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Seroprevalence of *Chlamydia trachomatis* Among Female Adults in the United States: The National Health and Nutrition Examination Surveys

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Background. *Chlamydia trachomatis* is the most common nationally notifiable sexually transmitted infection in the United States; however, the seroprevalence of *C. trachomatis* infection is unknown.

Methods. This cross-sectional study was conducted among 1725 females aged 18 to 39 years who provided serum and urine samples in the 2013 through 2016 National Health and Nutrition Examination Surveys. Presence of anti-*C. trachomatis* Pgp3 immunoglobulin G (IgG) was determined using both an enzyme-linked immunosorbent assay (ELISA) and multiplex bead array (MBA). Weighted seroprevalence estimates were calculated. Correlates of seroprevalence were examined by multivariable Poisson regression.

Results. In 2013 through 2016, overall seroprevalence of *C. trachomatis* Pgp3 IgG was 30.0% (95% confidence interval [CI], 25.5-35.0) as measured by ELISA and 29.4% (95% CI, 25.8-33.0) as measured by the MBA assay. Overall agreement between tests was 87.1% (1503/1725). There was a high positive agreement by the MBA assay with current detection of chlamydia in urine (86% [36/42]), a past-year diagnosis of chlamydia (81.8% [27/33]), and a history of treatment for pelvic inflammatory disease (60.7% [37/61]). Seroprevalence of *C. trachomatis* Pgp3 IgG, as measured by MBA, was significantly higher among non-Hispanic Blacks (68.0%; adjusted prevalence ratio (aPR) = 2.7 [95% CI, 2.3-3.3]), Mexican Americans (30.9%; aPR = 1.5 [95% CI, 1.2-1.9]), and other Hispanics (35.0%; aPR = 1.9 [95% CI, 1.4-2.5]) compared with non-Hispanic Whites (21.4%). A higher lifetime number of sexual partners and a younger age at sexual debut was also associated with higher seroprevalence.

Conclusion. Both the ELISA and MBA serologic assays revealed a high prevalence of antibodies to *C. trachomatis* Pgp3 in young adult females in the US household population. There were major racial/ethnic disparities in exposure to *C. trachomatis*, with increased vulnerability among non-Hispanic Black females.

Keywords. *Chlamydia trachomatis*; Pgp3 antibody; National Health and Nutrition Examination Surveys (NHANES); seroprevalence; enzyme-linked immunosorbent assay (ELISA); multiplex bead array (MBA).

Chlamydia trachomatis is a nationally notifiable infection in the United States and is reported more than any other sexually transmitted infectious disease, with more than 1.7 million infections in 2018 [1, 2]. Although easily treated, *C. trachomatis* infection is often asymptomatic. When left untreated, however, *C. trachomatis* infection can lead to negative health outcomes, such as pelvic inflammatory disease (PID) in women, congenital *C. trachomatis* infection via in utero transmission, and increased risk of human immunodeficiency virus acquisition [2–5]. People younger than age 25 years compose the majority of reported infections, and cases are higher in Black, Hispanic, American Indian/Native Alaskan, and Native Hawaiian/Other Pacific Islander persons compared with White persons [2, 6]. Over the past 20 years, *C. trachomatis* screening has substantially increased in the United States. It is currently recommended by the US Centers for Disease Control and Prevention (CDC) that women younger than age 25 years be screened annually, in addition to high-risk women 25 years or older [2]. Because infection is frequently asymptomatic, people may not know they are infected and therefore would not seek sexually transmitted infection testing and treatment [2, 7]. Therefore, surveillance-based case reporting of *C. trachomatis* infection may be biased.

Because tests for *C. trachomatis* infection by nucleic acid cannot determine past infection, serological assays are preferable to determine prevalence of prior exposure [8]. Serological testing for *C. trachomatis* can prove challenging because of cross-reactivity with other pathogens, especially other *Chlamydia* species, such as *Chlamydia pneumoniae* [9]. Immunoglobulin G (IgG) antibodies to plasmid gene

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product 3 (Pgp3) have been identified as the most reliable marker of exposure to *C. trachomatis* because Pgp3's genetic code is generally highly conserved across isolates and rarely found in *C. pneumoniae* [9–11]. In addition, *C. trachomatis* Pgp3 antibodies appear to persist for > 10 years in women [9, 12, 13]. Sensitivity and specificity of *C. trachomatis* Pgp3 antibody enzyme-linked immunosorbent assays (ELISAs) also tend to outperform ELISAs for other *C. trachomatis* antigens [8, 9, 12].

Estimating prevalence of prior exposure to *C. trachomatis* has many applications to *C. trachomatis* control, including determining potential vaccination strategies, as well as estimating the contribution of *C. trachomatis* for a variety of negative health outcomes [3, 8]. Nationally representative seroprevalence estimates of *C. trachomatis* in the United States have not been calculated previously. Therefore, the goal of this study was to estimate *C. trachomatis* seroprevalence among adult females in the noninstitutionalized, civilian population in the United States and describe the agreement of 2 serological assays that detect *C. trachomatis* Pgp3 antibodies.

METHODS

Data Source and Population

Data for this study are from the continuous National Health and Nutrition Examination Surveys (NHANES), a cross-sectional, complex survey conducted by the National Center for Health Statistics (NCHS) [14]. NHANES uses a stratified, multistage probability sampling design to generate representative estimates of the noninstitutionalized, civilian US population. The survey includes both in-person household interviews, where demographic and health-related information is collected through in person computer-assisted personal interviews and in-person visit to a medical examination center (MEC) with collection of additional health-related information via audio computer-assisted self-interviews, physical examinations, and biological specimens [15]. The overall response rate for the MEC component among females was 68.8% in 2013 and 2014 and 60.0% in 2015 and 2016 [16].

This analysis used data from females aged 18 to 39 years who participated in the MEC component of the 2013 through 2016 NHANES. Serum samples for *C. trachomatis* antibody testing were collected only among females who also provided urine samples for *C. trachomatis* nucleic acid detection. A total of 2250 females aged 18 to 39 years participated in the MEC component, of which 2195 provided urine samples, and 1725 females provided both urine and serum samples (Supplemental Figure 1).

Laboratory Testing

Urine and blood samples were frozen at –30°C and –80°C, respectively, and shipped to the CDC laboratories in Atlanta, Georgia, where they were tested [17]. Current *C. trachomatis* infections were detected with BDProbeTec *Chlamydia trachomatis* Amplified DNA Assay on urine samples [18, 19].

Detection of IgG antibody to *C. trachomatis* Pgp3 was performed on serum samples that had not undergone previous freeze thaw cycles, with 2 separate assays: the Luminex MAGPIX multiplex bead array (MBA [Luminex Corp., Austin, Texas]) and an indirect ELISA [3, 20–22]. Both assays were verified in house by the CDC's Laboratory Reference and Research Branch in the Division of Sexually Transmitted Disease Prevention. MBA assays were run once; unreadable results or tests that experienced error were rerun and the second test results were used. ELISA assays were run twice and the results averaged. In the case of error during the ELISA, it was rerun twice again, with the results averaged. Serum specimens were shipped to and analyzed at the CDC's Laboratory Reference and Research Branch [23].

Seropositivity for herpes simplex virus 2 (HSV-2) was detected at Emory University using a solid-phase enzymatic ImmunoDOT assay for gG-2, a glycoprotein that is not cross-reactive with HSV-1 [24–26].

Questionnaire Data

Self-reported data on age, sex, race/ethnicity, marital status, education, place of birth, and household income were all collected in the participant's household. Marital status was asked only of participants age 20 years or older; therefore a "not asked" category was created for participants ages 18 to 19 years. Participants age 20 years and older were categorized as either "never married," "married/living with partner," and "widowed divorced/separated." Poverty status was defined as either having an annual household income that was below the federal poverty line or having a household income that was at or above the poverty line. History of treatment for PID was collected at the MEC through computer-assisted personal interview. A sexual behavior questionnaire was administered in the MEC using an audio computer-assisted self-interview, including data on a self-reported diagnosis of chlamydia in the past 12 months. Lifetime and past-year sexual partners included both same and opposite sex partners, as well as anal, vaginal, and oral sex partners. If someone responded they had never had sex, values of "0" were imputed for sex partner in the past year and "no" for having a new sex partner in the past year. For those who reported not having a partner in the past year, but were sexually experienced, values of "no" were imputed for having a new sex partner in the past year.

Statistical Analysis

Concordance and discordance of results on the MBA and ELISA were described using unweighted observations. Positivity of the tests among those who had current detection, who reported being diagnosed with chlamydia in the past 12 months or been treated for PID previously were also

explored to evaluate test performance (ie, percent positive agreement). In a supplemental analysis, a univariable multinomial logistic regression was performed among females who were positive on at least 1 assay to explore factors associated with positive concordance and discordance on anti-*C. trachomatis* Pgp3 IgG assays. The possible outcomes were: ELISA positive and MBA negative, ELISA negative and MBA positive, and positive on both the ELISA or MBA.

Prevalence of anti-*C. trachomatis* Pgp3 IgG and factors associated with seropositivity were examined using MEC weights, which were calculated by the NCHS to account for nonresponse and unequal probability of selection. Additionally, NCHS poststratifies the weights to match the population counts from the Census Bureau's American Community Survey. Because this analysis combines data from 2 NHANES cycles, MEC weights were adjusted according to NCHS guidelines. Taylor series linearization was used for variance estimation. Korn-Graubard 95% confidence intervals (95% CI) were calculated for prevalence estimates [27].

Prevalence of anti-*C. trachomatis* Pgp3 IgG was defined 2 separate ways: (1) positive on ELISA and (2) positive on MBA. Sociodemographic and sexual behavioral factors associated with ELISA and MBA anti-Pgp3 IgG positivity were examined using univariable and multivariable Poisson regression. Prevalence estimates as well as prevalence ratios for age of sexual debut were calculated among only those who were sexually experienced, whereas all other prevalence estimates and prevalence ratios were estimated among all participants. As an ancillary analysis, age- and race-specific prevalence estimates of anti-*C. trachomatis* Pgp3 IgG (as measured by MBA) and anti-HSV-2 IgG were comparatively examined.

A sensitivity analysis was performed examining prevalence of anti-*C. trachomatis* Pgp3 IgG defined 2 additional ways: (1) positive on both the ELISA and MBA, and (2) positive on either the ELISA and MBA. Prevalence of being positive on both assays provides the most conservative (specific) population-level estimates for anti-*C. trachomatis* Pgp3 IgG positivity, whereas prevalence by being positive on either provides the most liberal (sensitive) population-level estimates. A third sensitivity analysis was performed using multiply imputed data to account for item nonresponse among female participants aged 18 to 39 years who participated in the MEC component, particularly for anti-*C. trachomatis* Pgp3 IgG and other variables of interest. Imputation for missing variables was done using predictive mean matching for continuous variables, logistic regression for binary variables, and random forests for categorical variables. Missingness by inclusion and exclusion in the main analysis is shown in Supplementary Table 1. All variables used in the primary analysis were added to the imputation model as well as additional auxiliary variables (smoking, alcohol use, drug use, insurance status, body mass index, healthcare use, pregnancy status, family size, military service, time of year of interview). Survey weights and stratum were also used in the imputation model.

All weighted analyses were performed in R version 3.6.1. using the "survey" package, whereas imputation was performed using the "mice" package [28, 29].

Ethics Statement

Data collection was approved by the NCHS Research Ethics Board. The analysis was conducted using deidentified publicly available data and was waived from review by Johns Hopkins University School of Medicine institutional review board.

RESULTS

Assay Concordance/Discordance

Of the 1725 females who were tested for anti-*C. trachomatis* Pgp3 IgG by both ELISA and MBA assays, 40.4% (697/1725) tested positive on at least 1 assay, 33.7% (582/1725) tested positive on the ELISA, whereas 34.2% (590/1725) tested positive on the MBA and 27.5% (475/1725) were positive on both. Among all participants, 87.1% (1503/1725) had concordant results, whereas 68.1% (475/697) of participants who tested positive on at least 1 assay, tested positive on both (Figure 1).

Among those who had current *C. trachomatis* infection (n = 42), the MBA assay had a slightly higher positive percentage agreement as opposed to the ELISA (85.7% [n = 36] vs. 78.6% [n = 33]). Across all definitions of anti-*C. trachomatis* Pgp3 IgG, a significantly higher prevalence of anti-*C. trachomatis* Pgp3 IgG was found among those who had current *C. trachomatis* infection than those who did not have current *C. trachomatis* infection (MBA, 85.7% vs. 32.9%, respectively). Similar findings were observed with a self-reported diagnosis of chlamydia in the past 12 months and those who have ever been treated for PID.

Distribution of sociodemographic and behavioral characteristics by results on both assays, including those who were concordant negative, are shown in Supplemental Table 2. Factors associated with being positive by only 1 assay compared with both assays are shown in Supplemental Table 3. Those who were married/living with partner were more likely to be positive on 1 assay, but not both (discordant), whereas non-Hispanic Black females and those with a higher number of lifetime sexual partners were more likely to be positive on both (concordant).

Seroprevalence by Sociodemographics

The overall population-level prevalence of anti-*C. trachomatis* Pgp3 IgG in females aged 18 to 39 years was similar when measured by ELISA (30.0% [95% CI, 25.5-35.0] and by MBA (29.4% [95% CI, 25.8-33.0]) (Table 1). Seroprevalence measured by being positive on both ELISA and MBA was expectedly lower at 23.0% (95% CI, 19.7-27.0), whereas it was expectedly higher when examined by being positive on either ELISA or MBA (36.3% [95% CI, 32.0-41.0]). Seroprevalence estimated

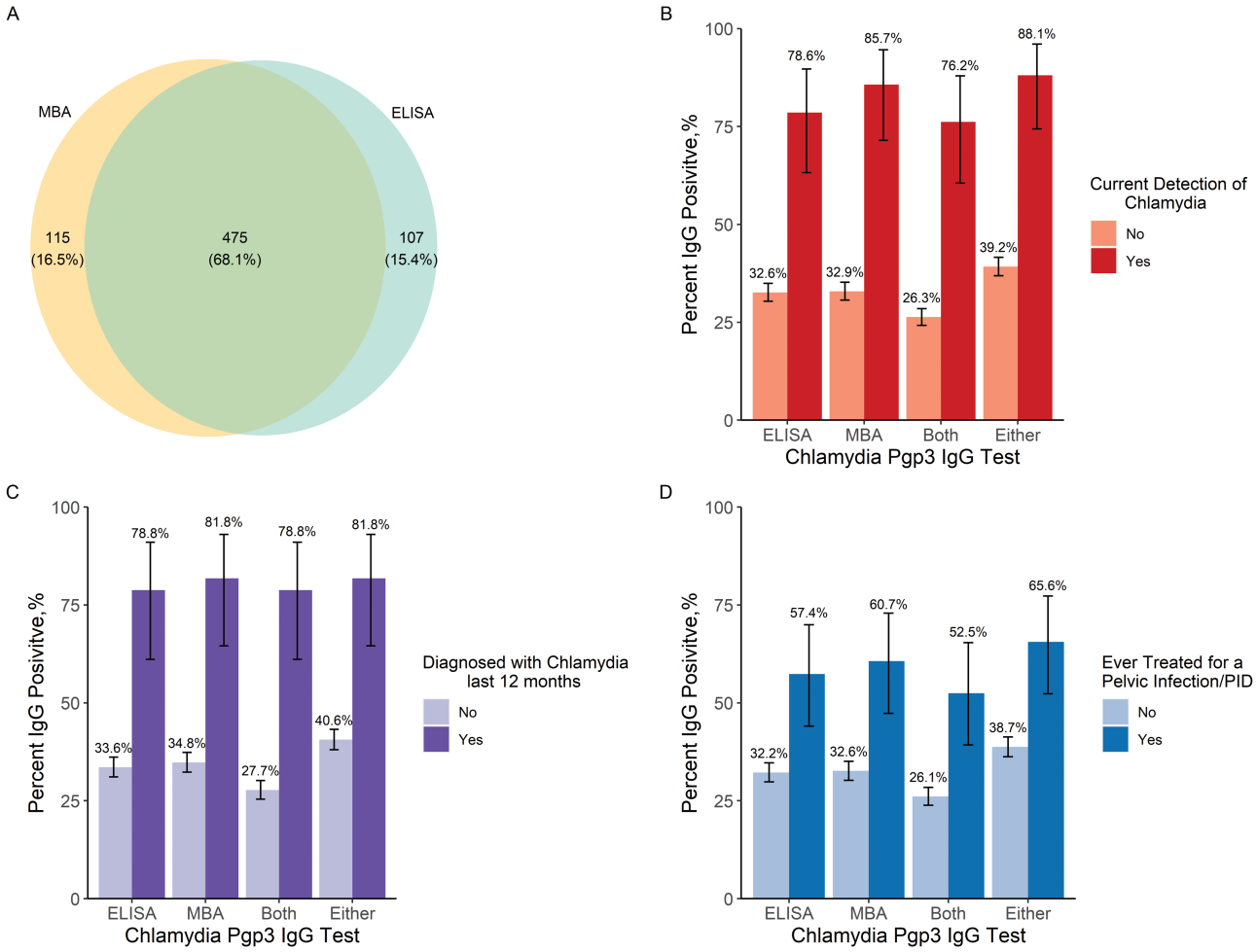

Figure 1. *Chlamydia trachomatis* seroprevalence among US females aged 18 to 39 years. *A*, Concordance and discordance of 2 anti-*C. trachomatis* Pgp3 IgG assays among females age 18–39 (n = 697). MBA positivity is represented in yellow, ELISA positivity represented in blue and concordance of MBA and ELISA in green. *B*, Anti-*C. trachomatis* Pgp3 IgG positivity among females with current chlamydia detection by urine NAAT test (n = 42) and without chlamydia detection (n = 1683). *C*, Anti-*C. trachomatis* Pgp3 IgG positivity among females diagnosed with chlamydia in the past 12 months (n = 33) and those not diagnosed (n = 1391). *D*, anti-*C. trachomatis* Pgp3 IgG positivity among females ever treated for PID or pelvic infection (n = 61) and those not diagnosed (n = 1471). Note: Data are unweighted. 95% confidence intervals are exact binomial confidence intervals. Definitions of positivity: ELISA, positive on the anti-pgp3 ELISA; MBA, positive on the anti-pgp3 MBA; Both, positive on both an anti-pgp3 MBA and ELISA; Either, positive on either the anti-pgp3 MBA and ELISA. Abbreviations: IgG, immunoglobin G; ELISA, enzyme-linked immunosorbent assay; MBA, multiplex bead format assay; NAAT, nucleic acid amplification test; Pgp3, plasmid gene product 3; PID, pelvic inflammatory disease.

by ELISA and MBA were fairly similar when stratified by individual characteristics.

By MBA, seroprevalence was highest among non-Hispanic Black females (68.0% [95% CI, 61.1-74.4]). Seroprevalence was also higher in Mexican Americans (30.9% [95% CI, 26.2-36.0]), other Hispanics (35.0% [95% CI, 28.1-42.4]), and people of other race/multiracial (35.9% [95% CI, 22.5-51.2]) compared with non-Hispanic Whites (21.4% [95% CI, 17.5-25.7]). ELISA underestimated the seroprevalence in non-Hispanic Black females in comparison to MBA.

There was an inverse relationship between seroprevalence and both education and poverty status. Those with educational attainment of less than a high school degree (MBA, 41.6% [95% CI, 34.7-48.7]) or who were under the federal poverty level (MBA, 42.5% [95% CI, 35.8-49.2]) had the highest seroprevalence, while those with a college degree or higher (MBA, 25.4% [95% CI, 22.0-29.0]) or who were at or above the poverty level (MBA, 25.0% [95% CI, 21.0-29.3]) had the lowest seroprevalence.

Seroprevalence by Sexual Behaviors

There were differences in seroprevalence by both reported lifetime and recent sexual behaviors. Seroprevalence increased with both an increase of lifetime sexual partners and increase of past years sexual partners. Among sexually experienced persons, seroprevalence was higher among those whose sexual debut was at 16 years or younger (MBA, 43.4% [95% CI, 38.7-48.2]) compared with those who initiated sex at 19 years or older (MBA, 10.2% [95% CI, 6.3-15.3]).

Table 1. Seroprevalence of *Chlamydia trachomatis* Overall and Stratified by Individual-Level Characteristics in US Females Ages 18-39, as Measured by 4 Definitions of Seropositivity[a]

	ELISA	MBA	Both	Either
Characteristics	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)	Weighted % (95% CI)
Overall	30.0 (25.5–35.0)	29.4 (25.8–33.0)	23.0 (19.7–27.0)	36.3 (32.0–41.0)
Age, y				
18–24	27.4 (22.4–32.9)	27.9 (22.9–33.2)	21.7 (17.8–25.9)	33.6 (27.8–39.8)
25–29	29.3 (23.3–35.8)	27.4 (22.6–32.6)	22.1 (17.3–27.4)	34.6 (28.8–40.8)
30–34	31.5 (24.6–39.1)	30.4 (23.9–37.5)	21.3 (16.0–27.4)	40.6 (34.4–46.9)
35–39	32.3 (25.3–40.0)	32.4 (25.2–40.3)	27.5 (21.2–34.5)	37.3 (29.1–45.9)
Race/ethnicity				
Non-Hispanic White	23.0 (18.1–28.5)	21.4 (17.5–25.7)	14.8 (11.6–18.5)	29.6 (24.9–34.6)
Non-Hispanic Black	60.5 (54.4–66.5)	68.0 (61.1–74.5)	59.7 (53.5–65.8)	68.8 (61.9–75.2)
Mexican American	31.8 (25.9–38.2)	30.9 (26.1–36.0)	25.4 (20.6–30.7)	37.4 (31.5–43.5)
Other Hispanic	35.1 (27.8–42.9)	35.0 (28.1–42.4)	28.7 (21.8–36.6)	41.3 (34.7–48.2)
Non-Hispanic Asian	17.0 (11.0–24.4)	15.5 (10.3–22.1)	9.9 (5.6–15.8)	22.6 (16.1–30.2)
Other race, including multiracial	43.0 (23.2–64.5)[a]	35.9 (22.5–51.2)	33.4 (21.2–47.6)	45.5 (24.2–68.1)[a]
Born in the 50 US states				
No	30.3 (25.3–35.7)	29.8 (25.5–34.5)	23.5 (19.7–27.6)	36.7 (31.6–42.0)
Yes	28.5 (23.0–34.4)	27.4 (23.4–31.7)	21.0 (16.7–26.0)	34.8 (29.7–40.2)
Poverty status				
Below the poverty level	39.2 (33.0–45.6)	42.4 (35.8–49.2)	35.1 (29.6–41.0)	46.4 (39.5–53.4)
At or above poverty level	26.3 (21.7–31.4)	25.0 (21.0–29.3)	19.0 (15.6–22.7)	32.4 (27.8–37.2)
Education				
Less than high school	36.8 (30.2–43.8)	41.6 (34.7–48.7)	32.2 (26.0–38.9)	46.2 (38.9–53.6)
GED/high school degree/some college	34.0 (27.2–41.2)	34.5 (27.5–42.1)	28.2 (21.9–35.2)	40.3 (33.2–47.8)
College degree of higher	27.4 (22.6–32.5)	25.4 (22.0–29.0)	19.6 (16.7–22.8)	33.1 (28.5–38.1)
Marital status				
Never married	34.7 (29.5–40.3)	33.9 (29.1–38.8)	29.2 (25.1–33.6)	39.4 (33.7–45.3)
Married/living with partner	25.8 (21.3–30.6)	26.2 (21.7–31.1)	18.6 (15.0–22.7)	33.3 (28.7–38.1)
Widowed/divorced/separated	48.6 (35.2–62.2)	45.7 (35.5–56.1)	38.2 (28.6–48.6)	56.1 (42.9–68.6)
Not asked[b]	23.4 (15.9–32.4)	18.9 (12.4–26.8)	15.3 (10.7–20.8)	27.0 (18.5–37.0)
Lifetime sexual partners				
0–1	11.2 (7.0–16.8)	7.4 (4.9–10.7)	4.8 (3.0–7.1)	13.9 (9.1–20.0)
2–3	23.9 (17.3–31.4)	18.6 (13.4–24.9)	14.3 (10.3–19.1)	28.2 (21.0–36.4)
4–7	37.1 (30.4–44.3)	40.7 (33.7–48.0)	33.1 (27.3–39.4)	44.7 (37.0–52.6)
8–15	37.4 (28.8–46.7)	38.5 (31.7–45.8)	29.8 (23.0–37.3)	46.2 (37.8–54.7)
> 15	43.2 (31.0–56.0)	47.2 (36.9–57.6)	38 (27.9–48.9)	52.3 (40.3–64.2)
Sexual partner over past 12 mo				
0	18.6 (10.0–30.3)	14.5 (8.8–21.9)	9.6 (4.9–16.3)	23.5 (14.3–34.9)
1	28.1 (23.8–32.8)	27.8 (23.9–32.0)	22.1 (18.8–25.7)	33.8 (29.2–38.8)
2	38.7 (30.4–47.5)	40.2 (34.0–46.7)	31.4 (25.0–38.3)	47.5 (39.9–55.2)
New sexual partner over past 12 mo				
No	28.1 (23.6 – 33.0)	27.7 (23.8–31.8)	21.9 (18.6–25.4)	33.9 (29.0–39.0)
Yes	34.9 (27.4 – 43.0)	34.9 (28.5–41.7)	26.8 (20.8–33.6)	43.0 (36.0–50.2)
Age of sexual debut, y[c]				
≤16	42.1 (36.9–47.4)	43.4 (38.7–48.2)	35.5 (31.6–39.6)	49.9 (44.1–55.7)
17–18	24.8 (17.6–33.3)	26.5 (21.7–31.8)	19.0 (14.2–24.6)	32.4 (25.3–40.1)
≥19	13.6 (9.1–19.2)	10.2 (6.3–15.3)	7.3 (4.7–10.6)	16.5 (12.0–22.0)

All data are weighted using pooled Medical Examination Center (MEC) survey weights provided by the National Center for Health Statistics.

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobin G; MBA, multiplex bead format assay; US, United States.

[a]Definitions of positivity: ELISA, positive on the anti-pgp3 ELISA; MBA, positive on the anti-pgp3 MBA; both, positive on both an anti-pgp3 MBA and ELISA; either, positive on either the anti-pgp3 MBA and ELISA.

[b]Not asked of participants ages 18 to 19 years.

[c]Only among those sexually experienced.

Associations with *C. trachomatis* Seroprevalence

Associations with anti-*C. trachomatis* Pgp3 IgG are shown in Table 2. Characteristics associated with being positive by ELISA were similar to characteristics associated with being positive by MBA. In multivariable analyses, racial/ethnic minorities were significantly associated with being seropositive. Compared with

non-Hispanic White females, non-Hispanic Black females had 2.7 (95% CI, 2.3-3.3, MBA) times the anti-*C. trachomatis* Pgp3 IgG prevalence. Having more lifetime sexual partners was also associated with being seropositive, as well as having a younger age at sexual debut. Although household poverty status and being widowed/divorced/separated (vs being never married) were significant predictors of anti-*C. trachomatis* Pgp3 IgG seropositivity in a univariable analysis, this was not observed in multivariable analysis.

Table 2. Factors Associated With Positivity for Anti-*C. trachomatis* Pgp3 IgG

	ELISA		MBA	
Characteristics	PR (95% CI)	adjPR[a] (95% CI)	PR (95% CI)	adjPR[a] (95% CI)
Age				
18–24	1	1	1	1
25–29	1.1 (0.8–1.4)	1.0 (0.8–1.2)	1.0 (0.8–1.3)	0.8 (0.7–1.0)
30–34	1.1 (1.0–1.4)	1.0 (0.9–1.2)	1.1 (0.8–1.4)	0.9 (0.7–1.2)
35–39	1.2 (1.0–1.5)	1.1 (0.9–1.4)	1.2 (0.9–1.5)	1.0 (0.8–1.2)
Race/ethnicity				
Non-Hispanic White	1	1	1	1
Non-Hispanic Black	**2.6 (2.1–3.3)**	**2.4 (1.8–3.1)**	**3.2 (2.7–3.8)**	**2.7 (2.3–3.3)**
Mexican American	**1.4 (1.1–1.8)**	**1.5 (1.2–2.1)**	**1.4 (1.1–1.8)**	**1.5 (1.2–1.9)**
Other Hispanic	**1.5 (1.2–2.0)**	**1.8 (1.3–2.5)**	**1.6 (1.3–2.1)**	**1.9 (1.4–2.5)**
Non-Hispanic Asian	0.7 (0.5–1.1)	1.0 (0.6–1.5)	0.7 (0.5–1.1)	1.1 (0.7–1.7)
Other race, including multiracial	**1.9 (1.3–2.8)**	1.6 (1.0–2.6)	**1.7 (1.1–2.6)**	1.3 (0.9–2.0)
Born in the 50 US states				
No	1	1	1	1
Yes	0.9 (0.8–1.2)	0.9 (0.7–1.2)	0.9 (0.7–1.1)	1.0 (0.8–1.2)
Poverty				
Below the poverty level	1	1	1	1
At or above poverty level	**0.8 (0.7–0.9)**	**0.9 (0.8–1.0)**	**0.7 (0.6–0.8)**	0.9 (0.8–1.0)
Education				
Less than high school	1	1	1	1
GED/high school degree/some college	**0.8 (0.7–0.9)**	0.8 (0.7–1.0)	**0.7 (0.6–0.8)**	**0.7 (0.6–0.9)**
College degree of higher	0.9 (0.8–1.1)	1.0 (0.8–1.2)	1.0 (0.8–1.1)	1.0 (0.8–1.2)
Marital status				
Never married	1	1	1	1
Married/living with partner	**0.7 (0.6–0.9)**	0.9 (0.7–1.1)	**0.8 (0.6–0.9)**	1.1 (0.9–1.3)
Widowed/divorced/separated	**1.4 (1.1–1.8)**	1.1 (0.9–1.5)	**1.3 (1.1–1.7)**	1.1 (0.8–1.5)
Not asked[b]	**0.7 (0.5–0.9)**	0.9 (0.7–1.3)	**0.6 (0.4–0.8)**	0.9 (0.6–1.2)
Lifetime sexual partners				
0–1	1	1	1	1
2–3	**2.1 (1.3–3.4)**	**1.9 (1.2–3.1)**	**2.5 (1.6–3.9)**	**2.2 (1.5–3.3)**
4–7	**3.3 (2.3–4.8)**	**2.8 (1.9–4.1)**	**5.5 (3.8–7.8)**	**5.0 (3.5–7.1)**
8–15	**3.3 (2.3–4.7)**	**3.0 (2.1–4.5)**	**5.2 (3.5–7.6)**	**5.4 (3.7–7.9)**
>15	**3.8 (2.5–6.0)**	**3.6 (2.2–6.0)**	**6.3 (4.3–9.5)**	**6.9 (4.7–10.3)**
Sexual partner over past 12 mo				
0	1	-	1	-
1	1.5 (0.9–2.4)	-	**1.9 (1.3–2.9)**	-
2	**2.1 (1.4–3.2)**	-	**2.8 (1.8–4.3)**	-
New sexual partner over past 12 mo				
No	1	1	1	1
Yes	1.2 (1.0–1.5)	0.9 (0.7–1.1)	1.3 (1.0–1.5)	0.9 (0.7–1.1)
Age of sexual debut, y[c]				
≤16	1	1	1	1
17–18	**0.6 (0.5–0.8)**	**0.7 (0.5–0.9)**	**0.6 (0.5–0.7)**	**0.8 (0.7–0.9)**
≥19	**0.3 (0.2–0.4)**	**0.5 (0.4–0.7)**	**0.2 (0.2–0.3)**	**0.4 (0.2–0.6)**

All data are weighted using pooled Medical Examination Center (MEC) survey weights provided by the National Center for Health Statistics. Prevalence ratios were estimated by Poisson regression. Boldface indicate significant association, where the 95% confidence interval does not cross the null value of 1.0.

Abbreviations: adjPR, adjusted prevalence ratio; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobin G; MBA, multiplex bead format assay; Pgp3, plasmid gene product 3; PR, prevalence ratio; US, United States.

[a]Multivariable model includes age, race, place of birth, poverty status, education, marital status, lifetime sexual partners, and a new sexual partner over the past 12 months. Multivariable model for age of sexual debut was a separate model that included an additional covariate for age of sexual debut.

[b]Not asked of participants ages 18 to 19 years.

[c]Only among those sexually experienced.

Age- and Race-Specific Seroprevalence of *C. trachomatis* and HSV-2

Figure 2 compares the seroprevalence of *C. trachomatis* measured by the anti-Pgp3 MBA assay with HSV-2 seroprevalence by race/ethnicity and age. In general, although the HSV-2 seroprevalence is lower than *C. trachomatis* seroprevalence, there appeared to be similar distributions by race/age categories that have higher prevalence of HSV-2 also have higher prevalence of *C. trachomatis*, with highest prevalence in non-Hispanic Black females. While HSV-2 seroprevalence increased with older age across race/ethnicity groups, *C.* trachomatis seroprevalence remained relatively stable for all race/ethnicity groups except non-Hispanic black females. Among non-Hispanic black females, *C.* trachomatis seroprevalence rapidly increased with older age until age 25 but then plateaued.

Sensitivity Analyses

Factors associated with being seropositive on both the ELISA and MBA, or by at least 1 of the assays were similar to those observed with the individual assays (Supplemental Table 4). Results from the multiple imputation are shown in Supplemental Tables 5 and 6. Seroprevalence estimates and measures of association were insensitive to imputation of missing data.

DISCUSSION

This study found a high prevalence of anti-*C. trachomatis* Pgp3 IgG (~30%) among young adult females in the US household population. In addition to providing the first national estimates, this study demonstrates that seroprevalence is dramatically higher among racial/ethnic minorities. This study also shows that, although the ELISA and MBA for Pgp3 IgG perform well, they do not provide consistent results and the MBA assay is likely superior.

In this study, seroprevalence of *C. trachomatis* was disproportionately higher among racial/ethnic minorities. Non-Hispanic Black females had the highest seroprevalence, with approximately two-thirds having prior exposure to *C. trachomatis* infection. Seroprevalence of *C. trachomatis* was also elevated among Mexican American, other Hispanic, and multiracial/other race females compared with non-Hispanic Whites. These are among the first nationally representative serological data demonstrating such striking racial/ethnic disparities in *C. trachomatis* infection in the United States. These data are consistent with findings of national surveillance data for reported current infections in minorities in the United States, as well as serosurveys of *C. trachomatis* Pgp3 conducted in other high-income settings including England [6, 30]. Some of the factors that may contribute to the observed racial disparities in *C. trachomatis* infection include differences in individual-level sexual behaviors, differences in sexual networks, differential access to sexually transmitted infection prevention and treatment services, and structural racism more broadly [6, 31, 32].

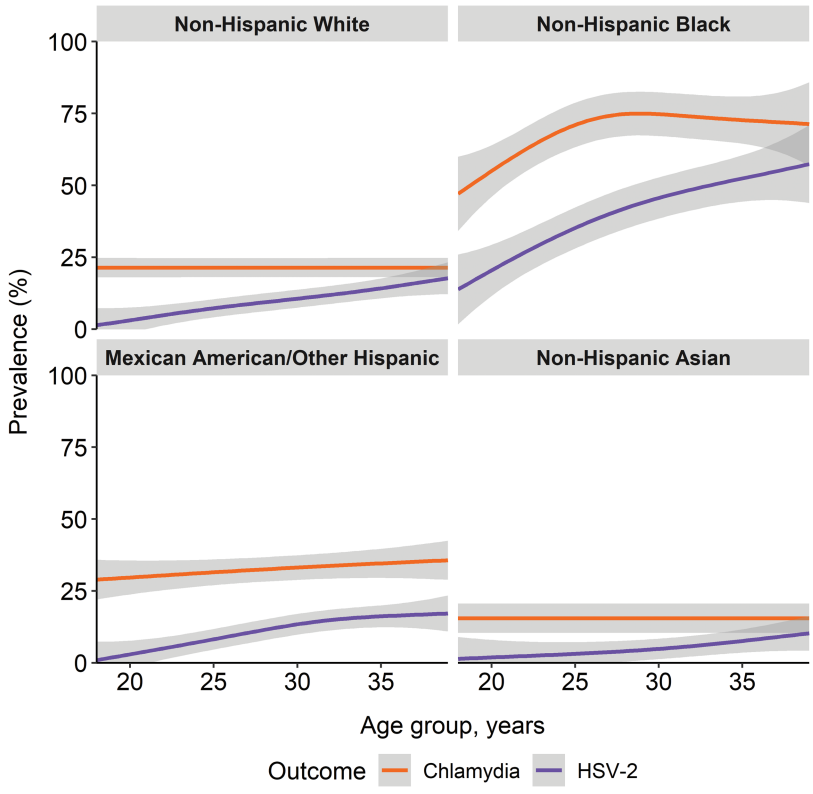

Figure 2. Seroprevalence of *Chlamydia trachomatis* (*C. trachomatis*) and herpes simplex virus 2 by age and race among US females age 18 to 39 years. Note: *C. trachomatis* prevalence as determined by a multiplex bead array (MBA). Abbreviations: HSV-2, herpes simplex virus 2; US, United States.

Seroprevalence was also elevated with an increase of lifetime sexual partners and high-risk sexual behaviors, indicating that these data are a good measure of the tremendous total burden of previous and current infection, which is significantly higher than current infections. Antibodies to HSV-2 have been thought of as a biological marker for sexual behavior and a way to indicate risk of other sexually transmitted infections [33–36]. As demonstrated in this study, HSV-2 seroprevalence is lower than *C. trachomatis* seroprevalence; however, they largely follow similar distributions by age and race with a large increase over younger ages in non-Hispanic Black females. Previous studies have demonstrated that *C. trachomatis* antibody detection decreases over time, but remain longer for women who have been infected multiple times [37, 38]. This suggests that the lifetime seroprevalence is generally underestimated using Pgp3-antibody assays and the racial disparities in *C. trachomatis* exposure may potentially be greater than that observed here. Sensitivity of Pgp3 ELISAs have also shown to decrease over time since infection [37].

A strength of this study is the comparison between the ELISA and MBA for Pgp3 IgG. Although both assays generally yielded similar associations and prevalence estimates, the lack of concordance between assays is concerning. The MBA assay is likely superior than the ELISA, as demonstrated by the slightly higher percentage agreement with currently detected *C. trachomatis* infection by urine nucleic acid amplification test (NAAT), females diagnosed with *C. trachomatis* during the past year, and also with females reporting being previously treated for PID. The higher concordance of the MBA with NAAT positivity compared with the Pgp3 ELISA has also been observed previously in women with PID [22]. The MBA assay has also been shown to be a superior method for detection of antibodies to *C. trachomatis* in trachoma [39].

There are several limitations to this study. The study population only included females between 18 and 39 years of age. It would have been ideal to include adolescent females as well as males, although Pgp3 testing is less sensitive in males [9, 12]. There is no widely accepted gold standard assay for chlamydia IgG, and this study used an indirect ELISA, which has been shown to be less sensitive than a double antigen ELISA [12]. Another weakness is that Pgp3 antibody may decrease over time, which may lead to underestimated seroprevalence estimates. In addition, urine testing in women has a lower sensitivity than vaginal swabs for *C. trachomatis* detection [40]. There is also possible selection bias because participants had to attend the MEC and agree to provide both urine and serum samples. Additionally, because high-risk persons including incarcerated individuals and homeless persons were not in the sampling frame, these data may not be generalizable to the entire US population. Nonetheless, NHANES is designed to be a nationally representative survey, and these data provide among the first population-based serological data for *C. trachomatis*.

This population-based study demonstrates that antibodies to *C. trachomatis* Pgp3 correlate with current detection of *C. trachomatis* infection, recent history of chlamydia diagnosis, and increased sexual partners, validating their use for serosurveillance, particularly using the MBA assay. As such, *C. trachomatis* serosurveillance data will be invaluable for performance and evaluation of future vaccine trials [3, 8], as well as other prevention measures including screening. Notably, these data also highlight stark racial/ethnic disparities in exposure to *C. trachomatis*. Increased public health interventions are needed to improve racial inequities in sexual health in the United States.

Supplementary Data

Supplementary materials are available at *Clinical 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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