Toxoplasma gondii seropositivity and cognitive functions in school-aged children

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SUMMARY

Toxoplasma gondii (*T. gondii*) infects one-third of the world population, but its association with cognitive functions in school-aged children is unclear. We examined the relationship between *Toxoplasma* seropositivity and neuropsychological tests scores (including math, reading, visuospatial reasoning and verbal memory) in 1755 school-aged children 12–16 years old who participated to the Third National Health and Nutrition Examination Survey, using multiple linear regressions adjusted for covariates. *Toxoplasma* seroprevalence was 7·7% and seropositivity to the parasite was associated with lower reading skills (regression coefficient [β] = −5·86, 95% confidence interval [CI]: −11·11, −0·61, $P = 0·029$) and memory capacities ($\beta = -0·86$, 95% CI: −1·58, −0·15, $P = 0·017$). The interaction between *T. gondii* seropositivity and vitamin E significantly correlated with memory scores. In subgroup analysis, *Toxoplasma*-associated memory impairment was worse in children with lower serum vitamin E concentrations ($\beta = -1·61$, 95% CI: −2·44, −0·77, $P < 0·001$) than in those with higher values ($\beta = -0·12$, 95% CI: −1·23, 0·99, $P = 0·83$). In conclusion, *Toxoplasma* seropositivity may be associated with reading and memory impairments in school-aged children. Serum vitamin E seems to modify the relationship between the parasitic infection and memory deficiency.

Key words: *Toxoplasma gondii*, infection, parasites, cognition, cognitive function, intelligence, memory, reading, math, school-aged children, vitamins, Vitamin E.

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is an intracellular parasitic protozoan hosted by cats capable of infecting all warm blooded animals including humans (Nicolle and Manceaux, 1908). About 30% of the world population lives with the parasite and in some countries, *Toxoplasma* prevalence is as high as 70% (Jones *et al*. 2001; Pappas *et al*. 2009). *T. gondii* is transmitted to humans through ingestion of contaminated undercooked meat, unwashed vegetables, contaminated drinking water, contact with infected cat feces, vertical transmission from mother to child, and occasionally via transplantation of infected organs (Jones *et al*. 2001).

Despite severe *Toxoplasma* encephalitis described in immunocompromised patients or ocular and neuropsychiatric illnesses reported in children with congenital *Toxoplasma* infection, latent toxoplasmosis is believed to be mostly asymptomatic (Gilbert *et al*. 2006). Yet, it has been suggested that the parasite could affect behaviour and cognition in immunocompetent humans (Havlíček *et al*. 2001; Flegr, 2007; Flegr *et al*. 2012; Guenter *et al*. 2012). It has even been hypothesized that the parasite could alter the host behaviour with the specific purpose of increasing transmission to its definitive cat host ('manipulation hypothesis'). The infection seems to make rodents lose their innate aversion for cat urine, rather developing an attraction to it; nonetheless, they preserve their aversion to odours of other predators which are not *Toxoplasma* definitive hosts (Vyas *et al*. 2007; Berenreiterová *et al*. 2011). Following an acute phase of tachyzoites proliferation in various organs, bradyzoites are formed in muscular and cerebral tissues during the latent infection and may persist for as long as a lifetime in neurons. This potentially affects neuronal function and/or increases neuronal cell death resulting in probable neurological impairments (Henriquez *et al*. 2009; Prandovszky *et al*. 2011). Previous studies have proposed the release of cytokines as a possible mediator of cognitive and behavioural changes associated with *Toxoplasma* infection (Stock *et al*. 2014). There is also evidence that the neurochemical changes induced by the parasite via its genome coding aromatic amino acid hydroxylases may account for most of the cognitive and behavioural disturbances (Stock *et al*. 2014). The neurobehavioral effects of *T. gondii* are apparently dependent on the gender of the secondary hosts for the parasitic infection may have an increasing effect on testosterone (Flegr *et al*. 2012). Higher testosterone levels

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have been found associated with greater cognitive performance in males, while in females a negative trend was seen (Thilers *et al*. 2006). Flegr also observed that *T. gondii* infected males tend to be expedient and dogmatic, while infected women tend to be warm-hearted, persistent and conscientious (Flegr, 2007). Besides gender, other factors such as vitamins have been reported to possibly influence the severity of *Toxoplasma* infection by enhancing defence against infections through regulation of antimicrobial peptide gene expression (e.g. vitamins A and D) and/or reduction of oxidative stress (e.g. vitamins E and C) (McCarthy and Davis, 2003; Rajapakse *et al*. 2005; Stephensen *et al*. 2006; Campbell *et al*. 2012).

Despite these experimental findings, only a few human studies have examined the relationship between *T. gondii* and cognitive functions, including older adults, young and middle-aged adults, or congenitally infected children (Alford *et al*. 1974; Caiaffa *et al*. 1993; Kusbeci *et al*. 2011; Flegr *et al*. 2012; Guenter *et al*. 2012; Gajewski *et al*. 2013; Mendy *et al*. 2014). No study has ever been done in school-aged children with latent *Toxoplasma* infection. Given the importance of cognitive skills in school-age, we investigated the association between *T. gondii* seropositivity and reading, mathematical aptitudes, visuospatial reasoning as well as verbal memory in children aged 12–16 years. We hypothesized that sex and serum vitamins may influence the potential relationships of *T. gondii* with cognitive skills and tested them for effect modification.

MATERIALS AND METHODS

Data source and study design

We used data from the Third National Health and Nutrition Examination Survey (NHANES III) conducted from 1988 to 1994 by the National Centre for Health Statistics (NCHS) of the Centres for Disease Control and Prevention (CDC) (CDC, 2006). The NHANES is an ongoing cross-sectional survey of the US non-institutionalized civilian population selected using a complex multistage sampling design to derive a representative sample of the US population. *T. gondii* antibodies were measured in participants 12 years and older and among them, cognitive functions were tested in those between the ages of 12–16. A total of 1786 NHANES participants aged 12–16 years who had data on *Toxoplasma* seropositivity underwent cognitive testing. After exclusion of 12 children with mental retardation reported by parents and 19 participants with missing data, the final sample included 1755 subjects. NHANES III datasets are publicly available at http://www.cdc.gov/nchs/nhanes/nh3data.htm. NHANES protocols were approved by the institutional review boards of the NCHS and CDC and informed consent was obtained from all participants.

T. gondii IgG antibodies

Serum *T. gondii* IgG antibodies were measured using the Platelia Toxo-G immunoglobulin G enzyme immunoassay (Sanofi Diagnostics Pasteur, BioRad, Hercules, California), according to the manufacturer's instructions. Before initiation of the study, the Platelia Toxo-G kit was evaluated by using a battery of 90 sera (23 negative and 67 positive) in comparison with various titers in the Centres for Disease Control and Prevention *Toxoplasma* immunofluorescence assay-immunoglobulin G test and dye test (Dr Jack Remington, Palo Alto, California); both the specificity and sensitivity were found to be 100% (Jones *et al*. 2001). Results were reported in International Units (IU) and, as per the instructions of the manufacturer, samples with titer of lower than 6 IU mL^{-1} were considered to be negative, while those with results of 6 IU mL^{-1} and higher were considered positive, indicating infection at some undetermined time.

Cognitive function measures

Cognitive function testing was performed in mobile exam centres by trained examiners using the reading and math parts of the Wide Range Achievement Test-Revised (WRAT-R), as well as the block design and digit span parts of the Wechsler Intelligence Scale for Children-Revised (WISC-R). The WISC-R test was administered first followed by the WRAT-R. In the WRAT-R reading subset, children were asked to read aloud each word from a list of 75 words sequenced by order of increasing difficulty, until 12 consecutive errors were made. The reading test assessed the ability to recognize and name letters, pronounce, spell and write words (Jastak and Wilkinson, 1984). In the WRAT-R arithmetic subtest, children were asked to solve as many problems as possible from a total of 56 in 10 min. If at least six problems were not completed within the allotted time, the child was asked to count aloud the 15 dots on the form, read five numbers, and solve three simple word problems. The math test assessed the ability to count and solve mathematical problems in limited amount of time (Jastak and Wilkinson, 1984). The WISC-R block design test required children to timely assemble blocks of different colours according to given patterns; it evaluates perceptional reasoning and executive functions (Wechsler, 1974). In the WISC-R digit span test children were given sequences of numbers at a rate of one per second and were asked to repeat them forward and backward; this test assessed verbal short-term memory (forward digit

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span) and working memory (backward digit span) (Wechsler, 1974). Scores were standardized by age and the tests were administered in the children primary language (English or Spanish). Raw scores from all exams were scaled to allow comparisons between the WISC-R and WRAT-R components. Continuous scores were utilized, with higher scores indicating superior performance.

Covariates and serum vitamins

Data on age, gender, race/ethnicity, family income, level of education, visual and hearing impairments, and main language spoken in family were collected during the NHANES using questionnaires. Family income to poverty ratio was calculated using guidelines and adjustment for family size, year and state. The presence of serum antibody against Hepatitis B and C, Herpes simplex virus 1 and 2, cytomegalovirus (CMV) as well as *Helicobacter pylori* was determined using immunodot assay (Hespesviruses) and enzyme linked immunosorbent assay (ELISA) (hepatitis, CMV, *Helicobacter pylori*).

Vitamin B-12 was measured using the Bio-Rad Laboratories 'Quantaphase Folate' radioassay kit which combines serum or a whole blood hemolysate sample with 125I-folate and 57Co-vitamin B12 in dithiothreitol and cyanide. Serum vitamin C was measured using isocratic high performance liquid chromatography with electrochemical detection at 650 mV. Vitamins A, E, and carotenoids were measured by isocratic high performance liquid chromatography with detection at three different wavelengths. The INCSTAR 25-OH-D assay consists of a two-step procedure involving a rapid extraction of 25-OH-D and assayed by equilibrium radioimmunoassay procedure. Detailed descriptions of the laboratory procedures are available at: http://www.cdc.gov/nchs/data/nhanes/nhanes3/cdrom/nchs/manuals/labman.pdf.

Statistical analysis

Descriptive analyses were performed and *P*-values for differences in proportions or means by *Toxoplasma* seropositivity status were calculated using chi-square test for categorical variables and student's *t*-test for continuous variables. Normal distribution of the data was tested using the Kolmogorov–Smirnov test and, given their significant skewness, serum vitamins were log-transformed to improve the Gaussian distribution of the data. Using linear regression modelling, the coefficients (β) with corresponding 95% confidence intervals (CI) were estimated for the association between *Toxoplasma* seropositivity and cognitive test scores, adjusting for age, gender, race/ethnicity, family income to poverty ratio, years of education, visual and hearing impairments, main family language and other infections (Hepatitis B and C, Herpes simplex virus 1 and 2, CMV, *Helicobacter pylori*). Effect modification by gender and log-transformed serum vitamins A, B 12, C, D and E as well as α and β carotenoids was tested by including a product term in the models and for significant interactions, effect sizes were reported for the associations between *Toxoplasma* and the cognitive test scores for each of the modifier categories. R squared was used to examine model fitness. Where applicable, a Bonferroni correction was applied to control for Type I error given the number of statistical tests (one for each of the four neuropsychological tests). Analyses were performed in STATA (Version 11, STATA Corporation, College Station, TX, USA). NHANES sampling weights and STATA survey commands were used in all statistical procedures to adjust for unequal selection probabilities, non-responses, over-sampling, post-stratification and sampling errors, so that estimates were nationally representative. *P*-values <0·05 were considered statistically significant.

RESULTS

The study sample consisted of 1755 participants with a mean age (±standard error (S.E.)) of 13·97 (±0·06) years (range 12–16). In the analysis weighted for complex survey design, survey non-response and post stratification, 51·8% of our population consisted of boys and 66·8% were non-Hispanic Whites. The prevalence of *T. gondii* seropositivity was 7·7% (8·5% in boys and 6·9% in girls). The proportion of children from families whose main language was not English was significantly higher in the infected than in the non-infected group. The family income to poverty ratio was lower in children infected with *Toxoplasma* and other infections were more frequent in this group (Table 1). The concentration of vitamins in serum was not different in seropositive and seronegative children. Overall and especially in girls, reading, visuospatial reasoning and memory scores were lower in children with *T. gondii* infection. In boys, no significant difference in scores was seen between *Toxoplasma* infected and non-infected groups (Table 1).

In adjusted linear regression, *T. gondii* seropositivity was negatively associated with reading skills ($\beta = -5{\cdot}86$, 95% CI: −11·11, −0·61, $P = 0{\cdot}029$) and memory scores ($\beta = -0{\cdot}86$, 95% CI: −1·58, −0·15, $P = 0{\cdot}017$), but not with math or visuospatial reasoning. The interaction between *Toxoplasma* and log-transformed serum vitamin E significantly correlated with math ($P = 0{\cdot}04$) and memory test scores ($P = 0{\cdot}03$). In stratified analysis, *T. gondii*-associated memory impairment was significantly worse in participants with log-transformed serum vitamin E concentration below the median ($\beta = -1{\cdot}61$, 95% CI: −2·44, −0·77, $P < 0{\cdot}001$) than in those with values at or above the median

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Table 1. Characteristics of study participants by Toxoplasma gondii (*T. gondii*) infection status, NHANES III (N = 1755)

	Boys ($N = 818$)				Girls ($N = 937$)				All (**N** = 1755)			
Characteristics	*T. gondii* –	*T. gondii* +	Total	*P*	*T. gondii* –	*T. gondii* +	Total	*P*	*T. gondii* –	*T. gondii* +	Total	*P*
Age, mean (S.E.), years	13·91 (0·09)	13·94 (0·25)	13·91 (0·09)	0·90	14·01 (0·08)	14·40 (0·20)	14·04 (0·07)	0·07	13·96 (0·06)	14·15 (0·17)	13·97 (0·06)	0·29
Race/ethnicity, %												
Non-hispanic whites	69·0	65·5	68·7	<0·001	65·0	59·9	64·6	<0·001	67·1	63·0	66·8	<0·001
Non-hispanic blacks	13·6	15·6	13·6		15·3	17·7	15·5		14·4	15·4	14·5	
Hispanics	8·1	6·6	7·9		9·0	5·5	8·7		8·5	6·1	8·3	
Other	9·3	14·2	9·7		10·7	16·9	11·2		10·0	15·5	10·4	
Education, mean (S.E.), years	7·43 (0·10)	7·54 (0·27)	7·44 (0·09)	0·69	7·71 (0·09)	7·88 (0·23)	7·72 (0·08)	0·49	7·56 (0·07)	7·69 (0·18)	7·57 (0·06)	0·50
Had trouble seeing, %	11·6	7·2	11·3	0·41	12·4	23·4	13·2	0·15	12·0	14·5	12·2	0·58
Had hearing trouble, %	6·2	4·4	6·0	0·69	5·9	0·4	5·5	0·01	6·0	2·6	5·8	0·28
Family language not English, %	5·7	9·3	6·0	0·11	6·0	20·7	7·2	0·02	5·8	14·5	6·5	0·01
Other infections, %	55·6	71·7	57·0	0·05	61·4	75·8	62·4	0·17	58·4	73·5	59·6	0·02
Income to poverty ratio, mean (S.E.)	2·30 (0·09)	1·84 (0·19)	2·26 (0·09)	0·04	2·31 (0·08)	1·68 (0·35)	2·26 (0·08)	0·15	2·30 (0·06)	1·77 (0·19)	2·26 (1·0·06)	0·02
Serum vitamins												
Vitamin B12, mean (S.E.), pg/ml	505·40 (14·30)	496·37 (40·59)	504·86 (13·94)	0·80	522·22 (18·49)	489·49 (87·49)	520·82 (18·38)	0·63	513·66 (11·46)	492·18 (50·34)	511·86 (11·21)	0·61
Vitamin C, mean (S.E.), mg dl^{-1}	0·80 (0·04)	0·87 (0·11)	0·81 (0·04)	0·37	0·84 (0·04)	1·00 (0·14)	0·85 (0·04)	0·19	0·82 (0·03)	0·93 (0·11)	0·83 (0·03)	0·13
Vitamin A, mean (S.E.), μg dl^{-1}	45·27 (0·88)	45·22 (2·72)	45·26 (0·85)	0·96	43·18 (0·76)	41·56 (0·92)	43·06 (0·71)	0·16	44·26 (0·60)	43·51 (1·54)	44·20 (0·57)	0·67
Vitamin E, mean (S.E.), μg dl^{-1}	715·83 (12·71)	697·65 (26·01)	714·35 (12·06)	0·45	749·55 (16·98)	750·75 (32·20)	749·64 (15·84)	0·95	731·75 (10·55)	721·40 (23·63)	730·94 (9·96)	0·55
α-carotene, mean (S.E.), μg dl^{-1}	2·45 (0·50)	2·77 (0·57)	2·48 (0·47)	0·38	2·64 (0·26)	3·72 (1·37)	2·71 (0·29)	0·14	2·54 (0·30)	3·18 (0·95)	2·59 (0·29)	0·11
β-carotene, mean (S.E.), μg dl^{-1}	12·37 (1·04)	12·71 (1·36)	12·40 (0·99)	0·68	12·76 (0·86)	13·71 (3·12)	12·83 (0·85)	0·60	12·55 (0·69)	13·16 (2·09)	12·60 (0·66)	0·52
Vitamin D, mean (S.E.), mg dl^{-1}	32·97 (1·07)	33·01 (5·54)	32·98 (1·07)	0·89	27·89 (0·92)	26·82 (1·73)	27·81 (0·85)	0·37	30·44 (0·74)	30·03 (3·14)	30·41 (0·72)	0·67
Cognitive test scores												
Standardized math score	92·97 (1·19)	92·27 (2·83)	92·91 (1·11)	0·82	93·55 (0·96)	85·01 (3·25)	92·93 (0·93)	0·01	93·25 (0·77)	89·09 (2·19)	92·92 (0·73)	0·07
Standardized reading score	94·49 (1·00)	87·60 (3·97)	93·91 (0·99)	0·09	93·87 (0·89)	82·56 (4·14)	93·05 (0·88)	0·008	94·19 (0·67)	85·39 (2·84)	93·50 (0·67)	0·003
Block design scaled score	9·58 (0·18)	9·28 (0·46)	9·56 (0·17)	0·54	9·05 (0·17)	7·62 (0·50)	8·94 (0·16)	0·007	9·32 (0·12)	8·55 (0·34)	9·26 (0·12)	0·03
Digit span scaled score	8·47 (0·17)	7·66 (0·47)	8·40 (0·17)	0·10	8·84 (0·15)	7·27 (0·61)	8·72 (0·15)	0·01	8·64 (0·12)	7·49 (0·37)	8·56 (0·11)	0·003

NHANES, National Health and Nutrition Examination Survey; S.E., standard error.
Geometric mean reported for serum vitamins and provitamins and calculated from the log-transformed estimates.
Other infections include hepatitis B and C, herpes simplex virus 1 and 2 seropositivity, helicobacter pylori seropositivity, CMV.
P-values indicate statistical significance of characteristics by *T. gondii* antibody seropositivity.

($\beta = -0{\cdot}12$, 95% CI: $-1{\cdot}23$, $0{\cdot}99$, $P = 0{\cdot}03$). There was no relationship between *Toxoplasma* and math score in general or in subgroup analysis by serum vitamin E levels (Table 2). R-squared for the different models ranged between 0·20 and 0·30.

DISCUSSION

In the present study, we used a representative sample of the US population to examine the relationship between *Toxoplasma* seropositivity and cognitive functions in children aged 12–16. The results suggest that *T. gondii* seropositivity is associated with poor reading performance and impaired verbal memory. Serum vitamin E seems to modify the relationship between the parasite and verbal memory with greater *Toxoplasma*-associated memory impairment found in participants with lower vitamin E level.

To our knowledge, no previous study has ever investigated latent *Toxoplasma* infection and cognitive function in school-aged children. However, findings of *Toxoplasma*-associated cognitive impairment have been reported in congenitally infected children and older adults. An association of congenital toxoplasmosis with reduced intellectual quotient measured by the Slosson Intelligence Test in toddlers has been described and mental retardation has been linked to congenital toxoplasmosis in school-aged children (Alford *et al*. 1974; Caiaffa *et al*. 1993). In older ages, the infection has recently been postulated to possibly weaken episodic and working memory functions (Gajewski *et al*. 2013; Mendy *et al*. 2014). Among adults, Guenter *et al*. found no relationship between latent toxoplasmosis and psychomotor speed and working memory, cognitive flexibility, or audio verbal and visuospatial working memory (Guenter *et al*. 2012). A secondary analysis of the data from this study stratified by gender was performed by Flegr *et al*. who noted that latent toxoplasmosis was significantly associated with worse verbal fluency in women. It found better working memory and cognitive flexibility in men with latent toxoplasmosis compared with non-infected ones (Flegr *et al*. 2012). These results were inconsistent with our results showing that gender did not modify the association between the parasite and cognitive deficiency. After extensive literature review, we found no research on the potential effects of vitamins on *Toxoplasma*-associated cognitive impairment; however, a few reports have investigated how vitamins may modify toxoplasmosis severity, producing contradictory results. In contrast with our findings of improved cognitive functions associated with vitamin E, McCarthy and Davis noted that vitamin E was detrimental to *T. gondii* infected mice by increasing tissue cyst concentrations, histologic damage and severe

Table 2. Linear regression coefficients (β) for the associations of *T. gondii* seropositivity and interaction between *Toxoplasma* and gender and vitamins with cognitive functions in children 12–16 years of age, NHANES III (N = 1755)

	Math score		Reading score		Block design score		Digit span score	
T. gondii	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
All participants	−0·99 (−5·39, 3·41)	0·66	**−5·86 (−11·11, −0·61)***	**0·029**	−0·41 (−1·18, 0·36)	0·30	**−0·86 (−1·58, −0·15)***	**0·017**
Interaction Toxoplasma*log-vitamin E	**18·00 (0·60, 35·40)***	**0·04**	5·82 (−18·54, 30·17)	0·64	1·56 (−1·97, 5·08)	0·39	**3·40 (0·24, 6·56)***	**0·03**
Participants with serum vitamin E < median	−3·87 (−9·97, 2·23)	0·21	**−8·92 (−16·65, −1·19)***	**0·024**	**−1·10 (−1·99, −0·21)***	**0·016**	**−1·61 (−2·44, −0·77)**[a]**	**<0·001**
Participants with serum vitamin E ≥ median	2·60 (−3,28, 8·47)	0·39	−1·96 (−8·09, 4·17)	0·53	0·26 (−1·01, 1·53)	0·69	−0·12 (−1·23, 0·99)	0·83

NHANES, National Health and Nutrition Examination Survey.
*$P < 0{\cdot}05$; **$P < 0{\cdot}001$.
[a] Remains significant after Bonferroni correction (critical alpha < .05/4 = 0·0125).
Linear regression models adjusted for age, gender, race/ethnicity, income to poverty ratio, education level, visual and hearing impairment, main language in family other than English, and other infections. Significant estimates (*P*-value < 0.05) presented in bold and underlined.

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meningo-encephalitis (McCarthy and Davis, 2003). Other vitamins such as vitamin D have been observed to reduce the survival rate of *Toxoplasma* infected mice (Rajapakse *et al*. 2005), while in a more recent study, vitamin D inhibited intracellular *T. gondii* parasite proliferation *in vivo* and *in vitro* (Rajapakse *et al*. 2007).

The mechanism by which *T. gondii* may affect reading abilities and verbal memory in children could be due to a modifying effect of neurotransmitter signal transduction by the parasite (Prandovszky *et al*. 2011). In neural cells, *Toxoplasma* has been suggested to alter the concentration and metabolism of dopamine, a critical modulator of neuronal activities in the frontal cortex and the hippocampus both involved in learning and working memory (McConkey *et al*. 2013). *T. gondii* infection also initiates a Th1 immune response with production of interferon-γ which could mediate cognitive impairment by increased neuronal death (Bate *et al*. 2006; McConkey *et al*. 2013). Furthermore, dopamine influences attentional processes in children leading to hyperactivity, inattention, and impulsive behaviour all resulting in cognitive deficit as a consequence of 'acting before thinking' (Nieoullon, 2002). Inattentive behaviour is known to be strongly associated with impairment of reading which is a cognitively complex process involving multiple skills, such as phoneme awareness, phonological decoding, orthographic coding and rapid automatized naming (Luca *et al*. 2007; Cornish *et al*. 2011). Previous studies have noted sex differences in the relationship between the infection and cognitive functions. Flegr *et al*. speculated that the increasing effect of the parasitic infection on testosterone among infected males could shift personality profiles, increasing motivation in boys and thus performance in several cognitive tests (Flegr *et al*. 2012). It has been proposed that an increase in testosterone level may have sex specific effects on cognition, as it could be associated with better visuospatial abilities, semantic and episodic memory in males but worst verbal fluency, semantic and episodic memory in females (Thilers *et al*. 2006). Gatkowska *et al* noted that toxoplasmosis induced a decreased noradrenergic activity in female mice, but increased activity in some brain areas of male mice (Gatkowska *et al*. 2013). Like dopamine, noradrenaline is a key neuromodulator involved in many cognitive functions including arousal, attention, cognitive flexibility, working and emotional memory (Chamberlain and Robbins, 2013). Moreover, Xiao *et al*. examined the effects of persistent *T. gondii* infection on gene expression in the frontal cortex of male and female mice. They realized that in females, the parasite altered the expression of genes involved in the forebrain development, neurogenesis, and sensory and motor coordination, while in males, it mostly modulated genes associated with olfaction (Xiao *et al*. 2012).

Intriguingly, we found no association of *Toxoplasma* infection with arithmetic abilities or visuospatial reasoning and the reason why is unclear to us. But, there seemed to be a trend toward a negative relationship for both of these tests with the parasitic infection. Research with regards to the effects of vitamins on *Toxoplasma*-related decline in cognition is lacking. In general, vitamin E, which forms the first line of defence against lipid peroxidation protects cell components from oxidation by reactive free radicals; it may have a stimulatory effect on the immune system by impacting cytokine production (McCarthy and Davis, 2003). The effect of antioxidants on severity of *Toxoplasma* infection is controversial, despite evidence that oxidative stress induced by the parasite may be a major factor for virulence and deteriorating cognition (McCarthy and Davis, 2003). Concordant with a beneficial effect of antioxidants on *T. gondii* infection, quercetin, a flavanol with antioxidant properties was found to suppress bradyzoites formation in a cell culture model, maybe by inhibiting heat shock protein synthesis (Weiss *et al*. 1998).

Our study had limitations. Given the cross-sectional study design, temporality between *T. gondii* infection and cognitive functions impairment cannot be assessed and causal inferences cannot be drawn. Some of our covariates such as visual and hearing impairments were self-reported and may have been subject to misclassification. Nevertheless, this study has major strengths. The large size representative sample of the US population makes findings generalizable and the broad range of covariates adjusted for increases the power of statistical inferences.

In conclusion, *T. gondii* may be associated with impaired reading ability and verbal memory in school-aged children between 12 and 16 years old as a result of cumulative effects of a latent infection. Vitamin E seems to modify the relationship. Future longitudinal studies are needed to confirm these findings and research could include trials to confirm a possible efficacy of vitamin E supplementation in improving cognitive impairment hypothetically associated with the infection.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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