ONLINE FIRST

Toxoplasma gondii Infection and Self-directed Violence in Mothers

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Context: Two studies based on clinical samples have found an association between *Toxoplasma gondii* infection and history of suicide attempt. To our knowledge, these findings have never been replicated in a prospective cohort study.

Objective: To examine whether *T gondii*–infected mothers have an increased risk of self-directed violence, violent suicide attempts, and suicide and whether the risk depends on the level of *T gondii* IgG antibodies.

Design: Register-based prospective cohort study. Women were followed up from the date of delivery, 1992 to 1995 until 2006.

Setting: Denmark.

Participants: A cohort of 45 788 women born in Denmark whose level of *Toxoplasma*-specific IgG antibodies was measured in connection with child birth between 1992 and 1995.

Main Outcome Measures: Incidence rates of self-directed violence, violent suicide attempts, and suicide in relation to *T gondii* seropositivity and serointensity.

Results: *T gondii*–infected mothers had a relative risk of self-directed violence of 1.53 (95% CI, 1.27-1.85) compared with noninfected mothers, and the risk seemed to increase with increasing IgG antibody level. For violent suicide attempts, the relative risk was 1.81 (95% CI, 1.13-2.84) and for suicide, 2.05 (95% CI, 0.78-5.20). A similar association was found for repetition of self-directed violence, with a relative risk of 1.54 (95% CI, 0.98-2.39).

Conclusion: Women with a *T gondii* infection have an increased risk of self-directed violence.

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SUICIDE IS A TRAGIC MULTIFACTORIAL outcome of mental illness, with complex biopsychosocial underpinning, leading to premature death of about 1 million individuals around the world each year, with an average annual mortality of 14.5 per 100 000 people, in effect 1 death every 40 seconds and 1.5% of all death.[1,2] For every suicide death, 10 to 20 persons are estimated to attempt suicide, with approximately 10 million people attempting suicide worldwide.[3]

Toxoplasma gondii is a widespread neurotropic protozoan parasite affecting approximately one-third of people worldwide, with symptoms ranging from none to minimal in the most common cases, to severe in rare cases, depending on the adequacy of the immune antiparasitic response. A relatively rare but devastating congenital infection occurs if the mother has a primary infection during pregnancy and passes the infection to the fetus. Felids have been identified as definitive hosts of *T gondii*, with the parasite multiplying sexually in the cats' gut and spreading oocysts. Humans are infected by ingestion of oocysts spread from feces of infected cats (eg, contaminated sandbox and ingestion of unwashed vegetables), eating undercooked meat infested with *T gondii* cysts, using knives used to cut infested meat to further cut vegetables, and, occasionally, drinking water from a contaminated water source. The ingestion of the parasite by various intermediate hosts, including humans, leads to the spread of the organism from the intestine to organs, predominantly muscles and the brain. There, under pressure from the immune system, the parasite hides within neurons and glial cells, in cystic structures, with minimal exposure to mediators of the immune system that succeed to contain but cannot eradicate it. Struc-

turally, previous reports on rodents have demonstrated that *T gondii* localizes in multiple brain structures, including the amygdala and the prefrontal cortex,[4] areas that take a leading role in emotional and behavioral regulation. *T gondii* has been implicated in behavioral, affective, and cognitive abnormalities in humans[5] and has been found consistently associated with schizophrenia.[6] In the current study cohort, we previously found that women with a high level of IgG antibodies had a significantly elevated risk of developing schizophrenia spectrum disorders.[7] Because these areas, occupied preferentially by *T gondii*, are among areas that show prominent histopathological changes in suicide victims,[3] it is plausible that *T gondii* infection disrupts affective and behavioral modulation and thus elevates risk of suicide.

A relationship between *T gondii* infection and suicidal self-directed violence was first reported by the group led by one of us (T.T.P.), who found greater titers of *T gondii* IgG antibodies in patients with mood disorders who attempted suicide as compared with both patients with mood disorders who did not attempt suicide and healthy controls.[8] A recent study of the same group, this time in a larger sample of schizophrenic patients, confirmed a significant relationship between *T gondii* infection and suicidal self-directed violence in younger patients (age groups with a particularly increased relative risk for suicide in schizophrenia), independent of symptom severity or antipsychotic dosage.[9] A limitation of these studies was that suicide attempts preceded testing for *T gondii* antibodies. We now investigated the prediction of subsequent self-directed violence and repeated self-directed violence by *T gondii* IgG antibody level at the time of delivery in women included in a population-based Danish study of neonatal screening for *T gondii* infection.[10] We also analyzed violent suicide attempts and suicides as separate outcomes.

METHODS

STUDY POPULATION

As was the case for our previous study of *T gondii* infection and schizophrenia,[7] the individuals included in the study population were originally recruited for a study of neonatal screening for *T gondii*.[10] Pregnant women living in 5 counties in Denmark from 1992 to 1995 were offered to have their child screened for *T gondii* shortly after birth. The 5 counties (the capital of Denmark including suburbs, the northeastern part of Zealand, and the southern part of Jutland) represented one-third of Danish deliveries during that period. Only 0.18% of the mothers refused to take part in the original study, and 9.26% of the mothers had no serum sample from the first trimester and were excluded from the study.

Our study population contains mothers born in Denmark who gave birth between May 15, 1992, and January 15, 1995, and whose child was screened for *T gondii* (N = 45 788). For mothers giving birth more than once during the study period, only the first delivery was included in the study.

All people living in Denmark from 1968 onward are registered in the Danish Civil Registration System.[11] Among many other variables, it includes information on personal identification number, sex, date and place of birth, continuously updated information on vital status, and personal identification number of parents. The personal identification number is used in all national registers, enabling accurate linkage between registers.

MEASURING OF *T GONDII* IgG ANTIBODY LEVELS

Performed in the context of the original study,[10] IgG levels in blood from the newborn were measured. In brief, a heel stick blood sample from the child was taken 5 to 10 days after birth and stored on filter paper for testing for phenylketonuria and other metabolic abnormalities. For children of mothers included in the original study, two 3.2-mm discs from the phenylketonuria card were analyzed by enzyme immunoassay for *T gondii* IgG antibodies.[12] The level of antibodies was expressed as a percentage of the optical density obtained for the World Health Organization international standard serum, and the mean of the 2 results measured the IgG level. In the present study, mothers of children with an IgG level more than 24 were regarded as *T gondii* positive from the time of delivery. The IgG antibodies measured in the blood from the child were maternal in origin, because IgG passes through the placenta, and infected newborn children will not begin producing *T gondii*–specific IgG until approximately 3 months of age.[13] For one-fourth of the women (n = 12 740) in the study population, data were also available on IgG level based on the first-trimester serum sample. The IgG levels measured in mothers and offspring were highly correlated (Spearman correlation = 0.76; $P < .001$).

ASSESSMENT OF SUICIDE, SELF-DIRECTED VIOLENCE, AND VIOLENT SUICIDE ATTEMPTS

The Danish Cause of Death Register[14] contains individual information on cause and date of all deaths in Denmark since 1967. From this register, we identified all persons in the study population who died of suicide (*International Classification of Diseases, Eighth Revision [ICD-8]* codes E950-E959 and *ICD-10* codes X60-X84) from 1992 to 2006 (n = 18).

The Danish National Hospital Register[15] includes the medical records of all patients treated in Danish general hospital inpatient departments since January 1, 1977, and outpatient clinics since January 1, 1995. The Danish Psychiatric Central Research Register[16] was computerized in 1969 and contains data on all admissions to Danish psychiatric inpatient facilities, and from 1995 on, information on visits to psychiatric outpatient clinics was included in the register. The recorded information in these 2 hospital registers includes the patient's personal identification number, reason for contacting the hospital, date of visit, and main diagnosis as well as auxiliary diagnoses. In both registers, the diagnostic system used until 1993 was the Danish modification of *ICD-8*,[17] and from 1994, the diagnostic system used was *ICD-10*.[18] Hospital treatments are free of charge for all residents in Denmark. For this study, we defined cases of self-directed violence as persons who fulfill at least 1 of the following 5 criteria[19]: (1) a contact with "suicide attempt/suicide" as the contact reason, (2) an *ICD-8* diagnosis with a code of E9500 to E9599, (3) a contact with the main diagnosis (*ICD-10*) in chapter F (mental illness) and an auxiliary diagnosis of poisoning (*ICD-10* codes T36-T50 and T52-T60) or lesions at the forearm, wrist, or hand (*ICD-10* codes S51, S55, S59, S61, S65, and S69), (4) a main diagnosis of poisoning with weak analgesics, epileptic drugs, sleeping medicine, Parkinson drugs, psychotropic drugs, or carbon monoxide (*ICD-10* codes T39, T42, T43, and T58), and (5) a diagnosis of suicide attempt or deliberate self-harm (*ICD-10* codes X60-X84). Searching through all records in the registers (including inpatient,

outpatient, and emergency department [somatic and psychiatric] contacts from 1977-2006), we identified 994 women with a history of self-directed violence and 7 of these ended up committing suicide. When considering self-directed violence, we used the date of the first contact for self-directed violence or the date of death from suicide (whichever came first) as the time of onset.

We also analyzed violent suicide attempts where we excluded poisoning as the method and unspecified methods and restricted to diagnoses coded as suicide attempts. In detail, we defined violent suicide attempts as suicide attempts using hanging and strangulation (*ICD-8* code E953 and *ICD-10* code X70.xx), drowning (*ICD-8* code E954 and *ICD-10* code X71.xx), shooting or explosive material (*ICD-8* code E955 and *ICD-10* codes X72.xx, X73.xx, X74.xx, and X75.xx), fire, steam, or hot objects (*ICD-10* codes X76.xx and X77.xx), sharp instrument (*ICD-8* code E956 and *ICD-10* code X78.xx), blunt instrument (*ICD-10* code X79.xx), jump from a high place (*ICD-8* code E957 and *ICD-10* code X80.xx), and collision with a vehicle or object in movement (*ICD-10* codes X81.xx-X82.xx) where xx could take the values 01, 10, and 11. Furthermore, the definition includes a diagnosis with an injury not due to chemical substances and with "suicide attempt/suicide" as a contact reason (*ICD-8* codes 800-959 and *ICD-10* codes S00-T32.9, T71, and T75-T75.8).

ASSESSMENT OF MENTAL ILLNESS

The study population was linked with the Danish Psychiatric Central Research Register[16] to obtain information on history of mental illness. Cohort members and their parents were classified with a psychiatric history (any diagnosis) if they had been admitted to a psychiatric hospital or had received outpatient care. Date of onset was defined as the first day of the first contact (inpatient or outpatient). We defined mood disorder as *ICD-8* codes 296, 298.09, 298.19, 300.49, and 301.19 and *ICD-10* code F3; schizophrenia spectrum disorder as *ICD-8* codes 295, 297, and 298.39 and *ICD-10* codes F20 to F29; and borderline disorder as *ICD-8* code 301.83 and *ICD-10* code F60.3.

STUDY DESIGN

Prevalence of *T gondii* at the time of delivery was estimated using a cross-sectional study design.[20] Prevalence and 95% likelihood ratio–based confidence intervals were calculated using the GENMOD procedure in SAS version 9.2.[21]

Incidence rate ratios of self-directed violence, referred to herein as relative risks, were estimated by the Cox proportional hazards model (Cox regression).[22,23] The proportional hazards assumption was evaluated by comparing estimated log-minus-log survival curves. The study population was divided into 2 groups according to history of self-directed violence prior to delivery. The 517 mothers with a history of self-directed violence prior to delivery were studied from the day they gave birth (May 15, 1992, to January 15, 1995) until date of first self-directed violence after delivery, date of death, date of emigration from Denmark, or December 31, 2006, whichever came first. The 45 271 mothers without a history of self-directed violence prior to delivery were studied from the day they gave birth (May 15, 1992, to January 15, 1995) until date of first episode of self-directed violence, date of death, date of emigration from Denmark, or December 31, 2006, whichever came first. Time since delivery was used as the underlying time scale. All relative risk estimates were adjusted for age at delivery and some estimates were further adjusted for time since first psychiatric contact and for psychiatric history and history of self-directed violence (including suicide) in the parents of the women. History of mental illness in women and parents were treated as time-dependent variables. Two categorical models were considered: a dichotomous model (seropositive and seronegative) and a model with seropositive IgG levels divided into groups according to the 25th, 50th, 75th, and 90th percentiles. We also investigated the effect of *T gondii* when stratifying by a history of mental illness (present or not present). All *P* values and 95% confidence intervals were based on likelihood ratio tests.[22]

Investigators were blinded to the identity of individuals in the study, and the study did not involve contact with the cohort members. The study was approved by the Danish Data Protection Agency.

RESULTS

The distribution of the *T gondii* IgG level measured in the newborn child showed a clear bimodal distribution (**Figure**). In accordance with Lebech and Petersen[12] and consistent with the bimodal distribution of IgG levels in the Figure, an IgG level more than 24 was considered seropositive. The prevalence of *T gondii* seropositivity was 26.80% (95% CI, 26.33-27.28) at the time of delivery.

RELATIVE RISK OF FIRST EPISODE OF SELF-DIRECTED VIOLENCE AFTER DELIVERY

Among the 45 271 mothers studied from delivery until 2006, 488 had a first contact for self-directed violence during the 595 306 person-years at risk, corresponding to a crude incidence rate of 8.20 per 10 000 person-years (**Table 1**). The follow-up was terminated before the end of the study period for 877 mothers (1.9%) because of death of other reasons than suicide (n=261) or emigration from Denmark (n=616). When the *T gondii* IgG level was treated as a dichotomous variable, comparing seropositive with seronegative women, mothers who were *T gondii* seropositive had a 1.53-fold (95% CI, 1.27-1.85; $P<.001$) significant relative risk of self-directed violence compared with *T gondii*–seronegative mothers (Table 1, 2 categories). When further subdividing the seropositive values according to the 25th, 50th, 75th, and 90th percentiles, the risk of self-directed violence seemed to increase with increasing IgG level (Table 1, 6 categories); women with an IgG level more than 83 had a relative risk of 1.91 (95% CI, 1.25-2.79) compared with seronegative women. Stratifying by psychiatric history, we found that among women with a history of mental illness, seropositive women had a relative risk of 1.25 (95% CI, 0.94-1.66) compared with seronegative women, and among women without a history of mental illness, seropositive women had a relative risk of 1.56 (95% CI, 1.21-2.00) compared with seronegative women (**Table 2**). The effect of *T gondii* infection was not significantly different in the 2 groups ($P=.25$), and therefore, the seemingly lower risk among women with a history of treated psychiatric disorders should be interpreted with caution. In a model adjusting for time since first psychiatric contact, the overall effect of *T gondii* was 1.41 (95% CI, 1.17-1.70) (**Table 3**). Further adjustment for psychiatric history in a parent or history of self-directed violence in a parent had only

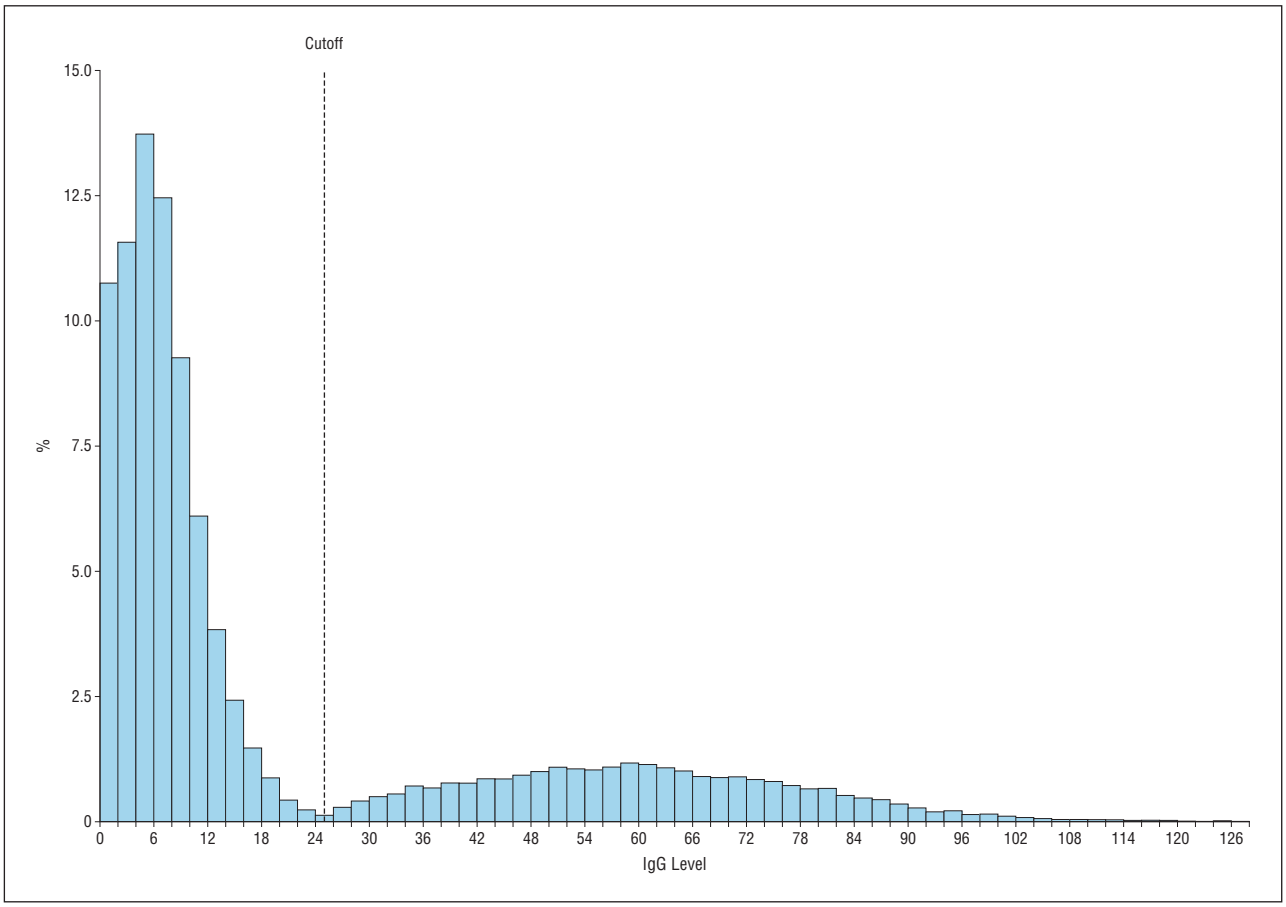

Figure. Distribution of *Toxoplasma gondii*–specific IgG level expressed as percentage of the optical density obtained for the World Health Organization international standard serum for the 45 788 Danish mothers in the cohort.

Table 1. Adjusted Relative Risk of Self-directed Violence Associated With *Toxoplasma gondii* IgG Antibody Level

Variable	No. of Cases	Incidence Rate[a]	Relative Risk (95% CI)[b]	*P* Value[c]
Total	488	8.20		
IgG level				
2 Categories				
0-24[d]	320	7.34	1 [Reference]	<.001
≥25	168	10.54	1.53 (1.27-1.85)	
6 Categories[e]				
0-24[d]	320	7.34	1 [Reference]	<.001
25-45	26	6.75	1.08 (0.70-1.58)	
46-58	40	9.87	1.49 (1.06-2.04)	
59-71	41	10.66	1.55 (1.10-2.12)	
72-83	35	14.01	1.87 (1.30-2.61)	
≥84	26	15.35	1.91 (1.25-2.79)	

[a] New cases per 10 000 person-years at risk.
[b] Estimates of relative risk when accounting for time since delivery and age at delivery.
[c] The *P* values measure the overall effect of each variable considered.
[d] Women with an IgG level less than 25 were designated as seronegative at the time of study entry.
[e] The *T gondii* seropositive values were divided according to the 25th, 50th, 75th, and 90th percentiles.

minor impact on the findings. Among the 214 cases with a psychiatric diagnosis prior to self-directed violence, 82 had at least once been diagnosed with mood disorder, 32 with schizophrenia spectrum disorder, and 37 with a borderline personality disorder, and of these, 34 had a diagnosis in more than 1 group.

RELATIVE RISK OF REPETITION OF SELF-DIRECTED VIOLENCE

The 517 women with self-directed violence prior to delivery were studied from delivery until 2006, and 84 met criteria for self-directed violence during the 6122 person-

Table 2. Adjusted Relative Risk of Self-directed Violence Associated With *Toxoplasma gondii* IgG Antibody Level Divided by Psychiatric History

	Without Psychiatric History			With Psychiatric History		
Variable	No. of Cases	Incidence Rate[a]	Relative Risk (95% CI)[b]	No. of Cases	Incidence Rate[a]	Relative Risk (95% CI)[c]
Total	274	4.85		214	71.41	
IgG level						
0-24[d]	181	4.36	1 [Reference]	139	67.74	1 [Reference]
≥25	93	6.20	1.56 (1.21-2.00)	75	79.37	1.25 (0.94-1.66)
P value			<.001			.12

[a] New cases per 10 000 person-years at risk.
[b] Estimates of relative risk when accounting for time since delivery and age at delivery.
[c] Estimates of relative risk when accounting for time since first psychiatric contact, time since delivery, and age at delivery.
[d] Women with an IgG level less than 25 were designated as seronegative at the time of study entry.

Table 3. Relative Risk of Self-directed Violence Associated With *Toxoplasma gondii* IgG Antibody Level Further Adjusted for Psychiatric History and Psychiatric History or Self-directed Violence in a Parent

Variable	Relative Risk (95% CI)[a]	*P* Value[b]	Relative Risk (95% CI)[c]	*P* Value[b]
IgG level				
2 Categories				
0-24[d]	1 [Reference]	<.001	1 [Reference]	<.001
≥25	1.41 (1.17-1.70)		1.40 (1.16-1.68)	
6 Categories[e]				
0-24[d]	1 [Reference]	.003	1 [Reference]	.003
25-45	0.99 (0.65-1.45)		0.97 (0.63-1.42)	
46-58	1.37 (0.97-1.88)		1.37 (0.97-1.89)	
59-71	1.46 (1.04-2.00)		1.45 (1.03-1.98)	
72-83	1.74 (1.21-2.43)		1.77 (1.23-2.47)	
≥84	1.65 (1.08-2.42)		1.57 (1.02-2.29)	

[a] Estimates of relative risk when accounting for time since delivery, age at delivery, and time since first psychiatric contact.
[b] The *P* values measure the overall effect of each variable considered.
[c] Estimates of relative risk when accounting for time since delivery, age at delivery, time since first psychiatric contact, and history of mental illness or history of self-directed violence in a parent.
[d] Women with an IgG level less than 25 were designated as seronegative at the time of study entry.
[e] The *T gondii* seropositive values were divided according to the 25th, 50th, 75th, and 90th percentiles.

years at risk. Among women with self-directed violence prior to delivery, seropositive women had a relative risk of self-directed violence after delivery of 1.54 (95% CI, 0.98-2.39; *P*=.06) compared with seronegative women.

RELATIVE RISK OF VIOLENT SUICIDE ATTEMPT AND RELATIVE RISK OF SUICIDE

Studying 45 745 women from delivery until 2006, 78 had a violent suicide attempt during 603 876 person-years at risk, corresponding to a crude incidence rate of 1.29 per 10 000 person-years. Women who were seropositive had a relative risk of a violent suicide attempt of 1.81 (95% CI, 1.13-2.84; *P*=.01) compared with seronegative women. In the cohort of 45 788 women, only 18 committed suicide during 604 844 person-years at risk. Based on 8 seropositive women, we estimated a relative risk of suicide of 2.05 (95% CI, 0.78-5.20; *P*=.14) for seropositive women compared with seronegative women. Suicides or violent suicide attempts were not accountable for the association between *T gondii* and self-directed violence since seropositive women had a relative risk of 1.42 (95% CI, 1.16-1.74) for self-directed violence, not categorized as suicide or violent suicide attempt, when censoring the women on the date of first violent suicide attempt.

COMMENT

We report a predictive association between *T gondii* IgG antibody titers shortly after delivery and self-directed violence in later life. This is consistent with associations emerging from 2 previous cross-sectional studies in smaller clinical samples in Maryland[8] and Turkey[24] and very recently in a larger study of schizophrenic patients in Germany.[9] The study is also consistent with an ecological report on a relationship between national suicide rates in Europe and *T gondii* seropositivity in older women after adjustment for socioeconomic factors.[25] The association was consistent for the outcomes self-directed violence, violent suicide attempts, repeated self-directed violence, and suicide, although not statistically significant for the last 2.

What are possible mechanisms underlying this association? A prominent candidate is a neuroimmune path, because in the latent prevalent form of infection, *T gon-*

dii is contained immunologically, and immunological alterations have previously been reported in individuals with a history of suicidal self-directed violence. For instance, Janelidze et al[26] reported increased peripheral levels of interleukin 6 (IL-6) and tumor necrosis factor α in suicide attempters relative to nonsuicidal depressed patients and healthy controls. The magnitude of difference in IL-6 levels between attempters and nonattempters and *Toxoplasma*-positive vs *Toxoplasma*-negative individuals is of a similar degree. For instance, Lindqvist et al[27] observed a mean difference in IL-6 level of 4.6 pg/mL between violent attempters and controls ($P < .001$) while Matowicka-Karna et al[28] observed a mean difference in IL-6 level between *Toxoplasma*-positive and *Toxoplasma*-negative individuals of 2.7 pg/mL ($P < .001$). While these elevations of IL-6 were reported in the "peripheral" blood, elevated levels of IL-6 have been found in the cerebrospinal fluid of suicide attempters,[27] also consistent with a report of elevated cytokine gene expression[29] and an increased number of microglia in suicide victims.[30]

Inflammatory cytokines are essential for resistance to *T gondii*[31,32] by activation of microglia and infiltrating macrophages in the central nervous system[33] and macrophages and lymphocytes[34] in the periphery. In addition to interferon γ, tumor necrosis factor α is involved in controlling *T gondii* replication,[35] and tumor necrosis factor α has been previously found elevated in suicide attempters[26] as well as *T gondii*–positive women.[36] Proinflammatory cytokines activate the enzyme indoleamine 2,3-dioxygenase (IDO) resulting in degradation of tryptophan toward kynurenines and a relative tryptophan deprivation of the microorganism, another mechanism of resistance.[32] In addition, the production of kynurenine secondary to IDO activation generates metabolites kynurenic acid and quinolinic acid,[37] known to be potent neuromodulators[38] of the *N*-methyl-D-aspartate receptors. A 7-fold increase in brain content of kynurenic acid was reported in mice chronically infected with *T gondii* accompanied by an increase in the levels of kynurenine, precursor of kynurenic acid, suggesting an activation of upstream enzymes involved in the kynurenine pathways, such as IDO.[39] Very recent associations support an activation of kynurenine pathways in suicidal self-directed violence. Specifically, kynurenic acid concentrations in the cerebrospinal fluid have been found to be associated with violent suicide attempts, history of major depression, and IL-6 levels,[40] while plasma kynurenine is elevated in patients with recurrent depression with a suicide attempt history as compared with patients with recurrent depression without a suicide attempt history.[41] Certainly, the absence of longitudinal studies allows alternative interpretations such as preexisting immune abnormalities leading to an increased risk for both *T gondii* infection as well as self-directed violence or, alternatively, a possibility of immune abnormalities in attempters associated with an increased risk of attempt (such as adverse developmental conditions and vulnerability to stress) that may have predated and potentially contributed to infection with *T gondii*. Unfortunately, these could not be formally tested in the current study, given that biological material and data were not sufficiently available.

The relationship showed a titer-response effect, with stronger associations at higher titers. Stronger titers may be the result of a more abundant *T gondii* cyst infestation or recent or more frequent reactivations. How can we explain a greater predictive association of *T gondii* infection with suicide attempts with increased antibody titers? One possibility is that the infection has been reactivated or that the woman was infected more recently. These hypotheses would require dynamic testing of IgG level and in the case of new infections, IgM level, which was not available in our data set. One could speculate that there is a direct effect of the IgG antibodies through molecular mimicry. IgG antibodies against several infectious agents, including *T gondii*, may cross-react with epitopes in neural tissue,[42,43] and from disorders such as systemic lupus erytemathosus[44] and paraneoplastic disorders,[45] it is known that neurological and psychiatric symptoms may be caused by antibodies crossing the blood-brain barrier. Also recently, anti–*N*-methyl-D-aspartate receptor antibodies as well as other specific central nervous system–related antibodies have been associated with psychosis as well as other neurological and psychiatric symptoms.[46,47] However, because the possible specific affinity of *Toxoplasma*-specific antibodies to epitopes in the brain has not been studied in detail, this remains speculative. The association with high titers could also be a result of a nonspecific activation of helper T cell type 2 immune response in individuals with self-directed violence, with a nonspecific elevation in antibody titers. To evaluate the latter possibility would involve testing titers for other pathogens, not done in our case. However, prior research[8,9] has found significant associations between suicidal self-directed violence and antibodies to *T gondii* but not to other neurotropic pathogens. A limitation of our study is the unavailability of early IgM antibody titers to document the timing of acute infection as well as the longitudinal course of IgG antibody titers allowing inferences regarding possible mediation of self-directed violence by reactivation of chronic infection with *T gondii*. Because behavioral effects of *T gondii* may be strain dependent,[48] there is a need for future strain-specific analysis as well as additional analysis for specific bradyzoite antibodies.

The estimated association between *T gondii* infection and self-directed violence was stronger among women without a psychiatric history than among women with a psychiatric history, although the difference was not significant. A possible implication is that some of the association could be mediated through mental illness. Because many patients with, for example, mood or substance use disorders may not have been treated as inpatients or outpatients in psychiatric clinics, we cannot exclude that some of the effect of *T gondii* infection in women without a psychiatric diagnosis is mediated by an elevation of risk for mental disorders. Nevertheless, in 2 previous publications in patients with mood disorders[8] and among younger patients with schizophrenia,[9] associations between *T gondii* serointensity and history of suicide attempt have been reported.

The strengths of the study include being, to our knowledge, the largest study of *T gondii* infection and self-directed violence. It is based on a unique population-

based cohort with almost complete follow-up data for up to 14 years. Women were included in the study irrespective of social status, and information on *T gondii* IgG level was collected prospectively and independently of the present study. Even though, for the first time to our knowledge, the *T gondii* antibody level was measured prior to first registered occurrence of self-directed violence, we cannot say with certainty whether the observed association between *T gondii* infection and self-directed violence is causal. *T gondii* infection is likely not a random event and it is conceivable that the results could be alternatively explained by people with psychiatric disturbances having a higher risk of becoming *T gondii* infected prior to contact with the health system. A study among pregnant women in Norway found an increased risk of recent maternal *T gondii* infection associated with consumption of raw or undercooked meat, incompletely washed fruits and vegetables, cleaning the cat litter box, and washing the kitchen knives infrequently after preparation of raw meat, prior to handling another food item.[49] Because of lack of information, we were not able to control for these risk factors. Instead we adjusted for psychiatric history, acknowledging that many mentally ill people are undiagnosed. Trying to account for some of the undiagnosed psychiatric illness, we adjusted for psychiatric history and history of self-directed violence in the parents, with only minor impact on the findings.

Other weaknesses of the study include the lack of representation of the entire Danish population in the study group. The cohort excluded men and nulliparous women who may have different risk factors for self-directed violence. Another issue is generalizing the results from the Danish study to the rest of the globe. Clearly, Denmark is a country with a relatively homogenous socioeconomic status, medical coverage, and socioeconomic security. However, findings from several epidemiological suicide studies based on Danish data have been in good accordance with the general international literature, even given the large differences in health care systems and social structure. One limitation is that many episodes of self-directed violence, especially those interrupted or aborted, may not be recorded and thus analyzed. In particular, before 1995, the number of unregistered suicide attempts was considerable because only admissions and not outpatient visits were included in the registers. We do not, however, have any reason to believe that the proportion of unregistered attempters is different in *T gondii*–positive vs *T gondii*–negative mothers. As a consequence of the underreporting of self-directed violence, some of the women included in the follow-up study of first self-directed violence may already have had an unregistered episode of self-directed violence at the time of delivery. When studying women with a diagnosis of self-directed violence before delivery, the association between *T gondii* infection and self-directed violence after delivery was the same size as among women without a diagnosis of self-directed violence prior to delivery. The mixture of incident and prevalent cases does therefore not seem to be a problem.

From the diagnoses registered in the Danish National Hospital Register and in the Danish Psychiatric Central Research Register, it was not always possible to conclude whether nonfatal self-directed violence was suicidal or nonsuicidal. For example, we did not include the *ICD-10* code T40, poisoning by neurotropic medications, as a main diagnosis in the definition of self-directed violence because this group may contain many poisonings by accident. When restricting to diagnoses specifically coded as suicide attempts and excluding poisoning as a method (violent suicide attempts), we found a higher relative risk associated with *T gondii* infection than for the broader definition (self-directed violence) as outcome. For the analysis of fatal suicidal self-directed violence (suicide), the power is limited (only 8 *T gondii*–positive women committed suicide), but for these, the relative risk was as high as 2.05. Suicides and violent suicide attempts were not accountable for the association between *T gondii* infection and self-directed violence.

Because information on the IgG level in the mothers' first-trimester sera samples was available for only a subset of the cohort, we based our analyses on IgG levels in the newborns. Although IgG levels in the child can be influenced by a number of factors (including placental transport), antibody levels in maternal sera from the first trimester and in the blood samples from infants were well correlated (Spearman correlation = 0.76; $P < .001$). We therefore have confidence in the results based on antibody levels measured in the newborns.

In conclusion, our results are consistent with the hypothesized association between *T gondii* infection and self-directed violence and, in concert with other converging evidence and better understanding of underlying mechanisms, if confirmed in future studies, may lead to new prognostic, prophylactic, and therapeutic approaches to suicide prevention.

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