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# Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014

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# Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014


## Abstract

**Objectives**: This study sought to examine the relationship between Toxoplasma gondii seropositivity and cognitive functioning in older adults.

**Design**: A cross-sectional study.

**Setting:** The National Health and Nutrition Examination Survey (NHANES) study took place at participants' homes and mobile exam centers.

**Participants**: A total of 2, 956 older adults aged 60 and above from the NHANES from 2011 to 2014 were included in the study.

Exposure of interest: Serum toxoplasma gondii antibody was analyzed in the lab. A value > 33 IU/mL was categorized as seropositive for toxoplasma gondii infection; <27 IU/mL was categorized as seronegative for toxoplasma gondii infection.

**Primary and secondary outcome measures**: Cognitive tests included the Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL) immediate and delayed memory, the Animal Fluency test (AFT), and the Digit Symbol Substitution Test (DSST).

**Results**: About half of the 2,956 participants (mean age 70.0) were female (51.0%), non-Hispanic White (48.3%), and completed some college or above (48.3%). A total of 703 participants were positive for toxoplasma gondii infection (23.8%). Adjusted linear regression showed that compared with participants with negative toxoplasma gondii infection, those with positive toxoplasma gondii infection had lower CERAD-WL immediate memory (beta [β] -0.16,

95% confidence interval [CI] -0.25, -0.07), CERAD-WL delayed memory (β -0.15, 95% CI -0.24, -0.06), AFT (β -0.15, 95% CI -0.24,-0.06), DSST (β -0.34, 95% CI -0.43, -0.26), and global cognition (β -0.24, 95% CI -0.32,-0.16) z scores after controlling for the covariates.

**Conclusions**: Toxoplasma gondii seropositivity is associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition in older adults. Public health measures aiming at preventing toxoplasma gondii infection may help preserve cognitive functioning in older adults.

**Keywords**: Toxoplasma gondii; cognitive functioning; older adults; neurotoxoplasmosis; neuropsychological; psychomotor performance

**Strengths and limitations:**

- This study is one of the few studies that examined the cognitive effect of Toxoplasma gondii seropositivity on cognitive outcomes in older adults.
- With the NHANES data, the study population was nationally representative of U.S. older adults.
- A wide range of sociodemographic, lifestyle, mental health, and physical health covariates was adjusted, reducing the possibility of residual confounding.
- A cross-sectional study hinders the assessment of longitudinal relationships.

## 1. Introduction

Alzheimer's disease and related dementia (ADRD) is a serious public health threat worldwide. In 2016, a total of 43.8 million people had dementia in the world. With a growing number of people with ADRD, families, communities, and healthcare systems around the world are heavily burdened (1). Although dementia is currently not curable, identifying modifiable risk factors associated with ADRD can help reduce the burden of the disease. Cognitive test performance in older adults is an important indicator of their cognitive functioning (2). By examining their correlations to cognitive tests, risk factors for cognitive decline can be identified and intervened.

Toxoplasma gondii is among the most prevalent human zoonoses (3) and affects about 30% of the global population (4). Toxoplasma gondii infection can happen prior to or after a person is born, with postnatal infection being more common. By consuming oocysts found in cat feces-contaminated soil or water (5, 6) or eating tissue cysts in undercooked meat (7, 8), humans can be infected with toxoplasma gondii after birth. While most people with a healthy immune system are asymptomatic following acute infection with toxoplasma gondii, some may experience unspecific symptoms lasting from several weeks to months, including fever, malaise, and lymphadenopathy (9).

A limited number of studies have found that toxoplasma gondii infection is associated with neurocognitive changes in humans (10-13). However, their direction of findings and effect sizes are inconsistent. In addition, this area is understudied, given the prevalence of toxoplasma gondii infection in humans. In a systematic review and meta-analysis of the association of toxoplasma gondii seropositivity and cognitive function in healthy people, only thirteen studies were included, most of which had small sample sizes (4). In addition, two included studies (14, 15) utilized the National Health and Nutrition Examination Survey (NHANES) 1988-1994 cycle

data and thus could not reflect the current epidemic of toxoplasma gondii infection. The findings of this study showed that seropositivity to toxoplasma gondii was modestly but significantly associated with poorer processing speed, working memory, verbal short-term memory, and executive functioning. However, studies using large, the latest, and nationally representative population-based data are needed to better elucidate the cognitive effects of toxoplasma gondii seropositivity.

In this study, taking advantage of the NHANES, we aimed to examine the relationship between toxoplasma gondii seropositivity and cognitive functioning in a nationally representative sample of U.S. older adults. The findings of this study will provide implications for understanding the cognitive effects of toxoplasma gondii infection and developing tailored public interventions to protect cognitive functioning in the growing number of older adults in the US.

## 2. Method

### *2.1 Study design and setting*

The NHANES is an ongoing, cross-sectional survey of civilian, non-institutionalized adults and children in the United States conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention. A nationally representative sample of children and adults across the country are surveyed bi-annually (16). Their sociodemographic, health, and nutritional status are evaluated using in-person interviews and physical exams. The interviews are conducted at participants' homes; health exams are conducted in specially equipped mobile exam centers. Health exams include laboratory testing of urine and blood specimens and medical, dental, and physiological assessments. Participants' serum toxoplasma gondii antibody

levels and cognitive functioning were measured in the NHANES 2011-2014 cycles. In this study, two survey cycles (2011-2012 and 2013-2014) were merged to increase sample size and power. Between 2011 and 2014, a total of 19,151 individuals participated in the NHANES. They were recruited from a selection of census blocks or clusters of census block area segments. The detailed sampling method has been published elsewhere (17).

Of the 19,151 individuals, we excluded individuals aged < 60 (n = 15,679) or had missing data on serum Toxoplasma gondii IgG (n=511). Participants with equivocal serum Toxoplasma gondii IgG (≥27 and <33 IU/mL) were further excluded (n=5). Finally, a total of 2,956 participants aged 60 and above were included in the analysis. The characteristics of the excluded participants due to missing data (n=516) were presented in the Appendix. Compared with the included participants, people who were excluded were more likely to be of other ethnicities than Non-Hispanic Whites, overweight/obese, completed lower education and had higher systolic blood pressure, lower Digit Symbol Substitution Test (DSST) score, and lower Animal Fluency test (AFT) score.

### *2.2 Ethical considerations*

The National Center for Health Statistics Research Ethics Review Board approved the NHANES. Participants in the NHANES provided written informed consent before enrolling in the study. The University of Houston-Downtown Committee for the Protection of Human Subjects granted this study an exemption because only publicly accessible and de-identified data were used.

### *2.3 Public and Patient involvement*

Patients or the public were NOT involved in the design, conduct, reporting, or dissemination plans of our research.

### *2.4 Independent* variable: Toxoplasma gondii seropositivity

Serological tests that detect Toxoplasma. gondii Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies are often used for clinical diagnoses of toxoplasmosis. While the IgM antibody test can validate acute phases, the IgG antibody test can identify acute or chronic phases (18). In the NHANES, an enzyme immunoassay (EIA) measuring IgG against toxoplasma gondii was used to measure the presence or absence of toxoplasma gondii (16). Toxoplasma gondii IgG was measured with two enzyme immunoassay kits (19). Strict quality control was implemented for every plate. A value between 27 and 33 IU/mL was deemed as "equivocal"; a value ≥33 IU/mL was deemed as "positive"; a value <27 IU/mL was deemed as "negative" (16). Samples with equivocal results (≥27 IU/mL and <33 IU/mL) were repeated twice and confirmed as negative. For our analysis, we categorized participants into "seropositive for toxoplasma gondii infection" or "seronegative for toxoplasma gondii infection". This cutoff is consistent with previous NHANES studies (20, 21).

### *2.5 Dependent variable: Cognitive functioning*

Three cognitive tests were used to assess participants' various domains of cognitive functioning, including the Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL), the AFT, and the DSST. The detailed method has been published elsewhere (22).

1) The CERAD-WL assessed participants' capacity for both immediate (immediate memory) and delayed (delayed memory) verbal learning (23). It consisted of a delayed recall after three consecutive learning trials. For each learning trial, participants must read aloud ten randomly selected words that are displayed on a computer screen in huge, bolded characters, one at a time. Following the presentation of the words, participants were encouraged to retain and recall as many words as they could. In each of the three trials, the order of the ten words was changed. There was a ten-point maximum for each trial. A participant's immediate memory score was the sum of their three trials' scores, ranging from zero to thirty. After the AFT and the DSST, participants took the delayed recall test, which asked them to recall as many words from the same ten-word list as they could. The delayed memory score, which varied from zero to ten, depended on how many accurate words a subject could recall.

2) Participants' language proficiency and executive function were assessed by the AFT (24). Each animal a participant named received one point, and they had one minute to name as many animals as they could. Participants were first prompted to identify three pieces of clothing as a warm-up.

3) The DSST measured the participants' working memory, sustained attention, and processing speed (25). A paper form with a top-mounted key that included nine numbers and paired symbols was used to conduct the exam. Participants were instructed to copy various symptoms to the matching symbols in the 133 boxes that were placed next to the numbers. They had two minutes to complete this task. The DDST score was based on the total number of correct matches (26). Before participants started the formal test, a sample practice test was provided. The possible score range of the DSST was between zero and 133 (27).

### *2.6 Covariates*

Covariates of this study were selected according to literature review and included age (years), sex (male or female), race/ethnicity (Mexican Americans, other Hispanics, non-Hispanic White, or non-Hispanic Black), education (below high school, high school graduate, or some college or above), depressive symptoms, smoking status (never, former, or current smokers), BMI (<18.5 kg/m$^2$, 18.5-24.9 kg/m$^2$, 25-29.9 kg/m$^2$, or ≥30 kg/m$^2$), prevalent coronary heart disease (CHD) (yes or no), stroke (yes or no), and systolic blood pressure (mmHg). The information was either self-reported or collected at health exams. The Patient Health Questionnaire (PHQ-9) was used to measure depressive symptoms (28). It is a nine-item screening tool on the frequency of depressive symptoms over the past two weeks and has a total score ranging from 0-27. A higher score indicates more severe depression symptoms. The PHQ-9 total score was used to indicate depressive symptoms.

### **2.7 Statistical analysis**

Means (standard deviation [SD]) were used to describe the characteristics of the study population for continuous data that followed a normally distributed distribution. Medians (interquartile range) were used for non-normally distributed continuous data. Data for categorical variables were summarized using frequency (percentages). Independent T-tests were used to compare group differences for continuous variables between the two groups. Chi-square tests were used to compare group differences for categorical variables between the two groups.

The CERAD-WL immediate memory, CERAD-WL delayed memory, AFT, and DSST were standardized with mean zero and variance one to compute cognitive test-specific z-scores. The cognitive test-specific z scores of the four tests were then averaged to calculate the global

cognition z scores. Linear regression models were constructed between toxoplasma gondii seropositivity (seronegative or seropositive) and each of the four cognitive test-specific and global cognition z scores. All models were adjusted for the covariates mentioned above. We considered a 95% confidence interval excluding one as statistically significant. All the analyses were performed using SPSS 25.0.

## 3. Results

The sociodemographic and health information of the study population, stratified by toxoplasma gondii seropositivity, was presented in **Table 1**. Of the 2,956 participants, 1, 403 were from the 2011-2012 cycle and 1,553 from the 2013-2014 cycle. A total of 703 participants were seropositive for toxoplasma gondii infection (23.8%). They had a mean age of 70.0 (SD 7.0). Most of the 2,952 participants (mean age of 70.0 [SD 7.0]) were female (51.0%), non-Hispanic White (48.3%), completed some college or above (48.3%), were never smokers (49.8%), and had a BMI ≥30 (35.6%) and an average of 8.7 (SD 10.7) hours of physical activity every week. Their mean total cholesterol and systolic blood pressure were 190.2 mg/dL and 133.5 mmHg. Their mean delayed memory, immediate memory, AFT, and DSST score were 5.8 (SD 2.4), 18.5 (SD 5.0), 16.4 (SD 5.6), and 45.8 (SD 17.5), respectively. Compared with participants with negative toxoplasma gondii seropositivity, participants with positive toxoplasma gondii seropositivity were older, less educated, and more likely to be male, current smokers, and had more physical activities. They were also more likely to have different ethnicities and lower CERAD W-L immediate recall, CERAD W-L delayed recall, AFT, and DSST scores.

Table 1. Characteristics of the participants by toxoplasma gondii seropositivity

| Variables | Negative (n=2,253) | Positive (n=703) | Total (n=2,956) | *P*-Value |
|---|---|---|---|---|
| Age, years | 69.7(6.9) | 70.9(7.0) | 70.0(7.0) | **<0.001** |
| Sex, n (%) | | | | **<0.001** |
| Male | 1,057(46.9%) | 390(55.5%) | 1,447(49.0%) | |
| Female | 1,196(53.1%) | 313(44.5%) | 1,509(51.0%) | |
| Race/ethnicity, n (%) | | | | **<0.001** |
| Mexican Americans | 231(10.3%) | 43(6.1%) | 274(9.3%) | |
| Other Hispanics | 161(7.1%) | 131(18.6%) | 292(9.9%) | |
| Non-Hispanic Whites | 1,111(49.3%) | 317(45.1%) | 1,428(48.3%) | |
| Non-Hispanic Blacks | 479(21.3%) | 165(23.5%) | 644(21.8%) | |
| Other | 271(12.0%) | 47(6.7%) | 318(10.8%) | |
| Education, n (%) | | | | **<0.001** |
| Below high school | 594(26.3%) | 256(36.4%) | 850(28.7%) | |
| High school graduate | 510(22.6%) | 165(23.5%) | 421(14.2) | |
| Some college or above | 1,147(50.9%) | 281(39.9%) | 1,428(48.3%) | |
| Depressive symptoms | 3.5(4.8) | 3.3(4.5) | 3.5(4.8) | 0.334 |
| Smoking, n (%) | | | | **0.043** |
| Never | 1,130(50.2%) | 342(48.6%) | 1,472(49.8%) | |
| Former | 846(37.5%) | 249(35.4%) | 1,095(37.0%) | |
| Current | 274(12.2%) | 111(15.8%) | 385(13.0%) | |
| Body mass index, n (%) | | | | 0.444 |
| <18.5 kg/m2 | 35(1.6%) | 9(1.3%) | 44(1.5%) | |
| 18.5-24.9 kg/m2 | 592(26.3%) | 166(23.6%) | 758(25.6%) | |
| 25.0-29.9 kg/m2 | 784(34.8%) | 262(37.3%) | 1,046(35.4%) | |
| ≥30 kg/m2 | 800(35.5%) | 251(35.7%) | 1,051(35.6%) | |
| Physical activity, hours/week | 8.2(10.3) | 10.6(12.1) | 8.7(10.7) | **0.025** |
| Total cholesterol, mg/dL | 190.7(42.5) | 188.4(43.4) | 190.2(42.7) | 0.209 |
| Systolic blood pressure, mmHg | 133.2(19.8) | 134.5(42.5) | 133.5(27.0) | 0.271 |
| CERAD W-L immediate recall | 18.7(5.0) | 17.9(4.8) | 18.5(5.0) | **<0.01** |
| CERAD W-L delayed recall | 5.9(2.4) | 5.5(2.4) | 5.8(2.4) | **<0.01** |
| Animal Fluency Test | 16.6(5.6) | 15.8(5.3) | 16.4(5.6) | **<0.01** |
| Digit Symbol Substitution Test | 47.2(17.4) | 41.2(16.8) | 45.8(17.5) | **<0.001** |

Data are presented as means (standard deviation) for continuous variables and n (%) for categorical variables. Bolded values mean statistical significance (P<0.05).

The means and 95% confidence intervals (CI)s of cognitive test-specific z-scores by toxoplasma gondii infection status were presented in **Table 2**. The mean of CERAD W-L immediate call, CERALD W-L delayed recall, AFT and DSST was 0.04 (95% CI -1.94, 2.01),

0.03 (95% CI -1.92, 1.99), 0.03 (95% CI -1.95, 2.01) and 0.08 (95% CI -1.88, 2.03), respectively, among participants with negative toxoplasma gondii infection. Among participants with seropositive toxoplasma gondii infection, the mean of CERAD W-L immediate call, CERALD W-L delayed recall, AFT and DSST was -0.12 (95% CI -2.01, 1.77), -0.12 (95% CI -2.07, 1.83), -0.11 (95% CI -1.99, 1.76) and -0.26 (95% CI -2.15, 1.63), respectively. For the global cognition z-scores, the mean was 0.06 (95% CI -1.92, 2.03) for participants with negative toxoplasma gondii infection and -0.18 (95% CI -2.06, 1.70) for those with positive toxoplasma gondii infection.

Table 2. Cognitive z-scores and 95% confidence intervals by toxoplasma gondii seropositivity

| | Negative (n=2,253) | Positive (n=703) |
|---|---|---|
| CERAD W-L immediate recall | 0.04 (-1.94, 2.01) | -0.12 (-2.01, 1.77) |
| CERAD W-L delayed recall | 0.03 (-1.92, 1.99) | -0.12 (-2.07, 1.83) |
| Animal fluency test | 0.03 (-1.95, 2.01) | -0.11 (-1.99, 1.76) |
| Digit Symbol Substitution Test | 0.08 (-1.88, 2.03) | -0.26 (-2.15, 1.63) |
| Global cognition | 0.06 (-1.92, 2.03) | -0.18 (-2.06, 1.70) |

Adjusted linear regression (**Table 3**) showed that compared with participants with seronegative toxoplasma gondii infection, those with seropositive toxoplasma gondii infection had lower CERAD-WL immediate memory (beta [β] -0.16, 95% CI -0.25, -0.07), CERAD-WL delayed memory (β -0.15, 95% CI -0.24, -0.06), AFT (β -0.15, 95% CI -0.24, -0.06), and DSST (β -0.34, 95% CI -0.43, -0.26) z scores controlling for age, race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. For the global cognition z score, which is calculated by averaging the four cognitive test-specific z-scores, the negative association remained (β -0.24, 95% CI -0.32, -0.16).

Table 3. The independent associations of toxoplasma gondii seropositivity (reference: Negative) with cognitive specific test and global cognition z-scores

| | Beta | 95% CI |
|---|---|---|

| | | |
|---|---|---|
| CERAD W-L immediate recall | -0.16 | **(-0.25, -0.07)** |
| CERAD W-L delayed recall | -0.15 | **(-0.24, -0.06)** |
| Animal fluency test | -0.15 | **(-0.24, -0.06)** |
| Digit Symbol Substitution Test | -0.34 | **(-0.43, -0.25)** |
| Global cognition | -0.24 | (-0.32, -0.16) |

95% CI means 95% confidence interval
Bolded values mean statistical significance (96% CI excluding zero).

## 4. Discussion

In this sample of 2,956 nationally representative older adults in the US, positive toxoplasma gondii infection is independently associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition. This relationship is independent of age, race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. Although our findings should be validated using longitudinal studies, they suggest that serum toxoplasma gondii seropositivity may be associated with cognitive impairment and that preventing toxoplasma gondii infection should be a target of public health interventions to protect cognitive functioning in older adults. This is very important given the high prevalence of toxoplasma gondii infection and the increasing population aging in the US.

A limited number of studies have examined the associations of toxoplasma gondii seropositivity with cognitive outcomes in humans. Two studies utilized the NHANES 1988-1994 cycle data and thus could not reflect the current epidemic of toxoplasma gondii infection (14, 15). In addition, one of them only included school-aged children (15). To the best of our knowledge, only two relevant studies exclusively targeted older adults (29, 30). In one study of 84 older adults aged 65 years and above in Germany, toxoplasmosis-positive participants showed impaired working memory, attention, and word fluency, but not processing speed measured by

DSST, compared with those who were toxoplasmosis negative (29). It is important to note that the sample size of that study is very small. Then, in another study including older adults in the US, while no statistically significant association was found between toxoplasma gondii IgG levels and memory performance or attention, toxoplasma gondii IgG levels were inversely associated with global cognition measured by Mini-Mental State Examination (MMSE) (30). However, in that study, researchers did not adjust BMI, exercise, or depressive symptoms. In another longitudinal study targeting adults aged >30 years with eleven years' follow-up, researchers found no associations of toxoplasma gondii infections with verbal fluency and verbal learning assessed by CERAD (3). However, their study population was middle-aged, which was different from our participants. Overall, the findings of cross-sectional or longitudinal studies are inconsistent. Most of these studies were based on relatively small sample sizes, had methodological limitations, or targeted a different age group. However, in our study, we took advantage of a nationally representative and relatively large sample and calculated global cognition, adding stronger evidence on the negative relationship between toxoplasma gondii infection and cognitive functioning.

The possible mechanisms that account for the association between toxoplasmosis gondii infection and worse cognitive functioning are complicated. T gondii infection has been shown to increase dopamine release in vitro and animal trials (31-34). Excess dopamine turnover has been associated with worse cognitive decline (35-37). Dysregulated dopamine may influence neuronal plasticity in the hippocampus in humans, a brain region important for memory and spatial orientation (38, 39). In addition, as a defense mechanism against toxoplasma gondii infection, the host may rapidly catabolize tryptophan and produce more kynurenine and quinolinic acid (40). It is reported that higher levels of dopamine, kynurenine, and quinolinic acid were

associated with increased neurotoxic effects and impulsive behavior incidence (41). Furthermore, toxoplasma gondii infection was associated with the dysbiosis of gut microbiota in mice, which may increase gut-blood-barrier permeability and induce mental disturbances and behavioral changes (42-44). Future studies are expected to explore the underlying mechanism of the cognitive effects of toxoplasmosis gondii infection in humans.

The major strength of this study is the relatively large, nationally representative sample of older adults in the US. Stringent quality control and assurance measures were implemented throughout the NHANES study, including the rigorous assessment of toxoplasma gondii IgG and the adoption of validated cognitive tests to assess multiple cognitive functioning domains, therefore guaranteeing the quality of data used in this study. Moreover, a comprehensive list of sociodemographic, lifestyle, mental, and physical health covariates were adjusted, minimizing residual confounding. Thus, the findings of our study are generalizable to U.S. older adults. Importantly, the cognitive effects of toxoplasma gondii infection in humans are understudied in the literature. Thus, our study fills in a research gap. Last but not least, the findings of lower DSST score associated with toxoplasma gondii infection is important as previous studies have shown that lower DSST scores were independently associated with a higher risk of dementia (45, 46).

The major limitation of this study is the cross-sectional design which prevented us from examining whether participants had long-term exposure to toxoplasma gondii or a recent exposure where the IgG immune response to toxoplasma gondii had just started (4). Reverse causation is also possible. Additionally, research has revealed that specific genes affect susceptibility and immune response to toxoplasma gondii infection (47). However, our study did not assess any genetic factors. Additionally, the participants were administered the AFT during

the CERAD-WL delay, which may interfere with their memory formation. Residual confounding is also likely, although we tried to adjust a comprehensive list of covariates. Finally, with three cognitive tests, we may not assess all domains of participants' cognitive functioning. In addition, the excluded people (n=516) were different from the included participants (n= 2,956) in several aspects; thus, selection bias is possible (48).

Future students are expected to 1) use more advanced methods for identifying specific strains and stages of toxoplasma gondii infection (49), 2) explore the pathophysiological mechanisms of cognitive effects of toxoplasma gondii infection, 3) include non-western populations, and 4) utilize longitudinal designs to assess the temporal relationship between toxoplasma gondii infection and cognitive functioning. These studies may enable the identification of new biomarkers for cognitive impairment and enlighten the development of toxoplasma gondii medications and vaccinations to protect people from toxoplasma gondii infection and its adverse effects.

In conclusion, toxoplasma gondii seropositivity is prevalent in U.S. older adults and is independently associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition in this population. Future studies are expected to examine the longitudinal relationship and pathophysiological mechanism between toxoplasma gondii infection and cognitive functioning. Public health measures are needed to prevent toxoplasma gondii infection, which may help preserve cognitive functioning in older adults.

## 5. Conflict of Interest

The authors have no conflict of interest to declare.

## 6. Data Availability Statement

The data that support the findings of this study are openly available on the NHANES website and can be accessed at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

## 7. Acknowledgment

We would like to thank NHANES participants for providing data for this study.

## 9. Authors' contributions

ZQ and CY performed data analysis; SG and XL drafted the original manuscript. All the authors significantly provided feedback on the manuscript.

## 10. Ethics approval and consent to participate

This study was exempted from the University of Houston-Downtown Committee for the Protection of Human Subjects because only public-available and de-identified data were used.

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Appendix. The characteristics of included and excluded participants due to missing data

| Variable | Excluded (n=516) | Included (n=2956) | *P* Value |
|---|---|---|---|
| Age, years | 69.6(7.0) | 70.0(7.0) | 0.173 |
| Sex, n (%) | | | 0.436 |
| Male | 243(47.1%) | 1447(49.0%) | |
| Female | 273(52.9%) | 1509(51.0%) | |
| Race/ethnicity, n (%) | | | **<0.001** |
| Mexican Americans | 47(9.1%) | 274(9.3%) | |
| Other Hispanics | 55(10.7%) | 292(9.9%) | |
| Non-Hispanic Whites | 152(29.5%) | 1428(48.3%) | |
| Non-Hispanic Blacks | 196(38.0%) | 644(21.8%) | |
| Other | 66(12.8%) | 318(10.8%) | |
| Education, n (%) | | | **<0.001** |
| Below high school | 170(33.0%) | 850(28.7%) | |
| High school graduate | 123(23.8%) | 421(14.2) | |
| Some college or above | 219(42.4%) | 1428(48.3%) | |
| Depressive symptoms | 3.1(4.2) | 3.5(4.8) | 0.136 |
| Smoking, n (%) | | | 0.578 |
| Never | 265(51.4%) | 1472(49.8%) | |
| Former | 189(36.6%) | 1095(37.0%) | |
| Current | 59(11.4%) | 385(13.0%) | |
| Body mass index, n (%) | | | **0.001** |
| <18.5 kg/m2 | 16(3.1%) | 44(1.5%) | |
| 18.5-24.9 kg/m2 | 134(26.0%) | 758(25.6%) | |
| 25.0-29.9 kg/m2 | 142(27.5%) | 1046(35.4%) | |
| ≥30 kg/m2 | 202(39.1%) | 1051(35.6%) | |
| Physical activity, hours/week | 8.1(10.0) | 8.7(10.7) | 0.558 |
| Total cholesterol, mg/dL | 192.5(45.9) | 190.2(42.7) | 0.385 |
| Systolic blood pressure, mmHg | 136.3(21.7) | 133.5(27.0) | **0.032** |
| Digit Symbol Substitution Test | 43.1(17.1) | 45.8(17.5) | **0.004** |
| CERAD W-L delayed recall | 5.6(2.5) | 5.8(2.4) | 0.093 |
| CERAD W-L immediate recall | 18.2(5.3) | 18.5(5.0) | 0.300 |
| Animal Fluency Test | 15.8(5.5) | 16.4(5.6) | **0.030** |

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# Toxoplasma gondii seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014

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# *Toxoplasma gondii* seropositivity and cognitive functioning in older adults: An analysis of cross-sectional data of the National Health and Nutrition Examination Survey 2011-2014

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**Abstract**

**Objectives**: This study sought to examine the relationship between *Toxoplasma gondii* seropositivity and cognitive function in older adults.

**Design**: An observational cross-sectional study.

**Setting:** The National Health and Nutrition Examination Survey (NHANES) study took place at participants' homes and mobile exam centers.

**Participants**: A total of 2, 956 older adults aged 60 and above from the NHANES from 2011 to 2014 were included in the study. Exposure of interest: Participants had serum *Toxoplasma gondii* antibody analyzed in the lab. A value > 33 IU/mL was categorized as seropositive for *Toxoplasma gondii* infection; <27 IU/mL was categorized as seronegative for *Toxoplasma gondii* infection.

**Primary and secondary outcome measures**: Cognitive tests included the Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL) for immediate and delayed memory, the Animal Fluency Test (AFT), and the Digit Symbol Substitution Test (DSST).

**Results**: About half of the 2,956 participants (mean age 70.0) were female (51.0%), non-Hispanic White (48.3%), and completed some college or above (48.3%). A total of 703 participants were positive for *Toxoplasma gondii* infection (23.8%). Adjusted linear regression showed that compared with participants with negative *Toxoplasma gondii* infection, those with positive *Toxoplasma gondii* infection had lower CERAD-WL immediate memory (beta [β] -0.16, 95% confidence interval [CI] -0.25, -0.07), CERAD-WL delayed memory (β -0.15, 95% CI -0.24, -0.06), AFT (β -0.15, 95% CI -0.24,-0.06), DSST (β -0.34, 95% CI -0.43, -0.26), and global cognition (β -0.24, 95% CI -0.32,-0.16) z-scores after controlling for the covariates.

**Conclusions**: *Toxoplasma gondii* seropositivity is associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition in older adults. Public health measures aiming at preventing *Toxoplasma gondii* infection may help preserve cognitive functioning in older adults.

**Keywords**: *Toxoplasma gondii*; cognitive functioning; older adults; neurotoxoplasmosis; neuropsychological; psychomotor performance

**Strengths and limitations:**

- This study is one of the few studies that examined the cognitive effect of *Toxoplasma gondii* seropositivity on cognitive outcomes in older adults.
- With the NHANES data, the study population was nationally representative of older U.S. adults.
- A wide range of sociodemographic, lifestyle, mental health, and physical health covariates was adjusted, reducing the possibility of residual confounding.
- A cross-sectional study hinders the assessment of longitudinal relationships.
- Unable to adjust for variables that were not evaluated in NHANES; thus, residual confounding could not be ruled out.

## 1. Introduction

Alzheimer's disease and related dementia (ADRD) is a serious public health threat worldwide. In 2016, a total of 43.8 million people had dementia in the world. With a growing number of people with ADRD, families, communities, and healthcare systems around the world

are heavily burdened (1). Although dementia is currently not curable, identifying modifiable risk factors associated with ADRD can help reduce the burden of the disease. Cognitive test performance in older adults is an important indicator of their cognitive functioning (2). By examining their correlations to cognitive tests, risk factors for cognitive decline can provide opportunities for interventions.

*Toxoplasma gondii* is among the most prevalent human zoonosis (3) and affects about 30% of the global population (4). Vertical *Toxoplasma gondii* infections can happen prior to birth resulting in congenital toxoplasmosis. Transmission after a person is born, postnatal infection, is the more common form of infection (5). By consuming oocysts found in cat feces-contaminated soil or water a person can develop toxoplasmosis (6, 7). Oocyst contaminated soil can also facilitate transmission via unwashed, nonheat-treated consumable products, like fruits and vegetables, to allow for transmission of this zoonotic disease (8). By eating tissue with cysts in undercooked meat (9, 10), humans can be infected with *Toxoplasma gondii* after birth. Another route of human exposure to *Toxoplasma gondii* can occur if a person experiences a blood transfusion or organ transplant. While most people with a healthy immune system are asymptomatic following acute infection with *Toxoplasma gondii*, some may experience nonspecific symptoms lasting from several weeks to months, including fever, malaise, muscle ache, lymphadenopathy, along with the miscarriage or stillbirth of a fetus (11, 12).

A limited number of studies have found that *Toxoplasma gondii* infection is associated with neurocognitive changes in humans (13-16). However, their direction of findings and effect sizes are inconsistent. In addition, this area is understudied, given the prevalence of *Toxoplasma gondii* infection in humans. In a systematic review and meta-analysis of the association of *Toxoplasma gondii* seropositivity and cognitive function in healthy people, only thirteen studies

were included, most of which had small sample sizes (4). In addition, two included studies (17, 18) utilized the National Health and Nutrition Examination Survey (NHANES) 1988-1994 cycle data and thus could not reflect the current epidemic of *Toxoplasma gondii* infection. The findings of this study showed that seropositivity to *Toxoplasma gondii* was modestly but significantly associated with poorer processing speed, working memory, verbal short-term memory, and executive functioning. However, studies using large, the latest, and nationally representative population-based data are needed to better elucidate the cognitive effects of *Toxoplasma gondii* seropositivity.

In this study, taking advantage of the NHANES, we aimed to examine the relationship between *Toxoplasma gondii* seropositivity and cognitive functioning in a nationally representative sample of U.S. older adults. The findings of this study will provide implications for understanding the cognitive effects of *Toxoplasma gondii* infection and developing tailored public interventions to protect cognitive functioning in the growing number of older adults in the US.

## 2. Method

### *2.1 Study design and setting*

The NHANES is an ongoing, cross-sectional survey of civilian, non-institutionalized adults and children in the United States conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention. A nationally representative sample of children and adults across the country are surveyed bi-annually (19). Their sociodemographic, health, and nutritional status are evaluated using in-person interviews and physical exams. The interviews are conducted at participants' homes; health exams are conducted in specially equipped mobile

exam centers. Health exams include laboratory testing of urine and blood specimens and medical, dental, and physiological assessments. Participants' serum *Toxoplasma gondii* antibody levels and cognitive functioning were measured in the NHANES 2011-2014 cycles. Medical conditions, including dementia or neurocognitive disorders, were not exclusion criteria of the NHANES. In this study, two survey cycles (2011-2012 and 2013-2014) were merged to increase sample size and power. Between 2011 and 2014, a total of 19,151 individuals participated in the NHANES. They were recruited from a selection of census blocks or clusters of census block area segments. The detailed sampling method has been published elsewhere (20).

Of the 19,151 individuals, we excluded individuals aged < 60 (n = 15,679) or had missing data on serum *Toxoplasma gondii* IgG (n=511). Participants with equivocal serum *Toxoplasma gondii* IgG (≥27 and <33 IU/mL) were further excluded (n=5) as the results were inconclusive. Finally, a total of 2,956 participants aged 60 and above were included in the analysis. The characteristics of the excluded participants due to missing data (n=516) were presented in the Appendix. Compared with the included participants, people who were excluded were more likely to be of other ethnicities than Non-Hispanic Whites, overweight/obese, completed lower education and had higher systolic blood pressure, lower Digit Symbol Substitution Test (DSST) score, and lower Animal Fluency test (AFT) score.

### *2.2 Ethical considerations*

The National Center for Health Statistics Research Ethics Review Board approved the NHANES. Participants in the NHANES provided written informed consent before enrolling in the study. The University of Houston-Downtown Committee for the Protection of Human

Subjects granted this study an exemption because only publicly accessible and de-identified data were used.

### *2.3 Public and Patient involvement*

Patients or the public were NOT involved in the design, conducting, reporting, or dissemination plans of our research.

### *2.4 Independent* variable: *Toxoplasma gondii* seropositivity

Serological tests that detect *Toxoplasma gondii* Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies are often used for clinical diagnoses of toxoplasmosis. While the IgM antibody test can validate acute phases, the IgG antibody test can identify acute or chronic phases (21). In the NHANES, an enzyme immunoassay (EIA) measuring IgG against *Toxoplasma gondii* was used to measure the presence or absence of *Toxoplasma gondii* (19). *Toxoplasma gondii* IgG was measured with two enzyme immunoassay kits (22). Strict quality control was implemented for every plate. A value between 27 and 33 IU/mL was deemed as "equivocal"; a value ≥33 IU/mL was deemed as "positive"; a value <27 IU/mL was deemed as "negative" (19). Samples with equivocal results (≥27 IU/mL and <33 IU/mL) were repeated twice and confirmed as negative. For our analysis, we categorized participants into "seropositive for *Toxoplasma gondii* infection" or "seronegative for *Toxoplasma gondii* infection". This cutoff is consistent with descriptions on the NHANES website and previous NHANES publications (23, 24).

### *2.5 Dependent variable: Cognitive functioning*

Three cognitive tests were used to assess participants' various domains of cognitive functioning, including the Consortium to Establish a Registry for Alzheimer's Disease Word Learning subtest (CERAD-WL), the AFT, and the DSST. The detailed method of assessing cognitive function has been published elsewhere (25).

1) The CERAD-WL assessed participants' capacity for both immediate (immediate memory) and delayed (delayed memory) verbal learning (26). It consisted of a delayed recall after three consecutive learning trials. For each learning trial, participants must read aloud ten randomly selected words that are displayed on a computer screen in huge, bolded characters, one at a time. Following the presentation of the words, participants were encouraged to retain and recall as many words as they could. In each of the three trials, the order of the ten words was changed. There was a ten-point maximum for each trial. A participant's immediate memory score was the sum of their three trials' scores, ranging from zero to thirty. After the AFT and the DSST, participants took the delayed recall test, which asked them to recall as many words from the same ten-word list as they could. The delayed memory score, which varied from zero to ten, depended on how many accurate words a subject could recall.

2) Participants' language proficiency and executive function were assessed by the AFT (27). Each animal a participant named received one point, and they had one minute to name as many animals as they could. Participants were first prompted to identify three pieces of clothing as a warm-up.

3) The DSST measured the participants' working memory, sustained attention, and processing speed (28). A paper form with a top-mounted key that included nine numbers and paired symbols was used to conduct the exam. Participants were instructed to copy various symbols to the matching symbols in the 133 boxes that were placed next to the numbers. They

had two minutes to complete this task. The DDST score was based on the total number of correct matches (29). Before participants started the formal test, a sample practice test was provided. The possible score range of the DSST was between zero and 133 (30).

### *2.6 Covariates*

Covariates of this study were selected according to literature review and included age (years), sex (male or female), race/ethnicity (Mexican Americans, other Hispanics, non-Hispanic White, or non-Hispanic Black), education (below high school, high school graduate, or some college or above), depressive symptoms, smoking status (never, former, or current smokers), BMI (<18.5 kg/m$^2$, 18.5-24.9 kg/m$^2$, 25-29.9 kg/m$^2$, or ≥30 kg/m$^2$), prevalent coronary heart disease (CHD) (yes or no), stroke (yes or no), and systolic blood pressure (mmHg). The information was either self-reported or collected at health exams. The Patient Health Questionnaire (PHQ-9) was used to measure depressive symptoms (31). It is a nine-item screening tool on the frequency of depressive symptoms over the past two weeks and has a total score ranging from 0-27. A higher score indicates more severe depression symptoms. The PHQ-9 total score was used to indicate depressive symptoms.

### **2.7 Statistical analysis**

Means (standard deviation [SD]) were used to describe the characteristics of the study population for continuous data that followed a normally dispersed distribution. Medians (interquartile range) were used for non-normally distributed continuous data. Data for categorical variables were summarized using frequency (percentages). Independent T-tests were used to

compare group differences for continuous variables between the two groups. Chi-square tests were used to compare group differences for categorical variables between the two groups.

The CERAD-WL immediate memory, CERAD-WL delayed memory, AFT, and DSST were standardized with mean zero and variance one to compute cognitive test-specific z-scores. The cognitive test-specific z-scores of the four tests were then averaged to calculate the global cognition z-scores. Linear regression models were constructed between *Toxoplasma gondii* seropositivity (seronegative or seropositive) and each of the four cognitive test-specific and global cognition z-scores. All models were adjusted for the covariates mentioned above. We considered a 95% confidence interval (CI) excluding one as statistically significant. All the analyses were performed using SPSS 25.0.

## 3. Results

The sociodemographic and health information of the study population, stratified by *Toxoplasma gondii* seropositivity, was presented in **Table 1**. Of the 2,956 participants, 1, 403 were from the 2011-2012 cycle and 1,553 from the 2013-2014 cycle. A total of 703 participants were seropositive for *Toxoplasma gondii* infection (23.8%). The participants had a mean age of 70.0 (SD 7.0). Most of the 2,952 participants (mean age of 70.0 [SD 7.0]) were female (51.0%), non-Hispanic White (48.3%), completed some college or above (48.3%), were never smokers (49.8%), and had a BMI ≥30 (35.6%) and an average of 8.7 (SD 10.7) hours of physical activity every week. Their mean total cholesterol and systolic blood pressure were 190.2 mg/dL and 133.5 mmHg. Around 63% of the participants had stroke. Their mean delayed memory, immediate memory, AFT, and DSST scores were 5.8 (SD 2.4), 18.5 (SD 5.0), 16.4 (SD 5.6), and 45.8 (SD 17.5), respectively. Compared with participants with negative *Toxoplasma gondii*

seropositivity, participants with positive *Toxoplasma gondii* seropositivity were older, less educated, and more likely to be male, current smokers, and had more physical activities. They were also more likely to have different ethnicities and lower CERAD W-L immediate recall, CERAD W-L delayed recall, AFT, and DSST scores.

Table 1. Characteristics of the participants by *Toxoplasma gondii* seropositivity

| Variables | Negative (n=2,253) | Positive (n=703) | Total (n=2,956) | *P*-Value |
|---|---|---|---|---|
| Age, years | 69.7(6.9) | 70.9(7.0) | 70.0(7.0) | **<0.001** |
| Sex, n (%) | | | | **<0.001** |
| Male | 1,057(46.9%) | 390(55.5%) | 1,447(49.0%) | |
| Female | 1,196(53.1%) | 313(44.5%) | 1,509(51.0%) | |
| Race/ethnicity, n (%) | | | | **<0.001** |
| Mexican Americans | 231(10.3%) | 43(6.1%) | 274(9.3%) | |
| Other Hispanics | 161(7.1%) | 131(18.6%) | 292(9.9%) | |
| Non-Hispanic Whites | 1,111(49.3%) | 317(45.1%) | 1,428(48.3%) | |
| Non-Hispanic Blacks | 479(21.3%) | 165(23.5%) | 644(21.8%) | |
| Other | 271(12.0%) | 47(6.7%) | 318(10.8%) | |
| Education, n (%) | | | | **<0.001** |
| Below high school | 594(26.3%) | 256(36.4%) | 850(28.7%) | |
| High school graduate | 510(22.6%) | 165(23.5%) | 421(14.2) | |
| Some college or above | 1,147(50.9%) | 281(39.9%) | 1,428(48.3%) | |
| Depressive symptoms | 3.5(4.8) | 3.3(4.5) | 3.5(4.8) | 0.334 |
| Smoking, n (%) | | | | **0.043** |
| Never | 1,130(50.2%) | 342(48.6%) | 1,472(49.8%) | |
| Former | 846(37.5%) | 249(35.4%) | 1,095(37.0%) | |
| Current | 274(12.2%) | 111(15.8%) | 385(13.0%) | |
| Body mass index, n (%) | | | | 0.444 |
| <18.5 kg/m2 | 35(1.6%) | 9(1.3%) | 44(1.5%) | |
| 18.5-24.9 kg/m2 | 592(26.3%) | 166(23.6%) | 758(25.6%) | |
| 25.0-29.9 kg/m2 | 784(34.8%) | 262(37.3%) | 1,046(35.4%) | |
| ≥30 kg/m2 | 800(35.5%) | 251(35.7%) | 1,051(35.6%) | |
| Physical activity, hours/week | 8.2(10.3) | 10.6(12.1) | 8.7(10.7) | **0.025** |
| Total cholesterol, mg/dL | 190.7(42.5) | 188.4(43.4) | 190.2(42.7) | 0.209 |
| Systolic blood pressure, mmHg | 133.2(19.8) | 134.5(42.5) | 133.5(27.0) | 0.271 |
| CERAD W-L immediate recall | 18.7(5.0) | 17.9(4.8) | 18.5(5.0) | **<0.01** |
| CERAD W-L delayed recall | 5.9(2.4) | 5.5(2.4) | 5.8(2.4) | **<0.01** |
| Animal Fluency Test | 16.6(5.6) | 15.8(5.3) | 16.4(5.6) | **<0.01** |
| Digit Symbol Substitution Test | 47.2(17.4) | 41.2(16.8) | 45.8(17.5) | **<0.001** |

Data are presented as means (standard deviation) for continuous variables and n (%) for categorical variables. Bolded values mean statistical significance (P<0.05).

The means and 95% confidence intervals (CI)s of cognitive test-specific z-scores by *Toxoplasma gondii* infection status were presented in **Table 2**. The mean of CERAD W-L immediate call, CERAD W-L delayed recall, AFT and DSST was 0.04 (95% CI -1.94, 2.01), 0.03 (95% CI -1.92, 1.99), 0.03 (95% CI -1.95, 2.01) and 0.08 (95% CI -1.88, 2.03), respectively, among participants with negative *Toxoplasma gondii* infection. Among participants with seropositive *Toxoplasma gondii* infection, the mean of CERAD W-L immediate call, CERAD W-L delayed recall, AFT and DSST was -0.12 (95% CI -2.01, 1.77), -0.12 (95% CI -2.07, 1.83), -0.11 (95% CI -1.99, 1.76) and -0.26 (95% CI -2.15, 1.63), respectively. For the global cognition z-scores, the mean was 0.06 (95% CI -1.92, 2.03) for participants with negative *Toxoplasma gondii* infection and -0.18 (95% CI -2.06, 1.70) for those with positive *Toxoplasma gondii* infection.

Table 2. Cognitive z-scores and 95% confidence intervals by *Toxoplasma gondii* seropositivity

| | Negative (n=2,253) | Positive (n=703) |
|---|---|---|
| CERAD W-L immediate recall | 0.04 (-1.94, 2.01) | -0.12 (-2.01, 1.77) |
| CERAD W-L delayed recall | 0.03 (-1.92, 1.99) | -0.12 (-2.07, 1.83) |
| Animal fluency test | 0.03 (-1.95, 2.01) | -0.11 (-1.99, 1.76) |
| Digit Symbol Substitution Test | 0.08 (-1.88, 2.03) | -0.26 (-2.15, 1.63) |
| Global cognition | 0.06 (-1.92, 2.03) | -0.18 (-2.06, 1.70) |

Adjusted linear regression (**Table 3**) showed that compared with participants with seronegative *Toxoplasma gondii* infection, those with seropositive *Toxoplasma gondii* infection had lower CERAD-WL immediate memory (beta [β] -0.16, 95% CI -0.25, -0.07), CERAD-WL delayed memory (β -0.15, 95% CI -0.24, -0.06), AFT (β -0.15, 95% CI -0.24, -0.06), and DSST (β -0.34, 95% CI -0.43, -0.26) z-scores controlling for age, race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. For the

global cognition z-score, which is calculated by averaging the four cognitive test-specific z-scores, the negative association remained (β -0.24, 95% CI -0.32, -0.16).

Table 3. The independent associations of *Toxoplasma gondii* seropositivity (reference: Negative) with cognitive specific test and global cognition z-scores

| | Beta | 95% CI |
|---|---|---|
| CERAD W-L immediate recall | -0.16 | **(-0.25, -0.07)** |
| CERAD W-L delayed recall | -0.15 | **(-0.24, -0.06)** |
| Animal fluency test | -0.15 | **(-0.24, -0.06)** |
| Digit Symbol Substitution Test | -0.34 | **(-0.43, -0.25)** |
| Global cognition | -0.24 | (-0.32, -0.16) |

95% CI means 95% confidence interval
Bolded values mean statistical significance (96% CI excluding zero).

## 4. Discussion

In this sample of 2,956 nationally representative older adults in the US, positive *Toxoplasma gondii* infection is independently associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition. This relationship is independent of age, race/ethnicity, education, depressive symptoms, smoking status, BMI, prevalent CHD, stroke, and systolic blood pressure. Although our findings should be validated using longitudinal studies, they suggest that serum *Toxoplasma gondii* seropositivity may be associated with cognitive impairment and that preventing *Toxoplasma gondii* infection should be a target of public health interventions to protect cognitive functioning in older adults. This is very important given the high prevalence of *Toxoplasma gondii* infection and the increasing population aging in the US.

A limited number of studies have examined the associations of *Toxoplasma gondii* seropositivity with cognitive outcomes in humans. Two studies utilized the NHANES 1988-1994 cycle data and thus could not reflect the current epidemic of *Toxoplasma gondii* infection (17,

18). In addition, one of them only included school-aged children (18). To the best of our knowledge, only two relevant studies exclusively targeted older adults (32, 33). In one study of 84 older adults aged 65 years and above in Germany, toxoplasmosis-positive participants showed impaired working memory, attention, and word fluency, but not processing speed measured by DSST, compared with those who were toxoplasmosis negative (32). It is important to note that the sample size of that study is very small. Then, in another study including older adults in the US, while no statistically significant association was found between *Toxoplasma gondii* IgG levels and memory performance or attention, *Toxoplasma gondii* IgG levels were inversely associated with global cognition measured by Mini-Mental State Examination (MMSE) (33). However, in that study, researchers did not adjust BMI, exercise, or depressive symptoms. In another longitudinal study targeting adults aged >30 years with eleven years' follow-up, researchers found no associations of *Toxoplasma gondii* infections with verbal fluency and verbal learning assessed by CERAD (3). However, their study population was middle-aged, which was different from our participants. Overall, the findings of cross-sectional or longitudinal studies are inconsistent. Most of these studies were based on relatively small sample sizes, had methodological limitations, or targeted a different age group. However, in our study, we took advantage of a nationally representative and relatively large sample and calculated global cognition, adding stronger evidence on the negative relationship between *Toxoplasma gondii* infection and cognitive functioning.

In this study, both groups demonstrated cognitive scores within the normal range, and while there was a statistically significant effect observed, its clinical relevance remains modest and somewhat ambiguous. The possible mechanisms that account for the association between *Toxoplasma gondii* infection and worse cognitive functioning are complicated. *Toxoplasma gondii* infection has been shown to increase dopamine release in vitro and animal trials (34-37).

Excess dopamine turnover has been associated with worse cognitive decline (38-40). Dysregulated dopamine may influence neuronal plasticity in the hippocampus in humans, a brain region important for memory and spatial orientation (41, 42). Furthermore, evidence suggests that dysregulation of neurotransmitters, particularly norepinephrine, is involved in the neuroimmune responses to brain infection (43). In the brains of animals infected with *Toxoplasma gondii* and in vitro studies involving infected human and rat neural cells, the noradrenergic system was shown to be suppressed with decreased norepinephrine levels. This reduction in norepinephrine levels was attributed to the downregulation of the dopamine β-hydroxylase gene expression, which encodes the enzyme responsible for synthesizing norepinephrine from dopamine (44). This altered synthesis of norepinephrine may partly explain the infection-related behavioral effects and the associations with mental illness. In addition, as a defense mechanism against *Toxoplasma gondii* infection, the host may rapidly catabolize tryptophan and produce more kynurenine and quinolinic acid (45). It is reported that higher levels of dopamine, kynurenine, and quinolinic acid were associated with increased neurotoxic effects and impulsive behavior incidence (46). Furthermore, *Toxoplasma gondii* infection was associated with the dysbiosis of gut microbiota in mice, which may increase gut-blood-barrier permeability and induce mental disturbances and behavioral changes (47-49). Future studies are expected to explore the underlying mechanism of the cognitive effects of *Toxoplasma gondii* infection in humans.

The major strength of this study is the relatively large, nationally representative sample of older adults in the US. Stringent quality control and assurance measures were implemented throughout the NHANES study, including the rigorous assessment of *Toxoplasma gondii* IgG and the adoption of validated cognitive tests to assess multiple cognitive functioning domains,

therefore guaranteeing the quality of data used in this study. Moreover, a comprehensive list of sociodemographic, lifestyle, mental, and physical health covariates were adjusted, minimizing residual confounding. Thus, the findings of our study are generalizable to U.S. older adults. Importantly, the cognitive effects of *Toxoplasma gondii* infection in humans are understudied in the literature. Thus, our study fills in a research gap. Last but not least, the findings of lower DSST score associated with *Toxoplasma gondii* infection is important as previous studies have shown that lower DSST scores were independently associated with a higher risk of dementia (50, 51).

The major limitation of this study is the cross-sectional design which prevented us from examining whether participants had long-term exposure to *Toxoplasma gondii* or a recent exposure where the IgG immune response to *Toxoplasma gondii* had just started (4). Reverse causation is also possible. Additionally, research has revealed that specific genes affect susceptibility and immune response to *Toxoplasma gondii* infection (52). However, our study did not assess any genetic factors. Additionally, the participants were administered the AFT during the CERAD-WL delay, which may interfere with their memory formation. Residual confounding is also likely, although we tried to adjust a comprehensive list of covariates. Finally, with three cognitive tests, we may not assess all domains of participants' cognitive functioning. In addition, the excluded people (n=516) were different from the included participants (n= 2,956) in several aspects; thus, selection bias is possible (53).

Future students are expected to 1) use more advanced methods for identifying specific strains and stages of *Toxoplasma gondii* infection (54), 2) explore the pathophysiological mechanisms of cognitive effects of *Toxoplasma gondii* infection, 3) include non-western populations, and 4) utilize longitudinal designs to assess the temporal relationship between

*Toxoplasma gondii* infection and cognitive functioning. These studies may enable the identification of new biomarkers for cognitive impairment and enlighten the development of *Toxoplasma gondii* medications and vaccinations to protect people from *Toxoplasma gondii* infection and its adverse effects.

In conclusion, *Toxoplasma gondii* seropositivity is prevalent in U.S. older adults and is independently associated with worse immediate and delayed verbal learning, language proficiency, executive functioning, processing speed, sustained attention, working memory, as well as global cognition in this population. However, the clinical relevance remains modest and somewhat ambiguous. Future studies are expected to examine the longitudinal relationship and pathophysiological mechanism between *Toxoplasma gondii* infection and cognitive functioning. Public health measures are needed to prevent *Toxoplasma gondii* infection, which may help preserve cognitive functioning in older adults.

## 5. Conflict of Interest

The authors have no conflict of interest to declare.

## 6. Data Availability Statement

The data that support the findings of this study are openly available on the NHANES website and can be accessed at https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

## 7. Acknowledgment

We would like to thank NHANES participants for providing data for this study.

## 9. Authors' contributions

WM, CS, QZ designed the project; ZQ and CY performed data analysis; SG, XL, ML, HC, and ZZ drafted the original manuscript. CS worked to revise the manuscript. All the authors significantly provided feedback on the manuscript.

## 10. Ethics approval and consent to participate

This study was exempted from the University of Houston-Downtown Committee for the Protection of Human Subjects because only public-available and de-identified data were used.

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Appendix. The characteristics of included and excluded participants due to missing data

| Variable | Excluded (n=516) | Included (n=2956) | *P* Value |
|---|---|---|---|
| Age, years | 69.6(7.0) | 70.0(7.0) | 0.173 |
| Sex, n (%) | | | 0.436 |
| Male | 243(47.1%) | 1447(49.0%) | |
| Female | 273(52.9%) | 1509(51.0%) | |
| Race/ethnicity, n (%) | | | **<0.001** |
| Mexican Americans | 47(9.1%) | 274(9.3%) | |
| Other Hispanics | 55(10.7%) | 292(9.9%) | |
| Non-Hispanic Whites | 152(29.5%) | 1428(48.3%) | |
| Non-Hispanic Blacks | 196(38.0%) | 644(21.8%) | |
| Other | 66(12.8%) | 318(10.8%) | |
| Education, n (%) | | | **<0.001** |
| Below high school | 170(33.0%) | 850(28.7%) | |
| High school graduate | 123(23.8%) | 421(14.2) | |
| Some college or above | 219(42.4%) | 1428(48.3%) | |
| Depressive symptoms | 3.1(4.2) | 3.5(4.8) | 0.136 |
| Smoking, n (%) | | | 0.578 |
| Never | 265(51.4%) | 1472(49.8%) | |
| Former | 189(36.6%) | 1095(37.0%) | |
| Current | 59(11.4%) | 385(13.0%) | |
| Body mass index, n (%) | | | **0.001** |
| <18.5 kg/m2 | 16(3.1%) | 44(1.5%) | |
| 18.5-24.9 kg/m2 | 134(26.0%) | 758(25.6%) | |
| 25.0-29.9 kg/m2 | 142(27.5%) | 1046(35.4%) | |
| ≥30 kg/m2 | 202(39.1%) | 1051(35.6%) | |
| Physical activity, hours/week | 8.1(10.0) | 8.7(10.7) | 0.558 |
| Total cholesterol, mg/dL | 192.5(45.9) | 190.2(42.7) | 0.385 |
| Systolic blood pressure, mmHg | 136.3(21.7) | 133.5(27.0) | **0.032** |
| Digit Symbol Substitution Test | 43.1(17.1) | 45.8(17.5) | **0.004** |
| CERAD W-L delayed recall | 5.6(2.5) | 5.8(2.4) | 0.093 |
| CERAD W-L immediate recall | 18.2(5.3) | 18.5(5.0) | 0.300 |
| Animal Fluency Test | 15.8(5.5) | 16.4(5.6) | **0.030** |

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No. | Recommendation | Page No. | Relevant text from manuscript |
|---|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 2-4 | abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2-4 | abstract |
| **Introduction** | | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4-5 | introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 6 | introduction |
| **Methods** | | | | |
| Study design | 4 | Present key elements of study design early in the paper | 6-7 | method |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 7-8 | method |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants | | |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case | | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6-10 | measure |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6-10 | *method* |
| Bias | 9 | Describe any efforts to address potential sources of bias | 15 | limitation |
| Study size | 10 | Explain how the study size was arrived at | 7 | method |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 10 | measure |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 10 | Statistical analysis |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 10 | Statistical analysis |
| | | (*c*) Explain how missing data were addressed | 10 | Statistical analysis |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy | | |
| | | (*e*) Describe any sensitivity analyses | | |
| **Results** | | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 11 | results |
| | | (b) Give reasons for non-participation at each stage | 11 | results |
| | | (c) Consider use of a flow diagram | | |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 11, 13 | results |
| | | (b) Indicate number of participants with missing data for each variable of interest | 12 | |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) | | |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time | | *results* |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure | | |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures | *13, 14* | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 13, 14 | results |
| | | (*b*) Report category boundaries when continuous variables were categorized | 13, 14 | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 13, 14 | |

Continued on next page

| | | | | |
|---|---|---|---|---|
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | | |
| **Discussion** | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 14 | discussion |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 17 | limitation |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 14 | |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 17 | |
| **Other information** | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 19 | |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.