and women. Abbreviation: Cq, cycle quantification.

samples from women. In brief, we quantified *C. trachomatis* load by an in-house TaqMan real-time PCR to quantify *C. trachomatis OmpA*-gene copies/mL [21]. A full description of the *C. trachomatis* load quantification has been described elsewhere [21].

For men, moderate correlation between Cq values of urine samples and urine *C. trachomatis* load (Pearson *r*, −0.61; n = 129; $P \leq .001$), and high correlation between Cq values of anorectal samples and anorectal *C. trachomatis* load (Pearson *r*, −0.93; n = 69; $P \leq .001$) were observed. For women, high correlation between Cq values of vaginal swabs and vaginal *C. trachomatis* load (Pearson *r*, −0.88; n = 403; $P \leq .001$), and high correlation between Cq values of anorectal swabs and anorectal *C. trachomatis* load (Pearson *r*, −0.96; n = 101; $P \leq .001$) were observed. In addition, Cobas Cq values are highly correlated (Pearson $R^2 > 0.98$) with known concentrations of *C. trachomatis* (inclusion forming units/mL) (personal communication, B. van der Veer, unpublished results). Therefore, we argue that Cq values are a valid proxy for *C. trachomatis* load in urogenital and extragenital samples used in this study.

Statistical Analyses

In the main analyses, the association between sample type and the outcome measure Cq values, as a proxy for *C. trachomatis* load, was assessed using univariable and multivariable linear regression analyses adjusting for sex and age (<25 years, ≥25 years). Anorectal samples were used as the reference group.

In the secondary analyses, association of sex (women, men), age (<25 years, ≥25 years), urogenital *N. gonorrhoeae* coinfection, anorectal *N. gonorrhoeae* coinfection, oropharyngeal *N. gonorrhoeae* coinfection, HIV coinfection, and a positive syphilis screening test (*Treponema pallidum* hemagglutination assay/*T. pallidum* particle agglutination assay [TPHA/TPPA]) were assessed with the *C. trachomatis* load per sample type, that is vaginal swab, urine sample, oropharyngeal swab, and anorectal swab.

In the linear regression analyses, determinants with $P < .05$ in the univariable model were included in the multivariable model. A *P* value of <.05 was considered statistically significant. Mean values, standard deviations, (adjusted) mean differences, and 95% confidence intervals (CI) were calculated. Mean differences represented the size of the associations found.

As Cq values represent values on a log scale, this is a less accessible and readily measure. To make it more accessible a "factor *C. trachomatis* load" that represents the number of times the *C. trachomatis* load was higher between studied groups was calculated for the statistically significant determinants using the formula: 2^(adjusted mean difference of Cq values). For example, the adjusted mean difference of Cq values between vaginal swabs and anorectal swabs was −2.87 Cq. Therefore, factor load was 2^2.87 = 7.3. Thus, vaginal swabs have a 7.3-times higher *C. trachomatis* load compared to anorectal swabs.

All analyses were performed using SPSS Version 24 (IBM SPSS Statistics for Windows, IBM Corporation).

Ethical Clearance

The Medical Ethics Committee of the Maastricht University Medical Center (Maastricht, the Netherlands) approved this study (METC 2017-0251) and waived the need for patient consent. Because the retrospective data originated from regular care and were analyzed anonymously, no further informed consent for data analysis was obtained.

RESULTS

C. trachomatis Load Per Sample Type, Estimated by Cycle Quantification Values

In multivariable analyses, vaginal swabs (−2.87 Cq) and urine samples (−1.33 Cq) had lower Cq values than anorectal swabs, which represented 7.3- and 2.5-times higher *C. trachomatis* load, respectively. Oropharyngeal Cq values were higher (2.73 Cq) compared to anorectal swabs, indicating a 6.6-times lower *C. trachomatis* load (Table 1).

The range of Cq values per sample type were comparable for men and women (Figure 2A). Men and women had similar urine ($P = .15$), anorectal ($P = .19$), and oropharyngeal ($P = .13$) Cq values indicative of similar *C. trachomatis* loads (Figure 2B). The mean Cq values from vaginal swabs (n = 3273, mean = 31.00 [SD 3.7]) and urine samples (n = 290, mean = 32.82 [SD 3.7]) in women were significantly different ($P \leq .001$).

Determinants Associated With *C. trachomatis* Load Stratified Per Sample Type

In multivariable analyses, women aged <25 years had lower vaginal Cq values (−0.69 Cq) compared to women aged ≥25 years, resulting in a 1.6-times higher *C. trachomatis* load in the former (Table 2). HIV-positive women had higher vaginal Cq values (6.86 Cq) compared to HIV-negative women, which represented a 116.2-times lower *C. trachomatis* load.

We additionally assessed whether or not the 3 HIV-infected women were repeatedly infected with *C. trachomatis*. All were not repeatedly infected with *C. trachomatis*. However, because the analyses included only 3 HIV-infected women this result was unreliable.

In multivariable analyses, patients aged <25 years had lower urine Cq values (−0.52 Cq) compared to patients aged ≥25 years, indicating a 1.4-times higher *C. trachomatis* load (Table 2). Patients coinfected with urogenital *N. gonorrhoeae* had higher urine Cq values (0.96 Cq) compared to urogenital *N. gonorrhoeae*-negative patients, representing a 1.9-times lower *C. trachomatis* load. HIV-positive patients had higher urine Cq values (0.95 Cq) compared to HIV-negative patients, which represented a 1.9-times lower *C. trachomatis* load.

We additionally assessed whether urogenital *N. gonorrhoeae* and HIV-coinfected patients with urine samples had repeat *C. trachomatis* infections (≥2 infections) or reported to have

Table 1. Main Analyses Including Different Sample Types and Associations With Sex and Age for *Chlamydia trachomatis* Cycle Quantification Values as a Proxy for *C. trachomatis* Load

Cq Values					
Determinants	% (n)	Mean Cq Value (SD)	Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Factor CT Load
Overall	100 (7743)				
Sample type					
Vaginal swab[a]	42.3 (3273)	31.0 (3.7)	**–3.02 (–3.24 to –2.80)**	**–2.87 (–3.13 to –2.62)[b]**	7.3
Urine sample	37.3 (2891)	32.5 (3.2)	**–1.50 (–1.74 to –1.28)**	**–1.33 (–1.58 to –1.08)[c]**	2.5
Oropharyngeal swab	1.6 (124)	36.8 (3.1)	**2.79 (2.14 to 3.44)**	**2.73 (2.08 to 3.38)**	6.6
Anorectal swab	18.8 (1455)	34.0 (3.8)	Ref	Ref	
Sex[d]					
Women	56.2 (4354)	31.6 (4.0)	**–1.24 (–1.40 to –1.07)**	0.21 (–.06 to .49)	
Men	43.8 (3389)	32.9 (3.4)	Ref	Ref	
Age[d]					
<25 y	60.3 (4666)	31.7 (3.7)	**–1.38 (–1.55 to –1.21)**	**–0.76 (–.93 to –.59)**	1.7
≥25 y	39.7 (3077)	33.1 (3.7)	Ref	Ref	

Statistically significant associations ($P < .05$) are depicted in bold.

Abbreviations: CI, confidence interval; Cq, cycle quantification; CT, *Chlamydia trachomatis*; Ref, reference.

[a]Only measured among women.

[b]When using oropharyngeal samples as the reference group the adjusted mean difference for vaginal swabs was –5.60 (95% CI, –6.26 to 4.95), factor CT load, 48.5.

[c]When using oropharyngeal samples as the reference group the adjusted mean difference for urine was –4.06 (95% CI, –4.70 to 3. 41), factor CT load, 16.7.

[d]Including all sample types, that is vaginal swabs, urine samples, oropharyngeal swabs, and anorectal swabs.

had an earlier STI (only among STI clinic patients). HIV-coinfected patients more often had repeat *C. trachomatis* infections compared to HIV-negative men (24.4% vs 6.4%; $P < .001$). Patients coinfected with HIV reported to have had an earlier STI more often compared to HIV-negative men (35.7% vs 16.0%; $P < .001$). Patients coinfected with *N. gonorrhoeae* had similar repeat *C. trachomatis* infections compared to urogenital *N. gonorrhoeae*-negative patients (11.2% vs 9.1%; $P = .69$). Patients coinfected with *N. gonorrhoeae* frequently reported to have had an earlier STI compared to *N. gonorrhoeae*-negative patients (31.7% vs 19.5%; $P = .07$).

No determinants were associated with oropharyngeal or anorectal *C. trachomatis* load in multivariable analyses (Supplementary Table 1).

DISCUSSION

This is the first and largest study to date providing a comparison of *C. trachomatis* load, estimated by Cq values, in urogenital and extragenital locations of men and women. The mean *C. trachomatis* load differed significantly per sample type and subsequently per anatomic location. Notably, no difference ($P > .05$) in *C. trachomatis* load was observed for men and

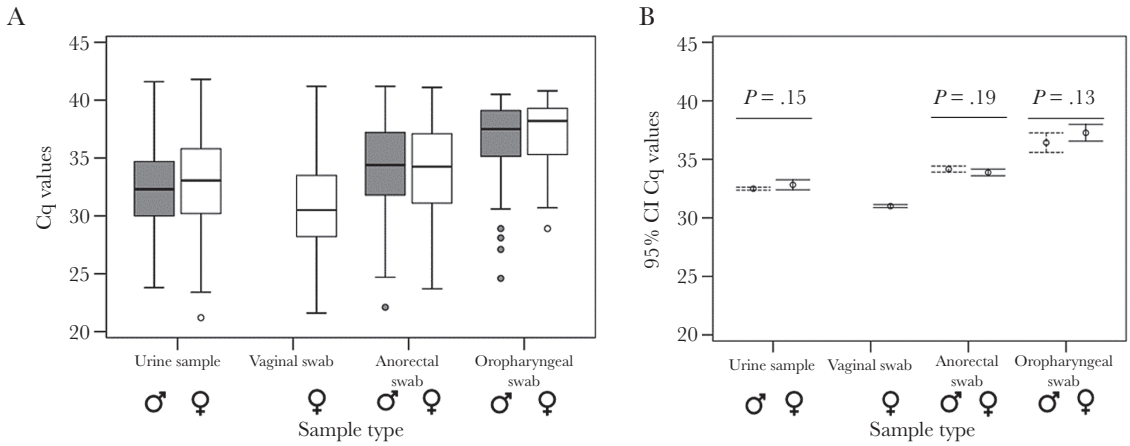

Figure 2. Boxplots and error bars showing the distribution of Cq values per sample type for men and women. *A*, Boxplots showing the distribution of Cq values as a proxy for *Chlamydia trachomatis* load per sample type for men (grey) and women (white). Middle lines are used to indicate median, boxes are used to indicate 25th and 75th percentile and whiskers indicate lowest and highest value. Outliers are indicated by circles. *B*, Mean Cq values and 95% CI (error bars) as a proxy for *C. trachomatis* load per sample type for men (dotted line) and women (solid line). Univariable regression analyses were performed to test unadjusted mean Cq value differences between men and women. Men and women had similar Cq values: urine (mean [SD] = 32.4 Cq [3.2] vs mean = 32.8 Cq [3.7]; $P = .15$), oropharyngeal (mean = 36.4 Cq [3.4] vs mean = 37.3 Cq [2.7]; $P = .13$), and anorectal (mean = 34.2 Cq [3.6] vs mean = 33.9 Cq [3.9]; $P = .19$). Abbreviations: CI, confidence interval; Cq, cycle quantification.

Table 2. Additional Analyses Including Potential Determinants Associated With Vaginal and Urine *Chlamydia trachomatis* Cycle Quantification Values as a Proxy for *C. trachomatis* Load

	Vaginal Cq Values					Urine Cq Values				
	% (n)	Mean Cq Value (SD)	Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Factor CT Load	% (n)	Mean Cq Value (SD)	Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)	Factor CT Load
Overall	100 (3273)					100 (2891)				
Sex										
Women	100 (3273)					10.0 (290)	32.8 (3.7)	0.33 (–0.06 to .72)		
Men						90.0 (2601)	32.5 (3.2)	Ref		
Age										
<25 y	75.4 (2468)	30.7 (3.6)	**–1.13 (–1.42 to –.84)**	**–0.69 (–1.00 to –.38)**	1.6	53.3 (1542)	32.2 (3.2)	**0.60 (–.83 to –.36)**	**–0.52 (–0.78 to –.27)**	1.4
≥25 y	24.6 (805)	31.9 (3.9)	Ref	Ref		46.7 (1349)	32.8 (3.3)	Ref	Ref	
Urogenital NG coinfection[a]										
Yes	2.9 (95)	31.8 (3.3)	**0.78 (.02 to 1.54)**			4.0 (116)	33.7 (3.2)	**1.20 (.60 to 1.81)**	**0.96 (0.32 to 1.59)**	1.9
No	95.0 (3108)	31.0 (3.7)	Ref			92.5 (2675)	32.5 (3.2)	Ref	Ref	
Anorectal NG coinfection[b]										
Yes	0.7 (22)	33.9 (2.2)	**2.28 (.71 to 3.85)**	1.36 (–0.33 to 3.04)		1.6 (45)	34.4 (3.5)	**1.27 (.26 to 2.28)**	0.87 (–0.34 to 2.09)	
No	22.3 (731)	31.6 (3.8)	Ref	Ref		10.8 (311)	33.1 (3.4)	Ref	Ref	
Oropharyngeal NG coinfection[c]										
Yes	0.5 (18)	33.9 (3.4)	1.04 (–0.71 to 2.80)	0.31 (–1.57 to 2.19)		0.9 (27)	34.0 (3.0)	0.98 (–0.29 to 2.24)	0.01 (–1.45 to 1.48)	
No	8.4 (275)	32.8 (3.8)	Ref	Ref		12.6 (364)	33.0 (3.4)	Ref	Ref	
HIV coinfection[d,e]										
Yes	0.1 (3)	37.8 (0.25)	**6.45 (2.26 to 10.64)**	**6.86 (2.72 to 10.99)**	116.2	1.4 (41)	33.8 (3.1)	**1.23 (.23 to 2.23)**	**0.95 (0.07 to 1.96)**	1.9
No	41.0 (1389)	31.4 (3.8)	Ref	Ref		57.5 (1663)	32.6 (3.3)	Ref	Ref	
TPHA/TPPA positive (syphilis screening test)[f]										
Yes	0.1 (4)	31.6 (15)	0.74 (–2.92 to 4.40)			0.7 (21)	33.3 (3.2)	0.48 (–0.92 to 1.88)	–0.19 (–1.62 to 1.24)	
No	26.3 (860)	30.9 (3.9)	Ref			32.8 (949)	32.8 (3.3)	Ref	Ref	

Statistically significant associations ($P < .05$) are depicted in bold.

Abbreviations: CI, confidence interval; Cq, cycle quantification; CT, *Chlamydia trachomatis*; HIV, human immunodeficiency virus; NG, *Neisseria gonorrhoeae*; Ref, reference; TPHA, *Treponema pallidum* hemagglutination assay; TPPA, *Treponema pallidum* particle agglutination assay.

[a]n = 70 patients with vaginal samples and n = 100 patients with urine samples were not tested for urogenital NG.

[b]n = 2520 patients with vaginal samples and n = 2535 patients with urine samples were not tested for anorectal *N. gonorrhoeae*.

[c]n = 2980 patients with vaginal samples and n = 2500 patients with urine samples were not tested for oropharyngeal *N. gonorrhoeae*.

[d]n = 1878 patients with vaginal samples and n = 1178 patients with urine samples were not tested for HIV.

[e]HIV result was missing for n = 3 patients with vaginal swabs and n = 9 patients with urine samples.

[f]n = 2409 patients with vaginal swabs and n = 1921 patients with urine samples were not tested for TPHA/TPPA (syphilis screening test).

women on extragenital locations, arguing for similar transmission potential, clinical relevance, and NAAT detection limits. Moreover, in women vaginal swabs had a higher *C. trachomatis* load compared to urine samples, confirming that vaginal swabs rather than urine samples should be used. A higher urogenital *C. trachomatis* load was observed in younger patients, whereas a lower urogenital *C. trachomatis* load was found in patients coinfected with HIV and *N. gonorrhoeae*.

The strength of the current study is the comparison of *C. trachomatis* load of different sample types of both men and women in 1 large study using the same diagnostic test (NAAT), allowing for the best comparison possible. *C. trachomatis* load can be quite variable in different patients depending on various factors, including time of diagnosis since acquiring the infection. However, our study group showed that the majority of patients had a stable *C. trachomatis* load in the time interval between diagnosis and treatment (66.3%, 73.1%, and 48.6% for vaginal swabs, urine samples, and anorectal swabs, respectively) [26]. The *C. trachomatis* load for each sample type might also vary as patients could apply the sampling instructions differently. Nevertheless, mean *C. trachomatis* load at a population level was quite stable, as seen in our results of anorectal and oropharyngeal samples across both sexes [6]. In earlier work we discussed the limitations of comparing different sampling methods (ie, urine vs swabs) and different sampling locations (vaginal swab vs anorectal or oropharyngeal swabs). For example, different sites have different epithelial cells, which might influence the *C. trachomatis* load. Some previous studies included the number of epithelial cells in assessment of the *C. trachomatis* load count but as anatomical sample sites differ in the number of cells in the samples, we argue that this hampers accurate comparison [14]. The Cq values of urine samples of men were moderately, but significantly, correlated with urine *C. trachomatis* load (expressed as copies/mL). Therefore, the results of urine Cq values could be a less stable indication of *C. trachomatis* load. This is an expected finding as we know concentration in urine can differ inter- and intraindividually and with time because infected cells from the male urethra may get washed out by first void urine. Another reason could be that the *C. trachomatis* major outer membrane protein breaks down faster in urine samples than in vaginal swabs leading to higher Cq values in the in-house qPCR (Taqman) compared to lower Cq values in the NAAT (Cobas 4800). Furthermore, we were unable to assess whether the amplified DNA was from viable or nonviable *C. trachomatis*. Previously, our study group developed a new viability technique (viability PCR) to discriminate between viable and nonviable *C. trachomatis* and showed that a substantial amount of *C. trachomatis* DNA originated from nonviable cells [27]. The nonviable *C. trachomatis* DNA would have no impact on *C. trachomatis* transmission and sequelae.

There is an ongoing discussion about what difference in *C. trachomatis* load is clinically or microbiologically relevant. Earlier, our group used a difference of 1 log load (3.3 Cq) as microbiology relevant to overcome potential technical variations when measuring the load within the same patient over time. However, in the current study, we averaged the Cq values over an entire group (eg, men, women, or patients aged <25 year). Therefore, potential technical differences between individuals were flattened out and had little impact on the results because these variations are randomly distributed over all samples tested within the particular group [26]. The precise clinical or microbiological relevance of the differences in *C. trachomatis* load between groups remains unknown. However, the fact that men and women had a similar extragenital *C. trachomatis* load indicates similar clinical relevance.

Nevertheless, of all sample types included, vaginal swabs had the highest *C. trachomatis* load, which could relate to the highest impact on transmission. Recently, our group showed a borderline significant association ($P = .54$) between a higher urogenital *C. trachomatis* load and microbiological *C. trachomatis* detection after treatment with azithromycin in women [28]. Our current study confirmed the presence of high urogenital *C. trachomatis* load in some patients, which might be clinically relevant when it hampers the treatment of a *C. trachomatis* infection with azithromycin.

Our research team and others have shown that the majority of women (summary estimate of 68.1%) diagnosed with urogenital *C. trachomatis* also have a concurrent anorectal *C. trachomatis* infection irrespective of reported anal sex or anal symptoms [29, 30]. The clinical relevance (risk of complications) and public health implications (transmission potential) associated with anorectal *C. trachomatis* detection in women are, however, under debate [31]. Anorectal infections in women could result from autoinoculation due to the close proximity between the vagina and anorectum [19, 32]. In our previous study, in a sample (n = 105) of concurrently infected STI clinic women showed that in the majority of cases (in 79% and 56% of women reporting anorectal intercourse and no anorectal intercourse, respectively), the anorectal *C. trachomatis* load was in the same range as the urogenital *C. trachomatis* load, suggesting similar clinical relevance [33]. Moreover, in the current study, we confirmed this finding and also showed that the anorectal *C. trachomatis* load in men equals that of women. We saw as many low as high anorectal loads in both men and women, which was suggested in an earlier smaller study based on *C. trachomatis* qPCR [12]. While diagnosis and treatment for anorectal *C. trachomatis* in MSM is recommended, whether this should apply in women is still under debate. However, the finding of a similar load in men and women suggests a similar clinical relevance. Relevance in women might be even more substantial as they bear the burden of reproductive morbidity caused by *C. trachomatis* infections [2]. Moreover, women concurrently infected with urogenital and anorectal *C. trachomatis* have an 8.5-times higher urogenital *C. trachomatis* load compared to anorectal-negative women [6]. Therefore,

women concurrently infected with urogenital and anorectal *C. trachomatis* might have higher chances of sequelae such as reproductive morbidity. Although, anorectal *C. trachomatis* infections in women might be coincidently treated with a urogenital infections due to the high concurrency, a recent study from our group suggested that the frequently used drug azithromycin may be less effective than doxycycline in clearing anorectal *C. trachomatis* infections in women [28]. Nevertheless, the question remains, whether and how substantial autoinoculation or other mechanisms for transmission from the female anorectal site to the female urogenital site, and vice versa, occur.

Studies have shown a lower *C. trachomatis* load among older patients and patients with repeat infections potentially due to development of partial immunity against *C. trachomatis* [8, 9, 34, 35]. Especially in HIV-infected men (a high-risk group) who were repeatedly infected with *C. trachomatis*, development of partial immunity might explain a lower load.

In conclusion, the mean *C. trachomatis* load estimated by Cq values differed per sample type and thus per anatomic location. Vaginal swabs had the highest *C. trachomatis* load, whereas oropharyngeal swabs had the lowest load. Notably, men and women had a similar *C. trachomatis* load on extragenital locations, arguing for similar transmission potential and clinical relevance. A lower load was associated with older age (>25) and coinfections with *N. gonorrhoeae* and HIV, which was suggestive of exposure to previous *C. trachomatis* and potential development of some kind of partial immunity reducing *C. trachomatis* load.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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