Tropical Biomedicine 36(2): 422–429 (2018)

Seroprevalence and molecular evaluation of *Toxoplasma gondii* in Schizophrenic patients hospitalized in Sistan and Baluchestan province, Southeast of Iran

Jafari Modrek, M.[1,2], Hasanzadeh, R.[2], Foroutan, M.[3], Mirahmadi, H.[1,2*], Rahmati-Balaghaleh, M.[1,2] and Zarean, M.[4,5*]

[1]Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

[2]Department of Parasitology and Mycology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[3]Abadan School of Medical Sciences, Abadan, Iran

[4]Department of Medical Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Cutaneous Leishmaniasis Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding authors e-mail: hmirahmadi59@gmail.com (Hadi Mirahmadi); zareanm@mums.ac.ir (Mehdi Zarean)

Received 8 August 2017; received in revised form 14 May 2018; accepted 24 May 2018

Abstract. Over one-third of the world's population are seropositive for *Toxoplasma gondii*. One of the important traits of *T. gondii* is its ability to alter and manipulating the behavior and personality of its intermediate host. The current study was aimed to determine the prevalence of acute and chronic toxoplasmosis in those persons suffer from schizophrenia using serological and molecular techniques. In this cross-sectional study, blood samples were taken from 118 Schizophrenia patients hospitalized in Sistan and Baluchestan province, southeast of Iran. IgM and IgG anti-*Toxoplasma* antibodies were measured using enzyme-linked immunosorbent assay (ELISA). Furthermore, the presence of parasite was evaluated using nested-PCR *B1* gene. Among 118 schizophrenic patients, 48 (40.67%), 4 (3.37%) and 14 (11.86%) were tested seropositive only for IgG, only for IgM and for both of IgG/IgM. So that, total prevalence was 66/118 (55.91%). All samples were also examined using nested-PCR and *T. gondii* DNA was found in 41 (34.74%) samples. Our study showed high seroprevalence of toxoplasmosis in southeast of Iran.

INTRODUCTION

Toxoplasmosis is a cosmopolitan zoonotic disease that caused by an obligate intracellular protozoan known as *Toxoplasma gondii* (*T. gondii*) which can infect a wide range of intermediate hosts, including livestock, birds, marine mammals and humans (Zarean *et al.*, 2017; Foroutan *et al.*, 2017; Robert-Gangneux and Darde, 2012; Wang *et al.*, 2017). *T. gondii* is characterized for the first time from the liver and spleen smears of a North African rodent known as *Ctenodactylus gondii* by Nicolle and Manceaux in 1908 (Nicolle and Manceaux, 1908). Over 1/3 of the world's population are seropositive for latent *Toxoplasma* (Foroutan-Rad *et al.*, 2016b; Robert-Gangneux and Darde, 2012). The main transmission routes for *T. gondii* are as follows: ingesting oocysts shed by infected cats, consuming raw or undercooked meat contaminated by tissue cysts, drinking water contaminated by oocysts, vertical transmission from mother to the fetus, blood transfusion, and organ transplantation

(Arab-Mazar *et al*., 2016; Arab-Mazar *et al*., 2014; Dubey, 2008; Foroutan-Rad *et al*., 2016a; Foroutan-Rad *et al*., 2016b; Foroutan and Majidiani, 2017; Khademvatan *et al*., 2017; Saki *et al*., 2016; Wang *et al*., 2017; Yousefi *et al*., 2017). *Toxoplasma* is mostly asymptomatic in immunocompetent persons, while in immunosuppressed persons can reactivate and causes some complications (Wang *et al*., 2017). Recently, a significant association between latent toxoplasmosis with some autoimmune diseases, neurodegenerative or mental disorders was shown (de Barros *et al*., 2017; Flegr, 2013; Flegr, 2015; Flegr *et al*., 2014; Majidiani *et al*., 2016; Monroe *et al*., 2015; Shapira *et al*., 2012; Sutterland *et al*., 2015).

Published papers have demonstrated that chronic toxoplasmosis can manipulate and alter the behavior, not only in animal models, but in men as well (Flegr, 2013; Flegr, 2015; HavlÍCek *et al*., 2001; Khademvatan *et al*., 2013; Kocazeybek *et al*., 2009; Sutterland *et al*., 2015; Webster *et al*., 1994; Witting, 1979; Yagmur *et al*., 2010). Schizophrenia is a severe psychiatric and destructive mental disorder which effects on patient's emotion, perception, personality, behavior and reduce quality of life. Also, it is considered as one of the most difficult syndromes in determination, etiology, treatment and has a wide range of clinical traits (Medeiros-Ferreira *et al*., 2013; Sutterland *et al*., 2015; Tandon *et al*., 2008). It is estimated that the prevalence of schizophrenia is one percent in the adult individuals and most of them suffer from the disease lifelong (Tandon *et al*., 2008).

In several investigations the seroprevalence of *T. gondii* were determined in schizophrenia patients in the Iran country ranged between 34–72.5% (Abdollahian *et al*., 2017; Ahmad *et al*., 2010; Alipour *et al*., 2011; Hamidinejat *et al*., 2010; Khademvatan *et al*., 2014a; Khademvatan *et al*., 2014b; Saraei-Sahnesaraei *et al*., 2009). Due to lack of any report on schizophrenia patients in southeastern Iran, thus the current study was aimed to determine the prevalence of acute and chronic toxoplasmosis in those persons suffer from schizophrenia using serological and molecular techniques.

MATERIALS AND METHODS

In this cross-sectional study that performed between June – November 2016, blood samples were taken from 118 Schizophrenia patients who referred to three different hospitals in Zahedan and Zabol cities, southeast of Iran (Baharan Psychiatric hospital, Psychiatric clinic of Ali-ibn-Abi-Talib hospital and Amir-al Momenin Psychiatric hospital). Inclusion criteria in our study were as follows: 1) Schizophrenic patients; 2) aged ≥ 18 years old; 3) persons who tend to participate in the study (all participants signed informed consent form); 4) clinical diagnosis was done by psychiatrist following the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV-TR) criteria (First *et al*., 1995). From each subject who met the above-mentioned inclusion criteria of the study, five mL venous blood was collected. All blood specimens transferred to the department of Medical Parasitology of Zahedan University. At first, the taken blood was centrifuged at 4000 rpm for 5 minutes. Then sera were separated to examine the specific anti-*Toxoplasma* IgM and IgG antibodies and stored in -20°C. All samples were examined for IgM and IgG anti-*Toxoplasma* antibodies using commercial available enzyme-linked immunosorbent assay (ELISA) kit (*Toxoplasma*-IgG/IgM-lot No: 95001–pishtazteb Iran) based on manufacturer's instruction by cut-off 0.262 and 0.214 for IgM and IgG, respectively. Furthermore, buffy coat separation was also done for all samples and kept in 20°C until DNA extraction for nested-PCR. DNA from each sample was extracted using DynaBio™ Blood/Tissue DNA Extraction Mini Kit (Takapoozist, Iran) based on manufacturer's protocol and kept in -20°C till tested. Nested-PCR was employed for molecular diagnosis. For this purpose, we used two specific set primers to detect *B1* gene as follows:

First stage primers

B1F1 : TCAAGCAGCGTATTGTCGAG

B1R1 : CCGCAGCGACTTCTATCTCT

Second stage primers

B1F2 : GGAACTGCATCCGTTCATGAG
B1R2 : TCTTTAAAGCGTTCGTGGTC

Ultimately, the PCR products were electrophoresed on 1.5% agarose gel and visualized using Gel Documentation System. The findings were analyzed by SPSS software (version 19) (SPSS Inc., Chicago, IL, USA).

RESULTS

In the current study, 118 schizophrenic patients were met the above-mentioned criteria to be participated. Among them, 83.9% (N=99) were male and 16.1% (N=19) were female. The mean age of patients was 38.02 years old (age range: 19–55 y). Total prevalence was 66/118 (55.91%); so that from these, 48 (40.67%), 4 (3.37%) and 14 (11.86%) were tested seropositive only for IgG, only for IgM and for both of IgG/IgM. All samples were also examined using nested-PCR and *T. gondii* DNA was found in 41 (34.74%) samples Fig. 1.

DISCUSSION

One of the important traits of *T. gondii* is its ability to alter and manipulating the behavior and personality of its intermediate host (Flegr, 2015; Khademvatan *et al.*, 2014a; Monroe *et al.*, 2015; Sutterland *et al.*, 2015). Cognitive dysfunction and behavioral changes were described in *T. gondii* infected-rats and mice (Webster *et al.*, 1994; Witting, 1979). Moreover, the researchers have found that infected persons with chronic toxoplasmosis may experience and show some changes in behavior traits, personality, psychomotor skills, psychotic symptoms, etc. (Flegr, 2013; Flegr, 2015; HavlÍCek *et al.*, 2001; Khademvatan *et al.*, 2013; Sutterland *et al.*, 2015).

In this study, the prevalence rate of *T. gondii* amongst schizophrenic patients was tested using both serological and molecular methods in southeast of Iran. The prevalence rate of toxoplasmosis is highly varied amongst different populations, depend upon geographical areas (Daryani *et al.*, 2014; Foroutan-Rad *et al.*, 2016a; Foroutan-Rad *et al.*, 2016b; Foroutan *et al.*, 2017). In this regards, in southeast of Iran low prevalence of *T. gondii* was reported in various human groups (22.7% in pregnant women, 25% in healthy blood donors, 22.8% the in general population), compared with other regions which is due to hot and dry climatic conditions that reduce *T. gondii* oocysts survival in the environment (Daryani *et al.*, 2014; Foroutan-Rad *et al.*, 2016a; Modrek *et al.*, 2014) In this research, the overall seroprevalence to *T. gondii* in

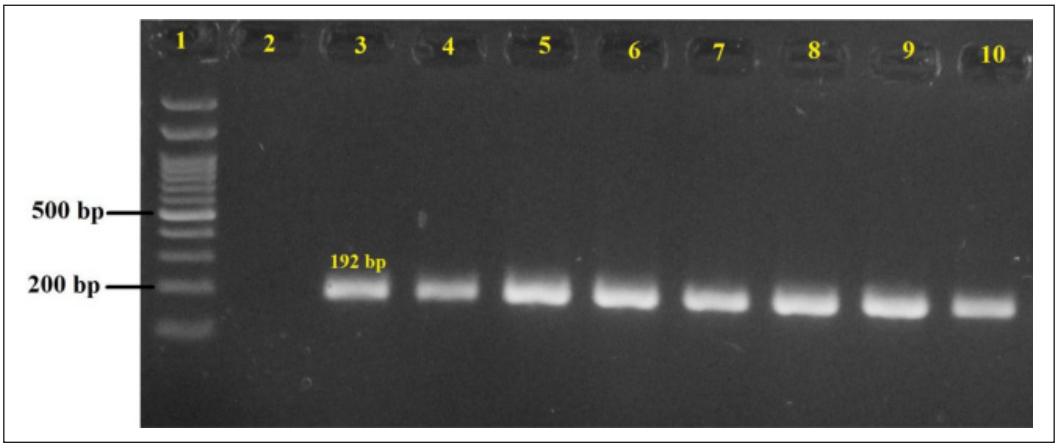

Figure 1. *B1*-nested PCR analysis of seropositive (IgM, IgG) samples from schizophrenia patients. Lane 1) 100 bp molecular weight marker; Lane 2) negative control; Lane 3) positive control; Lanes 4 to 10) randomly selected samples from patients.

schizophrenic patients was obtained 55.9% (66/118). The current findings are very higher than recent publications in this area amongst apparently healthy individuals (Daryani *et al*., 2014; Foroutan-Rad *et al*., 2016a; Modrek *et al*., 2014). The IgG seroprevalence (62/118; 52.54%) of the present study is in agreement with the result of other regions in Iran (Abdollahian *et al*., 2017; Hamidinejat *et al*., 2010; Saraei-Sahnesaraei *et al*., 2009) and some investigation in other countries (Dogruman-Al *et al*., 2009). However, Some reports are in conflict with us (Ahmad *et al*., 2010; Alipour *et al*., 2011; Alvarado-Esquivel *et al*., 2011; Cetinkaya *et al*., 2007; Esshili *et al*., 2016; Khademvatan *et al*., 2014a).

Previous reports have shown a higher prevalence of anti-*Toxoplasma* specific antibodies in schizophrenic patients than the healthy group. In this case, Esshili *et al*. (74.8% vs. 53.8%; $P = 0.00006$; OR: 2.54 with 95% CI = 1.6–4.04) (Esshili *et al*., 2016), Hamidinejat *et al*. (57.1% vs. 29.2%; $P < 0.05$; OR: 2.99 with 95% CI = 1.65–5.41) (Hamidinejat *et al*., 2010), and Alipour *et al*. (67.7% vs. 37.1%; $P < 0.01$) (Alipour *et al*., 2011) reported a significant association between toxoplasmosis and schizophrenia, while some researches failed to show the same association (Ahmad *et al*., 2010; Khademvatan *et al*., 2014a). The discrepancies among studies might be originated from the study population, preciseness in sampling and selecting the participants, the variable sensitivity and specificity of some serological methods, arbitrary cutoff value or antibody titer, timing of the infection, route of infection (tissue cyst or oocyst), the heterogeneous etiology of schizophrenia disorder, genetic susceptibility, varying degrees of neuropathogenecity among various strains/genotypes of *T. gondii* that has distinct capability, etc. Leweke *et al*. suggested that anti-schizophrenia drugs reduce the anti-*T. gondii* specific antibody levels (Leweke *et al*., 2004). Besides, as previously shown, antipsychotics inhibit *Toxoplasma* in cell culture (Jones-Brando *et al*., 2003). Although, a single reason that mentioned above may have little importance on results, but they can impress the association between *Toxoplasma* and schizophrenia disorder. Besides, more recently a systematic review and meta-analysis paper was published by Sutterland *et al*. and demonstrated that chronic toxoplasmosis is not only correlated with schizophrenia (OR 1.81, $P < 0.00001$), but also with addiction (OR 1.91, $P < 0.00001$), bipolar disorder (OR 1.52, $P = 0.02$), and obsessive–compulsive disorder (OR 3.4, $P < 0.001$) (Sutterland *et al*., 2015). This fact may be justified with greater tendency to eat inappropriate things (pica and coprophagia), insufficient personal hygiene, low self-care skills, abnormal behaviors, so on, exposed schizophrenic patients to acquiring toxoplasmosis.

Several methods have been employed for *T. gondii* diagnosis, which includes skin test antigen (or so-called toxoplasmin) (STA), serological tests to assess the specific antibodies such as Sabin and Feldman test (SFT), complement fixation test (CFT), indirect haemagglutination test (IHAT), latex flocculation test (LF), indirect immunofluorescence assay (IFA), ELISA, and detection of DNA using polymerase chain reaction-based techniques (PCR) (Robert-Gangneux and Darde, 2012). Among different serological methods, ELISA has high sensitivity and specificity for diagnosis of chronic and acute toxoplasmosis (Robert-Gangneux and Darde, 2012; Saki *et al*., 2013). Formerly, it has been reported that IgM antibodies may be remain for a long period up to several years after acute infection. Also the presence of rheumatoid factor (RF) in serum may lead to false positive in *T. gondii* diagnosis; accordingly, discernment of acute and chronic *T. gondii* infection will be possible using IgG avidity and/or PCR technique (Robert-Gangneux and Darde, 2012; Saki *et al*., 2013). In the present study, in 18 out of 118 (15.25%) samples, IgM antibody was detected using ELISA, while based on nested-PCR results, *T. gondii*-DNA was confirmed in 41/118 (34.74%) samples that indicates acute infection of toxoplasmosis is very high in schizophrenic patients.

Current study faced several limitations, including 1) Absence of healthy individuals as control group to evaluate the possible association between latent toxoplasmosis and schizophrenia disorder; 2) No questionnaire was designed by us in this study; therefore, we were unable to assess the related risk factors including gender, residence, history of blood transfusion or transplantation, education level, ethnicity, contact with cat, source of drinking water, consumption of raw/undercooked meat, exposure to soil, etc; 3) The present investigation was based on sampling from limited participant in a limited region.

In conclusion, our study showed high seroprevalence of toxoplasmosis in comparison to previous reports in the same region. There is evidence which suggests, despite the constant presence of anti-*Toxoplasma* IgM antibodies in serum, any positive IgM-ELISA could not surely be considered as an acute infection, thus the PCR technique to explore the DNA of parasite is required.

Acknowledgements. We are very grateful to the Baharan, Clinic of Psychiatric Ali-ibn-Abi-Talib hospital staffs in Zahedan city, and Amir-al Momenin Psychiatric hospital staffs in Zabol city, Sistan and Baluchestan Province, Iran, for their technical assistance. This study was performed as part of M.Sc student thesis of Raheleh Hasanzadeh, and it was financially supported by the grant No:7691, provided from Zahedan University of Medical Sciences, Zahedan, Iran.

Authors' Contributions:
M. Jafari Modrek and H. Mirahmadi conceived the study; R. Hasanzadeh performed the experiments; M. Foroutan wrote the manuscript; M. Zarean critically revised the manuscript. All authors read and approved the final manuscript.

Conflict of interests:
The authors declare no conflict of interests.

Funding/Support:
This study supported by the grant No:7691, provided from Zahedan University of Medical Sciences, Zahedan, Iran.

Ethical approval:
The study protocol was approved by the Research Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (No of Ethic code: (IR.ZAUMS.REC.1395.14). A written informed consent was obtained from all participants before blood sampling.

REFERENCES

Abdollahian, E., Shafiei, R., Mokhber, N., Kalantar, K. & Fata, A. (2017). Sero-epidemiological Study of *Toxoplasma gondii* Infection among Psychiatric Patients in Mashhad, Northeast of Iran. *Iranian Journal of Parasitology* **12**: 117-122.

Ahmad, D., Mehdi, S., Sayed, H.H., Sayed, A.K. & Shirzad, G. (2010). Serological survey of *Toxoplasma gondii* in schizophrenia patients referred to Psychiatric Hospital, Sari City, Iran. *Tropical Biomedicine* **27**: 476-482.

Alipour, A., Shojaee, S., Mohebali, M., Tehranidoost, M., Abdi Masoleh, F. & Keshavarz, H. (2011). *Toxoplasma* infection in schizophrenia patients: a comparative study with control group. *Iranian Journal of Parasitology* **6**: 31-37.

Alvarado-Esquivel, C., Urbina-Alvarez, J.D., Estrada-Martinez, S., Torres-Castorena, A., Molotla-de-Leon, G., Liesenfeld, O. & Dubey, J.P. (2011). *Toxoplasma gondii* infection and schizophrenia: a case control study in a low Toxoplasma seroprevalence Mexican population. *Parasitology International* **60**: 151-155.

Arab-Mazar, Z., Fallahi, S., Koochaki, A., Mirahmadi, H. & Tabaei, S.J. (2016). Cloning, expression and immuno-reactivity of recombinant *Toxoplasma gondii* GRA5 protein. *Iranian Journal of Microbiology* **8**: 331-337.

Arab-Mazar, Z., Seyyed-Tabaei, S.J. & Mirahmadi, H. (2014). Cloning of dense granular (GRA) 7 gene of *Toxoplasma gondii* into pTZ57RT vectors for sub-cloning in prokaryotic and eukaryotic plasmids. *Novelty in Biomedicine* **2**: 114-119.

Cetinkaya, Z., Yazar, S., Gecici, O. & Namli, M.N. (2007). Anti-*Toxoplasma gondii* antibodies in patients with schizophrenia – preliminary findings in a Turkish sample. *Schizophrenia Bulletin* **33**: 789-791.

Daryani, A., Sarvi, S., Aarabi, M., Mizani, A., Ahmadpour, E., Shokri, A., Rahimi, M.T. & Sharif, M. (2014). Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis. *Acta Tropica* **137**: 185-194.

de Barros, J.L., Barbosa, I.G., Salem, H., Rocha, N.P., Kummer, A., Okusaga, O.O., Soares, J.C. & Teixeira, A.L. (2017). Is there any association between *Toxoplasma gondii* infection and bipolar disorder? A systematic review and meta-analysis. *Journal of Affective Disorders* **209**: 59-65.

Dogruman-Al, F., Aslan, S., Yalcin, S., Kustimur, S. & Turk, S. (2009). A possible relationship between *Toxoplasma gondii* and schizophrenia: A seroprevalence study. *International Journal of Psychiatry in Clinical Practice* **13**: 82-87.

Dubey, J.P. (2008). The history of *Toxoplasma gondii* – the first 100 years. *The Journal of Eukaryotic Microbiology* **55**: 467-475.

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 Research* **245**: 327-332.

First, M.B., Spitzer, R.L., Gibbon, M. & Williams, J.B. (1995). Structured clinical interview for DSM-IV axis I disorders.

Flegr, J. (2013). How and why *Toxoplasma* makes us crazy. *Trends in Parasitology* **29**: 156-163.

Flegr, J. (2015). Neurological and neuropsychiatric consequences of chronic *Toxoplasma* infection. *Current Clinical Microbiology Reports* **2**: 163-172.

Flegr, J., Prandota, J., Sovickova, M. & Israili, Z.H. (2014). Toxoplasmosis – a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PloS one* **9**: e90203.

Foroutan-Rad, M., Khademvatan, S., Majidiani, H., Aryamand, S., Rahim, F. & Malehi, A.S. (2016a). Seroprevalence of *Toxoplasma gondii* in the Iranian pregnant women: A systematic review and meta-analysis. *Acta Tropica* **158**: 160-169.

Foroutan-Rad, M., Majidiani, H., Dalvand, S., Daryani, A., Kooti, W., Saki, J., Hedayati-Rad, F. & Ahmadpour, E. (2016b). Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis. *Transfusion Medicine Reviews* **30**: 116-122.

Foroutan, M., Dalvand, S., Daryani, A., Ahmadpour, E., Majidiani, H., Khademvatan, S. & Abbasi, E. (2017). Rolling up the pieces of a puzzle: A systematic review and meta-analysis of the prevalence of toxoplasmosis in Iran. *Alex J Med*. doi: 10.1016/j.ajme.2017.06.003.

Foroutan, M. & Majidiani, H. (2017). *Toxoplasma gondii*: are there any implications for routine blood screening? *International Journal of Infection* e62886.

Hamidinejat, H., Ghorbanpoor, M., Hosseini, H., Alavi, S.M., Nabavi, L., Jalali, M.H., Borojeni, M.P., Jafari, H. & Mohammadaligol, S. (2010). *Toxoplasma gondii* infection in first-episode and inpatient individuals with schizophrenia. *International Journal of Infectious Diseases* **14**: e978-981.

HavlÍCek, J., GaŠOvÁ, Z., Smith, A.P., ZvÁRa, K. & Flegr, J. (2001). Decrease of psychomotor performance in subjects with latent 'asymptomatic' toxoplasmosis. *Parasitology* **122**: 515-520.

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*. *Schizophrenia Research* **62**: 237-244.

Khademvatan, S., Foroutan, M., Hazrati-Tappeh, K., Dalvand, S., Khalkhali, H., Masoumifard, S. & Hedayati-Rad, F. (2017). Toxoplasmosis in rodents: A systematic review and meta-analysis in

Iran. *Journal of Infection and Public Health* **10**: 487-493.

Khademvatan, S., Khajeddin, N., Izadi, S. & Yousefi, E. (2014a). Investigation of anti-*Toxocara* and anti-T*oxoplasma* antibodies in patients with schizophrenia disorder. *Schizophrenia Research and Treatment* **2014**: 230349.

Khademvatan, S., Khajeddin, N., Saki, J. & Izadi-Mazidi, S. (2013). Effect of toxoplasmosis on personality profiles of Iranian men and women. *South African Journal of Science* **109**: 92-95.

Khademvatan, S., Saki, J., Khajeddin, N., Izadi-Mazidi, M., Beladi, R., Shafiee, B. & Salehi, Z. (2014b). *Toxoplasma gondii* Exposure and the Risk of Schizophrenia. *Jundishapur Journal of Microbiology* **7**: e12776.

Kocazeybek, B., Oner, Y.A., Turksoy, R., Babur, C., Cakan, H., Sahip, N., Unal, A., Ozaslan, A., Kilic, S., Saribas, S., Aslan, M., Taylan, A., Koc, S., Dirican, A., Uner, H.B., Oz, V., Ertekin, C., Kucukbasmaci, O. & Torun, M.M. (2009). Higher prevalence of toxoplasmosis in victims of traffic accidents suggest increased risk of traffic accident in *Toxoplasma*-infected inhabitants of Istanbul and its suburbs. *Forensic Science International* **187**: 103-108.

Leweke, F.M., Gerth, C.W., Koethe, D., Klosterkotter, J., Ruslanova, I., Krivogorsky, B., Torrey, E.F. & Yolken, R.H. (2004). Antibodies to infectious agents in individuals with recent onset schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience* **254**: 4-8.

Majidiani, H., Dalvand, S., Daryani, A., Galvan-Ramirez, M.L. & Foroutan-Rad, M. (2016). Is chronic toxoplasmosis a risk factor for diabetes mellitus? A systematic review and meta-analysis of case-control studies. *The Brazilian Journal of Infectious Diseases* **20**: 605-609.

Medeiros-Ferreira, L., Obiols, J.E., Navarro-Pastor, J.B. & Zuniga-Lagares, A. (2013). Metabolic syndrome and health-related quality of life in patients with schizophrenia. *Actas Esp Psiquiatr* **41**: 17-26.

Modrek, M.J., Mousavi, M. & Saravani, R., (2014). *Toxoplasma gondii* Seroprevalence Among Blood Donors in Zahedan, Southeastern Iran. *International Journal of Infection* **1**: e21111.

Monroe, J.M., Buckley, P.F. & Miller, B.J. (2015). Meta-Analysis of Anti-*Toxoplasma gondii* IgM Antibodies in Acute Psychosis. *Schizophrenia Bulletin* **41**: 989-998.

Nicolle, C. & Manceaux, L. (1908). Sur une infection à corps de Leishman (ou organismes voisins) du gondi. *CR Acad Sci* 147.

Robert-Gangneux, F. & Darde, M.L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews* **25**: 264-296.

Saki, J., Khademvatan, S., Soltani, S. & Shahbazian, H. (2013). Detection of toxoplasmosis in patients with end-stage renal disease by enzyme-linked immunosorbent assay and polymerase chain reaction methods. *Parasitology Research* **112**: 163-168.

Saki, J., Shafieenia, S. & Foroutan-Rad, M. (2016). Seroprevalence of toxoplasmosis in diabetic pregnant women in southwestern of Iran. *Journal of Parasitic Diseases* **40**: 1586-1589.

Saraei-Sahnesaraei, M., Shamloo, F., Jahani Hashemi, H., Khabbaz, F. & Alizadeh, S.-A. (2009). Relation between *Toxoplasma gondii* infections and schizophrenia. *Iranian Journal of Psychiatry and Clinical Psychology* **15**: 3-9.

Shapira, Y., Agmon-Levin, N., Selmi, C., Petrikova, J., Barzilai, O., Ram, M., Bizzaro, N., Valentini, G., Matucci-Cerinic, M., Anaya, J.M., Katz, B.S. & Shoenfeld, Y. (2012). Prevalence of anti-*Toxoplasma* antibodies in patients with autoimmune diseases. *Journal of Autoimmunity* **39**: 112-116.

Sutterland, A.L., Fond, G., Kuin, A., Koeter, M.W., Lutter, R., van Gool, T., Yolken, R., Szoke, A., Leboyer, M. & de Haan, L. (2015). Beyond the association. *Toxo-*

plasma gondii in schizophrenia, bipolar disorder, and addiction: systematic review and meta-analysis. *Acta Psychiatrica Scandinavica* **132**: 161-179.

Tandon, R., Keshavan, M.S. & Nasrallah, H.A. (2008). Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. *Schizophrenia Research* **102**: 1-18.

Wang, Z.D., Liu, H.H., Ma, Z.X., Ma, H.Y., Li, Z.Y., Yang, Z.B., Zhu, X.Q., Xu, B., Wei, F. & Liu, Q. 2017. *Toxoplasma gondii* Infection in Immunocompromised Patients: A Systematic Review and Meta-Analysis. *Frontiers in Microbiology* **8**: 389.

Webster, J.P., Brunton, C.F. & MacDonald, D.W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, Rattus norvegicus. *Parasitology* **109**(Pt 1): 37-43.

Witting, P.A. (1979). Learning capacity and memory of normal and *Toxoplasma*-infected laboratory rats and mice. *Zeitschrift für Parasitenkunde* **61**: 29-51.

Yagmur, F., Yazar, S., Temel, H.O. & Cavusoglu, M. (2010). May *Toxoplasma gondii* increase suicide attempt-preliminary results in Turkish subjects? *Forensic Science International* **199**: 15-17.

Yousefi, E., Foroutan, M., Salehi, R. & Khademvatan, S. (2017). Detection of acute and chronic toxoplasmosis amongst multi-transfused thalassemia patients in southwest of Iran. *Journal of Acute Diseases* **6**: 120-125.

Zarean, M., Shafiei, R., Gholami, M., Fata, A., Rahmati Balaghaleh, M., Kariminik, A., Tehranian, F., Hasani, A. & Akhavan, A. (2017). Seroprevalence of Anti-*Toxoplasma gondii* Antibodies in Healthy Voluntary Blood Donors from Mashhad City, Iran. *Archives of Iranian Medicine* **20**(7): 441-445.