

The Epidemiology of *Chlamydia trachomatis* Organism Load During Genital Infection: A Systematic Review

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Background. The role of organism load in *Chlamydia trachomatis* infection is not well understood. We conducted a systematic review to investigate the epidemiology of *C. trachomatis* organism load in human genital chlamydia infection.

Methods. Embase, PubMed, and Medline databases were searched for literature published through August 2014. English-language publications that quantified load in humans were eligible. Participant characteristics and laboratory data were extracted.

Results. A total of 737 records were identified, and 29 publications involving 40 883 participants were included. In women, load was highest for cervical swabs and lowest for urine specimens. In men, load was highest for rectal swabs and similar for urethral swabs and urine specimens. Evidence of any association between load and age, serovar, risk of transmission, hormone levels, and concurrent sexually transmitted infections was inconsistent. Eight of 9 culture-based studies found an association between load and signs and symptoms, in contrast with only 3 of 8 nucleic acid amplification test (NAAT)-based studies ($P = .03$).

Conclusion. *Chlamydia* organism load varies by specimen type and site of sampling, and viable chlamydia organism load may be a more important indicator of severity of infection than total load measured by NAAT.

Keywords. *Chlamydia trachomatis*; organism load; culture; nucleic acid amplification; systematic review.

Chlamydia trachomatis infection is the most commonly reported bacterial sexually transmissible infection (STI) in the world [1], and at any point in time, >100 million adults are known to be infected [1]. The majority of infections are asymptomatic [2], and if left untreated, chlamydia can cause fallopian tube scarring and contribute to ectopic pregnancy and tubal factor infertility in women [3] and to urethritis, epididymitis, and proctitis [4] in men. Any STI, including chlamydia infection,

increases one's susceptibility to human immunodeficiency virus (HIV) infection [5].

The clinical presentation and risk of acquiring or transmitting chlamydia may depend on several factors, including age or sex [6]. The number of chlamydia organisms present may also be associated with clinical presentation and transmission, as is observed with viral STIs, such as herpes simplex virus infection, in which a high viral load increases the risk of transmission [7]. Laboratory assays for quantifying organism load have changed over the last 20 years: early assays required the ability to culture the organism, but the development of nucleic acid amplification tests (NAATs) has provided opportunities to overcome the challenges associated with culture [8]. We conducted a systematic review to investigate the epidemiology of chlamydia organism load in human genital chlamydia infection and to evaluate evidence about the association of load with infection site or

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specimen type, chlamydia genotype, clinical presentation, and demographic characteristics.

METHODS

This systematic review was conducted in line with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement [9] and was registered with PROSPECT (Prospective Registration of Systematic Reviews; CRD42014007136).

Search Process

One author (R. M.) searched the electronic databases Embase, PubMed, and Medline for relevant literature published any time through to 31st August 2014. The search string was as follows: ((chlamydia OR "Chlamydia trachomatis") AND ("organism load" OR load OR quantification OR "inclusion forming unit" OR "IFU" OR "antigen load" OR EBs OR "elementary bodies") AND (*genital OR vagin* OR cerv* OR ureth* OR urine OR rect* OR anal)). Citation lists were searched by hand for additional references.

The outcome was *C. trachomatis* organism load. The term "load" is used hereafter to describe the chlamydia organism burden.

Studies were eligible if they had an analytical or observational study design, described human genital chlamydia infection, and were published in English. Studies were excluded if they were performed on animals or had the primary aim of evaluating the performance of a diagnostic test.

Data Extraction

We developed a data extraction spreadsheet; one author (R. M.) extracted data, and another (L. A. V.) checked data. Consensus for discrepancies was reached by discussion between 3 authors (L. A. V., R. M., and J. S. H.). The following information was extracted: (1) aim and study design, (2) sample characteristics (sample size, country, and recruitment site), (3) participant characteristics (sex, age, and race), (4) infection type (first vs subsequent infection) and site (cervical, urethral, and anorectal), (5) laboratory method and units of measurement, and (6) factors that were assessed for association with load (sex, age, race, hormonal contraceptive use, concurrent STI, signs and symptoms, immune response, and risk of transmission).

Data Analysis

Pooling of results using meta-analysis was not possible because of differences in laboratory assays. We conducted a frequency analysis of factors associated with load. The laboratory assays used were classified into 3 groups: NAATs (eg, quantitative polymerase chain reaction), direct fluorescent antibody (DFA) assays, and culture.

Assessment of Bias and Quality

We conducted an analysis of the quality of the research, using definitions and classifications based on the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) [10] guidelines. We assessed the risk of bias within studies and reported on (1) selection bias, (2) response bias, (3) measurement bias, (4) confounding, and (5) sample size.

RESULTS

Study Selection

The literature identification process is illustrated in Figure 1, and selected publications are summarized in Tables 1 and 2. Initial searches identified 1293 records from Embase (n = 393), PubMed (n = 425), and Medline (n = 475), and an additional 6 records were found by reviewing citation lists. Duplicate records (n = 556) were removed, giving 737 unique references. Of these, 671 were excluded by title and abstract review, and 42 were excluded by full-text review. Reasons for exclusion after full-text review were as follows: load was not reported (n = 18), test performance was evaluated (n = 20), animals were studied (n = 1), or the publication was a review only (n = 3). The remaining 29 publications were included. Two publications [13, 14] were from the same study. Another 2 publications from the same research team investigated immune responses to chlamydia in different subsets of the same population [22, 23]. These 4 publications reported different results and are treated as separate studies.

Study Characteristics

Of the 29 publications identified, 28 were peer-reviewed original articles, and 1 was a published conference abstract. NAAT-based assays measured load in 11 studies [11–21], 2 used DFA assays [38, 39], and 16 used culture [22–37]. Most (n = 25) were cross-sectional studies [11, 14–17, 19, 20, 22–39], 3 were cohort studies [12, 13, 21], and 1 was a case-control study [18]. One of the cohort studies presented both longitudinal [13] and cross-sectional [14] data. The studies comprised 40 883 individuals (22 108 were women, 10 202 were men, and 8573 had no sex data reported). Participants were aged 14–76 years. Nine studies [15, 18, 20, 34–39] were based in the United Kingdom, 4 were in Australia [11, 13, 14, 16], 9 were in the United States [17, 24–28, 30–32], 2 were in Canada [29, 33], 3 were in Europe [12, 19, 21], and 2 were in India [22, 23]. Most studies (n = 22) were set in STI/genitourinary medicine/family planning clinics [11, 12, 15, 16, 20, 21, 24–39]. Organism load was the primary outcome in 14 studies [12, 15, 17, 18, 20, 21, 23, 25, 26, 31, 34, 35, 38, 39] (Table 1).

Load Measurement

NAAT-based studies reported the number of copies per milliliter or swab [14–16, 18, 19], the number of inclusion-forming units

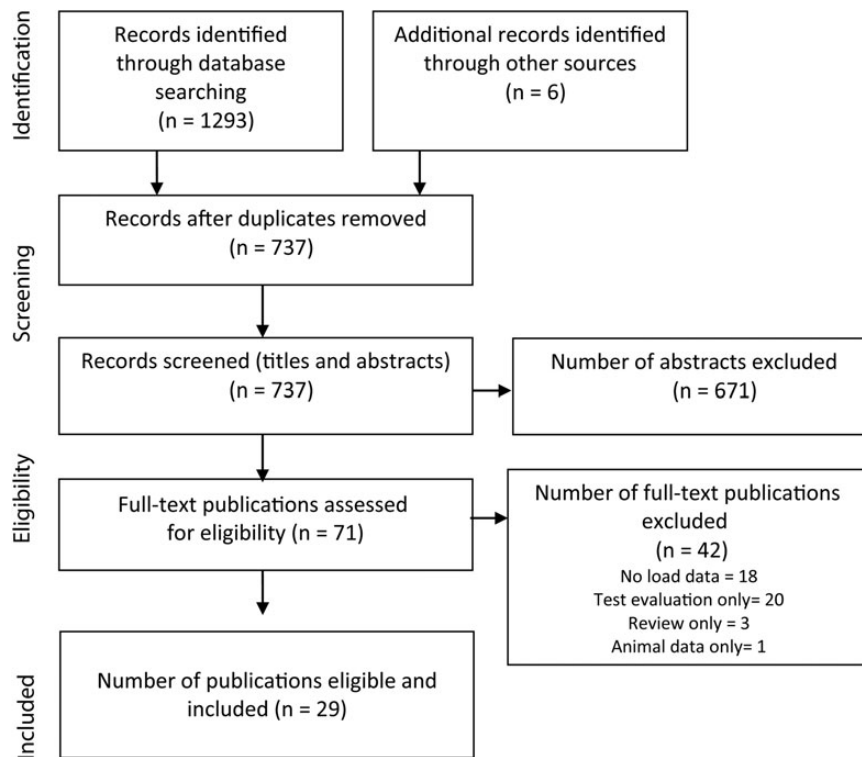


Figure 1. Summary of the process of identifying candidate publications and selecting publications for this report.

(IFU) per milliliter [12], the number of elementary bodies (EBs) per 100 μL [20], or the total number of copies [17], and 3 [11, 13, 21] accounted for sampling variability by standardizing their findings to the number of eukaryotic cells present. The 2 DFA-based studies reported load as number of EBs [38, 39]. Culture-based studies reported load as the total number of IFU or the IFU count [24–26, 28, 30], the number of inclusions [29, 35], the number of inclusions per well [27], the number of IFU per milliliter [22, 23, 31, 33], the number of inclusions per coverslip [32, 34, 36], and the number of IFU per 0.25 mL [37] (Table 1).

Specimen Type and Site of Infection

Twenty-three studies reported load for cervical infections [11–15, 18–23, 25, 26, 28, 30, 31, 33–39], 11 reported load for urethral infections [15, 18, 20, 21, 25, 26, 28, 30, 32, 34, 39], 5 reported load for anorectal infections [12, 16, 24, 27, 28], and 1 study did not specify the infection site [29]. Five NAAT-based [11, 12, 16, 18, 20], 2 DFA assay-based [38, 39], and 2 culture-based [28, 30] studies compared load between specimen types and sites (Table 2). One NAAT-based study compared load between specimen types within individuals and found that it was highest in cervical swabs, followed by vaginal swabs, urethral swabs, and urine specimens, in women, but there was no difference between urethral swabs and urine specimens in men [20]. Other NAAT-based studies compared load by specimen type between women and found that it was highest in cervical

swabs and lowest in urine specimens [11, 18] and higher for anorectal swabs, compared with vaginal swabs [12]. Among men, load was higher in anorectal swabs than in urine specimens [16]. One DFA assay-based study found that cervical swabs had higher EB counts than urine specimens in women [38], and 1 culture-based study found that load was highest in cervical swabs, followed by anorectal swabs and urethral swabs [28], in women.

Demographic Associations

Nine studies compared load between men and women [15, 17, 18, 21, 26, 29, 30, 34, 39], and of these, only 2, both NAAT-based, directly compared load in the same specimen type (urine) between men and women, finding no difference [18, 21]. One NAAT-based [15] and 4 culture- or DFA assay-based [26, 29, 30, 39] studies compared load in urethral swabs in men with that in cervical swabs in women and found that load was higher in women than men. The NAAT-based study found an almost 2-fold higher load in women, compared with men (median load, 5.6 log copies vs 3.5 log copies); this study also collected urethral swabs from women but did not compare urethral swabs between the sexes [15]. Two of the culture-based studies found that the median number of IFU was higher in women, compared with men (450 vs 60–72) [26, 30]. Malinson et al compared urethral swabs in men with cervical swabs in women and found that men had higher loads, but all men

Table 1. Description of Included Studies

Reference	Publication Year	Design	Location	Site(s)	Participants				Aim	Quantification Method	Units
					Females	Males	Total No.	Age, y			
Forcey et al [11]	2014	CS	Australia	STI	5055	0	5055	22–37	To investigate associations between <i>C. trachomatis</i> detection and stage of the menstrual cycle	NAAT	Total log ₁₀ no. of copies; no. of copies per 100 eukaryotic cells
Dukers-Muijers et al [12]	2013	Cohort	Netherlands	STI	44	8	52	21–28	To investigate <i>C. trachomatis</i> detection and organism load in azithromycin treated patients followed for up to 2 mo	NAAT	No. of IFU per mL
Walker et al [13] ^a	2012	Cohort	Australia	Primary care	1116	0	1116	16–25	To estimate chlamydia incidence and reinfection rates among young women	NAAT	No. of copies per 100 eukaryotic cells
Walker et al [14] ^a	2011	CS	Australia	Primary care	1116	0	1116	16–25	To estimate the prevalence of <i>C. trachomatis</i> among young women	NAAT	No. of copies per swab
Jalal et al [15]	2011	CS	UK	GUM	1744	1640	3384	15–62	To investigate the association between clinical features and organism load with and without genotype identification	NAAT	No. of copies per mL
Twin et al [16]	2011	CS	Australia	STI	0	612	612	18 to ≥40	To investigate chlamydia serovars and genotypic variants in Australian MSM	NAAT	No. of copies per swab or per mL
Van Der Pol et al [17]	2011	CS	US	NR	99	284	383	NR	To evaluate the use of organism load of <i>C. trachomatis</i> in clinical specimens as an epidemiologic tool	NAAT	Total log ₁₀ no. of copies
Wiggins et al [18]	2009	CC ^b	UK	GP	98	42	140	16–24	To estimate chlamydia organism load and investigate factors associated with load	NAAT	No. of copies per mL
Baczynska et al [19]	2008	CS	Denmark	Hospital clinic	102	0	102	18 to ≥25	To determine lower genital tract carriage rate of <i>M. genitalium</i> and compare it to the carriage of <i>M. hominis</i> and <i>C. trachomatis</i> in women seeking termination of pregnancy	NAAT	No. of copies per mL
Michel et al [20]	2007	CS	UK	GUM	1001	653	1654	16–69	To determine chlamydia load in matched specimens from different anatomic sites and examine its relation to clinical signs and symptoms	NAAT	No. of EBs per 100 μL
Gomes et al [21]	2006	Cohort	Portugal	GP, FP, STI	91	80	171	16–76	To investigate factors associated with <i>C. trachomatis</i> organism load	NAAT	No. of copies per 100 eukaryotic cells
Agrawal et al [22]	2009	CS	India	FP, hospital clinic	197	0	197	NR	To investigate the role of myeloid dendritic cells and plasmacytoid dendritic cells in the immunopathogenesis of cervical <i>C. trachomatis</i> infection	Culture	No. of IFU per mL

Table 1 continued.

Reference	Publication Year	Design	Location	Site(s)	Participants				Aim	Quantification Method	Units
					Females	Males	Total No.	Age, y			
Agrawal et al [23]	2009	CS	India	FP, hospital clinic	1548	0	1548	Median 26–28	To determine the association between infectious load and immune correlates to gain a better understanding of infectious load regulation in the female genital tract	Culture	No. of IFU per mL
Geisler et al [24]	2002	CS	US	STI	0	71	71	NR	To use serovar and auxotyping of strains to assess the epidemiology of anorectal chlamydia and gonococcal infections among MSM attending an STI clinic	Culture	No. of IFU count
Geisler et al [25]	2001	CS	US	STI	479	700	1179	NR	To investigate the association of <i>C. trachomatis</i> organism load with signs and symptoms	Culture	No. of IFU
Ekhert et al [26]	2000	CS	US	STI	6801	4233	11 034	<16 to ≥30	To investigate factors associated with <i>C. trachomatis</i> organism load	Culture	No. of IFU
Boisvert et al [27]	1999	CS	US	STI	0	618	618	Mean 27.4–29.4	To examine whether rectal infections with different serovars were associated with differences in clinical presentation as measured by symptoms, signs, number of leukocytes and inclusion counts	Culture	No. of inclusions per well
Workowski et al [28]	1994	CS	US	STI	155	0	155	Mean 21.7	To investigate whether the infecting <i>C. trachomatis</i> serovar was related to the type or severity of clinical manifestations among women	Culture	No. of IFU
Frost et al [29]	1993	CS	Canada	STI/FP	NR	NR	435	NR	To determine the chlamydia serovar in urogenital specimens	Culture	No. of inclusions
Workowski et al [30]	1992	CS	US	STI	581	934	1515	NR	To investigate factors associated with specific <i>C. trachomatis</i> serovars	Culture	No. of IFU
Barnes et al [31]	1990	CS	US	STI	1231	0	1231	<20 to ≥26	To investigate the association between epidemiologic variables and <i>C. trachomatis</i> organism load	Culture	IFU per mL
Singal et al [32]	1986	CS	US	STI	0	136	136	NR	To determine whether the use of a second urethral swab significantly improves rates of recovery of chlamydia from heterosexual men with urethritis	Culture	No. of inclusion counts per coverslip
Brunham et al [33]	1983	CS	Canada	STI	95	0	95	NR	To investigate correlations of the immune response with shedding or <i>C. trachomatis</i>	Culture	No. of IFU per mL
Mallinson et al [34]	1981	CS	UK	GUM	147	191	338	15 to ≥30	To investigate factors associated with <i>C. trachomatis</i> organism load	Culture	Inclusion counts per coverslip
Hobson et al [35]	1980	CS	UK	GUM	580	0	580	NR	To investigate clinical factors associated with <i>C. trachomatis</i> organism load	Culture	Inclusion counts
Tait et al [36]	1980	CS	UK	GUM	202	0	202	16–46	To investigate clinical factors associated with <i>C. trachomatis</i>	Culture	Inclusion counts per coverslip

Table 1 continued.

Reference	Publication Year	Design	Location	Site(s)	Participants			Age, y	Aim	Quantification Method		Units
					Females	Males	Total No.			Method	Method	
Hilton et al [37]	1974	CS	UK	GUM, FP	262	0	262	14 to >30	To investigate chlamydia in the female genital tract	Culture	No. of IFU per 0.25 mL	
Thomas et al [38]	1998	CS	UK	GUM	480	0	480	NR	To compare organism load between women with symptomatic and asymptomatic <i>C. trachomatis</i> infection	DFA	No. of EBs	
Thomas et al [39]	1990	CS	UK	GUM	NR	NR	8138	NR	To investigate the association of <i>C. trachomatis</i> and signs and symptoms and to determine the number of organisms recovered from the genital tract	DFA	No. of EBs	

Abbreviations: CC, case-control; CS, cross-sectional; *C. trachomatis*, *Chlamydia trachomatis*; DFA, direct fluorescent antibody assay; EB, elementary body; FP, family planning clinic and/or gynecology/perinatal clinic; GUM, genitourinary clinic; IFU, inclusion-forming unit; *M. genitalium*, *Mycoplasma genitalium*; *M. hominis*, *Mycoplasma hominis*; MSM, men who have sex with men; NAAT, nucleic acid amplification test; NR, no data reported; STI, sexually transmitted infection/sexual health clinic; UK, United Kingdom.

^a Same study.

^b Data available for 2 time points for each participant.

had urethritis, and only a proportion of women had signs or symptoms [34]. Eight NAAT-based and 6 culture-based studies investigated associations with age [13–16, 18–21, 26, 29–31, 34, 35]. Culture-based studies [26, 29–31, 35] were more likely than NAAT-based studies [18–20] to find that load increased with decreasing age (83% [5 of 6] vs 38% [3 of 8]; $P = .08$; Table 3). Three studies in the United States investigated associations between load and race/ethnicity, and all found that white participants had higher loads than black participants [26, 30, 31].

Serovar

Five NAAT-based [13–16, 21] and 5 culture-based [24, 26, 28–30] studies investigated associations with serovar. Walker et al found that load was highest for serovar D in their cross-sectional analysis [14] but found no associations in their cohort analysis [13]. Two culture-based studies found that load was significantly higher for B-complex serovars (B, D, and E) [26, 29], and 1 study found a nonsignificant trend toward an increased load with B-complex serovars [28]. Two other studies reported loads stratified by serovar but did not comment on the statistical significance of differences [24, 28].

Signs and Symptoms

Eighteen studies assessed associations with signs and symptoms, with 3 NAAT-based [15, 18, 20] and 8 culture-based [22–25, 27, 34–36] studies finding associations. In women, load was increased with the presence of cervical ectopy [34, 35], intermenstrual bleeding [25], vaginal discharge, and mucopurulent cervicitis [15, 18, 20, 22, 23, 25, 35, 36] and with pelvic pain or pelvic inflammatory disease (PID) [20, 25]. In men, load was increased with dysuria or urethritis [15, 20, 25]. One study among men found that proctitis was associated with a higher load [24]. In a multivariate analysis, Jalal et al found that the odds of signs and symptoms increased with increasing load in both sexes [15]. Culture-based studies were more likely to find an association with signs and symptoms than NAAT-based studies (89% [8 of 9] vs 38% [3 of 8]; $P = .03$; Table 3).

Load Over Time

Four studies investigated changes in load over time; 2 found that load was significantly lower for repeat infection [13, 21]. Wiggins et al tested participants again for chlamydia before treatment and found that, among women, load was significantly lower in the second specimen but that among men, there was no difference between specimens. However, no data were provided about the proportion of second specimens that were negative for chlamydia [18]. Dukers-Muijers et al investigated changes in load over time following treatment and found intermittent detection over an 8-week period [12].

Menstrual Cycle and Oral Contraceptive Pill (OCP) Use

Five studies investigated associations with menstrual cycle and found no association [11, 12, 31, 34, 35]. Two other studies

Table 2. Factors Investigated for Their Association With Chlamydia Organism Load in Genital Specimens

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥2 Time Points	Signs/Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
Forcey et al [11]															
Females	CVX, VAG, URINE	NAAT	+	NA	NA	NA	NA	NA	N	NA	NA	NA	NA	NA	Load highest in cervical swabs, followed by vaginal swabs and urine specimens; no association with stage of menstrual cycle
Dukers-Muijers et al [12]															
Females, males	VAG, ANAL	NAAT	+ ^a	NA	NA	NA	NA	N* ^a	NA	NA	NA	NA	NA	NA	Load higher in anorectal swabs than vaginal swabs; high pretreatment load in anorectal swabs only associated with load 23–51 d after treatment
Walker et al [13]															
Females	VAG	NAAT	NA	NA	N	N	+ ^b	NA	NA	NA	NA	NA	NA	NA	Load higher for prevalent than incident infection; suggestion that higher load may predict treatment failure; no association with age or serovar
Walker et al [14]															
Females	VAG	NAAT	NA	NA	N	+	NA	N	NA	NA	NA	NA	NA	NA	Load higher for serovar D vs E and for E vs F; no association with age or signs and symptoms
Jalal et al [15]															
Females	CVX, URTH ^c	NAAT	NA	+	N*	N*	NA	+*	NA	NA	NA	NA	NA	NA	Load higher in women than men; load higher with “clinical features”; no association between load and age or serovar
Males	URTH	NAAT	NA	+	N*	N*	NA	+*	NA	NA	NA	NA	NA	NA	...
Twin et al [16]															
Males	ANAL, URINE	NAAT	+	NA	N	N	NA	N	NA	NA	NA	NA	NA	NA	Load higher in anorectal swabs than urine samples; no association with age, serovar, or signs and symptoms

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥ 2 Time Points	Signs/ Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
Van Der Pol et al [17]															
Females, males	VAG for females, URINE for males	NAAT	NA	N	NA	NA	NA	N ^d	NA	NA	NA	NA	NA	NA	No associations found with sex or age
Wiggins et al [18]															
Females	VAG, URINE	NAAT	+	N ^e	+ ^f	NA	N	+* ^g	NA	NA	NA	NA	NA	NA	No difference in load between women and men; load higher in vaginal swabs than urine specimens in women; associations with age and signs and symptoms observed for women but varied by specimen type; no difference in load between 2 time points; no association with age or signs and symptoms in men
Males	URINE	NAAT	NA	N ^d	N	NA	N	N	NA	NA	NA	NA	NA	NA	...
Baczynska et al [19]															
Females	CVX	NAAT	NA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	Load higher among younger women
Michel et al [20]															
Females	URINE, CVX, VAG, URTH	NAAT	+	NA	+	NA	NA	+*	NA	NA	+*	NA	NA	NA	In women, organism load was highest for cervical swabs, followed by vaginal swabs, urethral swabs, and urine specimens; no difference in organism load between urethral swabs and urine specimens in men; load associated with age, signs and symptoms, and other STIs in women and with age and signs and symptoms in men
Males	URINE, URTH	NAAT	N	NA	+	NA	NA	+*	NA	NA	NA	NA	NA	NA	...

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥ 2 Time Points	Signs/Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
Gomes et al [21]															
Females, males	URINE	NAAT	NA	N	N	N	+	N	NA	NA	N	NA	NA	N	No difference in load by sex, age, serovar, signs and symptoms, or other STIs; load was higher for first infection, compared with subsequent infection; no association of load with partner concordance.
Agrawal et al [22]															
Females	CVX	CUL	NA	NA	NA	NA	NA	+	+	NA	NA	NA	+	NA	Load higher in women with MPC and in women with higher estradiol levels; plasmacytoid dendritic cell Nos. increased with increasing load
Agrawal et al [23]															
Females	CVX	CUL	NA	NA	NA	NA	NA	+	+	NA	NA	NA	±	NA	Load higher in women with MPC but lower in women with FD; load increased with higher estradiol levels; significant positive and negative correlations with immune factors and load observed, including associations with CD4 ⁺ and CD8 ⁺ T cells, IFN- γ , interleukins, and CRP
Geisler et al [24]															
Males	ANAL	CUL	NA	NA	NA	+	NA	+	NA	NA	NA	NA	NA	NA	Load lower for serovar G and highest for serovars D and J; abnormalities revealed by anal examinations and diagnoses of proctitis were more common among those with IFU counts ≥ 50 than among those with IFU counts <50
Geisler et al [25]															
Females	CVX	CUL	NA	NA	NA	NA	NA	+*	NA	NA	NA	NA	NA	NA	Among women, higher load associated with IMB, cervical exudate, cervical motion, uterine fundal or adnexal tenderness, MPC, and PID, adjusted

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥ 2 Time Points	Signs/Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
															for age, race, serovar, and OCP use; among men, higher load associated with dysuria, urethral discharge, and PMNLs, adjusted for age, race, serovar
Males	URTH	CUL	NA	NA	NA	NA	NA	+*	NA	NA	NA	NA	NA	NA	...
Ekheret et al [26]															
Females	CVX	CUL	NA	+	+*	+*	NA	NA	NA	NA	NA	+*	NA	NA	Univariate and multivariate analysis showed women had higher loads than men and that load was associated with serovar and decreasing age in both women and men; load was higher for B-complex serovars than for C-complex serovars; load higher for whites, followed by blacks and Native Americans
Males	URTH	CUL	NA	+	+*	+*	NA	NA	NA	NA	NA	+*	NA	NA	...
Boisvert et al [27]															
Males	ANAL	CUL	NA	NA	NA	+	NA	+	NA	NA	+	NA	NA	NA	Men coinfectd with <i>N. gonorrhoea</i> had higher loads; mean duration of symptoms was significantly longer for men with <100 inclusions than men with ≥ 100 inclusions; among men with serovar I, higher load was associated with symptoms
Workowski et al [28]															
Females	CVX, URTH, ANAL	CUL	+	NA	NA	+ ^h	NA	NA	NA	NA	NA	NA	NA	NA	Load highest for cervical swabs, followed by rectal swabs and urethral swabs; median no. of IFU in cervical isolates was 600 for class B isolates, 400 for group F,G isolates, and 800 for class C isolates, but unclear

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \ Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥ 2 Time Points	Signs/ Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
															whether findings were statistically significant
Frost et al [29]															
Females, males	UNK	CUL	NA	+	+	+	NA	NA	NA	NA	NA	NA	NA	NA	Load higher in women than men and higher in younger women; load higher with B-complex serovars
Workowski et al [30]															
Females	CVX	CUL	+	+	+	N	NA	NA	NA	NA	NA	+	NA	NA	Load higher for cervical swabs in women compared with urethral swabs in men; load higher for younger women; no associations with age in men; no associations with serovar; load higher in white men and women than black men and women in US
Males	URTH	CUL	+	+	N	N	NA	NA	NA	NA	NA	+	NA	NA	...
Barnes et al [31]															
Females	CVX	CUL	NA	NA	+	NA	NA	N	N	+	+	+	NA	NA	Higher load associated with decreasing age, OCP use, and concurrent gonorrhea or trichomoniasis; no association with menstrual cycle or signs and symptoms; load higher for white than black women in US
Singal et al [32]															
Males	URTH	CUL	NA	NA	NA	NA	NA	NA	NA	NA	-	NA	NA	NA	Load was lower with concomitant gonorrhea
Brunham et al [33]															
Females	CVX	CUL	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	NA	Higher load associated with increased serum IgM antibodies, but decreasing cervical IgM and IgA antibodies; higher load also associated with higher mean lymphocyte transformation stimulation index

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥ 2 Time Points	Signs/ Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
Mallinson et al [34]															
Females	CVX	CUL	NA	-	N	NA	NA	+	N	N	N	NA	NA	NA	Load higher in men (all symptomatic) than women (only some symptomatic); no association between load and age in men or women; no association with load and OCP use or menstrual cycle in women; women with cervical ectopy had higher load; no significant associations between load and other STIs for men or women; men with high load more likely to have infected sex partners
Males	URTH	CUL	NA	-	N	NA	NA	N	NA	NA	N	NA	NA	+	...
Hobson et al [35]															
Females	CVX	CUL	NA	NA	+	NA	NA	+	N	+	N	NA	NA	NA	Load higher in younger women and in those with cervical ectopy or MPC; load higher in those using OCP, but this may be due to cervical ectopy rather than OCP; load not associated with other STIs
Tait et al [36]															
Females	CVX	CUL	NA	NA	NA	NA	NA	+	NA	+	NA	NA	NA	NA	Load higher in women using OCP and in those with MPC
Hilton et al [37]															
Females	CVX	CUL	NA	NA	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	Load higher in women using OCP
Thomas et al [38]															
Females	CVX, URINE	DFA	+	NA	NA	NA	NA	N	NA	NA	NA	NA	NA	NA	Urine specimens more likely than cervical swabs to have a lower load; no association between signs and symptoms and load

Table 2 continued.

Reference, Sex	Specimen Type(s)	Quantification Method	Site \Sample Type	Sex (F>M)	Younger Age	Serovar	Load Measured at ≥2 Time Points	Signs/Symptoms	Menstrual Cycle /Estradiol Levels	OCP Use	Other STIs	Racial	Immune Factors	Transmission Risk	Summary
Thomas et al [39]															
Females	CVX	DFA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Load higher for cervical swabs from women than urethral swabs from men
Males	URTH	DFA	NA	+	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	...

Abbreviations: ANAL, anorectal swab; CRP, C-reactive protein; CUL, culture; CVX, endocervical swab; DFA, direct fluorescent antibody test; FD, fertility disorders; IFN- γ , interferon γ ; IgA, immunoglobulin A; IgM, immunoglobulin M; IMB, intermenstrual bleeding; MPC, mucopurulent cervicitis/discharge; N, no association; NA, not assessed; NAAT, quantitative nucleic acid amplification test; N, gonorrhoea, *Neisseria gonorrhoea*; no., numbers; OCP, oral contraceptive pill; PID, pelvic inflammatory disease; PMNL, polymorphonuclear leukocyte; STI, sexually transmitted infection; UKN, unknown specimen site; URINE, urine specimen; URTH, urethral swab; VAG, vaginal swab; +, positive association; -, negative association; *, based on multivariate analysis.

^a No differentiation between men and women in analysis. Women provided vaginal and anorectal swabs. Men provided anorectal swabs only.

^b Comparing load between prevalent and incident infection.

^c Cervical swabs plus urethral swabs collected from women and processed as a single specimen.

^d No differentiation between men and women in the analysis.

^e Comparison of load by sex was done on urine specimens only.

^f Association between load and age only observed for urine specimens in women.

^g Association between vaginal discharge and load only observed for vaginal swabs.

^h Unclear whether statistically significant.

reported a positive correlation between load and estradiol levels in women with mucopurulent cervicitis or fertility disorders [22, 23]. One culture-based study found no association between load and OCP use [34], and 3 found a significantly higher load with OCP use [31, 36, 37]. Hobson et al found that OCP use was indirectly associated with load, by causing cervical ectopy [35].

Concurrent STIs

Two NAAT-based studies gave conflicting results; one found no association with a concurrent STI [21], and the other found that load was increased among women with genital warts or *Molluscum contagiosum* [20]. Two culture-based studies found no association with concurrent STIs [34, 35], 1 culture-based study found that load was higher among men with concurrent gonorrhoea [27], and another culture-based study found that higher loads were associated with concurrent gonorrhoea or trichomoniasis in women [31].

Immune Markers

Three culture-based studies investigated associations with immune markers. One found that the number of plasmacytoid dendritic cells increased with load in women [22]. Another found significant positive correlations between load and the CD4⁺ T-cell count, myeloid dendritic cell count, interleukin 12 level, and interleukin 2 level in asymptomatic women and negative correlations with the CD8⁺ T-cell count and interleukin 8 level [23]. Women with fertility disorders had a positive correlation between load and the plasmacytoid dendritic cell count and interleukin 10 level and a negative correlation between load and the CD4⁺ T-cell count and IFN- γ level. A third study found that an increased load was associated with an increased serum immunoglobulin M level but a decreased cervical secretory immunoglobulin A level [33].

Risk of Transmission

Two studies investigated the risk of transmission between sex partners [21, 34]. One NAAT-based study found no difference in mean load between concordant and discordant partners [21]. One culture-based study found that men with a higher load were more likely to have chlamydia-infected partners [34].

Assessment of Study Bias

Several studies did not state whether recruitment was consecutive or participants were symptomatic and were at risk of selection bias [12, 19–24, 28–39] (Table 4 and Supplementary Table 1). Only 4 studies reported response rates [12–14, 31]. Three studies reported load by number of eukaryotic cells, to account for sampling variability, and had a low risk of measurement bias [11, 13, 21]. Five studies included multivariate analysis [15, 18, 25, 26, 31], 3 conducted stratified analyses [29, 34, 35], and 2 restricted eligibility to control for confounding factors [22, 23]. Load was the primary outcome in 14 studies, but none included sample size.

DISCUSSION

To our knowledge, this is the first systematic review of the epidemiology of genital chlamydia organism load, and it has shown that there is a considerable variability in how load is quantified and in the quality of studies. We found consistent evidence that load varies by specimen type and anatomical site. Within women, load is highest in cervical swabs, followed by vaginal swabs, and lowest in urine specimens. Among men, load is higher for anorectal swabs than for urethral swabs and is similar between urethral swabs and urine specimens. This means that the same specimen type and site of sampling should be used for any investigation of load and that any comparisons of load between men and women are of little value. Evidence for the association between load and age, serovar, hormone levels, concurrent STIs, and signs and symptoms varied considerably.

There are several explanations for this variability. First, it may be due to the use of different laboratory assays. NAAT-based assays are more sensitive for detecting chlamydia nucleic acid but are unable to distinguish between viable and nonviable organisms, thereby overestimating load. In contrast, culture will detect viable organisms but is less sensitive. This makes it difficult to compare culture results to NAAT results. Table 3 shows that culture-based studies were more likely to find associations with load, suggesting that the load of viable organisms may be more relevant than the total load (which comprises viable and nonviable organisms).

Sampling variability may also be a factor. Three studies accounted for sampling variability by reporting loads by the number of eukaryotic cells [11, 13, 21]. However, inflammatory cells attracted to the site of infection will be included among the sampled cells, thereby decreasing the load per eukaryotic cell [15]. Despite this limitation, median human β -globin gene expression, a measure of the number of eukaryotic cells, has been found to be similar between gonococci-positive and gonococci-negative samples [40], suggesting that their use to adjust for sampling variability is justified.

Study design and population group may contribute to this variability. In cross-sectional studies, it is not known when chlamydial infection was acquired, and because longitudinal studies suggest that load is lower for incident than prevalent infections [13, 21], it is unclear whether associations in cross-sectional studies are due to the type of infection (ie, incident or prevalent) or to factors such as participant age. The source population is also important. Several studies were based in STI clinics, where participants are more likely to have had previous chlamydia infection [14] and will have a lower load than if it was a first infection.

It was not always clear whether fresh or stored specimens were used, and chlamydia nucleic acid can deteriorate over time when stored [41]. Further, the quality of studies was highly variable; no sample size calculations for a load outcome were

included, so poor statistical power or uncontrolled confounding may have affected results.

Several studies found associations with younger age [18–20, 26, 29–31, 35]. It is possible that partial immunity acquired through past chlamydia infection may decrease shedding, leading to a lower load with increasing age [42]. In contrast, it is also possible that changes in the cervical epithelium, including cervical ectopy in young women, may contribute to increased chlamydial replication and shedding [26, 31]. Therefore, a higher load in younger women could be due to several different mechanisms.

Studies using culture were more likely to find associations with signs and symptoms [22–25, 27, 34–36] raising the possibility that viable organisms are more closely correlated with signs and symptoms than total load as measured with NAAT. The biological mechanisms behind this remain unclear, but evidence from *Chlamydia muridarum* in mice suggests that higher-load inocula produce a greater innate immune response, causing symptoms [43]. We found one study that identified correlations between load and immune factors [23]. This study was cross-sectional, so causal associations are difficult to confirm, but it suggested that the interplay between load and the immune response may be important for the clinical presentation of chlamydia infection.

The role of load in ascending infection and PID in women remains unclear, with only 1 study reporting on PID [25]. This study found that load was associated with PID independently of cervicitis, but it was cross-sectional, so it remains unclear whether a higher load caused PID. Data from animal models suggest that a higher load leads to ascending infection in mice [44].

Two studies found that load was lower for repeat infections [13, 21] and the study by Walker et al also reported that load was lower in incident [13] than prevalent infection, raising several hypotheses. First, load may be lower with repeat infection because past infection confers some protective immunity and influences organism replication but not chlamydia entry [21, 42]. Proof of this requires a longitudinal study with serial sampling, to detect incident infection, and detailed sexual behavior data, to identify the risk of reinfection. Second, load may increase over time, but this seems unlikely because chlamydia infection naturally clears over time without treatment [45]. A third hypothesis is that lower-load infections clear more quickly than higher-load infections, so prevalent cases may represent a biased sample of higher-load infections that have not cleared. This hypothesis is supported by mathematical models of chlamydia transmission [46].

Evidence for the association between load and serovar was conflicting. In their cross-sectional analysis, Walker et al found that the load was higher for serovar D [14], but this finding was not observed in their cohort analysis [13]. Although this difference may have been due to a lack of statistical power, it may have been due to uncontrolled confounding, such as not

Table 3. Summary of Factors Found to Be Associated With Chlamydia Organism Load in Genital Specimens, Stratified by Type of Laboratory Method Used

Factor Investigated	NAAT		Culture		DFA Assay	
	Studies Investigating an Association, Total No.	Studies Reporting an Association, ^a No. (%)	Studies Investigating an Association, Total No.	Studies Reporting an Association, ^a No. (%)	Studies Investigating an Association, Total No.	Studies Reporting an Association, ^a No. (%)
Female sex	4	1 (25)	4	3 (75)	1	1 (100)
Age	8	3 (38)	6	5 (83)	0	0 (0)
Serovar	5	1 (20)	6	4 (67)	0	0 (0)
Prevalence/incidence of infection	3	2 (67)	0	0 (0)	0	0 (0)
Signs and symptoms	8	3 (38)	9	8 (89)	1	0 (0)
Menstrual cycle/estradiol levels	1	0 (0)	5	2 (40)	0	0 (0)
OCP use	0	0 (0)	5	4 (80)	0	0 (0)
Other STI	2	1 (50)	4	2 (50)	0	0 (0)
First vs subsequent infection	3	2 (67)	0	0 (0)	0	0 (0)
Immune factors	0	0 (0)	3	3 (100)	0	0 (0)
Transmission risk	1	0 (0)	1	1 (100)	0	0 (0)

Abbreviations: DFA, direct fluorescent antibody; NAAT, nucleic acid amplification test; OCP, oral contraceptive pill; STI, sexually transmitted infection.

^a Either a positive or negative association.

accounting for previous infection [13, 21]. However, this result was supported by findings from culture-based studies, which found that load was higher for B-complex serovars [26, 29]. The clinical significance of this remains unclear, but others have found conflicting results for the association between serovar and clinical symptoms [28].

Three culture-based studies found associations with OCP use [31, 36, 37]. One hypothesis is that cervical ectopy induced by OCP use could increase chlamydia replication. Hobson et al conducted a stratified analysis to investigate the interaction between OCP use and cervical ectopy and found that OCP use was indirectly associated with load by causing cervical ectopy [35].

Three studies found that load was higher among US individuals with a race/ethnicity other than black. This is an interesting finding because STI rates are higher among blacks in the United States [47]. While there may be immunological or microbiological factors explaining this [48], confounding from past chlamydia infection may also be a factor.

From a clinical and public health perspective, there remain gaps in our knowledge about the role of load. First, its role in transmission remains unclear. Two studies investigated whether load was associated with transmission [21, 34]. Gomes et al found no difference in load between concordant or discordant partners [21]. However, the sample size was small and compared loads between males and females. The study by Mallinson et al was also based on small numbers, but it found that men with a higher load were more likely to have infected partners [34]. The ideal study to investigate the impact of load on transmission would include cases involving the same sex (ie, all

women or all men) and their partners, to minimize any differences in load being due to specimen site or type. Second, it is possible that higher-load infections are more susceptible to treatment failure [49]. Walker et al [13] found that women with a higher load were more likely to have a repeat infection of the same serovar, raising the possibility of treatment failure. Evidence from ocular trachoma studies suggests that azithromycin failure is more likely at higher loads [50]. Finally, it remains unclear whether a higher load leads to increased transmission of HIV infection or other STIs.

If load is important, then measuring it at the time of diagnosis may be needed. Our review suggests that viable organisms may be more relevant than the total load, so we may need to quantify chlamydia messenger RNA to measure replicating organisms, rather than chlamydia DNA or ribosomal RNA. Our existing NAATs are very sensitive, and in low-prevalence populations, the risk of false-positive results is high [8]. We may be diagnosing less clinically important infections with current NAAT-based assays, and given increasing concerns about antimicrobial resistance, the ability to reliably detect viable infections could lead to better health outcomes. If a higher load increases the risk of treatment failure, then different treatment regimens may be needed for higher-load infections. If higher loads increase the risk of ascending infection, then women with a high load may need to be monitored for PID to ensure that treatment is appropriate.

There are several limitations to our review. We reviewed English-language publications, which may limit the generalizability of our findings. The available data were of poor quality overall, with different laboratory assays and specimen types

Table 4. Summary of the Risk of Bias for Included Studies

Reference	Selection Bias (Population Sampled)	Response Bias	Measurement Bias (Outcome Ascertainment)	Confounding	Sample Size
Forcey et al [11]	+	+	+	+++	+
Dukers-Muijers et al [12]	+ / ++	+	+ / ++	+++	++
Walker et al [13]	+	++	+	+++	++
Walker et al [14]	+	++	+ / ++	+++	++
Jalal et al [15]	+	+	+ / ++	+	++
Twin et al [16]	+	+	+ / ++	+++	+
Van Der Pol et al [17]	?	?	+ / ++	?	?
Wiggins et al [18]	+	+ / ++	+ / ++	+ / ++	++
Agrawal et al [23]	+ / ++	++	+ / ++	+ / ++	+
Agrawal et al [22]	+ / ++	++	+ / ++	+ / ++	++
Baczynska et al [19]	+ / ++	++	+ / ++	+++	+++
Michel et al [20]	+ / ++	++	+ / ++	+++	++
Gomes et al [21]	+ / ++	++	+	+++	++
Geisler et al [24]	+ / ++	++	+	+++	++
Geisler et al [25]	+	+	+ / ++	+	+
Ekhert et al [26]	+	+	+ / ++	+	+
Boisvert et al [27]	+	+	+ / ++	+++	+
Thomas et al [38]	++	++	+ / ++	+++	++
Workowski et al [28]	+ / ++	++	+ / ++	+++	++
Frost et al [29]	+ / ++	++	+ / ++	+ / ++	++
Workowski et al [30]	+ / ++	++	+ / ++	+++	+
Thomas et al [39]	+ / ++	++	+ / ++	+++	++
Barnes et al [31]	+ / ++	++	+ / ++	+	+
Singal et al [32]	+ / ++	++	+ / ++	+++	++
Brunham et al [33]	+ / ++	++	+ / ++	+++	++
Mallinson et al [34]	++	+	+ / ++	+ / ++	++
Hobson et al [35]	+ / ++	++	+ / ++	+ / ++	++
Tait et al [36]	+ / ++	+	+ / ++	+++	++
Hilton et al [37]	+ / ++	++	+ / ++	+++	++

The risk of bias was classified as low (+), medium (++), high (+++), or unclear (?). See [Supplementary Table](#) for justification of the classification scheme. Based on the STROBE guidelines [10].

used, no sample size calculations, and minimal control for confounding.

In summary, this systematic review has identified that load varies by specimen type and the site of sampling and that it is possible that the load of viable organisms is more important than the total load. However, the role of load in chlamydia transmission, ascending infection, treatment failure, and STI transmission remains unclear. This will only be elucidated through well-designed cohort studies with serial sampling, to determine when chlamydia was acquired, and comprehensive data collection, to control for confounding.

Supplementary Data

[Supplementary materials](#) are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The

posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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