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Lack of circulating *toxoplasma gondii* DNA in seropositive patients with bipolar or schizophrenia spectrum disorders

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ABSTRACT

Toxoplasmosis has been previously associated with an increased risk of having Schizophrenia or Bipolar disorder in several epidemiological studies. The aim of this observational, cross-sectional study was to examine the seroprevalence of *Toxoplasma* infection in a cohort of Italian psychiatric inpatients and to verify the presence of circulating *Toxoplasma gondii* DNA in the seropositive subjects. Sixty-three patients affected by bipolar or schizoaffective disorders according to DSM-5 criteria were enrolled. The presence of *Toxoplasma* infection was firstly examined using an indirect serological method (ELFA), and three different direct PCR-based methods were performed to detect circulating DNA in the seropositive patients. The seroprevalence of infection was 28.6%, with a significant association between higher age and the infection status. PCR, nested-PCR and Real-Time PCR revealed no positive samples for *Toxoplasma gondii.* This result is in contrast with recent data from case-control studies that detected parasite genome in patients with different neuropsychiatric diagnosis without clinical evidence of acute toxoplasmosis. Our findings are to be interpreted with caution, because of the small sample size, the heterogeneity of enrolled patients and the observational nature of the study. Further studies are needed to better define the clinical features correlated to the seropositive status in neuropsychiatric patients.

1. Introduction

Toxoplasmosis is the most common neurotrophic protozoan infection in humans caused by the apicomplexan parasite *Toxoplasma gondii* (*T. gondii*), infecting around one-third of the human population (Flegr et al., 2014; Montoya and Liesenfeld, 2004). Disease manifestations can range from asymptomatic infection in immunocompetent individuals to severe forms of encephalitis, chorioretinitis, uveitis, or multi-organ involvement among immunocompromised individuals, congenital infection or infection with atypical or more virulent *Toxoplasma* genotypes (Boothroyd and Grigg, 2002; Montoya and Liesenfeld, 2004; Remington et al., 2001; Xiao et al., 2011). In Central Italy, a geographical area at intermediate prevalence of toxoplasmosis, the percentage of seropositivity is estimated to be in the 25–30% range (Mosti et al., 2013; Pinto et al., 2012, 2017), whereas prevalence rates up to 90% have been reported in areas of South America and Africa at high-risk of toxoplasmosis (Dubey and Jones, 2008; Gelaye et al., 2015; Montoya and Liesenfeld, 2004; Robert-Gangneux and Dardé, 2012). Humans, the intermediate hosts, usually acquire the infection by oral ingestion of tissue cysts containing bradyzoites through the consumption of raw or undercooked meat infected with *T. gondii*, or via ingestion of the parasite's oocysts spread by the feces of infected cats through consumption of contaminated water, raw fruits and vegetables (Bahia-Oliveira et al., 2003; Jones et al., 2009; Weiss and Dubey, 2009). Congenital infection is less frequent in Countries where prenatal program of surveillance is applied (Wallon and Peyron, 2018).

T. gondii has a particular tropism for muscle and brain tissues, where it remains localized in the form of cysts throughout life and establishes a chronic infection stimulating the production of a variety of cytokines by microglia, astrocytes and neurons (Carruthers and Suzuki, 2007; Prandovszky et al., 2011). In last decades, an increasing number of epidemiological studies has highlighted the potential involvement of *T. gondii* infection in the etiopathogenesis of several neuropsychiatric disorders. The role of this infectious agent in the emergence of major psychosis, such as Schizophrenia (SCZ) spectrum disorders, has been widely documented (Amminger et al., 2007; Esshili et al., 2016; Hinze-

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Selch et al., 2007; Torrey et al., 2007, 2012; Wang et al., 2006; Yolken et al., 2017) and, more recently, a strong association between *Toxoplasma* exposure and Bipolar disorder (BD) has been detected in several epidemiological studies (Dickerson et al., 2014a, 2014b; Hamdani et al., 2013, 2015; Pearce et al., 2012; Sutterland et al., 2015; Tedla et al., 2011). BD and SCZ are known to be the result of a complex interaction between genetic predisposition and environmental risk factors (Gershon et al., 2011; Kerner, 2014; Sullivan et al., 2012), including prenatal injuries and infectious insults like *T. gondii* (Arias et al., 2012; Brown and Derkits, 2010; Cannon et al., 2002; Torrey et al., 2012). The odds ratio (OR) of having SCZ among individuals with positive IgG anti-*T. gondii* has been calculated to be 2.7 in the meta-analysis of Torrey et al. (2007), while that of having BD among seropositive subjects was 2.4 in the case-control study of Hamdani et al. (2013). However, this evidence only suggests an epidemiologic correlation between toxoplasmosis and BD or SCZ, and no conclusive causal effect of *T. gondii* can be established so far.

It has been hypothesized that T. *gondii* might contribute to the onset and the progression of psychiatric symptoms by inducing brain function alterations including: neuroinflammation through the production of cytokines by microglia and astrocytes; increased dopamine production and imbalance of other relevant neurotransmitters (serotonin, glutamate, gamma-aminobutyric acid); promotion of neurodegenerative processes and impact on neuroplasticity (Prandovszky et al., 2011; Flegr, 2013; Fabiani et al., 2013, 2015; Parlog et al., 2015; Del Grande et al., 2017a; Yolken et al., 2009).

The large majority of studies investigating the association of *T. gondii* infection with neuropsychiatric disorders were based on serological assessments and produced controversial results (Alvarado-Esquivel et al., 2011; Arias et al., 2012; Cetinkaya et al., 2007; Dickerson et al., 2014b; Freedman et al., 2016; Hamdani et al., 2013; Pearce et al., 2012; Sutterland et al., 2015; Tedla et al., 2011; Torrey et al., 2007, 2012). Noteworthy, recent evidence demonstrated the presence of circulating *T. gondii* DNA in the blood of psychiatric or neurologic patients, which represents a direct evidence of reactivation of the parasite infection and could be useful to clarify the causative role of *T. gondii*. Del Grande et al. (2017b) described the case of a patient affected by recurrent ocular toxoplasmosis and BD with psychotic features in which parasite DNA was directly detected at the time of the occurrence of psychiatric symptoms through molecular analyses (nested-PCR).

Moreover, in three case-control studies a high positivity rate for circulating *T. gondii* DNA was found in patients with SCZ, Parkinson's disease (PD) and Alzheimer's disease (AD) without clinical evidence of toxoplasmosis (Fallahi et al., 2017; Omar et al., 2015; Rashno et al., 2017).

On the basis of such evidence from the literature, we aimed to investigate the seroprevalence of *T. gondii* infection in a sample of Italian psychiatric inpatients affected by BD or SCZ spectrum disorders, the psychiatric diseases predominantly associated to Toxoplasmosis. We particularly focused on the examination of the presence of circulating *T. gondii* DNA in the seropositive subjects. The findings would add evidence to the relationship between *T. gondii* and BD or non-affective psychosis.

2. Material and methods

2.1. Sample

In this observational, cross-sectional study we enrolled sixty three inpatients, all born in Italy, with a diagnosis belonging to SCZ spectrum disorders or BD with or without psychotic features, recruited among patients consecutively admitted at the hospital or day-hospital unit of the Psychiatric Clinic of the Department of Clinical and Experimental Medicine, University of Pisa, Italy. The diagnosis was made by a research team of psychiatrists (C.D.G., E.S., I.M.) according to DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) criteria by the use of the SCID-5-RV (Structured Clinical Interview for DSM-5, Research Version) (First et al., 2015). The study was approved by local institutional ethical committee (No. 333, approved on May 14th 2015) and all participants provided written informed consent after the explanation of the study procedures.

An anamnestic questionnaire was administered to the enrolled patients in order to collect socio-demographic characteristics (age, birthplace, place of residence, education level, marital status, working status, family environment), risk factors of exposure to the parasite (travel in regions at high-risk of toxoplasmosis, rural dwelling, usual consumption of raw or undercooked meat, habitual contact with cats during life), the presence of pathological conditions well known to be associated with toxoplasmic infection (lifetime episodes of lymphadenopathy, abnormal psychomotor development, epilepsy, eye diseases), family history of allergic or autoimmune diseases, family history of mental disorders and number of hospital admissions related to mental disorders. The Clinical Global Impression (CGI) Scale (Guy, 1976) was used to evaluate the severity of current episode.

At the time of hospitalization, peripheral blood of each patient was collected in a 10 ml Vacutainer tube without anticoagulant for serological analysis and in a 5 mL Vacutainer tube with EDTA (Vacutest, Kima) for molecular analysis. Samples for serological investigations were centrifuged at 1500 x g for 10 min before storage at −20 °C, while those allocated to molecular analysis were immediately stored at −20 °C.

2.2. T. gondii serological analysis

The serum samples were examined by the Enzyme Linked Fluorescent Assay (ELFA, Biomerieux, France) using the Vidas Toxo IgG II kit for IgG and Vidas Toxo IgM kit for IgM. The serological tests were all conducted in accordance with the manufacturer's instructions. The level of IgG was expressed in International Units/mL and samples were considered positive for IgG antibodies when the value was >8 IU/mL, indeterminate from ≥4 to ≤8 IU/mL and negative when <4 IU/mL. The IgM level was expressed as sample index (value of relative fluorescence of the sample/relative fluorescence value of the standard); ELFA results were positive when the reagent index was ≥0.65 IU/mL, indeterminate from <0.65 to ≥0.55 IU/mL and negative < 0.55 IU/mL.

2.3. T. gondii molecular analysis

Peripheral blood samples were collected in EDTA from patients with serologically positive results, DNA was extracted and molecular analysis was performed.

2.3.1. DNA extraction

DNA was extracted from 5 mL of peripheral blood using Qiamp Blood Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA was also extracted from *T. gondii* tachyzoites (kindly provided by Dr. Spano the ISS of Rome) was performed, as positive extraction control. DNA samples were stored in a freezer at −20 °C until polymerase chain reaction (PCR).

2.3.1. PCR

PCR was performed selecting a 529 bp fragment that is repeated 200–300-fold in the genome of *T. gondii*, as previously described by Homan et al. (2000). Polymerase chain reaction with the 529 bp fragment is more sensitive than with the 35-copy B1 gene. PCR was conducted in 0.5 mL microcentrifuge tubes containing a final volume of 50 μL composed by 60 mM Tris–HCl (pH 9.0), 2 mM $MgCl_2$, 15 MM of (NH4) 2 SO_4, 0.1% Triton X-100, 0.5 μM of each primer, 100 μM of Deoxyribonucleoside triphosphate, 0.5 U of Taq polymerase (Thermo Scientific) and 10 ng of DNA extracted.

The TOX4 primers (CGCTGCAGGGAGGAAGACGAAAGTTG) and TOX5 (CGCTGCAGACACAGTGCATCTGGATT) were selected respectively from the 5 'and 3′ ends of the fragment of 529 pb.

The PCR cycling conditions consisted of an initial denaturation step at 94 °C for 7 min, 35 amplification cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C with a final extension of 10 min at 72 °C in a thermocycler.

The amplification product ran by horizontal electrophoresis on agarose at 1.5%. The electrophoretic running occurred in TBE 1X buffer (Tris 89 mM, Boric acid 89 mM, EDTA 0.5 M, pH 8.8) for one hour to 100 V. Ethyl bromide (0.5 mg / mL) was incorporated into the agarose gel to allow for DNA highlighting by ultraviolet exposure. The molecular weight of the obtained bands was determined by comparison with markers of known molecular size (100 bp).

2.3.2. Nested-PCR

Nested-PCR was performed employing different primer pairs which amplify the MAG1 gene that encodes a 65-kDa matrix antigen strongly expressed by the bradyzoides. Although MAG1 protein was originally described as being expressed specifically during bradyzoite development, a study showed that this protein is expressed during both tachyzoite and bradyzoite development (Ferguson and Parmley. 2002). This method was previously tested with success by Contini et al. (2002) to improve the detection rate of toxoplasmosis reactivation in suspected HIV or HIV-susceptible patients.

Nested-PCR was initially performed in 0.5 mL microcentrifuge tubes in the final volume of 50 µL containing 1.5 mM MgCl2, 0.2 mM dNTP, 10 µm of each primer and 2 U of Taq and 10 ng of extracted DNA. The second amplification was performed using 2 µL of amplificate from the first reaction.

External primers M1OP1 (sense primer) and M1OP2 (antisense primer), and internal M1IP1 (Sense primer) and M1IP2 (antisense primer) had the following sequences, respectively: TGAGAACTCAGAG GACGTTGC (M1OP1), TCTGACTCAAGCTCGTCTGCT (M1OP2), GCAT CAGCATGAGACAGAAGA (M1IP1) and CCAACTTCGAAACTGATGTCG (M1IP2).

The amplification reaction performed by a thermocycler consisted of an incubation of 10 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 48 °C, one minute at 72 °C, and a final incubation of ten minutes at 72 °C.

The amplification product ran by horizontal electrophoresis on agarose at 2%. The electrophoretic running occurred in TBE 1X buffer (Tris 89 mM, Boric acid 89 mM, EDTA 0.5 M, pH 8.8) for one hour to 100 V. Ethyl bromide (0.5 mg / mL) was incorporated into the agarose gel to allow for the DNA highlighting by ultraviolet exposure. The molecular weight of the obtained bands was determined by comparison with markers of known molecular size (100 bp).

2.3.3. Real time-PCR

DNA extractions from IgG anti-*Toxoplasma* positive blood samples were tested for the presence of *T. gondii* through a Real-Time PCR protocol, using the SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). The target was a 200–300-fold repetitive 529 bp region of the parasite genome, detected using the primers TOX4 and TOX5, as described by Homan et al. (2000). The concentration for each primer was 0.25 µM in a final volume of 20 µl. Amplification protocol was characterised by a denaturation step of 7 min at 94 °C followed by 45 repeated cycles at 94 °C (30 s), 55 °C (30 s) and 72 °C (30 s). Fluorescence signals were collected at the end of every cycle and the presence of unspecific products was avoided through the analysis of the melting curve. Each sample was tested with at least four replicates.

The presence of the parasite DNA was quantified through a standard curve. The DNA deriving from *T. gondii* tachyzoites was used as template for the amplification of the 529 bp fragment. The PCR product was run on a 2% agarose gel, extracted using a commercial kit (JETQUICK Gel Extraction Spin Kit, Genomed), and quantified through spectrophotometry (Eppendorf). The standard curve was fitted within 4 points and ranging from 1.6 µg/µl to 1.6 pg/µl.

To confirm the presence or the absence of specific products, random amplifications were fractionated on a 2% agarose gel, stained with SYBR Safe DNA Gel Stain (Thermo Fischer Scientific), and visualized by UV transillumination.

2.4. Statistical analysis

Qualitative variables were expressed as numbers and percentage whereas quantitative variables were expressed as mean and standard deviation. The analysis of demographic, clinical and biological characteristics of seropositive patients, versus seronegative patients, was performed using Pearson's chi square test or Fisher's exact test, when appropriated, for categorical variables. Quantitative variables were compared using the Mann-Whitney test. A p-value <0.05 was considered significant. Power calculation for two-sample comparison of proportions was performed using a significance level (alpha) of 0.05. Statistical analysis was performed using GraphPad Stat Software. Statistical power and sample size were calculated using Epi Info ™ 7.

3. Results

The study population was composed of 43 patients with BD and 20 patients with SCZ spectrum disorders, all fulfilling the diagnostic criteria for schizoaffective disorder (SZA). Anti-*T. gondii* IgG were present in eighteen patients (28.6%) and none of them were in an acute phase of infection since IgM antibody detection was negative for the whole sample.

Comparing the socio-demographic, epidemiological and clinical characteristics of *T. gondii* seropositive and seronegative patients, a statistically significant difference in mean age was observed between the two groups (45.7 ± 9.4 years vs 38.2 ± 12.2 years, $p = 0.029$). Non-significant differences were observed for other two risk factors for *T. gondii* infection, rural dwelling (44.4% vs 24.4%, $p = 0.130$) and raw meat consumption (88.9% vs 66.7%, $p = 0.120$) while no evidence of association was observed for other subjects characteristics (Table 1).

With regard to the psychiatric clinical features of the subjects, there was no significant association between specific diagnostic categories and *T. gondii*-seropositive status (Table 2).

However, such negative results are to be interpreted with caution given the small sample size and therefore the limited statistical power of the study. For instance, the statistical power to detect a significant association between rural dwelling and *T.gondii* seropositivity was only 32.2%, and a larger sample size of 220 subjects would have been necessary to reach 80% power. Statistical power and sample size calculations for all other factors are shown in Supplementary Table 1.

The median titre of anti-*T. gondii* IgG was 46.5 IU/mL (range 9–300 IU/mL). Because of the great difference between the size of the two diagnostic categories of patients, it is difficult to evaluate the relevance of different serum intensity between the two groups.

PCR, nested-PCR and Real-Time PCR revealed no positive samples for *T. gondii*. Agarose gel runs with amplification products obtained with PCR assays are shown in Supplementary Fig. 1.

4. Discussion

This study aimed to investigate the seroprevalence of *T. gondii* infection in a sample of Italian psychiatric inpatients affected by BD or SCZ spectrum disorders using both serological and molecular techniques.

In our sample composed of patients affected by BD or SZA, we observed a seroprevalence rate for *T. gondii* of 28%, which is similar to what observed in the general population in Italy with the same age distribution, as previously reported in some epidemiological studies

Table 1
Socio-demographic, epidemiological and clinical characteristics of the sample: comparison between *Toxoplasma*-seropositive and *Toxoplasma*-seronegative patients.

	Total (*n* = 63)	Seropositive (*n* = 18)	Seronegative (*n* = 45)	*p*-value
Age (years, mean ± standard deviation)	40.4 ± 11.8	45.7 ± 9.4	38.2 ± 12.2	0.03[a]
Gender (female)	46% (*n* = 29)	50% (*n* = 9)	44.4% (*n* = 20)	0.78[b]
Educational level (secondary school and above vs lower than secondary school)	76.2% (*n* = 48)	77.8% (*n* = 14)	75.6% (*n* = 34)	1.00[b]
Rural dwelling (yes vs no)	30.2% (*n* = 19)	44.4% (*n* = 8)	24.4% (*n* = 11)	0.13[b]
Usual consumption of raw or undercooked meat (yes vs no)	73% (*n* = 46)	88.9% (*n* = 16)	66.7% (*n* = 30)	0.12[b]
Habitual contact with cats during life (yes vs no)	63.5% (*n* = 40)	66.6% (*n* = 12)	62.2% (*n* = 28)	0.78[b]
Ocular diseases (yes vs no)	6.3% (*n* = 4)	5.5% (*n* = 1)	6.7% (*n* = 3)	1.00[b]
Epilepsy (yes vs no)	11.1% (*n* = 7)	11.1% (*n* = 2)	11.1% (*n* = 5)	1.00[b]
Family history of allergic diseases (yes vs no)	17.5% (*n* = 11)	11.1% (*n* = 2)	20% (*n* = 9)	0.49[b]
Family history of autoimmune diseases (yes vs no)	4.7% (*n* = 3)	5.5% (*n* = 1)	4.4% (*n* = 2)	1.00[b]

p values calculated by Fisher's Exact Test.
[a] Mann–Whitney test.
[b] Pearson's chi-square test and correction of Yates.

(Mosti et al., 2013; Pinto et al., 2012, 2017). Although there was no statistically significant difference in the seropositive status within the two different diagnostic groups, a higher percentage of seropositivity has been detected in the group of bipolar patients (32.5%) with respect to schizophrenic patients (20%), supporting the evidence of a positive epidemiological relationship between toxoplasmosis and BD (Abdollahian et al., 2017; Dickerson et al., 2014b; Hamdani et al., 2013; Pearce et al., 2012; Tedla et al., 2011). However, BD is a multifactorial illness involving both genetic and environmental factors, and a causal etiopathogenetic effect of *T. gondii* cannot be established.

Our data also showed a different age distribution between seropositive and seronegative patients. The detection of a highest age within the seropositive group is in agreement with epidemiological data that shows an increase in the incidence of toxoplasmic infection associated with age, and a concomitant reduction in the rate of infection in youngest people in medium and high income populations in North Europe and USA (Dubey and Jones, 2008; Hofhuis et al., 2011; Jones et al., 2007, 2018; Mosti et al., 2013; Pinto et al., 2017).

Comparing the other socio-demographic, epidemiological and clinical characteristics of *T. gondii* seropositive and seronegative subjects we did not observe any statistically significant difference, although such negative results are partly attributable to the insufficient sample size of the present study, as shown by power calculations.

The main finding of our study is the lack of detection of circulating *T. gondii* DNA in the group of seropositive patients despite the use of three different molecular analysis methods. This result is in contrast with data reported in three previous case-control studies, in which the parasite genome was detected in patients with SCZ, PD and AD, respectively, without clinical evidence of acute toxoplasmosis (Fallahi et al., 2017; Omar et al., 2015; Rashno et al., 2017). Moreover, we reported the case of a Brazilian female affected by BD with psychotic features and recurrent episodes of toxoplasmic chorioretinitis and uveitis (Del Grande et al. 2017b). Noteworthy, this was the first case described in the literature in which infection reactivation was directly documented through molecular analyses (nested-PCR) and indirectly, by the high level of *T. gondii*-specific IgG, while ocular and psychiatric symptoms were present.

PCR-based methods for *T. gondii* detection in blood of infected patients can include many methods, like those described in the current study. Differences exist among these tests regarding sensitivity and specificity. Nested PCR is the usual method employed, but it was interesting to verify if a more rapid, but equally sensitive method, could be applied. Robert-Gangneux and Belaz (2016) described real-time quantitative PCR (qPCR) as a method with comparable sensitivity to nested PCR. The use of qPCR allowed a lower rate of contaminations and a more rapid *T. gondii* detection, although several studies showed that some strains could have deletions or mutations of the 529 bp fragment or a variable number of repeats, particularly atypical strains (Robert-Gangneux et al., 2015; Robert-Gangneux and Belaz, 2016). Besides, Wahab et al. (2010) showed about 5% of analyzed samples gave false-negative results compared to results from amplification of the B1 gene, that is highly conserved in different *Toxoplasma* strains. Moreover, PCR assays amplifying B1, other conserved genes or non-coding sequences (like 529 bp fragment) cannot distinguish easily acute and latent phases of infection, particularly during anti-*T. gondii* therapy or prophylaxis. Indeed, the application of more than one approach, with multiple targets (MAG1 and 529 bp) increased the possibility of DNA detection and render the negative results obtained more reliable. Ivovic et al. (2012) reported that parasite DNA detection in blood was dependent on parasite genotype (I, II, III) and/or infective dose. As data was not available for either of these criteria, it is difficult to hypothesize on the negative results in PCR.

The absence of circulating *T. gondii* DNA in seropositive patients in our sample, in contrast with previous evidence described in the literature, could be due to several factors: all patients were immunocompetent, not treated with immunosuppressive drugs and none of them had evidence of infection reactivation or acute infection, since specific IgM were negative in the whole sample, whereas in the case-control studies mentioned above some patients, as well as controls, were found to be IgM positive. Moreover, our sample is composed mainly of patients affected by a chronic long-term disease with several illness episodes treated with numerous antipsychotic and mood stabilizing drugs, which can cause a reduction in the level of anti-*T.gondii* IgG and DNA by inhibiting the replication of the parasite (Jones-Brando

Table 2
Psychiatric features of the patients: comparison between *Toxoplasma*-seropositive and *Toxoplasma*-seronegative patients.

	Total (*n* = 63)	Seropositive (*n* = 18)	Seronegative (*n* = 45)	*p*-value
DSM-5 diagnosis (SZA vs BD)	31.7% (*n* = 20)	22.2% (*n* = 4)	35.6% (*n* = 16)	0.38[b]
CGI (score 3–5 vs score 6–7)	41.3% (*n* = 26)	38.9% (*n* = 7)	42.2% (*n* = 19)	0.97[a]
Number of hospital admissions (<5 vs >5)	69.8% (*n* = 44)	66.7% (*n* = 12)	71.1% (*n* = 32)	0.96[a]
Family history of mental disorders (yes vs no)	0.3% (*n* = 18)	25.0% (*n* = 4)	31.8% (*n* = 14)	0.75[b]

CGI = Clinical Global Impression Scale.
[a] Pearson's chi-square test and correction of Yates.
[b] Fisher's Exact test.

et al., 2003; Webster et al., 2006). For this reason, the effects of antipsychotic medications, as well as timing of infection should be considered in these patients, since a temporal relationship between *Toxoplasma* exposure and disease onset has been recently suggested (Leweke et al., 2004; Omar et al., 2015; Yolken et al., 2017).

Toxoplasma-DNA detection in immunocompetent subjects, especially when IgM are negative, should be accurately investigated. Data from the literature are controversial, and some Authors (Omar et al., 2015) found circulating parasite DNA also in healthy, not schizophrenic, controls. Fallahi et al. (2017) explained the high sensitivity of PCR with a high rate of *T. gondii* infection in that Country. which can detect parasite DNA also in patients with chronic toxoplasmosis, as previously described by Silveira et al. (2011). Patients in that study were from Erechim, which is known to be an area with a high prevalence of ocular toxoplasmosis and where atypical *T. gondii* genotypes circulate (Carneiro et al., 2013). Non-II genotypes are common in Malaysia (Puvanesuaran et al., 2013) and Iran (Danechin et al., 2016) as well, the two Countries where studies mentioned above were carried out, whereas in Italy and Europe type II is the most frequent strain affecting humans (Ajzenberg et al., 2002; Peyron et al., 2006). The differences in genotype distribution between these different geographical areas might explain the discrepancy between our results and those obtained by other groups of study, although this hypothesis should be further investigated.

Therefore, these mechanisms might account for the contribution of *T. gondii* to the onset and maintaining of psychiatric disorders even in the absence of a reactivation of toxoplasmic infection.

The results of this study should be interpreted keeping in mind some limitations: the small sample size, that we are going to enlarge; the observational nature of the study, with the lack of a control group; and the heterogeneity of the sample, with patients at different stages of illness and under different treatments, that does not allow to draw conclusions about the etiophatogentic role of the parasite and its association with a specific diagnostic category.

However, according to our knowledge, to date this is the first study evaluating the *T. gondii* seroprevalence and the presence of circulating parasite DNA in a cohort of Italian psychiatric inpatients. A more comprehensive research in a larger sample of patients is required to consolidate these findings and to better define the clinical features correlated to the seropositive status in BD.

Finally, it seems to be of importance to consider that a better understanding of the contribution of infectious agents such as *T. gondii* in the pathophysiology of BD or other psychotic disorders could help in the identification of preventive measures and development of new pharmacological treatment approaches, with a significant impact on the disease course.

Disclosure statement

The authors declare that they have no conflict of interest with regard to the present work. The authors alone are responsible for the content and writing of the paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.01.104.

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