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Positive Association between Toxoplasma Gondii IgG Serointensity and Current Dysphoria/hopelessness Scores in the Old Order Amish: a Preliminary Study

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Positive association between *Toxoplasma gondii* IgG serointensity and current dysphoria/hopelessness scores in the Old Order Amish: a preliminary study

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Abstract: *Toxoplasma gondii* (*T. gondii*) IgG seropositivity and serointensity have been previously associated with suicidal self-directed violence (SSDV). Although associations with unipolar depression have also been investigated, the results have been inconsistent, possibly as a consequence of high heterogeneity. We have now studied this association in a more homogeneous population, [that is (i.e.) Old Order Amish (OOA)] with previously reported high *T. gondii* seroprevalence. In 306 OOA with a mean age of 46.1±16.7 years, including 191 (62.4%) women in the Amish Wellness Study, we obtained both *T. gondii* IgG titers (by enzyme-linked immunosorbent assay [ELISA]), and depression screening questionnaires (Patient Health Questionnaire [PHQ-9] [n=280] and PHQ-2 [n=26]). Associations between *T. gondii* IgG and dysphoria/hopelessness and anhedonia scores on depression screening questionnaires were analyzed using multivariable linear methods with adjustment for age and sex. Serointensity was associated with both current dysphoria/hopelessness (p=0.045) and current combined anhedonia and dysphoria/hopelessness (p=0.043), while associations with simple anhedonia and past/lifelong (rather than current) phenotypes were not significant. These results indicate the need for larger longitudinal studies to corroborate the association between dysphoria/hopelessness and *T. gondii* IgG-titers. Current hopelessness is a known risk factor for SSDV which responds particularly well to cognitive behavioral therapy, and may be a focused treatment target for *T. gondii*-positive individuals at high-risk for SSDV.

Keywords: anhedonia; hopelessness; Old Order Amish; *Toxoplasma gondii*.

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Introduction

Toxoplasma gondii (*T. gondii*) is a protozoan parasite that can infect most warm-blooded animals, including humans. More than 30% of the world's population carries *T. gondii* [1], with infection rates as high as 77% to as low as about 0.8% [2]. *Toxoplasma gondii* seroprevalence differs significantly between countries, diverse topographical regions within the same country and individuals of different ethnicities that live in similar regions [2–4]. Approximately, 11% of the United States population aged 6 years or older are infected with *T. gondii* [5].

Homoeothermic mammals, such as cattle and humans, serve as intermediate hosts for *T. gondii*. Its definitive hosts are members of the Felidae family, which includes household cats [3]. *Toxoplasma gondii* has three infectious stages (tachyzoites, sporozoites and bradyzoites). The sporozoites have the capability of infecting the intermediate hosts, but not the definitive hosts while the tachyzoites and bradyzoites have the capability to infect both the intermediate and definitive hosts [3, 6]. Tissue cysts contain bradyzoites and are predominantly found in skeletal and cardiac muscles, and neuronal tissues, e.g. brain and eye [7]. Cysts may also be located within other organs, e.g. kidneys, lungs and liver [7]. In immunosuppressed hosts (e.g. individuals with human immunodeficiency virus [HIV]/acquired immunodeficiency syndrome [AIDS]), the bradyzoites may convert into tachyzoites that penetrate other local and distant cells [8].

Toxoplasma gondii can be transmitted from mother to fetus during acute infection of a seronegative mother [9] or through reactivation of *T. gondii* infection during pregnancy [10]. The most common mode of infection in humans is through ingestion of contaminated food or water (e.g. ingestion of tissue cysts in raw or undercooked meat from intermediate hosts such as pigs, goats, cattle or oocyst in contaminated water). Rare, but medically relevant, ways of transmission include transplantation of an organ or blood transfusion from *T. gondii*-infected individuals [3, 11]. Acute infection with *T. gondii* typically produces a flu-like response that is often self-limiting in the immunocompetent host [6, 12], but the outcome of it is not a cure, rather a chronic latent infection develops with a potential for reactivation [6].

Multiple reports suggest links between chronic *T. gondii* infection and mental illnesses, including schizophrenia [13–21] and bipolar disorder [14, 22–25]. Chronic *T. gondii* infection has also been associated with depression in psychiatric patients [26], women Veterans [27] and pregnant women [28]. However, several other studies found no association between *T. gondii* infection and depression [23, 29–32]. One potential source of this discrepancy may be the heterogeneity of samples studied. Some of the heterogeneous risk factors in the study participants could be differences in lifestyles, co-occurring substance use disorders, discrete symptoms of depression being differentially associated with *T. gondii* infection, varying genetic vulnerability of the subjects to depression linked with *T. gondii* infection, infection with distinct strains of *T. gondii* or involvement of different mechanisms of infection with *T. gondii* (e.g. oocyst vs. tissue cyst), with all factors potentially having variable associations with depression.

The current study aimed to minimize the effects of possible confounders by investigating possible associations between *T. gondii* infection and two dominant symptoms of depression (i.e. dysphoria/hopelessness and anhedonia) in a sample of Old Order Amish (OOA), a population that is relatively homogeneous with similar lifestyles, socioeconomic status and cultural practices, has a low prevalence of substance use and a relatively high prevalence of *T. gondii* infection [33, 34]. We hypothesized that serological markers of *T. gondii* infection are positively associated with symptoms of depression, specifically, dysphoria/hopelessness and anhedonia. To our knowledge, this is the first time that relationships between specific symptoms of depression and *T. gondii* seropositivity and serointensity have been investigated.

Materials and methods

The protocol was approved by the University of Maryland Baltimore Institutional Review Board. Individuals were participants who had been previously enrolled in the Amish Wellness Study conducted by the University of Maryland School of Medicine. The Wellness Study is a community-based program that includes an assessment of cardiometabolic health (e.g. lipids, blood pressure, glycemia) as well as a broad panel of lifestyle factors collected from medical and family history questionnaires, and mood and sleep questionnaires. The Wellness Study recruitment process involves the participants aged 18 years and older being approached in their home by one of the registered nurses from the University of Maryland Amish Research Clinic and an Amish liaison. If the participant was interested, the nurse explained the study and obtained informed consent. The participants completed a panel of questionnaires at the home visit during which they were enrolled, and then received a clinic visit at either the Amish Research Clinic or in the Amish Wellness Mobile Clinic, where they underwent a clinical protocol that included a fasting blood draw. *Toxoplasma gondii* antibodies were subsequently measured from this sample.

Depression symptoms were obtained from the study participants using a mental health screening questionnaire. First, we used a questionnaire based on the Patient Health Questionnaire-9 (PHQ-9) [35], called the Amish Mood Questionnaire (n = 280), which was later replaced by a questionnaire based on the Patient Health Questionnaire-2 (PHQ-2) [36], called the Mental Health Depression

Questionnaire (n = 26). Each questionnaire asked: "during the last month have you had little feelings of interest or pleasure?", which represented anhedonia, and "during the last month have you often been bothered by feeling down, depressed, or hopeless?", which represented dysphoria/hopelessness. If the participants answered yes to both of these questions then they were categorized as currently experiencing both anhedonia and dysphoria/hopelessness. The questionnaires also asked if the participant had experienced either of these feelings in the past. Studies have reported that PHQ-9 has a sensitivity ranging from 80% to 81.3% and specificity ranging from 85.3% to 92% [37, 38] and the PHQ-2 sensitivity ranges from 83% to 89.3% and its specificity ranges from 75.9% to 92% [36, 38].

Participants' blood samples were tested for the presence of *T. gondii* IgG antibodies using an enzyme-linked immunosorbent assay (ELISA) (IBL International, Männedorf, Switzerland), which tests for the levels of immunoglobulin G (IgG) to whole tachyzoites. Standards for validation were employed for all assays. A preset cut-off value as stated by the kit manufacturer was used to define the status of the samples. Seropositivity was determined based on the IgG titer concentration (serointensity) of the antibodies. Participants with concentrations greater than or equal to 12 IU/mL were deemed seropositive. When the ELISA results indicated an equivocal concentration of *T. gondii* antibody (8–12 IU/mL), we repeated the ELISA. When the second ELISA remained in the equivocal range or showed a level less than 8 IU/mL, the result was considered negative. If the second ELISA showed a concentration in the positive range (>12 IU/mL), the result was considered positive. We did not measure IgM antibodies in these samples.

We included participants with both available *T. gondii* plasma titers and symptoms of depression (n = 306, 62.4% women; ages 18–87 years with a mean age of 46.1 ± 16.7 years). The demographic characteristics of the study population have been summarized in Table 1.

Logistic regression models were applied using depressive symptoms as dependent binary variables, and seropositivity or log-transformed serointensity as independent variables, adjusting for the covariates age and sex. The criterion two-tailed α was set at 0.05. No Bonferroni corrections for multiple comparisons were applied, considering the limited statistical power, given the need for adjustment for age (strong confounder based on cumulative effects) and sex (different exposure mechanisms to *T. gondii* and different vulnerability to depression). Statistics were completed using SAS/STAT 14.2 (Cary, NC, USA).

Results

All results presented are with adjustment for age and sex.

Serointensity (titers)

We found significant associations between serointensity and current dysphoria/hopelessness: p = 0.045, odds ratio (OR) = 1.26, 95% confidence interval (CI): [1.01, 1.58], and current combined anhedonia and dysphoria/hopelessness: p = 0.043, OR = 1.34, 95% CI: [1.01, 1.79].

There were no significant associations between serointensity and current anhedonia: p = 0.170, OR = 1.19, 95% CI: [0.93, 1.54], current anhedonia or dysphoria/hopelessness: p = 0.147, OR = 1.17, 95% CI: [0.95, 1.45], past anhedonia or dysphoria/hopelessness: p = 0.872, OR = 1.01, 95% CI: [0.86, 1.20], and ever-experienced anhedonia or dysphoria/hopelessness: p = 0.428, OR = 1.07, 95% CI: [0.91, 1.25].

Seropositivity

A low-grade significance/statistical trend was found for the association between seropositivity and current dysphoria/hopelessness: p = 0.058, OR = 2.31, 95% CI: [0.97, 5.50]. We also found a statistical trend for the association between seropositivity and current combined anhedonia and dysphoria/hopelessness: p = 0.056, OR = 2.99, 95% CI: [0.97, 9.15].

There were no significant associations between seropositivity and current anhedonia: p = 0.181, OR = 1.91, 95% CI: [0.74, 4.93], current anhedonia or dysphoria/hopelessness: p = 0.164, OR = 1.75, 95% CI: [0.80, 3.86], ever-experienced anhedonia or dysphoria/hopelessness: p = 0.136, OR = 1.56, 95% CI: [0.87, 2.79] and past anhedonia

Table 1: Demographic characteristics of the sample.

Variables	Total sample (n = 306)	Seropositive for *T. gondii* IgG (n, % = 141, 46%)	Seronegative for *T. gondii* IgG (n, % = 165, 54%)
Age, mean ± SD, years	46.1 ± 16.7	50.7 ± 16.2	42.2 ± 16.1
Gender, males, n (%)	115 (37.6%)	60 (42.6%)	55 (33.3%)
Current dysphoria/hopelessness, present, n (%)	26 (9.2%)	16 (12.7%)	10 (6.3%)
Current anhedonia, present, n (%)	20 (7%)	12 (9.6%)	8 (5%)
Current anhedonia and dysphoria/hopelessness, present, n (%)	16 (6%)	11 (9.3%)	5 (3.4%)
Current anhedonia or dysphoria/hopelessness, present, n (%)	30 (10.4%)	17 (13.3%)	13 (8.1%)
Ever anhedonia or dysphoria/hopelessness, present, n (%)	62 (21.5%)	33 (25.4%)	29 (18.2%)
Past anhedonia or dysphoria/hopelessness, present, n (%)	52 (17.5%)	27 (19.8%)	25 (15.5%)
Toxoplasma IgG antibody titers, mean ± SD, IU/mL	47.8 ± 104.3	99.8 ± 136.6	3.4 ± 2.8
Log *Toxoplasma* IgG antibody titers, mean ± SD	2.4 ± 1.9	4.1 ± 1	0.9 ± 0.9

Table 2: Associations between *T. gondii* IgG (seropositivity and serointensity) and cardinal symptoms of depression (anhedonia and dysphoria/hopelessness).

Variables	Odds ratio estimates adjusted for age and sex	95% CI adjusted for age and sex	p-Values adjusted for age and sex
Current dysphoria/hopelessness ~ seropositivity	2.31	[0.97, 5.50]	0.058[a]
Current dysphoria/hopelessness ~ log_serointensity	1.26	[1.01, 1.58]	0.045[b]
Current anhedonia ~ seropositivity	1.91	[0.74, 4.93]	0.181
Current anhedonia ~ log_serointensity	1.19	[0.93, 1.54]	0.170
Current anhedonia and dysphoria/hopelessness ~ seropositivity	2.99	[0.97, 9.15]	0.056[a]
Current anhedonia and dysphoria/hopelessness ~ log_serointensity	1.34	[1.01, 1.79]	0.043[b]
Current anhedonia or dysphoria/hopelessness ~ seropositivity	1.75	[0.80, 3.86]	0.164
Current anhedonia or dysphoria/hopelessness ~ log_serointensity	1.17	[0.95, 1.45]	0.147
Past anhedonia or dysphoria/hopelessness ~ seropositivity	1.39	[0.74, 2.59]	0.303
Past anhedonia or dysphoria/hopelessness ~ log_serointensity	1.01	[0.86, 1.20]	0.872
Ever anhedonia or dysphoria/hopelessness ~ seropositivity	1.56	[0.87, 2.79]	0.136
Ever anhedonia or dysphoria/hopelessness ~ log_serointensity	1.07	[0.91, 1.25]	0.428

p-Values, odds ratio estimates and confidence intervals, generated by multi-variable logistic regression models with the presence of symptoms of depression as dependent variables and *T. gondii* seropositivity and serointensity as independent variables, accounting for sex and age. [a]Statistical trend ($p < 0.10$). [b]Significant ($p < 0.05$). ~, related to.

or dysphoria/hopelessness: $p = 0.303$, OR = 1.39, 95% CI: [0.74, 2.59]. These results are summarized in Table 2.

Discussion

We found that serointensity for *T. gondii* was significantly associated with current dysphoria/hopelessness, and with current combined anhedonia and dysphoria/hopelessness, while associations with anhedonia scores alone, and lifelong symptoms, were not significant. There was also a marginal statistical trend for associations between *T. gondii* seropositivity and a combination of current dysphoria/hopelessness and anhedonia.

The results of our study point toward an association between certain cardinal depressive symptoms and anti-*T. gondii* IgG antibodies, which is consistent with the results of some previous studies in psychiatric patients [26], women Veterans [27] and women evaluated for prenatal depression [28].

Psychotropic medications have various degrees of anti-*T. gondii* activity *in vitro* [39]. This may be potentially clinically relevant considering a recent study that found that *T. gondii* seropositive patients with bipolar disorder, who were treated with medications with high *in vitro* anti-*T. gondii* activity, had fewer episodes of depression than those who were treated with medications with lower *in-vitro* anti-*T. gondii* activity [40]. Another clinically relevant report describes a *T. gondii*-seropositive patient with refractory symptoms of depression, who improved with antidepressant treatment only after being treated for toxoplasmosis [41].

However, our results are inconsistent with other studies that found no associations between *T. gondii* infection and symptoms of depression [14, 23, 29–32, 42–46]. Many of these studies had limitations in terms of not adjusting for confounders such as occupation, heritability, living environment, cultural practices, socioeconomic status and substance use disorders. The current study has likely minimized these confounders by evaluating the OOA, which is a relatively homogeneous population.

Toxoplasma gondii has also been previously associated with suicidal behavior, an important cause of death in patients with depression. Specifically, several coauthors of the current study first reported an association between *T. gondii* IgG antibody titers and a history of suicide attempts in individuals with recurrent mood disorders [47]. In addition, the seropositivity rate for anti-*T. gondii* IgG antibodies was significantly higher in psychiatric inpatients with a history of attempted suicide [48]. We have further confirmed this association between suicide attempts and *T. gondii* infection in patients with schizophrenia in Germany, in recent suicide attempters admitted to a psychiatry ward in Sweden, and in a large cohort of Danish mothers [49–51]. Recently, a study in patients with mental illness identified *T. gondii* IgM titers, rather than *T. gondii* IgG titers, being associated with suicide attempts [52]. However, the same group presented data at a recent professional meeting confirming *T. gondii* IgG titers as being predictively associated with death by suicide (unpublished observation).

Moreover, identification of specific clinical, endophenotypic or biomarker-based subgroups, may lead to individualized suicide prevention efforts. For instance, *T. gondii*-seropositive patients with increased hopelessness, a more robust predictor of suicide than syndromal depression [53], may differ in prognosis and therapeutic response relative to other patients.

Other unmeasured clinical traits may be important as well in the interface between *T. gondii* and suicidal behavior. For instance, impulsivity and aggression have been recognized as endophenotypes for suicidal behavior, and their presence has been associated with an increased risk of suicide [54]. Research suggests that *T. gondii* infection can lead to impaired regulation of impulse control, which includes risk-taking behavior and violence [55]. Relationships between chronic infection with *T. gondii* and greater impulsivity in younger psychiatrically healthy males, as well as greater trait reactive aggression among females, have been reported [56]. Coccaro et al. [57] reported an association between greater impulsivity and aggression scores with *T. gondii* seropositivity in patients with intermittent explosive disorder.

We are not aware of other studies that have been conducted using principal component analysis on various symptoms of depression in the context of associations with *T. gondii* infection. For practical reasons, we only chose to examine two symptoms of depression (dysphoria/hopelessness and anhedonia) in our study. The heavy schedule of staff and participant involvement in the Amish Wellness Study did not allow for more extensive interviewing, and the PHQ-9-based evaluation had to be changed to the shorter PHQ-2-based instrument (retaining only the anhedonia and dysphoria/hopelessness questions). Nevertheless, previous reports showed that asking even a solitary question related to dysphoria/hopelessness has a sensitivity for major depression of about 85–90%, which increases to 95% upon addition of another question related to anhedonia [36]. Most importantly, both dysphoria/hopelessness and anhedonia have been recognized as risk factors for suicide in depressed patients [58]. In addition, symptoms of dysphoria/hopelessness as well as anhedonia have been well modeled in animal studies [54], allowing application of research domain criteria principles to suicide research.

The top candidate mechanism underlying the association between depression and *T. gondii* includes perturbation of the serotonergic system in the brain of depressed individuals [59]. The biosynthesis of serotonin begins with the metabolism of tryptophan, which is the precursor amino acid for this neurotransmitter [60]. Tryptophan is metabolized through two pathways, which involve production of either serotonin or kynurenine. The rate-limiting enzymes involved in the metabolism of tryptophan toward kynurenine metabolites are indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) [61]. *Toxoplasma gondii* infection in hosts with intact immunity results in the release of interferon-γ (IFN-γ), which further leads to upregulation of IDO. Conversion of tryptophan into kynurenine by IDO ultimately hinders *T. gondii* replication by depleting tryptophan that is necessary for the development of *T. gondii* [62]. However, Engin et al. [63] reported that during infection with *T. gondii*, breakdown of tryptophan may be induced via other unknown pathways as well, which may involve interactions between TDO, IDO and nitric oxide (NO), with NO possibly inhibiting complete exhaustion of tryptophan in acute infection with *T. gondii*. Studies, including ours, in participants with depressive disorders, have previously linked increased plasma levels of kynurenine [64] and its metabolite quinolinic acid [65, 66] to a history of suicide attempts [67].

How could kynurenine production lead to symptoms of depression and suicidal behavior? One possibility is that kynurenine can act as an immunosuppressant [68–71] and thus, might promote reactivation of *T. gondii* intermittently. We previously reported that in patients with schizophrenia, only those who had both high kynurenine levels along with *T. gondii* seropositivity were at a higher risk of having a history of suicide attempts [72]. This brings up the possibility that higher kynurenine levels actuate the increased risk for suicidal self-directed violence (SSDV) by reactivation of latent *T. gondii* [72]. Another hypothesis is the excitotoxic N-methyl-D-aspartate (NMDA) receptor-mediated effects of quinolinic acid, a metabolite of kynurenine produced in the glial cells within the brain [61]. Both conceptual arguments and data support the hypothesis that depression may be induced via stimulation of NMDA receptors [73–77]. Dysfunction in NMDA glutamate receptors may also be implicated in the pathophysiology of suicide [75] as interventional studies, using intravenous ketamine (an NMDA antagonist) in patients with major depression, have demonstrated a rapid improvement in depression scores of these patients [78, 79] and a reduction in their suicidal ideation [80].

In contrast to dysphoria/hopelessness, we did not find a significant association between anhedonia scores and *T. gondii* serointensity. Anhedonia is defined as the inability to feel pleasure, which may be precipitated by a relative dopamine deficiency in the brain [81]. We hypothesize that the *T. gondii*-innate enzymatic capability to produce dopamine [82] may lead to a selective protection from anhedonia in *T. gondii*-linked depression, a hypothesis to be tested in future studies.

There are certain limitations to our study. Due to its cross-sectional nature, we cannot establish causality. Reverse causality has not been ruled out – it is possible that a higher *T. gondii* seropositivity in OOA with depressive symptoms (current dysphoria/hopelessness) could be the consequence, rather than the cause of depression. A possible explanation for depression causing increased exposure to *T. gondii* and higher *T. gondii* seropositivity includes less than optimal self-care (such as hand washing, vegetable cleaning and preparation of meat products) in depressed individuals.

Also, as our sample size was somewhat limited, we cannot rule out the possibility of a type II error with regard to the negative findings such as, the absence of associations between *T. gondii* and anhedonia (in contrast to dysphoria/hopelessness), and past symptoms (in contrast to present complaints). We did not use collateral information or standardized structured diagnostic instruments. Our PHQ-9 and PHQ-2 questionnaires were modified after feedback from members of the Amish community, and thus, the scores and thresholds on those questionnaires cannot be directly used to estimate diagnosis in the absence of thorough validation. Under any circumstances, meta-analyses have reported that both PHQ-9 and PHQ-2 scales cannot be used to clinically diagnose depression but they are useful for screening and referral for further clinical evaluation [37, 38].

There is a possibility that the participants might under-acknowledge and underreport certain symptoms of depression depending on how recently the episodes of low mood occurred, thus leading to recall or reporting bias. While there are a number of strengths in recruiting OOA, the Amish believe in limiting the expression of personal emotions. They value stoicism and expressing gratefulness rather than complaining, respond to hardships of daily life by yielding to divine providence and accepting it as God's will (*Gelassenheit*). They often attribute the resolution of past symptoms to divine intervention [33] and once past symptoms resolve, they may be particularly prone to underreport them. Therefore, finding significant associations between *T. gondii* and "current, but not past", hopelessness may be a result of a culturally based bias, and might not be generalizable to the non-OOA.

Nevertheless, we believe that the strengths of the study outweigh its limitations. Specifically, our study sample consisted of OOA, known to have a very low prevalence of substance use (which could be a major confounder in studies of depression), and a great deal of homogeneity in terms of food consumption, meal preparation, hygienic practices, social supports and occupation [33]. Recall and reporting biases notwithstanding, the Amish respond to questionnaires with well-known honesty and thoroughness that characterize the OOA culture [33], thereby increasing the reliability of the responses. Our past pencil-and-paper surveys of the Amish suggest that the OOA participants take the time to understand the questions being asked and to answer them with thoughtfulness and consistency [83]. Of major importance, the higher rate of seropositivity for *T. gondii* allows performing multivariable analyses with smaller sample sizes than in the general US population.

The findings from our study have a number of implications. Certain targeted treatment approaches might be able to benefit individual patients having both markers of chronic *T. gondii* infection and dysphoria/hopelessness, with/without anhedonia. For instance, cognitive behavioral therapy (CBT) has shown promise in reducing dysphoria/hopelessness in depressed patients [84, 85]. Brief supportive-expressive psychodynamic psychotherapy has also been shown to lower the serum levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin 6 (IL-6) [86], that might play a mediating role after *T. gondii* reactivation in chronically infected individuals, and have been implicated in the metabolism of neurotransmitters, activation of neuroendocrine pathways, altered neuroplasticity [87] and behavioral manifestations seen in depressed individuals [88]. In *T. gondii*-seropositive patients with elevated markers of inflammation, clinical trials on anti-inflammatory drugs such as celecoxib [89], infliximab [90], or other cytokine inhibitors and anti-cytokine monoclonal antibodies [91], may lead to novel interventions for refractory or persistently suicidal depression. It is likely that treatment with anti-inflammatory agents may not be helpful for all cases of depression as inflammation is likely involved in only a sub-group of depressed patients that might be easily identified using molecular markers of inflammation [92, 93]. Anti-parasitic interventions aimed at preventing reactivation of *T. gondii* infection may also have a role in refractory and suicidal depression in *T. gondii*-seropositive patients with intermittent recurrent elevations in titers, suggesting reactivation. Furthermore, primary strategies to prevent *T. gondii* infection such as education about food preparation, food storage, avoiding eating raw or undercooked meat and hand hygiene prior to food preparation or meals could prove useful in lowering the morbidity, economic and emotional burden of depression, and possibly, its premature mortality through suicide.

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