Toxoplasma gondii Infection in the United States, 2011–2014

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Abstract. *Toxoplasma gondii* can cause severe neurologic and ocular disease when transmitted congenitally and in immunosuppressed persons. Sera collected in the National Health and Nutrition Examination Survey 2011 through 2014 in 13,507 persons ≥ 6 years old were tested for *T. gondii* immunoglobulin (Ig) G and IgM antibodies, and in those both IgG and IgM antibody positive, for IgG avidity. Overall, 11.14% (95% confidence limits [CL] 9.88%, 12.51%) were seropositive for *T. gondii* IgG antibody (age-adjusted seroprevalence 10.42% [95% CL 9.19%, 11.76%]); in women aged 15–44 years, the age-adjusted *T. gondii* IgG seroprevalence was 7.50% (95% CL 6.00%, 9.25%). In multivariable analysis, risk for IgG seropositivity increased with age and was higher in males; persons living below the poverty level; persons with $\leq$ a high school education compared with those with $>$ a high school education; and non-Hispanic black, Mexican American, and foreign born non-Hispanic white persons compared with U.S.-born non-Hispanic white persons. Overall, 1.16% (95% CL 0.94%, 1.42%) were *T. gondii* IgM antibody positive and 0.71% (95% CL 0.54%, 0.92%) were both IgM and IgG antibody positive. In multivariable analysis, the significant risk factors for being both IgM and IgG positive were older age, crowding, and non-U.S. birth origin compared with U.S.-born persons. Among those positive for both IgM and IgG antibody, almost all had high avidity (all women aged 15–44 years had high avidity). *Toxoplasma gondii* antibody prevalence remains relatively low in the United States, although it is higher in non-U.S.-born persons, males, and some minority and socioeconomically disadvantaged groups.

INTRODUCTION

Toxoplasma gondii is one of the most common parasites that infect humans. Although infection is often asymptomatic in persons with a normal immune system, it is responsible for thousands of cases of encephalitis and systemic disease in immune-suppressed persons annually in the United States.¹ Infection can also lead to ocular disease in those with normal immunity or who are immunosuppressed, with up to 2% of those infected in the United States having ocular lesions.² In addition, congenital infection with *T. gondii* occurs in 400–4,000 infant births annually in the United States and can lead to neurological sequelae or ocular diseases.³ *Toxoplasma gondii* has also been found to be associated with mental illnesses^{4,5} and worse cognitive function in HIV-infected individuals⁶; however, there is not currently sufficient evidence to indicate these associations are causal.

The Centers for Disease Control and Prevention (CDC) monitors *T. gondii* infection in the United States by periodically testing sera for *T. gondii* antibodies from a representative sample of the population collected in the National Health and Nutrition Examination Survey (NHANES). In the present study, we assessed *T. gondii* immunoglobulin (Ig) G antibodies in NHANES 2011–2014, and in addition, we evaluated *T. gondii* IgM antibodies (an early marker of infection) and IgG avidity (a marker for persons infected longer than 4 months) in an attempt to examine demographic and risk factors among those more recently infected. *Toxoplasma gondii* IgG antibodies are thought to last throughout life with antibodies stimulated by chronic intracellular cysts; however, definitive life-long serologic studies have not been conducted.^{7,8} *Toxoplasma gondii* IgM antibodies usually persist for less than a year, although

studies have found that they can persist for 18 months or longer, and because false-positive tests occur, confirmation of *T. gondii* IgM test results at a reference laboratory is recommended for patient management.^{9,10}

MATERIALS AND METHODS

Serologic data from NHANES 2011–2014 were used for this study. The NHANES is a cross-sectional survey conducted by the National Center for Health Statistics (NCHS), CDC, that is based on a stratified, multistage probability cluster design to draw a representative sample of the civilian, noninstitutionalized U.S. population. The NHANES collects information on a wide variety of health measures and conditions through in-home interviews, standardized physical examinations, and collection of blood samples in mobile examination centers. Since 1999, data have been collected annually and released in 2-year cycles. In 2011–2014, low-income whites, non-Hispanic black persons, non-Hispanic Asian persons, and all Hispanic as well as Mexican American persons were sampled at higher frequencies to obtain more reliable and precise estimates for these subgroups. Protocols for the overall NHANES 2011–2012 and 2013–2014 and this study were reviewed and approved by the NCHS Research Ethics Review Board (ERB). Written informed consent for the NHANES study was collected from adults and assent was collected from children aged 7–17 years. More detailed information about the NHANES survey design and sampling methods have been published elsewhere.¹¹

Sera were tested for *T. gondii* IgG and IgM antibodies, and for those positive for both IgG and IgM antibodies, samples were further tested for IgG avidity. Tested sera were from surplus specimens of all participants ≥ 6 years of age in NHANES 2011–2014. Surplus sera came from those sample persons with sera still available after completion of all initial laboratory tests included in the original NHANES protocols. Therefore, serum samples may not have been available for all sample persons.

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The NHANES surplus sera program does not permit testing when the testing would produce clinically relevant results that would need to be reported to the participant because of the time lag between the signing of informed consent and the actual testing of the specimen. Therefore, the NCHS ERB required that pregnant women and those pregnant in the year before their participation in the NHANES be excluded from *T. gondii* IgM testing because these results could have clinical implications and we were unable to report results back to participants.

Laboratory testing. *Toxoplasma gondii* IgG antibodies were measured with the *Toxoplasma* IgG EIA (Bio-Rad, Redmond, WA); samples with results ≥ 33 IU/mL were considered positive, samples with results < 27 IU/mL were considered negative, and samples with results ≥ 27 IU/mL and < 33 IU/mL were considered equivocal. *Toxoplasma gondii* IgM antibodies were measured with the Platelia Toxo IgM (Bio-Rad); sample ratios < 0.8 were considered negative, sample ratios ≥ 1 were considered positive, and sample ratios ≥ 0.8 and < 1 were considered equivocal. For both IgG and IgM tests, equivocal results were categorized as negative for analytic purposes. The test results were normalized as index values, international units (IU/mL) or sample ratios, which are traceable to the WHO Anti-*Toxoplasma* Serum, 3rd International Standard Preparation, 1994. The *Toxoplasma* IgG EIA was previously evaluated by the CDC Parasitic Diseases Laboratory using an established serum panel and compared with the CDC *Toxoplasma* immunofluorescence assay and the Sabin-Feldman dye test and found to have a sensitivity and specificity of 100% in the serum panel tested.¹² Because samples that tested *T. gondii* IgM antibody positive and IgG antibody negative are likely to represent false-positive IgM results,^{9,10} we focused our main *T. gondii* IgM antibody analysis on samples that tested positive for both *T. gondii* IgG and IgM antibodies.

The avidity test was performed on samples positive for both *T. gondii* IgG and IgM antibodies by the Palo Alto Medical Foundation Toxoplasma Serology Laboratory using the VIDAS® TOXO IgG Avidity (TXGA) kit (bioMérieux, Marcy-l'Étoile, France).¹³ The VIDAS® TOXO IgG Avidity assay is an automated qualitative test for the determination of anti-*Toxoplasma* IgG avidity in human serum using the enzyme-linked fluorescent assay technique. The assay is intended for use in conjunction with results from the VIDAS TOXO IgG II and samples must have a positive titer ≥ 8 IU/mL. Samples with an index (ratio of high avidity antibodies to total IgG antibodies) of < 0.200 are coded as low avidity, samples ≥ 0.200 but < 0.300 are coded equivocal, and samples ≥ 0.300 are coded high. A high-avidity test is interpreted as indicating that *T. gondii* infection occurred longer than 4 months previously.^{14,15} A high-avidity result from a specimen collected during the first trimester of pregnancy can be helpful for ruling out a *T. gondii* infection as having occurred during pregnancy and, therefore, determining that there is minimal risk of transmission to the fetus. A low-avidity result, which can persist for 12 months or longer, is less clinically useful for determining the timing of *T. gondii* infection.

Statistical analysis. All estimates of seroprevalence were weighted using the NHANES examination weights to represent the total civilian noninstitutionalized U.S. population and to account for oversampling and nonresponse to the household interview and physical examination.¹⁶ Seroprevalence was calculated for the total population and by age groups (see paragraph below for age groups). Age-adjusted seroprevalence was compared across demographic and socioeconomic

subgroups. Race and Hispanic origin subgroups were based on the respondent's self-assessment and categorized as non-Hispanic white, non-Hispanic black, non-Hispanic Asian, Mexican American, and all Hispanic (i.e., Mexican American and all other Hispanics combined); subjects who did not self-select into these groups were classified as "other," which included individuals reporting multiple races, and were only included when all racial/ethnic groups were combined in analyses for the total population. Other demographic and socioeconomic subgroups included birth place (defined as U.S. born [born in the 50 U.S. states or D.C.] or non-U.S. born [not born in the 50 U.S. or D.C.]), poverty index (calculated by dividing family income by a poverty threshold specific for family size using the U.S. Department of Health and Human Services' poverty guidelines and categorized as either below poverty level or at or above poverty level),¹⁷ educational level of the head of household for those aged 6–19 years and individual education among those aged 20 years and older (measured as the last year of school completed and self-categorized as having less than a high school education, having completed high school or a general equivalency diploma, or having more than a high school education), and crowding index (measured as < 0.5 , 0.5 – 0.99 , and ≥ 1 person per room). More detailed information on each variable collected can be found in the NHANES file documentation.¹¹ Seroprevalence estimates were also calculated among U.S.-born persons to more accurately estimate transmission of *T. gondii* occurring in the United States. In many countries outside the United States, a higher percentage of persons are infected at an early age^{18,19} and, therefore, are already infected with *T. gondii* when immigrating to the United States.

Standard errors were calculated using Taylor series linearization using SUDAAN software to account for the complex sample design.²⁰ Age adjustment for IgG analysis was done using direct standardization with the 2000 projected U.S. census population as the reference population using the following age groups: 6–11, 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, and 70 and above; and 15–19, 20–29, 30–39, and 40–44 for analyses of those 15–44 years of age. Age standardization for *T. gondii* IgM analysis was similar, but because of smaller sample size, the age groups were collapsed into the following four groups: 6–19, 20–39, 40–59, and 60 and above. Estimates based on < 10 seropositive or seronegative persons or with a relative standard error (RSE) $> 30\%$ were considered unstable and when presented are accompanied by a note of caution. Pairwise differences in seroprevalence between subgroups were evaluated using a *t*-statistic, and tests for trends were conducted using a linear orthogonal contrast procedure, both in SUDAAN; *P* values < 0.05 were considered significant. No adjustments were made for multiple comparisons. Confidence intervals (CIs) were constructed using the approach described in Korn and Graubard.²¹ There was a significant interaction between race and Hispanic origin and birth place, so we divided the race and Hispanic origin subgroups into U.S. born and non-U.S. born for the multivariable models.

Response rates for *T. gondii* testing. Of the 23,832 persons, 6 years of age and older sampled for NHANES 2011–2014, 16,733 (70.2% of those sampled) were interviewed for the survey, 16,112 (96.3% of those interviewed) were examined, and 13,507 (83.8% of those examined) had sera available that was tested for *T. gondii* antibodies. The percent of those examined with sera available for testing

varied by age (68.0% among those aged 6–11 years and 80.2–89.4% among the remaining age groups), race and Hispanic origin (77.9% among non-Hispanic black persons, 88.5%, 85.5%, and 80.9% among non-Hispanic white persons, Mexican American persons, and Asian persons, respectively), was lower among those born in the United States (83.2%) than among non-U.S.-born persons (86.1%), and varied by educational level (83.6% among those with less than a high school education, 82.6% among those with a high school education, and 84.8% among those with more than a high school education), poverty index (81.6% for those below the poverty level and 85.4% for those at or above), and crowding level (81.0% for one or more persons per room, 83.0% for 0.5–0.99 persons per room, and 87.3% for less than 0.5 persons per room) ($P < 0.05$ from χ^2 analysis). After adjusting for age and race and Hispanic origin in a multivariable model examining *T. gondii* testing status as the outcome, only living below poverty level remained associated with not being tested (data not shown). Therefore, examination sample weights were adjusted using SAS PROC WTADJ (SAS Version 12.1, Cary, NC) to account for availability of sera for testing by age group, race

and Hispanic origin, and poverty index. Results using the original weights and the adjusted weights were compared. There were no substantial differences in conclusions reached using either the original weights or the adjusted weights. For ease of researchers attempting to duplicate the results presented here, we reported those based on the original examination weights. Of the 13,507 persons tested for *T. gondii* IgG antibodies, 13,194 persons were tested for IgM antibodies; 311 women pregnant or pregnant in the previous year were excluded from IgM testing as required by the ERB, and two samples were not tested for IgM because of lack of a sufficient sample volume.

RESULTS

***Toxoplasma gondii* IgG antibodies.** In NHANES 2011–2014, the weighted unadjusted prevalence of *T. gondii* IgG antibodies among persons 6 or more years of age was 11.14% (95% CL 9.88%, 12.51%) and the age-adjusted prevalence was 10.42% (95% CL 9.19%, 11.76%; Table 1) based on 13,507 participants tested. *Toxoplasma gondii* IgG prevalence increased with age ($P < 0.001$, linear test for trend),

TABLE 1

Age-specific and age-standardized¹ weighted prevalence of *Toxoplasma gondii* immunoglobulin G seropositivity among persons aged ≥ 6 years by demographic risk factors overall and among those U.S. born, NHANES 2011–2014

Category†	Overall			U.S. born		
	Prevalence	n	95% CL	Prevalence	n	95% CL
Total unadjusted	11.14	13,507	9.88, 12.51	8.64	10,254	7.40, 10.01
Total age standardized*	10.42	13,507	9.19, 11.76	7.89	10,254	6.66, 9.28
Age group						
6–11	0.91‡	1,757	0.52, 1.48	0.66‡**	1,651	0.29, 1.29
12–19	3.13	2,102	2.00, 4.64	2.44	1,810	1.32, 4.10
20–29	6.85	1,636	5.14, 8.92	4.24	1,271	2.84, 6.05
30–39	9.55	1,685	7.76, 11.59	5.35	1,130	4.01, 6.96
40–49	11.36	1,645	8.86, 14.26	7.46	1,049	4.53, 11.43
50–59	13.27	1,592	10.74, 16.16	9.61	1,076	7.10, 12.65
60–69	17.48	1,586	15.58, 19.51	16.18	1,066	14.07, 18.46
70+	24.58	1,504	21.49, 27.88	23.13	1,201	19.87, 26.65
Gender						
Male	11.25§	6,649	9.69, 12.97	8.45	5,066	6.91, 10.21
Female	9.67	6,858	8.50, 10.95	7.37	5,188	6.21, 8.68
Race and Hispanic origin††						
White, not Hispanic (ref)	8.26	4,971	6.77, 9.95	7.92	4,788	6.39, 9.69
Black, not Hispanic	12.04¶	3,113	10.54, 13.67	9.65	2,834	8.49, 10.90
Mexican American	13.88¶	2,010	11.17, 16.98	6.45	1,196	4.34, 9.16
All Hispanic	19.99#	3,337	16.68, 23.65	5.92	1,767	4.27, 7.97
Asian, non-Hispanic	8.81	1,580	6.97, 10.95	6.75**	432	2.89, 13.05
Place of birth						
Non-United States	22.52#	3,248	19.83, 25.39	NA	NA	NA
United States (ref)	7.89	10,254	6.66, 9.28	7.89	10,254	6.66, 9.28
Poverty index						
Below	16.14#	3,372	14.24, 18.19	11.61¶	2,546	8.85, 14.87
At or above (ref)	9.27	9,144	8.18, 10.46	7.42	7,099	6.39, 8.57
Education level						
< High School	17.93#	3,156	15.69, 20.35	11.41¶	2,026	8.33, 15.13
High School	11.86#	2,910	10.18, 13.71	9.80#	2,346	8.17, 11.63
> High School (ref)	8.07	7,325	7.04, 9.20	6.62§	5,781	5.64, 7.71
Crowding Index (persons per room)						
≥ 1	15.85#	3,124	13.44, 18.49	7.58	2,003	5.06, 10.84
0.5–0.99	11.20#	5,807	9.83, 12.68	8.71	4,399	7.42, 10.14
< 0.5 (ref)	8.00	4,472	6.70, 9.46	6.98	3,778	5.61, 8.57

CL = confidence limits; NA = not applicable; NHANES = National Health and Nutrition Examination Survey; Ref = reference group.

* All categories except age groups are age-standardized to the 2000 projected U.S. census population using the following age groups: 6–11, 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, and 70 years and above.

† Columns for categories may not add to totals because of item nonresponse.

‡ $P < 0.001$ for linear test for trend among age groups.

§ $P < 0.05$, ¶ $P < 0.01$, # $P < 0.001$ for comparison of subgroup to reference group for each cofactor.

** Relative standard error of the estimate $> 30\%$ and estimate may be considered unstable.

†† Total sample sizes for the reported race and Hispanic origin subgroups do not sum to the population total because population total includes those that did not self-select into any of the listed race and Hispanic origin groups or reported as multiracial.

was higher among males (11.25%) than females (9.67%) ($P = 0.02$), higher among non-Hispanic black (12.04%), Mexican American (13.88%), and all Hispanic (19.99%) persons than non-Hispanic white persons (8.26%) ($P = 0.001$, $P = 0.002$, and $P < 0.001$, respectively) or non-Hispanic Asian persons (8.81%) ($P = 0.012$, $P = 0.003$, $P \leq 0.001$, respectively), higher among non-U.S.-born (22.52%) than U.S.-born persons (7.89%) ($P < 0.001$), higher among those living below poverty level (16.14%) than those living at or above poverty level (9.27%) ($P < 0.001$), higher among those with less than a high school education (17.93%) or a high school education (11.86%) than those with greater than a high school education (8.07%) ($P < 0.001$ for both), and higher among those living in households with one or more persons per room (15.85%) or 0.5–0.99 persons per room (11.20%) than those living in households with less than 0.5 persons per room (8%) ($P < 0.001$ for both) (Table 1). The results were similar when restricting the analysis to U.S.-born persons, there was an increasing prevalence with older age ($P < 0.001$, linear trend) and prevalence was higher among those living below poverty compared with those living at or above poverty ($P = 0.001$), higher among persons with less than a high school education or just a high school education compared with those with greater than a high school education ($P = 0.003$ and $P < 0.001$, respectively), and higher among those living in households with 0.5–0.99 persons per room compared with those living in households with < 0.5 persons per room ($P < 0.05$). Among those U.S. born, non-Hispanic black persons had higher seropositivity than Mexican Americans or all Hispanics ($P < 0.05$ and $P < 0.01$); no other differences were found (Table 1).

Among women aged 15–44 years, the *T. gondii* IgG-unadjusted prevalence was 7.40% (95% CL 5.92%, 9.11%) overall, 4.34% (95% CL 3.06%, 5.95%) for U.S.-born women, and 20.29% (95% CL 15.90%, 25.26%) for non-U.S.-born women. The age-adjusted prevalence for women aged 15–44 years overall, among U.S.-born women, and among non-U.S.-born women was 7.50% (95% CL 6.00%, 9.25%), 4.54% (95% CL 3.13%, 6.34%), and 19.10% (95% CL 15.07%, 23.67%), respectively.

Multivariable analysis for *T. gondii* IgG-positive samples.

As noted in the methods, there was a significant interaction between race and Hispanic origin subgroups and birthplace, so we further divided the race and Hispanic origin subgroups into those U.S. born and non-U.S. born for the multivariable model. In the model non-U.S.-born non-Hispanic white (odds ratio [OR] = 2.31, $P = 0.002$), non-U.S.-born non-Hispanic black (OR = 6.03, $P < 0.001$), and non-U.S.-born Mexican American (OR = 2.79, $P < 0.001$) persons were more likely to be *T. gondii* seropositive than U.S.-born non-Hispanic white persons. In contrast, the likelihood of being seropositive was lower among U.S.-born Mexican American than U.S.-born non-Hispanic white persons (OR = 0.58, $P = 0.04$) (Table 2). Odds of being seropositive was also higher among males than females (OR = 1.24, $P = 0.01$) and those living below the poverty level compared with at or above (OR = 1.37, $P = 0.003$), and higher among those with a high school (OR = 1.41, $P = 0.001$) or less than a high school education (OR = 1.57, $P = 0.001$) compared with those with greater than a high school education (Table 2).

***Toxoplasma gondii* IgM antibodies.** Prevalence of *T. gondii* IgM antibodies was 1.16% (95% CL 0.94%, 1.42%)

TABLE 2

Risk factors for *Toxoplasma gondii* immunoglobulin G seropositivity, as estimated with a full logistic regression model for all persons ≥ 6 years of age, NHANES 2011–2014

Category	OR	95% CL	P value
Age groups (overall trend)	1.47	1.39, 1.55	< 0.001
Gender			
Male	1.24	1.05, 1.46	0.01
Female	Ref	—	—
Race/Hispanic origin and birthplace			
White, non-Hispanic, U.S. born (ref)	Ref	—	—
White, non-Hispanic, non-U.S. born	2.31	1.39, 3.87	0.002
Black, non-Hispanic, U.S. born	1.06	0.80, 1.41	0.662
Black, non-Hispanic, non-U.S. born	6.03	3.63, 10.02	0.000
Mexican American, U.S. born	0.58	0.34, 0.99	0.044
Mexican American, non-US born	2.79	1.70, 4.58	0.000
Asian, non-Hispanic, U.S. born	0.92	0.45, 1.88	0.824
Asian, non-Hispanic, non-U.S. born	1.22	0.89, 1.68	0.208
Poverty index			
Below	1.37	1.12, 1.68	0.003
At or above	Ref	—	—
Education level			
< High School	1.57	1.21, 2.03	0.001
High School	1.41	1.16, 1.70	0.001
> High School	Ref	—	—
Persons per room			
≥ 1	1.04	0.78, 1.38	0.801
0.5–0.99	1.11	0.89, 1.39	0.343
< 0.5	Ref	—	—

CL = confidence limits; NHANES = National Health and Nutrition Examination Survey; Ref = reference group.

based on 158 participants being IgM antibody positive among the 13,194 participants tested. Prevalence of being both IgM and IgG antibody positive was 0.71% (95% CL 0.54%, 0.92%), based on 93 samples positive for both IgG and IgM). Among those IgG antibody negative, 0.50% (95% CL 0.34%, 0.73%) were IgM positive (based on 65 participants being IgG negative but IgM positive out of 11,559 IgG-negative samples tested for IgM antibody).

Age-adjusted prevalence of those both *T. gondii* IgM and IgG antibody positive was 0.67% (95% CL 0.51%, 0.86%) and there was an increasing linear trend with age ($P < 0.001$; Table 3). Seropositivity was similar for males and females (0.68 and 0.66, $P = 0.86$) and higher among non-Hispanic Asian (1.28%) than both non-Hispanic white (0.68%) ($P = 0.04$) and non-Hispanic black persons (0.42%) ($P = 0.008$). Although prevalence was lower among Mexican American (0.22%) than non-Hispanic white persons ($P = 0.02$), the estimate of seropositivity for Mexican Americans was based on only three sample persons positive for both IgG and IgM with an RSE $> 60\%$ and is unstable; therefore, these results should be interpreted with caution. There were no differences in prevalence between all Hispanics and any of the other race and Hispanic origin subgroups. *Toxoplasma gondii* IgM and IgG prevalence was also higher among non-U.S.-born (1.16%) than U.S.-born persons (0.58%) ($P = 0.02$) and higher among those living in households with 0.5–0.99 (1.10%) persons per room than those living in households with < 0.5 persons per room (0.46%) ($P = 0.02$).

Among women aged 15–44 years, 0.58% were both IgM and IgG antibody positive (95% CL 0.34%, 0.92%), based on 13 positive samples among 2,501 women aged 15–44 tested for *T. gondii* IgG and IgM antibodies; this result was not significantly different for U.S.-born and non-U.S.-born women (0.47% and 1.04%, respectively; $P = 0.24$; note that these

TABLE 3

Age-specific and age-standardized¹ weighted prevalence of those both *Toxoplasma gondii* immunoglobulin (Ig) M positive and IgG positive among persons aged ≥ 6 years by demographic risk factors, NHANES 2011–2014

Category†	Prevalence	n	Lower 95% exact CL	Upper 95% exact CL
Total unadjusted	0.71	13,194	0.54	0.92
Total age standardized*	0.67	13,194	0.51	0.86
Age group				
6–19	0.28‡¶	3,814	0.06	0.82
20–39	0.48	3,072	0.29	0.75
40–59	0.88	3,219	0.49	1.45
60+	1.13	3,089	0.79	1.56
Gender				
Male (ref)	0.68	6,648	0.45	1.00
Female	0.66	6,546	0.47	0.89
Race and Hispanic origin#				
White, non-Hispanic (ref)	0.68	4,854	0.47	0.94
Black, non-Hispanic	0.42	3,039	0.21	0.74
Mexican American	0.22§¶	1,962	0.03	0.74
All Hispanic	0.63§	3,266	0.31	1.15
Asian	1.28¶	1,549	0.76	2.02
Place of birth				
Non-United States	1.16¶	3,167	0.78	1.66
United States (ref)	0.58	10,022	0.41	0.80
Poverty index				
Below	0.82	3,268	0.40	1.49
At or above (ref)	0.64	8,955	0.48	0.85
Education level				
< High School	0.91	3,087	0.52	1.47
High School	0.76	2,843	0.40	1.29
> High School (ref)	0.59	7,151	0.36	0.92
Persons per room				
≥ 1	0.55§	3,021	0.23	1.09
0.5–0.99	1.10¶	5,636	0.76	1.54
< 0.5 (ref)	0.46	4,437	0.25	0.78

CL = confidence limits; NHANES = National Health and Nutrition Examination Survey; Ref = reference group.

* All categories except age groups are age standardized to the 2000 U.S. census population using the following age groups: 6–19, 20–39, 40–59, and 60 years and above.

† Columns for categories may not add to totals because of item nonresponse.

‡ $P < 0.001$ for linear test for trend among age groups.

§ $P < 0.05$ for comparison of subgroup to reference group for each cofactor.

¶ Estimate based on < 10 positive sample persons or relative standard error (RSE) $> 30\%$ (eight positives and RSE $> 50\%$ for those aged 6–19 years, three positives and RSE $> 60\%$ for Mexican Americans, RSE $> 30\%$ for All Hispanics, and RSE $> 30\%$ for households ≥ 1 persons per room) so estimate may be unstable. Results should be interpreted with caution.

Total sample sizes for the reported race and Hispanic origin subgroups do not sum to the population total because population total includes those who did not self-select into any of the listed race and Hispanic origin groups or reported as multiracial.

results were based on five of 1,824 U.S.-born (RSE = 30%) and eight of 676 non-U.S.-born (RSE $> 40\% < 50\%$) women, respectively, and may be considered statistically unreliable and therefore, should be interpreted with caution).

Multivariable analysis for both *T. gondii* IgM-positive and IgG-positive samples. In multivariable analysis examining the risk for both *T. gondii* IgM and IgG seropositivity, the only statistically significant risk factors were age (continuous across increasing age groups, OR 1.31, 95% CL 1.18, 1.47), crowding (0.5–0.99 persons per room compared with < 0.5 persons per room, OR 2.26, 95% CL 1.04, 4.91), and being non-U.S. born compared with U.S. born (OR 2.27, 95% CL 1.26, 4.06).

IgG avidity. *Toxoplasma gondii* IgG avidity testing was performed on 92 samples that were both *T. gondii* IgM and IgG antibody positive (one sample was excluded because of lack of sufficient volume of serum remaining for avidity testing). Among 92 persons aged 6 and above and both IgM and IgG positive, almost all (92.94%, 95% CL 83.60%, 97.86%, 85 sample persons) had high avidity and few had low avidity

(1.87%, 95% CL 0.57%, 4.45%, RSE $> 40\% < 50\%$, four positive sample persons). Estimates were similar for males and females but because of small sample sizes and high RSEs, further analysis by gender could not be done. Thirteen women of childbearing age were tested for IgG avidity; avidity in all of these samples was high. All estimates of the prevalence of low avidity were based on small numbers with high RSE and may be statistically unreliable and, therefore, should be interpreted with caution.

DISCUSSION

In NHANES 2011–2014, the overall age-adjusted *T. gondii* IgG antibody prevalence was 10.4% (95% CI 9.2–11.8%), which is lower than the age-adjusted prevalence of 12.4% (95% CI 11.1–13.7%) in NHANES 2009–2010²² ($P = 0.03$). This is consistent with the decrease in *T. gondii* prevalence documented for the past two decades in the United States.^{22,23} Similar to findings in prior *T. gondii* antibody NHANES analyses,^{12,23} those more likely to be infected with *T. gondii* included older persons; males; persons of non-Hispanic black and Mexican American race/Hispanic origin, non-U.S.-born persons, those living in poverty, those with less education, and those living in more crowded households. Among women of reproductive age, the age-standardized seroprevalence appeared to decrease from 9.1% (95% CI 7.2–11.1%) in 2009–2010²² to 7.5% (95% CI 6.0–9.3%) in NHANES 2011–2014, but this decrease did not reach statistical significance ($P = 0.19$).

In multivariable analysis, U.S.-born Mexican Americans were less likely to be seropositive to *T. gondii* IgG than U.S.-born non-Hispanic white persons. This finding is consistent with lower estimates of *T. gondii* IgG prevalence shown in U.S.-born Mexican Americans compared with U.S.-born non-Hispanic whites in previous studies.^{12,22} However, the reasons that Mexican Americans in these U.S.-born groups have a lower prevalence of *T. gondii* antibodies than U.S.-born non-Hispanic whites are not known; there may be explanations related to culinary practices, such as thoroughly cooking meat, or environmentally related exposure factors.

Our results showed an increasing prevalence of IgG seropositivity with age, which is a result of cumulative seropositivity. We also found an increase in the prevalence of IgM seropositivity with age. There are two factors that might contribute to the increasing seroprevalence of IgM with age: 1) people eat an increasing amount of meat as they grow to adulthood, and, therefore, the chances increase for acquiring *T. gondii* infection from cysts in undercooked meat eaten either at home or in a setting away from home such as in a restaurant; and 2) older persons are more likely to garden in the United States and, therefore, may be more likely to be exposed to soil that contains *T. gondii* oocysts, for example, data collected by the National Gardening Association indicate that persons aged 55 years and older represent 36% of all gardening households,²⁴ yet this group represents only 28% of the U.S. population.²⁵ Unfortunately, a variable that summarizes a sample person's total long-term or lifetime meat ingestion was not available in NHANES 2011–2014, so we did not include it in our analysis. Gardening exposure was also not measured in NHANES 2011–2014. In addition, it is unlikely that the higher IgM seropositivity among persons who are IgG positive seen with increasing age is a result of reactivation due

to age-related immunosuppression because even in severely immunosuppressed HIV-infected persons with chronic *T. gondii* infection, IgM antibodies are rarely detected.

In the NHANES 2011–2014, we tested for IgG and IgM, then tested those IgM positive/IgG positive for avidity; almost all of these persons had “high” avidity, including all women of childbearing age, suggesting that the overall risk of maternal infection and congenital toxoplasmosis during pregnancy is presently low in the United States. Because *T. gondii* IgM can persist for variable periods of time (up to 18 months or longer),^{9,10} it is difficult to estimate the incidence of *T. gondii* infection from *T. gondii* IgM and IgG alone. Furthermore, because the NHANES is a cross-sectional study, there were no follow-up samples among the IgM negative/IgG negative and IgM positive/IgG negative persons to help determine the incidence of primary infection by seroconversion.

As noted in the methods, a high-avidity result indicates that a person has been infected for 4 months or longer. Low avidity indicates *T. gondii* infection could have occurred within the prior 4 months. Estimates of proportions of persons infected within the past 4 months based on low avidity should be considered maximum values because avidity can remain low for longer than 4 months (i.e., a year or longer); however, as previously noted, the estimates presented for low avidity are all based on small numbers of sample persons and have high RSEs and wide confidence limits and should therefore be interpreted with caution.

One strength of this analysis is that these estimates of *T. gondii* infection are derived from a representative sample of the U.S. population. However, there are a number of limitations to our study. U.S. birth status and socioeconomic information were self-reported and not independently validated. Also, availability of samples for testing varied by demographic groups; however, reanalysis of the data with sample weights adjusted for the lack of sera availability by age, race and Hispanic origin, and poverty index did not result in any different conclusions than those reported here. Any residual bias due to lack of serum availability that remains after weight adjustment and the effect on the results presented here is unknown. In addition, no pregnant women or women pregnant in the previous year were tested for *T. gondii* IgM antibodies in our study, so the *T. gondii* IgM antibody prevalence for this group is not directly known. However, men in the study were uniformly tested for *T. gondii* IgM antibodies and the prevalence for men and women in childbearing years was not statistically different ($P = 0.16$), so it is not likely that the exclusion of pregnant women and those pregnant in the year before significantly biased the IgM prevalence results for women.

The *T. gondii* IgG test used in this study has been validated against what is widely considered to be a gold standard,²⁶ but there is not a universally accepted gold standard for *T. gondii* IgM tests. The Platelia Toxo IgM test used in this study is a commonly used commercial test; a recent evaluation in France reported it had the highest sensitivity (97.9%) of nine tests evaluated, and it had a specificity of 92.6% when compared with two different validation methods (immunofluorescence assay-M and immunosorbent agglutination assay [ISAGA-M bioMerieux® SA, 89 Marcy l'Etoile, France]) at a reference laboratory.²⁷ However, in this French study population, the Platelia Toxo IgM predictive value positive was 63%, so it is possible that our results over estimate IgM antibody prevalence. *Toxoplasma gondii* IgM tests are designed by manufacturers to have high sensitivity

at the cost of specificity to limit the risk of missing acute infections. Alternatively, some patients who have a negative IgG test followed by a positive one have been reported to have either negative or transiently positive IgM test results,²⁸ which would lead to an underestimate of acute infections in our study. Additional serological tests can help further estimate the timing of infection and incidence of acute primary infection, such as the IgA, IgE, and AC/HS²⁹ but were not attempted in this study because of budget considerations.

As in our previous studies,^{12,22,23} the NHANES has made it possible to monitor the prevalence of chronic *T. gondii* infection in the U.S. population using IgG antibodies and, in addition, provided information using IgM antibodies and avidity to help determine which demographic groups are associated with a higher prevalence of more recent *T. gondii* infection. Prevention of *T. gondii* infection is important, especially in persons at higher risk for serious outcomes resulting from infection such as pregnant women or those immunosuppressed. *Toxoplasma gondii*-related preventive information is available at the CDC Web site (see link: <https://www.cdc.gov/parasites/toxoplasmosis/prevent.html>).

Received August 25, 2017. Accepted for publication October 11, 2017.
Published online December 18, 2017.

Financial support: The work was done by staff at the Centers for Disease Control and Prevention, Atlanta, Georgia, and Hyattsville, Maryland, and the Palo Alto Medical Foundation, Toxoplasma Serology Laboratory, Palo Alto, CA.

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