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## ***Toxoplasma gondii* Seropositivity and Suicide rates in Women**

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### **Abstract**

*Toxoplasma gondii* (*T. gondii*) is an intracellular protozoan parasite that infects roughly a third of the world population. In an immunocompetent host, infection is generally chronic and asymptomatic, as the immune system keeps *T. gondii* confined to cysts and the intracellular space within muscle and brain. Seropositivity has been linked to schizophrenia, car accidents, changes in personality, and more recently, suicidal attempts. Very recently, seroprevalence for 20 European countries was found to be associated with increased suicide rates. Although suicide rates were age-standardized, given that *T. gondii* seroprevalence increases with age, and blood samples were drawn in women, we now retested in women only the association between suicide and *T. gondii* seropositivity, stratified by age. Simple correlations between ranked *T. gondii* seropositivity and suicide rate identified statistically significant relationships in women 60 and older ( $p < 0.05$ ); adjusting for GDP, the statistical significance expanded to include women 45 and older. The strongest association was in the 60-74 group where, after adjustment for GDP, the relationship ( $p = 0.007$ ) resisted Bonferroni adjustment for multiple comparisons. In conclusion, the results suggest that a positive relationship between rates of infection with *T. gondii* and suicide is apparent in women of postmenopausal age. Prospective studies are necessary to further confirm this association predictively and explore mechanisms mediating this relationship.

### **Keywords**

Toxoplasmosis; *Toxoplasma gondii*; suicide; Europe

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## Introduction

*Toxoplasma gondii* (*T. gondii*) infects a high proportion of the population, although manifestations of infection generally are not apparent in the majority of individuals who are immunocompetent, as the immune system successfully controls *T. gondii* multiplication and spread. For instance, it is estimated that 22.5% of the U.S. population (60 million), and approximately 2.5 billion people worldwide, have contracted the parasite (Centers for Disease Control and Prevention, 2008). This intracellular protozoan parasite takes several forms: oocysts, tachyzoites (fast-growing), and bradyzoites (slow-growing, the most common form being confined to neurons, glial cells and cysts in the brain (Montoya & Leisenfeld, 2004).

While felids have been identified as definitive hosts of *T. gondii*, where the parasite multiplies sexually in the gut, this parasite needs intermediate hosts in any terrestrial warm blooded animals, where it localizes inside cells and cysts formed within the host's muscular and neural tissues. In most cases infection occurs with the accidental ingestion of a) soil-based oocysts (shed by hosts) found on unwashed vegetables/fruits and drinking contaminated water, or through handling litter boxes of infected cats b) tissue cysts which are found in undercooked meat (e.g. beef, pork, lamb, venison, etc.).

Although, in most cases, infection in immunocompetent hosts is considered asymptomatic, *T. gondii* seropositivity has been associated with higher rates of schizophrenia (meta-analysis: Torrey et al., 2007), personality disorders (Hinze-Selch et al., 2010), and car accidents (Flegr et al., 2002; Yereli et al., 2006). Moreover, with iatrogenic or pathogenic immunosuppression (e.g. organ transplant, AIDS, etc.), acute reactivation of toxoplasmosis may develop, typically presenting with encephalitis, ocular disease, and lymphadenopathy, (Montoya & Leisenfeld, 2004).

Recently, a first report of an association between lifelong history of suicide attempts and titers of IgG antibodies based on a study conducted at the University of Maryland School of Medicine (PI Postolache) on patients recruited in Baltimore and Washington DC (Arling et al., 2009) was shortly followed by an article linking history of suicide attempts with *T. gondii* seropositivity in Turkey (Yagmur et al., 2010). A very recent ecologic analysis (Lester, 2010) found an association between estimated *T. gondii* seropositivity and national average suicide rates across 20 European nations. Older age is a strong factor in the epidemiological occurrence of suicide, particularly in men (Hawton and Van Heeringen, 2009), and thus, appropriately, the national data for suicide used in the Lester et al. analysis were standardized for age. However, as aging is also related to increased seroprevalence of *T. gondii* (Hinze-Selch et al., 2010, Fromont et al., 2009), previously reported associations between *T. gondii* and human personality are gender-dependent (Flegr et al., 2000; Flegr, 2007; Lindova et al., 2006), and only women were sampled for the previously reported *T. gondii* seroprevalence data (Lester 2010), we are now retesting the hypothesis of a positive correlation between *T. gondii* seropositivity and national suicide rates in women only, stratified by age.

## Methods

As in Lester (2010), country seropositivity rates were estimated based on data available from pregnant women or women of child-bearing age (Lafferty, 2006). Assuming that differences between countries in this particular demographic group would be similar in other demographic groups, the twenty countries were designated rankings based on reported seroprevalence levels (1 - 20, with 1 designating the lowest).

Suicide rates by age group (0-14, 15-29, 30-44, 45-59, 60-74, 75+) were obtained from the European Mortality Database (World Health Organization, 2010). The year for which serum levels was reported varied for each country (1989-1999), so the year of the national suicide rates was matched to correspond to the relevant year of the serum analysis (Table 1). Our assumption of the validity of seropositivity ranks across age groups is rooted in the knowledge that *T. gondii* infection, once established, persists throughout life, but this assumption has not been tested and could be incorrect.

To reduce potential confounding by low socio-economical status, a risk factor for both *T. gondii* infection and suicide, the national gross domestic product (GDP) was obtained from the World Bank database (World Bank, 2010). Pearson product-moment correlations between *T. gondii* seropositivity rankings and suicide rates stratified by age group were performed, followed by linear regressions adjusting for GDP; the significance thresholds of regressions were primarily unadjusted (criterion alpha set at 0.05, two-tailed) and secondarily Bonferroni-adjusted for multiple comparisons.

In many European countries, it is part of pre-natal care to test pregnant women and women of child-bearing age for *T. gondii* seropositivity as a preventive measure against congenital toxoplasmosis in neonates. As such, *T. gondii* rates in this population are often the best available representation of rates in the overall population of a given country. Prior studies (c.f. Tenter et al, 2000; Lafferty, 2006; Lester, 2010) have conducted analyses based on this extrapolation. However, due to the inherent limitations of interpreting and drawing conclusions in men based on data from women, the analysis in this study was conducted for women only.

## Results

Characteristics of the countries are presented in Table 1. When stratified for age, seroprevalence ranking correlated positively with suicide rates for women, significantly in age groups 60 and older ( $r=0.51$  and  $0.54$  in women 60-74 and above 74 respectively,  $p<0.05$  in both groups, Table 2). After adjusting for GDP, the relationship between seroprevalence ranking and suicide rates became also statistically significant in the age group 45-59 years old ( $t=2.13$ , std coefficient  $0.47$ ,  $p=0.048$ ). In the age group 60-74 years old the association between *T. gondii* ranks and suicide rates after adjustment for GDP was statistically significant at a more stringent level, being able to survive a Bonferroni adjustment for multiple comparisons in ( $t=3.02$ , std coefficient  $0.62$ ,  $p=0.007$ , Figure 1). In the oldest group, 75 years old and above the correlation resisted linear regression adjustment for GDP ( $t=2.49$ , std coefficient  $0.54$ ,  $p=0.24$ ).

## Discussion

The main result of our study are that, although the representative seropositivity rates used in our analysis were collected from women in their child-bearing years, the relationship between *T. gondii* and suicide rates reaches significance in older age groups, roughly corresponding to postmenopausal years. Even in the immunocompetent host, seropositivity persists throughout life, as *T. gondii* successfully hides inside cells and cysts and avoids eradication by the immune system. Correspondingly, it is expected that older populations will have higher rates of seropositivity, and that a positive test at a younger age will remain positive with aging. Additionally, established suicide factors such as the accumulation of personal losses (e.g. loss of spouse or companion, loneliness, joblessness, medical illness, not having dependents at home) may act in concert with *T. gondii* seropositivity to contribute toward higher rates of suicide later in life. Immunological changes related to

aging could also act synergistically with immune activation necessary to contain *T. gondii* and possibly affect brain structures and function.

Post-mortem analysis of brain regions in infected rodents revealed that *T. gondii* cysts are found in greater density within the amygdale (Vyas et al., 2007) – structures typically implicated in the neurocircuitry of fear and anxiety. These cysts may contribute to the suppression of a rodent's typical aversion to feline odors (Berdoy et al., 2000) or even attraction to feline odors (Vyas et al., 2007), potentially leading to rodent self-destructive behaviors. This has been interpreted within the scope of parasite-induced behavioral manipulation of the host (Tenter et al., 2000), often occurring with a broad range of parasites that have a life cycle involving multiple hosts. There are also non-specific effects of *T. gondii* infection in rodents such as neophobia decrements and decreases in memory and learning (Webster, 2007). Decreased cognitive performance has also been found in humans with latent toxoplasmosis (Havlíček et al., 2001) evidenced with increased reaction times in psychomotor tasks. Increased reaction time can lead one to hypothesize increased risks for car accidents, a prediction that was confirmed in two previous studies. (Flegr et al., 2002; Yereli et al., 2006).

It is possible that immune response to infection with *T. gondii* exacerbates suicide risk factors such as depression – a potential precursor to suicide – and resultant cognitive deficits. Essential to containing infection from *T. gondii* is the production of proinflammatory cytokines (Aliberti et al., 2005, Miller et al., 2009). Proinflammatory cytokines, such as IL-6 in the cerebrospinal fluid (Lindqvist et al., 2009) and IL-6 and TNF in the plasma (Janelidze et al., 2010) have recently been associated with suicidal behavior.

Inflammatory mediators produced in response to *T. gondii* infection may contribute to depression. Previous research has linked production of pro-inflammatory cytokines with depression, the most common psychiatric condition associated with suicidal behavior in humans, and depressive-like behaviors in animal models (Dantzer et al., 2008). Supporting a possible role between depression and *T. gondii* infection, especially severe and refractory depression, a case report described a conversion from an antidepressant treatment-resistant to treatment-responsive case after the patient was treated for *Toxoplasma* infection (Kar and Misra, 2004).

It is also possible that elevations of kynurenine (KYNA) and its metabolites, secondary to activation of indoleamine 2,3-dioxygenase (IDO) by immune mediators targeting *T. gondii*, could result in changes in dopaminergic and glutaminergic neurotransmission, and thus contribute to affective and behavioral alterations leading to increased suicide risk (Dantzer et al., 2010; Khabazghazvini, 2010; Wonodi & Schwarcz, 2010; Capuron et al., in press). Very recent findings suggest that depressed patients with history of suicide attempts had greater kynurenine levels than depressed patients without history of suicide attempt and healthy controls (Sublette et al., 2011). Consistently, KYNA concentrations in cerebrospinal fluid have been found to be associated with violent suicide attempts, history of major depression, and IL-6 levels (Lindqvist et al 2010).

In fact interferon gamma, a cytokine critical for immunity against viral and bacterial intracellular infections, blocks the growth of *T. gondii* by activating macrophages and lymphocytes (Denkers and Gazzinelli, 1998), and specifically activating the enzyme indoleamine 2,3-dioxygenase (IDO) which restricts the amino acid tryptophan (Miller et al., 2009). And though tryptophan depletion inhibits the growth of the parasite, it also results in decreases in serotonin production in the brain. Potentially, as a consequence of lower serotonin production, experimentally-induced tryptophan depletion has been linked with

reversal of antidepressant effects (Delgado et al., 1990), increases in irritability (Russo et al., 2005), and increases in self-injurious behavior (McCloskey et al., 2009).

The results of are consistent with our previous findings on suicide attempts in mood disorders (Arling et al 2009), replicated by an independent group in a different setting (Yagmur et al 2010). These previously described relationship between *T. gondii* and suicide attempts suggests that it is *attempting* suicide that may be a consequence of latent toxoplasmosis, rather than *completion* per se. Future research may focus on specific risk factors of suicide, especially on clinical symptoms associated with suicidality (such as aggression, impulsivity, depression), specific strains of *T. gondii*, localization of *T. gondii*, and using bradyzoite vs. tachyzoite specific antibodies.

Limitations of our study include many of those inherent in ecological models. First, the results do not reflect individual data, and should not be interpreted as such. Data were not weighted for population size. Methods used to determine seropositivity may have differed among the different European countries, but there is no reason to believe that this would be linked with a systematic difference in reporting suicide. Another limitation is a lack of adjustment for the rural-urban distribution of population in each country. It is known from previous research that differences will exist where rural populations will demonstrate both higher rates of *T. gondii* seropositivity (Fromont et al., 2009) and higher rates of suicide (Hirsch, 2006) thus potentially leading to a spurious relationship. Due to limited availability of data from the WHO database, suicide rates are not available for rural and urban populations separately. Our analysis was based on the assumption that *rankings* of countries based on seroprevalence in peripartum holds across age groups and genders. This assumption has not been formally tested, and could be incorrect. Although previous studies have used this extrapolation to populations including both genders, (Tenter et al, 2000; Lafferty, 2006; Lester, 2010), we have only extrapolated across age groups, but not across genders.

Acknowledging these limitations, our results are consistent with a hypothesized association between a highly prevalent neurotropic parasite and suicide completion and suggest an increase of this association with age, particularly in peri and postmenopausal women. This work adds to the developing field studying the effect of the natural environment on affective and behavioral dysregulation. Additional longitudinal studies on the relationship between *T. gondii* and suicidal behavior may lead to discovering novel prognostic, preventative and therapeutic approaches in suicide prevention.

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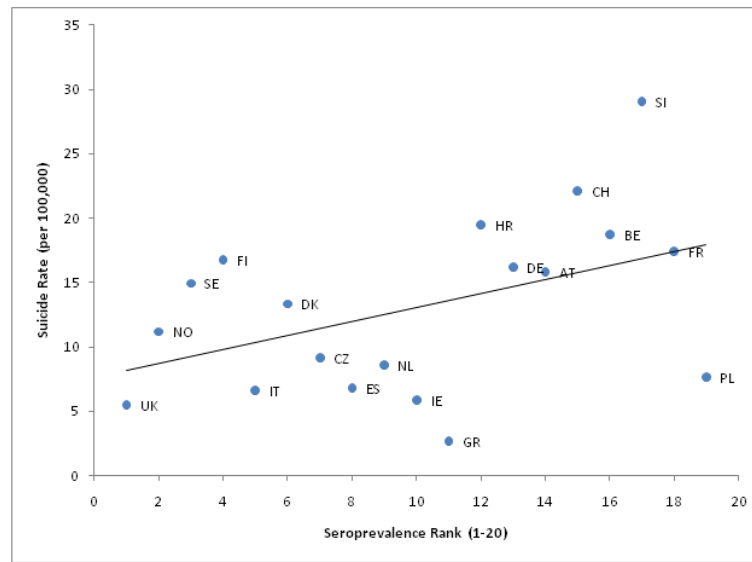
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**Figure 1. Relationship between suicide rates and *Toxoplasma gondii* seroprevalence rank in 20 European countries. Women 60-74 years old. Linear regression ( $t=2.54$ , std. coefficient 0.51,  $p=0.020$ ; after adjustment for GDP rank  $t=3.02$ , std. coefficient=0.33,  $p=0.008$ )**

AT: Austria, BE: Belgium, HR: Croatia, CZ: Czech Rep., DK: Denmark, FI: Finland, FR: France, DE: Germany, GR: Greece, HU: Hungary, IE: Ireland, IT: Italy, NL: Netherlands, NO: Norway, PL: Poland, SI: Slovenia, ES: Spain, SE: Sweden, CH: Switzerland, UK: United Kingdom.



Table 1

Seroprevalence and suicide rates and rankings among 20 European nations.

Country	Country Abbrev.	Year	Pop. (000)	GDP \$	Toxo %	Average Suicide Rate				TRk 1-20	SRk1-20	GDP Rank
						TSR per 100,000	MSR per 100,000	WSR per 100,000	MSR per 100,000			
Austria	AT	1997	8,072	25,953	42.0	17.8	28.6	8.5	7	7	7	
Belgium	BE	1990	9,987	20,323	50.0	17.5	25.8	10.5	5	8	10	
Croatia	HR	1995	4,776	4,737	38.0	18.6	30.3	8.8	9	5	18	
Czech Republic	CZ	1997	10,299	5,545	29.0	15.2	25.9	6.0	14	10	17	
Denmark	DK	1996	5,262	35,051	28.0	15.5	22.9	8.7	15	9	2	
Finland	FI	1989	4,964	23,518	20.0	27.5	45.7	11.1	17	3	9	
France	FR	1995	58,020	26,421	54.0	18.7	28.8	9.8	3	4	5	
Germany	DE	1992	80,594	25,577	39.0	14.7	22.6	8.0	8	11	8	
Greece	GR	1995	10,352	12,387	37.0	3.2	5.5	1.1	10	20	14	
Hungary	HU	1994	10,261	4,014	69.0	33.2	55.6	14.4	1	1	19	
Ireland	IE	1978	3,314	6,202	32.0	7.7	10.2	5.2	11	17	16	
Italy	IT	1995	57,301	19,809	23.0	6.9	11.2	3.3	16	18	11	
The Netherlands	NL	1999	15,812	26,033	31.0	9.0	12.4	5.9	12	15	6	
Norway	NO	1992	4,286	29,932	11.0	13.9	20.5	7.5	19	14	4	
Poland	PL	1991	38,245	1,998	59.0	14.7	25.8	4.6	2	12	20	
Slovenia	SI	1991	1,966	6,331	51.0	32.3	54.4	13.9	4	2	15	
Spain	ES	1992	39,008	15,680	30.0	6.6	10.8	3.0	13	19	13	
Sweden	SE	1992	8,692	30,820	14.0	14.5	20.4	8.9	18	13	3	
Switzerland	CH	1990	6,874	35,491	46.0	18.6	28.2	10.1	6	6	1	
United Kingdom	UK	1991	54,889	18,387	8.0	7.8	12.4	3.4	20	16	12	

Y: Year of sampling, Pop.: Population for the year of sampling, GDP: Gross domestic product, Age: Average age, Toxo: *T. gondii* seropositivity rate in women, TSR: Average suicide rate – overall, MSR: Average suicide rate – men, WSR: Average suicide rate – women, SRk: Overall suicide rank, TRk: *T. gondii* seropositivity rank, and GDP Rank: Per capita GDP rank.

**Table 2**

Pearson correlation coefficients for *Toxoplasma gondii* seroprevalence rank and suicide rates stratified across age groups for 20 European Nations (N=20)

	<b>0-14</b>	<b>15-29</b>	<b>30-44</b>	<b>45-59</b>	<b>60-74</b>	<b>75+</b>
Total	0.43	0.29	0.21	0.29	0.38 <sup>†</sup>	0.54 <sup>*†</sup>
Females	0.43	0.29	0.21	0.29	0.51 <sup>*††</sup>	0.54 <sup>*†</sup>

\* Significant at the .05 level (2-tailed), through Pearson correlation

<sup>†</sup> Significant at the 0.05 level (2-tailed), after multiple linear regression adjustment for the national gross domestic product (GDP)

<sup>††</sup> Significant at the 0.01 level (2-tailed), and resisting Bonferroni adjustment for multiple comparisons after linear regression adjustment for the national GDP.