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Differences in onset of disease and severity of psychopathology between toxoplasmosis-related and toxoplasmosis-unrelated schizophrenia

Holub D, Flegr J, Dragomirecká E, Rodriguez M, Preiss M, Novák T, Čermák J, Horáček J, Kodym P, Libiger J, Höschl C, Motlová LB. Differences in onset of disease and severity of psychopathology between toxoplasmosis-related and toxoplasmosis-unrelated schizophrenia.

Objective: Toxoplasmosis is a lifelong parasitic disease that appears to be associated to schizophrenia. However, no distinguishing attributes in *Toxoplasma*-infected schizophrenia patients have been described as yet.
Method: We searched for differences in symptom profile, cognitive performance and treatment response between 194 *Toxoplasma*-free and 57 (22.7%) *Toxoplasma*-infected schizophrenia patients treated in Prague Psychiatric Centre between 2000 and 2010.
Results: Infected and non-infected patients differed in severity of symptoms ($P = 0.032$) measured with the Positive and Negative Symptom Scale (PANSS). Infected patients scored higher in positive subscale of PANSS, but not in the general and negative subscales. Infected men scored higher also in Total PANSS score, and negative, reality distortion, disorganisation and cognitive scores. Higher PANSS scores of positive, negative and disorganised psychopathology were associated with the lower titres of anti-*Toxoplasma* antibodies suggesting that psychopathology deteriorates with duration of parasitic infection. Infected patients remained about 33 days longer in hospital during their last admission than uninfected ones ($P = 0.003$). Schizophrenia started approximately 1 year earlier in infected men and about 3 years later in infected women, no such difference was observed in uninfected subjects.
Conclusion: Latent toxoplasmosis in schizophrenia may lead to more severe positive psychopathology and perhaps less favourable course of schizophrenia.

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Key words: Schizophrenia; psychopathology; *Toxoplasma gondii*; infection theory; illness onset

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Significant outcomes

- *Toxoplasma*-infected schizophrenia patients showed more severe positive, disorganised and excitement psychopathology in the PANSS scores in comparison with uninfected ones and the impact of *Toxoplasma* infection was sex-related.
- Higher PANSS scores were associated with the lower titres of anti-*Toxoplasma* antibodies suggesting that psychopathology may deteriorate with duration of parasitic infection.
- *Toxoplasma*-infected patients spent more days in the hospital than uninfected ones during their last hospitalisation. Schizophrenia started about 1 year earlier in *Toxoplasma*-infected men and about 3 years later in *Toxoplasma*-infected women than in *Toxoplasma*-free patients.

Limitations

- It is likely that patients suffering from the most severe schizophrenic psychopathology refused to participate in the study, which made estimation of effects size impossible and increased risk of false-negative results in statistical testing.
- *Toxoplasma* status in subjects who refused to participate in the study was unknown, and therefore, it was not possible to assess the strength of the sieve effect resulting from the probable absence of subpopulation of subjects with the most severe disease in the study population and its impact on the result of present analyses.
- *Toxoplasma*-infected schizophrenia patients who agreed to participate in the study were underrepresented compared with published data.

Introduction

The protozoan *Toxoplasma gondii* has been repeatedly associated with schizophrenia (1–3). A renewed interest in infection theories of psychiatric disorders since the 1970s has resulted in a growing number of reports that supported the etiopathogenetic relevance of *Toxoplasma* infection to schizophrenia (overview see 4–6). The potential link between *Toxoplasma* infection and schizophrenia has been indicated by several epidemiological, neuropathological, serological, neurophysiological and pharmacological studies carried out in both schizophrenic and non-schizophrenic population samples in humans and also in animal models (7–10). *Toxoplasma* has been considered an infectious agent that can trigger psychotic disorder in predisposed subjects or modulate the course of the disease (4, 11). *Toxoplasma* causes lifelong latent infection in an immunocompetent host. The parasite remains dormant in the form of tissue cysts in predominantly neural and muscular tissue by the activity of the immune system. Immunosuppression or HIV infection leads to cysts reactivation (12). There are varied theoretical models that aim to explain the pathogenetic mechanism of *Toxoplasma* infection in the brain. They include direct and selective toxic impairment of neurons, glial cells and astrocytes (13), neuromodulatory impact of *Toxoplasma* metabolism (an excessive production of dopamine; 14, 15) or continuous production of proinflammatory cytokines that affect neuromodulation (16), especially the excessive inhibition of glutamatergic and nicotinergic neurotransmission (17). There is a limited number of studies that investigate specific psychopathology, course and onset of schizophrenia in patients who are lifelong carriers of *Toxoplasma* cysts in the brain in spite of the evidence that toxoplasmosis can modify human behaviour and personality traits (18, 19). A diverse scope of symptom manifestation was reported in *Toxoplasma*-positive schizophrenia patients (20–23). There was found a significant association of severe positive schizophrenia symptoms in ultra-high-risk individuals for psychosis and higher levels of anti-*Toxoplasma* IgG antibodies (24). A cognitive deterioration was detected in schizophrenic and non-schizophrenic population with positive antibodies against *Toxoplasma* (21, 25, 26). However, the questions of whether the clinical presentation of schizophrenia patients is modified by *Toxoplasma* infection, has not been answered as yet. The present study was conducted to search for differences in psychopathology and cognitive impairment between *Toxoplasma*-infected and non-infected patients with schizophrenia and related disorders.

Aims of the study

To determine whether schizophrenia patients with latent toxoplasmosis differed in severity of symptoms, cognitive performance, age of illness onset, number of admissions, duration of in-patient status and treatment response. Based on results showing a reduction of grey matter volume in *T. gondii*-positive patients, we hypothesised that *Toxoplasma*-infected patients with schizophrenia have more severe positive symptoms and worse cognitive performance.

Material and methods

Setting and participants

The final set of patients consisted of 251 individuals who were admitted to the Prague Psychiatric Centre's diagnostic and treatment programmes between 2000 and 2010 with the diagnoses of schizophrenia or schizophrenia spectrum disorders and agreed to participate in the study. Another 97 patients were excluded from the study: 5 patients

fulfilled exclusion criteria, 67 refused to participate in the study and 25 refused to give a blood sample. Six patients were excluded for uncertain serologic positivity (see below). The final sample of 251 patients from the capital city of Prague catchment area, 141 (56.18%) men and 110 (43.82%) women, were tested for *T. gondii* antibodies: 194 were *Toxoplasma*-negative and 57 were *Toxoplasma*-positive. Their mean age was 28.3 years (SD = 7.6 years). In *Toxoplasma*-free subgroup 84% (116) patients were single, 11% (15) married and 5% (7) separated/divorced and did not differ from *Toxoplasma*-positive subgroup: 88% (36) patients were single, 10% (4) married and 2% (1) separated/divorced. Exclusion criteria were psychoactive substance misuse (303.x, 304.x; DSM-IV-TR), neurological diseases, immunodeficiency and any medical condition interfering with PC performance (especially visual impairment). At the time of inclusion, all patients were between 17 and 53 years of age with a mean duration of illness of 54.9 (SD = 65.9) months. A number of hospital admissions ranged from 1 to 13 (mean = 2.64, SD = 2.76). Their *Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition)* diagnoses were schizophrenia in 144 patients (57.4%; 295.1, 295.2, 295.3 DSM-IV Codes), schizophreniform and brief psychotic disorders in 76 patients (30.3%; 295.4, 298.8), delusional disorder in 2 patients (0.008%; 297.1), schizoaffective disorder in 38 patients (11.2%; 295.7) and schizotypal personality disorder in 1 person (0.004%; 301.33). All patients were Caucasians and in a phase of clinical remission. The majority of our patients were on second generation antipsychotics from their first hospital admission, and it was considered as lifetime medication. The distribution of antipsychotic treatment was as follows: 105 (34.7%) patients took olanzapine 5 to 30 mg daily, 51 (16.8%) were on risperidone 2 to 8 mg, 28 (9.2%) were on clozapine 150 to 700 mg, 28 (9.2%) were on 200 to 1000 mg of daily dose of quetiapine and 20 patients (6.6%) did not take any medication. This project was reviewed and approved by the Ethical Committee of the Prague Psychiatric Centre and Mental Hospital Bohnice, and investigations were carried out in concordance with the latest version of the Declaration of Helsinki. The rationale of the study was explained verbally and in writing, and patients signed an informed consent form.

Procedure and diagnostic assessment

All patients were assessed at baseline before entering the research programmes. Diagnoses were reconfirmed by two psychiatrists based on the Structured Clinical Interview–Patient Version (SCID-I/P) according to DSM-IV criteria and sociodemographic descriptors were recorded (age of illness onset, illness duration, number of hospital admissions, length of the last admission stay, education). The medical history was taken, and a standard physical examination and biochemistry were performed to exclude severe medical and neurological disorders, drug and alcohol abuse. Severity of schizophrenia symptoms was evaluated by structured interview of the Positive and Negative Symptom Scale (PANSS) scale (27). The Conner's Continuous Performance Test (CPT) and the Auditory Verbal Learning Test (AVLT) were used to screen for neurocognitive abnormalities (28). CPT was chosen primarily to examine focused attention, vigilance, executive functioning (inhibitory control), and appropriate domains to study were as follows: reaction time, inattentiveness/vigilance, impulsivity, response speed consistency, capacity to discriminate difference between signal and noise distributions. In assessing verbal memory using AVLT, we examined the following domains: capacity to remember and learn in the course of the test, distractibility, short-term verbal memory and delayed recall. Both tests are compatible with other schizophrenia studies and are part of the standard assessment battery of the Prague Psychiatric Centre. Tests were carried out by a trained nurse and interpreted by a clinical psychologist (29, 30). Psychopathological assessment using PANSS ratings were completed for all patients by one of the attending psychiatrists (L.M.B., D.H. and J.Č.). Both patients and raters were blind to the patient's *Toxoplasma* status.

Toxoplasma assessment

We focused in our study on postnatal latent toxoplasmosis (asymptomatic 'dormant' stage of *Toxoplasma gondii* infection detectable by serologic positivity of specific IgG antibodies) and clinical relevance of acute toxoplasmosis (serologic positivity of IgM and IgA antibodies usually accompanied with clinical symptoms) was not considered. The latent toxoplasmosis is a lifelong disease in humans, and therefore, the presence of IgG anti-*Toxoplasma* antibodies is considered reliable indicator of viable parasites in tissue cysts that irreversibly persist in infected subjects for life (31). Blood samples were taken by a trained nurse who collected 2.5–10 ml blood specimen according to standard procedures in tubes without anticoagulant. After 10–25 min at room temperature, the upper plasma layer was carefully removed (with or without previous 1500 g centrifugation depending

on amount of sera after coagulation) and frozen. We tested the blood samples for anti-*Toxoplasma* IgG and IgM antibody concentrations by two methods: ELISA (Enzyme-Linked Immunosorbent Assay; IgG: SEVAC, Prague, IgM: TestLine, Brno) and the Complement Fixation Test (CFT; SEVAC, Prague) in the National Reference Laboratory for Toxoplasmosis of the Czech Republic. The decrease in CFT titres compared with ELISA method detects more reliably the 'old' *Toxoplasma* infection (32). CFT titres of antibodies to *Toxoplasma* were measured at dilutions between 1 : 4 and 1 : 1 024. All subjects testing IgG positive by IgG ELISA (positivity index >0.9) and those with CFT titres equal or higher than 1:8 were considered toxoplasmosis positive and were retested for possible acute toxoplasmosis by IgM ELISA tests. Six subjects who differed in CFT and ELISA tests were excluded from the study. No sample tested positive with IgM ELISA or had CFT equal or higher than 1 : 128. Our interpretation was that no subject had an acute toxoplasmosis in the course of the study. Distinction between acute and latent toxoplasmosis by IgG ELISA and CFT tests is sometimes difficult, however, detection of IgG *Toxoplasma*-specific antibodies is rarely problematic, and high sensitivity and specificity have been achieved by a variety of methods (33). The relapse of acute toxoplasmosis in immunosuppressed patients with positive titres of antibodies induced by past *Toxoplasma* infection suggests that the seropositivity, that is, the presence of specific serum IgG antibodies against *Toxoplasma,* always indicates the presence of viable parasites.

Data analysis

The normality of data distribution, normality of residuals and homogeneity of variances were evaluated. The total PANSS score and its standard subscale scores (positive, negative and general psychopathology) were calculated together with reality distortion, disorganisation and psychomotor poverty PANSS dimensions. We also calculated scores for the five component PANSS model: negative, positive, cognitive, excitement and depression/anxiety scores (for calculation and discussion see 34–36). For the confirmation of already detected difference in PANSS scores between infected and non-infected patients, more sensitive one-tailed tests without correction for multiple testing should be used. However, we used only the conservative two-tailed tests and also presented the Bonferroni's corrected results for all variables including the PANSS scores in the tables to allow the evaluation of the robustness of results. To characterise treatment response and treatment non-compliance, we established the following binary variables: narrowly defined schizophrenia (DSM-IV-TR Codes: 295.1; 295.2; 295.3) vs. schizophrenia spectrum disorders (DSM-IV-TR Codes: 295.4; 295.7; 297.1; 298.8; 301.33), high dose treatment (daily chlorpromazine equivalent dose above vs. below 1000 mg per day; 37), excessive dosage, that is, daily doses of antipsychotics exceeding vs. not exceeding those licensed as maximum by the British National Formulary 2010 (38), resistance targeting treatment (clozapine vs. standard antipsychotics), and depot form of antipsychotics administration (indicator of treatment non-adherence) vs. peroral administration of antipsychotic drugs.

The effect of toxoplasmosis on these binary variables was estimated by partial Kendall correlation with age as a covariate (39). The effect of toxoplasmosis on severity of symptoms was estimated with multivariate GLM test (General Linear Models) with the severity level of Positive, Negative and General symptoms as dependent variables and two binary variables, that is, toxoplasmosis status and sex of the patients as independent binary factors, and the age as continuous covariate. The distribution of some variables deviated from the normality and the deviance persisted even after log-transformation. Therefore, separate analyses for men and women were performed with conservative nonparametric test of partial Kendall correlation with age as covariate (40). Statistica® v 8.0 (StatSoft, Inc., Tulsa, OK, USA) was used for all statistical tests, that is, for contingency tables, General Linear Models (GLM) and nonparametric tests.

Results

The final sample included 57 *Toxoplasma*-infected patients with schizophrenia, which corresponds to 22.7% infection prevalence. There was no significant difference in the prevalence of seropositivity ($\chi^2 = 0.114$, $P = 0.737$, OR = 0.91, C.I.$_{95}$ = 0.50–1.67) between genders. Thirty-three men (23.4%) and 24 women (21.6%) were *Toxoplasma*-infected. Seropositive status did not differ significantly between schizophrenia patients (34; 23.6%) and other categories of psychoses (23; 21.5%), ($\chi^2 = 0.118$, $P = 0.854$, OR = 1.13, C.I.$_{95}$ = 0.62–2.07).

Effect of toxoplasmosis on psychopathology and cognitive impairment

Toxoplasma-infected patients displayed higher severity of symptoms measured with PANSS.

Tables 1 and 2 show results of univariate tests, namely the differences in particular PANSS subscales between *Toxoplasma*-infected and *Toxoplasma*-free patients measured by parametric and nonparametric tests, respectively. *Toxoplasma*-infected patients had higher scores on positive subscale ($P = 0.017$) and non-significantly higher five-factor model excitement component of the PANSS ($P = 0.089$). We did not find any significant difference in cognitive performance between *Toxoplasma*-infected and *Toxoplasma*-free subgroups in the Conner's Continuous Performance Test, and the Auditory Verbal Learning Test. A significant effect of *Toxoplasma*–sex interaction on cognitive performance was detected. After Bonferroni's correction for multiple testing, the impaired performance in CPT Commission ($P = 0.058$) in both, *Toxoplasma*-infected men and women, sex-dependent discrepancies in CPT Detectability ($P = 0.0496$) and also CPT SE Block T-score ($P = 0.041$) lost statistical significance.

Effects of toxoplasmosis on clinical characteristics

The relationship of toxoplasmosis status and clinical parameters (the time of onset in schizophrenia, length of the last hospital stay, number of hospital admissions and the mean daily dose of antipsychotics in chlorpromazine equivalents) were estimated by GLM with toxoplasmosis, sex and age as independent variables. Table 1 and Fig. 1 show the relationship of toxoplasmosis and the time of onset in schizophrenia, length of the last hospital stay, number of hospital admissions and the mean daily dose of antipsychotics. Results of more robust nonparametric tests performed separately for men and women are presented in Table 2. The binary variables high doses of antipsychotics ($P = 0.022$) and excessive doses of antipsychotics ($P = 0.004$) showed a significant positive association with toxoplasmosis. The continuous variable daily dose in mean Chlorpromazine Equivalent (CE) was insignificantly higher (600 vs. 487 mg; $P = 0.329$) in *Toxoplasma*-infected patients.

Correlation between length of infection and symptom severity

The concentration of anti-*Toxoplasma* antibodies decreases with duration of infection (41). Therefore, the negative correlation between PANSS scores and the concentration of antibodies may indicate the cumulative effect of latent toxoplasmosis over time, while positive correlation would indicate the carry-over effect of past acute toxoplasmosis. The partial Kendall correlation between PANSS scores and concentration of antibodies, with age of patients as covariate showed that four PANSS subscales and composite scores (Positive Subscale, Disorganisation Subscale, Five Component Positive and Five Component Excitement) correlated negatively with the concentration of anti-*Toxoplasma* IgG antibodies (Table 3, Fig. 2) in the *Toxoplasma*-infected subpopulation, even when the age of patients was controlled. The results also showed a non-significant positive association between the diagnoses of schizophreniform or brief psychotic disorder and anti-*Toxoplasma* antibodies level in the Kendall's rank correlation analysis (Table 3). The separate analyses by gender found highly significant correlation only for women (Table 3).

Discussion

Psychopathological severity and cognitive impairment

We confirmed the association of *Toxoplasma* infection with excitement and positive psychopathology reported by Wang et al. (23). The score of the PANSS-positive subscale and excitement component scores were significantly higher in our *Toxoplasma*-infected subgroup. Unlike Wang et al., we did not confirm higher PANSS cognitive and negative scores in *Toxoplasma*-infected patients. However, we detected changes that the Wang et al. study did not report: higher total PANSS score and composite score of disorganisation in infected patients. In the Chinese study, the number of patients had been almost twice as high as in our study, which increased the sensitivity of tests and therefore the probability to detect the weaker effects of toxoplasmosis. Wang et al. did not control for the effects of age and sex in their analyses. Previously reported gender-related differences in the impact of latent toxoplasmosis on human behaviour (42–44) and also disparity in clinical variables linked with gender in our study (Fig. 1) suggest that there may be gender-associated differences in *Toxoplasma*-induced effects. Our analysis of men and women subpopulations revealed that only the infected men scored significantly higher in negative PANSS scores, reality distortion, disorganisation and cognitive composite scores (Tables 1and 2).

Our findings indicate that schizophrenic psychopathology correlated negatively with the concentration of anti-*Toxoplasma* IgG antibodies in the *Toxoplasma*-infected patients. This suggests that the toxoplasmosis modifies psychopathology features, rather than the severity of symptom influences the probability of being infected by

Table 1. Effect of sex, age, toxoplasmosis and sex–toxoplasmosis interaction on characteristics of schizophrenia disease (Descriptive statistics and GLM analyses)

	Descriptive statistics: (means)								Results of GLM analyses: significances (P) and effect sizes (η^2)							
	Men-negative	SD (men −)	Men-positive	SD (men +)	Women-negative	SD (women −)	Women-positive	SD (women+)	P sex	η^2 -sex	P toxo	η^2 toxo	P sex-toxo	η^2 sex-toxo	P age	η^2 age
Onset of psychosis (age)	22.67	6.08	23.48	6.30	24.36	6.54	27.03	7.45	**0.006****	0.031	0.227	0.006	**0.026***	0.020	**0.000****	0.466
Length of last hospitalisation (days)	53.02	38.93	64.85	54.94	46.89	34.77	80.25	126.28	0.544	0.002	**0.006****	0.031	0.219	0.006	0.420	0.003
Number of hospitalisations	2.92	4.40	3.00	2.62	2.78	2.92	2.08	1.59	0.158	0.008	0.263	0.005	0.700	0.001	**0.000****	0.140
Chlorpromazine equivalent (mg)	498.34	332.68	582.30	430.10	489.29	310.00	534.62	406.76	0.588	0.001	0.258	0.006	0.739	0.000	0.732	0.001
PANSS-positive scale	12.98	4.45	14.88	4.19	12.07	4.00	13.92	7.80	0.152	0.008	**0.017****	0.023	0.900	0.000	**0.013****	0.025
PANSS-negative scale	17.85	6.27	19.34	5.94	15.19	6.46	13.67	5.88	**0.000****	0.073	0.942	0.000	0.129	0.009	0.507	0.002
PANSS general scale	31.77	7.45	33.19	7.05	29.89	8.07	31.08	12.25	**0.089**	0.012	0.400	0.003	0.957	0.000	**0.037***	0.018
PANSS total score	62.90	15.62	67.41	13.69	57.48	16.28	58.67	23.16	**0.004****	0.035	0.360	0.003	0.614	0.001	**0.023***	0.021
PANSS reality distortion	7. 12	2.86	8.34	3.02	6.71	2.72	7.25	4.80	**0.074**	0.013	0.114	0.010	0.606	0.001	**0.001****	0.042
PANSS disorganisation	8.09	2.63	8.75	2.38	7.58	2.67	8.08	3.57	0.115	0.010	0.257	0.005	1.000	0.000	**0.006****	0.031
PANSS psychomotor poverty	17.72	5.93	18.84	5.75	14.96	6.30	13.46	5.79	**0.000****	0.077	0.603	0.001	0.183	0.007	0.173	0.008
PANSS 5_negative	22.50	7.38	23.68	6.99	19.23	7.53	17.33	6.95	**0.000****	0.072	0.673	0.001	0.204	0.007	0.248	0.006
PANSS-5_positive	16.77	5.60	18.13	5.40	15.107	5.38	17.08	10.06	**0.099**	0.011	0.136	0.009	0.574	0.001	**0.001****	0.044
PANSS 5_cognitive	8.32	2.59	9.23	2.60	7.68	2.58	7.46	2.96	**0.003****	0.037	0.464	0.002	0.192	0.007	0.245	0.006
PANSS 5_excitement	5.07	1.70	5.48	2.47	4.80	1.46	5.33	2.04	0.439	0.002	**0.089**	0.012	0.818	0.000	0.809	0.000
PANSS 5_depressive	10.24	3.07	10.29	3.56	10.67	3.45	11.46	4.90	0.161	0.008	0.522	0.002	0.428	0.003	0.117	0.010

The table shows mean values of dependent variables (columns 1-4) and results, that is, significances (P) and effect sizes (η^2 in GLM) of parametric tests (GLM) (columns 5–12). The significant results ($P < 0.05$) are denoted with asterisk, the results significant after Bonferroni's correction are denoted with ** and trends ($P < 0.1$) are printed in bold.

Table 2. Effect toxoplasmosis on characteristics of schizophrenia disease – nonparametric test results

	Results of Kendall analyses: effect (τ) and significances (*P*)					
	τ all	*P* all	τ men	*P* men	τ women	*P* women
Onset of psychosis (age)	0.054	0.204	−0.004	0.947	0.169	**0.043***
Length of last hospitalisation	0.102	**0.017***	0.059	0.306	0.157	**0.058**
Number of hospitalisations	0.025	0.553	0.081	0.158	−0.052	0.529
Chlorpromazine equivalent	0.043	0.329	0.068	0.247	0.004	0.961
High dose	0.100	**0.022***	0.137	**0.019****	0.034	0.684
Clozapine	−0.022	0.616	−0.011	0.852	-0.032	0.695
Excessive dose	0.127	**0.004****	0.146	**0.013****	0.080	0.336
Depot form administration	0.032	0.463	0.055	0.346	−0.001	0.990
Brief psychotic disorder	−0.021	0.615	-0.030	0.598	−0.012	0.890
PANSS-positive scale	0.099	**0.020***	0.157	**0.006****	0.010	0.901
PANSS-negative scale	0.009	0.836	0.105	**0.067**	−0.100	0.226
PANSS general scale	0.037	0.382	0.078	0.172	−0.027	0.756
PANSS total score	0.062	0.147	0.123	**0.032***	−0.023	0.783
PANSS reality distortion	0.071	**0.098**	0.142	**0.014****	−0.037	0.661
PANSS disorganisation	0.080	**0.063**	0.115	**0.046***	0.034	0.700
PANSS psychomotor poverty	-0.010	0.824	0.070	0.225	−0.106	0.234
PANSS 5_negative	-0.001	0.981	0.076	0.188	−0.098	0.274
PANSS 5_positive	0.064	0.137	0.092	0.110	0.016	0.862
PANSS 5_cognitive	0.052	0.229	0.140	**0.015***	−0.055	0.539
PANSS 5_excitement	0.087	**0.043***	0.078	0.174	0.097	0.276
PANSS 5_depressive	0.004	0.924	−0.009	0.883	0.023	0.794

The table shows results, that is, significances (*P*) and effect sizes (τ) of nonparametric (partial Kendall correlation with age as a covariate) tests. The significant results ($P < 0.05$) are denoted with asterisk, the results significant after Bonferroni's correction are denoted with ** and trends ($P < 0.1$) are printed in bold.

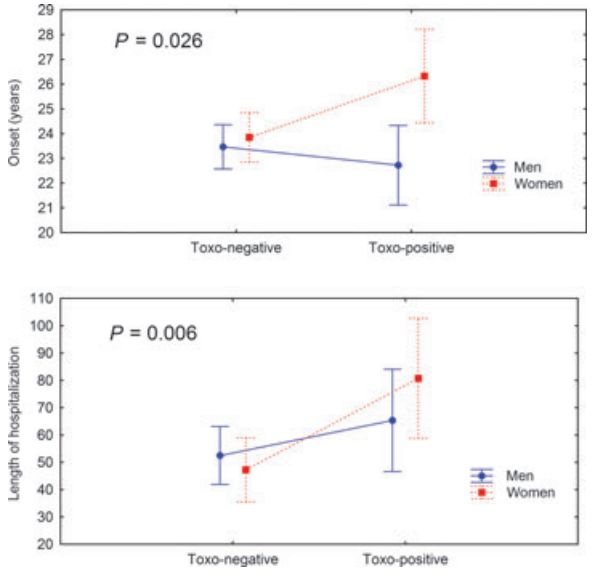

Fig. 1. Differences in onset of schizophrenia disease and in the length of last admission hospital stays in days between *Toxoplasma*-free and *Toxoplasma*-infected men and women. The presented values differ from raw data listed in Table 1 because the age of onset and the length of hospital stay has been controlled for the age of patient, that is,. the age of onset/length of hospital stay has been computed for covariate (age) as its mean. Vertical bars denote 0.95 confidence intervals. The *P*-value shows significance of toxoplasmosis–sex interaction (above) and effect of toxoplasmosis (below) estimated with GLM.

Toxoplasma. We interpret this finding as a cumulative effect of latent toxoplasmosis on individual psychopathology. Positive symptoms, excitement and disorganisation were more prevalent among patients in our study than deficit ones. The link between positive schizophrenic symptoms and *Toxoplasma* infection is supported by case reports of schizophrenia-like symptoms in both acute toxoplasmosis and in *Toxoplasma* encephalitis in HIV-positive patients (45, 46). Paranoid and bizarre delusions, auditory and visual hallucinations, disorganised speech and thought disorders in patients may be misdiagnosed for schizophrenia or schizophreniform disorder (6, 23). Although cognitive impairment was reported in both, *Toxoplasma*-infected non-schizophrenic (25) and schizophrenic subjects (21), we found no significant differences in the cognitive performance between *Toxoplasma*-infected and *Toxoplasma*-free patients. However, the observed trends (the significant results of two sided tests before the Bonferroni's corrections) suggest that possible effects of toxoplasmosis on cognitive performance of schizophrenic patients deserve future study.

The different psychopathological symptom profiles in *Toxoplasma*-infected patients may be associated not only with the immune response to a pathogen but also with the infection impact on brain morphology. Specific behavioural alterations in rodents can be explained by selective localisation of *T. gondii* tissue cysts in specific brain areas (47). Chronic *T. gondii* infection led to the loss of brain parenchymous tissue particularly in the hippocampus, periaqueductal and periventricular

	τ all	*P* all	τ men	*P* men	τ women	*P* women
PANSS-positive scale	−0.188	**0.041***	−0.197	0.114	−0.199	0.174
PANSS-negative scale	0.026	0.774	−0.045	0.717	0.146	0.319
PANSS general scale	−0.090	0.329	−0.127	0.308	−0.093	0.524
PANSS total score	−0.080	0.384	−0.102	0.412	−0.020	0.890
PANSS reality distortion	−0.157	**0.087**	−0.141	0.256	−0.167	0.254
PANSS disorganisation	−0.241	**0.009****	−0.288	**0.021***	−0.162	0.266
PANSS psychomotor poverty	0.027	0.770	−0.050	0.687	0.133	0.364
PANSS 5_negative	0.018	0.844	−0.064	0.612	0.111	0.448
PANSS 5_positive	−0.198	**0.033***	−0.195	0.123	−0.159	0.276
PANSS 5_cognitive	−0.074	0.424	−0.105	0.407	0.010	0.944
PANSS 5_excitement	−0.197	**0.033***	−0.063	0.616	−0.361	**0.013***
PANSS 5_depressive	0.038	0.678	0.087	0.491	−0.065	0.656
Brief psychotic disorder	0.096	0.291	−0.090	0.457	0.414	**0.004****

Table 3. Partial Kendall correlation between PANSS scores and concentration of anti-*Toxoplasma* antibodies in *Toxoplasma*-infected patients

The significant results ($P < 0.05$) are denoted with asterisk, the results significant after Bonferroni's correction are denoted with ** and trends ($P < 0.1$) are printed in bold, the confounding variable age of patients was controlled. The last line shows similar partial correlation between probability of the brief psychotic disorder diagnosis and the concentration of anti-*Toxoplasma* antibodies.

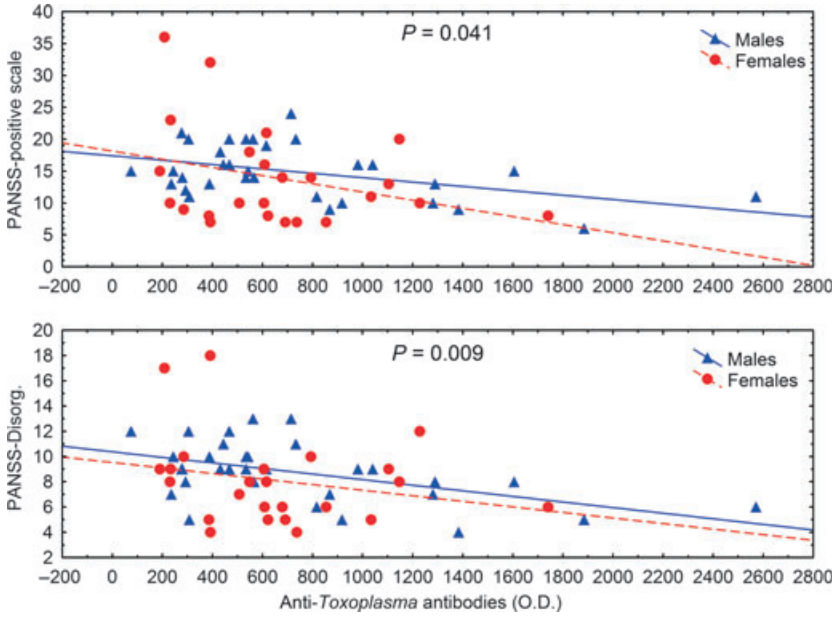

Fig. 2. Correlation between concentration of anti-*Toxoplasma* IgG antibodies and PANSS-positive scale, and between concentration of anti-*Toxoplasma* IgG antibodies and PANSS Disorganisation Symptom Dimension. The negative correlation of symptoms severity with concentration of IgG most probably reflects positive correlation of symptoms severity with length of the infection. The *P*-value shows significance of toxoplasmosis effect estimated with GLM.

areas (48). Recently, Horáček et al. (49) found in a MRI study a significant reduction of grey matter volume in *T. gondii*-positive patients with schizophrenia in the caudate, median cingulate, thalamus and occipital cortex bilaterally and also in the left cerebellar hemispheres. This finding corresponds with the reported involvement of these areas in positive, excitement and disorganisation symptoms (50, 51). We could not exclude completely the impact of antipsychotic treatment on psychopathology features due to its antiinflammatory and antiinfectious properties (52).

Clinical and demographic features of psychotic illness

To our knowledge, this is the first published study that reports a relationship between *Toxoplasma* infection and duration of schizophrenia. The difference in the age of schizophrenia onset between men and women appear to be amplified in our population of infected patients (Table 1). Raw data presented in Table 1 suggested that in comparison with *Toxoplasma*-free patients, schizophrenia started about one and 2.5 years later in *Toxoplasma*-infected men and *Toxoplasma*-infected women, respectively. However, when the age of patients was statistically controlled, we found that schizophrenia started about 1 year earlier in *Toxoplasma*-infected men and about 3 years later in *Toxoplasma*-infected women compared with *Toxoplasma*-free patients, see Fig. 1. It is indicative that no gender-related differences in the onset of schizophrenia were observed in non-infected patients (Fig. 1). The majority of *Toxoplasma*-infected individuals in the Czech Republic become infected at age 9 or younger. However,

further incidence increase is seen in women during the peak fertility between the age of 25 and 35 (41). Statistically, women get infected with *T. gondii* later than men in the Czech Republic. Difference in mean age of *Toxoplasma* infection in men and women may be one of the factors that can help to explain the gender-associated difference in onset of schizophrenia. The difference in onset of schizophrenia between men and women was reported in many previous studies (53, 54). We suppose that certain forms of schizophrenia can be triggered by the *Toxoplasma* infection. The peak of age of onset of toxoplasmosis-unrelated schizophrenia is about 24 years in both men and women. However, the *Toxoplasma*-infected sample consists of subjects with toxoplasmosis-unrelated schizophrenia, with mean onset of 24 year, and toxoplasmosis-related schizophrenia, with mean onset earlier in men (infected with *Toxoplasma* in childhood) and later in women (infected both in childhood and around age 30). Two peak distribution of toxoplasmosis incidence in women in Czech Republic might be responsible for the delayed age of psychotic illness onset in women. It would be theoretically appropriate to study age of psychotic illness onset in women in countries showing only one peak toxoplasmosis seroprevalence incidence (55).

Alternatively, the shift in onset of schizophrenia in *Toxoplasma*-infected men and women can be caused by differences in effect of toxoplasmosis on a physiology of men and women and following differences in course of schizophrenia disease between sexes. The sex-related differences in sensitivity to dopaminergic challenge including neuroprotective role of oestrogens to neurotoxicity of tryptophan metabolites are reported to exist (56). Increased concentration of testosterone in men and decreased concentration of testosterone in women has been observed in *Toxoplasma*-infected subjects (57). Indirect evidence also exists for increased concentration of oestrogens in the *Toxoplasma*-infected women (58, 59). Kaňková et al. (58) and James (59) reported that infected women are more likely to produce sons than uninfected mothers and that the offspring sex ratio (proportion of males) increases with the concentration of anti-*Toxoplasma* antibodies in *T. gondii*-positive mothers. Experiments on infected female mice showed similar results (39). Steroids are known to modulate not only the sex ratio (59) but also the symptoms of various psychotic disease, including schizophrenia. Epidemiological and clinical evidence suggests an influence of oestrogens on the vulnerability threshold for schizophrenia and modulating its symptoms (60, 61).

Our results, namely the absence of any difference in the age of onset between *Toxoplasma*-free men and women, suggest that latent toxoplasmosis could be the major case of sex-specific disease onset. Therefore, separate analyses for *Toxoplasma*-infected and non-infected patients should be performed, and findings replicated in larger cohort studies. We do not assume that the early identification of schizophrenia factors such as a greater awareness of the illness, improved outreach and/or access to care (62) differ between men and woman in the Czech population.

Toxoplasma-infected patients spent more days in the hospital than uninfected ones during their last hospitalisation. Also, their mean daily dose of antipsychotics was higher. Latent toxoplasmosis in our schizophrenic population appears to be associated with signs of poor treatment response. The prolonged inpatient stay among our *Toxoplasma*-infected subgroup raised the annual direct costs of schizophrenia treatment. Even though latent toxoplasmosis is not curable, it is justifiable to administer anti-*Toxoplasma* medication to augment standard medication regimes (8, 63). Or *Toxoplasma*-inhibitory features of antipsychotic and mood stabilising drugs can be utilised in treatment of *Toxoplasma*-infected patients (64, 65) to modify the course of disease (66). Anti-*Toxoplasma* immune response modulation also warrants further attention because it may improve the clinical course of schizophrenia or prevent chronicity in first-episode patients (67).

Limitations

The prevalence of *Toxoplasma*-infected subjects was lower than that observed in other studies (1). It is known that *Toxoplasma*-infected men are more suspicious than *Toxoplasma*-free controls [for review see (11)]. Total of 40 (14%) patients refused to provide blood samples and higher suspiciousness of infected subjects could have been among the reasons for their refusal to participate in this study (11).

Three psychiatrists performed PANSS scoring and underwent the same PANSS training. Possible differences between their ratings (as well as all other potential confounding variables such as the type of antipsychotic medication) could result in false-negative results in performed statistical tests. The existence of confounding variables cannot be a source of any systematic bias and a cause of false-positive results. Still, future studies should aim at better control of these sources of variance to decrease a risk of false-negative results.

Concluding remarks

Toxoplasmosis was associated with more severe positive, disorganised and excitement symptoms in our schizophrenia patients. Latent toxoplasmosis can act either as a symptom modifier or an independent risk factor (as suggested by sex-specific differences of onset of the psychotic illness and perhaps also different treatment response). We believe that the long-term effect of toxoplasmosis in schizophrenia needs a more careful investigation because it may result in higher risk of relapse, poorer treatment response, an insufficient adherence to treatment or poor psychosocial functioning. The toxoplasmosis-associated differences in the course of schizophrenic illness may provide a rationale for the inclusion of anti-*Toxoplasma* antibodies screening programmes and more assertive preventative, educational and diagnostic measures.

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Declaration of interest

All authors confirmed their agreement to submission this article and declared that they have no conflict of interest in the last 2 years.

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