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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 examination centres.

Participants A total of 2956 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 analysed in the laboratory. A value>33 IU/mL was categorised as seropositive for *Toxoplasma gondii* infection; <27 IU/mL was categorised 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 2956 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% CI −0.25 to −0.07), CERAD-WL delayed memory (β −0.15, 95% CI −0.24 to −0.06), AFT (β −0.15, 95% CI −0.24 to −0.06), DSST (β −0.34, 95% CI −0.43 to −0.26), and global cognition (β −0.24, 95% CI −0.32 to −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.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ 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 National Health and Nutrition Examination Survey (NHANES) data, the study population was nationally representative of older US 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.

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

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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 non-specific 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 13 studies were included, most of which had small sample sizes.[4] In addition, two included studies[17 18] utilised 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 US 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.

METHOD

Study design and setting

The NHANES is an ongoing, cross-sectional survey of civilian, non-institutionalised adults and children in the USA conducted by the National Centre for Health Statistics of the Centres for Disease Control and Prevention. A nationally representative sample of children and adults across the country are surveyed biannually.[19] Their sociodemographic, health, and nutritional status are evaluated using in-person interviews and physical examinations. The interviews are conducted at participants' homes; health examinations are conducted in specially equipped mobile examination centres. Health examinations 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 2956 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.

Ethical considerations

The National Centre 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 deidentified data were used.

Public and patient involvement

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

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 EIA 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

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'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 categorised participants into 'seropositive for *Toxoplasma gondii* infection' or 'seronegative for *Toxoplasma gondii* infection'. This cut-off is consistent with descriptions on the NHANES website and previous NHANES publications.[23 24]

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 10 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 10 words was changed. There was a 10-point maximum for each trial. A participant's immediate memory score was the sum of their three trials' scores, ranging from zero to 30. After the AFT and the DSST, participants took the delayed recall test, which asked them to recall as many words from the same 10-word list as they could. The delayed memory score, which varied from zero to 10, 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 1 min 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 examination. Participants were instructed to copy various symbols to the matching symbols in the 133 boxes that were placed next to the numbers. They had 2 min 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]

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), Body Mass Index (BMI<18.5, 18.5–24.9, 25–29.9, or ≥30 kg/m^2), prevalent coronary heart disease (CHD) (yes or no), stroke (yes or no), and systolic blood pressure (mm Hg). The information was either self-reported or collected at health examinations. 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 2 weeks and has a total score ranging from 0 to 27. A higher score indicates more severe depression symptoms. The PHQ-9 total score was used to indicate depressive symptoms.

Statistical analysis

Means (SD) were used to describe the characteristics of the study population for continuous data that followed a normally dispersed distribution. Medians (IQR) were used for non-normally distributed continuous data. Data for categorical variables were summarised using frequency (percentages). Independent T-tests were used to compare group differences for continuous variables between the two groups. χ^2 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 standardised 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% CI excluding one as statistically significant. All the analyses were performed using SPSS V.25.0.

RESULTS

The sociodemographic and health information of the study population, stratified by *Toxoplasma gondii* seropositivity, was presented in table 1. Of the 2956 participants, 1403 were from the 2011–2012 cycle and 1553 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 2952 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 mm Hg. 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

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

Variables	Negative (n=2253)	Positive (n=703)	Total (n=2956)	P value
Age, years	69.7 (6.9)	70.9 (7.0)	70.0 (7.0)	**<0.001**
Sex, n (%)				**<0.001**
Male	1057 (46.9)	390 (55.5)	1447 (49.0)	
Female	1196 (53.1)	313 (44.5)	1509 (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	1111 (49.3)	317 (45.1)	1428 (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	1147 (50.9)	281 (39.9)	1428 (48.3)	
Depressive symptoms	3.5 (4.8)	3.3 (4.5)	3.5 (4.8)	0.334
Smoking, n (%)				**0.043**
Never	1130 (50.2)	342 (48.6)	1472 (49.8)	
Former	846 (37.5)	249 (35.4)	1095 (37.0)	
Current	274 (12.2)	111 (15.8)	385 (13.0)	
Body Mass Index, n (%)				0.444
<18.5 kg/m^2	35 (1.6)	9 (1.3)	44 (1.5)	
18.5–24.9 kg/m^2	592 (26.3)	166	758 (25.6)	
25.0–29.9 kg/m^2	784 (34.8)	262	1046 (35.4)	
≥30 kg/m^2	800 (35.5)	251	1051 (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, mm Hg	133.2 (19.8)	134.5 (42.5)	133.5 (27.0)	0.271
CERAD-WL immediate recall	18.7 (5.0)	17.9 (4.8)	18.5 (5.0)	**<0.01**
CERAD-WL 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 (SD) for continuous variables and n (%) for categorical variables. Bolded values mean statistical significance (p<0.05).
CERAD-WL, Consortium to Establish a Registry for Alzheimer's Disease Word Learning.

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-WL immediate recall, CERAD-WL delayed recall, AFT, and DSST scores.

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

Adjusted linear regression (table 3) showed that compared with participants with seronegative *Toxoplasma*

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Table 2 Cognitive z-scores and 95% CIs by *Toxoplasma gondii* seropositivity

	Negative (n=2253)	Positive (n=703)
CERAD-WL immediate recall	0.04 (–1.94 to 2.01)	–0.12 (–2.01 to 1.77)
CERAD-WL delayed recall	0.03 (–1.92 to 1.99)	–0.12 (–2.07 to 1.83)
Animal Fluency Test	0.03 (–1.95 to 2.01)	–0.11 (–1.99 to 1.76)
Digit Symbol Substitution Test	0.08 (–1.88 to 2.03)	–0.26 (–2.15 to 1.63)
Global cognition	0.06 (–1.92 to 2.03)	–0.18 (–2.06 to 1.70)

CERAD-WL, Consortium to Establish a Registry for Alzheimer's Disease Word Learning.

gondii infection, those with seropositive *Toxoplasma gondii* infection had lower CERAD-WL immediate memory (beta (β) –0.16, 95% CI –0.25 to –0.07), CERAD-WL delayed memory (β –0.15, 95% CI –0.24 to –0.06), AFT (β –0.15, 95% CI –0.24 to –0.06), and DSST (β –0.34, 95% CI –0.43 to –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 to –0.16).

DISCUSSION

In this sample of 2956 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 ageing in the USA.

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

	Beta	95% CI
CERAD-WL immediate recall	–0.16	**(–0.25 to –0.07)**
CERAD-WL delayed recall	–0.15	**(–0.24 to –0.06)**
Animal Fluency Test	–0.15	**(–0.24 to –0.06)**
Digit Symbol Substitution Test	–0.34	**(–0.43 to –0.25)**
Global cognition	–0.24	(–0.32 to –0.16)

Bolded values mean statistical significance (96% CI excluding zero).
CERAD-WL, Consortium to Establish a Registry for Alzheimer's Disease Word Learning.

A limited number of studies have examined the associations of *Toxoplasma gondii* seropositivity with cognitive outcomes in humans. Two studies utilised 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 USA, 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.[33] However, in that study, researchers did not adjust BMI, exercise, or depressive symptoms. In another longitudinal study targeting adults aged>30 years with 11 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

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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 synthesising norepinephrine from dopamine.[44] This altered synthesis of norepinephrine may partly explain the infection-related behavioural effects and the associations with mental illness. In addition, as a defence 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 behaviour 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 behavioural 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 USA. 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, minimising residual confounding. Thus, the findings of our study are generalizable to US 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 are 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=2956) 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) utilise 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.

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