National Seroprevalence and Risk Factors for Zoonotic *Toxocara* spp. Infection

Kimberly Y. Won,* Deanna Kruszon-Moran, Peter M. Schantz, and Jeffrey L. Jones

*Division of Parasitic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, CCID, Centers for Disease Control and Prevention, Atlanta, Georgia; Division of Health and Nutrition Examination Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, Maryland*

Abstract. To estimate the prevalence of *Toxocara* spp. infection in a representative sample of the United States population ≥6 years of age, sera from participants in the Third National Health and Nutrition Examination Survey (1988–1994) were tested for antibodies to *Toxocara*. Among the 30,930 persons selected for the survey, 82% (N = 25,733) were interviewed, and 91% (N = 23,527) of those interviewed underwent physical examination of which 87% (N = 20,395) were tested. The age adjusted *Toxocara* seroprevalence was 13.9% (95% confidence intervals [CI] 12.5, 15.3), and was higher in non-Hispanic blacks (21.2%) than non-Hispanic whites (12%) or Mexican Americans (10.7%; P < 0.001). Increased *Toxocara* seropositivity was associated with head of household level of education (low versus high) (odds ratio [OR]: 2.2; CI: 1.8, 2.8), poverty (OR: 1.5; CI: 1.3, 1.8), elevated blood lead concentrations (OR: 1.4; CI: 1.1, 1.9), and dog ownership (OR: 1.2; CI: 1.1, 1.4). *Toxocara* infection is widespread and associated with specific risk groups.

INTRODUCTION

Larval stages of *Toxocara canis* and *Toxocara cati*, common intestinal roundworms of dogs and cats, respectively, frequently infect humans worldwide. *Toxocara* eggs are passed unembryonated in the feces of these animals, become infectious in suitable environments, and can remain infective in the soil for many years. Human infection can result in a variety of syndromes with different clinical manifestations. Two commonly described syndromes, visceral larva migrans and ocular larva migrans, can include abdominal pain, hepatomegaly, persistent eosinophilia, visual impairment, and retinal scarring. 

A condition known as covert toxocariasis may be the most common form of the disease and can include symptoms such as headache, cough, fever, and wheezing. Individuals with covert toxocariasis may or may not have elevated eosinophil counts. However, many *Toxocara* infections remain asymptomatic and therefore remain underdiagnosed and underappreciated.

High human seroprevalence has been shown in areas with documented soil contamination, and the risk for transmission may be increased in proportion to the degree of environmental contamination. In studies of clinical cases of *Toxocara* infections in humans, pet ownership and geophagia or pica have consistently been identified as important risk factors for zoonotic transmission of *Toxocara*. Factors such as age, sex, and geographic location may also be important risk factors, and reports have shown that toxocariasis disproportionately affects socioeconomically disadvantaged populations. Because only limited information is available on the persistence of *Toxocara* antibodies after infection, it is difficult to determine whether high human seroprevalence is indicative of persistent antibodies or re-infection. However, because *Toxocara* infection can cause human morbidity, it is valuable to understand the level of *Toxocara* exposure among populations.

In this study, serum samples collected as part of the Third National Health and Nutrition Examination Survey (NHANES III) were tested for *Toxocara* antibodies to estimate the seroprevalence of *Toxocara* infection in the United States and to identify characteristics associated with human infection. Examining seroprevalence differences will aid in identifying groups to target for health education messages.

METHODS

The NHANES III was a nationally representative, cross-sectional survey conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) between 1988 and 1994. It was designed to obtain health statistics of the United States population through household interviews, standardized physical exams, and collection of blood samples in mobile examination centers. The NHANES III consisted of a stratified, multistage, probability cluster sample of 33,994 people older than two months of age, representative of the civilian, non-institutionalized general U.S. population. People between two months and five years of age, those older than 60 years of age, Mexican Americans, and non-Hispanic blacks were oversampled to assure adequate sample size for these groups. A more detailed description of the survey design, informed consent procedure and the sample has been published elsewhere. For our study, only surplus sera were examined, and there was no link between existing study data, biological specimens, and patient identifiers. Our evaluation was determined to be exempt from human subjects review.

Race/ethnicity was based on self-reported information and classified as non-Hispanic white, non-Hispanic black, or Mexican American. Those who were not classified into one of these three categories were placed in the “other” racial/ethnic group and were only analyzed within the total population. Although children as young as two months of age were included in NHANES III, many of the serum samples from young children were not available for testing. For our study, surplus sera from individuals ≥6 years of age were tested for antibodies to *Toxocara*. Multivariate analyses were conducted for persons ≥6 years of age with the exception of one predictor variable (occupation) that was only applicable to individuals ≥20 years of age. Age was grouped into eight categories (6–11, 12–19, 20–29, 30–39, 40–49, 50–59, 60–69, and 70+ years) and was entered into logistic regression models accordingly. Poverty index was calculated by dividing total

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* Address correspondence to Kimberly Y. Won, Division of Parasitic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, CCID, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Mailstop F-36, Atlanta, GA 30341-3724. E-mail: kfw7@cdc.gov
family income by the U.S. poverty threshold and adjusted for family size. A crowding index was calculated by dividing the total number of residents in a household by the number of rooms in the household. This index was expressed as number of persons per room and grouped into three categories (<0.5, 0.5–0.99, and ≥1 persons per room). Head of household education was measured as the last year of schooling completed by the head of household and was initially grouped into four categories (no high school, some high school, high school graduate, and some college). In the logistic regression models, education was collapsed into three categories (less than high school, high school graduate, and some college). Blood lead levels were considered above normal at concentrations of 10 μg/dL or higher. Metropolitan residence was defined as living in an area with a population of ≥1 million persons. All other areas, including rural areas, were considered to be non-metropolitan. Pet ownership, as well as dog and cat ownership specifically, was defined as ownership of an animal(s) at the time of the survey and did not reflect ownership in the past. Working in a soil-related occupation was according to the longest held job and included farm workers, farm operators, farm managers, and related agricultural occupations. Regions were defined as Northeast (Connecticut [CT], Massachusetts [MA], Maine [ME], New Hampshire [NH], New Jersey [NJ], New York [NY], Pennsylvania [PA], Rhode Island [RI], Vermont [VT]); South (Alabama [AL], Arkansas [AR], Delaware [DE], Washington [DC], Florida [FL], Georgia [GA], Kentucky [KY], Louisiana [LA], Maryland [MD], Mississippi [MS], North Carolina [NC], Oklahoma [OK], South Carolina [SC], Tennessee [TN], Texas [TX], Virginia [VA], West Virginia [WV]); Midwest (Iowa [IA], Illinois [IL], Indiana [IN], Kansas [KS], Michigan [MI], Montana [MT], Missouri [MO], North Dakota [ND], Nebraska [NE], Ohio [OH], South Dakota [SD], Wisconsin [WI]); West (Arkansas [AK], Arizona [AZ], California [CA], Connecticut [CT], Hawaii [HI], Idaho [ID], Montana [MT], New Mexico [NM], Nevada [NV], Oregon [OR], Utah [UT], Washington [WA], Wyoming [WY]).

Laboratory Testing. All specimens were tested during the years 2005–2006 using one dilution, enzyme immunoassay (EIA) with sensitivity and specificity of 78% and 92%, respectively.14 Specimens were tested using 96-well Immulon II HB flat bottom plates sensitized with *Toxocara canis* excretory-secretory (TES) antigen at a dilution of 1:2000 using a 0.1M NaHCO3/Na2CO3 buffer. Although this assay used a T. cati antigen, it was not able to distinguish between *T. canis* and *T. cati* infections. All sera were diluted 1:100 with a phosphate buffered saline (PBS) + 0.05% Tween solution. An anti-IgG enzyme conjugate was used to detect antigen-antibody complexes, and 3,3′, 5,5′-tetramethylbenzidine (TMB) was the substrate used to visualize any reaction. The plates were read at 450 nm using a VMax microplate reader (Molecular Devices Corp., Menlo Park, CA) with a computer equipped with SOFTmax software (Molecular Devices Corp., Menlo Park, CA) for reader control and data analysis. For each individual test run, a positive cutoff ratio value was calculated. Because results from the standard EIA are considered positive at a titer of ≥1:32, four low-positive control specimens with this titer and a high positive control, run in duplicate, were used as quality control for each run. The cutoff ratio was calculated by averaging the optical density (O.D.) readings for the low positive controls and dividing this value by the mean of the high positive control O.D. values. Each sample ratio was calculated by dividing the specimen O.D. value by the mean of the high positive control and was compared with the cutoff ratio to determine if the sample was positive or negative.

A battery of 49 specimens (25 positive, 24 negative) originally tested using the standard titration EIA were evaluated using the one dilution EIA. Comparative sensitivity and specificity were 96% and 100%, respectively. Reproducibility of results was also evaluated midway through the study. Eighty-one samples (11 positive, 70 negative) were randomly chosen for retesting. Upon retesting, results were consistent with the initial results except for one of the 11 positive samples that tested negative, and one of the 70 negative samples that tested positive. Both of the samples with inverted results were close to the respective positive cutoff ratios when initially tested.

**Statistical Analysis.** Estimates were weighted to represent the total U.S. population and to account for oversampling and nonresponse to the household interview and physical examination.15 Statistical analyses were conducted using SUDAAN, a family of statistical procedures for analysis of data from complex sample surveys.16 Standard error estimates were calculated using the Taylor Series Linearization method in SUDAAN to account for the complex sample design, and prevalence estimates were age-adjusted by the direct method to the 2000 U.S. population when seroprevalence was compared across population subgroups. Screening for possible predictors for *Toxocara* seropositivity was done by evaluating differences in seroprevalence without correcting for multiple comparisons and examining 95% confidence intervals (CI) generated by SUDAAN. The P values were determined from a univariate t-statistic generated from a general linear contrast procedure in SUDAAN. Multivariate logistic regression was used to further determine independent predictors. Modeling was conducted for the combined population and variables that had a Satterthwaite-adjusted F statistic with a P value < 0.05 from the logistic model were considered significant.

**RESULTS**

Of the 30,930 persons 6 years of age and older selected for the NHANES III survey, 83% (N = 25,733) were interviewed and 91% of those interviewed (N = 23,527) underwent physical examination. Of those examined, 87% (N = 20,395) were tested for *Toxocara* antibodies. The percentage of sera tested for *Toxocara* within designated age categories ranged from 84–95% except for the 6 to 11 year old group, for which 74% were available and tested. The availability of a specimen for antibody testing among those examined varied by age, race, sex, region, foreign birth, residence in a metropolitan area, poverty, crowding index, and head of household education (P < 0.05). It did not vary by dog or cat ownership or blood lead concentration.

The overall age-adjusted *Toxocara* seroprevalence for individuals ≥ 6 years of age as 13.9% (95% CI: 12.5, 15.3) and varied significantly among racial/ethnic groups. *Toxocara* seropositivity was significantly higher for non-Hispanic blacks 21.2% (95% CI: 19.7, 22.8) than any other racial/ethnic group (Table 1). Seroprevalence differed by sex, foreign birth, poverty level, household crowding, education, and blood lead levels. Persons ≥ 20 years of age who reported being involved
in a soil occupation, such as farming and agriculture, had a seroprevalence of 25.5% (95% CI: 18.0, 33.0) compared with 13.5% (95% CI: 12.2, 14.9; *P* < 0.001) for those persons not involved in these types of occupations (Table 1). *Toxocara* seroprevalence varied by age among racial/ethnic groups as shown in Figure 1.

In the overall multivariate model for persons 6 years of age or older, *Toxocara* seroprevalence was significantly higher for non-Hispanic blacks (compared with non-Hispanic whites), and significantly lower for Mexican Americans (compared with non-Hispanic whites). Seroprevalence was higher for persons 12–19, 20–29, and 30–39 years of age as compared with 6–11 years of age, was higher among males, those living in poverty, individuals born outside of the United States, those living in non-metropolitan areas, those with above normal blood lead concentrations, dog owners, and those living in the three geographic regions outside of the West. Seroprevalence was also higher for those persons whose head of household had less than or at least a high school education compared with those with more than a high school education. Although crowding index was found to be a significant factor in the univariate analysis, it was no longer significant in the multivariate analysis (Table 2).

### DISCUSSION

In our national population-based study, the overall age-adjusted *Toxocara* seroprevalence for individuals ≥ 6 years of age was 13.9%. Reported seroprevalence estimates vary worldwide, but many of these estimates were determined from convenience samples of various populations. 17–20 The
wide range of seroprevalence estimates may be a reflection of a difference in characteristics (i.e., socioeconomic status, race, soil exposure, pet density, etc.) of the sampled populations. Because of the different serologic assays used to determine these figures and the potential bias introduced when using convenience samples, it is difficult to compare seroprevalence estimates. However, estimates from the NHANES III data were determined from a nationally representative sample, and confirm that there is substantial exposure to *Toxocara* in the United States.

Studies have reported age, both young and old, as a risk factor for *Toxocara* infection. However, young age is widely considered to be a particularly vulnerable time for acquiring infection because children are likely to play in contaminated environments and then put their fingers and hands in their mouths either incidentally or intentionally. In a case control study conducted in Bogotá, Columbia, children between one and four years of age were found to have higher *Toxocara* seropositivity compared with older children. In the NHANES III population sample, seroprevalence in children 6–11 years of age ranged from a low of 10.1% among non-Hispanic whites to a high of 18.9% among non-Hispanic blacks. In a study conducted by Hermann and others, sera from children 6 to 11 years of age who had participated in the first Health and Nutrition Examination Survey (HANES I), 1971–1973, were tested for *Toxocara* antibodies. Seroprevalence was > 20% for black children, but < 5% for white children. This earlier report noted that the evaluated group was not representative of the U.S. population because sera of more white children were available for testing when compared with the entire HANES I population of the same age. The absolute differences in the number of children tested were relatively small, but reached statistical significance because of the large sample size. A similar study conducted with the same HANES I population reported *Toxocara* seroprevalence > 15% for black female children and > 20% for black male children between 6 and 11 years of age, but < 5% for white children in the same age category.

Among the entire NHANES III population, *Toxocara* seroprevalence was significantly higher for non-Hispanic blacks than other race/ethnicity groups. Prevalence among non-Hispanic blacks was greatest during adolescence, whereas prevalence among non-Hispanic whites and Mexican Americans did not show the same pattern. These differences may reflect behaviors and/or environmental factors, which placed...
these different groups at risk of exposure to Toxocara at different stages of life. Because there is limited information on the duration of persistence of Toxocara antibodies and the incidence of reinfection, it is difficult to determine whether seroprevalence in older age groups is a reflection of previous infections with persistent antibodies or newly acquired infections. A few reports suggest that Toxocara antibodies can persist for years, even after anthelmintic treatment, but most of these reports are based on relatively small sample sizes. These reports also show that antibody levels tend to decrease over time.\textsuperscript{27–29} In the NHANES III population, Toxocara seroprevalence did not increase with age as was seen with Toxoplasma seroprevalence.\textsuperscript{30} Both Toxocara and Toxoplasma can be transmitted through soil, but Toxoplasma can also be transmitted through undercooked contaminated meat and antibodies to Toxoplasma are long lasting. However, the relatively stable seroprevalence across age groups may suggest that individuals are susceptible to reinfection with Toxocara.

Many studies have evaluated pet (most commonly dog and cat) ownership as a potential risk factor for Toxocara infection, and have shown associations with human Toxocara seroprevalence.\textsuperscript{1,26} In contrast, other studies worldwide have not shown the same association.\textsuperscript{25,31–33} The different findings may be indicative of the incidence of infection relative to the time the testing was done. Differences may also be related to the type of population evaluated. Individuals tested as part of a clinical case-control study may be significantly different than individuals in population-based studies. In our study, dog ownership was associated with Toxocara seropositivity in the multivariate analysis.

In the NHANES III population, head of household education was also found to be associated with Toxocara seroprevalence. Individuals who had not completed high school had the highest seroprevalence and seropositivity decreased steadily with higher levels of completed education. Lower education levels are often associated with lower socioeconomic status and may also be associated with occupations involving soil exposure, which was also associated with Toxocara seropositivity in the overall population. Those persons with lower education may also be more likely to live in areas with high environmental contamination. In addition, individuals with high blood lead levels had significantly higher Toxocara seropositivity. Elevated blood lead levels in children have often been associated with pica, a widely accepted risk factor for Toxocara infection.\textsuperscript{34–37} Although increased lead exposure in adults may result from routes of exposure different from those associated with Toxocara infection, it may be indicative of lower socioeconomic status, which has been shown to be associated with Toxocara infection. The findings of this study confirm that Toxocara infection is widespread in the United States. Although the sensitivity of the assay used was not perfect, and the positive predictive value was relatively low (61%), even a 39% reduction of the overall seroprevalence would still yield a Toxocara seroprevalence of >8% in the general population. Furthermore, when this assay is used in conjunction with a presumptive diagnosis to confirm Toxocara infection, the predictive values are raised.\textsuperscript{28} Although this assay does not distinguish between acute and chronic Toxocara infections and progress has been made on the development of highly specific recombinant antigen tools,\textsuperscript{38–40} this test is still the most widely used serologic tool in nontropical settings. Because NHANES III was not designed specifically to evaluate risk factors associated with Toxocara infection, further studies should be conducted to confirm results obtained in this study.

Despite the limitations encountered in this study, NHANES III has provided the best national estimates to date of the prevalence of Toxocara infection in the United States. With an estimated 72 million dogs and 82 million cats in the United States,\textsuperscript{41} there is potential for widespread environmental contamination with Toxocara spp. eggs. Further studies under controlled conditions are necessary to further define potential morbidity associated with Toxocara infection. Prevention efforts such as hand washing after soil contact, prevention of soil contamination in public areas by dog and cat feces, and preventive anthelmintic treatment of puppies and kittens can help minimize exposure to Toxocara spp.\textsuperscript{42,43} and help control potential morbidity associated with Toxocara infection. Continued monitoring of Toxocara seroprevalence could be done through future NHANES surveys.

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Authors’ addresses: Kimberly Y. Won, Peter M. Schantz, and Jeffrey L. Jones, Division of Parasitic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, CCID, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Mailstop F-36, Atlanta, GA 30341-3724, Tel: 770-488-4415, Fax: 770-488-3115, E-mails: kwf7@cdc.gov, pmsl1@cdc.gov, and jjlj@cdc.gov. Deanna Kruzon-Moran, Division of Health and Nutrition Examination Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention, HYAT Building IV, Room 4308, Mailstop P-08, Hyattsville, MD 20782, E-mail: ddk0@cdc.gov.

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