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Original article

Toxoplasma-infected subjects report an Obsessive-Compulsive Disorder diagnosis more often and score higher in Obsessive-Compulsive Inventory

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ABSTRACT

Background: Latent toxoplasmosis, the life-long presence of dormant stages of *Toxoplasma* in immunoprivileged organs and of anamnestic IgG antibodies in blood, affects about 30% of humans. Infected subjects have an increased incidence of various disorders, including schizophrenia. Several studies, as well as the character of toxoplasmosis-associated disturbance of neurotransmitters, suggest that toxoplasmosis could also play an etiological role in Obsessive-Compulsive Disorder (OCD).
Methods: The aim of the present cross-sectional study performed on a population of 7471 volunteers was to confirm the association between toxoplasmosis and OCD, and toxoplasmosis and psychological symptoms of OCD estimated by the standard Obsessive-Compulsive Inventory-Revised (OCI-R).
Results: Incidence of OCD was 2.18% ($n = 39$) in men and 2.28% ($n = 83$) in women. Subjects with toxoplasmosis had about a 2.5 times higher odds of OCD and about a 2.7 times higher odds of learning disabilities. The incidence of 18 other neuropsychiatric disorders did not differ between *Toxoplasma*-infected and *Toxoplasma*-free subjects. The infected subjects, even the OCD-free subjects, scored higher on the OCI-R.

Limitations: Examined subjects provided the information about their toxoplasmosis and OCD statuses themselves, which could result in underrating the strength of observed associations.
Conclusions: The results confirmed earlier reports of the association between toxoplasmosis and OCD. They also support recent claims that latent toxoplasmosis is in fact a serious disease with many impacts on quality of life of patients.

1. Introduction

About one-third of the human population of developed countries have latent toxoplasmosis, i.e., they carry the dormant stage of the coccidian parasite *Toxoplasma gondii* in their immunoprivileged organs, e.g., brain, eyes, and testes, for the rest of their lives [1]. For a long time, latent toxoplasmosis was considered to be asymptomatic and therefore harmless for immunocompetent subjects. Within the past 15 years, more and more analytic studies show that *Toxoplasma*-infected subjects have higher incidences of certain disorders [2], especially neuropsychiatric disorders [3]. The strongest evidence for a direct etiological role of toxoplasmosis exists for schizophrenia. The association between schizophrenia and toxoplasmosis has been reported in at least 50 studies, including some prospective longitudinal studies [4–6]. These longitudinal studies showed that *Toxoplasma* infection had preceded the onset of schizophrenia for 6–36 months [7]. The presence of genes for rate-limiting enzymes of dopamine synthesis in *Toxoplasma* genome [8] as well as their expression in the brain of an infected host [9,10] suggest that an increased concentration of this neurotransmitter could be responsible for the increased incidence of schizophrenia.

Increased brain levels of dopamine in conjunction with a disturbance in the concentration of serotonin is known to play an important role not only in schizophrenia, but also in Obsessive-Compulsive Disorder (OCD) [11]. OCD is a chronic, heritable, and debilitating neuropsychiatric disorder and its incidence in the general population is about three times higher than that of schizophrenia [12]. OCD is characterized by recurrent thoughts (obsessions) and repetitive behaviors (compulsions), the latter

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often reported to "neutralize" obsessions and reduce anxiety [13].

It has been documented by two case control studies [14,15] that OCD patients have higher seroprevalence of toxoplasmosis than *Toxoplasma*-free subjects. Based on these two studies the overall Odds Ratio (OR) for OCD was computed as 3.40 (CI_{95} = 1.73–6.68) [6]. In addition, our ecological regression study performed on a set of 88 countries showed a strong correlation between the prevalence of toxoplasmosis and the Obsessive-Compulsive Disorder-related burden in European and especially in non-European countries [2]. The prevalence of toxoplasmosis explained 18 and 36% of the between-countries variability in OCD-related burden in Europe and outside Europe, respectively. Recently, another internet-based cross-sectional study was performed on a cohort of 2619 subjects tested for toxoplasmosis. This study was not primarily focused on the relation between toxoplasmosis and mental disorders; however, it also monitored the prevalence of certain mental health disorders, including OCD. The number of OCD patients in 2248 *Toxoplasma*-free subjects was 62 while in 371 *Toxoplasma*-infected subjects it was 15, suggesting that the OR is equal to 1.63 for women and to 1.73 for men [16].

1.1. Aims of the study

The present study had three aims: (1) to test whether *Toxoplasma*-infected subjects have a higher probability of reporting an OCD diagnosis, (2) to test whether *Toxoplasma*-infected subjects in a normal (non-clinical) population express a higher intensity of OCD symptoms, (3) to assess the specificity of the toxoplasmosis-mental health disorder association. For these purposes, a large-scale cross-sectional internet study on a cohort of 7471 normal subjects was carried out. In the confirmatory part of the study, we tested hypotheses 1 and 2, while in the exploratory part of the study we searched for an association between toxoplasmosis and 20 mental health disorders.

2. Methods

2.1. Participants and procedure

Subjects were invited to participate in the study using a Facebook-based snowball method [17]. An invitation to participate in "*an experiment searching for associations between keeping dogs and cats and health status and personality of a subject*" was posted on the wall of the Facebook page "Guinea pigs", a page for people willing to take part in diverse psychological and psychopathological experiments (www.facebook.com/pokusnikralici) [18]. This community consists of people who understand Czech language, i.e. Caucasians of Czech and Slovak origin, of various ages, education levels, occupations, and places of residence. To keep the study blind and avoid possible bias, no form of the term toxoplasmosis or *Toxoplasma* was mentioned during the recruitment, not even in the Informed Consent form. This omission was approved by the Institutional Review Board (IRB). The participants were informed about the general aims of the study (to study the relations of keeping dogs and cats with health status and personality traits) on the first page of the questionnaire. They were also provided with the following information: "*The questionnaire is anonymous and obtained data will be used exclusively for scientific purposes. Your cooperation in the project is voluntary and you can terminate it at any time by closing this web page. Please share the link to this questionnaire with your friends, for example on Facebook*". The share button was pressed by 906 participants, which resulted in data from a total of 7500 responders from 20th August 2014 to 23rd December 2015.

2.2. Serological tests for toxoplasmosis

Pregnant women are not subjected to obligatory testing for toxoplasmosis neither in Czechia, nor Slovakia. Some blood donors as well as subjects who were suspected of having acute or chronic toxoplasmosis were tested for the presence of anti-*Toxoplasma* antibodies in various clinical facilities. However, most of the women and nearly all of the men who know their *Toxoplasma*-infection status were tested for toxoplasmosis by us during systematic research of behavioral effects of latent toxoplasmosis, which has been running at the Faculty of Science for 20 years. Within the past 3 years, we have also regularly offered such testing to registered members of the "Guinea pigs" community 2–3 times a year; about 1000 of them have already been tested. The complement-fixation test, which determines the overall levels of IgM and IgG antibodies of particular specificity and Enzyme-Linked Immunosorbent Assays (ELISA) (IgG ELISA: SEVAC, Prague), were used to detect the toxoplasmosis status of the subjects. Only subjects with clearly positive results of CFT and IgG ELISA tests were diagnosed as *Toxoplasma*-infected (or conversely *Toxoplasma*-free).

In a similar study performed recently on the "Guinea pigs" population, we also asked the responders when they had been tested for toxoplasmosis. The results showed that *Toxoplasma*-free participants had been tested relatively recently (mode = 1 year, median = 3.0 years, mean = 5.4 years). The highest rate of infection is in early childhood in Czechia and Slovakia. Therefore, the fraction of subjects with negative tests who were *Toxoplasma*-infected at the time of our study was probably relatively low.

2.3. Electronic questionnaire

The questionnaire was distributed as a Qualtrics (Provo, UT) survey. Responders used a 5-point Likert scale (1: never, 2: minimally (1–2 times in life), 3: rarely, 4: from time to time, 5: very often), to rate their past contacts with six known or suspected toxoplasmosis-associated risk factors (eating or tasting raw meat, touching soil during gardening, eating root vegetables that were not washed properly, drinking water from suspicious sources like creeks, having sex without a condom with numerous people). The responders were asked about the size of the communities they grew up in (1: less than 1000 inhabitants, 2: 1–5 thousand inhabitants, 3: 5–50 thousand inhabitants, 4: 50–100 thousand inhabitants, 5: 100–500 thousand inhabitants, 6: more than 500 thousand inhabitants). The subjects were also asked to rate the intensity of their life-long contact with cats (definitive hosts of *Toxoplasma*) using a 7-point scale (1 – never, 2 – we kept a cat only in the past and only for a short time, 3 – we kept a cat only in the past but for a long time, 4 – we have one cat, 5 – we have two cats, 6 – we have three cats, 7 – we have more than three cats) and whether they are *Toxoplasma*-infected. They were reminded that *Toxoplasma* is "*a parasite of cats, dangerous especially to pregnant women*". The response "I do not know, I am not sure" was set as a default answer which the responders could change by selecting either "*No, I was tested by a doctor and the result of my laboratory tests was negative*" or "*Yes, I was tested by a doctor and I had antibodies against Toxoplasma*". The responders of our questionnaires had three options: they could complete any questionnaire absolutely anonymously, they could sign the finished questionnaire by a code obtained after the anonymous registration, or they could sign the finished questionnaire by a code obtained after the non-anonymous registration. When we checked the information about toxoplasmosis status provided in 190 signed questionnaires with the corresponding information in our records, we found a perfect (100%) agreement.

Our electronic questionnaire contained a checklist of 20 mental health disorders, which included obsessive compulsive disorder (see Table 3), and the Obsessive-Compulsive Inventory-Revised (OCI-R), a highly effective 18-item theoretically driven self-report measure with high specificity for symptoms of OCD compared with symptoms of other anxiety disorders, as well as with a non-clinical population [19]. The questionnaire took subjects 20–40 min to complete and about 76% of subjects who started the study finished the whole questionnaire.

2.4. Statistical analysis

Univariate tests (Chi^2 test), ANCOVA (Effective hypothesis decomposition, Type VI method of main effect construction, Sigma-restricted parametrization), and logistic regressions (case-wise deletion of missing data, Quasi-Newton estimation method, asymptotic data errors) were performed by the statistical software Statistics v. 10.0. The effect of toxoplasmosis on OCI-R score was assessed by multivariate analysis of covariance with toxoplasmosis and age as independent variables. The associations between binary and ordinal predictors and OCD or toxoplasmosis were studied by logistic regressions. The Fisher exact test was calculated using the on-line statistical calculator at http://www.socscistatistics.com/tests/fisher/default2.aspx. Before the analyses, records of 29 subjects younger than 15 years and 8 other subjects with suspicious data (mostly too short in stature or the same responses to all questions) were excluded from the data set.

3. Results

The questionnaire was completed by 7471 subjects, including 2682 men (mean age 35.6, SD = 12.4) and 4789 women (mean age 32.9, SD = 12.3). Information about mental health was provided by 1790 men and 3646 women and the OCI-R questionnaire was completed by 1802 men and 3666 women. For the age structure of the population see Fig. 1. Among the men, 281 subjects were *Toxoplasma*-free (mean age 34.4, SD = 13.4) and 65 *Toxoplasma*-infected (mean age 33.1, SD = 9.3), while in women, 831 were *Toxoplasma*-free (mean age 31.7, SD = 11.4) and 350 *Toxoplasma*-infected (mean age 35.8, SD = 11.1). The prevalence of toxoplasmosis was significantly higher in women (29.6%) than in men (18.8%), $Chi^2 = 16.1$, $P < 0.0001$. Information about mental health was provided by 289 men and 1043 women and the OCI questionnaire was completed by 272 men and 1003 women, who also provided information about their toxoplasmosis status. Incidence of OCD was 2.18% ($n = 39$) in men and 2.28% ($n = 83$) in women in the whole set and 2.08% ($n = 6$) in men and 1.92% ($n = 20$) in women in the subjects who also provided information about their toxoplasmosis status.

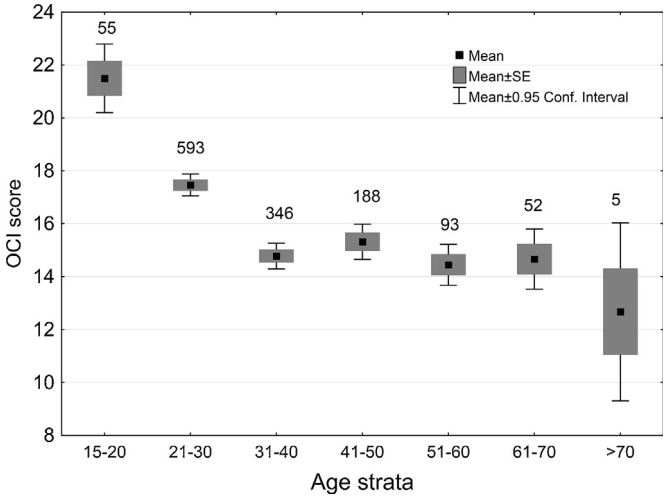

Fig. 1. OCD symptoms in different age groups. The intensity of symptoms (OCI score) was measured with Obsessive-Compulsive Inventory-Revised. The numbers indicate numbers of subjects in particular age groups.

The effect of toxoplasmosis on OCI-R score was assessed by multivariate analysis of covariance with toxoplasmosis and age as independent variables. The *Toxoplasma*-infected subjects had significantly higher OCI-R scores (17.2) than *Toxoplasma*-free subjects (16.1) ($F_{1,1334} = 6.722$, $P = 0.007$). The effect of toxoplasmosis was also significant for the subpopulation of 1303 subjects who did not reportedly suffer from OCD (16.8 vs. 15.9, $F_{1,1300} = 6.47$, $P = 0.014$) but not for subpopulation of 26 subjects who reported suffering from OCD (27.7 vs. 32.7, $F_{1,23} = 0.48$, $P = 0.494$). OCI-R score decreased with the age of subjects ($F_{1,1332} = 59.4$, $P < 0.0001$), however, a more detailed inspection of data showed that it remained practically stable after the age of 30, Fig. 1. Neither sex, nor sex-toxoplasmosis interaction had any effect on OCI-R.

Logistic regression with toxoplasmosis, sex, and age as predictors showed a positive association between OCD and toxoplasmosis (OR = 2.27, $C.I._{95} = 1.01–5.09$, $P = 0.047$). To test whether toxoplasmosis or certain risk factor(s) of toxoplasmosis were responsible for the observed association, we also analyzed the more complex model containing toxoplasmosis, sex, age, the size of the communities in which the respondents grew up, intensity of life-long contact with cats, eating or tasting of raw or undercooked meat, touching soil during gardening, eating root vegetables that were not washed properly, drinking water from suspicious sources like creeks, and the risk of acquiring sexually transmitted diseases (by having sex without a condom with numerous people). Only toxoplasmosis status, not any other suspected risk factors for *Toxoplasma* infection, or age, were associated with OCD (OR = 2.55, $C.I._{95} = 1.10–5.94$, $P = 0.030$), see also Table 1. To check the integrity of the data, we repeated the logistic regression with toxoplasmosis as the dependent variable and all other predictors of infection as independent variables. Table 2 shows that toxoplasmosis was positively associated with higher age of subjects, female sex, spending childhood in smaller communities, contact with soil, and eating root vegetables that were not washed properly. There was also a nearly significant positive association with frequently having sex without a condom with various people (OR (range) = 1.56, $C.I._{95} = 0.99–2.44$, $P = 0.053$).

The specificity of the observed effect of toxoplasmosis on the probability of being OCD positive was relatively high. Of the list of 20 neuropsychiatric disorders, only the occurrence of OCD and

Table 1
Association of OCD with toxoplasmosis and potential risk factors for toxoplasmosis.

	Chi^2	P	OR	−95%CL	+95%CL
Toxoplasmosis	4.72	0.030	2.55*	1.10	5.94
Age	1.87	0.171	0.11	0.00	2.61
Sex	0.25	0.615	0.78	0.30	2.03
Size of place of living in childhood	0.01	0.940	0.96	0.30	3.03
Number of cats at house	0.83	0.363	0.50	0.12	2.21
Eating raw meat	0.17	0.677	0.76	0.21	2.75
Touching soil	1.14	0.285	0.34	0.05	2.45
Eating unwashed vegetables	0.72	0.397	2.25	0.34	14.77
Drinking suspicious water	0.73	0.394	1.96	0.42	9.27
Having risky sex	2.84	0.092	3.10	0.83	11.53

Associations were measured with one multivariate Logistic regression with all listed factors as independent variables. Significant Odds Ratio (OR) (in two side tests) is denoted with asterisk.

Table 2
Association of toxoplasmosis with its potential risk factors.

	Chi^2	*P*	OR	−95%CL	+95%CL
Age	12.80	0.000	4.01*	1.87	8.59
Sex	15.64	0.000	2.01*	1.42	2.84
Size of place of living in childhood	12.18	0.000	0.52*	0.36	0.75
Number of cats at house	0.87	0.352	1.23	0.80	1.88
Eating raw meat	0.46	0.499	0.87	0.58	1.30
Touching soil	9.35	0.002	3.14*	1.51	6.54
Eating unwashed vegetables	5.50	0.019	1.88*	1.11	3.20
Drinking suspicious water	2.36	0.124	0.68	0.41	1.11
Having risky sex	3.75	0.053	1.56	0.99	2.44

Associations were measured with one multivariate Logistic regression with all listed factors as independent variables. Significant Odds Ratios (OR) (in two side tests) are denoted with asterisk. *P* value 0.000 means $P < 0.0005$.

learning disabilities were significantly correlated with toxoplasmosis, Table 3. A strong trend was also observed for schizophrenia; however, due to the low number (4) of schizophrenia patients in the dataset, this association was not significant ($P = 0.066$, Fisher exact test).

4. Discussion

Results of the present study suggested that latent infection with the parasite *T. gondii* could be a risk factor for acquiring Obsessive Compulsive Disorder (OCD). Our data support the association between toxoplasmosis and OCD in two ways. First, infected subjects have about a 2.5 times higher oddsof reporting of having been previously diagnosed with OCD than *Toxoplasma*-free subjects. Second, the *T. gondii* positive respondents score higher on the OCI-R even when nine potential confounding factors were controlled. Our results also confirmed that spending childhood in small communities, touching soil during gardening, eating unwashed root vegetables, and possibly also having sex without condoms with many partners were risk factors for acquiring *Toxoplasma* infection (but not for OCD) in the modern Czech and Slovak population.

The present results are in agreement with already published data, namely with results of two case control studies [14,15], and with one ecological regression study [2]. They also agree with preliminary results of another cross-sectional study performed on a cohort of 25,000 internet users [16]. It must be emphasized that this study, as well as the current study, has been performed on members of the same internet community and therefore a large fraction of subjects participated in both studies. This fact will be of particular interest for authors of future meta-analyses. Here, we showed that *Toxoplasma*-infected subjects achieved higher OCI-R score than the *Toxoplasma*-free subjects. This was also true for subjects who reported not suffering from OCD, suggesting that toxoplasmosis could also be associated with certain obsessive and compulsive symptoms in a non-clinical population. This seems to agree with the observation of some *Toxoplasma*-infected subjects that "in certain situations, they are doing something with intense feeling of inappropriateness and astonishment about what they are doing" [20].

Table 3
Association between toxoplasmosis and various neuropsychiatric disorders.

	N	OR	−95%CL	+95%CL	*P*
Unipolar depression	78	1.38	0.84	1.02	0.203
Bipolar disorder	16	1.73	0.61	1.02	0.306
Schizophrenia	4	9.39	NA	NA	NA
Anxiety disorder	85	1.30	0.80	1.02	0.286
Alcohol use disorder	9	0.97	0.19	1.05	0.967
Gambling	4	2.52	NA	NA	NA
Parkinson disease	1	NA	NA	NA	NA
Alzheimer disease	2	1.87	NA	NA	NA
Drug use disorder	2	NA	NA	NA	NA
Posttraumatic disorder	20	1.54	0.62	1.06	0.354
Obsessive compulsive disorder	26	2.27*	1.01	5.09	0.047
Panic disorder	41	1.11	0.55	1.03	0.770
Insomnia primary	21	1.11	0.55	1.03	0.770
Learning disabilities	43	2.57*	1.37	1.00	0.003
Borderline personality disorder	10	0.85	0.17	1.01	0.845
Antisocial personality disorder	13	1.39	0.37	0.93	0.628
Attention deficit hyperactivity disorder	12	3.16	0.95	0.99	0.061
Bulimia, anorexia	16	1.30	0.44	1.01	0.633
Burn-out syndrome	58	0.97	0.53	1.05	0.911
Sexuological disorder	11	1.18	0.30	1.05	0.810

Associations were measured with separate Logistic regressions with presence/absence of particular disorder as dependent variables and toxoplasmosis, age, and sex as independent factors. This part of questionnaire, the mental disorder checklist, was completed by 1332 toxoplasmosis-tested subjects. Significant Odds Ratios (OR) (in two side tests) are denoted with asterisk.

The existence of a statistical association does not prove causality. Toxoplasmosis could be a cause (or rather co-cause) of OCD or OCD could increase the risk of *Toxoplasma* infection. It is also possible that some unknown, independent third factor positively influences both the risk of OCD and toxoplasmosis. It should be noted, however, that the former scenario seems to be more plausible, as the OCD-associated behavioral changes such as fear of contamination, repetitive hand washing, and social avoidance decrease rather than increase the risk of *Toxoplasma* infection. Similarly, no known toxoplasmosis risk factors were associated with OCD in our study. It has been demonstrated that approximately 47% of the variance in the risk of OCD is explained by genetic factors and the remaining variance is dependent on some environmental factors, such as infection and traumas in childhood [21]. All these influences have effects on specific anatomical circuits and neurotransmitter systems. The traditional concept was based mainly on the clinical effects of serotonergic drugs, such as SSRIs and clomipramine, indicating that OCD might be associated with a dysfunction of the serotonin system [22]. However, some evidence also pointed to the role of dopamine, based on clinical studies showing an augmented effect of atypical antipsychotics in treatment as well as on the quinpirole (agonist of dopaminergic D2 and D3 receptors) pharmacological animal model of compulsive checking [23]. Congruently, a meta-regression of 13 randomized controlled trials on add-on antipsychotic drugs to SSRIs documented a significant association between D2/D3 dopamine receptor affinities of antipsychotics and effectiveness in OCD [24]. In addition, recent evidence has emphasized the role of dysregulations of glutamate in fronto-striatal systems, including hyperactivity of the glutamatergic system in the orbito-frontal cortex and left caudate nucleus [25] as well as decreased glutamate-glutamine levels in prefrontal cortex and subcortical regions including the thalamus [26–28]. In line with this concept, several glutamatergic agents have been tested in OCD as augmentation therapy, such as riluzole, memantine, and ketamine [29].

Both dopaminergic and glutamatergic systems are substantially affected by latent toxoplasmosis. First, *Toxoplasma* is known to carry two genes for the dopamine synthesizing enzyme, tyrosine hydroxylase, in its genome [8] and induces the synthesis of this neurotransmitter in the brain tissue of infected animals [9,10] (but see also [30]), and probably also humans [31,32]. The increased dopaminergic activity could then explain the higher risk of both schizophrenia and OCD in *T. gondii* infected subjects. Second, *T. gondii* infection induces in astrocytes a production of indoleamine-2,3-dioxygenase (IDO), a rate-limiting enzyme in catabolism of tryptophan [33]. Tryptophan is degraded by IDO to kynurenine, which is either metabolized to kynurenic acid, an antagonist of the glutamate NMDA (*N*-methyl-D-aspartate) receptor [34] or hydroxylated to quinolinate, a potent NMDA neurotoxic agent [35]. Hence, both dopaminergic and glutamatergic systems are affected by *T. gondii* and could represent the mediating factors between toxoplasmosis and OCD.

It is important to remember, however, that toxoplasmosis also induces other neurological and endocrinological changes, such as an increase of testosterone in male rats and humans [36,37], and these changes could be also responsible for toxoplasmosis-associated behavioral changes and a different (more serious) clinical picture of certain mental health disorders (bipolar disorder and schizophrenia) in *Toxoplasma*-infected patients [38,39].

Up to now, the psychiatric consequences of toxoplasmosis have been studied mainly in schizophrenia. The effect of *T. gondii* on the risk of this psychosis has been confirmed in many studies, (for review see [4–6]). *Toxoplasma*-infected schizophrenia patients express characteristic gray matter changes in caudate, median cingulate, thalamus, and other regions [40]. This finding, together with the results of several prospective studies (surveyed in [6]), support the notion that toxoplasmosis plays a causal role in the etiology of psychosis. It is possible to speculate that an analogous neurotoxic effect of *T. gondii* on gray matter (mediated by glutamate and dopamine dysregulation) could contribute to the brain abnormalities related to the OCD development. Neural abnormalities in OCD are represented by structural gray matter changes in orbitofrontal cortex, anterior cingulate, prefrontal and parietal cortices, and the caudate nucleus [41,42] as well as by functional alterations within the same regions [43,44].

4.1. Limitations

The main limitation of the present study was that the examined subjects provided the information about their toxoplasmosis and OCD statuses themselves. It is possible that some of the subjects provided inaccurate or obsolete information. For example, some of them probably acquired *Toxoplasma* infection after being serologically tested for the presence of anti-*Toxoplasma* antibodies. The presence of this sort of false negative subject could be mostly avoided by serological examination of participants of future studies. It should be noted, however, that such an experimental design would result in many false negative subjects of another sort. There is strong evidence that the anti-*T. gondii* antibodies level decreases with age of infected subjects [45] and that a large fraction of serologically *Toxoplasma*-negative subjects after the age of 50 are in fact *Toxoplasma*-infected [46]. Fortunately, the stochastic noise, e.g., the presence of false negative or false positive subjects, could cause only false negative results of studies, i.e., failure to detect an existing association, not false positive results of the study, i.e., the detection of non-existing associations.

We were able to check whether participants of our studies correctly reported their toxoplasmosis status and found 100% correspondence of reported toxoplasmosis status with that in our records, see Section 2. However, there are only indirect indices that responders also correctly reported the status of clinically diagnosed OCD. First of all, the prevalence of 2% within the population of our subjects corresponds well with reported prevalence 0.7–3% in a general population [12,13]. The OCI-R score of subjects who reported suffering from OCD was nearly two times higher (30.2) than that of subjects who did not report this disorder (15.9). Again, the presence of both false OCD positive and false OCD negative subjects in our dataset could only increase the risk of false negative, not false positive results of the study see the Appendix.

The effect of toxoplasmosis on OCI-R score is relatively small. It explained only about 0.5% of the variability in OCI-R score of the whole population. This is mostly because a great deal of variability in this trait exists in the general population – even the presence of OCD explained only 3.5% of the variability in OCI-R score despite the fact that the subjects who reported an OCD diagnosis had scores about 2 times higher than other subjects. A possible explanation for this pattern is that nonclinical populations contain a large fraction of subjects with undiagnosed OCD.

4.2. Conclusions

Latent toxoplasmosis seems to be associated with numerous disorders. For some of them, the existence of a causal relationship between the infection and the disorder has already been proven beyond any reasonable doubt (schizophrenia); for other disorders, the existence of such a relationship is only suggested. The present study provides relatively strong support for the hypothesis of an association between OCD and toxoplasmosis. It is still necessary to repeat this study on different populations.

Ethical standards

The entire study and method of acquiring electronic Informed consent were approved by the IRB of the Faculty of Science, Charles University (No. 2014/07, 2015/08).

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Declaration of interest

The authors declare that they have no competing interest.

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Appendix

To analyze possible effects of the presence of false negative subjects, e.g. subjects who acquired *Toxoplasma* infection after they had been serologically tested for toxoplasmosis or who misreported their infection status, we performed the following Monte-Carlo test: we generated 50,000 sets of 1500 *Toxoplasma*-free and 500 *Toxoplasma*-infected subjects. The score of each *Toxoplasma*-free subject was drawn from the normal distribution (mean = 15, background variability standard deviation (s.d.) = 10) while the score of each *Toxoplasma*-infected subjects were generated from the same distribution and increased by a random contribution of toxoplasmosis (normal distribution, mean 1.5, s.d. = 1, 2,5, 5, 10, and 25, respectively). Then mean statistical significance and mean effect size of these differences for all 50,000 random sets were computed after 0%, 5%, 15%, 20%, 25%, and 30% of randomly selected *Toxoplasma*-infected individuals from each of 50,000 set were relocated from *Toxoplasma*-infected to *Toxoplasma*-free subset (false negative subjects). Table 4 shows mean *P* values computed with Student *t*-test (part a) and Cohen's *d* (part b) for all combinations of s.d. The computation was performed with the R 3.3.1 package for Windows. The script for modeling is available at https://figshare.com/s/f30b0e69da224acada17. The results showed that the presence of false negative subjects never increased the probability of false positive results of the statistical analyses (probability of Type I error) and usually strongly increased the probability of false negative results of the statistical analyses (probability of Type II error). The probability of Type II error increased with increasing the fraction of false negative subjects in

Table 4
Effect of contamination of data set with false negative subjects.

	0%	5%	10%	15%	20%	25%	30%
Effect on statistical significance (P values)							
1.0	0.020	0.023	0.028	0.033	0.038	0.045	0.052
2.5	0.023	0.026	0.030	0.036	0.041	0.048	0.056
5.0	0.030	0.035	0.040	0.046	0.053	0.061	0.068
10.0	0.061	0.068	0.076	0.084	0.094	0.103	0.115
25.0	0.193	0.203	0.214	0.224	0.233	0.245	0.256
Effects on Cohen's d (effect size)							
1.0	0.150	0.148	0.145	0.142	0.140	0.138	0.136
2.5	0.148	0.146	0.144	0.142	0.140	0.137	0.135
5.0	0.145	0.143	0.140	0.138	0.136	0.134	0.132
10.0	0.134	0.131	0.129	0.128	0.125	0.123	0.121
25.0	0.094	0.093	0.091	0.089	0.088	0.087	0.085

The row headings show standard deviations of the random effect of toxoplasmosis on the variable under study, e.g. on OCD score. The column headings show the fraction of false negative subjects in the subset of serologically negative subjects.

the population and increasing the s.d. of contributions of toxoplasmosis.

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