



Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi*Toxoplasma gondii* and anxiety disorders in a community-based sampleAdam A. Markovitz^a, Amanda M. Simanek^b, Robert H. Yolken^c, Sandro Galea^d, Karestan C. Koenen^d, Shu Chen^e, Allison E. Aiello^{f,*}^a Department of Health Management and Policy, School of Public Health, University of Michigan, Ann Arbor, MI 48109, United States^b Joseph J. Zilber School of Public Health, University of Wisconsin–Milwaukee, Milwaukee, WI 53201, United States^c Department of Pediatrics, Stanley Division of Developmental Neurovirology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, United States^d Department of Epidemiology, Columbia University Mailman School of Public Health, New York, NY 10032, United States^e Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI 48109, United States^f Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC 27599, United States

ARTICLE INFO

Article history:

Received 7 May 2014

Received in revised form 28 July 2014

Accepted 4 August 2014

Available online 12 August 2014

Keywords:

Toxoplasma gondii

Mental health

Generalized anxiety disorder

Depression

PTSD

Infection

ABSTRACT

A growing body of literature suggests that exposure to the neurotropic parasite *Toxoplasma gondii* (*T. gondii*) is associated with increased risk of mental disorders, particularly schizophrenia. However, a potential association between *T. gondii* exposure and anxiety disorders has not been rigorously explored. Here, we examine the association of *T. gondii* infection with both anxiety and mood disorders. Participants ($n = 484$) were drawn from the Detroit Neighborhood Health Study, a population-representative sample of Detroit residents. Logistic regression was used to examine the associations between *T. gondii* exposure (defined by seropositivity and IgG antibody levels) and three mental disorders: generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD) and depression. We found that *T. gondii* seropositivity was associated with a 2 times greater odds of GAD (odds ratio (OR), 2.25; 95% confidence interval (CI), 1.11–4.53) after adjusting for age, gender, race, income, marital status, and medication. Individuals in the highest antibody level category had more than 3 times higher odds of GAD (OR, 3.35; 95% CI, 1.41–7.97). Neither *T. gondii* seropositivity nor IgG antibody levels was significantly associated with PTSD or depression. Our findings indicate that *T. gondii* infection is strongly and significantly associated with GAD. While prospective confirmation is needed, *T. gondii* infection may play a role in the development of GAD.

© 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

1. Introduction

Anxiety and mood disorders contribute substantially to the burden of disease and disability in the United States. A recent national study estimates that generalized anxiety disorder (GAD), posttraumatic stress disorder (PTSD), and major depressive disorder affect 5.7%, 6.8%, and 16.6% of adults in their lifetime, respectively (Kessler et al., 2005). Studies have established a genetic contribution to these mental disorders (Hettema et al., 2001; Sullivan et al., 2000; Xian et al., 2000). Yet, the mapping of direct paths from gene to mental disorders has been slow and inconsistent, as only a few genome-wide association studies have detected risk genes and many putative gene findings have failed replication (Hamer, 2002). More fundamentally, a large proportion of variation in mental

health remains unexplained by genetic factors. For these reasons, discovery of new risk factors for mental disorders is crucial.

A growing body of epidemiologic literature has implicated infections as novel risk factors for development of mental disorders (Benros et al., 2013; Dalman et al., 2008). One pathogen of particular interest is the neurotropic parasite *Toxoplasma gondii* (*T. gondii*). *T. gondii* is capable of reproducing asexually within any warm-blooded animal but must return to its definitive host, the cat, to undergo sexual reproduction, develop into infectious oocysts, and return to the environment through fecal shedding (Carruthers and Suzuki, 2007). Infection is transmitted to an intermediate host (e.g., a rodent) or a dead-end host (e.g., a human) via ingestion of tissue cysts in undercooked meat or oocysts in cat feces or contaminated soil, whereupon the parasite progresses to form latent cysts in muscle and neural cells, including neurons, glial cells, and astrocytes (Carruthers and Suzuki, 2007).

As *T. gondii* does not complete its life cycle until passing from its intermediate rodent host to its definitive feline host, the “manipulation hypothesis” posits that the parasite may be under selective

* Corresponding author. Address: Department of Epidemiology, The University of North Carolina at Chapel Hill, 135 Dauer Drive 2101, McGavran–Greenberg Hall, CB #7435, Chapel Hill, NC 27599-7435, United States. Tel.: +1 919 966 7430.

E-mail address: aaello@email.unc.edu (A.E. Aiello).

pressure to influence rodent behavior to promote predation by and transmission to the definitive feline host (Lafferty, 1999). Indeed, *T. gondii* has been shown to profoundly alter anxiety in rodents, as evidenced by increased activity, decreased fear of novel stimuli, and diminished predator vigilance (Berdoy et al., 2000; Webster, 1994; Webster et al., 1994). Meanwhile, a broad range of other behaviors related to learning (Vyas et al., 2007), social status (Berdoy et al., 1995), and olfaction (Vyas et al., 2007) remain unaffected.

While the neurologic effects of toxoplasmosis in congenitally-infected or immunocompromised humans are well-established (e.g., encephalitis in AIDS patients), infection among the immunocompetent is generally considered relatively benign: the parasite is never cleared from the nervous system but cell-mediated immune response suppresses pathogenic activity (Montoya and Liesenfeld, 2004). This “no harm done” assumption is now being reconsidered, as growing evidence links *T. gondii* to several mental disorders (Fekadu et al., 2010). Decades of serological investigations have corroborated a relationship between *T. gondii* and schizophrenia (Torrey et al., 2012). More recently, studies have implicated the infection in mood disorders (e.g., depression, bipolar disease) and suicidal behavior (Fekadu et al., 2010), while a small case-control study suggests an association with obsessive-compulsive disorder (Miman et al., 2010). To our knowledge, no previous study has examined the association between *T. gondii* and either GAD or PTSD, and none has investigated the parasite’s association with any diagnosed anxiety disorder among individuals living in the community setting.

To address these gaps in the literature, we used data from the Detroit Neighborhood Health Study (DNHS), a prospective, population-based study of residents of Detroit, Michigan. The purpose of this study was to examine whether *T. gondii* seropositivity and IgG antibody levels were associated with three different mental disorders, GAD, PTSD, and depression, in persons 18 years of age and older living in Detroit, Michigan.

2. Materials and methods

2.1. Study population

The DNHS is a longitudinal, population-based study designed to investigate correlates of mental disorders in the city of Detroit. A probability sample of 1547 individuals (aged ≥ 18 years) living within the Detroit city limits participated in a baseline telephone survey in 2008–2009. The DNHS was approved by the institutional review board at the University of Michigan, and all participants provided written, informed consent. Participants were administered a 40 minute assessment via a telephone survey, which included questions on socio-demographic characteristics and a standardized assessment of GAD, PTSD, and depression. Wave 1 survey participants were representative of the Detroit population in terms of age, gender, race, income, and educational attainment (for more detailed information, see Uddin et al., 2010). All respondents were invited during the phone interview to participate in the biospecimen component of the study and 484 (31.3%) participants provided venipuncture blood specimens that were tested for *T. gondii* IgG antibodies. The socio-demographic characteristics of the biospecimen sample were comparable to the overall study sample with the exception of income and education levels, which were lower among those who provided a biospecimen. In addition, past year GAD, PTSD, and depression were statistically significantly more prevalent among those who provided biospecimens tested for *T. gondii*-specific IgG versus those in the overall study sample, where 11.4% vs. 7.7% had GAD ($p = 0.01$), 13.4% vs. 9.4% had PTSD ($p = 0.01$), and 15.8% vs. 11.4% had depression ($p = 0.01$) in the past year at baseline.

2.2. Laboratory analyses

Serum samples were analyzed for *T. gondii* infection by standard procedures. Sera were frozen and stored at -70°C , then shipped on dry ice (within four weeks) to the Stanley Laboratory of Developmental Neurovirology, Baltimore, Maryland. The presence and quantity of immunoglobulin G (IgG) serum antibodies to *T. gondii* were measured by solid phase enzyme-linked immunosorbent assays and with laboratory personnel unaware of the status of the study participants (Wang et al., 2011; Yolken et al., 2011). Reagents for these assays were obtained from IBL Laboratories, Hamburg, Germany.

2.3. Measures

Participants were categorized in the following manner: (1) Seropositivity: participants with *T. gondii* IgG values <10 International Units (IU) were dichotomized as seronegative and those with IgG values ≥ 10 IU were categorized as seropositive; (2) Serointensity: continuous IgG antibody levels were standardized such that a one unit increase in *T. gondii* IgG antibody level represents the effect of 1 standard deviation change in *T. gondii* IgG antibody level; and (3) Antibody level category: IgG antibody level was categorized as high level (≥ 20.2 IU), low level (10–20.2 IU), or seronegative (<10.0 IU).

History of GAD, PTSD, and depression during the past year was assessed during the baseline telephone survey with validated instruments based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association, 2000) as previously described (Uddin et al., 2010). Briefly, past-year GAD was assessed using the seven-item generalized anxiety disorder scale (GAD-7) (Spitzer et al., 2006). Each of the seven symptoms was scored from 0 (not at all) to 3 (nearly every day), with total scores ranging from 0 to 21. Respondents who scored ≥ 10 were categorized as having past-year GAD. Past-year PTSD was assessed using a modified version of the PTSD checklist (PCL-C), a 17-item measure of DSM-IV symptoms of PTSD (Weathers, 1996). Participants identified past exposure to 19 potential traumatic events (PTE) and described PTSD symptoms related to two traumatic events: (1) the event identified by the participant as the most traumatic and (2) a randomly selected PTE experienced by the participant. PTSD was considered present if all six DSM-IV criteria were met in reference to either the worst event or the random event. Past-year depression was assessed with the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001). The nine items on the PHQ-9 were scored from 0 (not at all) to 3 (nearly every day), with total scores ranging from 0 to 27. Past-year depression was considered present if participants reported depressed mood or anhedonia and the co-occurrence of at least one additional symptom for “more than half the days” in a 2-week period over the past year. One symptom, “thoughts that you would be better off dead or of hurting yourself in some way,” was included in the depression score if present, regardless of symptom duration. A clinical reappraisal study ($n = 51$) demonstrated that the identification of individuals with GAD, PTSD, and depression by the survey screening scales displayed high concordance for diagnoses of GAD, PTSD, and depression obtained via in-person clinical interviews (Uddin et al., 2010).

Covariates: Age in years was self-reported and treated as a continuous variable. Race was self-reported and individuals were categorized as White, African-American, and Hispanic/Other. Gender was dichotomized as female and male. Household income was self-reported as pre-tax family income and was categorized as (1) less than \$25,000, (2) \$25,000–\$50,000, or (3) greater than \$50,000. Marital status was categorized as married, divorced, separated, widowed, or never married. Medications were classified according to the Center for Disease Control and Prevention Ambu-

latory Care Drug Database System (Centers for Disease Control and Prevention, 2009) and medication use was dichotomized as currently taking anti-parasitic (i.e., antiprotozoals, antimalarials), anti-microbial (i.e., tetracyclines, sulfonamides and trimethoprim, antiviral agents), immunologic (i.e., immunomodulators), and/or central nervous system (i.e., antianxiety agents, antipsychotic/antimanics, antidepressants) medications, or not.

2.4. Statistical analysis

Statistical analyses were conducted using SAS, version 9.2 (SAS, 2008). Two-sided *T*-tests and chi-square tests were used to examine bivariate associations between *T. gondii* serostatus, mental disorders, and covariates of interest. Covariates were considered confounders based on *a priori* hypotheses regarding covariates that are associated with *T. gondii* infection and predictive of the outcomes of interest. Logistic regression models were used to estimate the crude and confounder-adjusted odds ratio (OR) and 95% confidence intervals (CI) for the associations between the *T. gondii* seropositivity and serointensity (continuous and dichotomized IgG antibody levels) and each mental disorder. The fully adjusted model included age, gender, race, income, marital status, and use of medications thought to alter both immune function and mental disorders.

3. Results

3.1. Demographic and clinical characteristics by *Toxoplasma gondii* serostatus

Demographic and clinical characteristics by *T. gondii* serostatus are shown in Table 1. Of the 484 participants, approximately 26% ($n = 128$) were *T. gondii* seropositive. Age and marital status were statistically significantly associated with *T. gondii* seropositivity. There were no significant associations between *T. gondii* seropositivity and gender, race, income, education, or medication use. Of the 448 participants, 55 (11.4%) had GAD, 65 (13.4%) had PTSD, and 76 (15.8%) had depression in the past year at baseline.

3.2. Association between *T. gondii* seropositivity and serointensity and mental disorders

The crude and covariate-adjusted associations between *T. gondii* seropositivity and GAD, PTSD, and depression are shown in Table 2. In unadjusted models, there was no statistically significant association between *T. gondii* seropositivity and GAD, PTSD or depression. After adjusting for age, gender, race, income, marital status, and medication use, seropositivity for *T. gondii* was associated with a 2.25 times greater odds (95% CI, 1.11–4.53) (Table 2). *T. gondii* seropositivity was not significantly associated with PTSD or depression after adjustment.

We next examined the relationship between serointensity (i.e., continuous IgG antibody levels) and each mental disorder. For every one standard deviation increase in *T. gondii* antibody level, there was a marginal increase in the odds of GAD (OR 1.13; 95% CI, 0.99–1.28) in the adjusted model. When we restricted the analyses to only seropositive subjects ($n = 128$), the trend remained the same for GAD, but did not reach statistical significance, potentially due to small sample size. Serointensity was not significantly associated with increased odds of PTSD or depression.

3.3. Association between *T. gondii* antibody level category and mental health disorders

To examine whether *T. gondii* antibody levels exerted a non-linear effect, we examined the association between high or low anti-

Table 1

Demographic and clinical characteristics by *Toxoplasma gondii* serostatus, $n = 484$ (DNHS, 2008–2009).

	Distribution of <i>T. gondii</i> exposure		<i>P</i> -value ^a
	Seropositive ($n = 128$)	Seronegative ($n = 356$)	
Age, mean (sd)	57.2 (14.7)	49.5 (16.4)	<0.01
Gender, n (%)			
Female	69 (53.9)	217 (61.0)	0.16
Male	59 (46.1)	139 (39.0)	
Race/ethnicity, n (%)			
White	20 (15.6)	36 (10.2)	0.25
African–American	100 (78.1)	292 (82.5)	
Hispanic/Other	8 (6.3)	26 (7.3)	
Income, n (%)			
Less than \$25,000	63 (49.2)	184 (51.7)	0.79
\$25,000–\$50,000	28 (21.9)	78 (21.9)	
Greater than \$50,000	28 (21.9)	68 (19.1)	
Education, n (%)			
Less than high school	23 (18.0)	64 (18.0)	0.73
High school completed/GED	42 (32.8)	106 (29.8)	
Some college	45 (35.2)	121 (34.0)	
College graduate or graduate work	18 (14.1)	65 (18.3)	
Marital status, n (%)			
Married	37 (28.9)	74 (20.8)	0.03
Divorced	29 (22.7)	74 (20.8)	
Separated	4 (3.1)	25 (7.0)	
Widowed	22 (17.2)	41 (11.5)	
Never been married	36 (28.1)	142 (39.9)	
Medication use ^b			
Yes	21 (16.4)	46 (13.5)	0.41
No	107 (83.6)	296 (86.5)	

^a *T*-tests (two-tailed) for difference in means, Pearson chi-square tests of independence for proportions, tests for demographic trends were calculated.

^b Medications include: central nervous system medications, anti-parasitic and anti-microbial medications, and/or immunologic.

Table 2

The association between *Toxoplasma gondii* seropositivity and mental health disorders in the Detroit Neighborhood Health Study.

Mental disorder	Toxo sero-status	Odds ratio (95% confidence interval)	
		Unadjusted	Adjusted ^a
GAD	Pos vs. Neg	1.41 (0.77–2.57)	2.25 (1.11–4.53)
PTSD	Pos vs. Neg	1.18 (0.66–2.09)	1.68 (0.88–3.21)
Depression	Pos vs. Neg	0.77 (0.42–1.37)	1.09 (0.57–2.10)

Abbreviations: GAD, generalized anxiety disorder; PTSD, post-traumatic stress disorder.

^a Adjusted model includes age, gender, race, income, marital status, and medication use.

body levels compared to seronegative status with each mental disorder (Table 3). In fully adjusted models, individuals in the high *T. gondii* antibody level category had an OR of 3.35 (95% CI, 1.41–7.97) for GAD as compared to seronegative subjects. The OR for GAD for individuals categorized in the low antibody level category compared to those who were *T. gondii* seronegative was not statistically significant in the fully adjusted model. Neither high nor low *T. gondii* antibody level category was significantly associated with PTSD or depression when compared to seronegative subjects.

4. Discussion

Our study is the first to examine the association between *T. gondii* infection and any diagnosed anxiety disorder among individuals participating in a population-based study. We found that seropositive individuals had more than twice the odds of reporting GAD compared to seronegative individuals. Strikingly, individuals in

Table 3

The association between *Toxoplasma gondii* antibody level categories and mental health disorders in the Detroit Neighborhood Health Study.

Mental disorder	Level category	Odds ratio (95% confidence interval)	
		Unadjusted	Adjusted ^a
GAD	Pos Low vs. Neg	0.89 (0.36–2.20)	1.43 (0.54–3.81)
	Pos High vs. Neg	1.98 (0.97–4.05)	3.35 (1.41–7.97)
PTSD	Pos Low vs. Neg	0.96 (0.43–2.15)	1.29 (0.54–3.07)
	Pos High vs. Neg	1.40 (0.68–2.87)	2.19 (0.96–5.04)
Depression	Pos Low vs. Neg	0.61 (0.27–1.41)	0.86 (0.35–2.09)
	Pos High vs. Neg	0.93 (0.45–1.92)	1.40 (0.60–3.25)

Abbreviations: GAD, generalized anxiety disorder; PTSD, post-traumatic stress disorder.

^a Adjusted model includes age, gender, race, income, marital status, and medication use.

the highest antibody level category had more than 3 times the odds of GAD as compared to seronegative individuals, suggesting a graded relationship between immune response to *T. gondii* and odds of GAD. By examining the association between *T. gondii* and GAD, PTSD, and depression, we were uniquely positioned to examine whether *T. gondii* was related to multiple anxiety and mood disorders. Our novel finding that *T. gondii* was associated with GAD but neither PTSD nor depression suggests that *T. gondii* is specifically associated with GAD in our study population.

Despite compelling evidence that *T. gondii* infection profoundly alters the manner in which rodents perceive and respond to stressful stimuli (Webster, 2007), only two previous studies have investigated whether *T. gondii* is related to human anxiety (Groer et al., 2011; Miman et al., 2010). Groer et al. assessed whether *T. gondii* seropositivity and serointensity were associated with anxiety among a cohort of pregnant women enrolled in a study of postpartum thyroiditis, as assessed by the Profile of Mood Disorder States (POMS), a non-clinical diagnostic screening instrument (Groer et al., 2011). Similar to our study, the authors found a positive correlation between *T. gondii* serointensity and the POMS tension-anxiety subscale score ($r = 0.31$, $p < 0.04$). However, use of the POMS limited Groer et al. to scoring participants on a 5-point anxiety scale, whereas our study utilized a validated survey instrument that enabled us to assign subjects clinical diagnoses of GAD. In addition, generalizability of their findings were limited to pregnant women enrolled in a study of postpartum thyroiditis (Groer et al., 2011), whereas we included a subset of individuals drawn from a population-based sample in our study.

To our knowledge, only one prior study has examined associations between *T. gondii* and any anxiety disorder as diagnosed by DSM-IV criteria (Miman et al., 2010). In a case-control study of 142 subjects, Miman et al. found that individuals with psychiatrist-diagnosed obsessive-compulsive disorder (OCD) were more likely to be seropositive for *T. gondii* than were healthy controls (chi-square 12.12, $p < 0.01$). However, the authors did not report continuous or categorical antibody levels. Overall, our study is the first to demonstrate that, in addition to a positive association between *T. gondii* seropositivity and GAD, there may be a graded relationship between *T. gondii* IgG antibody levels and odds of GAD.

While the underlying mechanisms by which *T. gondii* specifically affects GAD but not PTSD or depression remain uncertain, potential anxiogenic pathways include histopathological, immunological, and neuromodulatory alterations (Webster, 2007). Rodent studies have failed to uncover a highly selective tropism of *T. gondii* for a specific brain region; tissue cysts have been detected throughout the brain, with observed distribution patterns varying both between (Berenreiterova et al., 2011; Haroon et al., 2012; Vyas et al., 2007) and within (Berenreiterova et al., 2011) studies. However, cyst density does not appear homogenous across brain regions (Berenreiterova et al., 2011), while a recent study suggests

that cysts may preferentially persist and increase in number in limbic regions known to mediate anxiety, including the amygdala and hypothalamus (Haroon et al., 2012). *In vivo* studies of chronically infected rodents indicate that *T. gondii* cysts may impede neuronal function, as neurons harboring cysts demonstrate dendritic retraction (Mitra et al., 2013) and reduced uptake of the potassium analogue thallium (Haroon et al., 2012).

Another intriguing possibility is that *T. gondii* may directly activate the dopaminergic system of its host. The *T. gondii* genome contains an ortholog of tyrosine hydroxylase (Etkin et al., 2009), the rate-limiting enzyme in dopamine biosynthesis. Brains of infected mice demonstrate increased levels of dopamine and parasitic tyrosine hydroxylase, both of which localize to the cysts themselves (Prandovszky et al., 2011). As dopamine is known to powerfully potentiate anxiety expression in the amygdala (de la Mora et al., 2010), the capacity to augment local dopaminergic signaling could allow *T. gondii* to inappropriately activate anxiety circuitry even in the absence of a highly specific tropism. This model is supported by reports that infected rats demonstrate greater activation in amygdalar and hypothalamic nuclei (House et al., 2011) and that the dopamine receptor antagonist haloperidol suppresses behavioral changes in infected rats (Webster et al., 2006). As human studies have implicated both amygdalar (Etkin et al., 2009) and dopaminergic (Koenen et al., 2009; Rowe et al., 1998) disruptions in GAD, parasitic neuromodulation in these limbic structures may activate anxiety circuitry and precipitate the development of human GAD. It is important to note that these pathways are shared by both PTSD and GAD and that the association between high *T. gondii* antibody level category and PTSD approached statistical significance in fully adjusted models. By definition, however, PTSD requires the occurrence of an external, traumatic event to trigger this outcome in individuals and is therefore less likely to be associated with *T. gondii* infection compared to GAD, a diagnosis that does not require an exogenous event.

Prior studies of the association between *T. gondii* and depression have been derived primarily from small case-control studies in which subsamples of individuals with depressive disorders were included secondarily as controls to the primary outcome of interest, e.g., schizophrenia or history of suicidal behavior (Arling et al., 2009; Cetinkaya et al., 2007; Hamidinejat et al., 2010; Hinz-Selch et al., 2007). In the study by Groer et al., the authors found that while *T. gondii* seropositivity was not associated with higher depressive symptoms in their cohort of pregnant women, among those seropositive for *T. gondii*, IgG titer was positively correlated with the POMS depression ($r = 0.37$, $p < 0.01$) subscale score after controlling for age and race (Groer et al., 2011). More recently, however, Pearce et al. examined the association between *T. gondii* seropositivity and history of depression as among a U.S. population-based sample of persons age 15–39 years of age using data from the National Health and Nutrition Examination Survey III (Pearce et al., 2012). Consistent with our findings among individuals drawn from a population-based sample of Detroit residents, neither *T. gondii* seropositivity nor serointensity was associated with depression.

Our study design was cross-sectional and we are therefore limited in our ability to assess causality. While a convergence of evidence suggests that *T. gondii* exposure may contribute to anxiety, it is possible that the altered behavior of individuals with GAD increases the risk of exposure to *T. gondii*. To our knowledge, however, no data exist to suggest that GAD increases exposure to undercooked meat or cat ownership, two main routes of *T. gondii* infection. In addition, it is also possible that GAD-related stressors could suppress host immunity, permit *T. gondii* reactivation, and result in elevated *T. gondii* antibody levels. However, the specificity of the observed relationship between high *T. gondii* antibody level category and GAD but not PTSD or depression argues against non-

specific immunosuppression resulting from poor mental health. Another limitation is our measurement of *T. gondii* exposure, as we were unable to assess parasite strain, route, or timing of infection. Although it is difficult to measure some of these parameters in a population-based study, future research should strive to include this information in assessment of *T. gondii* exposure in the community setting. Last, reporting of comorbid conditions were only available for 74% of our participants (360/484). Using this subset, we conducted sensitivity analyses to examine whether comorbidity was a potential confounder of the associations of interest in this study. First, we created a modified Charlson comorbidity index using data from the subset of participants who had complete data on 10 available health conditions included in the original Charlson index (Charlson et al., 1994, 1987). The modified Charlson comorbidity index was not significantly associated with either *T. gondii* serostatus or any of the mental health outcomes. Therefore, the comorbidity index did not meet the criteria for considering a confounder in our data (Rothman et al., 2012). Nonetheless, we conducted a sensitivity analysis by adding in the comorbidity index in the fully adjusted models for each of our outcomes. We observed that the odds of having GAD among seropositive individuals decreased slightly from 2.25 (95% CI, 1.11–4.53) to 2.16 (95% CI, 0.92–5.08). Among those in the highest antibody level category, the odds of having GAD increased from 3.35 (95% CI, 1.41–7.97) to 3.92 (95% CI, 1.41–10.87), suggesting that the association between high antibody levels to *T. gondii* and GAD are robust to control for comorbid conditions.

Our novel findings suggest that *T. gondii* exposure, particularly among the highest antibody level category, is associated with GAD but not PTSD or depression even after adjusting for important covariates. Given the tremendous personal and societal burden of GAD in the United States (Kessler et al., 2005), identifying a modifiable risk factor for GAD would have major public health implications. Current interventions for reducing *T. gondii* infection, such as sanitation of consumer meat, proper meat cooking, and hygienic cat feces handling, have helped to lower prevalence in the United States; yet, 1 in 10 people remain infected with *T. gondii* nationally (Jones et al., 2007). Further reducing the incidence of infection and reactivation will require an effective vaccine and safer chemotherapeutics (Jongert et al., 2009). Future research is needed to elucidate underlying biological mechanisms and to prospectively confirm and investigate the observed relationship between *T. gondii* exposure and GAD.

Conflict of interest

We have no commercial or other association that might pose a conflict of interest.

Meeting(s) where information has previously been presented

An abstract entitled, “*Toxoplasma gondii* and anxiety disorders in a population-based sample” was presented at the 47th annual Society for Epidemiological Research meeting on June 24–27, 2014 in Seattle Washington.

Acknowledgments

We gratefully acknowledge Helen Meier for coordinating the DNHS project, Caroline Cheng for statistical consultation, Fuller Torrey for manuscript review, and the many Detroit residents who chose to participate in the DNHS. This work was supported by the Stanley Medical Research Institute [AEA and RY]; and the National Institutes of Health [grant numbers R01DA022720, R01DA022720-Revision, R01DA022720-S1, and R01AG040115 to

AEA]. The role of the sponsors was to fund research only. The study sponsors played no role in each of the following: the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

References

- American Psychiatric Association, 2000. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, D.C.
- Arling, T.A., Yolken, R.H., Lapidus, M., Langenberg, P., Dickerson, F.B., Zimmerman, S.A., Balis, T., Cabassa, J.A., Scrandis, D.A., Tonelli, L.H., Postolache, T.T., 2009. *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with recurrent mood disorders. *J. Nerv. Ment. Dis.* 197, 905–908.
- Benros, M.E., Waltoft, B.L., Nordentoft, M., Ostergaard, S.D., Eaton, W.W., Krogh, J., Mortensen, P.B., 2013. Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. *JAMA Psychiatry* 70, 812–820.
- Berdoy, M., Webster, J.P., Macdonald, D.W., 1995. Parasite-altered behaviour: is the effect of *Toxoplasma gondii* on *Rattus norvegicus* specific? *Parasitology* 111 (Pt. 4), 403–409.
- Berdoy, M., Webster, J.P., Macdonald, D.W., 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. Biol. Sci.* 267, 1591–1594.
- Berenreiterova, M., Flegel, J., Kubena, A.A., Nemec, P., 2011. The distribution of *Toxoplasma gondii* cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PLoS One* 6, e28925.
- Carruthers, V.B., Suzuki, Y., 2007. Effects of *Toxoplasma gondii* infection on the brain. *Schizophr. Bull.* 33, 745–751.
- Centers for Disease Control and Prevention, 2009. Center for Disease Control and Prevention Ambulatory Care Drug Database System.
- Cetinkaya, Z., Yazar, S., Gecici, O., Namli, M.N., 2007. Anti-*Toxoplasma gondii* antibodies in patients with schizophrenia—preliminary findings in a Turkish sample. *Schizophr. Bull.* 33, 789–791.
- Charlson, M., Szatrowski, T.P., Peterson, J., Gold, J., 1994. Validation of a combined comorbidity index. *J. Clin. Epidemiol.* 47, 1245–1251.
- Charlson, M.E., Pompei, P., Ales, K.L., MacKenzie, C.R., 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J. Chronic Dis.* 40, 373–383.
- Dalman, C., Allebeck, P., Gunnell, D., Harrison, G., Kristensson, K., Lewis, G., Lofving, S., Rasmussen, F., Wicks, S., Karlsson, H., 2008. Infections in the CNS during childhood and the risk of subsequent psychotic illness: a cohort study of more than one million Swedish subjects. *Am. J. Psychiatry* 165, 59–65.
- de la Mora, M.P., Gallegos-Cari, A., Arizmendi-Garcia, Y., Marcellino, D., Fuxe, K., 2010. Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. *Prog. Neurobiol.* 90, 198–216.
- Etkin, A., Prater, K.E., Schatzberg, A.F., Menon, V., Greicius, M.D., 2009. Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Arch. Gen. Psychiatry* 66, 1361–1372.
- Fekadu, A., Shibre, T., Cleare, A.J., 2010. Toxoplasmosis as a cause for behaviour disorders—overview of evidence and mechanisms. *Folia Parasitol. (Praha)* 57, 105–113.
- Groer, M.W., Yolken, R.H., Xiao, J.C., Beckstead, J.W., Fuchs, D., Mohapatra, S.S., Seyfang, A., Postolache, T.T., 2011. Prenatal depression and anxiety in *Toxoplasma gondii*-positive women. *Am. J. Obstet. Gynecol.* 204 (433), e431–437.
- Hamer, D., 2002. Genetics. Rethinking behavior genetics. *Science* 298, 71–72.
- Hamidnejat, H., Ghorbanpoor, M., Hosseini, H., Alavi, S.M., Nabavi, L., Jalali, M.H., Borojeni, M.P., Jafari, H., Mohammadaligol, S., 2010. *Toxoplasma gondii* infection in first-episode and inpatient individuals with schizophrenia. *Int. J. Infect. Dis.* 14, e978–981.
- Haroon, F., Handel, U., Angenstein, F., Goldschmidt, J., Kreutzmann, P., Lison, H., Fischer, K.D., Scheich, H., Wetzel, W., Schluter, D., Budinger, E., 2012. *Toxoplasma gondii* actively inhibits neuronal function in chronically infected mice. *PLoS One* 7, e35516.
- Hettema, J.M., Neale, M.C., Kendler, K.S., 2001. A review and meta-analysis of the genetic epidemiology of anxiety disorders. *Am. J. Psychiatry* 158, 1568–1578.
- Hinze-Selch, D., Daubener, W., Eggert, L., Erdag, S., Stoltenberg, R., Wilms, S., 2007. A controlled prospective study of *Toxoplasma gondii* infection in individuals with schizophrenia: beyond seroprevalence. *Schizophr. Bull.* 33, 782–788.
- House, P.K., Vyas, A., Sapolsky, R., 2011. Predator cat odors activate sexual arousal pathways in brains of *Toxoplasma gondii* infected rats. *PLoS One* 6, e23277.
- Jones, J.L., Kruszon-Moran, D., Sanders-Lewis, K., Wilson, M., 2007. *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. *Am. J. Trop. Med. Hyg.* 77, 405–410.
- Jongert, E., Roberts, C.W., Gargano, N., Forster-Waldl, E., Petersen, E., 2009. Vaccines against *Toxoplasma gondii*: challenges and opportunities. *Mem. Inst. Oswaldo Cruz* 104, 252–266.
- Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry* 62, 593–602.

- Koenen, K.C., Amstadter, A.B., Ruggiero, K.J., Acierno, R., Galea, S., Kilpatrick, D.G., Gelernter, J., 2009. RGS2 and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. *Depress. Anxiety* 26, 309–315.
- Kroenke, K., Spitzer, R.L., Williams, J.B., 2001. The PHQ-9: validity of a brief depression severity measure. *J. Gen. Intern. Med.* 16, 606–613.
- Lafferty, K.D., 1999. The evolution of trophic transmission. *Parasitol. Today* 15, 111–115.
- Miman, O., Mutlu, E.A., Ozcan, O., Atambay, M., Karlidag, R., Unal, S., 2010. Is there any role of *Toxoplasma gondii* in the etiology of obsessive–compulsive disorder? *Psychiatry Res.* 177, 263–265.
- Mitra, R., Sapolsky, R.M., Vyas, A., 2013. *Toxoplasma gondii* infection induces dendritic retraction in basolateral amygdala accompanied by reduced corticosterone secretion. *Dis. Models & Mechanisms* 6, 516–520.
- Montoya, J.G., Liesenfeld, O., 2004. Toxoplasmosis. *Lancet* 363, 1965–1976.
- Pearce, B.D., Kruszon-Moran, D., Jones, J.L., 2012. The relationship between *Toxoplasma gondii* infection and mood disorders in the third National Health and Nutrition Survey. *Biol. Psychiatry* 72, 290–295.
- Prandovszky, E., Gaskell, E., Martin, H., Dubey, J.P., Webster, J.P., McConkey, G.A., 2011. The neurotropic parasite *Toxoplasma gondii* increases dopamine metabolism. *PLoS One* 6, e23866.
- Rothman, K.J., Greenland, S., Lash, T.L., 2012. *Modern Epidemiology*, third ed. Lippincott Williams & Wilkins, Philadelphia, PA.
- Rowe, D.C., Stever, C., Gard, J.M., Cleveland, H.H., Sanders, M.L., Abramowitz, A., Kozol, S.T., Mohr, J.H., Sherman, S.L., Waldman, I.D., 1998. The relation of the dopamine transporter gene (DAT1) to symptoms of internalizing disorders in children. *Behav. Genet.* 28, 215–225.
- SAS, 2008. SAS Statistical Software, Version 9.2. SAS Institute, Inc, Cary, NC.
- Spitzer, R.L., Kroenke, K., Williams, J.B., Lowe, B., 2006. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch. Intern. Med.* 166, 1092–1097.
- Sullivan, P.F., Neale, M.C., Kendler, K.S., 2000. Genetic epidemiology of major depression: review and meta-analysis. *Am. J. Psychiatry* 157, 1552–1562.
- Torrey, E.F., Bartko, J.J., Yolken, R.H., 2012. *Toxoplasma gondii* and other risk factors for schizophrenia: an update. *Schizophr. Bull.* 38, 642–647.
- Uddin, M., Aiello, A.E., Wildman, D.E., Koenen, K.C., Pawelec, G., de Los Santos, R., Goldmann, E., Galea, S., 2010. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9470–9475.
- Vyas, A., Kim, S.K., Giacomini, N., Boothroyd, J.C., Sapolsky, R.M., 2007. Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6442–6447.
- Wang, H., Yolken, R.H., Hoekstra, P.J., Burger, H., Klein, H.C., 2011. Antibodies to infectious agents and the positive symptom dimension of subclinical psychosis: the TRAILS study. *Schizophr. Res.* 129, 47–51.
- Weathers, F.F.J., 1996. Psychometric review of PTSD checklist (PCL-C, PCL-S, PCL-M, PCL-R). In: Stamm, B.H. (Ed.), *Measurement of Stress, Trauma, and Adaptation*. Sidran Press, Lutherville, MD.
- Webster, J.P., 1994. The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology* 109 (Pt. 5), 583–589.
- Webster, J.P., 2007. The effect of *Toxoplasma gondii* on animal behavior: playing cat and mouse. *Schizophr. Bull.* 33, 752–756.
- Webster, J.P., Brunton, C.F., MacDonald, D.W., 1994. Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* 109 (Pt. 1), 37–43.
- Webster, J.P., Lamberton, P.H., Donnelly, C.A., Torrey, E.F., 2006. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour. *Proc. Biol. Sci.* 273, 1023–1030.
- Xian, H., Chantarujikapong, S.I., Scherrer, J.F., Eisen, S.A., Lyons, M.J., Goldberg, J., Tsuang, M., True, W.R., 2000. Genetic and environmental influences on posttraumatic stress disorder, alcohol and drug dependence in twin pairs. *Drug. Alcohol Depend.* 61, 95–102.
- Yolken, R.H., Torrey, E.F., Lieberman, J.A., Yang, S., Dickerson, F.B., 2011. Serological evidence of exposure to Herpes Simplex Virus type 1 is associated with cognitive deficits in the CATIE schizophrenia sample. *Schizophr. Res.* 128, 61–65.