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**CONTRIBUTIONS TO THE STUDY OF
TOXOPLASMOSIS IN THE UNITED STATES
AND ROMANIA**

- A B S T R A C T -

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**Timișoara
2024**

Toxoplasma gondii is a widespread obligate intracellular protozoan, which infects approximately 30% of humanity. It has the ability to infect different types of cells, to spread across different hosts (humans, birds, terrestrial and aquatic mammals) and within all ecosystems. *T. gondii* is considered one of the most well-adapted and successful parasites.

T. gondii has three developmental stages: tachyzoite, bradyzoite and sporozoite. The life cycle of *T. gondii* is complex and functions in a prey–predator system, with the participation of the definitive and intermediate hosts. There have been described four main ways by which humans can become infected with *T. gondii*, involving all developmental stages of the parasite: (i) zoonotic transmission through the consumption of food and/or water contaminated with sporulated oocysts containing sporozoites; (II) foodborne transmission through the consumption of primary offal and raw / undercooked meat containing bradyzoites inside tissue cysts; (III) vertical transmission, from the acutely infected mother to the fetus (the tachyzoites have the ability to cross the placenta); (iv) blood transfusion (from a recently infected donor which is parasitemic at the time of blood sampling) or organ transplant (if organs taken from an infected donor contain tachyzoites or tissue cysts).

Toxoplasmosis, a systemic and cosmopolitan disease, is considered one of the most damaging zoonotic diseases in the world. With a loss of 2–8 million disability-adjusted life years, toxoplasmosis exerts a significant impact on individual and health care services costs. The course and severity of *T. gondii* infection depend on different factors: (i) the stage of the parasite (sporozoites vs. bradyzoites) and the inoculated dose; (ii) the parasite genotype; (iii) the host's immune status; and (iv) the genetic characteristics of the host.

Various techniques can be used to confirm the diagnosis of toxoplasmosis, depending on the patient's immune background, the disease setting or clinical signs. There are indirect methods (based on detecting the presence of specific antibodies) and direct methods (based on identifying the presence and isolation of the parasite or its DNA). The diagnosis of *T. gondii* infection is crucial for the surveillance, prevention and control of toxoplasmosis.

Toxoplasmosis must be addressed as a multidisciplinary collaboration and future studies should be integrated in new partnerships aimed to find reliable solutions to a complex disease. Results of past and future studies should strengthen

the collaboration between scientists and physicians, to a better clinical management of patients infected with *T. gondii*, resulting in an improved health of these patients. The awareness of toxoplasmosis is a critical point in understanding, promoting and implementation of prevention and control strategies.

Studies conducted in the United States of America aimed to evaluate the clinical and serologic findings in both infants born to mothers who were not treated for *T. gondii* during gestation and in infants born to mothers treated during pregnancy. Moreover, we investigated the usefulness of testing the cerebrospinal fluid by polymerase chain reaction for the diagnosis of congenital toxoplasmosis, the impact of maternal anti-*Toxoplasma* treatment during pregnancy on sensitivity of diagnostic tests for congenital infection in the neonate and the genotype of *Toxoplasma gondii* among humans living in North America.

Congenital Toxoplasmosis in the United States of America

Even though *Toxoplasma gondii* infection is largely asymptomatic (except for immunocompromised individuals), in case of a primary infection acquired during pregnancy, congenital toxoplasmosis can occur. Congenital toxoplasmosis can cause significant neurologic manifestations and other untoward sequelae.

Diagnosis of congenital toxoplasmosis is based on a combination of serological and molecular tests. Maternal screening and treatment differ according to national policies and may impact the sensitivity of diagnostic methods in infants at birth. In the United States, national screening programs at the prenatal or postnatal level have not been implemented. Therefore, there are no accurate data regarding the disease burden or the actual incidence of congenital toxoplasmosis in the United States.

Severe Congenital Toxoplasmosis in the United States.

Clinical and Serologic Findings in Untreated Infants

The Palo Alto Medical Foundation Toxoplasma Serology Laboratory database was searched for data on infants 0 to 180 days old, in whom congenital

toxoplasmosis had been confirmed and who had been tested for *Toxoplasma gondii*-specific immunoglobulin G (IgG), IgM, and IgA antibodies, between 1991 and 2005. Their clinical findings were confirmed at the National Collaborative Chicago-based Congenital Toxoplasmosis Study center. Available clinical data and laboratory profiles of 164 infants with congenital toxoplasmosis whose mothers had not been treated for the parasite during gestation have been reviewed.

One or more severe clinical manifestations of congenital toxoplasmosis were reported in 84% of the infants and included eye disease (92.2%), brain calcifications (79.6%), and hydrocephalus (67.7%). In 61.6% of the infants, eye disease, brain calcifications, and hydrocephalus were present concurrently. *T. gondii*-specific IgM, IgA, and IgE antibodies were demonstrable in 86.6%, 77.4%, and 40.2% of the infants, respectively. Testing for IgM and IgA antibodies increased the sensitivity of making the diagnosis of congenital toxoplasmosis to 93% compared with testing for IgM or IgA individually. IgM and IgA antibodies were still present in 43.9% of infants diagnosed between 1 and 6 months of life.

This study revealed that severe clinical signs of congenital toxoplasmosis including hydrocephalus, eye disease, or intracranial calcifications occurred in 85% infants whose sera were referred to the reference Toxoplasma Serology Laboratory during a period of 15 years. Laboratory tests, including serologic and polymerase chain reaction tests, were critical for diagnosis in the infants. These results were remarkably in contrast with those of European investigators who rarely observed severe clinical signs in infants with congenital toxoplasmosis in countries where screening programs have been implemented.

Congenital Toxoplasmosis in the United States: Clinical and Serologic Findings in Infants Born to Mothers Treated during Pregnancy

In this study were assessed clinical and serologic findings in 25 infants with congenital toxoplasmosis born to mothers treated during pregnancy in the United States. Results indicate a lower prevalence of eye findings and hydrocephalus in the group of infants born to treated mothers (62.5% and 38.5%, respectively) compared to results on the same pathologies reported in a previous cohort of infants born to untreated mothers (92.2% and 67.7%, respectively). The sensitivity of the IgM

ISAGA and IgA ELISA in the present study were lower (44% and 60%, respectively) compared to sensitivity of these methods in the previously studied group of infants born to untreated mothers (86.6% and 76.5%, respectively). These findings provide further evidence that anti-parasitic treatment if administered during pregnancy can contribute to better clinical outcomes, even in countries where systematic screening and treatment have not been routinely implemented.

Polymerase Chain Reaction in Cerebrospinal Fluid for the Diagnosis of Congenital Toxoplasmosis

In this study, was evaluated the potential of the polymerase chain reaction (PCR) in the cerebrospinal fluid (CSF) for diagnosis of congenital toxoplasmosis. Both congenitally infected (diagnosed clinically and serologically) and noninfected infants born to untreated mothers were investigated.

CSF PCR was positive in 27 of the 58 (46.5%) congenitally infected infants and was negative in each of the 103 infants without congenital toxoplasmosis. The frequency of positive CSF PCR varied according to whether infants had major clinical signs of the disease: PCR was positive in 70.9%, 53.3% and 50.9% of those with hydrocephalus, cerebral calcifications and/or eye disease, respectively. Of six infants who were negative for both IgM and IgA antibodies, three had a positive PCR in their CSF as the confirmatory test for diagnosis of congenital toxoplasmosis. IgM and IgA antibodies and CSF PCR, when combined, yielded a higher sensitivity for diagnosis of congenital toxoplasmosis when compared with the performance of each test alone.

These findings revealed that in infants with clinical and serologic findings suggestive of congenital toxoplasmosis and born to untreated mothers, CSF PCR has the potential to increase the frequency of cases in which the diagnosis is confirmed.

Maternal Anti-*Toxoplasma* Treatment during Pregnancy Is Associated with Reduced Sensitivity of Diagnostic Tests for Congenital Infection in the Neonate

In this multicenter study, 115 neonates born to 61 treated (53%) and 54 (47%) untreated women were retrospectively included in three centers (France, Serbia, and the United States) to assess the impact of maternal anti-*Toxoplasma* treatment on the performance of neonatal workup at birth (neosynthesized anti-*Toxoplasma* IgM, IgA, and IgG and quantitative PCR). Independently of the time of maternal seroconversion, the serological techniques were impacted differently by maternal treatment. The detection of IgM by immunosorbent agglutination assay (ISAGA) and Western blotting (WB) dropped from 90.7% and 88.2% in untreated neonates to 53.3% and 51.9% in treated neonates ($p < 0.05$), whereas IgM enzyme-linked immunosorbent assay (ELISA) and IgA ISAGA were not significantly affected by maternal treatment. A 2-fold reduction in the sensitivity of neosynthesized IgG by WB was also observed in the case of treatment during pregnancy (37.7% versus 82.3%). Interestingly, the effect of treatment was shown to be duration dependent, especially for IgM detection, when the treatment course exceeded 8 weeks, whatever the therapy. The sensitivity of *Toxoplasma* PCR in blood was also lowered by maternal treatment from 39.1% to 23.2%. These results highlight that anti-*Toxoplasma* therapy during pregnancy may set back biological evidence of neonatal infection at birth and underline the need for a careful serological follow-up of infants with normal workup.

The Role of Molecular Methods for the Genetic Characterization of *Toxoplasma gondii* in North America

Human pathogenesis of *T. gondii* is influenced by the number and genetic diversity of the parasite. Molecular techniques are used for genotyping of *T. gondii* in different populations and regions. Whereas in Europe most of *Toxoplasma gondii* genotypes belong to the type II lineage, in Latin America, type II is rare and atypical strains predominate. In North America, data on *T. gondii* genotypes in humans are scarce.

Genetic Characterization of *Toxoplasma gondii* DNA Samples Isolated From Humans Living in North America

In this study, *T. gondii* DNA samples from 67 patients with diagnosed toxoplasmosis in the United States were available for genotyping. Discriminant analysis of principal components was used to infer each atypical genotype to a geographic area where patients were probably infected. Associations between genotype, disease severity, immune status, and geographic region were also estimated. Of 67 DNA samples, 41 were successfully genotyped: 18 (43.9%) and 5 (12.2%) were characterized as types II and III, respectively. The remaining 18 genotypes (43.9%) were atypical and were assigned to a geographic area. Ten genotypes originated from Latin America, 7 from North America, and 1 from Asia (China). In North America, unlike in Europe, *T. gondii* atypical genotypes are common in humans and, unlike in Latin America, type II strains are still present with significant frequency.

Clinicians should be aware that atypical genotypes are common in North America and have been associated with severe ocular and systemic disease and unusual presentations of toxoplasmosis in immunocompetent patients.

Role of *Toxoplasma* IgA antibodies for the Diagnosis of Acute Toxoplasmosis

Toxoplasma gondii is able to cross the placenta and infect the fetus if the pregnant women gets infected with this parasite for the first time during pregnancy. The gestational age at which the maternal infection was acquired influence the risk of transmission and the severity of congenital disease. Confirming a diagnosis of acute infection with *T. gondii* in pregnant women is not always easy. Therefore, there is a need to identify serologic tests that may contribute to confirming the diagnosis of an acute toxoplasmosis.

The role of *Toxoplasma* IgA in the diagnosis of acute toxoplasmosis was evaluated in two studies, one conducted in pregnant women from USA and the other in women of childbearing age from Western Romania.

Role of *Toxoplasma* IgA as Part of a Reference Panel for the Diagnosis of Acute Toxoplasmosis in Pregnant Women from USA

This study evaluated the usefulness of adding the *Toxoplasma gondii* IgA antibody enzyme-linked immunosorbent assay (ELISA) to the serologic panel of tests done for the diagnosis of acute toxoplasmosis in pregnant women in a reference laboratory in the United States. In this retrospective study, were investigated 690 consecutive pregnant women with positive *T. gondii* IgG antibody test results who also had *T. gondii* IgA and IgM antibody tests performed. The study participants were defined as acutely or chronically infected with *T. gondii* based on a panel of serologic tests performed at the Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL). Among the 81 women who were positive by *T. gondii* IgA antibody ELISA testing, 61 (75.3%) were acutely infected with *T. gondii*, while of the 547 who were negative by IgA testing, only 24 (4.4%) were acutely infected ($p < 0.001$). Among the 71 women who were positive by both IgA and IgM antibody tests, 61 (85.9%) were acutely infected, whereas 24 (19.2%) of the 125 women who were positive by only the IgM ELISA were acutely infected ($p < 0.001$). These results demonstrate that pregnant women with *T. gondii* IgA antibodies are more likely than pregnant women without *T. gondii* IgA antibodies to have had a recent infection with *T. gondii*. *Toxoplasma* IgA antibody testing can therefore improve the accuracy of a serologic panel for the diagnosis of acute toxoplasmosis during pregnancy.

Role of *Toxoplasma* IgA for the Diagnosis of Acute Toxoplasmosis in Women of Childbearing Age from Western Romania

In this study was assessed the value of adding a *T. gondii* IgA test to the serologic panel for the diagnosis of toxoplasmosis, including the detection of a recently acquired infection. Serologic testing was conducted in 1317 females aged 15–45 years. *T. gondii* IgM and IgA antibody tests were performed in those with detectable IgG antibodies and IgG avidity test was performed if IgM and/or IgA screening test results were positive. Of the 607 persons with detectable *T. gondii* IgG antibodies, *T. gondii* IgM antibodies were demonstrated in 8.90% (95%CI: 6.88–11.43), *T. gondii* IgA in 1.65% (95%CI: 0.90–3.01) and both *T. gondii* IgM and

IgA in 0.99% (95%CI: 0.45–2.14). The prevalence of IgA antibodies tended to decrease with increasing avidity, from 75% (95%CI: 19.41–99.37) in samples with low avidity to 11.76% (95%CI: 4.44–23.87) in those with high avidity ($p = 0.01$). Of the study participants who were positive for both *T. gondii* IgM and IgA antibodies, 66.67% had low or equivocal IgG avidity test results compared to 6.25% who tested positive for IgM, were negative for IgA and in whom low or equivocal IgG avidity test results were noted ($p = 0.001$). This study indicates that *T. gondii* IgA antibodies may be rarely detected during a serologic screening. However, in individuals with demonstrable *T. gondii* IgG and IgM antibodies, testing for *T. gondii* IgA may improve the rate for the detection of a recently acquired toxoplasmosis.

Epidemiological Studies Conducted in Romania: Seroprevalence and Risk Factors Associated with *Toxoplasma gondii* Infection

Seroprevalence studies

Globally, it is estimated that one third of the human population is infected with *T. gondii*, and the prevalence among different population groups varies widely, between 0.5% and 87.7%. There are many factors that are shown to influence the prevalence of *T. gondii* infection. However, the real value of *T. gondii* seroprevalence is not known precisely due to the lack of data from certain areas of the globe.

In international literature there were few data regarding the prevalence of *T. gondii* infection in Romanian population and studies conducted in Western Romania aimed to evaluate the magnitude of infection with *T. gondii* in this area.

Seroprevalence of *Toxoplasma gondii* Infection in Western Romania

The seroprevalence of *T. gondii* was evaluated among 304 individuals (aged 15-84 years) using the Pastorex Toxo test, which allows the simultaneous detection of *T. gondii* IgG and/or IgM antibodies. *T. gondii* antibodies were demonstrated in 197 individuals (64.8%) and the prevalence increased with age: 35.0% in those < 20 years versus 76.8% in those ≥ 70 years ($p < 0.001$). There was a higher prevalence

of *T. gondii* antibodies in rural areas (76.9%) than in urban regions (55.3%) ($p < 0.001$). The prevalence of *T. gondii* antibodies in women of reproductive age (16 – 49 years) was 55.5% (30/54 women). These results suggest a high prevalence of *T. gondii* antibodies in Western Romania.

Seroprevalence of *Toxoplasma gondii* Infection in Children from Western Romania

In this serological survey serum samples of 441 children (aged 1-18 years) were screened for *T. gondii* immunoglobulin G and immunoglobulin M antibodies. The overall *T. gondii* seroprevalence was 16.6% and tended to increase with age. Seroprevalence was 18.4% in children from rural regions and 14.7% in those from urban regions. *T. gondii* antibodies were demonstrated in 19.5% of females and 13.3% of males. Results suggest that exposure to *T. gondii* started during childhood.

Seroprevalence of *Toxoplasma gondii* Infection in Women of Childbearing Age from Western Romania

In Romania, limited data were available regarding *T. gondii* seroprevalence among women of childbearing age: (i) only small-scale studies have been conducted; (ii) there is no national screening program for pregnant women and congenital toxoplasmosis implemented.

Seroprevalence in women of childbearing age from Arad County

In this study, serum samples from 2626 women were analyzed. *Toxoplasma gondii* IgG antibodies were demonstrated in 1081 women (41%) and prevalence tended to increase with age, from 32% in women aged 15–19 years to 62% in women aged 40–45 years. There was a higher prevalence in rural areas (46%) than in urban areas (36%).

*Seroprevalence in women of childbearing age
from Bihor County*

Of the 1935 females aged 15–45 years included in this study, *T. gondii* IgG antibodies were found in 706 females (36.48%) and the proportion of IgG positive females tended to increase with age. The seroprevalence was higher in females residing in rural areas (47.79%) compared with those from urban areas (30.95%).

*Seroprevalence in women of childbearing age
from Timis County*

Serologic testing to demonstrate the presence of *T. gondii* IgG antibodies was conducted in 1317 females aged 15–45 years. *T. gondii* IgG were detected in 607 (46.09%; 95%CI: 43.41–48.79) and IgG seroprevalence tended to increase with age from 35.44% (95%CI: 29.89–41.30) in age group 15–24 years to 62.85% (95%CI: 56.57–68.82) in age group 35–45 years, showing a significant age-associated increase ($p < 0.001$).

The results observed in these studies bring new epidemiological data regarding the prevalence of *T. gondii* infection among women of childbearing age in Western Romania. The prevalences of *T. gondii* infection observed in this population group are among the highest values observed in European countries.

**Seroprevalence of *Toxoplasma gondii* Infection in Pregnant Women
from Western Romania**

T. gondii seroprevalence was evaluated in 208 pregnant women (aged 12–41 years) by demonstration of serum antibodies using the commercial Vitros anti-*Toxoplasma* immunoglobulin G (IgG) and IgM assays. *T. gondii* antibodies were demonstrated in 116 (55.8%) of 208 pregnant women: both anti-*T. gondii* IgG and IgM antibodies were demonstrated in two (0.9%) cases, and IgG antibodies alone in 114 (54.8%) women. The presence of *T. gondii* antibodies tended to increase with age from 51.8% (14/27) in pregnant women aged 12–20 years, to 52.8% (66/125) in those aged 21–30 years, and 64.3% (36/56) in those aged 31–41 years. These results showed a high prevalence of *T. gondii* antibodies in pregnant women in

Romania, underlining once again the importance of implementing a national surveillance system for *T. gondii* infection in pregnant women in Romania.

Seroprevalence of *Toxoplasma gondii* Infection in Blood Donors from Western Romania

Serologic testing to demonstrate the presence of *T. gondii* antibodies was conducted in 1347 healthy blood donors who presented to the Regional Blood Transfusion Center in Timisoara between 19 November–21 December 2018. The overall prevalence of *T. gondii* antibodies was 45.9%, with a significant age-associated increase ($p < 0.001$) from 32.6% in age group 18–25 years to 67.6% in age group 56–63 years. *T. gondii* antibodies were detected in 43.2% (213/493) females of childbearing age. This study brings new and valuable data regarding the prevalence of *T. gondii* infection in Romania, revealing a high prevalence of *T. gondii* antibodies in blood donors. These findings may serve as a starting point for further epidemiological studies that should lead to implementation of prevention programs for toxoplasmosis.

Seroprevalence of *Toxoplasma gondii* Infection in Patients with Cardiovascular Diseases from Western Romania

This case–control study aimed: (i) to assess the presence of specific IgG and IgM anti-*T. gondii* antibodies in both cardiovascular patients and control subjects; (ii) to evaluate the potential relationship between cardiovascular diseases and *T. gondii* infection. Serologic testing to demonstrate the presence of *T. gondii* antibodies was conducted in 256 patients with cardiovascular diseases and 261 matched blood donors. The overall seroprevalence of *T. gondii* antibodies was 64.06% in patients with cardiovascular diseases and 52.88% in blood donors and tended to increase with age in both groups. The seroprevalence of *T. gondii* antibodies was significantly higher in cardiovascular male patients (69.94%) compared to male blood donors (55.69%) ($p = 0.006$). When compared to the control group, a significantly higher prevalence of *T. gondii* antibodies was found among patients with hypertension (82.35%; $p = 0.01$) and unstable angina (67.56%; $p = 0.02$).

This study brings new epidemiological information on the prevalence of *T. gondii* in Romanian cardiovascular patients. *T. gondii* seroprevalence was significantly higher in patients with hypertension and unstable angina, suggesting that individuals with these diagnoses may be more frequently infected with *T. gondii*. This study may be a valuable starting point for further research to better evaluate the impact of *T. gondii* exposure on patients with cardiovascular diseases.

Seroprevalence of *Toxoplasma gondii* Infection in Psychiatric Patients from Western Romania

This study assessed for the first time the prevalence of *T. gondii* infection in psychiatric patients and healthy individuals with no known psychiatric disorders in Western Romania. The presence of specific IgG anti-*T. gondii* antibodies was evaluated in 308 psychiatric patients and 296 control subjects. Overall, the seroprevalence of IgG antibodies was higher in psychiatric patients (67.86%; 209/308), compared to controls (54.05%; 160/296) ($p < 0.001$). These results revealed a significantly higher prevalence of *T. gondii* antibodies among patients with schizophrenia (69.77%), organic (personality and behavior) disorders (76.74%), and mental disorders concerning alcohol abuse (84.62%), compared to controls ($p = 0.009$, $p = 0.005$, $p = 0.043$, respectively). This study provided new and important information on the seroprevalence of *T. gondii* in Romanian psychiatric patients and may serve for further scientific research regarding the status of *T. gondii* infection in patients with psychiatric disorders.

Risk Factors Associated with *Toxoplasma gondii* infection

Transmission of *T. gondii* infection can change over time due to the complex socioeconomic, dietary and environmental factors. Therefore, a considerable number of studies have been conducted over the years to evaluate the factors potentially involved in the wide variation of *T. gondii* seroprevalence between countries or between different regions in the same country. Exactly how risk factors are associated to the different routes of *T. gondii* transmission to humans remains under

debate. A risk factor analyzed alone may have a minor impact on the epidemiology of *T. gondii* infection, but when combined together, risk factors have the power to influence the global distribution patterns of this parasitic disease.

In Romania, there was an urgent need for studies to evaluate the potential risk factors associated with *T. gondii* infection, considering that this information was completely lacking.

Risk Factors Associated with *Toxoplasma gondii* in Pregnant Women from Western Romania

Risk factors of *Toxoplasma gondii* infection were assessed among 208 pregnant women in Western Romania using a survey questionnaire created with input from physicians, veterinarians, and clinical laboratory specialists at the Municipal and County Clinical Emergency Hospitals in Timisoara, Romania.

Lower level of education and working with meat were found to be risk factors for *T. gondii* seropositivity. Pet owners (cats and/or dogs) had a higher *T. gondii* seroprevalence than those who did not report having any pet ($p = 0.032$). Women with ≥ 4 live births were more frequently *T. gondii* seropositive than those without previous births ($p < 0.002$). Women with histories of spontaneous abortions were more frequently *T. gondii* seropositive than those without such a history ($p = 0.036$).

Risk Factors Associated with *Toxoplasma gondii* in Blood Donors from Western Romania

The epidemiological questionnaire filled out by 1347 healthy blood donors from Western Romania provided information regarding the risk factors associated with *T. gondii* infection. The *T. gondii* seroprevalence showed a significant age-associated increase ($p < 0.001$) and decreased with increasing level of education, from 64.3% in individuals who graduated from elementary/middle school to 40.4% in those who graduated from university ($p < 0.001$). The multiple logistic regression analysis revealed that age, level of education and having pets (cats and/or dogs) were significantly associated with *T. gondii* infection.

Risk Factors Associated with *Toxoplasma gondii* in Patients with Cardiovascular Diseases from Western Romania

A structured questionnaire was used to identify the potential risk factors associated with *T. gondii* among 1205 patients with cardiovascular diseases. Patient's area of residence, gender, educational level, owning dogs, owning any pet, and toxoplasmosis awareness were significantly associated with *T. gondii* seropositivity in multiple logistic regression analyses. Identifying toxoplasmosis awareness as a risk factor for *T. gondii* infection in Romanian cardiovascular patients underlines the importance of health education to increase awareness of the multiple possibilities of infection with *T. gondii* and how this parasitic disease can be prevented.

Risk Factors Associated with *Toxoplasma gondii* in Psychiatric Patients from Western Romania

The questionnaire used to assess the potential correlation between risk factors and *Toxoplasma gondii* seropositivity showed a higher likelihood of positive IgG antibodies against *T. gondii* in older individuals, patients residing in rural areas, and females. The statistical analysis also revealed associations between *Toxoplasma gondii* infection and certain risk factors, like activities that involve contact with soil, low-income levels, and limited educational attainment.

These findings reiterate the importance of toxoplasmosis awareness and health education for better control and prevention of infection with *T. gondii*. Public health authorities should promote information regarding the epidemiology of *T. gondii* in order to reduce transmission. These data may serve as a starting point for further studies that should lead to implementation of prevention programs for toxoplasmosis.

The considerable number of studies conducted globally so far, describe *Toxoplasma gondii* as the world's most "successful parasite" and human toxoplasmosis as one of the most significant parasitic diseases.

In my future research activity, I will continue to evaluate the impact of *T. gondii* infection in human population. The main objectives of research will include:

- assessment of the burden of toxoplasmosis in the United States and worldwide
- assessment of the prevalence and severity of congenital toxoplasmosis among Romanian children
- evaluation of the prevalence of *T. gondii* infection among Romanian population to confirm the potential decreasing trend observed in our previous studies
- genotyping *Toxoplasma gondii* in Romanian pregnant women
- investigation of the possible association between *T. gondii* and psychiatric disorders, such as bipolar disorder and depression

My further research activities will strengthen comprehensive and continuous training for both medical students and health care providers will help us understanding the ever-changing world we live in, by improving education and thus offering the opportunity for a better healthcare.