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MAJOR REVIEW

Toxoplasma gondii Infection and Clinical Characteristics of Patients With Schizophrenia: A Systematic Review and Meta-analysis

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Schizophrenia is associated with an increased prevalence of IgG antibodies against *Toxoplasma gondii* (*T. gondii* seropositivity), whereby the infection seems to precede the disorder. However, it remains unclear whether a *T. gondii* infection affects clinical characteristics of schizophrenia. Therefore, a systematic review and meta-analysis was conducted following PRISMA guidelines examining the association between *T. gondii* seropositivity and severity of total, positive, or negative symptoms or age of onset in schizophrenia. PubMed, Embase, and PsycInfo were systematically searched up to June 23, 2019 (PROSPERO #CRD42018087766). Random-effects models were used for analysis. Furthermore, the influence of potential moderators was analyzed. Indications for publication bias were examined. From a total of 934 reports, 13 studies were included. No overall effect on severity of total, positive, or negative symptoms was found. However, in patients with a shorter duration of illness *T. gondii* seropositivity was associated with more severe positive symptoms (standardized mean difference [SMD] = 0.32; *P* < .001). Similar but smaller effects were seen for total symptoms, while it was absent for negative symptoms. Additionally, a significantly higher age of onset was found in those with *T. gondii* seropositivity (1.8 y, *P* = .015), although this last finding was probably influenced by publication bias and study quality. Taken together, these findings indicate that *T. gondii* infection has a modest effect on the severity of positive and total symptoms in schizophrenia among those in the early stages of the disorder. This supports the hypothesis that *T. gondii* infection is causally related to schizophrenia, although more research remains necessary.

Key words: symptomatology/positive symptoms/toxoplasmosis/PANSS/age of onset

Introduction

Both genetic factors and environmental factors are involved in schizophrenia.[1] Among the environmental factors, infectious agents have repeatedly been implicated in schizophrenia. Of these infectious agents, *Toxoplasma gondii (T. gondii)* is the most studied and was found to be associated with schizophrenia in a large meta-analysis.[2]

Toxoplasma gondii is an obligate intracellular protozoan that can infect all warm-blooded mammals.[3] Apart from pregnant and immunocompromised people, usually the infection merely causes flu-like symptoms for a brief period, after which it resides in the body in a latent stage.[4] Prevalence of latent *T. gondii* infection in humans is estimated to be around 30% globally, although it varies widely across the world (0%–80%), depending on regional environmental and socioeconomic conditions.[3,5,6] Various studies have shown that a latent *T. gondii* infection could potentially affect the behavior of its host,[7,8] although some of these earlier findings (such as reduced fear specifically to cat urine by mice) have been called into question.[9] It is hypothesized that the parasite induces this behavioral changes in order to promote its own survival, as it increases the predation chance of intermediate hosts such as mice by their definite host, felines (such as cats), since it can solely sexually reproduce inside the felines' intestine.[10]

Several mechanisms are suggested how the parasite accomplishes this behavioral change in the host. Firstly, evidence suggests *T. gondii* can influence neurotransmitter levels in the brain of intermediate hosts.[7] *Toxoplasma gondii* has been reported in vitro to increase dopamine release in neurons,[11] probably by self-expression of genes encoding for the rate-limiting enzyme for synthesis of dopamine,[12] although this finding was recently challenged.[13] Furthermore, *T. gondii* infection may also alter glutamate signaling in the brain by inducing the kynurenine pathway.[14] Another possible mechanism is through immunological pathways. Pro-inflammatory cytokines associated with *T. gondii* infection can induce the activation of apoptosis through microglial activation and facilitate neurodegeneration. These suggested mechanisms of how *T. gondii* infection could influence its host may play a role in the expression of psychotic symptomatology, as these neurotransmitters and immunological disturbances have also been implicated in the pathophysiology of psychosis and schizophrenia.[7]

These findings, together with indications that the infection proceeds the onset of psychosis, that the association seems to be more pronounced in the early stages of the disorder and gets stronger with higher antibody levels,[2] suggests that toxoplasmosis could be a causal etiological factor in schizophrenia.[15] However, more evidence remains necessary to provide further credence to this hypothesis. One line of evidence that has been explored is whether *T. gondii* influences the clinical phenotype of patients with schizophrenia.[16–20] It has been reported in a large study that patients with first-episode schizophrenia show higher scores on the positive and lower scores on the negative subscale of the PANSS when seropositive to *T. gondii*.[21] However, later studies reported no or other associations with the severity of symptoms.[22–29] Whether this is caused by chance or by factors moderating the overall effect, such as duration of illness, study quality, or symptom severity, remains unexplored. A recently published meta-analysis on *T. gondii* infection in bipolar disorder indicated that only in younger age, probably serving as proxy for shorter duration of illness, a significant association could be demonstrated with the presence of the disorder.[30] A reason for this could be that potential neurotoxic effects of *T. gondii* infection contributing to the development of psychiatric illness would be more evident in recent onset cases. Assuming causality, *T. gondii* infection would precede the onset of psychiatric illness such as schizophrenia or bipolar disorder. On the other hand, prevalence of *T. gondii* infection increases with age in people,[31] also in individuals with psychiatric illness that have already developed this disorder, probably due to other etiological factors (genetic factors, childhood trauma, drug abuse).[1,32] It is uncertain whether recent *T. gondii* infection exerts detectable behavioral changes in individuals that already have a psychotic disorder. Therefore, the contribution of *T. gondii* infection to the psychiatric disorder or severity of symptomatology could be harder to distinguish later in life.

If clinical characteristics do differ in schizophrenia patients with or without *T. gondii* infection, this would lend further support to the hypothesis that *T. gondii* infection is causally related to schizophrenia.[33] One could argue that these differences would probably be more apparent with regard to negative symptoms, as these are considered to be more stable throughout the course of a psychotic disorder while positive symptoms tend to fluctuate. Alternatively, it can be argued that hyperdopaminergic states are related more to positive symptoms, which is one the main proposed mechanisms by which *T. gondii* increases the risk of developing a psychosis. It should be noted that ideally longitudinal assessment of severity of symptoms is needed, especially since the severity of psychotic symptoms tends to fluctuate during the course of the disorder. Therefore, another strategy to find support for the hypothesis that *T. gondii* infection may influence the risk to pass the psychosis threshold is to study its association with a stable clinical characteristic like age of onset.[34]

So far, no systematic review has been performed investigating the potential effect on clinical characteristics by *T. gondii* infection in patients with schizophrenia.

Aims of the Study

The primary objective of this meta-analysis is to evaluate whether the presence of antibodies against *T. gondii* is associated with clinical characteristics of schizophrenia. Secondly, we analyzed factors potentially influencing the observed effects, including duration of illness, age, and gender.

Methods

We followed the systematic review guidelines provided by the PRISMA statement.[35] The study was registered at Prospero (#CRD42018087766).[36]

Search Strategy

PubMed, Embase, MEDLINE databases were used for the systematic search, see supplementary appendix 1 for search strategy. Titles and abstracts of studies retrieved were screened independently by 3 authors (D.M., J.R., and B.K.) to identify studies that potentially met the inclusion criteria outlined above. Full texts of potentially eligible studies were screened and independently assessed for eligibility by 2 authors (D.M. and B.K.). Any disagreement about the eligibility of particular studies was resolved through discussion with a fourth reviewer (A.S.).

Inclusion Criteria

We conducted a systematic search using the following inclusion criteria: (1) original research papers; (2) published in

any language; (3) analysis of *T. gondii* infection; (4) patients with a disorder in the schizophrenia spectrum defined by a DSM-III (or ICD-9) or higher classification method; (5) measurement of severity of symptoms by means of PANSS or BPRS and/or measurement of age of onset of schizophrenia; (6) use of one of the following diagnostic assays: Sabin-Feldman dye test, complement fixation (CF), immune hemagglutination (IHA), immune fluorescence (IF), or enzyme-linked immunosorbent assay (ELISA). (7) providing data on clinical characteristics in cases with and without *T. gondii* infection. Exclusion criteria were studies that included immunocompromised patients.

Quality Assessment and Data Extraction

A standardized form was used to extract data from the included studies for assessment of study quality and evidence synthesis (supplementary appendix 2). Two review authors (D.M. and B.K.) extracted data independently; discrepancies were identified and resolved through discussion (with A.S. if necessary). Authors were approached for additional data, if necessary, to reach inclusion criteria or on data possible moderators. Also, all authors were asked whether data of published work overlapped and whether they were aware of relevant (un)published work.

Study quality was assessed by 2 review authors (D.M. and A.S.) independently following Cochrane criteria of quality on case-control or cohort studies.[37] If there was a difference in quality score, the difference was discussed. If consensus could not be reached, a third opinion was asked (L.dH.) for a final decision.

Outcome Measures and Moderators

As main outcome measures we selected the average positive, negative and overall symptom scores as measured by PANSS or BPRS. Average scores with their respective standard deviation were converted to standardized mean differences (SMDs). Furthermore, average age of onset was measured in years.

Since *T. gondii* infection is more prevalent with increasing age,[3] mean age at the time of assessment was analyzed as a possible moderator. Gender, age of onset, and duration of illness have shown correlations with the severity of symptoms.[16,28,34,38] Therefore, we used data on all these variables for moderator analysis.

Meta-analysis of Eligible Studies

The random-effects method, which assumes differences among the effect sizes as a result of variations in study characteristics, was chosen since considerable heterogeneity between studies was expected.[2] Heterogeneity between studies was assessed using the I^2 statistic. Meta-analytical calculations were carried out with Comprehensive Meta-analysis Software 3.0.[39]

To assess by which extent moderators influence variance of the true effect, meta-regression analyses were performed for continuous data (using methods of moments analysis) or subgroup analysis for categorical data (with the mixed-effects model). The amount of variance caused by the moderators was expressed by R^2.

Evidence of publication bias was assessed by examination of funnel plots and by the Egger's test (which was considered significant if the one-sided P value was ≤ .10). If applicable, Duval and Tweedie's trim and fill method was used to statistically adjust for indications of publication bias.

Results

Following our systematic search up to June 23, 2019 we found 934 studies, from which 13 studies were finally included (see flowchart in figure 1).[16,18,21,25,27,28,34,38,40–45] Of these studies, one was unpublished.[44,45] In total, 2368 cases, of which 741 were seropositive to *T. gondii* were analyzed. See table 1 for a summary of the included studies.

Total Symptoms

Overall, no association between *T. gondii* infection and severity of total symptoms was found (SMD = 0.06; P = .35). Moderate heterogeneity was observed (I^2 = 39%). Indications for publication bias were observed by the Egger's test (P = .04) and by inspecting the funnel plot (supplementary appendix 3). Remarkably, studies had to be imputed to the right of the mean to correct for this statistical bias with the Duval and Tweedie's trim and fill method, resulting in a corrected nonsignificant SMD of 0.09 (95% confidence interval [CI]: −0.03 to 0.21, P = .14).

When effect of moderators on the heterogeneity was examined, we found no significant difference concerning gender, but we did observe effects of other moderators (table 2).

Firstly, the quality of the study seemed to have a large influence on the variance of observed effects between studies (R^2 = 100%, P = .002). The scatterplot indicated that a higher score on study quality rendered a higher SMD. When grouping the studies into a high and low study quality (High quality being a score of 4 and above), a significant difference between groups was found (Q-between = 14.16; P < .001). The high-quality group showed higher total severity of symptomatology in the group seropositive to *T. gondii* (toxo-positive), whereas in the low-quality group a significant negative association was found (SMD_{High} = 0.22; P < .001 vs SMD_{Low} = −0.16, P = .05).

Furthermore, regression analysis indicated that some of the variance of observed effects could be explained by

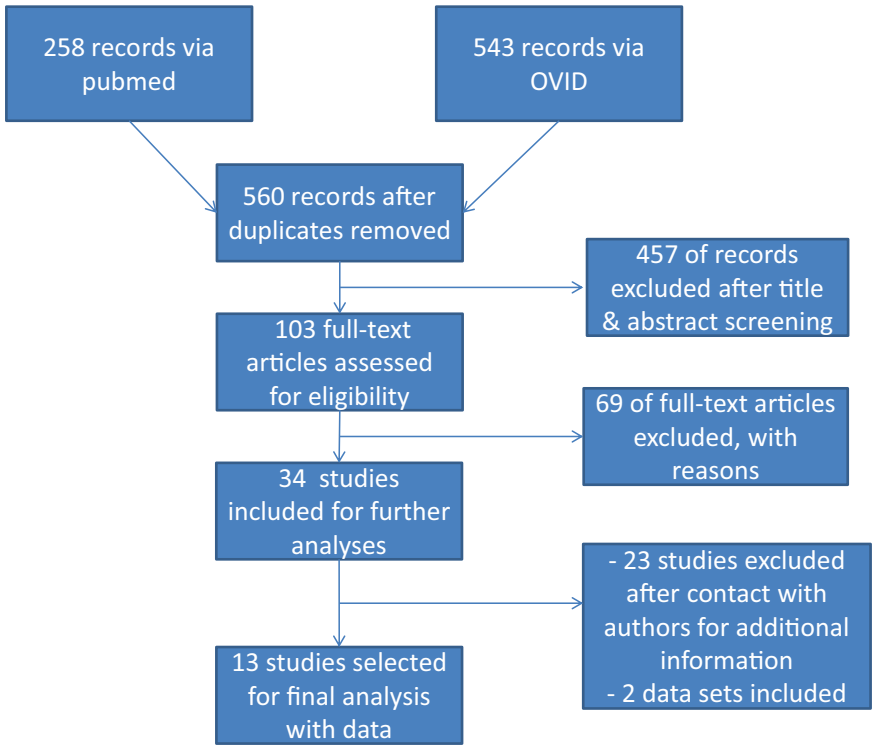

Fig. 1. Flowchart of selection process.

age of the study population (R^2 = 53%, P = .053). The scatterplot showed that a younger mean age rendered a higher SMD. As a secondary analysis, the studies were grouped into 2 categories (cutoff age 35 y), whereby a nonsignificant difference between groups was found (Q-value in between= 1.08; P = .30). Studies with a relatively young mean age did show significantly higher severity of total symptomatology in the toxo-positive group (SMD = 0.13; P = .05), whereas in the older group no association was found (SMD = −0.02; P = .88). Therefore, even though duration of illness (DOI) did not seem to explain a substantial part of the variance, as it is closely related to mean age of the study population, we performed a group analysis for this potential moderator as well. A shorter DOI corresponded with higher severity of total symptomatology at a trend level (DOI < 10 y; SMD = 0.13, P = .06).

Lastly, age of onset was indicated to be responsible for 65% of the variance (P = .03). When grouped into 2 categories, there was no significant difference between groups, neither did one category show a significant SMD.

Positive Symptoms

Overall, no association between *T. gondii* infection and severity of positive symptoms was found (SMD = 0.07; P = .34). Moderate heterogeneity was observed (I^2 = 47%). Indications for publication bias were observed by the Egger's test (P = .08) and by inspecting the funnel plot (supplementary appendix 4). Just as with total symptomatology, Duval and Tweedie's trim and fill method imputed 2 studies that had to be added to the right of the mean, indicative for publication bias underrepresenting studies with more severe positive symptoms in the toxo-positive group. The adjusted effect size showed a nonsignificant small effect: SMD = 0.1 (95% CI: −0.06 to 0.25, P = .21).

When effect of moderators was examined, we found no significant difference concerning gender, overall symptom severity or age of onset, but we did observe effects of other moderators (table 2).

Regression analysis showed that the average mean age of the study population statistically had a large effect on variance in positive symptoms between studies (R^2 = 100%, P = .02). The scatterplot showed that a younger mean age rendered a higher SMD. As a secondary analysis, the studies were grouped into 2 categories (cutoff age 35 y), whereby a significant difference between groups was found (Q-between groups = 10.41; P < .001). Studies with a relatively young mean age showed significantly more severe positive symptoms for the toxo-positive group (SMD = 0.24; P < .001), whereas in the older group no significant association was found.

DOI equally showed to have a large effect on the variance as well (R^2 = 100%, P = .05). A shorter DOI corresponded with a higher SMD (figure 2). Like with mean age, for a secondary analysis, the studies were grouped into 2 categories (cutoff DOI of 10 y), whereby a significant difference between groups was found (Q-value in between = 10.41; P < .001). Toxo-positive

Table 1. Study Characteristics of Studies Reporting *T. gondii* Infection and Schizophrenia Phenotype

Study	Year	Country	Case Population	Questionnaire	Mean Age (y)	Male/ Female	Mean Age of Onset (y, SD)	Average Total Symptom Score (PANSS Score Unless Otherwise Stated)	Mean Duration of Illness (y)
Esshili et al.	2016	Tunisia	Treated patients with schizophrenia (schizophreniform and schizoaffective disorder excluded), recruited at Psychiatric Hospital (n = 246)	BPRS, SANS, SAPS	40.5	186/54	24.51 (6.71)	44 (BPRS)	Unknown
Fond et al.	2015	France	Patients with schizophrenia and schizoaffective disorder, with no history of substance abuse, mental retardation or head trauma. Recruited at admission in 2 university-affiliated psychiatric departments (n = 114)	PANSS	35.7	82/32	25.01 (7.75)	69	10.5
Fond et al.	2018	France	Clinically stable patients (defined as no hospitalization 8 wk prior and no treatment changes with schizophrenia or schizoaffective disorder (n = 250)	PANSS	32.0	184/66	21.8 (6.7)	71	10.6
Holub et al.	2013	Czech Republic	Patients with schizophrenia and schizophrenia spectrum disorders admitted to the Psychiatric Centre, substance use and neurological disorders excluded (n = 251)	PANSS	28.3	141/110	23.77 (6.50)	61	4.6
Horacek et al.	2012	Czech Republic	Patients with schizophrenia that were in clinical remission and had no severe medical illness, alcohol or drug abuse recruited at Prague psychiatric center (n = 44)	PANSS	30.8	22/22	24.31 (8.0)	61	6.6
James et al.	2013	Nigeria	Individuals presenting to the Federal Psychiatric Hospital with a psychosis for the first time (n = 140)	BPRS	28.2	84/56	Unknown, +/- 24.6	47 (BPRS)	3.6
Karabulut et al.	2015	Turkey	Patients with schizophrenia that did not have a neurologic disorder, alcohol or drug abuse or immunodeficiency recruited at Elazig mental health hospital (n = 85)	PANSS	41.7	46/39	unknown	86	unknown
Park et al.	2012	Korea	Patients with schizophrenia that were hospitalized and or treated at outpatient services at psychiatry department of University Hospital (n = 96)	BPRS and PANSS	46.1	62/34	32.07 (12.52)	82	13.8
Perron et al.	2012	France	Patients with schizophrenia, with no history of substance abuse, mental retardation or head trauma. Recruited during or after admission in 2 university-affiliated psychiatric departments (n = 45)	PANSS	34.8	34/11	23.80 (7.55)	65	10.9

Table 1. Continued

Study	Year	Country	Case Population	Questionnaire	Mean Age (y)	Male/ Female	Mean Age of Onset (y, SD)	Average Total Symptom Score (PANSS Score Unless Otherwise Stated)	Mean Duration of Illness (y)
Sutterland et al.	2020	Netherlands	Group 1: Patients with recent onset schizophrenia spectrum disorders recruited at outpatient department of university hospital (n = 155) & Group 2: Stable outpatients with schizophrenia spectrum disorders recruited at multiple psychiatric centers in the Netherlands (n = 354) (GROUP cohort)	PANSS	25.4 (1) & 28.3 (2)	103/46 (1) & 270/84 (2)	Unknown & 22.53 (6.25)	63 (1) & 48 (2)	Unknown (1) & 7.4 (2)
Tanaka et al.	2017	United States	Patients with schizophrenia and schizoaffective disorder recruited at outpatient psychiatric clinic of John Hopkins Hospital (n = 28)	PANSS	39.0	18/10	22.56 (9.27)	63	15.3
Vlatkovic et al.	2017	Croatia	Male inpatients with schizophrenia with an illness duration of at least 5 y, both non-treatment resistant (non-TRS) as treatment resistant schizophrenia (TRS) (n = 210)	PANSS	44.9	210/0	Non-TRS: 23.82 (5.34) TRS: 22.84 (5.39)	109	21.5
Wang et al.	2006	China	Inpatients with first-episode schizophrenia spectrum disorders with PANSS score above 60, substance use and neurological disorders excluded (n = 600)	PANSS	22.6	291/309	22.01 (5.46)	78	0.5

Table 2. Analysis of the Association of *T. gondii* Infection With Clinical Phenotype in Schizophrenia Subjects, Including Moderator Assessment

Clinical Phenotype Measurement	Moderator	Available Datasets	Method of Analysis	Heterogeneity (I^2)	Coefficient and R^2 or Q-Between Groups (P-value)	SMD (P-value)
Total symptoms	None	13	Random-effects	39%	-	0.06 (P = .35)
	Gender	Female (5) Male (7)	Mixed-effects	0% 30%	Q = 0.587 (P = .30)	0.07 (P = .50) −0.03 (P = .84)
	Mean age	13	MofM	-	Coefficient = −0.016 R^2 = .53 (P = .053)	-
	Age of onset	10	MofM	-	**Coefficient = −0.13** **R^2 = .65 (P = .03)**	-
	Duration of illness	10	MofM	-	Coefficient = −0.016 R^2 = .11 (0.20)	-
	Study quality	12	MofM	-	**Coefficient = 0.079** **R^2 = 1.00 (P = .002)**	-
	Grouped by mean age	<35 y (6) >35 y (7)	Mixed-effects	0% 61%	Q = 1.08 (P = .30)	**0.13 (P = .05)** −0.02 (P = .88)
	Grouped by duration of illness	<10 y (5) >10 y (5)	Mixed-effects	0% 73%	Q= 0.89 (P = .35)	0.13 (P = .06) −0.06 (P = .76)
	Grouped by study quality	3 or lower (7) 4 or higher (6)	Mixed-effects	0% 0%	**Q = 14.16 (P < .001)**	**−0.16 (P = .05)** **0.22 (P < .001)**
	Grouped by age of onset	<23.5 y (5) >23.5 y (5)	Mixed-effects	68% 0%	Q = 0.34 (P = .56)	0.13 (P = .31) 0.04 (P = .67)
Positive symptoms	None	13	Random-effects	47%	-	0.07 (P = .34)
	Gender	Female (5)Male (7)	Mixed-effects	0% 31%	Q = 0.92 (P = .34)	0.18 (P = .23) 0.03 (P = .80)
	Mean age	13	MofM	-	**Coefficient = −0.025** **R^2 = 1.00 (P < .001)**	-
	Overall symptom severity	13	MofM	-	Coefficient = −0.007 R^2 = .27 (P = .11)	-
	Age of onset	10	MofM	-	Coefficient = −0.037 R^2 = .20 (P = .21)	-
	Duration of illness	9	MofM	-	**Coefficient= −0.029** **R^2 = 1.00 (P < .001)**	-
	Study quality	13	MofM	-	**Coefficient = 0.098** **R^2 = .84 (P = .004)**	-
	Grouped by duration of illness	<10 y (4) >10 y (5)	Mixed-effects	0% 0%	**Q = 8.02 (P = .005)**	**0.32 (P < .001)** 0.003 (P = .98)
	Grouped by mean age	<35 y (7) >35 y (6)	Mixed-effects	7% 0%	**Q = 12.49 (P < .001)**	**0.24 (P < .001)** −0.13 (P = .11)
	Grouped by study quality	3 or lower (6) 4 or higher (7)	Mixed-effects	0% 44%	**Q = 5.64 (P = .02)**	−0.12 (P = .19) **0.17 (P = .04)**
Negative symptoms	None	13	Random-effects	57%	-	0.02 (P = .84)
	Gender	Female (5) Male (7)	Mixed-effects	16% 10%	Q = 0.33 (P = .57)	−0.04 (P = .80) 0.07 (P = .43)
	Mean age	13	MofM	-	Coefficient= 0.008 R^2 = .00 (P = .51)	-
	Overall symptom severity	12	MofM	-	Coefficient = −0.001 R^2 = .00 (P = .92)	-

Table 2. Continued

Clinical Phenotype Measurement	Moderator	Available Datasets	Method of Analysis	Heterogeneity (I^2)	Coefficient and R^2 or Q-Between Groups (P-value)	SMD (P-value)
	Age of onset	10	MofM	-	Coefficient= 0.030 R^2 = .00 (P = .43)	-
	Duration of illness	9	MofM	-	Coefficient = 0.008 R^2 = .00 (P = .67)	-
	Study quality	13	MofM	-	Coefficient = 0.078 R^2 = .21 (P = .07)	-
	Grouped by study quality	3 or lower (6) 4 or higher (7)	Mixed-effects	0% 64%	**Q = 5.65 (P = .02)**	−0.18 (P = .052) 0.15 (P = .15)
Age of onset	None	10	Random-effects	63%	-	**0.25 (P = .02)**
	Study quality	10	MofM	-	Coefficient = −0.75 R^2 = .07 (P = .07)	-
	Grouped by study quality	3 or lower (4) 4 or higher (6)	Mixed-effects	0% 66%	**Q = 6.33 (P = .01)**	**0.6 (P < .001)** 0.12 (P = .24)
	Mean age	10	MofM		Coefficient = 0.018 R^2 = .17 (P = .22)	
	Duration of illness	9	MofM		Coefficient = 0.036 R^2 = .22 (P = .14)	

Note: SMD, standardized mean difference; MofM, methods of moments; significant P-values of <.05 are highlighted in bold.

cases with short DOI had more severe positive symptoms (SMD = 0.32; P < .001). The other group showed no association. Since both DOI and mean age were statistically responsible for all of the variance, a correlation between these moderators was expected. Analyzing the correlation between duration of illness and average mean age with the Spearman's rank test indeed showed a very high correlation (rho = 0.950; P < .001).

Lastly, the quality of studies significantly moderated the variance of the observed effect between studies (R^2 = 75%, P = .02). The scatterplot indicated that a higher score on quality rendered a higher SMD. When grouping the studies into a high and low study quality, a significant difference between groups was found (Q-between groups = 4.27; P = .04). The high-quality group showed more severe positive symptoms in the toxo-positive group (SMD = 0.17; P = .04), whereas in the low-quality group no significant association was found.

Negative Symptoms

No association between a *T. gondii* infection and the severity of negative symptoms was found (SMD = 0.02; P = .84). Moderate heterogeneity was found between studies (I^2 = 57%). There was no indication for publication bias by the Egger's test (P = .27) or inspection of the funnel plot.

The impact of potential moderators was assessed. Of all the assessed moderators, only study quality showed an indication that it caused part of the variance: R^2 = 21% (P = .07). When grouping the studies into a high and low study quality, a significant difference between groups was found (Q-between groups= 5.65; P = .02). The low-quality group showed a trend for less severe negative symptoms in the toxo-positive group (SMD = −0.18; P = .052), whereas in the high-quality studies no significant association was found (table 2).

Age of Onset

Overall, a significantly higher age of onset in toxo-positive cases with schizophrenia was found with an average absolute difference of 1.78 years (95% CI: 0.35–2.44, P = .015). Significant heterogeneity was observed between studies (I^2 = 63%). Indications of publication bias were seen by the Egger's test (P = .02) and by funnel plot inspection. Using Duval and Tweedie's trim and fill, 3 studies were imputed on the left side of the mean. This is suggestive for publication bias with an underrepresentation of studies with a lower age of onset in the toxo-positive group. The adjusted difference became non-significant (P = .14).

Quality of the study potentially explained some of the variance of the true effect (R^2 = 7%, P = 0.07). In the scatterplot, a higher quality of the study meant a lower difference for age of onset. When studies were grouped

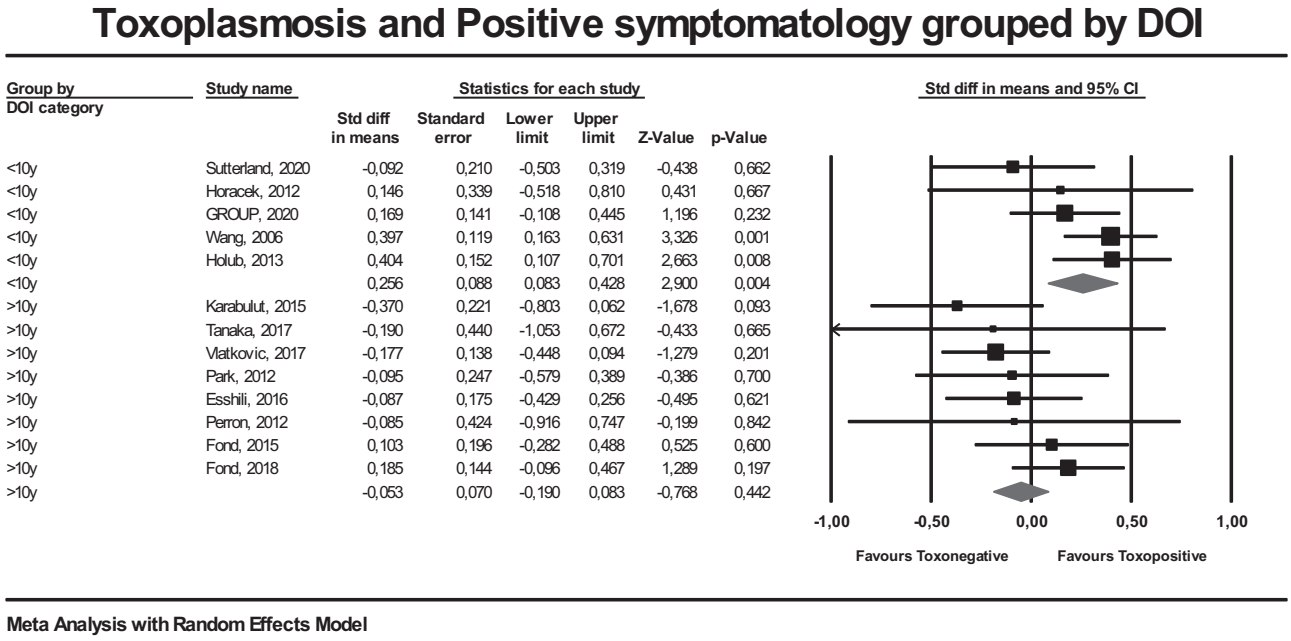

Fig. 2. Forest plot of *T. gondii* infection and positive symptomatology in individuals with schizophrenia grouped by duration of illness (DOI).

according to quality score as previously described, a significant difference was found between groups (Q-between = 6.33, P = .01). Only in the group with lower quality studies a significant difference in mean age between the toxo-positive group and toxo-negative group was observed (table 2).

Discussion

This study was the first to systematically investigate the potential influence of *T. gondii* on clinical characteristics in schizophrenia by means of a meta-analysis. We did not find an association of *T. gondii* infection and severity of symptomatology overall. However, when addressing the heterogeneity between studies we did find significantly increased severity of total and positive symptoms in toxo-positive individuals with schizophrenia with an average age under 35 years and a duration of illness of under 10 years. Also, indication of an older age of onset was found among toxo-positive individuals with schizophrenia, although this finding appeared to be influenced by publication bias and study quality.

Our findings seem to indicate that *T. gondii* infection is associated with severity of symptomatology in the early stage of schizophrenia, whereby the observed effect on total symptomatology seems to be driven by an increase in positive symptoms. In our moderator analysis we found (not surprisingly) a very high correlation between a shorter DOI and younger mean age, making it impossible to determine which factor is driving the moderation effect. It is fair to reason that DOI as an explanation would be more plausible. When presuming *T. gondii* is an etiological factor contributing to the risk of schizophrenia, it could be expected that the effect on symptom severity is more prominent in those with recent onset psychosis: the risk of contracting an infection by *T. gondii* increases with age, also in patients who already have schizophrenia due to other etiological factors such as genetic and environmental factors including drug abuse and childhood trauma.[1,46] This could result in a dilution of the effect of seropositivity for *T. gondii* on clinical characteristics in an older sample, as in these patients with established schizophrenia the contribution of *T. gondii* infection might not be discernable.[32] A recent meta-analysis on *T. gondii* infection and bipolar disorder also found an association only in the studies studying a younger population.[30] Moreover, during time there is also an increasing variation due to comorbidity, antipsychotic treatment and pathophysiological changes during illness progression into chronic stages, affecting severity of symptomatology.[46–48] With regard to the association of total symptomatology and seropositivity to *T. gondii* infection, we also found that average age of onset moderated the variance between studies, where a younger age of onset rendered higher symptoms in individuals with schizophrenia who were toxo-positive. We believe this because the average age of onset will be influenced by the population characteristics age at inclusion as well as DOI, as these variables are intercorrelated.

No association was found between *T. gondii* infection and negative symptom severity. Assessed moderators also did not change this finding, indicating that *T. gondii* probably specifically influences positive symptoms in the early stages of schizophrenia. As negative symptomatology is

relatively stable during the course of disease,[49] a false-negative finding is considered less likely. These findings could point at the influence of *T. gondii* on proposed dopamine pathway since a hyperdopaminergic state is connected more to positive rather than negative symptoms.[50]

Remarkably, our analysis showed indications for publication bias, suggesting that studies that show an association of *T. gondii* infection with total and positive symptomatology in schizophrenia are underrepresented. In an era where incentives greatly support reporting positive over negative findings, this finding at first glance appears unlikely. However, when considering the overall effect sizes we found (SMD = 0.1), it could be that there have been studies that did find a positive association, but lacked sufficient power to prove the significance and subsequently remained unpublished. An extra indication that *T. gondii* infection could truly be associated with higher positive symptomatology is that high-quality studies showed a significant association when analyzed separately. In addition, studies with lower scores on study quality tended to show a negative association with symptomatology, even reaching significance for total symptomatology. It is unclear why these studies tended to have opposite results. One possible explanation could be that studies that did not perform age-matching or correction tended to have an older population of toxo-positive individuals with schizophrenia (as the risk of toxopositivity increases with age). Since we have found that studies that included, on average, a younger population with a shorter DOI did show a positive association as opposed to older individuals with a longer DOI, this could be a resemblance of that finding. Another explanation that needs to be addressed is the possibility that studies of lower methodological quality have a higher chance of reporting chance findings and, therefore, a higher risk of contributing to publication bias.[51] However, these explanations seem unlikely as the statistical indication of publication bias pointed at a lack of studies with positive associations. More high-quality studies with a large sample size targeting individuals with recent-onset schizophrenia are needed to solidify or reject current findings.

Lastly, it appeared from our meta-analysis that individuals with schizophrenia had a later age of onset when they are toxo-positive. However, this finding should be treated with great caution, since we found both indications for publication bias as for indications that studies with lower methodological quality were responsible for this finding. Moreover, a significantly later age of onset could well be an epiphenomenon of the fact that older patients have an increased chance of being seropositive to *T. gondii*. As studies with lower quality less often performed age-matching or correction, their individuals with schizophrenia who were toxo-positive were on average older, increasing the chance of finding a higher age of onset. We, therefore, feel reluctant to interpret this finding. However, one could speculate that some individuals with schizophrenia only pass the psychosis-threshold because of the *T. gondii* infection. Since with increasing age, more people acquire a latent *T. gondii* infection,[3] the average age of onset for people who develop psychosis because of the *T. gondii* seroconversion is higher than for those who develop psychotic symptoms due to other etiological factors that often occur earlier in life.[52] Nevertheless, we deem the current evidence concerning an effect on the age of onset as insufficient to support the latter hypothesis.

Limitations

Certain analyses could not be performed in our study. Since all included studies were cross-sectional in design, it was impossible to assess whether the infection preceded illness onset in toxo-positive individuals. In addition, when people have contracted *T. gondii* infection, this remains a fairly stable characteristic during life. Conversely, clinical symptoms fluctuate over time and are influenced by treatment response, drug use, stage of illness, and other factors.[46,53] Results from an investigation concerning an association between a trait and state variable cross-sectionally, therefore, needs to be interpreted with caution. Longitudinal studies are needed in order to better determine if a *T. gondii* infection truly influences symptomatology.

Another limitation is that the number of studies is still relatively modest and some studies with data on this matter had to be excluded because they did not provide sufficient data that would make it suitable for our analysis. Policies making data available for the scientific community after publication of a study are increasingly supported and would help increase quality of future meta-analyses. Nevertheless, other authors did provide additional data, and we were able to include hitherto unpublished data. This enabled us to analyze several moderators, contributing to a better understanding why certain studies did find an effect while others did not.

As discussed earlier, our study showed indications for publication bias. This seemed especially problematic with respect to the association of *T. gondii* infection with age of onset but not with regard to the associations found with symptomatology. Making data publicly accessible after completion of a study is increasingly advocated and could help combat this issue.[54]

Conclusion

The findings in this meta-analysis suggest that *T. gondii* may influence severity of symptomatology (mainly positive symptoms) of schizophrenia in the early stages of the disorder. These findings are in line with other evidence that this infection could play a causal role in schizophrenia.[2,7,10]

This may imply that the global morbidity caused by this infection is much larger than hitherto assumed. More studies are necessary to investigate the hypothesis of *T. gondii* infection affecting human behavior, whereby longitudinal studies are needed to assess the effect of *T. gondii* on the course of symptomatology. Nevertheless, it may be prudent to direct more research at preventing the infection as well as its possible deleterious consequences.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin Open* online.

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