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Seroprevalence of antibodies to Toxocara species in the United States and associated risk factors, 2011– 2014

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Abstract

Background—Toxocariasis results from infection with larval stages of a dog and cat intestinal nematode and causes human morbidity. The current US estimate of *Toxocara* exposure is 13.9% (NHANES III 1988–1994).

Methods—We used a multiplex bead based assay (Tc-CTL-1MBA) with purified *Toxocara canis* antigen to estimate *Toxocara* antibody seroprevalence in serum of 13,509 persons six years and older from the National Health and Nutrition Examination Survey (NHANES), 2011–2014 and identified seropositivity risk factors. We tested a subset of 500 samples with the *T. canis* enzyme immunoassay used in NHANES III to estimate prior seroprevalence had samples from NHANES III been tested by Tc-CTL-1MBA.

Results—The age standardized estimate of *Toxocara* seroprevalence was 5.0% (95% confidence interval [CI], 4.2%–5.8%), lower than previously reported even adjusting for increased Tc-CTL-1MBA specificity. Risk factors for seropositivity from multiple logistic regression were older age (odds ratio [OR], 2.1; 95%CI, 1.1–3.9 in persons 50–59 years old; OR, 1.7; 95%CI, 1.0–2.8 in persons 60–69; and OR, 2.6; 95%CI, 1.5–4.7 in persons ≥70 versus persons 6–11), non-Hispanic Black race/Hispanic origin (OR, 1.4; 95%CI, 1.0–2.0) versus non-Hispanic White, male sex (OR, 1.9; 95%CI, 1.6–2.2), living below poverty level (OR, 1.9; 95%CI, 1.4–2.6), households with ≥0.5 persons per room (OR, 1.3; 95%CI, 1.0–1.6), less than college education (OR, 1.9; 95%CI, 1.5–2.4), and birth outside the United States (OR, 3.6; 95%CI, 2.6–5.1).

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Disclaimer:
The findings and conclusions in this report are those of the authors and do not necessarily represent the view of the Department of Health and Human Services or the Centers for Disease Control and Prevention.

Conflict of Interest:
None of the authors declare a conflict of interest.

Author Contributions:
SHS and HMC performed Tc-CTL-1MBA. HMC also performed TES-AgEIA. JLJ and SH designed the study, wrote the protocol, and assisted in discussion of the analysis, interpretation of the results, and preparation of the manuscript. EWL analyzed the NHANES data, with assistance from RW and DKM, and wrote the manuscript.

Conclusions—*Toxocara* seroprevalence estimates in 2011–14 were lower than in a study from NHANES III, 1988–94, but seropositivity risk factors remained the same and should continue to be the focus of prevention efforts.

Keywords

seroprevalence; antibodies; *Toxocara canis*; *Toxocara cati*; United States

Background

Human toxocariasis is caused by inadvertent infection and migration of *Toxocara* species larvae. The larvae are immature *Toxocara canis* and *cati*, intestinal nematodes of dogs and cats. Severity ranges from covert with non-specific asthma-like symptoms to marked eosinophilia, fever, and hepatomegaly in visceral toxocariasis (VT), retinal scarring and visual impairment in ocular toxocariasis (OT), and cerebral vasculitis, meningitis, encephalitis, myelitis, and seizures in neurotoxocariasis (NT)[1]. Prevalent in the tropics and sub-tropics and in less industrialized countries, toxocariasis is also associated with socioeconomically disadvantaged populations in industrialized countries [1].

In the US National Health and Nutrition Examination Survey (NHANES) I (1971–1973), seroprevalence of antibody to *T. canis* excretory-secretory antigen (TES-Ag) expressed by infective larvae was 4.6%–7.3% among children aged 1–11 years [2]; seroprevalence was 13.9% among persons aged ≥6 years in NHANES III (1988–1994)[3]. In NHANES III, ages 20–39, non-Hispanic Black race/ethnicity, male sex, living below the poverty level, high school education or less compared with at least some college, elevated blood lead levels, dog ownership, rural residence, birth outside the US, and residence in regions outside the West were predictors of *Toxocara* seropositivity. These results suggest elevated exposure to *Toxocara* in the US population, particularly in certain subpopulations, but must be interpreted with caution. TES-Ag enzyme immunoassay (EIA) used in NHANES I and III is reliable [4] but cross-reacts with other helminths [5, 6]. Furthermore, these estimates are over 17 years old. To provide current estimates of national seroprevalence using improved laboratory methods and to identify subpopulations with higher risk, we employed a multiplex bead-based assay with purified recombinant Tc-CTL-1 antigen (Tc-CTL-1MBA) to test a nationally representative sample of individuals surveyed in 2011–2014.

Methods

Study design and participants

NHANES is a nationally representative, cross-sectional survey conducted by the National Center of Health Statistics (NCHS). Since 1999, approximately 5000 individuals have been interviewed each year and have undergone health examination and laboratory testing. In 2011–2014, non-Hispanic Asians, Hispanics, non-Hispanic blacks, as well as non-Hispanic whites and others at ≤130% poverty level were oversampled to increase reliability and precision of health status indicator estimates for these subgroups. Survey design details are found elsewhere [8].

Serum samples

Tested sera were from individuals ≥6 years old in the NHANES 2011–2014 survey that were previously used for other tests, and if sera remained, were returned as surplus specimens to the NCHS repository.

Ethical approval

The overall NHANES 2011–2014 survey (Protocol #2011-17) and this study (Protocol #2014-13) were approved by the NCHS Ethics Review Board.

Detection of Toxocara antibody

Samples were probed for antibodies against recombinant *T. canis* excretory-secretory antigen, Tc-CTL-1, by multiplex bead-based assay (Tc-CTL-1MBA). Details of antigen identification, expression, and antibody probing are described elsewhere [4]. Briefly, Tc-CTL-1 was coupled to MagPlex Magnetic Microspheres (Luminex) using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride-N-hydroxysulfosuccinimide reactions. Next, 50 μL of these beads were incubated with 50 μL of test sera diluted 1:100 in phosphate-buffered saline (PBS)–0.3%Tween/5% milk in each well of a Costar 96-well black, round-bottom plate (Fisher Scientific) at room temperature, with shaking at 800 rpm for 30 minutes. Beads underwent 2 minutes of magnetic separation followed by a wash of 2 cycles of instillation of 100 μL of PBS–0.3% Tween for 40 seconds in a Biotek magnetic washer ELx50. Detection of antibody bead complex was performed through 30 minutes of incubation with 50μL per well of 1:200 biotinylated mouse anti-human immunoglobulin G (clone H2, affinity purified, Southern Biotech) in PBS-1% BSA, 0.05% NaN3, followed by wash as before, and 30minutes of incubation with 50μL per well of 1:250 Streptavidin, R-phycoerythrin conjugate (Invitrogen) diluted in PBS-1% BSA, 0.05% NaN3. Beads were resuspended with 100 μL per well of PBS-1% BSA, 0.05% NaN3. Mean fluorescence intensity (MFI) for each well was determined by BioPlex manager software, version 6.02 (BioRad). A cut-off (23.1 MFI) based on ROC curve analysis determined positive and negative results.

Recognizing TES-AgEIA (used in NHANES III) has 78% sensitivity and 92% specificity [7], whereas Tc-CTL-1MBA has 90% sensitivity for VT and 99% specificity [4], to compare seroprevalence estimates in NHANES III and this study, we randomly selected 250 samples within each group of sera determined positive or negative by Tc-CTL-1MBA from those available in NHANES 2013–2014, and probed for antibodies using TES-AgEIA. Detailed methods are found elsewhere [3]. In brief, sera diluted 1:100 in PBS-0.05%Tween was placed into 96-well Immulon II HB flat bottom plates sensitized with TES-Ag diluted 1:2000 in 0.1M $NaHCO_3/Na_2CO_3$. Antigen-antibody complexes were detected using anti-IgG enzyme conjugate and visualized with tetramethylbenzidine substrate read at 450nm. Cutoffs were determined by averaging optical density (OD) readings for four standards at a 1:32 titer (determined to be the cutoff for being positive from a reference ELISA), and dividing this value by the mean of four high positive control OD values. For each sample, a ratio was calculated by dividing the sample OD value by the mean of the high positive control. This ratio was compared with the cutoff; values above the cutoff were considered positive.

Statistical Analysis

Analysis was performed with R statistical software [9] and the *survey* package [10, 11]. We analyzed data on available demographic characteristics putatively related to *Toxocara* exposure as in NHANES III [3], and also a self-reported asthma diagnosis given the hypothesized association between *Toxocara* exposure and asthma symptoms [19]. We used definitions from NHANES III for factors; age was categorized into those aged 6–11, 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, and ≥70 years. Self-reported race/Hispanic origin was categorized as non-Hispanic white, non-Hispanic black, non-Hispanic Asian (not available in NHANES III), Mexican-American, and all others (individuals not self-identifying into any previous group including other Hispanics and those reporting multiple races). Poverty level (the ratio of family income divided by a poverty threshold specific for family size using guidelines from the US Department of Health and Human Services) was categorized as <1 (below poverty) or ≥1 (at or above poverty) [12]. Crowding index (the total number of household residents divided by rooms in the household) was categorized as <0.5, 0.5–0.99, or ≥1 persons per room (PPR). Head of household education (the last year of school completed by the head of household) was grouped into no high school (<9^{th} grade), some high school (9–12 grade), high school graduate, and at least some college.

The complex survey design was specified using survey design variables for the number of strata and primary sampling units. Examination weights were incorporated to account for oversampling and nonresponse to interview and examination. The outcome was defined as antibody assay positive or negative. Estimates were standardized by age groups mentioned above to the 2000 US census population and calculated for each level of the previously described factors for the total population and stratified by race/Hispanic origin. Seroprevalence and 95% confidence intervals were estimated using the Korn and Graubard method [13]. Estimates based on <10 sample persons positive for Toxocara, a relative standard error (RSE) >30%, or standard errors with <10 degrees of freedom were designated potentially unstable. To increase stability of the estimates, those age 60–69 and ≥70 were combined when comparing age specific estimates and when examining a linear test for trend across age groups. To screen for independent predictors of *Toxocara* seropositivity, t tests were performed for each factor level relative to the reference level (the level with lowest risk) [14] for the total population and stratified by race/Hispanic origin using age-standardized estimates. Factors with significant differences in the previous analyses were included in a multiple logistic models to identify potential independent predictors of positive antibody. P-values <0.05 were considered statistically significant. No multiple comparison corrections were made.

Of 23,832 persons ≥6 years old in NHANES 2011–2014, 70.2% agreed to the interview and 96.3% of those interviewed were examined. Of those persons examined, 83.8% (13,509) had serologic specimens obtained and available for testing. Differences in availability were found by age group ($p<0.001$), race/Hispanic origin ($p<0.001$), poverty level ($p<0.001$), crowding index ($p<0.001$) and place of birth ($p<0.001$) by χ^2 testing (Supplemental Table 1). These factors were used to calculate adjusted sampling weights [15]. All analyses were repeated using the adjusted weights and compared to the original results to determine the impact of unavailable samples.

To determine degree of correlation between Tc-CTL-1MBA and TES-AgEIA, we performed χ^2 testing from each assay for the 250 samples randomly selected from those designated positive or negative by Tc-CTL-1MBA. Sample size was determined by χ^2 power calculation (effect size w=0.2, df=1, α=0.05, and β=0.2 resulting in n=196, rounded up to 250).Within samples positive by TES-AgEIA, the proportion positive by Tc-CTL-1MBA was multiplied against seroprevalence by TES-AgEIA as the most conservative estimate of seroprevalence had Tc-CTL-1MBA been used.

Results

Estimates of *Toxocara* seroprevalence by age and race/Hispanic origin

Estimated Toxocara seroprevalence among US persons ≥6 years old (n=13,509) was 5.1% (95%CI 4.3%–5.9%) unadjusted and 5.0% (95%CI 4.2%–5.8%) age standardized to the 2000 US census population. Seroprevalence increased significantly with age from 3.0% in those age 6–11 to 4.8%–6.4% in all age groups ≥30 years ($p<0.05$, test for linear trend with age group $p<0.001$) (Table 1). When stratified by race/Hispanic origin, prevalence increased with older age among non-Hispanic Blacks (3.5% age 6–11 compared with 10.4% among those age 50–59 and those age ≥60, $P<0.001$; test for linear trend $p<0.001$), and in some age strata for non-Hispanic Asians (2.1% in those age 6–11 compared with 6.1%, $P<0.05$ and 19.7%, $P<0.001$; in those age 40–49 and age ≥60, respectively, linear test for trend $p<0.001$). Among non-Hispanic whites, there was no significant differences between individual age groups and the reference group ($p>0.20$) and no significant linear trend with age group ($p=0.05$). Among Mexican Americans there were some individual differences (3.0% in those age 6–11 compared with 8.7%, $P<0.05$; and 8.9%, $P<0.05$ in those age 20–29 and 40–49, respectively) but no significant linear trend with age group. There is insufficient sample size to make stable estimates for some age and race and Hispanic origin subgroups (unstable estimates are noted in Table 1) and insufficient power for some subgroup comparisons.

Predictors of *Toxocara* seropositivity on univariate analysis

Age standardized seroprevalence varied by race/Hispanic origin (6.6% in non-Hispanic Blacks, 7.3% in non-Hispanic Asians, and 6.1% in Mexican Americans compared with 3.7% in non-Hispanic Whites; $P<0.001$, $P<0.001$, and $P<0.05$ respectively), male sex (6.2% vs 3.8% among females, $P<0.001$), income below poverty level (10.2%) compared with at or above the poverty level (3.9%, $P<0.001$), greater household crowding (0.5–0.99 PPR: 5.6% and ≥1 PPR: 10.5% vs <0.5 PPR: 3.5%, $P<0.001$), less than a college education (high school graduate: 6.4%, some high school: 7.8%, and less than high school: 11.1% vs at least some college: 3.4%, $P<0.001$), and birth outside the US (11.9% vs 3.7%, $P<0.001$) (Table 2). Within race/Hispanic origin strata, male sex ($P<0.01$) remained associated with elevated seroprevalence except in Mexican Americans. Similarly, income below poverty level, high school graduation, and less than high school education ($P<0.05$) compared to at least some college remained associated with elevated seroprevalence except in Non-Hispanic Asians. Birth outside the US ($P<0.05$) was associated with elevated seroprevalence except in Non-Hispanic Whites. Household crowding with ≥1 PPR compared to households with <0.5 PPR was associated with higher seroprevalence in non-Hispanic Whites and Mexican Americans ($P<0.05$). An asthma diagnosis was associated with lower prevalence compared to those

without asthma only in non-Hispanic Blacks (4.0% vs 7.1%, $P<0.001$). The sample size was insufficient to calculate stable estimates for some subgroups and power was insufficient for some subgroup comparisons (Table 2).

Independent risk factors for *Toxocara* seropositivity

To identify independent factors associated with seropositivity, we performed multiple logistic regression on factors associated with seropositivity in univariate analysis. Persons 50–59, 60–69, and ≥70 years old were more likely to be seropositive (OR, 2.1; 95%CI, 1.1–3.9; OR, 1.7; 95%CI, 1.0–2.8; and OR, 2.6; 95%CI, 1.5–4.7; respectively) compared to those 6–11 years old (Table 3). Non-Hispanic Blacks were more likely (OR, 1.4; 95%CI, 1.0–2.0) and Mexican Americans less likely (OR, 0.6; 95%CI, 0.4–1.0) to be seropositive compared to non-Hispanic Whites. Of the remaining factors, male sex (OR, 1.9; 95%CI, 1.6–2.2), living below poverty level (OR, 1.9; 95%CI, 1.4–2.6), household crowding with ≥0.5 PPR (OR, 1.3; 95%CI, 1.0–1.6), head of household having less than a college education (OR, 1.9; 95%CI, 1.5–2.4), and birth outside the US (OR, 3.6; 95%CI, 2.6–5.1) were associated with higher seroprevalence.

Impact of availability of sera by subgroup

A comparison between each set of seroprevalence estimates using weight adjustments for each factor with significant differences in availability within levels was made against unadjusted weights. A maximal difference of 1.3% amongst all comparisons was observed (Supplemental Results).

Correlation between results of testing with TES-AgEIA and Tc-CTL-1MBA

Results from samples tested with both TES-AgEIA and Tc-CTL-1MBA were significantly correlated (P<0.0001). Of 249 available sera positive for *Toxocara* antibody by Tc-CTL-1MBA, 208 (83.5%) were positive by TES-AgEIA. Of 250 sera negative for *Toxocara* antibody by Tc-CTL-1MBA, 178 (71.2%) were negative by TES-AgEIA. Of those TES-AgEIA positive, 74.3% were positive by Tc-CTL-1MBA (Table 4). This proportion positive was multiplied against the seroprevalence estimate (13.9%) from NHANES III resulting in a conservative estimated seroprevalence of 10.3% among persons age ≥6 years if the current assay had been used.

Discussion

This study updates estimates of seroprevalence of *Toxocara* antibody in the US and identifies subpopulations at risk for exposure by examining *Toxocara* specific antibodies in a nationally representative sample of individuals surveyed during 2011–2014 with a specific assay utilizing purified recombinant Tc-CTL-1 antigen. Overall age standardized prevalence of 5.0% (95%CI 4.2%–5.8%) is lower than 13.9% (95%CI 12.5%–15.3%) observed in NHANES III among those age 6 and older measured by TES-AgEIA [3]. Similarly, the age specific estimate of 3.0% (95%CI 1.9%–4.5%) in children 6–11 years old is also lower than the estimates ranging from 4.6% to 7.3% in children 1–11 years old in a previous study from NHANES I measured by TES-AgEIA [2]. When accounting for the difference in test characteristics between the two assays, overall seroprevalence was estimated to be 10.3% in

NHANES III among those age 6 and older if the current assay had been used. Even with this conservative adjustment that does not account for samples both TES-AgEIA negative and Tc-CTL-1MBA positive, these estimates remain higher than the 2011–14 estimate of 5.0% overall, suggesting decreased exposure to *Toxocara* over 17 years in the US population.

In the current study, older adults (≥50 years of age) had higher *Toxocara* seroprevalence than children 6–11 years old, whereas in NHANES III, younger adults 20–39 years old had higher seroprevalence compared to children 6–11 years old. If *Toxocara* antibodies are long-lived as previous studies have inferred [16, 17], the apparent shift towards greater seroprevalence among older age groups in this study could reflect aging of a cohort of individuals with greater past exposure to *Toxocara*. Further supporting this possible cohort effect is decreased *Toxocara* seroprevalence in adults 40–49 years old relative to contiguous age groups in NHANES III with a corresponding decrease in prevalence among adults 60–69 years old in this current study (Table 3) occurring 17 years after completion of NHANES III.

Although we observed lower overall seroprevalence and differences in age groups at higher risk in this study, we identified similar risk factors for *Toxocara* seropositivity amongst shared factors described in NHANES III. Prevalence remains higher among non-Hispanic Blacks and lower among Mexican Americans. Furthermore, birth outside of the US, male sex, poverty, and less than college education are again identified as possible risk factors.

This study introduces new findings. It estimates *Toxocara* seroprevalence among non-Hispanic Asians in the US for the first time. On univariate analysis, seroprevalence was higher among non-Hispanic Asians compared to non-Hispanic Whites, but this association was confounded by place of birth. Over 75% of the non-Hispanic Asian population based on NHANES data were born outside the US and in multiple logistic models adjusted for place of birth, non-Hispanic Asian race/ethnicity was no longer associated with higher seroprevalence. We note crowding, a possible correlate of lower socioeconomic status [18], as a risk factor for seropositivity, which was not noted on multiple logistic regression in NHANES III. Contrasting with a previous study that found higher seroprevalence with an asthma diagnosis in children 4–6 years old [19], we found lower seroprevalence in those with an asthma diagnosis among non-Hispanic Blacks. While consistent with the “hygiene hypothesis” [20] in an older population, this finding must be interpreted with caution as it is limited to one subgroup.

This antibody-based study is unable to differentiate between recent or remote infection [17]. Furthermore, 16.2% of subjects undergoing examination did not have specimens obtained and available for testing. In particular, differences in availability of specimens varied significantly in subgroups by age, race/Hispanic origin, poverty, crowding, and place of birth (Supplemental Table 1). Sample weights were adjusted to account for nonresponse but the adjustment had minimal impact on seroprevalence estimates (differences ≤1.3%), suggesting minimal nonresponse bias.

To facilitate comparison of results, we estimated the seroprevalence for NHANES III if samples had been tested using Tc-CTL-1MBA. Adjustments are based on a subset of

specimens, which may not represent the entire US population. Furthermore, higher specificity of Tc-CTL-1MBA compared to TES-AgEIA[4] may reflect detection of only *T. canis* specific antibody in Tc-CTL-1MBA in contrast to non-specific *Toxocara* antibody reflecting infection with *T. canis* or *cati* in TES-AgEIA. TES-AgEIA has not been tested against sera with antibodies exclusive to *T. cati*, whose role in human infection is unknown, but thought to be important. In addition, variables such as pet ownership and location of residence were only available in NHANES III. Blood lead level and occupation were available in NHANES 2011–2014, but these data were too small of a subset or nonspecific to allow inclusion in the current study.

Despite the above mentioned caveats, we confirm risk factors associated with exposure that can cause a disease with severe morbidity. These risk factors can guide healthcare providers in evaluation of possible toxocariasis in patients as well as inform public health interventions to reduce exposure in populations at risk, such as encouraging hand washing after contact with soil, reducing soil contamination by dog and cat feces where at-risk populations live, and treating dogs and cats with antihelminths to reduce *Toxocara* burdens [3].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Summary

Evidence of human exposure to *Toxocara*, an intestinal nematode of dogs and cats, was lower based on a national serosurvey conducted from 2011 through 2014 than in a survey conducted 17 years prior, but remained associated with specific risk groups.

Table 1

Estimated *Toxocara* seroprevalence in the United States by age group and race/Hispanic origin in persons ≥ 6 years of age, NHANES 2011–2014

		entire study population (n=13509)	Non-Hispanic White (ref) (n=4972)	Non-Hispanic Black (n=3114)	Non-Hispanic Asian (n=1581)	Mexican American (n=2010)
age group	**sample size**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
total	13509	5.1 (4.3, 5.9)	3.8 (3.0, 4.7)	6.7 (5.5, 8.1) $^{+++}$	7.4 (5.9, 9.2) $^{+++}$	6.1 (4.4, 8.3) $^{+}$
6–11 (ref)	1758	3.0 (1.9, 4.5)	3.0 (1.4, 5.7) $^{\int}$	3.5 (1.7, 6.4)	2.1 (0.4, 6.1) $^{\ddagger\int\int\int}$	3.0 (1.3, 5.9) $^{\int}$
12–19	2103	3.9 (2.9, 5.0)	2.8 (1.5, 4.5)	5.4 (3.4, 8.2)	4.4 (2.0, 8.2) $^{\int}$	3.8 (1.9, 6.8)
20–29	1636	4.6 (3.3, 6.2)	2.6 (1.4, 4.5)	4.0 (1.9, 7.2)	4.0 (1.4, 8.8) $^{\ddagger\int}$	8.7 (4.8, 14.2) *
30–39	1686	4.8 (3.7, 6.0) *	3.6 (2.4, 5.3)	5.3 (3.1, 8.5)	4.5 (2.2, 8.0)	6.1 (3.1, 10.5)
40–49	1645	5.4 (4.2, 6.8) **	3.7 (2.2, 5.6)	6.4 (3.9, 9.7)	6.1 (3.4, 9.9) *	8.9 (5.1, 14.2) *
50–59	1592	6.4 (4.7, 8.6) **	5.3 (3.1, 8.6)	10.4 (7.5, 13.8) ***	5.6 (2.0, 12.2) $^{\int}$	6.8 (2.9, 13.3) $^{\int}$
≥60	3089	5.7 (4.5, 7.1) **	4.1 (2.8, 5.7)	10.4 (8.0, 13.3) ***	19.7 (13.9, 26.6) ***	3.9 (2.0, 6.8)

*** $P < 0.001$,

** <0.01,

* <0.05 from a two sample t-test comparing each age group to the age 6–11 reference group

$^{+++}$ $P < 0.001$,

$^{++}$ <0.01,

$^{+}$ <0.05 from a two sample t-test comparing each race/Hispanic origin subgroup to the non-Hispanic White reference group for the total population

† estimate with <10 degrees of freedom

‡ estimate based on <10 positive sample persons

$^{\int}$ relative standard error (RSE) >30% and <40%,

$^{\int\int}$ RSE >40% and <50%,

$^{\int\int\int}$ RSE >50% and <60%.

Elevated RSE may result in unstable estimates.
95% confidence interval estimated by Korn and Graubard method [13]
Estimates are weighted to represent the total U.S. population and account for oversampling and nonresponse to the household interview and physical examination

Table 2

Estimated age adjusted *Toxocara* seroprevalence in the United States among persons ≥ 6 years of age by factor and race/Hispanic origin, NHANES 2011–2014

			entire study population (n=13509)	Non-Hispanic White (ref) (n=4972)	Non-Hispanic Black (n=3114)	Non-Hispanic Asian (n=1581)	Mexican American (n=2010)
factor		**sample size**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
race		13509	5.0 (4.2, 5.8)	3.7 (2.9, 4.5)	6.6 (5.4, 8.0) $^{+++}$	7.3 (5.9, 9.0) $^{+++}$	6.1 (4.4, 8.2) $^{+}$
sex							
	female (ref)	6859	3.8 (3.1, 4.6)	2.5 (1.9, 3.3)	4.9 (3.8, 6.3)	5.6 (4.1, 7.4)	5.1 (3.6, 7.1)
	male	6650	6.2 (5.3, 7.2) ***	4.8 (3.8, 6.0) ***	8.8 (6.9, 11.0) ***	9.5 (7.1, 12.3) **	7.3 (4.9, 10.5)
poverty level							
	at or above (ref)	9144	3.9 (3.3, 4.5)	3.0 (2.5, 3.6)	5.9 (4.5, 7.5)	6.3 (4.8, 8.0)	4.6 (3.0, 6.6)
	below	3374	10.2 (8.4, 12.1) ***	8.0 (5.0, 12.1) **	8.6 (6.5, 11.0) *	11.5 (6.6, 18.2)	10.4 (7.0, 14.7) **
crowding index							
(persons per room)	<0.5 (ref)	4471	3.5 (3.0, 4.2)	3.1 (2.4, 3.8)	6.8 (4.5, 9.8)	5.4 (2.8, 9.4)	4.0 (1.3, 9.3) $^{\int\int}$
	0.5–0.99	5810	5.6 (4.7, 6.7) ***	4.3 (3.2, 5.6)	7.2 (5.6, 9.0)	8.7 (5.9, 12.3)	3.8 (2.3, 5.9)
	≥1	3124	10.5 (8.3, 13.0) ***	8.3 (3.9, 15.1) $^{*\int}$	8.4 (5.2, 12.7)	9.3 (6.0, 13.5)	11.0 (7.4, 15.5) **
head of household							
education	some college (ref)	7115	3.4 (2.8, 4.0)	2.6 (2.0, 3.2)	5.2 (4.0, 6.5)	7.1 (5.7, 8.8)	3.0 (1.4, 5.4)
	high school graduation	2863	6.4 (5.3, 7.6) ***	5.4 (3.9, 7.3) **	7.7 (5.6, 10.3) *	5.7 (2.7, 10.5)	6.5 (4.3, 9.5) **
	some high school	1872	7.8 (6.3, 9.5) ***	5.9 (3.8, 8.6) **	7.8 (5.3, 11.0)	5.8 (1.7, 13.8) $^{\dagger\ddagger\int}$	7.9 (4.6, 12.3) *
	<high school	1210	11.1 (8.5, 14.2) ***	9.0 (3.7, 17.7) $^{*\int}$	18.4 (8.5, 32.8) *†	8.2 (3.3, 16.3) $^{\dagger\int}$	8.1 (5.0, 12.3) **
birthplace							
	US (ref)	10257	3.7 (3.1, 4.4)	3.5 (2.8, 4.4)	5.5 (4.5, 6.8)	1.1 (0.3, 2.6) $^{\int}$	2.7 (1.5, 4.4)
	non-US	3247	11.9 (9.7, 14.4) ***	7.8 (4.0, 13.4)	15.3 (8.3, 24.9) *	8.1 (6.3, 10.2) ***	8.6 (6.2, 11.7) ***
asthma diagnosis							
	no (ref)	11285	5.1 (4.3, 5.9)	3.6 (2.8, 4.5)	7.1 (5.8, 8.7)	7.4 (5.8, 9.3)	6.2 (4.4, 8.4)
	yes	2215	4.5 (3.5, 5.5)	4.0 (2.8, 5.5)	4.0 (2.6, 5.9) ***	7.2 (3.7, 12.5)	4.9 (2.6, 8.4)

*** $P < 0.001$,

**<0.01,

*<0.05 by two sample t-test for each covariate level relative to the reference group

^{+++}P < 0.001,

$^{++}$<0.01,

$^{+}$<0.05 from a two sample t-test comparing age adjusted estimate for each race/Hispanic origin subgroup to the non-Hispanic White reference group for the total population

†estimate with <10 degrees of freedom

‡estimate based on <10 positive sample persons

$^{\int}$relative standard error (RSE) >30% and <40%,

$^{\int\int}$RSE >40% and <50%,

$^{\int\int\int}$RSE >50% and <60%.

Elevated RSE may result in unstable estimates.
95% confidence interval estimated by Korn and Graubard method [13]
Estimates are weighted to represent the total U.S. population and account for oversampling and nonresponse to the household interview and physical examination.

Table 3

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

Factor	OR (95% CI)
Age (years)	
6–11	Ref
12–19	1.1 (0.6, 1.9)
20–29	1.4 (0.8, 2.4)
30–39	1.3 (0.7, 2.3)
40–49	1.6 (0.9, 2.7)
50–59	2.1 (1.1, 3.9) *
60–69	1.7 (1.0, 2.8) *
≥70	2.6 (1.5, 4.7) **
Race/Hispanic Origin	
Non-Hispanic White	Ref
Non-Hispanic Black	1.4 (1.0, 2.0) *
Non-Hispanic Asian	0.7 (0.5, 1.1)
Mexican American	0.6 (0.4, 1.0) *
Other Hispanic and Other Race	1.1 (0.8, 1.8)
Sex	
female	Ref
male	1.9 (1.6, 2.2) ***
Poverty level	
below	1.9 (1.4, 2.6) ***
at or above	Ref
Crowding index (persons per room)	
≥0.5	1.3 (1.0, 1.6) *
<0.5	Ref
Head of household education	
less than college	1.9 (1.5, 2.4) ***
at least some college	Ref
Place of birth	
other	3.6 (2.6, 5.1) ***
United States	Ref

*** P < 0.001,

** <0.01,

* <0.05

Table 4

2 × 2 table of results of comparative testing with the TES-AgEIA in each group of 249 and 250 randomly sampled sera found positive or negative, respectively, by Tc-CTL-1MBA.

		Tc-CTL-1MBA		
		+	−	
TES-AgEIA	+	208 74.3%† 83.5%‡	72 25.7%† 28.8%‡	280
	−	41 18.7%† 16.5%‡	178 81.3%† 71.2%‡	219
		249	250	
		χ^2=150 P<0.0001		

† Percentage positive or negative by Tc-CTL-1MBA within a row specifying all positive or negative TES-AgEIA results.

‡ Percentage positive or negative by TES-AgEIA within a column specifying all positive or negative Tc-CTL-1MBA results.