



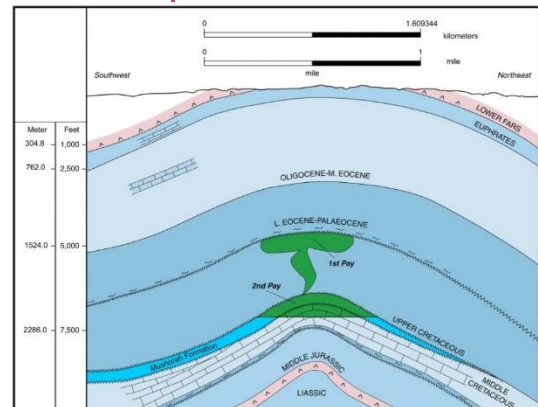
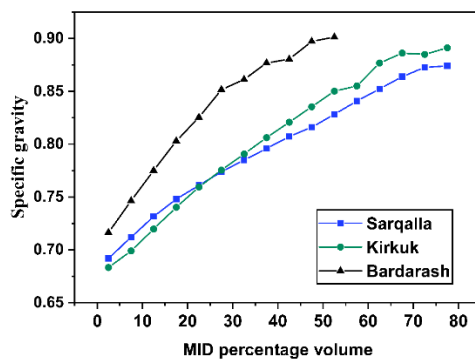
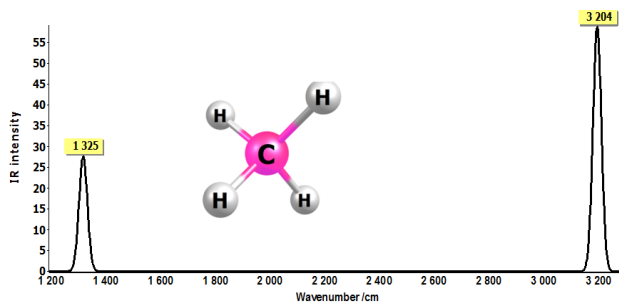
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Seroprevalence of *Toxoplasma gondii* among pregnant women in the Turkish Republic of Northern Cyprus

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Abstract

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* and is generally asymptomatic, affecting almost all organs. Serological techniques are applicable for diagnosing *T. gondii* since it is challenging to demonstrate under the microscope or culture in the laboratory. This study aimed to determine *Toxoplasma* seroprevalence in pregnant women at Near East University Hospital in the Turkish Republic of Northern Cyprus (TRNC) between 2016 and 2018. Antibodies of *Toxoplasma* Immunoglobulin M (Toxo IgM) and *Toxoplasma* Immunoglobulin G (Toxo IgG) were analyzed in pregnant women retrospectively by enzyme-linked immunosorbent assay (ELISA). Toxo IgG avidity was tested by ELISA in pregnant women to differentiate between acute and chronic infections. The pregnant women were between 17 and 51 years. Of the 1348 participants, 572 (42.43%) were from the TRNC, 746 (55.34%) were from Turkey, and 30 (2.23%) were from other countries. In this study, the prevalence rates were 1.4% and 12.7% for IgM and IgG among pregnant women. High Toxo IgG avidities were observed in 75% of the IgM seropositive women. The study demonstrated that Toxo IgG prevalence rates were low compared to many countries.

Introduction

Toxoplasmosis is a protozoan disease caused by *Toxoplasma gondii*, causing morbidities and mortalities in humans, domestic animals, and terrestrial and aquatic wildlife (1). Because of the parasite's life cycle, *T. gondii* is a global zoonosis that is more prevalent in hot and humid areas. Felids are the definitive hosts of *T. gondii* and are infected by taking the sporulated oocysts or, sometimes, infected animals such as mice or rats (2). The oocysts infect most warm-blooded animals, including mammals and birds. *T. gondii* infection spreads by ingesting uncooked or poorly cooked meat containing the cysts, drinking water, eating food containing oocysts, and from pregnant mothers to the fetus. Other less common transmission methods include blood transfusion, organ transplantation, and insects such as flies (through mechanical transmission) (3).

The infectious *T. gondii* stages, tachyzoites, bradyzoites (tissue cysts), and sporozoites (oocytes) are all highly infectious to humans (4). After intake, oocysts develop into tachyzoites, localizing in the nervous and muscular

tissues, and convert into tissue cysts (bradyzoites). Also, ingesting undercooked meat containing the tissue cyst may result in human infection (5).

Congenital toxoplasmosis takes place via transmission to the fetus during gestation. Contaminated water and food with infected cats' feces cause *Toxoplasma* infection. The host cell is invaded and eventually destroyed during the incubation period of 5–18 days (6). A severer congenital toxoplasmosis form affects the retina and causes chorioretinitis visual disorder. Congenital toxoplasmosis and toxoplasmosis in immunodeficient patients can cause nervous disorders (7).

Toxoplasma may induce abortions, stillbirths, and different anomalies in infants. Congenital toxoplasmosis severely threatens pregnancies and neonates and significantly impacts public health. Although *Toxoplasma* infection is mostly symptomless in healthy people, morbidity and mortality are primarily observed in immunosuppressive individuals, fetuses, and newborns (8). Hence, screening pregnant women for *Toxoplasma* is vital in reducing disease occurrence.

Toxoplasmosis prevalence varies from country to country and has been shown on all continents except for Antarctica (9). Several studies have elucidated the relationship between toxoplasmosis and parameters such as lifestyle, eating habits, and socioeconomic position (10, 11). Finding such factors' effect on seroprevalence provides essential information regarding public health.

Immunoglobulin G (IgG) monoclonal gammopathy and raised IgM levels have been described in innately infected infants with congenital toxoplasmosis. Glomerulonephritis was characterized by IgM, fibrinogen, and *Toxoplasma* antigen deposits in people infected innately (12).

Toxoplasmosis affects nearly one-third of global pregnant women, and prevalence rates as high as 56.2% were recorded in South American women (13). The fluctuation is due to geographical, socioeconomic, and environmental causes such as host age, status, and genetic factors (14). Vertical transmission of *T. gondii* infection occurs between one to four months of colonization in the placenta, negatively impacting pregnancies and newborns (15).

As pregnancy progresses, the likelihood of mother-to-child transmission rises (16). Infection with *T. gondii* gained during early pregnancy is more likely to lead to clinical conditions. In cases of toxoplasmosis during pregnancy, congenital toxoplasmosis can be observed in 20% to 50% of infants if the therapeutic methods are incompatible (17).

T. gondii is accredited as one of the most successful protozoan parasites; although it was discovered over a century ago, toxoplasmosis is still one of the most common parasitic infections, infecting 2.3 billion people and other warm-blooded animals (11). However, pregnant women do not receive adequate screening for toxoplasmosis, which could be due to neglecting its importance. This study retrospectively assessed the hazard of toxoplasmosis in the TRNC in public health terms, particularly in pregnant women, from 2016 to 2018.

Materials and Methods

Study site and sample collection

This study retrospectively collected demographic data from pregnant women admitted to the Department of Obstetrics and Gynecology Outpatient Clinic at the Near East University Hospital and went to the Clinical

Microbiology Laboratory in Near East University/Faculty of Medicine Hospital in Nicosia, Northern Cyprus. It was executed between 2016 and 2018 at the Medical Microbiology Laboratory of the Near East University Hospital. The Near East University Hospital's Head Physician granted the required permit for the retrospective study.

Samples were taken from 1348 pregnant women, 17–51 years old, who were followed during pregnancy visiting the hospital. About 5 mL of venous blood was taken from each participant and aseptically drawn to serum separator tubes. The blood samples were numbered, put in centrifuge tubes, and centrifuged at 1000 rpm for 10 minutes for serum separation. Serum samples used within a few hours after separation were kept at room temperature, while samples used the next day were kept refrigerated at 4°C until use. Sera used after a few days of the collection were stored at -20°C.

Sample analyses

Toxo IgM and Toxo IgG were measured in the pregnant women's blood samples by ELISA in the Clinical Microbiology Laboratory at Near East University Hospital. The Toxo IgG avidness test was executed by an ELISA technique to diagnose acute or chronic disease in Toxo IgG and Toxo IgM positive cases. IgG and IgM antibodies were quantitated in patient sera following the manufacturer's instructions using a microplate reader (ABBOTT Architect i1000, USA). Serum samples were visually examined, and non-homogeneous sera were vortexed until homogenization, then studied.

In the Toxo IgM test evaluation, a reading of < 0.5 was considered negative for toxoplasmosis, while values between 0.5 and 0.6 were considered the gray zone. On the other hand, values ≥ 0.6 were considered positive. Blood was recollected after two weeks from women whose samples were detected as a gray zone. In the Toxo IgG test evaluation, antibody concentrations < 1.6 IU/mL were considered negative, 1.6–3.0 IU/mL were in the gray zone, and ≥ 3.0 IU/mL were positive. Moreover, a low Toxo IgG avidity denotes infection in the last three months, and high avidity refers to a six-month-old infection.

Results

Of the 1348 participants, 572 were from the TRNC, 746 were Turkish, and 30 were from other nationalities. Also, 266 women were 17–24 years old, 936 were 25–34, and 146 were between 35 and 51 years (Figure 1).

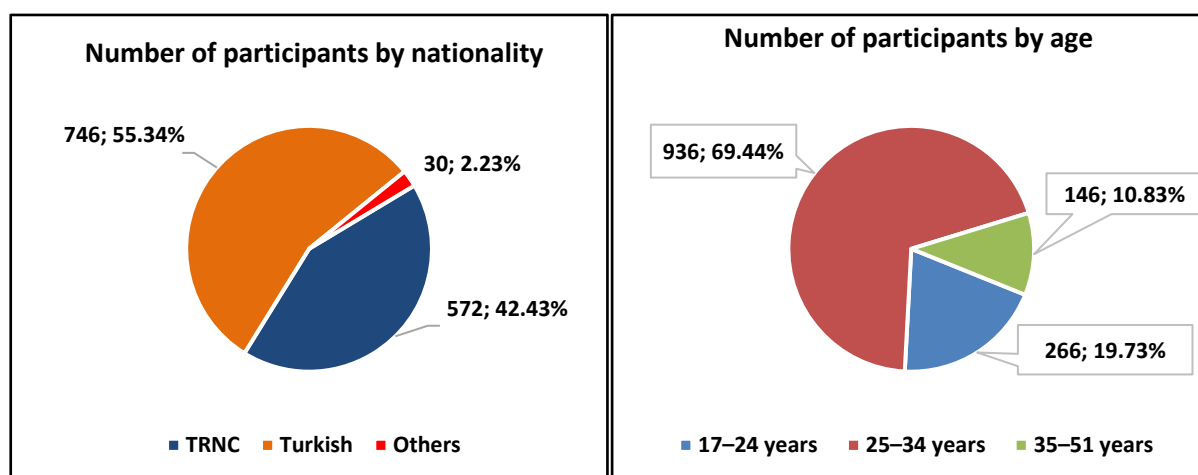


Figure 1. Number and percentage of different participant groups. The total number of participants was 1348.

Nineteen patients (1.4%) were Toxo IgM reactive, while 1329 patients (98.6%) were Toxo IgM non-reactive. Results showed that 171 (12.7%) patients were Toxo IgG reactive, while 1168 (86.6%) were Toxo IgG non-reactive, and nine (0.7%) patients were in the gray zone (Figure 2). Twelve of the 19 IgM-positive patients were from TRNC, and seven of the 746 Turkish women were IgM-positive. Moreover, 74 patients from TRNC were Toxo IgG-positive, and 96 Turkish patients were IgG seropositive. Also, one of the patients from other nationalities was positive (Figure 1). Five of the nine patients in the gray zone were from TRNC, and four were Turkish. About 12.7% of the patients were Toxo IgG reactive; the infected patients' numbers per age group are shown in Figure 2.

Toxoplasma avidity was studied in only 12 of the 19 pregnant women reactive to Toxo IgM because seven of the women did not follow up. The results showed high avidity in nine (75%) pregnant women, which means chronic infection. The high avidity indicates that the *Toxoplasma* infection was acquired six months ago. However, low IgM reactivity was recorded in one patient only (8.3%), meaning that the infection occurred within the last three months. Borderline avidity occurred only in two pregnant patients (16.7%). The ratio of Toxo IgM reactive samples was 13/536 (2.43%) in 2016 and 5/470 (1.06%) in 2017 and declined to 1/342 (0.29%) in 2018.

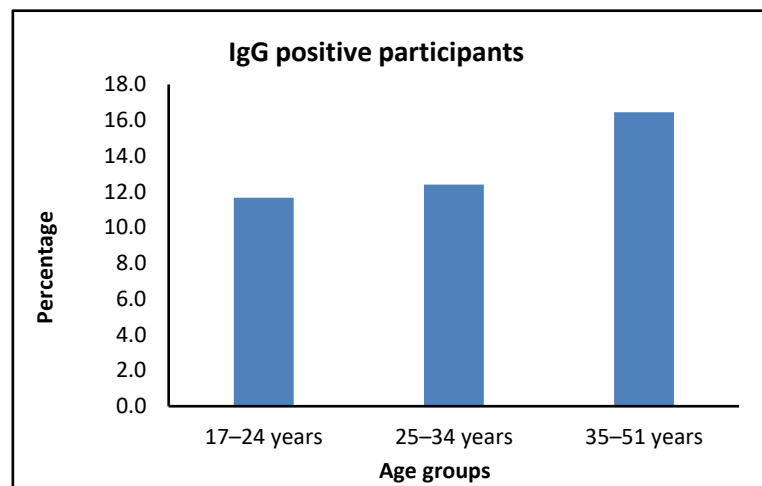


Figure 2. Percentage of IgG reactive participants according to their ages.

Discussion

Cyprus is an island in the Mediterranean Sea with highly humid and warm weather. *T. gondii* oocysts are more resistant to warm and humid weather conditions (18), which makes these oocysts survive and remain infective for long periods in Cyprus. In Turkey, most retrospective studies of seropositivity rates reported 44% to 85% IgG positivity (19-21). This study showed that the IgG seroprevalence in Cyprus is lower than in other parts of Turkey. The exact reasons for the lower *Toxoplasma* prevalence rate need to be addressed in future research.

The global *T. gondii* IgM seroprevalence was estimated to be 1.9%. The prevalence was highest in the Eastern Mediterranean region (4.1%) and lowest in The Americas (1.1%). The IgM seroprevalence in this study was 26% lower than the global estimate of the disease and 66% lower than in Eastern Mediterranean areas. This decline in seroprevalence can be attributed to the establishment of animal shelters, which have increased in number in recent years. Also, veterinary controls of all pets are adequately accomplished, and endectocides are used for pet animals. According to the data from veterinarians, the number of pet animals has risen in the last decade. Most owners feed their pets with commercially available uncontaminated food with *Toxoplasma*.

Moreover, the global IgG seroprevalence was 32.9%, the highest in The Americas (45.2%) and the lowest in the Western Pacific (11.2) (22). In comparison, the overall seroprevalence in our study was 12.7%, indicating a 61.4% lower prevalence than the global estimate and close to the lowest prevalence regions.

Seropositivities of 34.69%, with a Sabin–Feldman dye test, and 30.61%, with an indirect fluorescent antibody (IFA) test, were reported in cattle in the TRNC (23). Also, a prevalence rate of *T. gondii* tissue cysts as high as 36% was reported in sheep and water buffalo meats destined for human consumption (24). A prevalence rate of *T. gondii* as high as 25% was reported in a study on 101 stray dogs in three regions of TRNC (25). These reports indicated that toxoplasmosis is a severe problem in ruminants and canines in this country, facilitating transmission of the infection among mammalian species and humans. For this reason, countrywide studies should be conducted to determine toxoplasmosis's additional dimensions; hence, the ways of fighting the disease should be decided.

The prevalence of *Toxoplasma* IgM in Syrian refugees living in Kahramanmaraş in 2012 and 2013 was 4.76% and 4.84%, respectively. *Toxoplasma* IgG prevalence was 80% and 62.6%, respectively. In the same years, in contrast, *Toxoplasma* IgM rates were 1.96% and 2.34% in Kahramanmaraş's local population, whereas *Toxoplasma* IgG seroprevalence was 49.7% and 45.7% in 2012 and 2013 (26). Our results show a decline in the incidence of the disease in the region in the later years. The reduction in *Toxoplasma* prevalence is probably the outcome of the animal control strategy mentioned earlier in the discussion.

In the neighboring countries of Cyprus, *Toxoplasma* seroprevalence varies from country to country. *Toxoplasma* IgG seropositivity rate was 67.5% among pregnant women in Egypt (27), a prevalence higher than the current study's results. Many factors contribute to *Toxoplasma* prevalence rates, such as contact with cats and soil, place of residence (urban or rural), socioeconomic status, educational level, consumption of undercooked meat and contaminated raw vegetables, drinking raw milk, and consuming homemade cheese (28).

The rate of toxoplasmosis is lower in Northern Cyprus than in some countries, although the climatic conditions are preferable for the oocysts. In this study, the IgM rate and avidity were lower than in other studies. In this analysis, only 63.2% of patients who acquired infection finished pregnancy follow-up at the Near East University Hospital. High *Toxoplasma* IgG avidity was observed in nine of the 12 patients (75%) and was determined as a chronic infection. Marginal avidity occurred in two patients (16.7%), and low avidity occurred in one patient (8.3%). According to the hospital records, healthy babies were delivered as an outcome of the pregnant women's follow-up. The measures taken in some countries have reduced the seroprevalence of toxoplasmosis. In countries with low seroprevalence, cases of congenital toxoplasmosis will be low.

Conclusions

Toxo IgM reactivity was assessed by IgG avidity test in a specialized reference lab in Turkey to differentiate between acute and chronic infections. The study showed that Toxo IgG and IgM reactivities were 12.7% and 1.4%, respectively. Our retrospective data showed that the incidence of IgM decreased over the years from 2016 to 2018. Since the risk factors of toxoplasmosis have not been validated and discussed, further studies are advocated, particularly amongst pregnant women in TRNC, to determine the factors that increase the risk of *Toxoplasma* infections.

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