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The role of latent toxoplasmosis in the aetiopathogenesis of schizophrenia – the risk factor or an indication of a contact with cat?

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Abstract: We assessed IgG antibody to *Toxoplasma gondii* in 300 inpatients with schizophrenia (SG), 150 outpatients with anxiety and depressive disorders (PCG), and 150 healthy blood donors (HCG). Seropositivity rates were 60.7% for SG, 36.7% for PCG, and 45.3% for HCG ($p<0.001$). The seropositivity rate for anti-*Toxoplasma* IgG antibodies in SG was significantly higher that in PCG ($X^2 = 23.11$, OR = 2.66, $p = 0.001$) and HCG ($X^2 = 9.52$, OR = 1.86, $p = 0.002$). Among SG, 85% of those who reported close cat contact had IgG antibodies to *T. gondii*. Close cat contacts were reported by 59% of SG, 6% of PCG, and 9% of HCG ($p<0.001$). There was a nonsignificant positive association between toxoplasmosis and schizophrenia for people with a contact with a cat (OR = 2.221, $p = 0.127$, $CI_{95} = 0.796–6.192$), and significant negative association between toxoplasmosis and schizophrenia for people without contact with a cat (OR = 0.532, $p = 0.009$, $CI_{95} = 0.332–0.854$). Close cat contact (OR = 2.679, $p<0.001$), 51–65-year age group (OR = 1.703, $p<0.001$) and education [illiterate+primary (OR = 6.146, $p<0.001$) and high school (OR = 1.974, $p = 0.023$)] were detected as independent risk factors in multivariate logistic regression. The effect of toxoplasmosis on risk of schizophrenia disappeared in the complex model analyzed with multivariate logistic regression. In conclusion, our data suggest that the toxoplasmosis has no direct effect on the risk of schizophrenia in Turkey but is just an indication of previous contacts with a cat.

Keywords: schizophrenia, *Toxoplasma gondii*, latent toxoplasmosis, Turkey

Schizophrenia is a complex chronic neuropsychiatric disease of the central nervous system, thought to have multiple aetiologies. Accumulating evidence from many studies has shown that genetic factors play a role in its aetiopathogenesis, and specific candidate predisposing genes have been identified. Environmental exposures have also been identified as increasing risk factors for the disease. Epidemiologic studies have established that winter-spring birth, urban birth, and perinatal and postnatal infection are all risk factors for the disease developing in later life (Yolken et al. 2001, Torrey and Yolken 2003, Brown 2006, Torrey et al. 2006).

Multiple epidemiological and neuropathological studies linking an infectious agent to schizophrenia have involved *Toxoplasma gondii.* Toxoplasmosis can vary from being an asymptomatic, self-limiting infection to being a fatal disease, and it has also been associated with increased rates of schizophrenia in offspring when the infection takes place during pregnancy (Brown et al. 2005).

In vitro neuropathologic studies of *T. gondii* in cell cultures and postmortem studies of the brains of individuals who had schizophrenia have both shown many glial abnormalities, especially in astrocytes, which were selectively affected. Decreased numbers of astrocytes have been reported. Similarly, animal studies of *T. gondii* infections have demonstrated that this organism affects the levels of dopamine, norepinephrine, and other neurotransmitters (Stibbs 1985, Skallová et al. 2006, Hodková

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et al. 2007) as well as indolamine 2,3-dioxygenase and kynurenine (Silva et al. 2002, Guidetti et al. 2006). The *T. gondii* genome has genes for two aromatic amino acid hydroxylases that could directly affect the dopamine and/or serotonin biosynthesis (Gaskell et al. 2009, Henriquez et al. 2009). Increased levels of dopamine and kynurenine have been suggested to exist in the brains of both *Toxoplasma*-infected subjects (Flegr et al. 2003, Miller et al. 2004, Novotná et al. 2005) and patients with schizophrenia (Carlsson 1988, 2001, Schwarcz et al. 2001, Miller et al. 2004). Disturbance in dopaminergic neurotransmission and in kynurenine are suspected of playing a key role in the pathogenesis of schizophrenia (Carlsson 1988, Schwarcz and Hunter 2007).

Neurophysiological mechanisms of *Toxoplasma* infections that may lead to an alteration in animal behaviour have not yet been defined. One hypothesis is that the metabolic products released from the cyst form of the parasite in the brain cause inflammation and encephalitis with an associated alteration of behaviour (Hay et al. 1983). There was also an evidence of focal inflammation with disrupted tissue cysts in mice (Sims and Hay 1995). *Toxoplasma*-infected mice have impaired motor performance (Hutchison et al. 1980), longer muscle reaction times (Hrdá et al. 2000), and deficits in learning capacity and memory (Witting 1979, Hodková et al. 2007). The neurophysiological mechanisms of these changes may be related to increased concentrations of dopamine observed in the brains of chronically infected mice (Stibbs 1985). Other theories involve marijuana-like compounds known as endocannabinoids. This hypothesis predicts that there is a subset of individuals with schizophrenia for whom an appropriate antibiotic regime might control the inflammatory infection to the degree that the brain would produce lower levels of endocannabinoids and hence provide symptomatic relief. The endocannabinoid system is a homeostatic regulator of all body systems, including the nervous system. Thus, imbalances in the endocannabinoid system have been considered a possible cause of various forms of mental illness and abnormal behaviour (Melamede 2009).

Yolken and Torrey (2006) reviewed the role of *T. gondii* in the aetiopathogenesis of schizophrenia. They discussed the epidemiological evidence for a role for this agent and potential mechanisms of gene-environmental interaction. They suggested that the potential role of *T. gondii* as a causative agent of a complex disorder such as schizophrenia was not consistent with Koch's postulates. They also suggested a modification of Koch's postulates and added Hill's criteria (strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, and experimental evidence) (Hill 1965). They also added additional criteria such as genetic compatibility and analogy. Toxoplasmosis satisfies many of the criteria that have been proposed to assess associations between environmental factors and complex human diseases. Except for specificity, all of the above criteria such as plausibility, experimental evidence, and analogy satisfy Hill's and other criteria.

The outstanding questions regarding infectious theories concern timing and causality. Attempts are underway to address the former by examining the sera of individuals prior to the onset of illness and to address the latter by using anti-infective medications to treat individuals with psychosis (Yolken and Torrey 2008). Anti-parasitic agents as well as antipsychotics are effective in treating parasitoses. Both classes of drugs have been shown to exert dopaminergic activity. New chemical entities that are liable to alter neurochemical changes related to the brain's perception of the risk of predation secondary to parasites may result in new approaches for the treatment of psychosis (Treuer et al. 2007). To prove the above hypothesis, some efforts to modify phenothiazines to develop an antimalarial agent failed but did lead to novel antiemetic-sedative antihistamines, including promethazine, promazine, and eventually chlorpromazine, the first effective treatment for schizophrenia and mania. Chlorpromazine has antipsychotic and antimanic properties, and it revolutionized the therapeutics of psychotic illnesses (Frankenburg and Baldessarini 2008).

In an effort to prove this hypothesis, a study was conducted by Shibre et al. (2009) in which patients with schizophrenia received trimethoprim as an adjuvant treatment but did not show any significant improvement in symptoms compared with those on placebo. The assumption is that if the symptoms of schizophrenia are caused by *T. gondii* infection, it may be possible to improve the symptoms through inhibition of *Toxoplasma* replication (Shibre et al. 2009).

Forty-two studies were subjected to a meta-analysis to define the association between *T. gondii* exposure and the risk of schizophrenia (Torrey and Yolken 2003, Torrey et al. 2007). The authors found that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii*, with a combined odds ratio of 2.73. The authors observed three major problems with the plausibility of *T. gondii* being aetiologically linked to schizophrenia. One is that these studies are serological in nature and not based on direct detection of *Toxoplasma* organisms or DNA. The second problem is epidemiological: schizophrenia has not been found to be unusually prevalent in countries like France and Ethiopia, where undercooked or raw meat is regularly consumed. A third problem is that a majority of individuals with schizophrenia do not have measurable antibodies to *T. gondii*.

Based on a large number of studies that have examined the role of environmental factors and genetic determinants of susceptibility, especially studies linking perinatal and postnatal infections to schizophrenia, there has been a renewed interest in the possible role of some viruses (influenza, rubella, HSV-2) and parasitic infections in the aetiopathogenesis of schizophrenia (Brown 2006).

Latent toxoplasmosis, the life-long presence of *T. gondii* cysts and anti-*Toxoplasma* antibodies in immunocompetent subjects, has generally been considered to be asymptomatic and harmless (Remington and Krahenbuhl 1982). Two previous studies regarding toxoplasmosis and schizophrenia have been carried out in Turkey. Cetinkaya et al. (2007) reported that individuals with schizophrenia had significantly more IgG antibodies to *T. gondii* than the patients with depressive disorders or healthy controls. Tanyuksel et al. (reported in Torrey et al. 2007), by contrast, did not find any significant difference in seroprevalence of toxoplasmosis between individuals with schizophrenia and healthy controls.

The aim of this study was to assess the prevalence of seropositivity for latent toxoplasmosis in individuals with schizophrenia and to discuss the relationship of latent infection with *Toxoplasma gondii* to the aetiopathogenesis of schizophrenia.

MATERIALS AND METHODS

This is a cross-sectional, retrospective study of 300 individuals who had been admitted to the inpatient units of the Psychiatry Department of Cerrahpasa Medical Faculty, University of Istanbul (Turkey), and Bakirkoy Mental Health Hospital (Istanbul, Turkey) between July and December 2007. All individuals had been diagnosed with schizophrenia according to the *Structured Clinical Manual of Mental Disorders*, Fourth Edition (First et al. 1997). Our study included 120 males (40%) and 180 females (60%) of the ages 25 to 65 (mean 42.6) years.

We included two control groups. The first control group consisted of 150 patients, including 56 males (37%) and 94 females (63%) with a mean age of 44.5 years who were admitted to the outpatient units of the Psychiatry Department during the same period as the study group. Their diagnoses included panic attack (97 patients), depression (97 patients), anxiety disorders (29 patients), and obsessive compulsive disorders (26 patients).

The second control group consisted of 150 potential blood donors who were tested in the Department of Clinical Microbiology of the Cerrahpasa Medical Faculty. This group included 58 males (39%) and 92 females (61%) with a mean age of 42.6 years. All of them declared having no history of schizophrenia or any clinical symptoms of acute toxoplasmosis.

The patient group with schizophrenia and the two control groups were matched for age, gender, and residence (Table 1). All study participants were lifelong residents of the European section of Istanbul.

A questionnaire was verbally administered to all individuals in the study including information about their age, education, occupation, marital status, family history, medical history, and family ownership of dogs, cats, and other pets up to the subject's age of 13. Individuals with schizophrenia were also asked about their diagnosis, onset of symptoms, and medications.

Serological techniques. Five millilitres of blood were taken from the patients under sterile conditions. The blood samples were centrifuged at 1000 rpm and the sera stored at –70°C until analyzed. A commercial Micro Enzyme Immuno Assay (mEIA) kit (Meddens Diagnostica BV, Netherlands) was used for the detection of anti-*T. gondii* IgG and IgM antibodies. The titre of anti-*Toxoplasma* antibodies in the sera was measured at dilutions of 1:20 and evaluated according to the guidelines of the National Committee on Clinical Laboratory Standards (Garcia et al. 2004). For ELISA assays, absorbance values of <0.9 were considered to be negative, and values between 0.9 and 1.1 were considered to be threshold. Threshold cases were repeated after 2 to 3 weeks or another test was used for confirmation. Absorbance values of >1.1 were considered to be positive. Latent toxoplasmosis was defined as being positive for IgG antibodies and negative for IgM antibodies by ELISA. To confirm the result, the Sabin-Feldman Dye Test (SFDT), a gold standard, was used, in which the positivity index was >16.

Statistics. Statistical analyses were performed with the UNISTAT 5.0 statistical package for Windows (UNISTAT Ltd., London, UK) and SPSS 10.0 for Windows TM (SPSS Inc., Chicago, IL). The univariate data analysis was done using the contingence tables. A logistic regression method was used for the multivariate analysis. The forward conditional method was used for selection of the final model. All statistical tests were two-sided and a p-value lower than 0.05 was considered statistically significant.

Table 1. Demographical properties of schizophrenia patients and control groups.

Demographical properties	Schizophrenia n (%)	Patient control n (%)	Healthy control n (%)
Gender			
Male	120 (40%)	56 (37%)	58 (39%)
Female	180 (60%)	94 (63%)	92 (61%)
Age (years)			
25–34	51 (17%)	52 (35%)	39 (26%)
35–50	91 (30%)	62 (41%)	49 (33%)
51–65	158 (53%)	36 (24%)	62 (41%)
Cat contact history			
Yes	176 (59%)	9 (6%)	14 (9%)
No	124 (41%)	141(94%)	136 (91%)
Dog contact history			
Yes	23 (8%)	5 (3%)	6 (4%)
No	277 (93%)	145 (97%)	144 (96%)
Disease duration	8–57 years	–	–
Education			
Illiterate+Primary school	222 (74%)	52 (35%)	23 (15%)
High school	54 (18%)	59 (39%)	58 (39%)
University	24 (8%)	39 (26%)	69 (46%)
Marital status			
Single	207 (69%)	34 (23%)	37 (25%)
Married	35 (11%)	78 (52%)	83 (55%)
Divorced	58 (20%)	38 (25%)	30 (20%)
Job status			
Have a job	25 (8%)	87 (58%)	117 (78%)
Jobless	275 (92%)	63 (42%)	33 (22%)
Psychiatric disease at least in one relative			
Yes	136 (45%)	32 (21%)	24 (16%)
No	164 (55%)	118 (79%)	126 (84%)
Schizophrenia history in family			
Yes	94 (31%)	3 (2%)	2 (1%)
No	206 (69%)	147 (98%)	148 (99%)
Underlying disease			
Diabetes	7 (2%)	4 (3%)	3 (2%)
Cardiac disease	12 (4%)	11 (7%)	7 (5%)

RESULTS

The percentages of anti-*T. gondii* IgG antibody positivity in the patients with schizophrenia, in the control group with depression and anxiety disorders, and in the healthy controls were found to be 60.7%, 36.7%, and 45.3%, respectively. The difference in IgG antibody positivity between the schizophrenia group (182/300) and the healthy control group (68/150) was significant ($X^2 = 9.52$, OR = 1.86, p = 0.002); the difference between the schizophrenia group and the patient control group (55/150) was highly significant ($X^2 = 23.11$, OR = 2.66, p = 0.001). The difference in IgG antibody positivity between the schizophrenia group and the control groups was significant or nearly significant when analyzed separately for men and women (see Tables 2a and 2b for results for particular age groups and genders).

Close cat contact, less education and 51–65-year age group were found to be risk factors of schizophrenia in the univariate logistic analyses (Table 3).

The results of univariate logistic analyses of toxoplasmosis × schizophrenia interaction in schizophrenia patients calculated separately for subjects with and without cat contact were as follows. There was a nonsignificant positive association between toxoplasmosis and schizophrenia for individuals with contact with a cat (OR = 2.221, p = 0.127, CI_{95} = 0.796–6.192), and significant negative association between toxoplasmosis and schizophrenia for individuals without contact with a cat (OR = 0.532, p = 0.009, CI_{95} = 0.332–0.854).

The results of multivariate logistic regression with dependent factor schizophrenia (schizophrenia × pooled controls) and independent factors latent toxoplasmosis, cat contact, age, gender, and education are shown in Table 4. Close cat contact (OR = 2.679, p<0.001), 51–65-year age group (OR = 1.703, p<0.001) and education [illiterate+primary (OR = 6.146, p<0.001) and high school (OR = 1.974, p = 0.023)] were observed as independent risk factors. Close years cat contact, defined as the family having owned a cat in the subject's childhood up to 13 years, was reported by 176/300 (59%) of individuals with schizophrenia, 9/150 (6%) of the patient control group, and 14/150 (9%) of the healthy controls. The difference was statistically highly significant (p<0.001) (Table 1). Among individuals with schizophrenia, 152 of the 176 (86%) patients who had had close contact with cats were seropositive for *Toxoplasma* IgG antibody. The difference in *Toxoplasma* IgG seropositivity between individuals with and without close cat contact was statistically highly significant in subjects with schizophrenia (p<0.0001, OR = 19.078, CI_{95} = 10.556–34.480). No significant difference in seropositivity was detected between individuals with and without close cat contact in the patient control group (p = 0.054, OR = 3.755, CI_{95} = 0.900–15.669) but significant difference was detected between individuals with and without close cat contact in the healthy control group (p = 0.009, OR = 5.082, CI_{95} = 1.356–19.048). Close dog contact was reported by 23/300 (8%) of individuals with schizophrenia, 5/150 (3%) of the patient controls, and 4/150 (2.6%) of the healthy controls. Among individuals with schizophrenia, 15 of the 23 (65%) patients who had close contact with dogs in their childhood were seropositive for *Toxoplasma* IgG antibody. We did

Table 2a. The distribution of latent toxoplasmosis according to the age and gender in schizophrenia patients and patient control group.

	Schizophrenia	Patient control	X^2	p	OR	CI_{95}
Age						
25–34	23/51 (45.1%)	15/52 (28.8%)	2.921	0.087	2.026	(0.89–4.57)
35–50	50/91 (54.9%)	23/62 (37.1%)	4.709	0.030	2.068	(1.06–4.00)
51–65	109/158 (69%)	17/36 (47.2%)	6.101	0.014	2.486	(1.19–5.19)
Gender						
Male	78/120 (65%)	25/56 (44.6%)	6.519	0.011	2.303	(1.20–4.39)
Female	104/180 (57.8%)	30/94 (31.9%)	16.530	<0.001	2.919	(1.72–4.93)

Abbreviations: X^2 – chi square; p – probability; OR – odds ratio; CI_{95} – confidence interval.

Table 2b. The distribution of latent toxoplasmosis according to the age and gender in schizophrenia patients and healthy control group.

	Schizophrenia	Healthy control	X^2	p	OR	CI_{95}
Age						
25–34	23/51 (45.1%)	19/39 (48.7%)	0.116	0.733	0.865	(0.37–1.99)
35–50	50/91 (54.9%)	23/49 (46.9%)	0.818	0.366	1.379	(0.68–2.76)
51–65	109/158 (69%)	26/62 (41.9%)	13.744	<0.001	3.080	(1.67–5.65)
Gender						
Male	78/120 (65%)	24/58 (41.4%)	8.917	0.003	2.631	(1.38–5.00)
Female	104/180 (57.8%)	44/92 (47.8%)	2.431	0.119	1.493	(0.90–2.47)

Abbreviations: see Table 2a.

Table 3. Result of univariate logistic regressions.

	B	SE	Sig	OR	CI_{95} for OR	
					Lower	Upper
Cat contact	2.825	0.247	**0.0001**	16.861	10.399	27.338
Toxoplasma IgG	0.797	0.167	**0.0001**	2.220	1.601	3.076
Age						
25–34 (r)						
35–50	0.38	0.225	0.091	1.463	0.941	2.273
51–65	1.057	0.217	**0.001**	2.877	1.880	4.402
Gender						
Female (r)						
Male	0.084	0.167	0.616	1.088	0.783	1.510
Education						
Illiterate+Primary school	2.589	0.262	**<0.001**	13.320	7.967	22.270
High school	0.731	0.279	**0.008**	2.077	1.201	3.590
University (r)						

Separate analyses were performed for dependent factor schizophrenia (schizophrenia × pooled controls) and particular independent variables, i.e. age, cat contact, gender, *Toxoplasma* IgG positivity and education. Significant results are printed in bold. *Abbreviations*: B – beta regression coefficient; SE – standard error; Sig – significant; OR – odds ratio; CI_{95} – confidence interval.

Table 4. The results of multivariate logistic regression.

	B	SE	Sig	OR	CI_{95} for OR	
					Lower	Upper
Toxoplasma IgG	−0.030	0.122	0.806	0.971	0.765	1.232
Cat contact	0.985	0.136	**<0.001**	2.679	2.051	3.499
Age						
25–34 (r)						
35–50	−0.165	0.145	0.255	0.848	0.638	1.126
51–65	0.532	0.144	**<0.001**	1.703	1.284	2.259
Education						
Illiterate+Primary school	1.816	0.299	**<0.001**	6.146	3.419	11.04
High school	0.680	0.298	0.023	1.974	1.100	3.54
University (r)						

Dependent factor schizophrenia (schizophrenia × pooled controls) and seven independent binary variables (latent toxoplasmosis, cat contact, age 35–50 years, age 51–65 years, gender, no or primary school education, high school education) entered the analysis and the forward conditional method was used for selection of the final model. Significant results are printed in bold. *Abbreviations*: B – beta regression coefficient; SE – standard error; Sig – significant; OR – odds ratio; CI_{95} – confidence interval.

not evaluate the role of close contact with dog and other pets in the pathogenesis of schizophrenia, because there was a low rate of dog and other pet ownership in all of the groups.

DISCUSSION

Our results are consistent with the meta-analysis of Torrey et al. (2007), since we detected a statistically significant difference for *Toxoplasma* IgG seropositivity in serological tests (EIA, SFDT) between the schizophrenia and control groups. The OR was detected as 2.66 (comparing the schizophrenia patients to those without schizophrenia) and 1.86 (comparing the schizophrenia patients to healthy controls). *Toxoplasma* IgG positivity was detected as a risk factor by univariate regression analysis. However, the effect of *Toxoplasma* IgG positivity disappeared in the complex model (multivariate logistic regression).

No significant difference was detected between males and females with schizophrenia for IgG positivity, and age and gender did not show any significant differences individually in the groups for *Toxoplasma* IgG positivity. Similar results for cases of schizophrenia were also reported by Alvarado-Esquivel et al. (2006) in Mexico.

On the other hand, Dickerson et al. (2007) reported that among 358 patients with schizophrenia who had serological evidence of toxoplasmosis, more were female. The opposite result, i.e., higher seropositivity in male than female students, was reported by Lindová et al. (2006).

In the present study for the 51–65-year age group, anti-toxoplasmosis IgG antibodies were significantly more prevalent in the schizophrenia group compared to the patient controls ($X^2 = 6.101$, OR = 2.486, $p = 0.014$) or healthy controls ($X^2 = 13.744$, OR = 3.080, $p<0.001$). The 51–65-year age was found to be risk factor in the univariate analysis.

To be a member of the education groups illiterate +primary and high school was also found to be a risk factor (in comparison with a university education) in univariate analysis and the effect of illiterate+primary and high school did not disappear in the complex model. The reason of this may depend on the number of schizophrenia patients in this group (55% of the schizophrenia patients were graduated from primary school and only 19% of the schizophrenia patients were illiterate). We selected our patients from the Bakirkoy Mental Hospital. This hospital is a well-known and modern hospital that specializes in mental diseases. For this reason, it is possible to diagnose schizophrenia in all age groups. We suggest that patients were diagnosed with schizophrenia when their symptoms were started. Similar to our results, *Toxoplasma* IgG seropositivity was reported to be significantly higher in individuals older than 45 years when compared with individuals younger than 45 years in 277 cases with schizophrenia and 465 cases with major depression by Selch et al. (2007). By contrast, Dickerson et al. (2007) reported that 358 individuals with schizophrenia who had serological evidence of *Toxoplasma* infection were more likely to be female but did not differ in age, and they suggested that the absence of an age effect for *Toxoplasma* seropositivity may represent a type 2 error that can be avoided with a larger sample size.

The results of the present study from Istanbul can also be compared with two previous studies done elsewhere in Turkey. In the present study, *T. gondii* IgG positivity was 61% in patients with schizophrenia, 37% in patients with anxiety and depression, and 45% in healthy controls. This is very similar to the study of Cetinkaya et al. (2007) from Afyon, in which the *T. gondii* seropositivity rate was 66% in patients with schizophrenia, 24% in patients with depression, and 22% in healthy controls. Tamer et al. (2008) also reported that seropositivity to *Toxoplasma* IgG antibodies was significantly higher in schizophrenia patients than controls ($p<0.05$). By contrast, the study by Tanyuksel et al. (2007) from Ankara reported *T. gondii* seropositivity as being 36% in patients with schizophrenia and 33% in healthy controls, which was not significantly different.

The prevalence of close cat contact in the individuals with schizophrenia (59%) compared to the patient controls (6%) or healthy controls (9%) is one of the most striking findings from this study. The number of contacts with dog and other pets was too low for any analyses. There was a significant negative association between toxoplasmosis and schizophrenia for individuals without contact with a cat (OR = 0.532, $p = 0.009$, $CI_{95} = 0.332–0.854$). This result (as well as the absence of effect of toxoplasmosis on risk of schizophrenia in multivariate logistic regression method of the model containing independent factors toxoplasmosis and cat) suggests that toxoplasmosis has no effect on risk of schizophrenia in Turkey and may be just an indicator of a passed contact with a cat. If this is true,

then possibly some other pathogen (common in Turkey) transmitted from the cat to human could be responsible for higher probability of schizophrenia. There are other infectious agents that have been reported to sporadically cause psychotic symptoms: viruses such as herpes simplex viruses (HSV-1 and HSV-2), Epstein-Barr virus, cytomegalovirus (CMV), influenza, measles, rubella, mumps, polio, vaccinia, enteroviruses such as Coxsackie B4, arboviruses such as Eastern equine encephalitis virus, retroviruses such as human immunodeficiency virus (HIV), endogenous human retroviruses, and Borna disease virus (BDV) (Torrey 1986, Little and Sunderland 1998, Caroff et al. 2001).

The results also suggest that schizophrenics had really been more often in contact with a cat in their past, not just had higher tendency to report a contact with a cat. There was a nonsignificant positive association between toxoplasmosis and schizophrenia for individuals with contact with a cat (OR = 2.221, p = 0.127, CI_{95} = 0.796–6.192).

Close cat contact was significant in all analyses, including the multivariate analysis with cat and toxoplasmosis as independent factors. This is in line with results of several other studies. For example, significant differences were detected between patient and control groups in exposure to the cats in two studies reported by Torrey and Yolken. They reported that among the individuals with serious mental illnesses, 84 of the 165 (51%) had a house cat in childhood versus 65 of the 165 case controls (38%) (Torrey and Yolken 1995). They also carried out a case-control telephone survey of 264 mothers of individuals with a severe psychiatric disorder (schizophrenia, schizoaffective disorder, or bipolar disorder) and 528 mothers of matched controls. They reported that cat ownership between birth and age 13 was significantly more common in cases than in controls (p = 0.0072). They also detected that dog (p = 0.0937) and other pet ownership (p = 0.0424) between birth and age 13 was not significantly different after a Bonferroni's correction for multiple tests (Torrey et al. 2000). Owning a cat has been shown to be a risk factor for human toxoplasmosis in some previous studies (MacKnight and Robinson 1992, Kapperud et al. 1996, Kortbeek et al. 2004), although other studies have reported that cat contact is not a risk factor for *Toxoplasma* infection in some populations (Wallace et al. 1993, Bobic et al. 1998, Cook et al. 2000, Ertug et al. 2005). Cat contact (as well as low education) may, in fact, be a proxy for a certain lifestyle, and the real source of infection may be, for example, eating or tasting raw meat or fresh (unwashed) vegetables, drinking contaminated water, or other factors. Blood groups A, B, or AB, compared with blood group 0, have also been associated significantly with *Toxoplasma* infection (Kolbekova et al. 2007). Contact with soil, gardening, and soil-related occupations have been shown to be risk factors for toxoplasmosis in a number of studies (Jeannel et al. 1988, MacKnight and Robinson 1992, Jones et al. 2001, Kortbeek et al. 2004). The association between residence in a small town/village and having toxoplasmosis probably reflects more frequent contact with soil through gardening and farming in rural areas (Kolbekova et al. 2007). There are many immigrants in Istanbul from small towns/villages, and many live in low socio-economic conditions. They may continue such habits as having frequent contact with soil and close contact with animals. We suggest that the high rate of latent toxoplasmosis could be due to such factors.

The present study has several limitations. The study population included relatively older individuals (53%) and the findings may therefore differ from findings in younger populations. Self-reported data obtained from questionnaires could potentially introduce a bias. Several factors thought to be risk factors for toxoplasmosis were not assessed; consumption of drinking water other than bottled water, drinking unfiltered water, and eating unwashed raw vegetables, meat, or fruit. This study was a case-control and cross-sectional study; the issue of temporality is problematic in such studies, since it is possible that these individuals were exposed to *Toxoplasma* after the onset of their symptoms. For this reason, there is a pressing need for prospective cohort studies that assess exposure in individuals prior to the onset of psychiatric symptoms (Yolken and Torrey 2006).

In conclusion, close cat contact and the following education levels, illiterate+primary and high school, toxoplasmosis and 51–65-year group were risk factors for schizophrenia according to the univariate analyses. However, the effect of toxoplasmosis on risk of schizophrenia disappeared in the complex model (multivariate logistic regression) while the others were still found to be as independent risk factors. Based on the results of our study, the toxoplasmosis has no direct effect on the risk of schizophrenia (is not a risk factor for schizophrenia in Turkey) and is just an indication of previous contacts with a cat. This result is confirmed by much stronger effect of contact with cat than that of toxoplasmosis in univariate analyses and by negative effect of toxoplasmosis on the risk of schizophrenia in subjects without the contact with a cat. We suggest that in future research, large extended serial studies are needed, including other pets (dogs and others) and based on molecular techniques or isolation of *T. gondii*, together with serological assays, that would define the relationship between *Toxoplasma* infection and schizophrenia.

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REFERENCES

Alvarado-Esquivel C., Sifuentes-Alvarez A., Narro-Duarte S.G., Estrada-Martinez S., Diaz-Garcia J.H., Liesenfeld O., Canales-Molina A. 2006: Seroepidemiology of *Toxoplasma gondii* infection in pregnant women in a public hospital in northern Mexico. BMC Infect. Dis. 6: e113.

Bobic B., Jevremovic I., Marinkovic J., Sibalic D., Djurkovic-Djakovic O. 1998: Risk factors for *Toxoplasma* infection in a reproductive age female population in the area of Belgrade, Yugoslavia. Eur. J. Epidemiol. 14: 605–610.

Brown A.S. 2006: Prenatal infection as a risk factor for schizophrenia. Schizophr. Bull. 32: 200–202.

Brown A.S., Schaefer C.A., Quesenberry C.P. Jr., Liu L., Babulas V.P., Susser E.S. 2005: Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. Am. J. Psychiat. 162: 767–773.

Carlsson A. 1988: The current status of the dopamine hypothesis of schizophrenia. Neuropsychopharmacology. 1: 179–186.

Carlsson A. 2001: Does dopamine have a role in schizophrenia? Biol. Psychiat. 13: 3–21.

Caroff S.N., Mann S.C., Gliatto M.F., Sullivan K.A., Campbell E.C. 2001: Psychiatric manifestations of acute viral encephalitis. Psych. Ann. 31: 193–204.

Cetinkaya Z., Yazar S., Gecici Ö., Namli M. 2007: Anti-*Toxoplasma gondii* antibodies in patients with schizophrenia – preliminary findings in a Turkish sample. Schizophr. Bull. 33: 789–791.

Cook A.J.C., Gilbert R.E., Buffolano W., Jufferey J., Petersen E., Jenum P.A., Foulon W., Semprini A.E., Dunn T.E. 2000: Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. Br. Med. J. 321: 142–147.

Dickerson F., Boronow J., Stallings C., Origoni A.,Yolken R. 2007: *Toxoplasma gondii* in individuals with schizophrenia: association with clinical and demographic factors and with mortality. Schizophr. Bull. 33: 737–740.

Ertug S., Okyay P., Turkmen M., Yuksel H. 2005: Seroprevalence and risk factors for *Toxoplasma* infection among pregnant women in Aydin province, Turkey. BMC Public Health 5: e66.

First M.B., Spitzer R.L., Gibbon M., Williams J.B.W. 1997: Structured Clinical Interview for DSM-IV Axis I Disorders – Clinical Version (SCIDCV). American Psychiatric Press, Washington, D.C., 84 pp.

Flegr J., Preiss M., Klose J., Havlíček J., Vitáková M., Kodym P. 2003: Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii*. Dopamine, a missing link between schizophrenia and toxoplasmosis? Biol. Psychol. 63: 253–268.

Frankenburg F.R., Baldessarini R.J. 2008: Neurosyphilis. malaria, and the discovery of antipsychotic agents. Harv. Rev. Psychiat. 16: 299–307.

Garcia L.S., Fritsche T., Gardy K., Healy G., McAuley J., Rocha A., Wilson M., Wong J. 2004: Clinical Use and Interpretation of Serologic Tests for *Toxoplasma gondii*; Approved Guideline. NCCLS document M36-A, Pennsylvania. 24(6), 1–21.

Gaskell E.A., Smith J.E., Pinney J.W., Westhead D.R., McConkey G.A. 2009: A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. PLoS ONE 4: e4801.

Guidetti P., Bates G.P., Graham R.K., Hayden M.R., Leavitt B.R., MacDonald M.E., Slow E.J, Wheeler V.C, Woodman B., Schwarcz R. 2006: Elevated brain 3-hydroxykynurenine and quinolinate levels in Huntington disease mice. Neurobiol. Dis. 23: 190–197.

Hay J., Aitken P.P., Autchinson W.M., Graham D.J. 1983: The effect of congenital and adult-acquired *Toxoplasma* infection on activity and responsiveness to novel stimulation in mice. Ann. Trop. Med. Parasitol. 77: 483–495.

Henriquez S.A., Brett R., Alexander J., Pratt J., Roberts C.W. 2009: Neuropsychiatric disease and *Toxoplasma gondii* infection. Neuroimmunomodulation 16: 122–133.

Hill A.R. 1965: The environment and disease: association or causation? Proc. R. Soc. Med. 58: 295–300.

Hodková H., Kolbeková P., Skallová A., Lindová J., Flegr J. 2007: Higher perceived dominance in *Toxoplasma* infected men – a new evidence for role of increased level of testosterone in toxoplasmosis-associated changes in human behavior. Neuroendocrinol. Lett. 28: 110–114.

Hrdá Š., Votýpka J., Kodym P., Flegr J. 2000: Transient nature of *Toxoplasma gondii*-induced behavioral changes in mice. J. Parasitol. 86: 657–663.

Hutchison W.M., Aitken P.P., Wells B.W. 1980: Chronic *Toxoplasma* infections and motor performance in the mouse. Ann. Trop. Med. Parasitol. 74: 507–510.

Jeannel D., Niel G., Costagliola D., Danis M., Traore B.M., Gentilini M. 1988: Epidemiology of toxoplasmosis among pregnant women in the Paris area. Int. J. Epidemiol. 17: 595–602.

Jones J.L., Kruszon-Moran D., Wilson M., McQuillan G., Navin T., McAuley J.B. 2001: *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. Am. J. Epidemiol. 154: 357–365.

Kapperud G., Jenum P.A., Stray-Pedersen B., Melby K.K., Eskild A., Eng J. 1996: Risk factors for *Toxoplasma gondii* infection in pregnancy. Results of a prospective case-control study in Norway. Am. J. Epidemiol. 144: 405–412.

Kolbekova P., Kourbatova E., Novotna M., Kodym P., Flegr J. 2007: New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic. Clin. Microbiol. Infect. 13: 1012–1017.

Kortbeek L.M., De Melker H.E., Veldhuijzen I.K., Conyn-van Spaendonck M.A. 2004: Population-based *Toxoplasma* seroprevalence study in The Netherlands. Epidemiol. Infect. 132: 839–845.

Lindová J., Novotná M., Havlíček J., Jozífková E., Skallová A., Kolbeková P., Hodný Z., Kodym P., Flegr J. 2006: Gender differences in behavioral changes induced by latent toxoplasmosis. Int. J. Parasitol. 36: 1485–1492.

Little J.T., Sunderland T. 1998: Psychosis secondary to encephalitis and encephalopathies. Semin. Clin. Neuropsychiat. 3: 4–11.

MacKnight K.T., Robinson H.W. 1992: Epidemiologic studies on human and feline toxoplasmosis. J. Hyg. Epidemiol. Microbiol. Immunol. 36: 37–47.

Melamede R. 2009: Parasitic brain infection, endocannabinoids, and schizophrenia. Med. Hypotheses 72: 220–222.

Miller C.L., Llenos I.C., Dulay J.R., Barillo M.M., Yolken R.H., Weis S. 2004: Expression of the kynurenine pathway

enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. Neurobiol. Dis. 15: 618–629.

Novotná M., Hanusova J., Klose J., Preiss M., Havlicek J., Roubalová K., Flegr J. 2005: Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. BMC Infect. Dis. 5: e54.

Remington J.S., Krahenbul J.L. 1982: Immunology of *Toxoplasma gondii*. In: A.J. Nahmias, and J. O'Reilly (Eds.), Immunology of Human Infection. Part II. Plenum Publishing Corporation, New York, pp. 327–231.

Schwarcz R., Hunter C.A. 2007: *Toxoplasma gondii* and schizophrenia: linkage through astrocyte-derived kynurenic acid? Schizophr. Bull. 33: 652–653.

Schwarcz R., Rassoulpour A., Wu H.Q., Medoff D., Tamminga C.A., Roberts R.C. 2001: Increased cortical kynurenate content in schizophrenia. Biol. Psychiat. 50: 521–530.

Selch D.H., Däubener 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.

Shibre T., Alem A., Abdulahi A., Araya M., Beyero T., Medhin G., Deyassa N., Negash A., Fekadu A. 2009: Trimethoprim as adjuvant treatment in schizophrenia: a double-blind, randomized, placebo-controlled clinical trial. Schizophr. Bull. Published online June 16, 2009.

Silva N.M., Rodrigues C.V., Santoro M.M., Reis L.F.L., Alvarez-Leite J.I., Ricardo T., Gazzinelli R.T. 2002: Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. Infect. Immun. 70: 859–868.

Sims T.S., Hay J. 1995: Host-parasite relationship between congenital *Toxoplasma* infection and mouse brain: role of small vessels. Parasitology 110: 123–127.

Skallová A., Kodym P., Frynta D., Flegr J. 2006: The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. Parasitology 133: 525–535.

Stibbs H.H. 1985: Changes in brain concentrations of catecholamines and indoleamines in *Toxoplasma gondii* infected mice. Ann. Trop. Med. Parasitol. 79: 153–157.

Tamer G.S., Dundar D., Yalug I., Caliskan S., Yazar S., Aker A. 2008: The schizophrenia and *Toxoplasma gondii* connection: infectious, immune or both? Adv. Ther. 25: 703–709.

Tanyuksel M., Uzun O., Araz E., Koru O., Babur C. 2007: Investigation of possible role of toxoplasmosis in patients with first episode schizophrenia. 56th American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting, 4–8 November, Philadelphia, USA.

Torrey E.F. 1986: Functional psychoses and viral encephalitis. Integr. Psychiat. 4: 224–236.

Torrey E.F., Bartko J.J., Lun Z.R., Yolken R.H. 2007: Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. Schizophr. Bull. 32: 729–736.

Torrey E.F., Leweke M.F., Schwarz M.J., Mueller N., Bachmann S., Schroeder J., Dickerson F., Yolken R.H. 2006: Cytomegalovirus and schizophrenia. CNS Drugs 6: 879–885.

Torrey E.F., Rawlings R., Yolken R.H. 2000: The antecedents of psychoses: a case-control study of selected risk factors. Schizophr. Res. 46: 17–23.

Torrey E.F., Yolken R.H. 1995: Could schizophrenia be a viral zoonosis transmitted from house cats? Schizophr. Bull. 21: 167–171.

Torrey E.F., Yolken R.H. 2003: *Toxoplasma gondii* and schizophrenia. Emerg. Infect. Dis. 9: 1375–1380.

Treuer T., Martenyi F., Karagianis J. 2007: Parasitosis, dopaminergic modulation and metabolic disturbances in schizophrenia: evolution of a hypothesis. Neuroendocrinol. Lett. 28: 535–540.

Wallace M.R., Rossetti R.J., Olson P.E. 1993: Cats and toxoplasmosis risk in HIV-infected adults. JAMA 269: 76–77.

Witting P.A. 1979: Learning capacity and memory of normal and *Toxoplasma*-infected laboratory rats and mice. Z. Parasitenkd. 61: 29–51.

Yolken R.H., Bachmann S., Rouslanova I., Lillehoj E., Ford G., Torrey E.F., Schroeder J. 2001: Antibodies to *Toxoplasma gondii* in individuals with first-episode schizophrenia. Clin. Infect. Dis. 32: 842–844.

Yolken R.H., Torrey E.F. 2006: Infectious agents and gene-environmental interactions in the etiopathogenesis of schizophrenia. Clin. Neurosci. Res. 6: 97–109.

Yolken R.H., Torrey E.F. 2008: Are some cases of psychosis caused by microbial agents? A review of the evidence. Mol. Psychiat. 13: 470–479.

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