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EVALUATION OF TOXOPLASMA SEROPREVALENCE AND IgG AVIDITY RESULTS IN PREGNANT WOMEN IN NEAR EAST UNIVERSİTY HOSPİTAL

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CLINICAL MICROBIOLOGY AND MEDICAL

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THESIS APPROVAL CERTIFICATE

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DECLARATION

I am a master student at the Medical and Clinical Microbiology department hereby declare that this dissertation entitled "Evaluation of toxoplasma seroprevalence and IgG avidity results in pregnant women in Near East University Hospital" has been prepared by myself under the guidance and supervision of "Assoc. Prof. Dr. Kaya SÜER'' in partial fulfillment of the Near East University, Graduate School of Health Sciences regulations and does not to the best of my knowledge breach and law of copyrights and has been tested for plagiarism and a copy of the result can be found in the thesis.

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DEDICATION

I dedicate my thesis to my beloved parents

Pakiza and Hoshyar

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ABSTRACT

Objective: Toxoplasmosis is a protozoa infection caused by Toxoplasma gondii (T gondii), which is generally asymptomatic and can affect all organs. Serological methods are preferred in the diagnosis of T gondii because it is very difficult to show microscopically or to produce in culture. IgM antibodies in patients with acute toxoplasmosis may be positive in the serum for a long time. This leads to false diagnoses of acute infection. In the researches, the results of low avidity indicate that infection has occurred in the last 3-4 months and high avidity results have occurred at least 6 months ago. It is used in the differential diagnosis of acute and chronic toxoplasmosis when avidity tests are required. In this study, we aimed to determine toxoplasma seroprevalence and avidity values in pregnant women followed up in our hospital.

Material and Methods: Toxoplasma tests of pregnant women admitted to the gynecology outpatient clinic of Near East University Hospital between 2015-2018 were evaluated retrospectively. The results of Toxoplasma Immunglobuline M (Toxo IgM) and Toxoplasma Immunglobuline G (Toxo IgG) antibodies of 1348 healthy pregnant women were analyzed retrospectively from microbiology laboratory records. Toxo IgM and Toxo IgG antibodies were studied by ELISA (Abbott i1000). Toxoplasma Immunglobuline G avidity (Toxo IgG avidity) tests were performed by ELISA in pregnant women who required differential diagnosis of acute and chronic toxoplasmosis.

Results: In this study, the average age of pregnant women 29.03 ± 5.095, Cyprus 572 (42.4%), Turkey 746 (55.3%), and 30 in other countries (2.3%) were found to be nationals. In this study, Toxo IgM positivity was 1.4% and Toxo IgG positivity was 17.5% in pregnant women. Toxo IgG low avidity positivity was detected in only one pregnant woman.

Conclusion: In our study, Toxo IgG positivity in pregnant women; 17.5%, Toxoplasma IgM; 1.4% and Toxo IgG avidity; high avidity: 75%, border avidity:

16.7%, low avidity: 8.3%, as found. As a result of the study, Toxo IgG positivity rates were found to be low compared to many countries.

Keywords: Toxoplasmosis, pregnancy, seroprevalence, avidity

ÖZET

Amaç: Toksoplazmoz genellikle asemptomatik seyreden, tüm organları etkileyebilen, Toxoplasma gondii'nin neden olduğu protozoal bir enfeksiyondur. T gondii'nin tanısında mikroskopik olarak gösterilmesi veya kültürde üretilmesi çok zor olduğu için serolojik yöntemler tercih edilmektedir. Akut toksoplazmoz geçiren kişilerde ortaya çıkan IgM antikorları serumda çok uzun süre ile pozitif olarak saptanabilir. Bu durum yanlış akut enfeksiyon tanılarına yol açmaktadır. Yapılan araştırmalarda, düşük aviditenin sonuçları son 3-4 ay içinde enfeksiyonun ortaya çıktığını, yüksek avidite sonuçlarının ise en az 6 ay önce enfeksiyonun meydana geldiğini göstermektedir. Akut ve kronik toksoplazmozun ayrıcı tanısında avidite testleri kullanılmaktadır. Çalışmamızda hastanemizde takip edilen gebelerde toksoplazma seroprevalansı ve avidite değerlerini saptamayı amaçladık.

Gereç ve Yöntemler: 2015-2018 yılları arasında Yakın Doğu Üniversitesi Hastanesi Kadın Hastalıkları Polikliniği'ne başvuran gebelerin toksoplazma testleri retrospektif olarak incelendi. 1348 sağlıklı gebenin Toksoplazma Immunglobulin M (Toxo IgM) ve Toksoplazma Immunglobulin G (Toxo IgG) antikor sonuçları retrospektif olarak mikrobiyoloji laboratuvarı kayıtlarından incelendi. Tokso IgM ve Tokso IgG antikorları ELISA (Abbott i1000) ile çalışıldı. Akut ve kronik toksoplazmozun ayırıcı tanısı gereken gebelerde Toksoplazma Immunglobulin G avidite (Tokso IgG avidite) testleri ELISA ile yapıldı.

Bulgular: Bu çalışmada, gebelerin ortalama yaşı 29.03 ± 5.095, 572' sinin (% 42.4) KKTC, 746'sının (% 55.3) Türkiye ve 30'unun (% 2.3) diğer ülkeler uyruklu olduğu belirlenmiştir. Bu çalışmada gebe kadınlarda Toxo IgM pozitifliği % 1.4, Toxo IgG pozitifliği % 17.5 olarak saptanmıştır. Toxo IgG düşük avidite pozitifliği sadece bir gebe kadında saptanmıştır.

Sonuç: Çalışmamızda gebe kadınlarda Toxo IgG pozitifliği; % 17.5, Toxo IgM; % 1.4 ve Toxo IgG aviditesi; yüksek avidite:% 75, sınırda avidite:% 16.7 ve düşük Avidite:% 8.3 olarak bulundu. Çalışmanın sonucunda tokso IgG pozitifliği oranları bir çok ülkeye göre düşük olarak saptandı.

Anahtar Sözcükler: Toksoplazmoz, gebelik, seroprevalans, avidite

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LIST OF ABBREVIATIONS

TRNC	Turkish Republic of North Cyprus
NEU	Near East University
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgA	Immunoglobulin A
Ab	Antibody
Ag	Antigen
Toxo IgG	Toxoplasma Immunoglobulin G
Toxo IgM	Toxoplasma Immunoglobulin M
T. gondii	Toxoplasmosis gondii
PBS	Phosphate Buffer Saline
HRP	Horse Radish Peroxidase

CHAPTER 1

1. INTRODUCTION

1.1. Background and history of the study

Toxoplasmosis is an infection caused by a parasite with the protozoan Toxoplasma gondii (T gondii). The infection results in different types of proven syndromes in humans, environmental mammals, and many bird classes (Rayan, R., et,al. 2004).There has been progress throughout time about toxoplasmosis and they have been mentioned below in sequential order T gondii was first detected in North Africa from a rodent (Manceaux, N., 1909). Castellani in 1914, is believed to be the first to define T gondii organisms in human blood via a 14 year Ceylon child who passed away due to illness characterized by strong anemia, splenomegaly and fever (Dubey, J., 2009) The first human case of ocular toxoplasmosis testified by Janku in Prague, in 1923, the case was noticed in an infant of 11 months of age. Later on intrauterine spread was described in a new born baby by Wolf and Cowen in 1937. Toxoplasmosis can be deadly in adults found by Pinkerton and Weinmann in 1940. Asymptomatic persons were also part of the detection of T gondii cysts in 1945 by Kean and Grocott. Hogan, in 1951, established the first medical descriptions of ocular toxoplasmosis. Beverley and Beattie, in 1958, confirmed the above findings in 39 reported cases (Dubey, J., 2009). Beverly found regular inherited transmission in mice. Jacobs, Remington and Melton, in 1960, stated that meat of infected animals might be the source of contamination. Georges Desmonts, in 1960, started examining transformation of T gondii to women's fetus in Paris. Moreover prophylactic method of treatment was practiced on pregnant women with developed seroconversion. Years later, Frankel and his fellow experts showed that the experiences in the past were on felines not on humans, as Vietzke clarified the case as an infectious driver among individuals in 1968. It's important to mention that toxoplasmosis was first detected in 1950 in Turkey by Akcay and the first human case was identified by Unat in 1953 (Onul, B., 1980)

Two of the main forms parasite are tissue cyst and oocysts which play important role in the transmission. A study by Jones in 2003 shows that hot areas with low altitudes and moist environment helps to increase the infection of T gondii (Jones, J.,et,al. 2003).

Human Infection can arise via:

i. Tissue absorption cysts in uncooked meat
ii. Food absorption or polluted water with mature oocysts fecal-orally
iii. Mother to fetus transmission called transplacental or vertical transmission
iv. Donors can also rarely transmit the infection via needle stick wounds, blood transfusion or orange transplantation.

Approximately one in three people in the world may get infected by toxoplasmosis (Ayeh-Kumi, et al., 2010, Monotoya, J.,et al. 2004). Ayeh-Kumi claims that between 30% and 65% of people are already infected by toxoplasmosis (Ayeh-Kumi,, et al., 2010).

If unborn fetus gets infected with T gondii via placenta then inherited toxoplasmosis may happen. A type of transmission of T gondii can also occur between one to four months of maternal-fetal occurrence. They also have stated that there are opposing health problems, on infants and pregnancies, coming from inherited toxoplasmosis (Dubey, J.,et al. 2011, Stray-Pedersen, 1993).

Claim that inherited contamination creates risk through acute T. gondii in a rate starting from 20% and ends in 50% if a severe treatment is not taking place (Jones, J.,et al. 2003, Dunn, D.,et al. 1999, Stray-Pedersen, 1993). An article, describes that T. gondii mostly causes infection when it's gained congenitally (Torok, O.,et al. 2013).

Toxoplasmosis is transmitted to humans by oral ingestion of cysts in infected animal tissues or sporocysts in their extracts, produced by T gondii protozoa. Reticulo endothelial system, muscle, eye and brain tissue, especially in the formation of cysts in many tissues or manifests with acute infection. Toxoplasmosis is an infectious disease that can transplacental pass from infected pregnant to fetus, resulting in

congenital infection, anomalies and abortion (Desmonts, G.,et al. 1974).

Infections transmitted by the congenital tract can cause chorioretinitis, blindness, strabismus, hydrocephalus, microcephalus and cerebral calcifications in stillbirths of infants. It is thought that the transmission of infection from mother to fetus will almost always be possible if the mother is infected during pregnancy. Rarely, an immunocompromised woman with an acute infection 6-8 weeks before pregnancy can transmit the infection to the fetus. Therefore, it is important to diagnose acute infections using appropriate diagnostic methods and to investigate the fetus when necessary (Garcia, J., et al. 1999, Montoya, J., & Remington, J., 2000).

1.2 Geographic Distribution

Toxoplasmosis is believed to infect more than one billion people worldwide. Toxoplasmosis can be described as one of the widest infectious parasites in the world. The infections act the most when the environment is warm, moist and low in terms of altitude comparing to the cool climate in the regions where there are mountains. Toxoplasmosis infection reaches 50% in places like Asia, Africa, South Europe and South America. Due to uncooked and raw meat, France is known to be more common for toxoplasmosis infection compared to other European countries. Cats have caused more infection in Central America comparing to other parts of United States (Remington, et al. 2001).

1.3 Etiology

The seroprevalence reports of toxoplasmosis in various countries and continents differ significantly. Some variations are found when the genotypes of the parasite isolated from human and animal. Type I strain are often linked with inherited diseases in humans according to genotypical characteristics.

In clinical cases, Type II was largely excluded from instances of toxoplasmosis and

often correlated with recurrent disease reactivation and in 65 percent of AIDS cases, this type is removed. Type III strains are frequently isolated from animal cases. The variation is thought to be as a result of geographical, socio-economic and environmental factors such as hostage, genetic and immune status of the host. Among the other factors include parasite genotype, parasite load and stage of parasite development (Montoya, J.,& Remington, J.,2000). Such parasites are essential intracellular parasites, they are developed in-vitro media and need a living structure of organisms, for instance laboratory mice, embryonic eggs or culture of tissue (Furtado, J., et al. 2013).

According to the sort of host and therefore the duration of infection, T gondii is outlined in 3 totally different forms. These forms within the life cycle of the parasite are referred to as oocysts, tachyzoites and bradyzoites.

1.4.1 Oocysts

This parasite is originated in felines with the shape of an oval, thick and resistant wall and a measurement of 10x12 μm. An infected cat can steal up to 10 million oocysts a day (Dubey, J., et al. 2011). Oocysts that are not yet infectious when cat feces come out, become infectious as a result of sporulation in the presence of appropriate heat and humidity. If there is not enough heat and moisture, oocysts extracted with cat droppings are not contagious. Sporulation time depends on the temperature and oxygen of the environment. Sporulation was not shown to be less than 4 ° C and higher than 37 ° C (Kuman, A.,2002). The oocyst comprises 2 sporocysts, each of which produces 4 sporozoites. In moist soil, mature oocysts can last for 18 months. Such oocytes are contagious and play a role in spreading the infection.

Tissue cyst (bradyzoite) and parasite taken tachyzoite , invasion of the cat bowel epithelial cells, and first asexual reproductive behavior by splitting it into two merozoites (schizogony), which evolved at the end of this period. Zygote formed by microgamete and macrogamete immature oocyst fertilization is produced by feces

(Torok, E., et. al.2013),(Töre, O.,2001). Every natural oocyst is transferred to all vertebral bodies, in particular herbivores, the oocysts enter the digestive tract and the released sporozoites first propagate in the intestinal epithelium and then disperse parasitemia to the entire body. Tissue cysts form and the parasite get dormant after this period when acute toxoplasmosis develops. As cats consume tissue-cyst food (birds, rodents, and herbivores), the intestinal cysts and therefore the normal T gondii cycle of life release.

1.4.2 Tachyzoite

It is a rapidly growing, aggressive and somatic type of parasite, classified as trophozoites and endozoides and is present in the acute infection cycle. The type tachyzoites is oval or fluffy, 2-3 wide and 5-7 long and needs an intracellular environment even if all eukaryotic organelles are required for reproduction. In the cells of the infected vertebral tachyzoites, vacuoles can cause the infection of all types of cells and can form a rosette every 6 to 8 hours (Dubey, J.,1998), (Radke, et al. 2001). The host cells then erupt into the environment, infecting new cells to create fake cysts or tissue cysts. Giemsa and Wright stain are well colored. Microscopy of electron shows a highly developed organelle structure (Kuman, A.,2002). It was isolated from human milk, saliva, urine, seminal and vaginal fluids and tears. Trophozoites have been shown to survive 4-7 days in these fluids and are transmitted by mucosal surface with 10 trophozoids (Foch B., et al. 2000).

1.4.3 Bradyzoites

Upon colonization of the intended cell, tachyzoites produces a tissue cyst called a bradyzoite. Tachyzoites proliferate rapidly to shape the rosette at the same time, which contributes to cell disruptions. Bradyzoites, on the other hand, develop slowly growing, tissue cysts. Tissue cysts produced by goal tissue tachyzoites remain divided during development and the amount of tissue cysts grows slowly. The young

and old tissue cysts of 20-200 micron diameter, with more or less bradyzoite number, can be observed depending on the period of the infection. The Wright, Giemsa, Gomori's Methamine Silvering and Immunoperoxidase are very well colored tissue cysts with different dimensions. Throughout histological parts already done on the 8th day of infection, tissue cysts can be seen. When the tissue cyst is found in the brain, the tissue cyst is spherical and adapts to the shape of the heart's and skeletal muscles. Even if tissue cysts can be found in every organ, the chronic phase of infection is most common in the brain, the skeletal muscle, and heart muscle. It is somewhat immune to stomach acid and other natural factors, so the main source of infection is fresh or uncooked beef or other types of red meat. Infected meat tissue cysts are not likely to survive by heating up to 67 degrees, gamma irradiance (0.4 kGy), freezing and thawing at –20 degrees Celsius but are never dead from heating in a micro oven (Montaya, J.,& Remington, J.,2000).

1.5 Evolution between form

The last host cat and every living organ that can be tainted with toxoplasma is present in trophozoites and bradyzoites of T gondii. The proliferation of the parasie sporogony (sexual proliferation) takes place in Felidae family only. The main organ is the host animal. The cat may be infected with any form of toxoplasma gondii from the digestive tract. T gondii reach the small intestine epithelial cells through consuming rodent, mouse and worm. Scale of 10-16 merozoidal agents finishes schizogony (asexual proliferation) in the small intestinal epithelium. As a product of oocysts there is sporogony (sexual proliferation). Gametocytogenesis in 3–15 days is used to create macro-gametocytes and microgametocytes by oocysts. Macrogametocytes and microgametocytes develop and evolve into macrogametes. Zygote is formed as a result of macrogamete fertilization by microgamete. Zygote is immature oocysts in 4 days on average and is excreted first in the digestive cavity, then excreted with the feces. In the first instance, oocysts are two sporoblasts, and in 1–5 days, sporozoites of 4 haploids. In about three weeks when the cat is obtaining mature oocysts through the digestive tract, the excretion of immature oocysts takes

1-2 months. A contaminated cat can produce 10^7 – 10^9 oocysts each day in an intense phase.

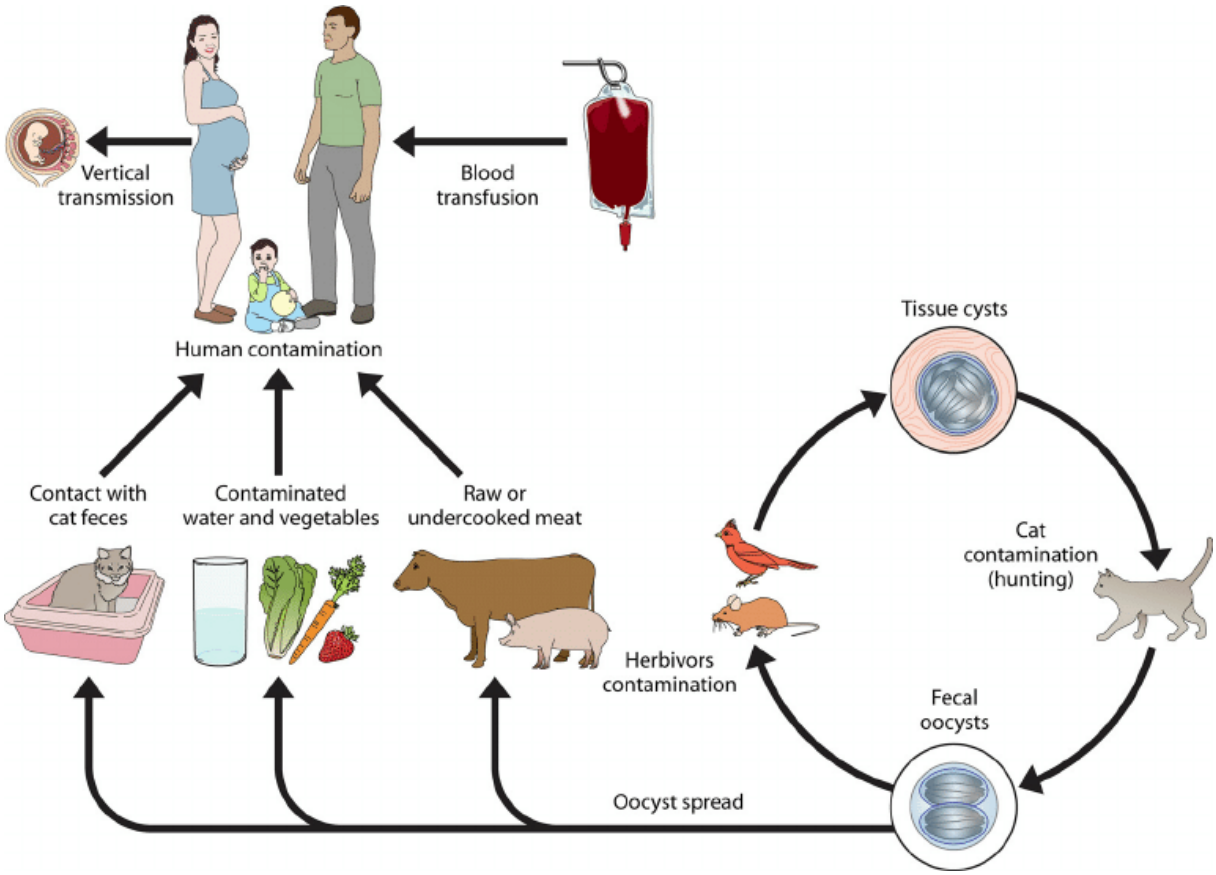


Figure: 1.1 Toxoplasmosis transmission .

www.betterboxllc.com

1.6 Problem Statement

Ayeh-Kumi, et al. (2010) reported that one third of the world population will be affected. According to Jones, J., et al.(2001) Toxoplasma is measured to be one of the three leading infectious disease after listeriosis and salmonellosis. Garweg, et al. (2005) and Liesenfield, O.,et al.(1997) reported that, inherited toxoplasmosis has opposing health problems on pregnancies and newborns. Another issue is that pregnant women don't get the screening process for whatever reason, which could be due to neglecting the importance of it.

1.7 Aim of the study

This study was useful to determine the seroprevalence of toxoplasmosis in pregnant women in Near East University (NEU) Hospital. It was showed the level of necessity for informing the society in terms of infection risk.

(a) Detection of the prevalence of toxoplasmosis seroprevalence at NEU Hospital

(b) Provided information on the rates of abortus and sequelae births.

(c) Ensure the assessment of measures to be taken in terms of Public Health.

1.8 Research Scope

This study was carried out with a purposive random sampling of pregnant women attending NEU clinic in the city of Lefkosa in the Northern Cyprus. The study covered a period of Four years from 2015-2018.

1.9 Significance of the study

This study investigated the risks of pregnant women in terms of toxoplasmosis and reveal the necessity of the necessary precautions. In this study of seropositive rates in dogs related to toxoplasmosis in the TRNC, the data was helpful in determining the effect on pregnant women.

1.10 Research question

What is the risk of toxoplasmosis in the TRNC in terms of public health, especially in pregnant women?

CHAPTER 2

2. LITERATURE REVIEW

2.1 Forms of T gondii

There are three main forms of T gondii. Torok,,O.,et al. (2013) claims that the forms include:

i. Oocysts: they are communicable and sporozoites

ii. Tachyzoites: nonsexual type accountable the attack of cells, semicircular shaped and are present while acute phase of the contamination which occupies every mammalian cell type, except non nucleated RBCs.

The tissue cyst/Bradyzoites, consist of intracellular trophozoites also grow in the cytoplasm of host.

Cyst and oocysts are the two key parasite types which are believed to be responsible to transmit the infection.

2.2 Transmission of T. gondii

T. gondii is the global zoonosis, which can kill almost everyone and everything. It is located more in hot and humid areas than in cold and dry places because of the life-cycle of the parasite. Cats, which are the definitive hosts of T. gondii, become infected by ingesting the sporulated oocysts or in some cases infected animals like rats or mice (Baron, S.,1996). The oocysts are highly contagious to most mammals, including humans and birds. Infection of T. gondii spread by one of the following four known routes.

Below are the most usual cases of human infection;

- Raw red or undercooked meat, including the cysts.
- Drinking water and consuming water or food that is infected with oocysts.
- Also from infected pregnant women to the fetus.

Least common transmission paths;

- Contaminated organ been transplanted
- Dogs as vector play a role in transmission
- Transfusion blood from an infected person
- Infected needles getting touched with flesh
- Via exposing wound and damaged skin
- Insects can also be a source of transportation of the disease (flies, cockroaches, worms, and slugs) (Montaya, P,&Remington, J.,2000).

Epidemiological scholars have shown that cats are mainly essential for the transmission of parasites in many parts of the world. Tissue cysts are highly prevalent in human-consuming food. Around 1% of cats have been identified in excreted oocysts in various parts of the world (Töre, O,. 2001).

2.3 Foodborne contamination

Can be transferred to humans through food contaminated with oocysts of the parasite Eating raw meat or selfish (oyster , mussel) infected with T gondii .it may possible to cause oral exposure by not washing hands when touch raw or uncooked food or selfish

In the spread of toxoplasmosis, cats play the most important role. Cats transmit oocysts for three weeks following contamination of their feces. The infected cat makes its feces in the cat litter and may cause the owner to become infected. It may

contaminate the ground or water with its feces if the cat is permitted to go outside. A woman infected by T gondii may transmit the infection to the unborn child (congenital infection) via placenta during pregnancy. The pregnant female may have no signs but may have significant consequences, such as infections of the nervous system and skin, to unborn children. Neurological, neurocognitive and chorioretinitis findings may be detected in infants with congenital toxoplasmosis.

2.4 Morphology of T gondii

Three major stages of infectious Toxoplasma gondii exist. Tachyzoites, bradyzoites (tissue cysts) and sporozoites (oocytes) occur at different stages of the disease (Dubey,J.,1998). They are all highly infectious to humans. T gondii consisting of sporozoites and tachozoites in terms of their appearance, cell inclusions and organelles with a similar number of rhopters, they are very similar in all three forms (Baron, S,.1996). Bradyzoites differ in structure from tachyzoites according to studies by (Dubey, J,.1998). At the rear end is located the nucleus of bradyzoites, while at the central end of the nucleus is tachyzoite. In addition, rhoptry contents are labyrinthine in tachyzoites, while bradyzoites are often electron-dense (Kwofie, k,. 2012).

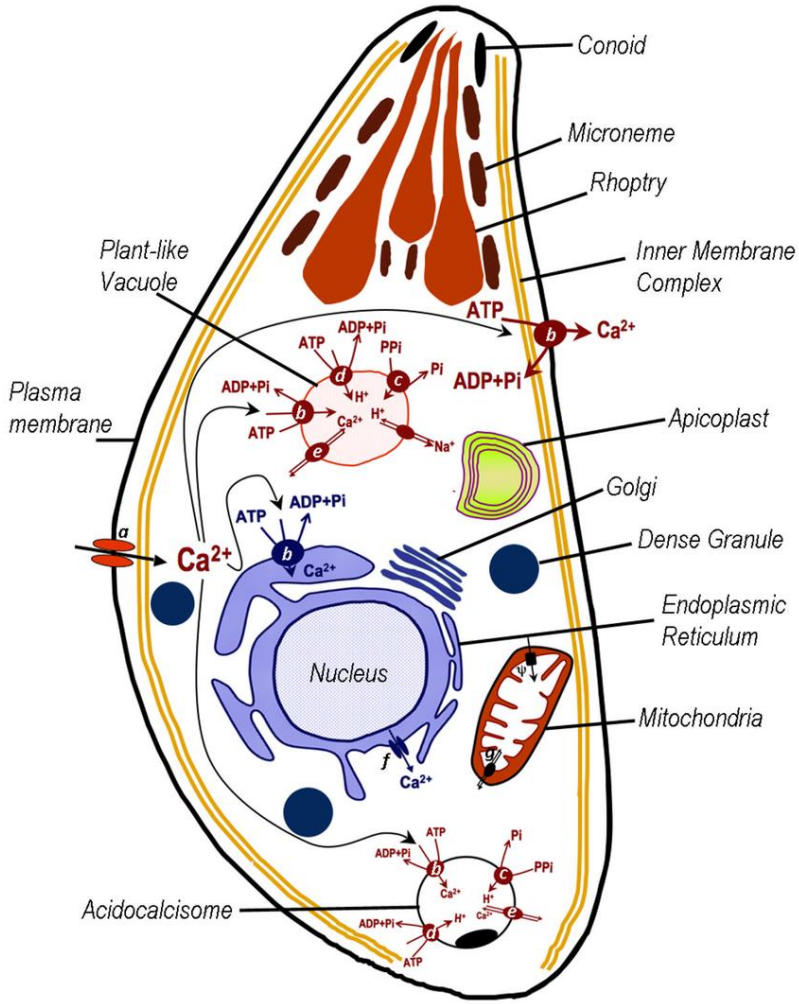


Figure: 2.1 Ultrastructure of Toxoplasma gondii as seen under the electron microscope. Source: Baum, J. et al.2008.

2.5 Life cycle of T gondii

The authoritative host, like domestic cats, has now been identified in Toxoplasmosis as representatives of the Felidae family. According to Baron (1996), T gondii was enacted in 1970. Different hospitality is offered by other hot homeothermic-blooded animals including humans and birds. T gondii infection of domestic cats (Dubey, j,. 2011). The parasites persist and move into the stomach where they infect cat's epithelial cells. The parasites then develop into sexual activity and replicate several

oocytes. The soil is contaminated with oocysts spilled by cat's faeces. Thus, the host becomes infected by consuming vegetables produced in contaminated soil. Likewise, contaminated water resources can cause the host to become infected (Dubey, J., 1998).

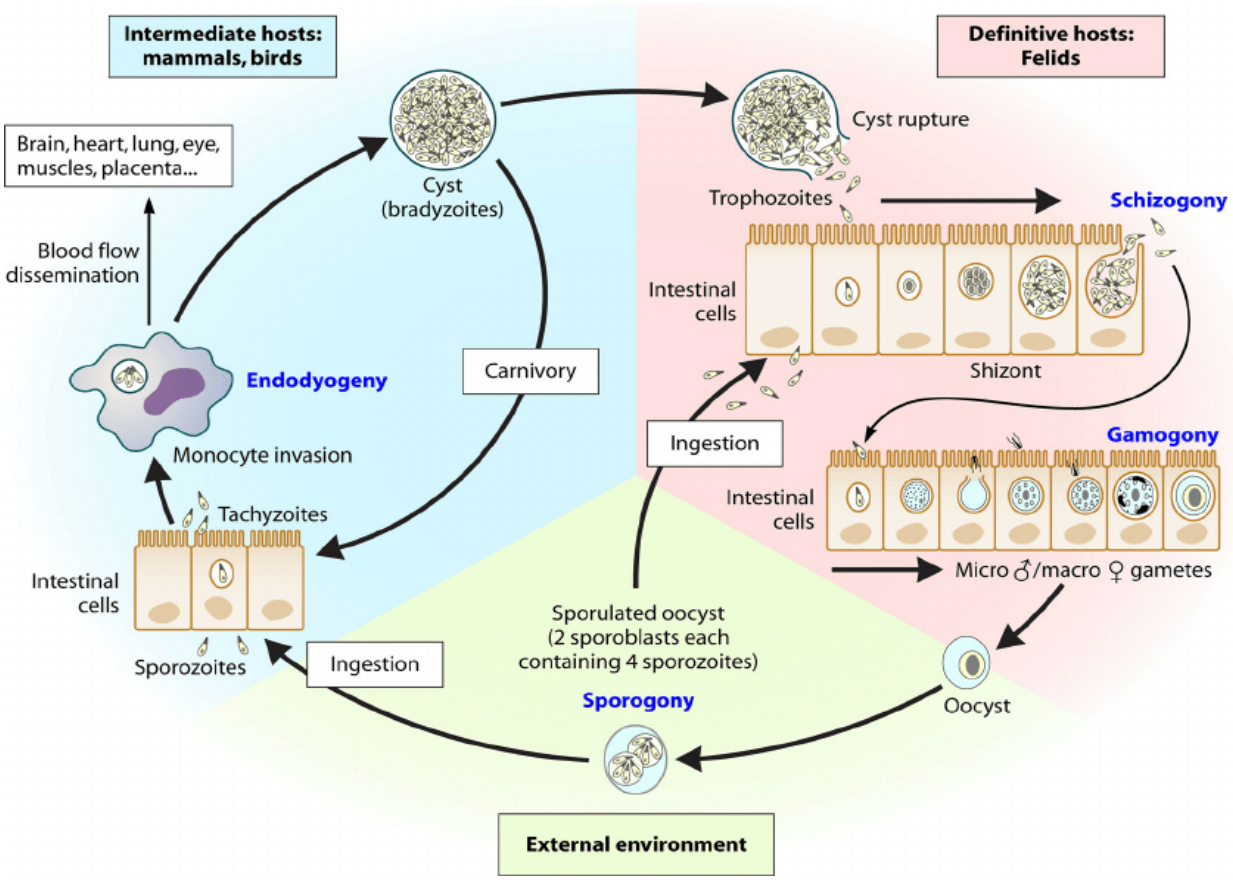


Figure: 2.2 Life cycle of T gondii

Source: Baum et al. 2006

Human beings are contaminated with unclean vegetables or polluted water or cat litters. A while after ingestion, oocysts convert into tachyzoites. These tachyzoites localize in the neural and muscle tissue, and grow into a tissue cyst called bradyzoites, containing the cysts of the tissue. Ingest crude or poorly cooked meat that contains the tissue cyst results in human infection by the parasite (Baron,S., 1996).

2.6 Pathogenesis of toxoplasmosis

The peculiar genotypes for congenital toxoplasmosis are more extreme than that attributed to typical genotypes. Some infants suffering from a more severe congenital infection appear to experience a toxoplasma antigen, which may be important for their disease's pathogenesis. In congenitally infected infants, immunoglobulin G (IgG) monoclonal gammopathy has been described and IgM levels may increase in congenital toxoplasmosis newborns. Among congenitally infected people, glomerulonephritis was identified with deposits of IgM, fibrinogen, and Toxoplasma antigen (Hokelek, M., 2019).

Congenital toxoplasmosis occurs as a result of transmission to the baby during pregnancy. Consumption of water and food contaminated with faeces of infected cats leads to the development of toxoplasmosis. The host cell is engaged and finally destroyed during incubation from 5 to 18 days by active cell propagation. (Dubey, 1998) . Stray-Pedersen (1993) and Dunn et al. (1999) reported that a more severe form of congenital toxoplasmosis involves retinal infection and causes visual impairment chorioretinitis. Tocoplasmosis seen in congenital toxoplasmosis and mainly in immunocompromised patients can cause brain damage (Dunn, D,. 1999) .

2.7 Survival Mechanisms of T gondii

Toxoplasma gondii has shown to trigger the trophoblast cell apoptosis, a single-cell parasite commonly found in animals, and ultimately inflict fetal harm and abortion. Dense granule protein 15 (GRA15) is a key ingredient in the innate immunity to Toxoplasmosis. However, host-cell apoptosis appears unclarified in T gondii infection and its pathogenesis.

T gondii experiences other mechanisms to prevent human disease and eventually host immune system damage following unauthorized entry. An example is the use of plasmids to invade the host immune system (Henrik, V,. 1999).

This helps the immune system of the host to withstand cell death. The application of

the anti-apoptotic mechanism is a T gondii mechanism against host immune system to escape apoptosis (Hippe, D., 2009). They also note that the pro-apoptosis effector proteins such as Bak and Bax are being affected. When these proteins are disrupted, toxo pro-apoptosis effector proteins have changed shape and structure of T gondii. This results in the inability of proteins to be moved to the host cells and apoptosis is initiated during this process. Another mechanism used by T gondii is host cell autophagy. This is because of toxoplasmosis ability to autophagy inhanced . This reduces host cell volumes, reducing the ability of the host immune system to kill T gondii (Wang, Y., 2009).

2.8 Epidemiology of T gondii

Toxoplasmosis is regarded as the third largest infectious disease that causes eat-borne deaths following the tradition of listeriosis and salmonellosis (Jones, J., et al 2003, Dubey, J., et al 2011).

The variation is thought to be the result of geographical, socioeconomic, and environ mental matters such as the host age, genetic status, and host status (Furtado,J., 2013). Transmission of mother-fetal T gondii occurs within one to four months of placenta colonization by Dubey,J,. et al. (2009) and Stray-Pedersen (1993) as reported. This has a negative health effect on pregnancies and newborns (Garweg, et al 2005, Liesenfeld, O.,et al 1997). Furtado et al. (2013) research stated that, as pregnancy progresses, the likelihood of mothers-to-children transmission rises. Infection with Toxoplasma gondii gained during early pregnancy is more likely to lead to clinical conditions. In addition, the risk of congenital acute toxoplasma infection is estimated (Jones, J,.et al 2003, Stray-Pederson 1993, Dunn, D,.et al 1999). In cases of toxoplasmosis detected during pregnancy, congenital toxoplasmosis can be seen between 20% and 50% if treatment regimens are not compatible (Dunn, D,. 1999). Estimates in France, for the period 1987 to 1995, that the risk of mother-to-child transmission was 6% at the age of 13 weeks, 40% at 26 weeks and 72% at 36 weeks. In the case of fetuses and neonates, congenital toxoplasmosis can cause severe to fatal sequelae.

2.9 Clinical manifestations of toxoplasmosis

Toxoplasmosis can be defined as acute and chronic form. Asymptomatic form is usually seen in the course of the disease. The immune system is generally asymptomatic in healthy adults and children. In patients with clinical findings, no specific examination findings are detected. The symptomatic finding is 20% lymphadenopathy in people with normal immune system. The symptomatic finding is 20% lymphadenopathy in people with normal immune system. Fever, night sweats, muscle pain, sore throat, maculo papular rashes, hepatosplenomegaly, abdominal pain may also be seen.

Toxoplasmosis may be fatal in immunocompromised individuals. The causes of mortality are central nervous system involvement, myocarditis and pulmonary involvement, respectively.

In immunocompromised individuals, chorioretinitis is usually subclinical. It can rarely cause sudden vision loss and glaucoma. The clinical course is more severe in immunocompromised people. Retroorbital examination shows multifocal or bilateral necrotizing lesions and vitreous involvement.

In pregnancy, the expectant mother is usually asymptomatic. The symptoms in the baby vary according to the month of pregnancy. The rate of transmission to the fetus in the first trimester of pregnancy is around 10-25%. This rate is between 30-54% in the second trimester and 60-65% in the third trimester. As the gestational week increases, the risk of transmission to the fetus increases, while the damage to the fetus decreases. T gondii contamination threatens fetuses and newborns in the time of pregnancy. The incidence of reactivation increases in individuals receiving high doses of immunosuppressive therapy such as malignancy and connective tissue diseases or in pregnant women infected with HIV (Remington, J,.et al 1995, Mitchell et al 1990). T gondii that can be spread for disease to the fetus. According to a Montoya and Remington study (2010), this could cause severe encephalitis, myocarditis, hepatitis or pneumonitis. Congenital intracranial calcification, microcephalus, hydrocephalic convulsions and severe intrauterine growth restrictions also include clinical manifestations of congeal toxoplasmosis (Dubey et al 2011).

Comprehensive studies in neonates conducted by Di Carlo et al. (2008) and Brown et al. (2009) have shown that delays in treatment of toxoplasmosis can lead to severe sequelae, including neurological and mental impairment.

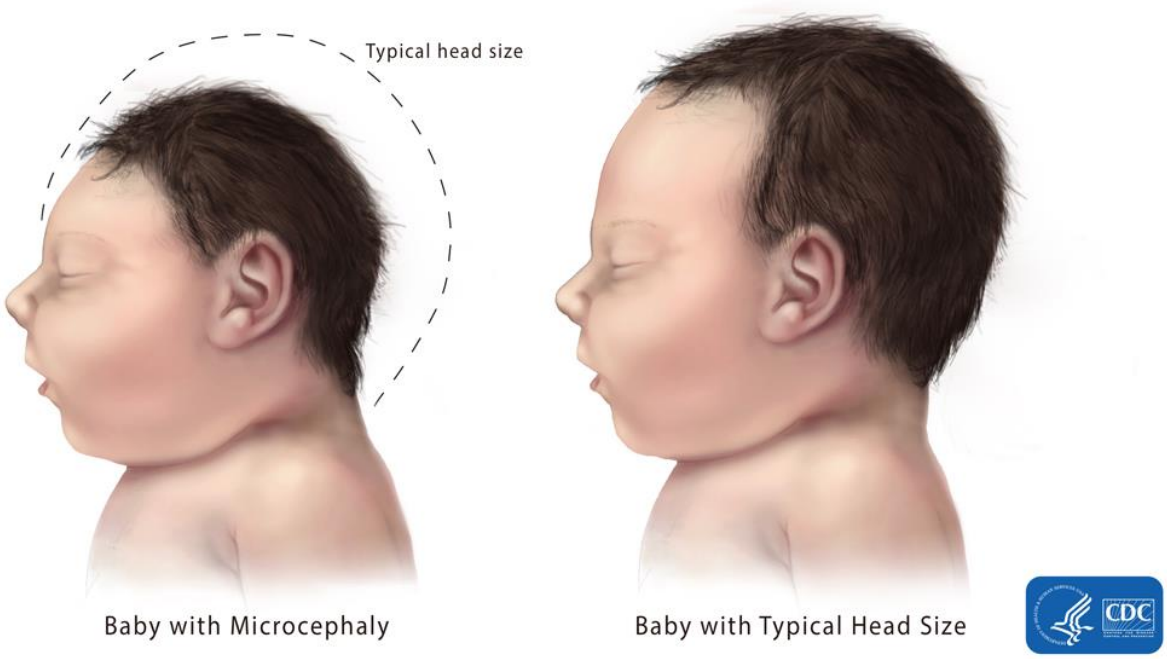


Figure: 2.3 microcephalus. Source: CDC, 2013

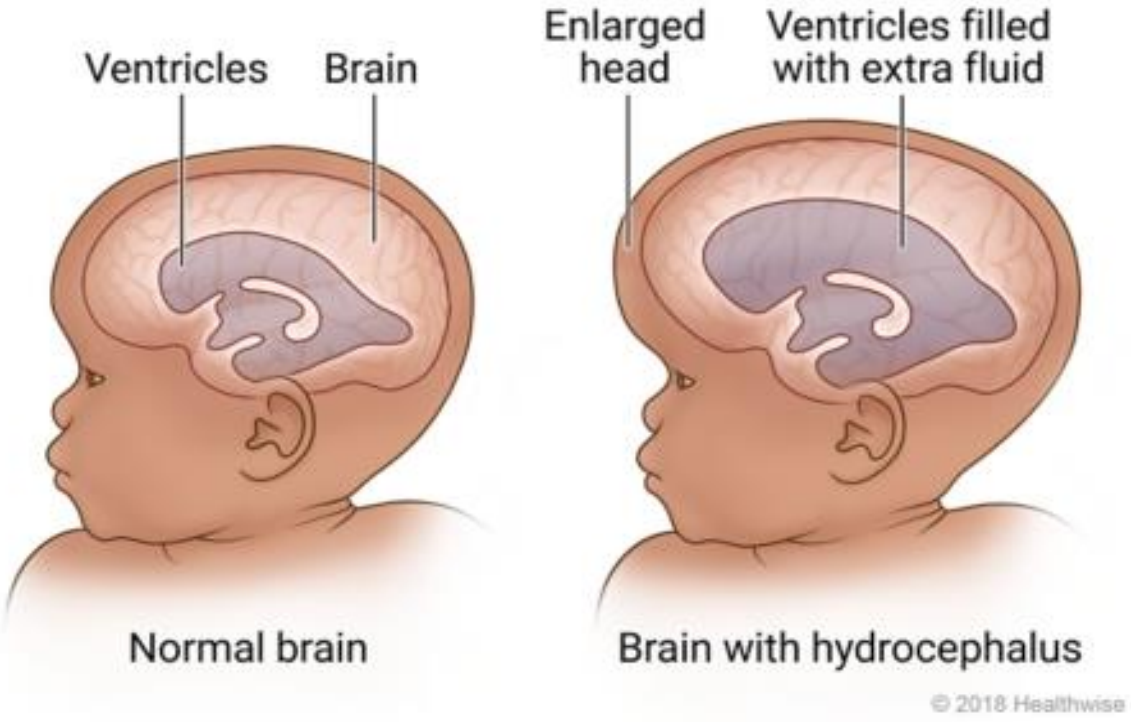


Figure: 2.4 Hydrocephalus: Source: Parvaneh, 2012.

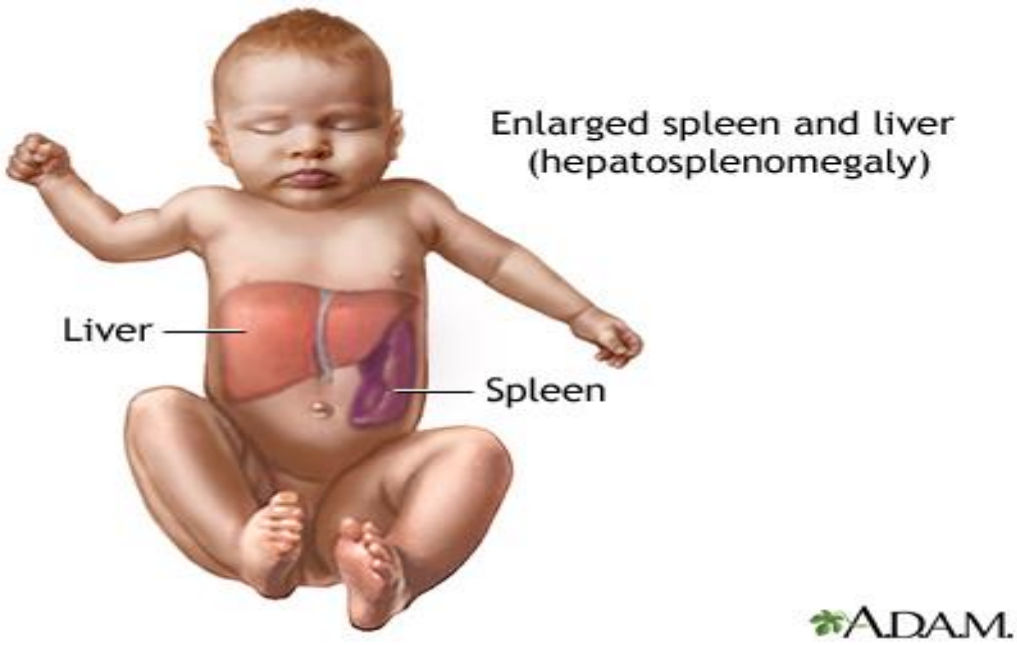


Figure: 2.5 Hepatosplenomegaly

Source: A.D.A.M. 2017

Figure: 2.6 chorioretinitis: Source: Smith, JR, 2002.

It is vital to realize that T. gondii is due to the possibility of determining the risk of congenital infection and of ensuring adequate treatment of the parent and child in the childhood. High mortality and maternal morbidity of the first quarter gestation is often due to serious medical conditions, such as severe neurological impairment or fetal death (Thiebaut, R., et al. 2007).

Disease later in the second or third quarter will most certainly be asymptomatic at conception, usually leading to slightly less severe infant and subsequent child injury (Moncada & Montoya, 2007). This disease may be more prevalent in pregnancy. In order to begin relatively efficient anti-parasite therapy, quick and accurate diagnosis is necessary (Stray-Pederson, 1992). The toxoplasma involvement of IgM is disadvantageous because it can continue for years much more than is usually defined and therefore cannot be used as an acute stage marker (Bobic B.,et al. 1991). This is an issue because fetuses are mostly transmitted to women who get acute infection during pregnancy, as previously identified (Liesenfeld, et al., 1997). This is a problem. This in convent has led several authors to develop an assay to detect the infections in the toxoplasma of the patients, called the IgG avidity test, described by Hedman in 1989 and which is mainly based on the differences found in the union forces which arise in the interaction between the antigen-antibody. Nevertheless, IgG anti-T.gondii antibodies of low eagerness are developed in the earliest stages,

showing high eagerness in the chronic process (Hedman et al. 1989). The analysis of IgG avidity ELISA consists of an immunoenzyme assay that is used to isolate a destabilizing agent of hydrogen bridges such as urea and thiocyanate between a particular IgG and antigen, in order to almost fully dissociate low-avidity IgGs from the antigen-antibody network in recent infections, whereas high-avidity persistent inflammations are most frequently seen Antiquities gondii. (Figure below)

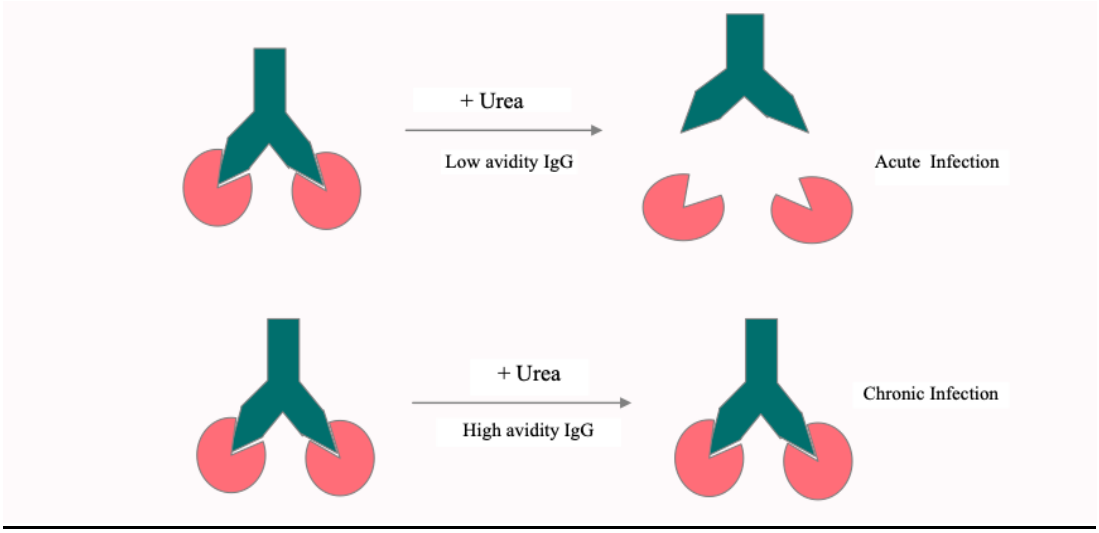


Figure: 2.7 Diagram showing the characteristics of a test of low avidity IgG and another of high avidity IgG.

2.10 Toxoplasmosis in Meat animals

The major economic importance of the disease for meat animals and for people as consumers is often considered to be an issue of medical importance and public health concern. Toxoplasmosis transmission was suggested by this way. (Fayer, R., 1981). Tissue cysts were distributed between carnivores through T gondii transmission. The T.gondii is relatively higher for herbivorous and omnivorous meat. Studies by Arko-According Gilbert,G.,(2002). showed that, in ingestion of raw or poorly cooked food contaminated with cyst contamination resulted with increase infection in population.

2.11 Global Seroprevalence of toxoplasmosis

Studies showed that T gondii have severe health consequences on pregnancies or newborns (Garweg, j.,et al. 2005, Liesenfield, O.,et al 1997). Claim that inherited contamination creates risk through acute T. gondii in a rate starting from 20% and ends in 50% if a severe treatment is not taking place (Jones, J., et al. 2003, Dunn et al. 1999, Stray-Pedersen, 1993). Recorded a low risk of congenital toxoplasmosis transmission of acute maternal infections in the first trimester (Remington J.,et al. 2001, Dunn, D.,et al. 1999). Toxoplasmosis studies showed that seroprevalence rate is different at geographically (Jacquire, P.,1995). It is estimated that nearly one third of the global population are affected by toxoplasmosis (Ayeh-Kumi, et al. 2010, Montoya, J., and Liesenfeld, O., 2004). Ayeh-Kumi, et al. (2010) also reported that toxoplasmosis infects between 30% and 65% of the world population. It is estimated that global seroprevalence is 46.1% (Jacguire,P. 1995). According to Partisani, (1991) in Europe, Africa and Latin America the seroprevalence of latent toxoplasma infection is measured at 75% to 90%. The Seroprevalence of toxoplasma infection in El Salvador was estimated high by Montoya and Liesenfeld. Increased mortality of toxoplasmosis has been reported in immunosuppressed people, such as people with HIV / AIDS and pregnant women (Dupont, C., et al 2012). In a study, seroprevalence of toxo IgG in ethiopia was 81.1% (Gebremedhin, E., et al. 2014). In a study investigating the seroprevalence of Toxoplasma IgG antibodies in Nigeria, 40.8% positivity was found (Akınbami, A., et al 2010). In the study conducted in the USA between 1999 and 2003, the seroprevalence was found to be 10.8% in 11 million women between the ages of 15-44 at childbearing age (Jones et al 2003). In a Brazilian study, Toxo IgG seroprevalence was found to be increased in women of childbearing age (Garcia, J., et al 1999)

2.12 Diagnosis of toxoplasmosis

Typically, serological tests are used to diagnose toxoplasmosis. Serological antibodies against toxoplasmosis are investigated to determine whether a person is infected. Investigating toxoplasmosis in pregnant women is of particular importance.

The tissue cyst form of the parasite can also be demonstrated microscopically as a result of staining of biopsy materials with Giemsa or Sabin Fieldman. However, these techniques are less widely used because these examples are difficult to obtain. Molecular techniques can also be used to detect T gondii DNA. CDC recommends screening with serological methods for the risk of congenital toxoplasmosis in pregnant women (CDC, 2013).

2.13 Serological Detection of T. gondii

It includes the detection of T gondii In the serum of infected patients antibodies. Serological methods used in the diagnosis of toxoplasmosis are listed below: Sabin-Feldman Dye test, Indirect Hemagglutination test, Indirect Fluorescent antibody test (IFA), Direct agglutination test (DAT), Latex agglutination test (LAT), Enzyme Linked Immunosorbent assay (ELISA) and Immunosorbent agglutination analysis test (IAAT). Toksoplazmoz tanısında en etkili ve tercih edilen yöntem ve dolayısıyla altın standart Sabin-Feldman Boya testidir (Hill et al 2002). Ancak günümüzde moleküler yöntemler altın standard tanı yöntemi olarak kullanılmaktadır.

2.14 Histologic Detection of T gondii

The diagnosis of toxoplasmosis can be histologically diagnosed by biopsy or necropsy of the host tissue. It may be preferred to use this method, especially in immunocompromised patients (Baron, S.,1996).

2.15 Molecular detection of T gondii

Acoording Kwofie, K,.(2012), DNA identification of t gondii in biological samples is part of this procedure. By isolation of DNA from the sample, the DNA molecule is identified by Polymerase Chain Reaction (PCR). PCR is best suited to immunodeficiency.

2.16 Treatment of toxoplasmosis

Spiramycin and Pyrimethamine are the main medicines of choice widely used for human toxoplasmosis. Spiramycin, a macrolide antibiotic, is the drug of choice in pregnant women for the treatment of toxoplasmosis (Montaya, J., and Remington, J.,2000). Primethmamine and sulfodiazine may be preferred in non-pregnant patient groups (Caroline, P., et al 2013). However, because of the potential teratogenicity of pyrimethamine and sulphonamide therapy should not be administered in a first trimester of pregnancy (Montaya, j., and Remington,J.,2000). A combination of folic acid is added to prevent thrombocytopenia and leukopenia which may develop during sulfonamide and pyrimethamine treatment (Baron 1996).

2.17 Prevention of toxoplasmosis

Despite the complexity of the route of transmission of toxoplasmosis, improved hygiene practices can be used to prevent this. The basic principle of the prevention of toxoplasmosis depends on the prevention of contact with the oocysts found in the environment (Lappalainen, M.,2004). Institutionalization of education and public health programs can reduce toxoplasma infection, especially in pre-natal care (Fabiana, M.,et al. 2007). These programs include simple rules such as the use of gloves when cleaning domestic cat litter, and the proper washing of hands after contact with raw meat (Hill, D., et al 2002).

Pregnant women and immunosuppressed patients should avoid contact with such soil, cat, raw meat or unpasteurized milk products (Fabiana, M., et al. 2017). Vegetable consumption should be used by cooking above 67 ° C. It is recommended to store meat and meat products at -20 ° C. Meat consumption should be well cooked (Hill, D.,2002).

2.18 Antibodies

Antibodies are made of light chain and heavy chain proteins, which form a structure shaped like an Y. While the base of the y-form structure is retained and therefore similar to all antibodies, each antibody is distinguished by its tips in the forks of the Y-shaped structure (Selamawit, D.,2004). The tips are antigen-based, while the preserved region is immune-based (Litman G., et al.,1993). In response to antigenic stimulation, antibodies are generally secreted and therefore represent approximately 20% of plasma protein (Selamawit, D., 2004).

2.19 Types of antibodies/Immunoglobulin

Five main types of antibodies in the body are developed, they are:

2.19.1 IgG:

According to Selamawit, D.,(2004) IgG is the most ample immunoglobulin that is unseen in the body and forms around 80% of the entire antibodies It spreads more easily in additional vascular areas in relation to other immunoglobulin and neutralized toxin while it binds to the extra-vascular areas of microorganisms. Thus, it is the only antibody that can pass the placenta in humans where it gives the fetus and newborn immunity (Pier et al. 2004). In the first 6-12 months of life the infant receives protection immunity against infection, while the baby's own immune system matures. IgG is capable of preventing systemic infection spread.

2.19.2 IgM:

It forms of about 10% of all blood serum immunoglobulins, according to .Plasma cel ls are synthesized in primary infections at early stage to protect the spread of pathoge ns in the early stages of infection (Pier, et al., 2004).

2.19.3 IgA:

It constitutes nearly 20% of all blood vessels (Selamawit, D.,2004). Where IgA is secreted are listed below: serum, tears, sweat, milk, colostrum, saliva, and other mucosal surfaces. (Pier, G.,et al., 2004). In the early life stages of newborns, pathogen microorganisms are prevented from passing through the gastrointestinal tract due to IgA in breast milk. (Selamawit, D., 2004).

2.19.4 IgD:

This constitutes less than 1% of all immunoglobulins through monovalent immunoglobulin (Selamawit, D.,2004). The lymphocyte occurs on the surface of B-cells and joins monomeric IgM. It renders it antigen receptor for basophils and mast cells to generate antimicrobial factors(Geisberger, G.,et al. 2006).

2.19.5 IgE:

The monomer contains 0.004% of all serum immunoglobulins (Selamawit,D., 2004). It was reported that IgE molecules, particularly mast cells and basophils, bind to allergens and to tissue cells. IgE receptor reactions contribute to allergic reactions, for example asthma, hives, and hay fever.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Study site

This study was conducted as (retrospectively data collection). demographic data of pregnant applied from Department of Obstetrics and Gynecology outpatient clinic at the Near East University Hospital and attended in Clinical Microbiology Laboratory in Near East University Faculty of Medicine Hospital in the city of Nicosia in Northern Cyprus.

3.2 Subjects

This study was conducted between 2015 and 2018 at the Near East University Hospital Medical Microbiology Laboratory. The sample composed of 1348 women aged between (29.03±5.095) 17 to 51-year-old pregnant women visiting the hospital. Inclusion requirements for the study participants had to be met.

3.3 Nature of the Study

There are Three main possibilities for a study

Quantitative research: this is the type of research which aims to measure or quantify data and also to compare data with previous data and sometimes makes future projection.

Qualitative study this is the type of research which requires just observation data coll ection, evaluation and explanation. It type of investigation is a little exploratory open. Information or data collection tools include discussion and interviews by the focus gr oups.

Mixed methodology analysis: this research type merges qualitative and quantitative research designs in data collection, analysis and explanation.
It strives to eliminate the weakness of both methods.

-The research design of this study is quantitative method.

3.4 Data Consideration and Source

In gathering data for this study, primary data and more specifically first-hand data or information was obtained by the researcher conducting the study. Blood samples were obtained from every pregnant woman who was followed up for pregnancy.

3.5 Study Population

This study included pregnant women in mean age (29.03±5.095) 17-51 ,who were followed up at the Near East University Hospital in Northern Cyprus.

3.6 Sample size

In Near East University Hospital Laboratory a total of 1348 blood samples from pregnant women were taken .

3.7 Research Design

Explanatory research design is used to determine the seroprevalence of T gondii infection in pregnant women attending Near East Hospital.

3.8 Blood sample collection

Each participating pregnant woman was aseptically drawn to serum separator tubes approximately 5ml of venous whole blood. The blood will then be isolated on numbered cryotubes and processed at-20C^{o}for use at 1000 rpm for ten minutes.

3.9 Analysis of Samples

Toxo IgM and Toxo IgG blood samples taken from pregnant women were studied by ELISA in the Near East University Hospital Clinical Microbiology Laboratory. Toxo IgG Avidity test was performed by ELISA method in order to make differential diagnosis of acute or chronic disease in cases with Toxo IgG and Toxo IgM positivity.

3.9.1 Precautions, method and concepts of ELISA

Quantitative determination of Toxo IgG and Toxo IgM antibodies in patient sera according to manufacturer's protocol was performed using ELISA (Architect i1000 SR ABBOTT, USA).

3.9.2 Specimen Collection And Preparation For Analysis

SPECIMEN TYPES

This study was carried out using serum samples of pregnant women, which collected in collection tube (serum separate tube).

PREPARATION OF ANALYSIS:

The study was carried out following the manufacturer's instructions. Samples with serum separation completed were vortexed at low speed before starting the study. Serum samples were examined visually and homogenous samples were studied.
Non-homogeneous sera were vortexed until homogeneous.
Serums separated from blood samples can be stored at room temperature if they are to be taken into operation within 3 hours. Serums separated from blood samples can be stored at 2-8 degrees if they are to be taken into the study within 14 days. Serums separated from blood samples can be stored at -20 ° C if the study is to be performed for more than 14 days.

3.9.3 Assay Procedure

The microparticle bottle was resuspended before loading the reagent kit into the instrument. . If microparticles are still adhere to bottle, continue to invert the bottle until the microparticle have been completely resuspended. Calibration of the device is done with control sera. The Architect Toxo IgM or IgG control value must be within the acceptable ranges as specified in the control package insert. If a control out of its specific range the associated test result are invalid and sample must be retested recalibration may be indicated . Abbott Architect i1000 automated system prepared for the study of pregnant women's serum is loaded into the device. Minimum sample cup volume is calculated by system and printed on the order list report to minimize the effect of evaporation, verify adequate sample cup volume is present prior to running.

Specimen cannot be diluted for the ARCHITECT TOXO IgM assay. If the Toxo IgG result is> 2000 IU / ML, an automatic dilution protocol is applied. The system performs 1:10 dilution of specimen and automatically calculate concentration of specimen before dilution and reports the result. When test is conduct using TOXO IgG assay file specimen flagged as > 2000IU/ML will be automatically retested in 1 :10 dilution.

3.9.4 Interpretation of Results

In the evaluation of Toxo IgM test: <0.5 index negative, 0.5-0.6 index grayzone and> 0.6 index positive. The result unit for t Serum samples detected as grayzone are re-tested after 2 weeks.

In the evaluation of Toxo IgG test: <1.6 IU/ml. negative, 1.6-3.0 IU/ml. grayzone and ≥3 IU/ml. positive.

3.10 Toxo IgG Avidity

It is vital to realize that T. gondii is due to the possibility of determining the risk of congenital infection and of ensuring adequate treatment of the parent and child in the

childhood. High mortality and maternal morbidity of the first quarter gestation is often due to serious medical conditions, such as severe neurological impairment or fetal death (Thiebaut, R.,et. al.).Evaluation of Toxo IgG avidity results: Low avidity refers to infection in the last three months, and high avidity refers to a disease that has passed before six months.

Avidity test method

After Eliza diagnosis of blood serum of pregnant women IgG antibody of toxoplasma to positive and toxo IgM antibody positive ,toxoplasma IgG avidity test will be evaluate to determine acute and chronic toxoplasma infection in pregnant women .

Procedure of avidity

1-Microtiter plates previously coated with toxoplasma antigen

2- Washed 3 time with PBS plus 0.05% tween (20PBST)

3- Serum sample were dilute 1/200 and add 100ul/well o 2 rows of a plate (row A and row B)

4- Incubation 45 min at temperature 37

5- Row B washed 3 time with PBST and row A washed 3 time with modified PBST buffer contain 6 ml urea found time with PBST

6- The antihuman IgG conjugated with horseradish peroxidase (HRP)was added with dilute of 1/1,000 in PBST

7- Incubate and wash

8- Add O phenylenediamine (OPD)

9- Reaction stopped by sulfuric acid

10- Automated ELIZA reader used

3.11 Data Analysis

In order to evaluate the answers from the completed survey, Microsoft Excel and the Scientific Program for Social Sciences (SPSS, version 13.0 IL, USA) program were used.

3.12 Ethical Consideration

Necessary permission was obtained from the Near East University Hospital Chief Physician for retrospective study. The information collected from the hospital records were used in this study for academic purposes only.

CHAPTER 4

4. RESULTS

4.1 General characteristics of study participants

This study was taken from the records of 1348 pregnant women retrospectively from the records of the Near East University Hospital. Demographic information of pregnant women applied from obstetrics and gynecology outpatient clinic of the Near East University Medical Faculty Lab in Near East Hospital between 2015-2018 have been registered. Toxo IgG and Toxo IgM results were determined by ELISA test in Microbiology Laboratory of Near East University Hospital.

Toxo IgG avidity test results were studied in a private reference laboratories in Turkey. The differential diagnosis of IgM result positivity toxoplasmosis was evaluated by Toxo IgG avidity test in pregnant women. Seroprevalence in pregnant women was evaluated only in patients with positive toxo IgG.

SPSS version 13.0 INC, Chicago, IL, USA was used for statistical analysis. Mean differences were analyzed using T-test. $p < 0.05$ was considered significan

4.2 Result according average of age and nationality

It was determined that 572 (%42.4) are TRNC, 746(%55.3) are Turkey, and 30 (%2.3) are other nationalities ,showed in (Figure 4.2.1.).

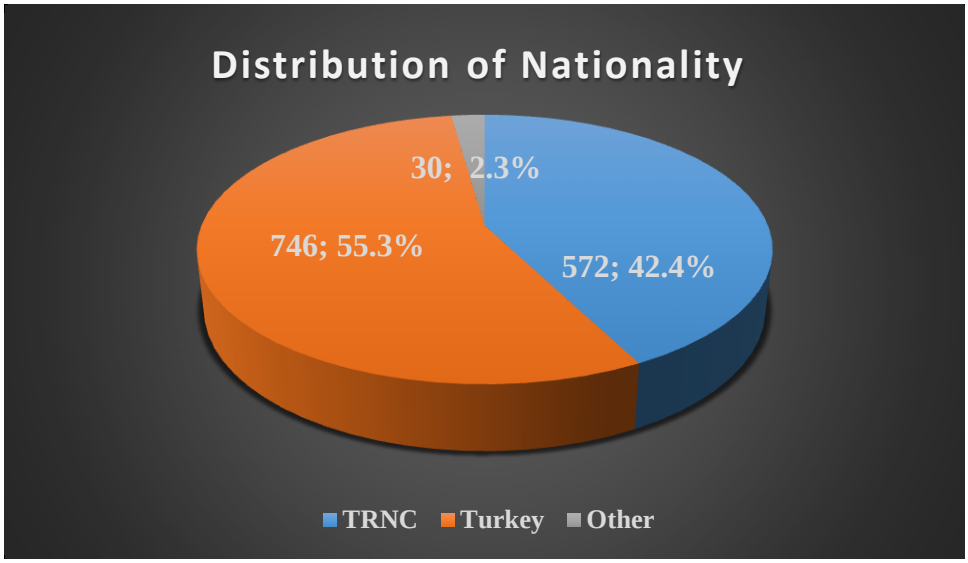


Figure 4.2.1. Distribution of pregnant women in the 17-51 age range by nationality

4.3 Seroprevalence of Toxo IgG and Toxo IgM antibody by ELIZA

1.Seropositivity rates of Toxo IgM on the total of 1348 pregnant women in 17-51 age , result was showed that 19 patient ,1.4% Toxo IgM reactive ,while 1392 patient 98.6% Toxo IgM nonreactive , range are given in Figure 4.3.1.

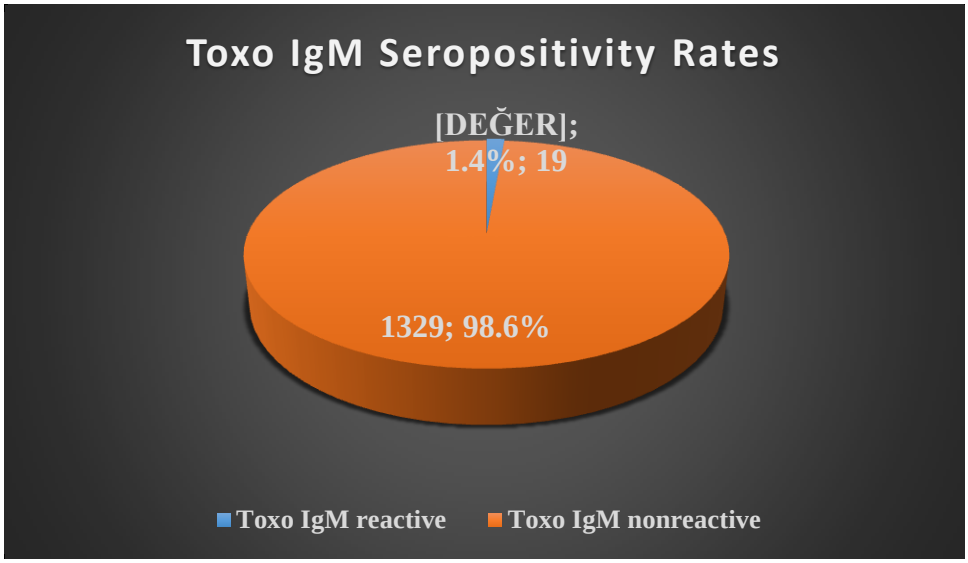


Figure 4.3.1. Seropositivity rates of Toxo IgM

2.Seropositivity rates of Toxo IgG on the total of 972 pregnant women in 17-51 age , result was showed that 171 patient number, 17.5% Toxo IgG reactive. while, 81.5% Toxo IgG nonreactive which ,number of patient 792 , Grayzone 9 patient %1, range are given in Figure 4.3.2.

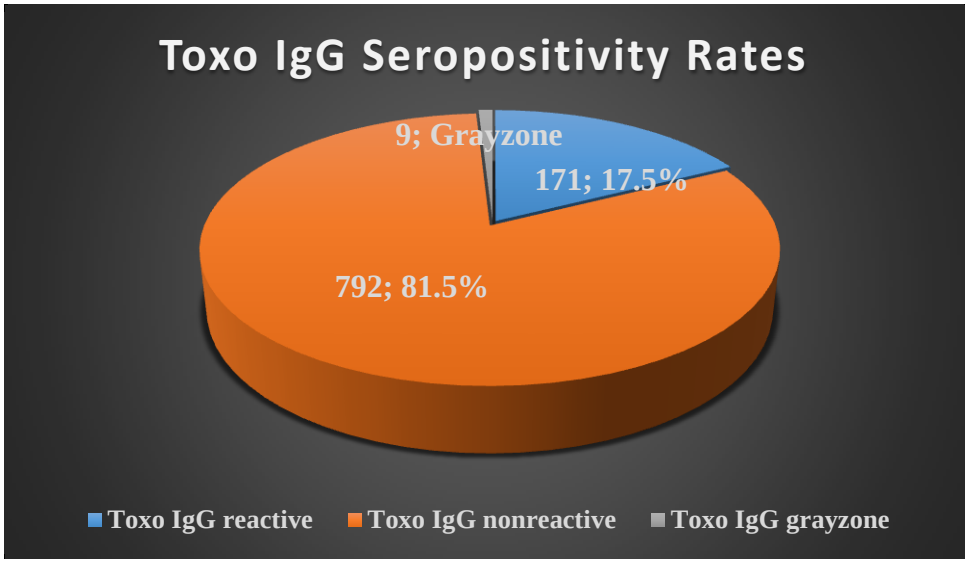
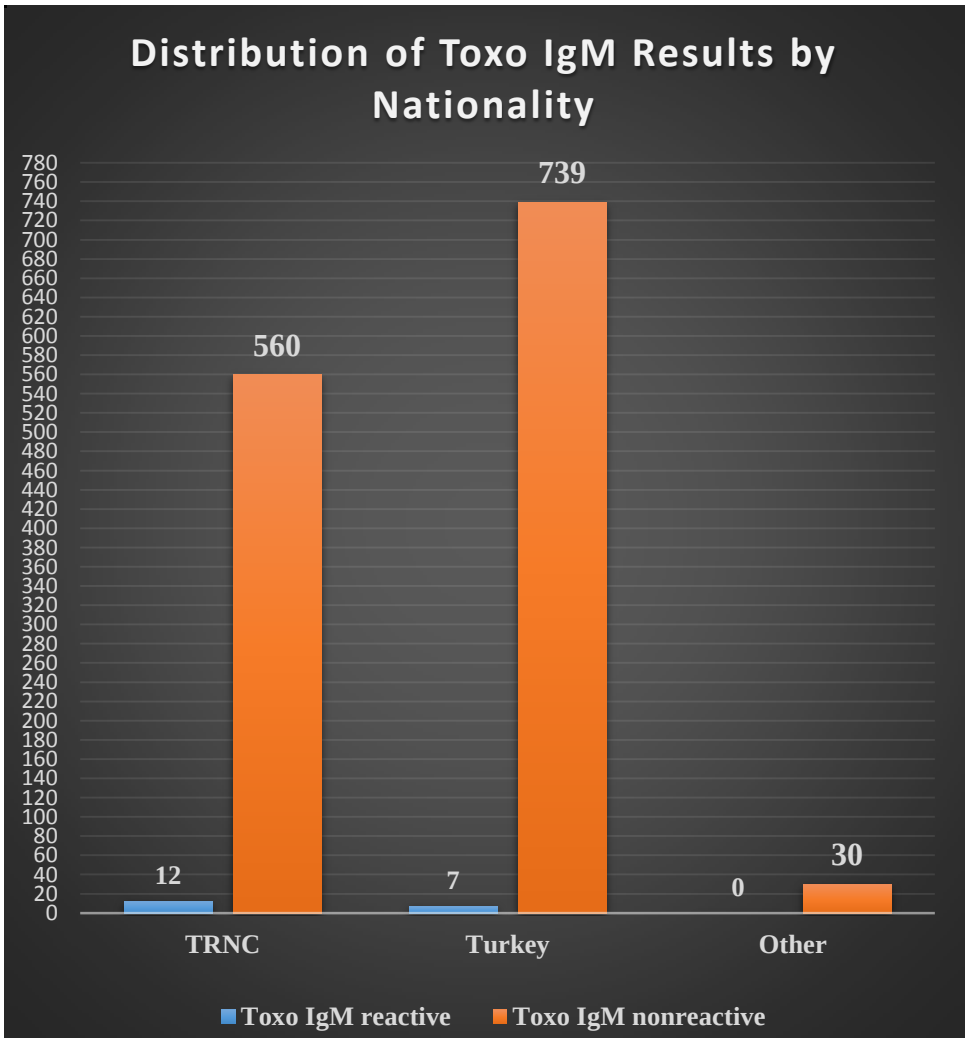


Figure 4.3.2. Seropositivity rates of Toxo IgG on pregnant women

4.3.3 & 4.3.4 Distribution of toxoplasma IgM and IgG results by nationality :

4.3.3 Distribution of toxoplasma IgM results by nationality

on the total of 1348 pregnant women in 17-51 age range.range showed in

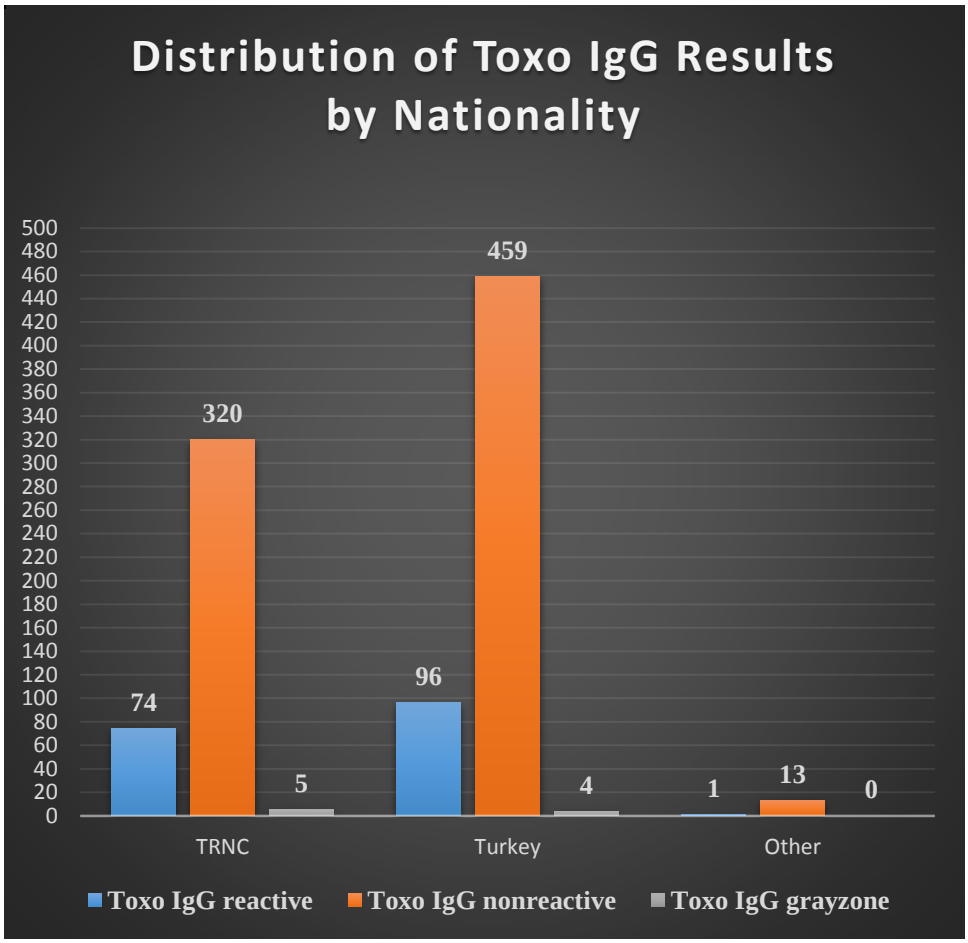


p<0.05; p=0.168

Figure 4.3.3 There is no statistically significant difference between the toxoplasma IgM and nationalities.

4.3.4. Distribution of toxoplasma IgG results by nationality

Toxoplasma IgG was studied in 972 out of a total of 1348 pregnant women showed in Figure 4.3.4.



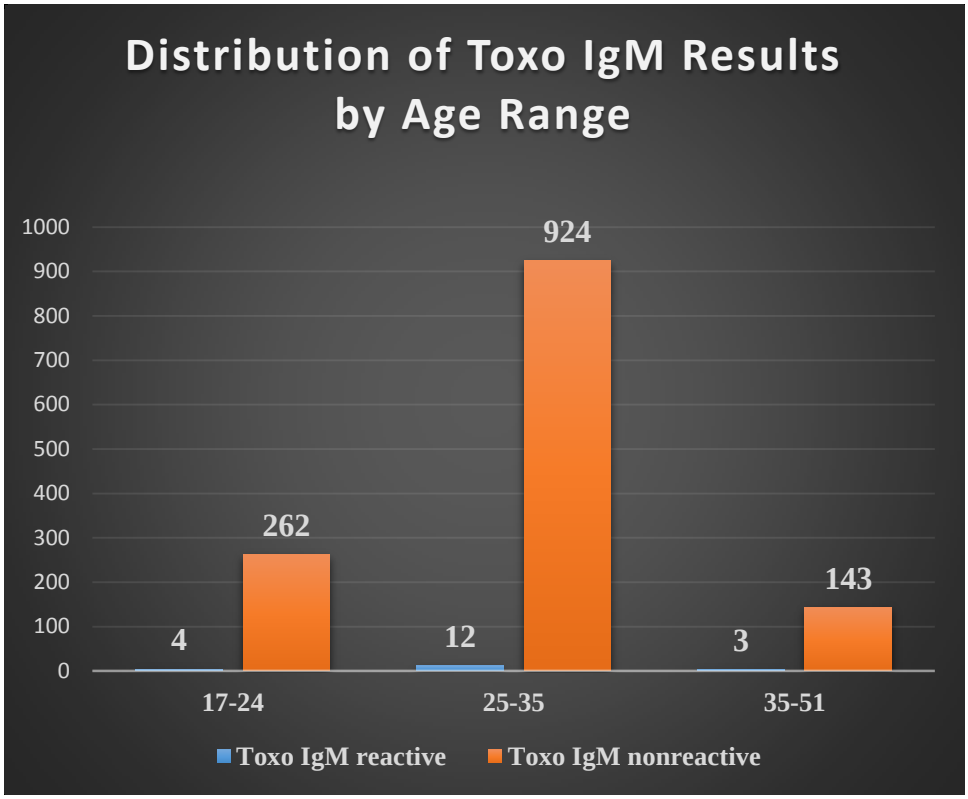
p<0.05; p=0.677

Figure 4.3.4 There is no analytical difference in the distribution of toxoplasma IgG results according to nationalities.

4.3.5 & 4.3.6 Toxo IgM and IgG positivity was statistically significant in the 25-35 age group

4.3.5 Toxo IgM positivity was statistically significant in the 25-35 age group

Figure 4.3.5. Distribution of toxoplasma IgM results according to age groups in total 1348 pregnant women

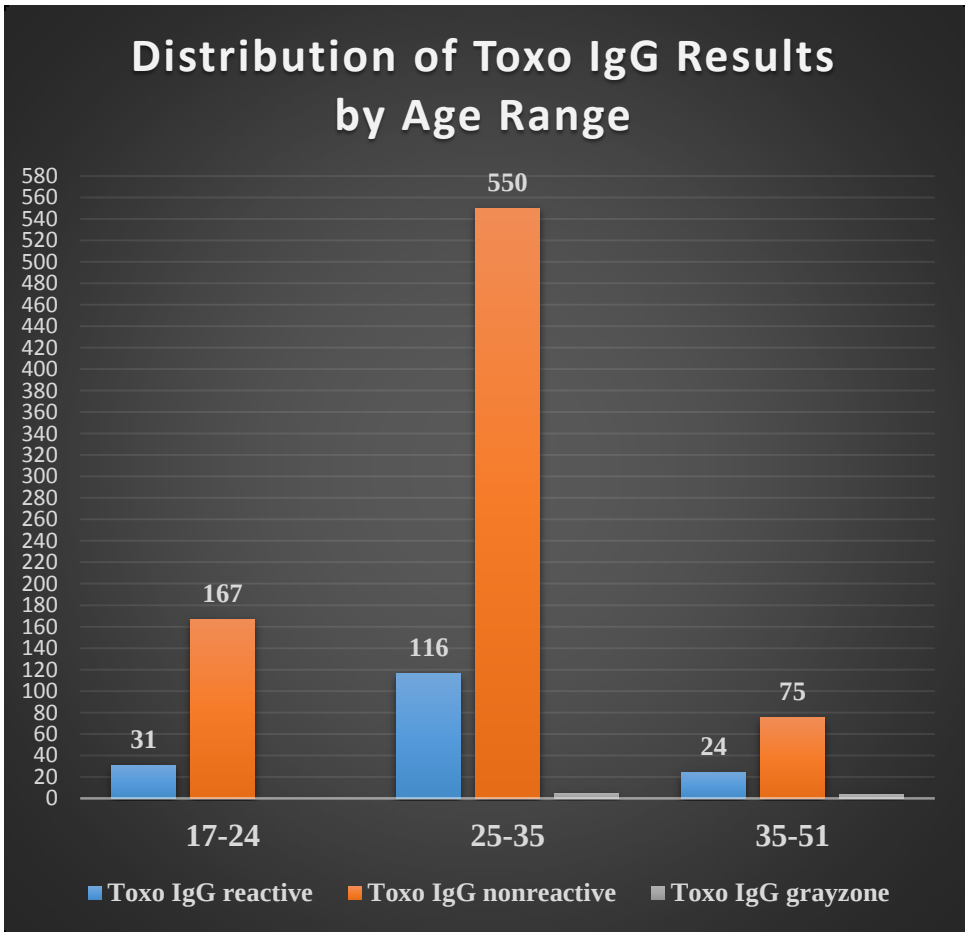


p<0.05; p=0.752

Figure 4.3.5 There was no statistically significant difference in toxoplasma IgM results according to age groups

4.3.6 Toxo IgG positivity was statistically significant in the 25-35 age group

4.3.6. Distribution of toxoplasma IgG results according to age groups in total 972 pregnant women Toxo IgG was studied in 972 out of a total of 1348 pregnant women showed in Figure 4.3.6.



p<0.05; p=0.004

Figure 4.3.6 Toxo IgG positivity was statistically significant in the 25-35 age group (p=0.004).

4.4. Toxoplasma avidity test result

Avidity tests studied in only 12 pregnant women from 19 pregnant women with toxo IgM positivity the result appear high avidity (75%) which consist of 9 pregnant women which means chronic infection, mean that toxoplasma infection acquired before six months ago but low avidity of IgM positivity only 1(8.3%) which means infection was acquired within the last three months, avidity on the borderline which mean infection at an indeterminate only 2 pregnant women patient (16.7%) in total 12 patient pregnant women .

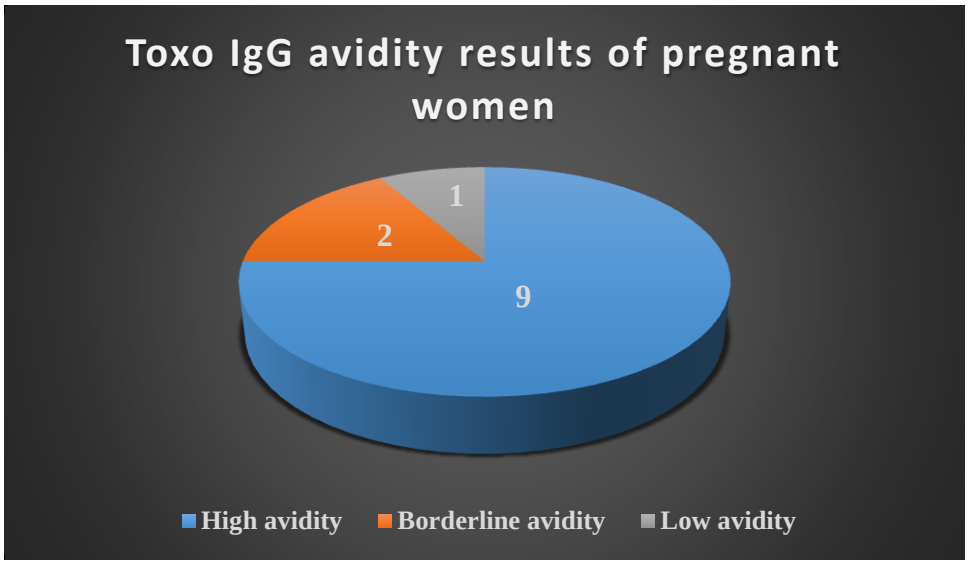
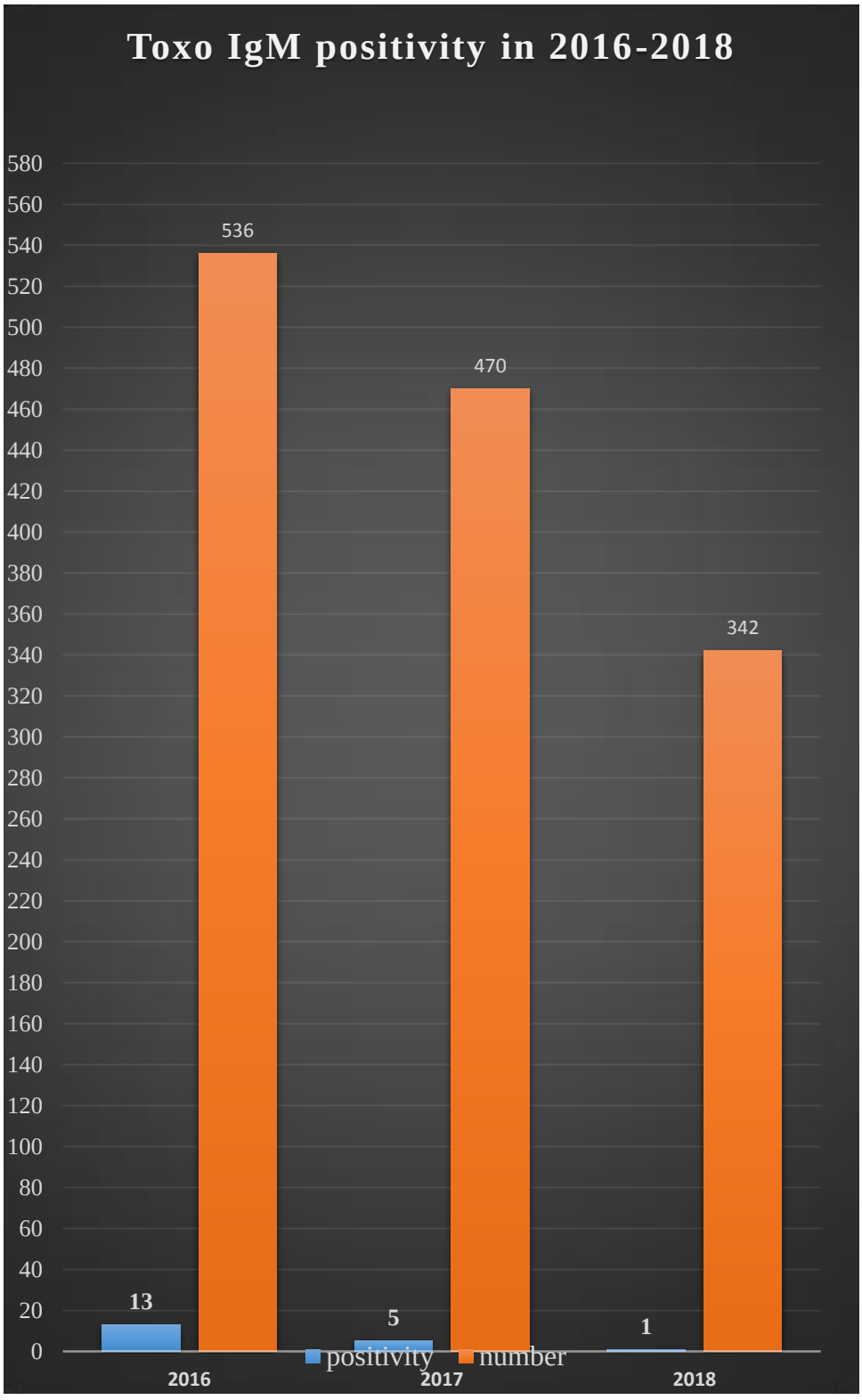


Figure 4.4.1. Toxo IgG avidity results of pregnant women

4.5 Toxo IgM positivity per year

While the ratio was determined as 13/536 (75%) in 2016 and 5/470 (16.7%) in 2017, this ratio was determined as 1/132 (8.3%) in 2018.



(p<0.05, p=0.024)

Figure 4.5.1. Toxo IgM positivity ratio in 2016-2016.

CHAPTER 5

5.DISCUSSION

Congenital toxoplasmosis is a serious risk in pregnancy and newborns and therefore plays an important role in public health. Although the disease is generally asymptomatic in healthy individuals, the main groups that can be seen due to mortality and disease are immunosuppressive individuals, fetus and newborns. The prevalence of toxoplasma infection, which is common in the world, varies from country to country and from region to region. In the world, except for the Antarctic continent has been shown in all continents.

The aim of this study was to investigate the seropositivity rates of toxoplasma infection which may cause miscarriages, stillbirths and various anomalies in the baby after pregnancy, the effect of various risk factors and IgG avidity values in seropositive pregnant.

Many studies have examined the relationship between toxoplasma seropositivity and some indicators such as eating habits, lifestyle and socioeconomic status. Determining the effect of such indicators on seropositivity allows to obtain important information especially in terms of public health.

Serological diagnostic methods are used routinely in the early diagnosis of toxoplasma infection during pregnancy. Toxoplasma infection in the first trimester of pregnancy causes congenital malformations in 90% of fetuses (Linguissi, L., et.al.2012). Infection in the pre-pregnancy period does not cause sequelae in the baby (Liu, Q., et.al, 2009). The island of Cyprus is located in the Mediterranean Sea and generally has warm weather and high humidity. T gondii oocysts are known to be more resistant in warm and humid weather conditions (Caballero-Ortega, et.al 2012,).

In Turkey, most of the studies conducted retrospectively to determine seropositivity rates between 30% and 79% of IgG positivity of parasites were found (İnci, M., et,al.2009, Kuk, S.,et.al 2012, Sütçü, A.,et.al 1998).

Between 1986 and 1999, a study evaluating the seroprevalence of toxoplasma in fertile women in 53 countries was found to be 42%. According to this study, it is stated that approximately 2.5 billion people in the world are infected with T. gondii (Hill, D.,2002). However, there are few studies on the seroprevalence of toxoplasma in Northern and Southern Cyprus in the literature. In our study, Toxo IgG positivity in pregnant women; 17.5%, Toxo IgM; 1.4% and Toxo IgG Avidity; High Avidity: 75%, Border Avidity: 16.7%, Low Avidity: 8.3%, as found.

Seropositivity of 34.69% with SF test and 30.61% with IFA test in cattle in Turkish Republic of Northern Cyprus indicated that toxoplasmosis may be a problem in cattle breeding in this country; for this reason, the researches that are not enough to date, and the countrywide researches should be done to determine the additional dimensions of the disease and thus the ways of combating the disease should be determined (Nalbantoğlu, 2002).

In this study, 101 dogs (68 females and 33 males) from TRNC were examined by SF dye test and 25 dogs (24.75%) were found to be seropositive for T. Gondii (Ergene, O.,et al. 2019) Regarding seroprevalence of toxoplasma gondii, toxoplasma IgG positivity was found to be 41.5% and toxoplasma IgM positivity was found to be 0.43% in 462 pregnant women in a study conducted in TRNC between 2010-2012 (Süer, K.,et al 2012) A retrospective evaluation of toxoplasma gondii IgM, IgG and avidity results in pregnant women in the Turkish Republic of Northern Cyprus; toxoplasma IgG; 19.9%, toxoplasma IgM; 1.2%, toxoplasma IgM grayzone; 0.2% and toxoplasma IgG avidity; 70.6% high avidity, 17.6% borderline avidity and 11.8% low avidity positivity were detected (Güler, E.,et al. 2018)

A study conducted in Southern Cyprus, Toxoplasma IgG seroprevalence was found to be 6.5% in female students, 18% in pregnant women and 40.1% in ruminants (Liassides, M.,et al. 2016) In a limited number of studies in Cyprus, since 2012, seropositivity of toxoplasma IgG has been reduced.

In other countries in the geographical area where Cyprus is located, the results of the studies on seroprevalence of toxoplasma may differ from country to country.

Toxoplasma IgG positivity was found to be 47.8% by ELISA in 301 pregnant women in Ankara (Ustaçelebi, S.,et al. 1986). 428 pregnant women found 43% toxoplasma IgG positivity by ELISA in their study(Polat, E,.et al, 2002). Söyletir et al. found that seroprevalence of toxoplasma was 46.3% in 548 women of childbearing age (Söyletir, G.,et al. 1989). Toxoplasma IgG was found to be 43.6% positive in 204 women aged 18-35 years with ELISA (Türkmen, L.,et al. 2001). In a study performed in 540 pregnant women in Afyon, Toxoplasma IgG was found to be 28.9% positive by ELISA (Altındiş, M.,et al. 2002). Turkey also made at different research toxoplasma IgG seropositivity, generally it showed higher positivity than Cyprus.

Toxoplasma IgM seropositivity of Syrian refugees living in Kahramanmaraş in 2012 and 2013 was found to be 4.76% and 4.84%, respectively. Toxoplasma IgG seropositivity in Syrian refugees was 80% and 62.6%, respectively. In the same years, Toxoplasma IgM seropositivity rates were 1.96% and 2.34% in the local population in Kahramamaraş in 2012 and 2013, whereas Toxoplasma IgG seropositivity in the same population was 49.7% and 45.7%, respectively (Bakacak,K.,et al. 2015). Toxoplasma seroprevalence was found to be high in both toxoplasma IgM and toxoplasma IgG compared to Cyprus.

Toxoplasma IgG and IgM positivity were 82.6% and 1.8%, respectively, in Lebanese pregnant women (n: 2456). In this study, IgG seropositivity was found to be the highest among the 35-44 age group (87.81). n this study, IgG seropositivity was found to be the highest among the 35-44 age group (87.81%), two abortions due to T. gondii infection were recorded during pregnancy and 64 cases had seroconversion (Nahouli, et al. 2017). Toxoplasma IgG positivity was higher in Lebanese pregnant women than in pregnant women of Cyprus.

Toxoplasma IgG seropositivity rate was found to be 67.5% among pregnant women in Egypt. In the last 20 years, the rate of toxoplasmosis has increased in the studies

conducted in Egypt. This prevalence is higher than other global rates (Hala, M.,et al. 2012).

In Greece, the prevalence of Toxoplasma infection in women of reproductive age (15-39 years) was 35.6% in 1984, 25.6% in 1994 and 20% in 2004. In the last 30 years, the prevalence of Toxoplasma infection in Greece has decreased significantly. These seropositivity rates are significantly lower than those reported in central European countries, but higher than those reported in the UK and Scandinavian countries. The prevalence of the Toxoplasmosis in Europe is women of reproductive age, France > 50%, Switzerland 52.4% , Germany 59%, Finland 20.3%, Norway10.9% and England 8.1% it has been reported. Differences in the prevalence of Toxoplasma infection by country warm and humid climatic conditions are associated with cooking habits and the number of cats living outside, but the exact roles of these factors are not fully understood. The gradual decline in seroprevalence in Greece can be explained by the improvement in the socio-economic situation. Decreasing consumption of home-grown fruits and vegetables, increased consumption of pasteurized milk and increased use of freezers at home, increased awareness and training, eating enough cooked meat, keeping cats, preparing hygienic vegetables and hand washing are the factors that cause infection in humans (Diza, E.,et al. 2005) The factors that lead to a reduction in toxoplasma seroprevalence in Cyprus and Greece are similar.

As a result of the studies conducted in France in the last 17 years, the toxoplasma IgG positivity of 21480 people was 64.5% in 1997 and 54.7% in 2013. Seroprevalence decreased by 1.3% on average annually. The decrease in seroprevalence according to age groups shows acceleration, especially in those younger than 20 years. According to these data, seroprevalence in France is expected to decrease even more in all age groups by 2030. Considering the transmission path of T. gondii, this decrease can be explained by a reduction in the intake of salads and fresh vegetables contaminated by oocytes released by cats. In 2016, the federation of ready-made food manufacturers for dogs, cats, birds and other pets reported that the French owned cat population was 10 million in 2006 and 13.5

million in 2016. Domestic cats are largely fed with dry food and therefore a reduction in the rate of transmission to humans is observed. An increase in bottled water consumption and improvement of the quality of tap water are also seen as factors that reduce oocyst intake. The most important explanation for the overall decrease in seroprevalence is that the consumption of contaminated meat decreases over time. Consumption of mutton, the main meat containing T. gondii cysts, in France while it was 5.4 kg / year per person in 1990, it decreased to 2.7 kg / year per person in 2013. Thus, the decrease in seroprevalence over time can be explained by both reduced meat consumption and reduced parasites in commercial meat. The organic meat production method, which is grown without allowing animals to circulate outdoors, can reverse the rate of seroprevalence, reducing the risk of contamination from environmental sources. Increased seroprevalence with respect to the age of the population shows a cumulative increase in relation to consumption of infected meat, the main possible route of infection in Europe. In France, the prevention program for reducing the consumption of undercooked meat for women of childbearing age may have had a positive effect (Guigue, et al. 2018). Seroprevelance in Cyprus was found to be much lower compared to France. The consumption of bottled water in Cyprus has accelerated rapidly over the last decade. Another factor that would explain the difference between seroprevalence in these two countries, where meat consumption is high, is higher consumption of well-cooked meat in Cyprus.

The incidence of toxoplasmosis in Northern Cyprus is low compared to some countries, although it has favorable climatic conditions for the life of the oocyst.

In a study conducted in Northern Cyprus in 2012, toxoplasma IgG positivity was found to be 41.5% in pregnant women. In the study conducted in 2018, it was found to be 19.9%. As stated in our results, the proportion of pregnant women with toxoplasma IgG positivity over the years tends to decrease. (Süer, k,.et.al 2012, Güler, E.,et.al 2018)

Toxoplasmosis infection is an asymptomatic and self-limited disease in immunocompromised individuals (Crucerescu, E.,et al. 2002). However, in both cases it is potentially severe; congenital toxoplasmosis as a result of acute

toxoplasmosis during pregnancy and in patients receiving immunosuppressive treatment such as transplantation, malignancies and AIDS infection (Candolfi, E.,et al. 2007). Serological tests are routinely used for the diagnosis of toxoplasmosis. Toxoplasma IgM and toxoplasma IgG are evaluated in serological diagnosis. Toxoplasma IgG avidity test provides information about the process of infection in cases where both of them are positive. However, in some cases the results may be misleading. For example, in children and adolescents with ocular findings of congenital toxoplasmosis, an increase in IgG titer may not be detected. Toxoplasma IgM antibody positivity may remain positive in some cases as residual IgM in the years following primary infection. Some patients with positive rheumatoid factor and antinuclear antibodies (ANA) may be found to be false positive (Hedman, K.,et al. 1989).

To clarify these suspicious conditions, toxoplasma IgG avidity test is applied. Toxoplasma IgG avidity test results indicate that high avidity means that Toxoplasma chronic infection infection was taken before 3 months, while borderline avidity means infection in an indefinite period, while low avidity means acute infection in which the infection was acquired in the last 3 months.

In a study, 1737 pregnant women were examined for toxoplasmosis. Toxoplasma IgG positivity was 24.2% (n: 421) and toxoplasma IgG was 73.9% (n: 1284). In the same study, Toxoplasma IgM was 99.3% negative (n: 1724), while Toxoplasma IgM positivity was 0.7% (n: 13). High avidity was found in 9 of 13 cases in the avidity test applied to these patients. Low avidity was positive in only 3 cases (Selek,D.,et al. 2015).

After serological screening in 128 pregnant women in Morocco, 54 women (42.4%) were tested positive for IgG antibodies and five women (3.9%) tested positive for both anti-Toxoplasma IgG and IgM antibodies. Only two of the five cases had low avidity (Laboudi, M.,et al. 2017).

In our study, toxoplasma IgM positivity and low avidity were found in low rates as in other studies. In this retrospective study, only 12 of 19 patients who required toxoplasma IgG avidity study completed pregnancy follow-up in our hospital.

Toxoplasma IgG avidity was detected in 75% (n: 9) of the 12 patients with high avidity and was evaluated as chronic infection. Borderline avidity was 16.7% (n: 2) and low avidity was 8.3% (n: 1) pregnant women. As a result of the follow-up of pregnant women, healthy infants were obtained from hospital records.

The seroprevalence of the toxoplasmosis has been reduced by the measures taken in some countries. In countries with low seropositivity, cases of relatively innate toxoplasmosis will be reduced.

The reasons for this change in seropevalence can be considered as follows:
Animal shelters, which have started to be implemented on the island and have been increasing in number in recent years, may have reduced the rate of spread of the disease.

The veterinary controls of all owned pets are carried out appropriately. Internal and external parasites are applied in veterinary control. According to the information received from veterinarians, the number of owned pets has increased in the last decade. Most of the owner animals are fed with commercial food that is not contaminated with toxoplasma.

Although the water used by the public for daily cleaning needs is tap water, it is seen that the water they drink is bottled water.

5.2 Conclusion And Recommendations

Our study was retrospectively data collection that determined seroprevalence of Toxoplasma gonadii infection between pregnant women in mean age(29.03±5.095)(17-51) conducted in Near East Laboratory Hospital in Northern Cyprus in 2015-2018 on 1348 patient , and evaluated Toxo IgM positivity results by IgG avidity test in Particular Reference Lab in Turkey to differentiated between chronic and acute infection.

In this retrospective study showed that most of pregnant women who had toxoplasmosis in total 1348 was from Turkish which was the ration 55.3% while in TRNC 42.4% and other country patient who live in Northern Cyprus only 2.3 % .

While by ELISA test which evaluated Toxo IgM and Toxo IgG it was found to be Toxo IgG reactive was 17.5% n:171 in total 972 patient and Toxo IgM reactive was 1.4% n:9 patient on total 1348 patient, It mean according evaluation seroprevalence for by ELIZA for Toxoplasmosis infection chronic infection of Toxoplasmosis with mean IgG reactive is higher than acute infection in TRNC.

And between evaluated Toxo IgM and Toxo IgG by ELIZA according statistically data analysis in our study showed that there was not statistically significant difference for distribution of Toxo IgM reactive and Toxo IgG reactive by nationality which determined in our study ,While in distribution of Toxo IgM reactive according age group between 3 groups (17-24), (25-35), (35-51) which determined in our study it was not statistically significant difference in Toxo IgM, but results showed statistically significant difference for Toxo IgG reactive in age 25-35 among age groups ,It mean in TRNC between age 25-35 the ration of IgG is high for pregnant women which indicate chronic infection if pregnant women in good immunity there is not high risk for abortion . In this retrospective study, only 12 of 19 patients who had Toxo IgM posistivity and required toxoplasma IgG avidity study completed pregnancy follow-up in Near East hospital and evaluated in Special Lab in Turkey . Toxoplasma IgG avidity was detected in 75% (n: 9) of the 12 patients with high avidity and was evaluated as chronic infection. Borderline avidity was 16.7% (n: 2) and low avidity was 8.3% (n: 1) pregnant women which enhanced acute infection. As a result of the follow-up of pregnant women.

According our retrospectively data collection showed that over year (2016-2017-2018) there was decrease in toxoplasma IgM positivity infection in TRNC which statistically significant showed decrease IgM positivity over years , It mean acute toxoplasmosis infection which cause abortion ,stillbirth , anomalies for fetus in TRNC over years reduced.

5.3 recommendations

1-Since the toxoplasmosis risk factors have not been assessed and discussed, a further study is recommended, especially among pregnant women in a TRNC, in determining the risk factors for toxoplasmosis infections.

2- the provision of health services, it is recommended that pregnant women should be routinely screened for toxoplasmosis during prenatal follow-up

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