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Abstract

Toxoplasmosis has been previously associated with an increased risk of having schizophrenia in several epidemiological studies. The aim of this prospective study was to examine for the first time a possible association between positive serology to *Toxoplasma gondii* (*T. gondii*) and schizophrenia in the Algerian population. Seventy patients affected by schizophrenia according to DSM-5 criteria and seventy healthy controls were enrolled in the study. We found a significant association between schizophrenia and the infection status with a seroprevalence of 70% in patients with schizophrenia compared to 52.9% in controls and a calculated odds ratio of 2.081. In addition, while *T. gondii* seroprevalence increases significantly with age in controls, this association was not observed in patients with schizophrenia, which display a high percentage of seropositive subjects under 38 years of age, suggesting that *T. gondii* infection may promote the onset of schizophrenia. Moreover, our analysis also revealed that patients with schizophrenia had significantly lower levels of serum immunoglobulins G (IgG) to *T. gondii* compared to controls. Thus, this study adds to previous research questioning the asymptomatic aspect of chronic toxoplasmosis and the etiology of schizophrenia.

Keywords: Toxoplasmosis, Schizophrenia, Algerian population, IgG antibody levels.

1. Introduction

Schizophrenia is a chronic mental illness with a world prevalence of 1% (Marder and Cannon, 2019). Patients with schizophrenia demonstrated psychotic disorders including hallucinations, delusions and disorganized speech, negative symptoms such as reduced motivation and expressiveness as well as cognitive deficits including memory impairment and reduced cognitive processing speed (Marder and Cannon, 2019). First symptoms are generally observed in the late adolescence. Interactions between environmental factors including obstetric complications, early social adversity, residence in urban areas during childhood, consumption of psychotropic drugs, as well as genetic predispositions impacting

signaling pathways associated with the immune system and synaptic functioning were shown to be linked to the etiology of the disease (Brown, 2011; Marder and Cannon, 2019). Failure in the identification of genes with major role in schizophrenia establishment has refocused attention on non-genetic factors, including infectious agents such as influenza virus and herpes simplex virus type 1 and 2 (Kim, 2007; Hamdani et al., 2017; Burgdorf et al., 2019). Notably, several studies revealed an association between schizophrenia and the protozoan parasite *Toxoplasma gondii* (*T. gondii*) responsible for toxoplasmosis (Torrey et al., 2007). This wild-spread anthropozoonosis affects approximately one-third of the world's population. *T.gondii* is an obligatory intracellular protozoan parasite belonging to the phylum apicomplexa. Humans are contaminated by ingestion of tissue cysts present in undercooked meat, ingestion of oocysts present in water and soil contaminated with cats feces or by vertical transmission from a pregnant woman to her fetus (Carruthers and Suzuki, 2007). *T. gondii* is considered as an opportunistic parasite as severe symptoms developed in immuno-suppressed patients. However, infection of immuno-competent individuals with type II strains, which cause most infections in humans, is characterized by cyst development in the brain, leading to life-long chronic infection. In the central nervous system (CNS), the parasite infects astrocytes, microglia and neurons but only persists in neurons (Carruthers and Suzuki, 2007). Until recently, parasite persistence was considered clinically asymptomatic. However, latent cerebral toxoplasmosis is now considered as a risk factor to develop behavioral and mental disorders such as schizophrenia (Torrey et al., 2012) and bipolar disorder (Del Grande et al., 2017).

Schizophrenia has been correlated with alterations in the levels of several neurotransmitters including dopamine, serotonin, glutamate and GABA (Fuglewicz et al., 2017) as well as increased kynurenic acid (KYNA) in the cerebrospinal fluid (Erhardt et al., 2001) and the CNS (Plitman et al., 2017). Current therapeutic approaches to treat positive schizophrenia symptoms aim to reduce the hyperactivity of the dopaminergic system by blocking the dopamine D1/D2 receptors using the first generation (typical) antagonist agents chlorpromazine and haloperidol (Taly, 2013; Karakuła-Juchnowicz et al., 2014;; Uno and Coyle, 2019). Second generation atypical antipsychotic agents (clozapine, quetiapine, risperidone, olanzapine, sertindole) target a broader spectra of receptors (5-HT1A agonists, D1/D2, D4 and 5-HT2A/C antagonists) (Taly, 2013). However, they have negligible effects on negative and cognitive symptoms with the possible exception of clozapine that restores the N-methyl-d-aspartate receptor (NMDA-R) hypofunction (Uno and Coyle, 2019). The NMDA-R hypofunction observed in patients with schizophrenia has been proposed to be the result of KYNA abnormal accumulation (Zádor et al., 2019). Accordingly, inhibition of KYNA formation improves neurotransmitter signaling and cognitive impairments (Zádor et al., 2019).

Interestingly, increased levels of KYNA, 3-Hydroxykynurenine (3-HK) and quinolinic acid (QUIN) have been observed in the brain of mice chronically infected with *T. gondii* (Notarangelo et al., 2014). Treatment with anti-parasitic drugs restored normal levels of these metabolites demonstrating the direct effect of brain infection on the activation of the kynurenine pathway (KP) (Notarangelo et al., 2014). Activation of the KP during latent toxoplasmosis is presumably triggered by the establishment of a low-grade chronic neuroinflammation characterized by the up-regulation of pro-inflammatory cytokines such as tumor necrosis factor, Interleukin-12, Interleukin-1β and Interferon gamma (IFNγ) (Tedford and McConkey, 2017). Notably, IFNγ stimulates the expression of the rate-limiting

enzyme indoleamine 2,3-dioxygenase (IDO) in astrocytes and microglia, leading to degradation of tryptophan, an amino acid essential for the development of the parasite (Ellen Tedford and Glenn McConkey, 2017). KP activation also results in down-stream release of the neuroactive compounds KYNA and QUIN, which affect neuronal functions (Braidy and Grant, 2017). Brain dopamine level is also up-regulated by *T. gondii* infection in mice (Henriquez et al., 2009). Consistent with this result, increase in dopamine release has been monitored in neuronal cultures containing parasite cysts (Prandovszky et al., 2011). Finally, chronic toxoplasmosis has been correlated with decreased levels of the glutamate transporter GLT-1 in astrocytes and increased concentrations of extracellular glutamate (David et al., 2016). Elevated levels of glutamate (Chang et al., 2007) and decreased hippocampal volume (Kraguljac et al., 2013) are also observed in patients with schizophrenia. Therefore, latent cerebral *T. gondii* infection by inducing a chronic inflammatory response in the brain and modulating neuronal functions may lead to increased risks of developing schizophrenia.

In 2007, Torrey et al. conducted a meta-analysis of twenty-three studies led over 50 years in 17 countries that covered a total of 3873 patients with schizophrenia and 7046 healthy controls, most of them from China where Chinese 1 is the most predominant atypical *T. gondii* genotype, and where the highest odds ratio (OR) of 14.22 was found. The combined OR obtained was 2.73 (95 CI ,2.10-3.60; $p<0.00001$) (Torrey et al., 2007). This meta-analysis was updated in 2012 by the same team and fifteen additional studies were included however comprising only two African countries (Egypt and Ethiopia). The OR obtained was very similar (OR= 2.71, 95% CI: 1.93-380) (Torrey et al., 2012). More recent studies led in Tunisia (Esshili et al., 2016), France (Hamdani et al., 2017) and Denmark (Burgdorf et al., 2019) confirmed an association between toxoplasmosis and schizophrenia. The obtained OR values exceed other environmental and genetic factors previously investigated for their link with schizophrenia (Fuglewicz et al., 2017), reinforcing the assumption that *T. gondii* exposure represents a risk factor to develop schizophrenia (Xiao et al., 2018). In line, in a low prevalence population, *T. gondii* seropositivity was associated with schizophrenia only in individuals with a recent onset of psychosis but not in patients with established schizophrenia (Yolken et al., 2017).

Here, we explore for the first time a possible link between positive serology for *T. gondii* and schizophrenia in the Algerian population characterized by a high prevalence of *T. gondii* type II strains (Bachi et al., 2018), which was previously estimated to be 53.2% (Schneider et al., 1977) in the whole population and more recently to be 47,8% in pregnant women (Messerer et al., 2014).

2. Methods

2.1. Characteristics of the cohort

This prospective study was carried out at the specialized establishment for psychiatric disorders (Etablissement Hospitalier spécialisé en psychiatrie «Kraria Slimen») in Ain Abessa Sétif, Algeria. A total of 70 healthy volunteers and 70 inpatients with diagnosed schizophrenia and under treatment with antipsychotic drugs including typical antipsychotics: Haloperidol (HALDOL), Fluphenazine (MODECATE) or Chlorpromazine (LARGACTIL) and atypical antipsychotics: Risperidone (RISPERDAL), Olanzapine (ZYPREXA), Amisulpride (SOLIAN) were enrolled in the study. The clinical diagnosis of the patients with schizophrenia

has been confirmed by the 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, 2015). Patients with a history of alcoholism, smoking, substance abuse, epilepsy, mental retardation, head trauma, brain surgery, meningitis, and encephalitis were excluded from the study. The control group consisted of 70 healthy volunteers recruited within the hospital staff. They had no history of schizophrenia or any psychiatric disorders. These volunteers were chosen because they shared the same environment (including meals) as the patients with schizophrenia. A questionnaire was duly filled by all participants of the study, including information about their age, gender, inbreeding of their parents, socioeconomic conditions, family history, medical history, education level, marital status, professional status, eating habits (in particular regarding the consumption of uncooked or undercooked meat), and shared environment with domestic animals, notably cats for their role in parasite transmission, in particular during their childhood. All participants were at least 18 years of age and provided written informed consent after explaining the study procedures. The study was approved by the Scientific Council of Sétif University and the Ethics Committee (Ref. CED/03/2020).

2.2. Serological analysis

Blood samples dry tubes were taken by venipuncture under sterile conditions from all participants. The serum was then separated directly after collection by centrifuging for 15 min at 30,000 rpm. The commercial enzyme-linked immune-sorbent assay (ELISA) kits MAGLUMI Toxo IgG CLIA (Snibe diagnostic, Shenzhen, CHINA) was used to quantitatively measure anti-Toxoplasma IgG antibody levels using the chemiluminescence immunoassay system (CLIA) (MAGLUMI 1000, Shenzhen, CHINA) following the manufacturer's instructions. The interpretation of the results (according to the manufacturer's criteria) was as follows: ≤ 2 AU / ml was considered negative and ≥ 2 AU/ ml was considered positive. The sensitivity of the test is less than 0,25 AU / ml. No cross reaction with IgG or IgM antibodies of Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Human Immunodeficiency virus, syphilis, Epstein-Barr virus is detected.

2.3. Statistical analysis

Statistical analyses were performed with the software R version 3.6.1. The statistical tests are two-sided and the level of statistical significance was set at 5%.

Means between patients with schizophrenia and healthy participants were compared with a t-test (t.test function) considering whether variances were equal or not.

We performed a ROC analysis (roc function) to define the threshold age that discriminates the most the schizophrenia status. Thus, different thresholds of age were used to classify individuals according to schizophrenia. Individuals younger than the age threshold were classified non-affected while the older ones were set as affected. The obtained classification was compared to the observed one and sensitivity and specificity were computed. So we got as many sensitivity and specificity values as tested ages. These values were used to plot the ROC curve and to compute the Youden index as the sum of sensitivity and specificity computed for each tested threshold. The age associated with the highest Youden index has the best ability to identify true affected and healthy people.

The Chi^2 test was used to compare samples in contingency tables (chisq.test function). When at least one expected sample is included in the interval [5-10], the Yates correction was applied. The Fisher exact test was preferred over the Chi^2 test when any expected sample

was below 5 (fisher.test function). The odds ratio (OR) and its 95% confidence interval were estimated with the function oddsratio.
The power of the Chi² test to compare schizophrenia and toxoplasmosis in individuals younger than 38 years, as well as the required sample to achieve a power of 80%, was estimated with the function pwr.chisq.test. The proportions of individuals tested positive for toxoplasmosis in patients with schizophrenia and healthy participants aged between 20 and 30 years are compared with the function prop.test with Yates correction to adjust for small sample size. Although there are 2.6 times more seropositive individuals in patients compared to controls, this difference is not statistically significant (p= 0.171 with continuity correction). This could be explained by a low power (45%) achieved to detect any difference with these small sample groups. As expected, no statistical significant differences for the other groups are also observed (in [30-40] years, p= 0.396; in [40-50] years, p= 0.875; for 50 years and more, p= 1; p values given with continuity correction).

3. Results

We evaluated the seroprevalence of anti-*T. gondii* IgG antibodies in 140 subjects including 70 patients with schizophrenia (56 men and 14 women) with an average age of 40.76 ±9.3 and 70 healthy controls (56 men and 14 women) with an average age of 37.97 ± 9.3 (Table 1). The mean age (p= 0.078) and gender composition (p= 1) of the two groups did not differ significantly (Table 1).

3.1. Association between schizophrenia and infection by *T. gondii*

Toxoplasmosis was diagnosed in 49/70 (70%) patients and 37/70 (52.9%) controls (Table 2). The difference in these seroprevalences was statistically significant (p= 0.037) with a calculated OR of 2.081 (95% confidence interval (CI): [1.040-4.165]) (Table 3). Interestingly, we noticed that the subjects under 40 years of age display a higher percentage of positive serology for *T. gondii* compared to healthy controls of the same range of age (Table 2 and Figure 1). Accordingly, after constructing the receiver operating characteristic (ROC) curve (Suppl. Fig 1), which uses the age as a threshold to rule an individual as schizophrenia negative (age < threshold) or schizophrenia positive (age > threshold), the maximum value of the Youden index was observed with the age 38. We therefore defined 38 as the discriminant age and divided the cohort into two homogenous groups (<38yr, n=65 and >38 yr, n=75) for further analysis. We found that individuals under 38 years of age affected by schizophrenia are 2.715 times more likely to have an anti-*T. gondii* positive serology than those without schizophrenia (OR= 2.715, 95% CI: [0.970-7.602]) (Table 3). Our results tend to be statistically significant (p = 0.054) but show only a power of 48.6% to reach the significance threshold of 0.05. We calculated that a larger sample size of 138 subjects would have been necessary to reach 80% power. By contrast, individuals over 38 years of age affected by schizophrenia are only 1.270 times more likely to have an anti-*T. gondii* positive serology than the controls (OR= 1.270, p= 0.834, 95% CI: [0.465- 3.464]) (Table 3).

Reflecting these findings, a significant association was found between *T. gondii* seropositive and the age status. The mean age of the seropositive subjects (including patients with schizophrenia and healthy controls) was 41.267 ± 9,720. Our analysis indicates that individuals over 41 years of age are 3.1 more likely to be seropositive for *T. gondii* compared

to individuals under 41 years of age (p= 0.002, OR= 3.133, 95% CI= [1.508-6.511]), reflecting the higher frequency of exposure to the parasite with time. However, when considering patients with schizophrenia independently of healthy controls, no association was found between the *T. gondii* infectious status and the age group (p= 0.23, OR= 2.167, 95% CI: [0.761-6.172]) consistent with the high percentage of *T. gondii* seropositive patients under 41 years of age in this group (Figure 1 and Table 2).

By contrast, our results did not show a significant relation between the *T. gondii* infection status and gender (p= 0.340, OR= 1.501, 95% CI: [0.650-3.464]), the socio-economic status (p= 0.152, OR= 4.657, 95% CI: [0.571-215.367]), contacts with cats (p= 0.961, OR= 1.018, 95% CI: [0.503-2.058]) and the job status (p= 0.270, OR= 0.676, 95% CI: [0.337-1.357]).

3.2. Comparison of anti- *T. gondii* IgG levels in patients with schizophrenia and control subjects

Interestingly, in seropositive participants, patients with schizophrenia have significant (p <0, 0001) lower levels (12.892 AU/ml) of serum IgG antibodies to *T. gondii* compared with health y controls (334.212 AU/ml) (Figure 2). When examining the anti-*T. gondii* IgG level distributio n in seropositive subjects enrolled in the study, we observed that the majority (n=54/86) has levels under 100 AU/ml (Figure 2). However, while 100% of patients with schizophrenia displ ay levels under the concentration of 100 IU/ml, 86.48% of healthy individuals have IgG levels over 100 AU/ml (p= 5.548e-15, OR= 0, 95% CI: [0-0.022]) (Table 4). Noteworthy, the control g roup shows a higher variability in terms of antibody titers (Figure 2).

3.3. Association between schizophrenia and history of psychological problems in the family

No significant association was found between the schizophrenia status and age (p= 0.078) or gender (p=1, OR=1, 95% CI: [0.437-2.289]) (Table 1). However, schizophrenia was strongly associated with family history of psychological problems (p= 2.162e-11, OR= 20.222; 95% CI: [7.219-56.650]) and at a lesser extend to parent inbreeding (p= 0.019, OR= 2.622, 95% CI: [1.154-5.961]). In addition, patients with schizophrenia are predominantly single (p= 1.012e-08) and jobless individuals (p< 2.2e-16) presumably due to invalidating conditions of their disorder. By contrast, we did not find any statistically significant relation between schizophrenia and contacts with cats (p= 0.579, OR= 1.214, 95% CI: [0.612-2.410]). In addition, a too low number of patients displaying comorbid disorders (e.g. arterial hypertension (AH) and diabetes (DIAB)) were included in our study to draw any conclusion on a putative association between comorbidity and schizophrenia.

4. Discussion

The aim of this study was to examine the association between positive serology to *T. gondii* and schizophrenia in a sample of Algerian psychiatric inpatients. Toxoplasmosis was diagnosed in 52.9% of healthy controls in agreement with a previous study reporting a seroprevalence of 53% in the Algerian population (Schneider et al., 1977). The rate of infection by *T. gondii* in patients with schizophrenia (70%) was statistically higher than in controls (p= 0.03789) with a calculated OR of 2.081. Thus, our result suggests that *T. gondii* infection increases by 2-fold the risk of developing schizophrenia, which is consistent with

previous studies performed in Tunisia and Egypt (El-Sahn et al., 2005; Esshili et al., 2016) and with the OR found in two meta-analyses (Sutterland et al., 2015; Torrey et al., 2012). Our study revealed that this association was mainly the result of the higher incidence of *T. gondii* in patients with schizophrenia under 38 years of age (OR= 2.715) compared to patients over 38 years of age (OR= 1.270). In line, in healthy controls, we found a significant increase of *T. gondii* seropositivity for subjects over 41 years of age (p= 0.002) consistent with an expected increased frequency of exposure to the parasite with time. By sharp contrast, no association was found between *T. gondii* seropositivity and the age status in patients with schizophrenia (p= 0.23) due to the high percentage of *T. gondii* positive subjects under 41 years of age in this group, suggesting that infection may promote the onset of schizophrenia in susceptible individuals. Consistent with this hypothesis, a recent large-scale study led in Denmark revealed that *T. gondii* seropositivity is associated with schizophrenia (OR= 1,47) but an even stronger association (OR= 2,78) was observed after excluding participants whose diagnosis preceded blood sample collection, suggesting that *T. gondii* infection might be a causal factor for schizophrenia (Burgdorf et al., 2019). Moreover, our study indicates a strong association between the schizophrenia status and previous history of psychological problems in the family (OR= 20.222, p= 2.162e-11) and at a lesser extend to inbreeding of parents (OR= 2.622, p= 0.019).

T. gondii type II strains establish lifelong persisting infections in the brain of their host forming intracellular cysts in neurons. It has been hypothesized that the low-grade chronic inflammation monitored during cerebral toxoplasmosis combined with neuronal functional alterations notably due to imbalance in neurotransmitter levels may increase the risk of developing schizophrenia (Sorlozano-Puerto and Gutierrez-Fernandez, 2016). However, our study relies on the measurement of anti-*T. gondii* antibodies in the serum of patients, which provide a marker of past exposure to the parasite but not of the presence of cysts in the CNS, thus of undergoing chronic infection. RH Yolken's team has developed a method for detecting the presence of tissue cysts in *T. gondii* seropositive mice and individuals consisting in measuring antibodies against MAG1, a parasite protein abundantly expressed within the cyst and the cyst wall (Ferguson and Parmley, 2002; Xiao et al., 2016). Importantly, in humans, only a fraction of individuals with serological evidence for past exposure to *T. gondii* displayed a positive response for MAG1 antibodies and thus, presumably had cysts in the CNS (Xiao et al., 2013). Thus, it may be of relevance in future studies to examine the association between schizophrenia and anti-MAG1 IgG levels in an attempt to determine the percentage of cysts carriers in seropositive patients with schizophrenia compared to seropositive controls.

The established association between schizophrenia and *T. gondii* seroprevalence motivated clinical trials aiming to evaluate the therapeutic efficacy of antiparasitic agents (azithromycin, trimethoprim, artemisinin, and artemether) in patients with schizophrenia (Chorlton, 2017). None of these drugs were found to be effective to ameliorate psychopathological symptoms. However, none of these commonly administrated anti-*T. gondii* drugs showed efficacy to eradicate intraneuronal cysts in chronically infected mice,

presumably due to reduced metabolism of the slow-growing bradyzoites, and limited passage of the drugs to the CNS (Cerutti et al., 2020). Therefore, it might be important to re-evaluate the efficacy of new developed drugs effective in reducing cyst viability *in vitro* or in mice (Cerutti et al., 2020; Martynowicz et al., 2019; Rutaganira et al., 2017) for the treatment of schizophrenia.

Interestingly, we found that the levels of anti-*T. gondii* IgG in patients with established schizophrenia are significantly reduced compared to healthy controls (Figure 2). It is possible that some of the medications used to treat schizophrenia impact on antibody levels as previously observed (Leweke et al., 2004) or on parasite persistence in the brain. Lowered antibody levels may be a consequence of the global immune-suppressive effect of antipsychotic treatments (May et al., 2019). Supporting a direct effect on parasites, the antipsychotic haloperidol and the mood stabilizer valproic acid were demonstrated to inhibit *T. gondii* growth *in vitro* (Jones-Brando et al., 2003). Besides, antipsychotic treatments may also have an impact on cyst burden by indirectly modulating brain homeostasis. Indeed, *T. gondii* persistence in the CNS has been reported to correlate with increased dopamine, glutamate and KYNA levels, thereby altering neuronal functions (Parlog et al., 2015). It is not yet elucidated whether some of these metabolic changes are induced by the parasite via secreted effectors but antipsychotic medications by targeting similar pathways may influence *T. gondii* persistence in the CNS. Additional evidence of a possible interference between *T. gondii* and antipsychotic medications is supported by the observation of increased *T. gondii* seroprevalence in individuals with treatment-resistant forms of schizophrenia (Vlatkovic et al., 2018) compared to treatment-responsive patients.

Thus, an improved understanding of the relationship between *T. gondii* infection and the pathophysiology of schizophrenia could help in preventing this devastating disease by developing new pharmacological treatment approaches.

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References:

Bachi, F., Yebbous-Bensaid, S.A., Gourbdji, E., Dardé, M.L., 2018. Première caractérisation moléculaire de deux souches algériennes de Toxoplasma gondii isolées de deux cas de toxoplasmose congénitale. Médecine Mal. Infect. 48, 78–80. https://doi.org/10.1016/j.medmal.2017.09.019

Braidy, N., Grant, R., 2017. Kynurenine pathway metabolism and neuroinflammatory disease. Neural Regen. Res. 12, 39–42. https://doi.org/10.4103/1673-5374.198971

Brown, A.S., 2011. Exposure to prenatal infection and risk of schizophrenia. Front. Psychiatry. 2, 63. https://doi.org/10.3389/fpsyt.2011.00063

Burgdorf, K.S., Trabjerg, B.B., Pedersen, M.G., Nissen, J., Banasik, K., Pedersen, O.B., Sørensen, E., Nielsen, K.R., Larsen, M.H., Erikstrup, C., Bruun-Rasmussen, P., Westergaard, D., Thørner, L.W., Hjalgrim, H., Paarup, H.M., Brunak, S., Pedersen, C.B., Torrey, E.F., Werge, T., Mortensen, P.B., Yolken, R.H., Ullum, H., 2019. Large-scale study of Toxoplasma and Cytomegalovirus shows an association between infection and serious psychiatric disorders. Brain. Behav. Immun. 79, 152–158. https://doi.org/10.1016/j.bbi.2019.01.026

Carruthers, V.B., Suzuki, Y., 2007. Effects of Toxoplasma gondii infection on the brain. Schizophr. Bull. 33, 745–751. https://doi.org/10.1093/schbul/sbm008

Cerutti, A., Blanchard, N., Besteiro, S., 2020. The Bradyzoite: A Key Developmental Stage for the Persistence and Pathogenesis of Toxoplasmosis. Pathogens. 9, 234. https://doi.org/10.3390/pathogens9030234

Chang, L., Friedman, J., Ernst, T., Zhong, K., Tsopelas, N.D., Davis, K., 2007. Brain Metabolite Abnormalities in the White Matter of Elderly Schizophrenic Subjects: Implication for Glial Dysfunction. Biol. Psychiatry. 62, 1396–1404. https://doi.org/10.1016/j.biopsych.2007.05.025

Chorlton, S.D., 2017. Toxoplasma gondii and schizophrenia: a review of published RCTs. Parasitol. Res. 116, 1793–1799. https://doi.org/10.1007/s00436-017-5478-y

David, C.N., Frias, E.S., Szu, J.I., Vieira, P.A., Hubbard, J.A., Lovelace, J., Michael, M., Worth, D., McGovern, K.E., Ethell, I.M., Stanley, B.G., Korzus, E., Fiacco, T.A., Binder, D.K., Wilson, E.H., 2016. GLT-1-Dependent Disruption of CNS Glutamate Homeostasis and Neuronal Function by the Protozoan Parasite Toxoplasma gondii. PLOS Pathog. 12, e1005643. https://doi.org/10.1371/journal.ppat.1005643

Del Grande, C., Galli, L., Schiavi, E., Dell'Osso, L., Bruschi, F., 2017. Is Toxoplasma gondii a Trigger of Bipolar Disorder? Pathogens. 6, 3. https://doi.org/10.3390/pathogens6010003

El-Sahn, A.A., Shatat, H.Z., Ghitany, E.M., 2005. Seropositivity of toxoplasmosis in patients with schizophrenia. J. Egypt. Public Health Assoc. 80, 509–524.

Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindström, L.H., Engberg, G., 2001. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci. Lett. 313, 96–98. https://doi.org/10.1016/s0304-3940(01)02242-x

Esshili, A., Thabet, S., Jemli, A., Trifa, F., Mechri, A., Zaafrane, F., Gaha, L., Juckel, G., Babba, H., Bel Hadj Jrad, B., 2016. Toxoplasma gondii infection in schizophrenia and associated clinical features. Psychiatry Res. 245, 327–332. https://doi.org/10.1016/j.psychres.2016.08.056

Ferguson, D.J.P., Parmley, S.F., 2002. Toxoplasma gondii MAG1 protein expression. Trends Parasitol. 18, 482. https://doi.org/10.1016/s1471-4922(02)02349-8

First, M.B., 2015. Structured Clinical Interview for the *DSM* (SCID), in: Cautin, R.L., Lilienfeld, S.O. (Eds.), The Encyclopedia of Clinical Psychology. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 1–6. https://doi.org/10.1002/9781118625392.wbecp351

Fuglewicz, A.J., Piotrowski, P., Stodolak, A., 2017. Relationship between toxoplasmosis and schizophrenia: A review. Adv. Clin. Exp. Med. Off. 26, 1031–1036. https://doi.org/10.17219/acem/61435

Hamdani, N., Daban-Huard, C., Godin, O., Laouamri, H., Jamain, S., Attiba, D., Delavest, M., Lépine, J.-P., Le Corvoisier, P., Houenou, J., Richard, J.-R., Yolken, R.H., Krishnamoorthy, R., Tamouza, R., Leboyer, M., Dickerson, F.B., 2017. Effects of Cumulative Herpesviridae and Toxoplasma gondii Infections on Cognitive Function in Healthy, Bipolar, and Schizophrenia Subjects. J. Clin. Psychiatry. 78, e18–e27. https://doi.org/10.4088/JCP.15m10133

Jones-Brando, L., Torrey, E.F., Yolken, R., 2003. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of Toxoplasma gondii. Schizophr. Res. 62, 237–244. https://doi.org/10.1016/s0920-9964(02)00357-2

Karakuła-Juchnowicz, H., Flis, M., Szymona, K., Kuczyńska, M., Stelmach, E., Kowal-Popczak, A., 2014. New prospects for antipsychotic treatment - the role of the kynurenine pathway. Psychiatr. Pol. 48, 1167–1177. https://doi.org/10.12740/PP/25520

Kim, J.J., 2007. Viral Infections as Etiological Factors of Schizophrenia. Psychiatry investig. 4, 61–65.

Kraguljac, N.V., White, D.M., Reid, M.A., Lahti, A.C., 2013. Increased Hippocampal Glutamate and Volumetric Deficits in Unmedicated Patients With Schizophrenia. JAMA Psychiatry 70, 1294. https://doi.org/10.1001/jamapsychiatry.2013.2437

Leweke, F.M., Gerth, C.W., Koethe, D., Klosterkötter, J., Ruslanova, I., Krivogorsky, B., Torrey, E.F., Yolken, R.H., 2004. Antibodies to infectious agents in individuals with recent onset schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci. 254, 4–8. https://doi.org/10.1007/s00406-004-0481-6

Marder, S.R., Cannon, T.D., 2019. Schizophrenia. N. Engl. J. Med. 381, 1753–1761. https://doi.org/10.1056/NEJMra1808803

Martynowicz, J., Augusto, L., Wek, R.C., Boehm, S.L., Sullivan, W.J., 2019. Guanabenz Reverses a Key Behavioral Change Caused by Latent Toxoplasmosis in Mice by Reducing Neuroinflammation. mBio 10. https://doi.org/10.1128/mBio.00381-19

May, M., Beauchemin, M., Vary, C., Barlow, D., Houseknecht, K.L., 2019. The antipsychotic medication, risperidone, causes global immunosuppression in healthy mice. PLOS ONE. 14, e0218937. https://doi.org/10.1371/journal.pone.0218937

Messerer, L , Bouzbid S , Gourbdji E, Mansouri R, Bachi F. 2014. Seroprevalence of Toxoplasmosis in Pregnant Women in Annaba, Algeria. Rev Epidemiol Sante Publique.62:160-5. doi: 10.1016/j.respe.2013.11.072.

Notarangelo, F.M., Wilson, E.H., Horning, K.J., Thomas, M. a. R., Harris, T.H., Fang, Q., Hunter, C.A., Schwarcz, R., 2014. Evaluation of kynurenine pathway metabolism in Toxoplasma gondii-infected mice: implications for schizophrenia. Schizophr. Res. 152, 261–267. https://doi.org/10.1016/j.schres.2013.11.011

Parlog, A., Schlüter, D., Dunay, I.R., 2015. Toxoplasma gondii-induced neuronal alterations. Parasite Immunol. 37, 159–170. https://doi.org/10.1111/pim.12157

Plitman, E., Iwata, Y., Caravaggio, F., Nakajima, S., Chung, J.K., Gerretsen, P., Kim, J., Takeuchi, H., Chakravarty, M.M., Remington, G., Graff-Guerrero, A., 2017. Kynurenic Acid in Schizophrenia: A Systematic Review and Meta-analysis. Schizophr. Bull. 43, 764–777. https://doi.org/10.1093/schbul/sbw221

Prandovszky, E., Gaskell, E., Martin, H., Dubey, J.P., Webster, J.P., McConkey, G.A., 2011. The neurotropic parasite Toxoplasma gondii increases dopamine metabolism. PloS One 6, e23866. https://doi.org/10.1371/journal.pone.0023866

Rutaganira, F.U., Barks, J., Dhason, M.S., Wang, Q., Lopez, M.S., Long, S., Radke, J.B., Jones, N.G., Maddirala, A.R., Janetka, J.W., El Bakkouri, M., Hui, R., Shokat, K.M., Sibley, L.D., 2017. Inhibition of Calcium Dependent Protein Kinase 1 (CDPK1) by Pyrazolopyrimidine Analogs Decreases Establishment and Reoccurrence of Central Nervous System Disease by Toxoplasma gondii. J. Med. Chem. 60, 9976–9989.https://doi.org/10.1021/acs.jmedchem.7b01192

Schneider, R., Tabet-Derraz, O., Dedet, J.P., Belkaid, M., Lamri, I., 1977. [Serodiagnosis of 2,438 cases of toxoplasmosis by immunofluorescence at the Pasteur Institute of Algeria. Epidemiological and clinical corollaries]. Arch. Inst. Pasteur Algerie. 52, 95–104.

Sorlozano-Puerto, A., Gutierrez-Fernandez, J., 2016. Toxoplasma gondii and Schizophrenia: A Relationship That Is Not Ruled Out. Schizophrenia Spectrum Disorders and Their Treatments. Chapter: 4. Publisher: InTech. Rijeka, Croatia. https://doi.org/10.5772/66018

Sutterland, A.L., Fond, G., Kuin, A., Koeter, M.W.J., Lutter, R., van Gool, T., Yolken, R., Szoke, A., Leboyer, M., de Haan, L., 2015. Beyond the association. Toxoplasma gondii in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. Acta Psychiatr. Scand. 132, 161–179. https://doi.org/10.1111/acps.12423

Taly, A., 2013. Novel approaches to drug design for the treatment of schizophrenia. Expert Opin. Drug Discov. 8, 1285–1296. https://doi.org/10.1517/17460441.2013.821108

Tedford, E., McConkey, G., 2017. Neurophysiological Changes Induced by Chronic Toxoplasma gondii Infection. Pathogens. 6:19. https://doi.org/10.3390/pathogens6020019

Torrey, E.F., Bartko, J.J., Lun, Z.-R., Yolken, R.H., 2007. Antibodies to Toxoplasma gondii in patients with schizophrenia: a meta-analysis. Schizophr. Bull. 33, 729–736. https://doi.org/10.1093/schbul/sbl050

Torrey, E.F., Bartko, J.J., Yolken, R.H., 2012. Toxoplasma gondii and Other Risk Factors for Schizophrenia: An Update. Schizophr. Bull. 38, 642–647. https://doi.org/10.1093/schbul/sbs043

Uno, Y., Coyle, J.T., 2019. Glutamate hypothesis in schizophrenia. Psychiatry Clin. Neurosci. 73, 204–215. https://doi.org/10.1111/pcn.12823

Vlatkovic, S., Sagud, M., Svob Strac, D., Sviben, M., Zivkovic, M., Vilibic, M., Vuksan-Cusa, B., Mihaljevic-Peles, A., Pivac, N., 2018. Increased prevalence of Toxoplasma gondii seropositivity in patients with treatment-resistant schizophrenia. Schizophr. Res. 193, 480–481. https://doi.org/10.1016/j.schres.2017.08.006

Xiao, J., Li, Y., Prandovszky, E., Kannan, G., Viscidi, R.P., Pletnikov, M.V., Yolken, R.H., 2016. Behavioral Abnormalities in a Mouse Model of Chronic Toxoplasmosis Are Associated with MAG1 Antibody Levels and Cyst Burden. PLoS Negl. Trop. Dis. 10. https://doi.org/10.1371/journal.pntd.0004674

Xiao, J., Prandovszky, E., Kannan, G., Pletnikov, M.V., Dickerson, F., Severance, E.G., Yolken, R.H., 2018. Toxoplasma gondii: Biological Parameters of the Connection to Schizophrenia. Schizophr. Bull. 44, 983–992. https://doi.org/10.1093/schbul/sby082

Xiao, J., Viscidi, R.P., Kannan, G., Pletnikov, M.V., Li, Y., Severance, E.G., Yolken, R.H., Delhaes, L., 2013. The Toxoplasma MAG1 peptides induce sex-based humoral immune response in mice and distinguish active from chronic human infection. Microbes Infect. 15, 74–83. https://doi.org/10.1016/j.micinf.2012.10.016

Yolken, R., Torrey, E.F., Dickerson, F., 2017. Evidence of increased exposure to Toxoplasma gondii in individuals with recent onset psychosis but not with established schizophrenia. PLoS Negl. Trop. Dis. 11, e0006040. https://doi.org/10.1371/journal.pntd.0006040

Zádor, F., Nagy-Grócz, G., Kekesi, G., Dvorácskó, S., Szűcs, E., Tömböly, C., Horvath, G., Benyhe, S., Vécsei, L., 2019. Kynurenines and the Endocannabinoid System in Schizophrenia: Common Points and Potential Interactions. Molecules. 24, 3709. https://doi.org/10.3390/molecules24203709

Table 1: Demographic characteristics of patients with schizophrenia and healthy controls.

		Healthy controls n (%)	Patients with Schizophrenia n (%)	*p*	OR	CI95
Number of participants		70	70			
Age (mean ± SD)		37.97 ± 9.326	40.76 ± 9.3	0.078 [1]	NA	NA
Sex	Female	14 (20)	14 (20)	1 [2]	1	0.437-2.289
	Male	56 (80)	56 (80)			
Job status	No	0 (0)	60 (85.7)	**< 2.2e-16 [2]**	0	0-0.012
	Yes	70 (100)	10 (14.3)			
Marital Status	Single	21 (30)	48 (68.6)	**1.012e-08 [3]**	NA	NA
	Married	47 (67.1)	13 (18.6)			
	Divorced	2 (2.9)	9 (12.9)			
Psychological problem in family	No	65 (92.9)	27 (39)	**2.162e-11 [2]**	**20.222**	**7.219-56.650**
	Yes	5 (7.1)	42 (60)			
	Unspecified	0 (0)	1 (1)			
Inbreeding of parents	No	59 (84.3)	45 (64.3)	**0.019 [2]**	**2.622**	**1.154- 5.961**
	Yes	11 (15.7)	22 (31.4)			
	Unspecified	0 (0)	3 (4.3)			
Socio-economic status	Average	70 (100)	62 (88.6)	0.006 [3]	>1	1.826-ND
	Precarious	0 (0)	8 (11.4)			
Contact with cats	No	35 (50)	28 (40)	0.579 [2]	1.214	0.612-2.410
	Yes	35 (50)	34 (48.6)			
	Unspecified	0 (0)	8 (11.4)			
Underlying disease AH/DIAB	No	66 (94.3)	66 (94.3)	1 [3]	1	0.178-5.608
	Yes	4 (5.7)	4 (5.7)			

SD = standard deviation; CI = confidence interval; OR = odds ratio; NA = not applicable; ND = not determined; AH = arterial hypertension; DIAB = diabetes.
1 T test
2 Chi^2 test
3 Fisher exact test

Table 2: Seroprevalence of *T. gondii* in the studied populations.

		Patients with schizophrenia		Healthy controls	
		IgG positive n (%)	IgG negative n (%	IgG positive n (%)	IgG negative n (%)
Overall		49 (70)	21 (30)	37 (52.9)	33 (47.1)
Sex	Male	41 (73.2)	15 (26.8)	30 (53.6)	26 (46.4)
	Female	8 (57.1)	6 (42.9)	7 (50)	7 (50)
Age group	<30	6 (60)	4 (40)	3 (23.1)	10 (76.9)
	30-39	14 (70)	6 (30)	15 (53.6)	13 (46.4)
	40-49	20 (71.4)	8 (28.6)	13 (65)	7 (35)
	≥50	9 (75)	3 (25)	6 (66.7)	3 (33.3)

IgG = immunoglobulin G

Table 3: Seroprevalence of *T. gondii* in the studied populations with statistical analyses.

		Patients with schizophrenia		Healthy controls		*p-value* χ^2	OR	CI_{95}
		IgG positive n (%)	IgG negative n (%)	IgG positive n (%)	IgG negative n (%)			
Overall		49 (70)	21 (30)	37 (52.9)	33 (47.1)	0.037	2.081	1.040-4.165
Age group	< 38	17 (65.4)	9 (34.6)	16 (41)	23 (59)	0.054	2.715	0.970-7.602
	≥ 38	32 (72.7)	12 (27.3)	21 (67.7)	10 (32.3)	0.834 [1]	1.270	0.465-3.464

[1] With Yates correction

Table 4: Dichotomized anti-*T. gondii* IgG levels in patients with schizophrenia and control subjects.

IgG rate	Healthy controls	Patients with schizophrenia	*p-value* χ^2	OR	CI_{95}
[2-100] AU/ml	13.52%	100%	5.548e-15	0	0-0.022
> 100 AU/ml	86.48%	0%			

Figure Legends

Figure 1: Percentage of seropositive individuals for *T. gondii* (Tg+) in patients with schizophrenia (schizo+) and healthy controls (Schizo-) subdivided into the indicated age groups.

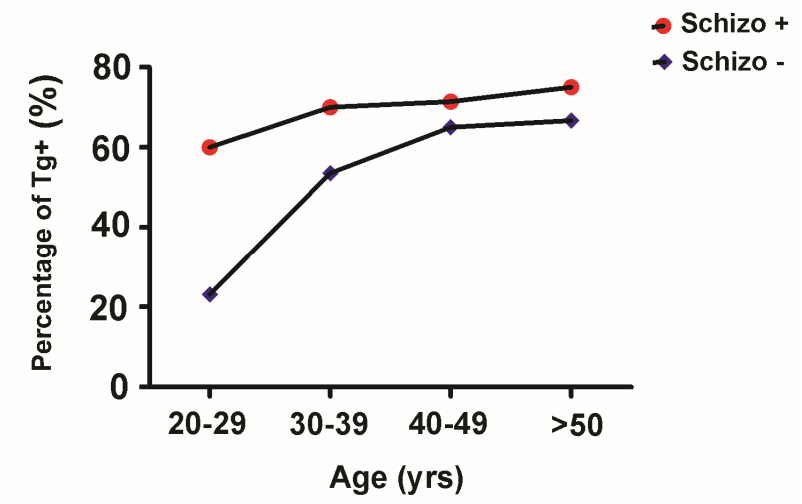

Figure 2: A- Histograms indicating the number of individuals (y axis) displaying the indicated **range** (x axis) of IgG levels in *T. gondii* seropositive subjects for patients with schizophrenia (right histogram) and healthy controls (left histogram)). **B**- Anti-*T. gondii* IgG levels (AU/ml) in seropositive patients with schizophrenia (schizo+, n=49) and seropositive healthy controls (schizo-, n=37). Student t- Test, ****p<0.0001.