Seroprevalence of anti-*Toxoplasma gondii* IgM antibody and risk factors among pregnant and non-pregnant women referred to health centers of Khyber Pakhtunkhwa, Pakistan

By
Shanza Kiran

Department of Zoology
Faculty of Biological Sciences
Quaid-i-Azam University Islamabad, Pakistan
2021

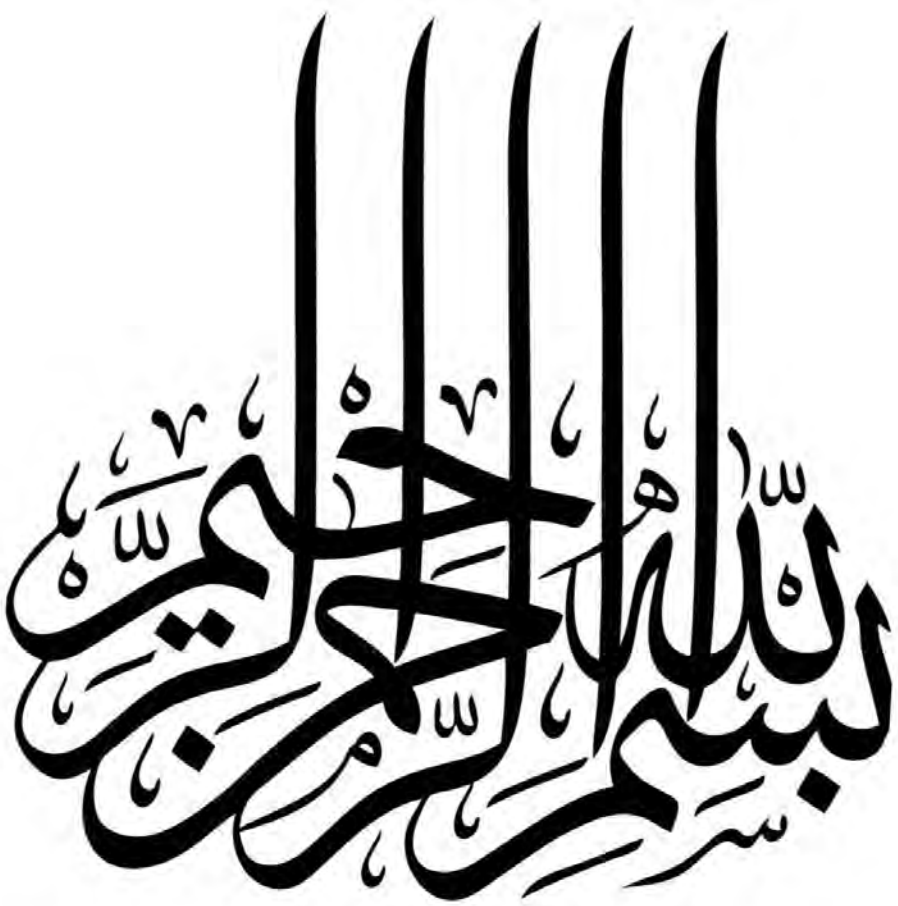
Seroprevalence of anti-*Toxoplasma gondii* IgM antibody and risk factors among pregnant and non-pregnant women referred to health centers of Khyber Pakhtunkhwa, Pakistan

A dissertation submitted in the partial fulfillment of the requirements for the degree of Master of Philosophy.
In Parasitology

By
Shanza Kiran

Department of Animal sciences
Faculty of Biological Sciences
Quaid-i-Azam University Islamabad, Pakistan
2021

“In the name of ALLAH, the most Beneficent, the most Merciful”



CERTIFICATE

This dissertation –Seroprevalence of anti- *Toxoplasma gondii* IgM antibody and risk factors among pregnant and non-pregnant women referred to health centers of Khyber Pakhtunkhwa, Pakistan" is submitted by Ms. Shanza Kiran is accepted in its present form by the department of Animal sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Parasitology.

Supervisor _____

Assistant Prof. Dr. Kiran Afshan

External Examiner_____

DECLARATION

I hereby declare that the material contained in this thesis –Seroprevalence of anti-*Toxoplasma gondii* IgM antibody and risk factors among pregnant and non-pregnant women referred to health centers of Khyber Pakhtunkhwa, Pakistan" is my original work. I have not previously presented any part of this work elsewhere for any other degree.

Shanza Kiran

February 2021

Dedicated to my Janji, Ammi Jan,
Husband Usman and my Only Brother
(Zeeshan Khan Awan (Anu)).

Contents

ACKNOWLEDGEMENTS

All the praises for ALLAH, the lord of the heavens and the earth. The lord and cherisher of all the worlds. To Him be glory throughout the heavens and the earth, and He is exalted in power, full of wisdom. All the praises for Almighty ALLAH, who created us Muslim and gave us the power of knowledge to differentiate the right and wrong. Many thanks to Him as He blessed us with the Holy Prophet Hazrat Muhammad (Peace be upon him) for whom the whole universe is created. Many thanks to Prophet Muhammad (Peace be upon him) who enabled us to worship only one Allah and brought us out of darkness and enlightened the way of Heaven. I would like to thank my supervisor **Dr. Kiran Afshan** for her affection, continuous support, guidance, and encouragement throughout my research work. She not only helped me in achieving my research goals but also taught me scientific writing, independent thinking, and strong research ethics. I once again thank her for always being so nice with me.

I wish to express my sincere thanks to **Chairman, Dr. Sajid Malik** Department of Animal Sciences, Quaid-i-Azam University Islamabad for providing necessary research facilities. I would further like to thank to another faculty member, **Dr. Sabika Firasat** for being a source of inspiration and enlightenment for me.

I would like to acknowledge my family for all their moral support and undying encouragement, especially my **mother and father** for their continuous prayer and affections, **Usman** for their love, support, encouragement, and hope. More generally, I would like to thank all my well-wisher, who are always praying for my bright and successful future. Most importantly, I would like to thank my beloved brother, **Zeeshan khan** for his love, help and support at every stage in my career.

And finally, I would like to acknowledge all my fellow lab-mates and colleagues ***Nadir Akhtar, Asim Khan , Ayesha, Raza Ullah, Noor Ullah, Samreen Tariq, Khola ,Sobia, Ayesha, Jamil, Seemi, Rutaba, Imad khan, Imtiaz, Muhammad ihrar, Maria Komal, Raeesa and Waheed*** for their cooperation.

Shanza Kiran

List of Figures

List of Tables

List of Abbreviations:

Abbreviations	Full form
CFT	**Complement fixation test**
CNS	**Central Nervous System**
DALY	**Disability adjusted life year**
ELISA	**Enzyme linked immunosorbent assay**
FAST- ELISA	**Falcon assay screening test- Enzyme linked Immunosorbent assay**
GBD	**Global burden of disease**
GIS	**Geographic information system**
HRP	**Horseradish peroxidase**
HIV	**Human Immuno deficiency virus**
IHA	**Indirect haemmagglutination test**
ID	**Immunodiffusion test**
OD	**Optical density**
RDTS	**Rapid antigen detection system**
LAT	**Latex agglutination test**
MAT	**Modified agglutination test**
PCR	**Polymerase chain reaction**
T.gondii	***Toxoplasma gondii***

Abstract

Background: Intracellular parasite *Toxoplasma gondii* which causes toxoplasmosis in humans is a common parasite. It infects one third of the world's population. Infection is normally asymptomatic in most adults, some people, especially women in early pregnancy, may experience serious complications. Toxoplasmosis is not reported very often and its prevalence is estimated based on regional studies. In Pakistan, few studies have been carried out in different parts of the country which show quite high prevalence. The aim of this study was to evaluate the seroprevalence of anti-*Toxoplasma gondii* IgM antibodies in women of childbearing age and associated risk factors referred to different health centers of Khyber Pakhtunkhwa, Pakistan. Serological test by Enzyme Linked Immunosorbent Assay (ELISA) for the detection of anti- *Toxoplasma* IgM antibodies was carried out to address the associated risk factors which possess potential burden socially and economically.

Methods: A total of 423 respondents between the age group 18 and >40 years were included in our study. Subjects were pregnant and non-pregnant ladies from the different districts of Khyber Pakhtunkhwa province, Pakistan Many factors were analyzed such as pregnancy status of women, age group, gestation period, profession and the residency of the respondent. Highly sensitive and specific sero-diagnostic technique, Enzyme-linked immunosorbent assay (ELISA) was used to detect anti- *Toxoplasma* IgM antibodies from the blood sera. Chi-square test and Odd ratios (OR) were used to analyze the data. Simple logistic regression analysis was performed to find association between seroprevalence of anti-Toxoplasma IgM antibodies and associated risk factors.

Results: Different outcomes were seen, in all the study an effort was made to evaluate and compare the seroprevalence with already reported seroprevalence. The overall seroprevalence was found 56.26% with highest seropositivity in Mansehra 55 (13.0%), followed by Abbottabad 51 (12.0%), Batagram 43 (10.1), Peshawar 43 (10.1%) and lowest in Haripur district 35 (8.27%). Statistically a significant association was observed among the five districts of Khyber Pakhtunkhwa (χ^2=9.982, p=0.045).The prevalence rate was higher in pregnant women 34.70% (147) as compared to the non-pregnant women 18.6% (79). A statistically significant association was observed between the pregnancy status and the seropositivity (χ^2=27.06, p=0.001). Among different age groups the highest seroprevalence was observed at 25-29 years of age group n=58 (42%) followed by 20-24 years of age group n=51 (36.9%), 30-34 years of age group n=15 (10.86%) , 35-39,15-19 years of age group

n=11 (7.97%) and the least prevalence was at age group >40 years of age group n=3 (2%) among the pregnant women .Whereas in non-pregnant ladies highest seroprevalence was observed at age group >40 years (41.4%) n=29 followed by 25-29 years (18.57%), 30-34 years (14.28%), 20-24 years (12.85%), 35-39 years (10%) and the lowest prevalence was observed in age group 15-19 (2%) years. Statistically non signification association was found among different age groups of suspected women of child bearing age (χ^2=2.099, p=0.842). Among three trimesters of gestation period highest seroprevalence was observed at 3rd trimester 33.20% n=88 followed by 2nd trimester 17.7% n=47 and lowest at 1st trimester 4.90% n=13. In our study when trimester factor was analyzed non-significant association was observed (χ^2=3.432, p=0.185).When profession factor was analyzed higher seroprevalence was found among housewives 29.53% followed by teachers 16.54%, students 8.4% and other working ladies including nurses 3.87% and lowest in bankers 2.42%.Statistical non-significant association was the outcome of the profession factor (χ^2=6.10, p=0.227)

Conclusion: Compared to the already reported seroprevalence, in our study we noted the increase in the seroprevalence among the women of childbearing age population. The study showed that pregnant women are more infected and are at great risk of being infected with drastic outcomes as the vertical transmission of the parasite can result in defective outcomes in the foetus. Through our investigation we came to conclusion that there is a much need of public awareness regarding the disease transmission and life cycle of the parasite. There is a need of timely screening of the suspected people especially the pregnant women population, they should be facilitated at the health centers with proper detection techniques so that the higher prevalence can be controlled in time, in this regard serological screening is important to reduce the risk of congenital transmission. Further studies are needed to evaluate whether this trend is same throughout the country or is just localized to this province.

INTRODUCTION

The world's population will hit around 9.7 billion by 2050, according to World Health Organization (WHO). Different parasitic diseases affect health, causes some of the most debilitating and widespread illness. Among some 1500 human infectious agents, 66 are protozoans and 287 are infectious helminths (Chomel *et al.,* 2006). Zoonotic disease poses a significant disease burden (Macpherson *et al.,* 2005) calculated by a financial cost, mortality morbidity and other measures on society (Lopez *et al.,* 2006).Monetary costs include the reduction of animal population and the expenses of treatment, and the loss of income of people affected by the disease. However due to differences in medical and labor rates, the absolute cost of recovery period of human patients is higher in economically developed countries than in developing countries. Toxoplasmosis has higher economic strain in developed countries than the same disease in a low-income country. Correspondingly the chosen matric of the WHO is the Disability Adjusted Life Year (DALY), which was used to determine the estimates of Global Burden of Disease (GBD) (Paul *et al.,* 2011).

In the decade, food and water borne infections have gained sufficient attention. Some of these infections are well known but are considered to emerge because they have become more widespread recently or are more modified due to improved diagnostic tools and increased awareness (WHO, 2002). The growth in the number of highly susceptible people due to ageing, malnutrition, HIV infection and other underlying medical conditions are other factors that can reflect the emergence of certain zoonotic parasitic diseases and change in lifestyle such as increase in the number of people eating meals cooked in restaurant, canteens and fast food outlets as well as from street footage (WHO, 2002). 75 percent of emerging human pathogens are estimated to be zoonotic (Woolhouse, 2002).

The main food borne parasites include *Toxoplasma gondii*, *Sarcocystic* species, *Taenia spp*, and *Trichenella spp* and by eating raw or under cooked meat contaminated with the cyst phases of these parasites, humans get infected. In most nations, actions are taken by examining the meat in the slaughterhouse or laboratory to prevent humans from being contaminated with meat borne parasites. No unique meat inspection is performed for toxoplasmosis or sarcocystosis. The sensitivity of meat inspection for cysticercosis is poor, leading to a high number of contaminated carcasses entering the food chain (Chomel,. 2008). Toxoplasmosis burden close to that of salmonellosis and campylobacteriosis is still ignored and underreported disease (Kijlstra and Jongert, 2009). This zoonotic parasite is widespread

in the world (Fallah *et al.*, 2008). The intake of undercooked meat is the possible source of infection in Western and Asian countries with a significant effect on public health (Cook *et al.*, 2000, Fallah *et al.*, 2008, Kijlstra and Jongert, 2009). The seroprevalence of *T.gondii* is lower in pure vegetarians (Hall *et al*., 1999, Roghmann *et al*., 1999). In most epidemiological studies, however, eating unwashed raw vegetables or fruits is recognized as a significant risk factor (Antoniou *et al*., 2007, Fallah *et al*., 2008, Liu *et al*, 2009). Toxoplasmosis in pregnant women is commonly considered as a significant health problem. Prevention should be aimed to women who may transmit the infection to a developing embryo and a newborn (Kijlstra and Jongert, 2009). Many animals used for the processing of meat reveal evidence of *T.gondii* infection, as measured by the response of serum antibodies. The viable parasites were isolated from the meat of game cattle, goats, chicken, and pork of pigs but not from beef (Tenter *et al*., 2000). The most effective method to destroy the tissue cysts of *T.gondii* are heating and freezing of meat. However, in countries where the meat chain is well established, measures to avoid the introduction of *Toxoplasma* infected meat into the food chain would be theoretically feasible. Monitoring of farms and adjusting farm management can play an important role in the control of infection (Kijlstra and Jongert, 2009).

1.1 Toxoplasmosis

Toxoplasmosis is one of the main zoonotic diseases worldwide (Torgerson PR *et al.*, 2011). *Toxoplasma gondii* triggers it, an obligate protozoan parasite from Apicomplexa (Tenter *et al.*, 2000), with domestic cats as definitive host and warm-blooded animals as intermediate host (Dubey *et al*., 2010) and most prevalent parasite of human population (Mitra Sharbatkhori *et al.,* 2011). One third of the world's adult population is estimated to be infected with the parasite (Rober-Gangneux and Darde, 2012). Depending on many sociogeographical variables prevalence of *Toxoplasma gondii* infections in human differs across the globe. Between different ethnicities and diverse regional locations, the source of infection could vary widely. *T.gondii* infection is usually attained by ingestion of bradyzoite containing tissue cysts, or by ingestion of sporozoite containing cysts (Tenter *et al.,* 2000). This parasite has three main stages: trachyzoites, bradyzoites and sporozoite (Tenter *et al.,* 2000). A higher incidence of infection among expectant mothers and women of childbearing age has been documented from various parts of the world (Pappas *et al.,* 2009).

Over one third of the population of humans is infected with *T.gondii*, which leads to microcephaly, anencephaly, chorido-retinitis, and other mental health disorders, extreme congenital pathology and spontaneous or premature birth abortion (Krueger *et al.,* 2018).

Humans procure *T.gondii* infection by intake or handling of undercooked or raw meat carrying tissue cysts or by the consumption of oocysts contaminated food or water in the faeces of infected cat (Frenkel, 1973). Both foodborne and waterborne sources have been correlated with outbreaks (Bahia- Oliveira *et al.,* 2003, Dawson, 2005,). In general, primary infections are minimal in length and are characterized by fever, swollen lymph nodes and muscle weakness; they are typically regulated by the immune system and seldom require care (Ho – Yen, 2001). Primary infections inevitably lead to semi dormant tissue cysts that are not removed by treatment with antibiotics (Benenson, 1982).

Consequentially, persistent infections predispose people to the danger of being reactivated. Long term infections remain clinically inconspicuous in most cases. The link between seropositivity and unusual mental problem in human has been identified in several studies (Torrey *et al*., 2007). There is also a high risk of congenital infection when primary infection occurs in a previously immunologically naïve mother during pregnancy (MacLeod *et al*., 2000). In infants born to asymptomatic mothers, human toxoplasmosis has long been commonly accepted as a serious congenital disorder. It is widely agreed that the parasite from a pregnant woman whose infection has recently been acquired is Trans placental to the fetus (Desmonts and Couvreur, 1974)

1.2 Classification

Phylum Apicomplexa comprises of unicellular, spore forming, and obligate intracellular parasite species for some part of their life cycle with some infecting two separate hosts for their asexual and sexual stages. Characteristic organelles are present which serves an adaptation for their penetration with the host cells, certain motile organelles are also present during certain stages of reproduction (Slapeta *et al.,* 2019). Species of class Conoidasida are the parasites of vertebrates. They commonly infect the epithelial cells of the gut but may also parasitize the other tissues. Their life cycle possesses three different stages merogony, gametogony, and sporogony (Dubey *et al.,* 2017).

Kingdom: Protista

Infra kingdom: Alveolata

Phylum: Apicomplexa

Class: Conoidasida

Order: Eucoccidiorida

Family: Sarcocystidae

Sub-Family: Toxoplasmatinae

Genus: *Toxoplasma*

Species: *T. gondii* (Overdulve *et al*., 1970)

1.3 General morphology

1.3.1 Trophozoite

A crescent shape, proliferative, feeding form also known as tachyzoite was discovered by Nicolle and Manceaux (1909) in gundi (Polomoshnoy, 1979). It can parasitize almost every cell and divides by a process known as endodyogeny (Dubey, 2014).

1.3.2 Cystozoites

They are also known as bradyzoites. *T. gondii* can remain as cysts in tissues for several months (Freppel *et al.,* 2019).

1.4 Life Cycle

Toxoplasma gondii is one of the most booming parasites in the world due to its worldwide distribution ranging from arctic to hot deserts as well as remote islands and cities (Levine , 1961). Universally it is among the most prevalent parasites in the worldwide human population (Frenkel, 2000). Life cycle of *T. gondii* is voluntarily heteroxenous. All warm-blooded species, including most livestock and humans are likely to be intermediate hosts. Natural hosts are members of the Felidae family, such as domestic cat (Evans, 1992). Two asexual growth stages are experienced by the *T. gondii* in intermediate hosts. In the first step, in several different types of the host cells, tachyzoites proliferate rapidly via a repeated endodyogeny. The second step of growth that results in the development of tissue cysts is initiated by tachyzoites of the last generation. Bradyzoites propagate slowly by endodyogeny

within the tissue cyst (Dubey, 1998). For neural and muscular tissues, tissue cysts have strong specificity. They are found primarily in the central nervous system (CNS) as well as in the eyes, skeletal and heart muscles. However, they can also be located to a lesser degree in visceral organs, such as liver, lungs and kidneys (Dubey, 1998). Tissue cysts are the last stage of life cycle in the intermediate host and are infectious instantly. They may survive in some intermediate host species for lifetime. Their longevity period mechanism is still unknown.

Many researchers, however, claim that tissue cysts frequently break down, with bradyzoites changing into tachyzoites that reinvade the host tissues and again convert to bradyzoites inside new tissue cysts (Dubey, 1998). The bradyzoites initiate another asexual multiplication process if consumed by a definitive host, which consists of initial replication by endodyogeny preceded by recurrent endopolygeny in small intestine epithelial cells. The terminal phases of the asexual multiplication trigger the life cycle's sexual phase. In the epithelium of the small intestine, gamogony and oocysts transformation occurs as well. Unsporulated oocysts are deposited in the intestinal lumen and released with the faeces into the atmosphere. Infectious oocysts are formed outside the host in external environment which comprises of two sporocysts having four sporozoites. This process is known as sporogony (Gross, 1996).

The life cycle of *T.gondii* has three infectious phases, i.e., tachyzoites, tissue cyst bradyzoites, and sporozoites found in sporulated oocysts. All three stages are contagious to both the intermediate and definitive hosts, which are able to receive *T.gondii* mainly by one of the following routes, (i) Horizontal transmission by fecal-oral consumption of infective oocysts from the environment. (ii) Horizontal transmission by the oral intake of tissue cyst found in raw or undercooked meat or main offal from intermediate hosts, (iii) vertically by Trans-placental tachyzoite transmission (Gross, 1996). In addition, tachyzoites may also be transferred from mother to newborn during lactation period (Astrid M Tentera, 2000).

T. gondii can be transmitted from definitive host to intermediate host and vice versa also between definitive and between intermediate hosts. Which of the different routes of transmission is more epidemiologically relevant is currently unknown. *T. gondii* infections are not associated with the presence of specific species; their transmission can continue unabated (Astrid M Tentera, 2000).

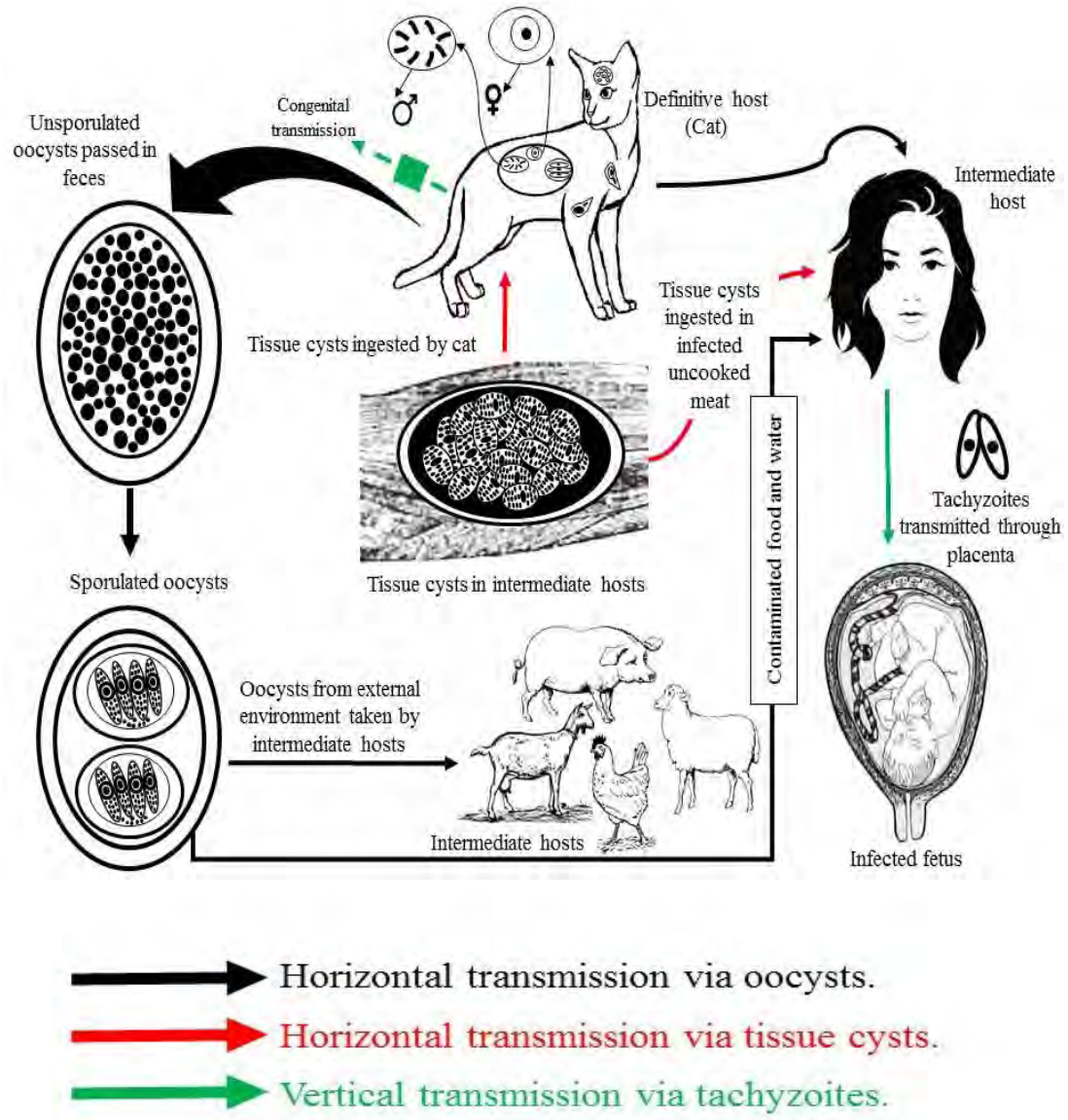


Figure1. 1: Life cycle of *Toxoplasma gondii* and its routes of transmission.

1.5 Global prevalence of Toxoplasmosis

In the period of five years from January 2011- December 2016, 381 waterborne outbreaks were recorded in the online databases (Artemis Efstratiou *et al.,* 2017).

Serological evidence of toxoplasmosis is present in a significant proportion of world`s population. Prevalence of disease ranges from 0 percent to 90 percent in human population (Dubey, 2010). Seroprevalence is highest in temperate regions and lowest in cold regions.

Prevalence is reflected by the environmental factors that benefit or are inappropriate for the growth of *T.gondii* oocysts. Prevalence of *T.gondii* varies between different ethnicity. Variations in prevalence in ethnic communities are correlated with hygienic behaviors unique to lifestyle, and above all diet (Desmonts, 1961). Epidemiological research demonstrates that in most countries people become infected by consuming undercooked meat containing viable bradyzoites. A serological study of pregnant women from Chile, for example, indicated that 43 percent of seropositive expecting women were also positive for oocysts antigen (Munoz – Zanzi *et al.,* 2010). Complications of toxoplasmosis during gestation can result in spontaneous abortion, perinatal mortality and other multiple congenital defects. A recent study suggested an occurrence of 2 cases in 1000 births of congenital toxoplasmosis. This contributes to approximately 2300 DALYs annually (Kortbeek, 2009). A recent analysis estimates the worldwide seroprevalence of toxoplasmosis in expecting mothers or women of child bearing age ranges from 5.3 percent to 78 percent growing with age (Sadaruddin, 1991). The seropositivity of *T.gondi*i infections in women of child bearing age ranges between 7.7%-76.7% in different countries, France 71% (Jeannel *et al.,* 1988), United kingdom 7.7-9.1% (Allain *et al.,* 1998, Nah *et al.,* 2009), Spain 18.8% (Gutierrez-Zufiaurre *et al.,* 2000), Norway 10.9% (Jenum *et al.,* 1998), Iran 51.9% (Assmar *et al.,* 1997), Sweden 14-25.7% (Peterson *et al.,* 2000),India 45% (Sigh *et al.,* 2004),Nigeria 75.4% (Onadeko *et al.,* 1996), Brazil 50-76 % (Ricciardi *et al.,* 1978).In western region of Turkey seropositivity for anti-Toxoplasma anti IgM in pregnant women is 0.4% and in whole Turkey the overall seropositivity for T.gondii antibodies ranges between 43-85%, and this high seroprevalence is attributed to the presence of high numbers of wander cats and the diet they consume is also rich in wild vegetables, salads and medium to rare cooked meat (Gulden Sonmez Tamer *et al.,* 2009). Among Persian women eating raw and undercooked meat, the highest reported seroprevalence rate is 93 percent (John and Petri, 2007).In Cameron the overall seroprevalence of *Toxoplasma gondii* antibodies in pregnant women were 45.5% in 2019, among this seroprevalence the prevalence of IgM were 9.7% (Guemgne Todjom, 2019). According to new findings in China the seroprevalence of *Toxoplasma gondii* infection rate in women of child bearing age is approximately 10% (GAO, *et al.,* 2012).

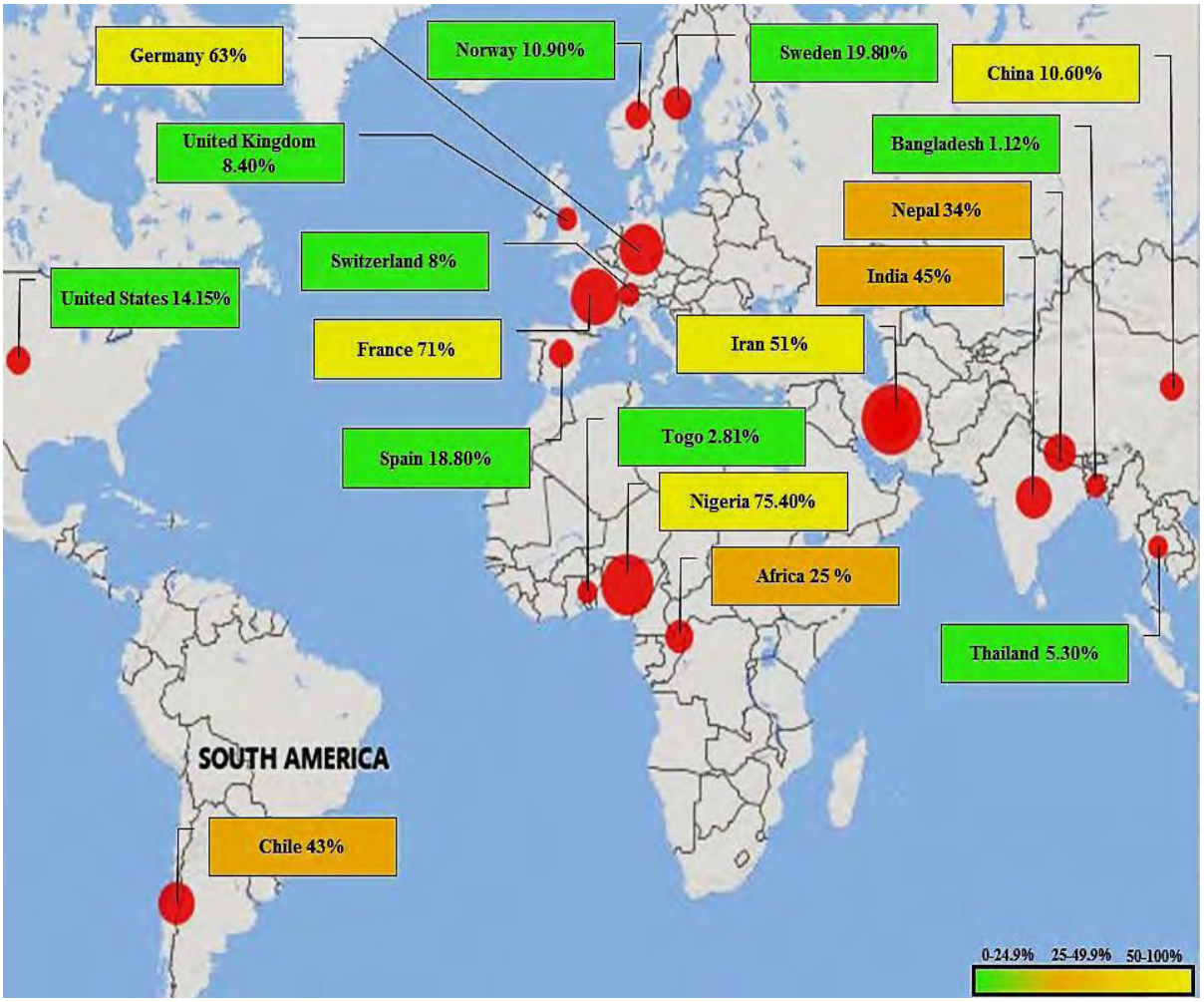


Figure1. 2: Illustrates the Global prevalence of *Toxoplasma gondii* infection in pregnant and non-pregnant women. Prevalence ranges are shown by different color schemes and is indicated in colored legend each color represents different ranges of prevalence given in percentages.

1.6 Prevalence in Pakistan

A met-analysis between cat interaction and the prevalence of antibodies of *T.gondii* was performed and found a conflict while being significant, interaction with cats might not be the most important risk factor of *T.gondii* infection (Wei *et al.,* 2016). The occurrence of *T.gondii* IgG antibodies was found to have a prevalence of 17.6 percent in pregnant and non-pregnant women with and without a background of reproductive problems from Multan, Pakistan. A comparative prevalence of 17.4 percent (Saddaruddin *et al.,* 1991) and 17 percent (Pal *et al.,* 1996) was reported from Islamabad, Pakistan. A significant higher rate of 29.5 percent was found in the general public of Southern Punjab, Pakistan by using latex agglutination test (Tasawar *et al.,* 2012), from Swabi, Khyber Pakhtunkhwa, Pakistan 19.3 percent in pregnant women was reported using latex agglutination test. In Kashmir 47.5

percent of IgM antibodies was prevalent in women with a history of recorded miscarriage (Zargar *et al.,* 1996).

Multiple causes, eating of semi cooked meat, usage of homemade ice could be the possible reason of variations in the results reported from different parts of the world (Asgari Q *et al.,* 2009). The existence of animals may indicate the possibility of a contaminated atmosphere posing a risk to feline and human population, possession of dogs, drinking non boiled goat milk, taking raw vegetables and fruits, trans placentation and organ transplantation, blood donation (Sroka *et al.,* 2010). People working in the fields are found to have higher prevalence as compared to the ones in the urban areas it relates to the degree of exposure, drinking rain water, bad hygiene of the kitchen (Petersen *et al,* 2010).

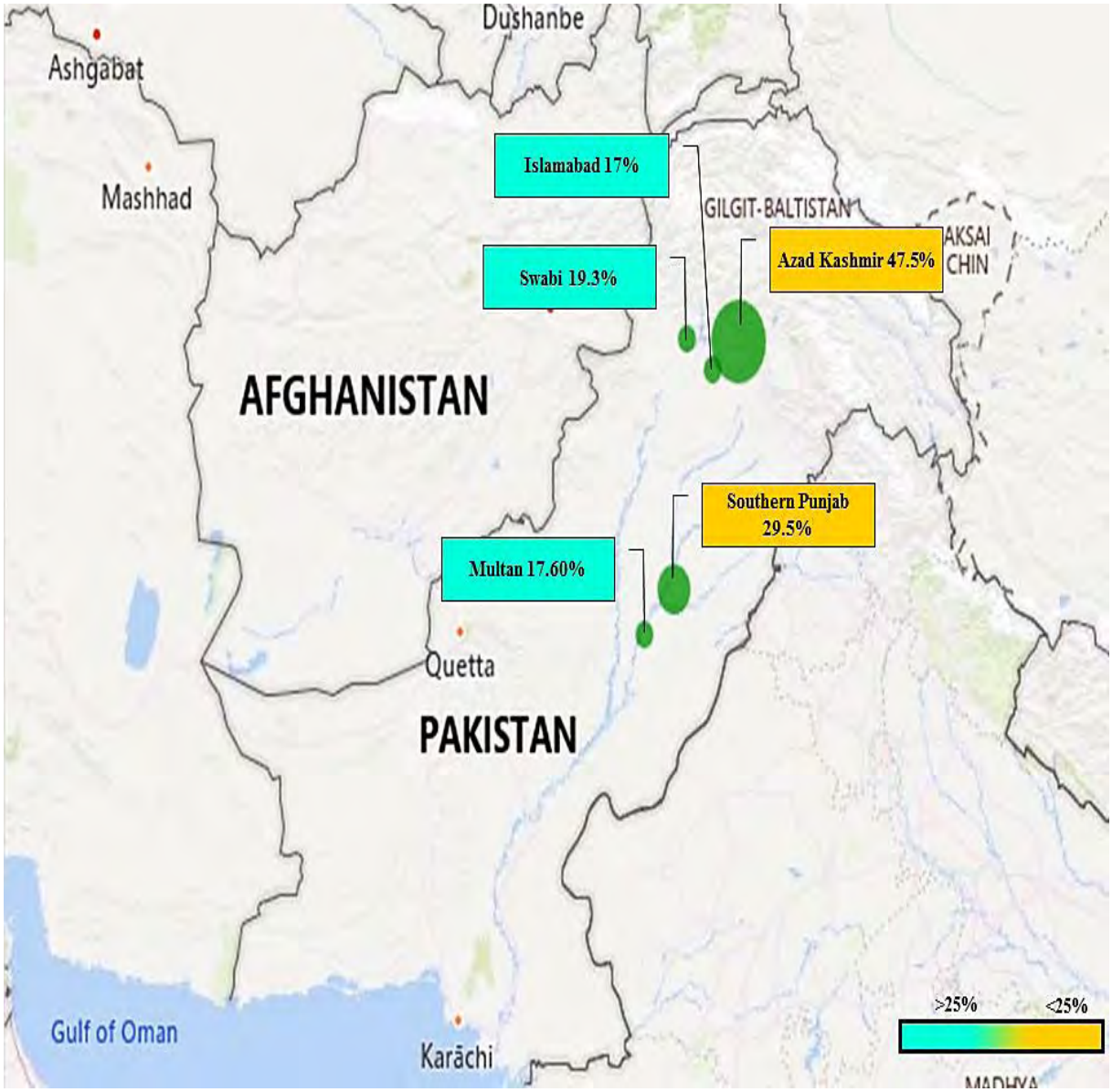


Figure 1.3: Displays the seroprevalence of anti-Toxoplasma gondii antibodies IgM in pregnant and non-pregnant women from different cities of Pakistan using variable approaches.

1.7 Pathology and clinical aspects of Toxoplasmosis

Asymptomatic toxoplasmosis is usually accompanied by lesions which appear symptomatic later. Symptomatic infections may be diagnosed in one of the several stages followed by lesions and clinical patterns relying on when it has been transferred in utero and at the time after the postpartum diagnosis is done (Couvreur *et al.,* 1984).

Toxoplasmosis is commonly acquired by mouth; sources are cysts in meat or through oocysts from cats and soil. The developing embryo becomes infected in utero via umbilical vein and organisms from placenta make their way to the liver first. Congenital disease in premature infants results in a relapsing condition (Frenkel, 1974).

Toxoplasma gondii exists in two different forms in human tissues (i) a proliferative form or tachyzoites (ii) bradyzoites and many intermediated feeding forms known as trophozoites. Bradyzoites develop from the merozoites (Frenkel, 1966). Tachyzoites actively divide and intra cellular tachyzoites can be seen in large number in the vacuoles of host cell, this stage is conveniently known as ―Group stage". Tachyzoites actively multiply when enter a new cell. Bradyzoites multiply slowly and become surrounded by an argyrophilic membrane and transforms into a cyst stage (Frenkel, 1966). Cysts remain persistent for a long period of time, from months to years and support the chronic stage of infection. The change from group stage to cyst stage is usually associated with a mark of immunity (Alfrod *et al.,* 1974).

Majority of immunocompromised individuals affected with *T.gondii* are asymptomatic (Hill, 2005). Lymphadenitis is the most commonly diagnosed type of toxoplasmosis which is followed by many non-specific symptoms (Frenkel, 1998).

The infection risk with congenital toxoplasmosis is lowest in first trimester of gestation and highest in the last trimester, on the other hand if infection occurs in 1-12 weeks of gestation, the disease is more potentially serious (Dubey, 2020).

1.8 Current investigation

The diagnosis of toxoplasmosis is particularly important in four classes of individuals: pregnant women who became infected during conception, embryos and newborns who are congenitally infected immunosuppressed patients and those with ocular pathology (Bastien, 2002).

The infection status of *Toxoplasma gondii* has been detected by different methods. The simple microscopic detection methods have serval drawbacks of low sensitivity, but immunological detection plays a crucial role because it is focused on the detection of antibodies. Many detection methods such as complement fixation test (CFT), immunodiffusion test (ID), indirect hemagglutination test (IHA), various forms of enzyme linked immunosorbent assay (ELISA), Rapid antigen detection system (RDTS), modified agglutination test (MAT), latex agglutination test (LAT), test paper, PCR, isolation methods and histopathology are routinely used detection methods. Among them serological tests are fast, budget friendly and most sensitive and specific but their sensitivity, specificity and predictive values vary (Greiner and Gardner, 2000; Hill e*t al.,* 2006; Dubey, 2008; Dard *et al.,* 2016). According to World Health Organization WHO the standard and the specific test for *T. gondii* detection in human is Sabin and Feldman dye test (DT), but due to certain shortcomings such as it is laborious and requires live parasite many laboratories replaced this with other tests. Dubey developed MAT (modified agglutination test) it has been extensively used for the detection of *T. gondii* antibodies in many species including human (Cubas-Atienzar *et al.,* 2019; Khan and Noordin, 2010, Sabin and Feldman, 1948). Serological and clinical analysis is the main approaches in the diagnosis of *T. gondii*. In the serum of conceiving women *T. gondii* specific immunoglobulin are detected within 14 days of infection, however the serological outcomes cannot differentiate between acute and chronic infections. Acute state of infection is indicated by the presence of IgM antibodies or both IgM and IgG antibodies on the other hand a negative report suggests either a recent infection or no infection at all (Dubey *et al.,* 1995, 1996, 2015; Gamble *et al.,* 2005; Hill *et al.,* 2006; Gardner *et al.,*2010). Additional confirmatory assays such as seroconversion and IgG avidity tests are recommended because IgM can remain detectable in the serum after the acute infection has ended (Dubey, 2010).

It is very important to choose the appropriate method of diagnosis at the affordable level (Stillwagon *et al.,* 2011), however the approach chosen is influenced by the guidelines given for adoption and the prevalence rate of a country as well as on the prevailing clinical situation such as in expecting ladies, immunocompromised patients or the ones with lower vision. Performance of the diagnostic methods varies from the old detection approaches to the most recent ones such as immunoblot (Villard *et al.,* 2016).There are two main categories of the diagnostic methods one that are low on expenses and can be used on the limited to small amount of serum these are the screening methods which include methods such as

hemagglutination and agglutination and larger screening methods such as ELISA (enzyme-linked immunosorbent assay) and CLIA (Chemiluminescence immuno assay), second method is confirmatory tests such as dye test, immunoblot and immunoglobulin M immunosorbent agglutination assay (ISAGA) these methods are complex and expensive and are mostly referred in reference centers (Villard *et al.,* 2016).

In our study we used the fast screening method ELISA for the serological detection in order to interpret the prevalence scenario in Khyber Pakhtunkhwa. Our respondents include the women of child bearing age from different backgrounds. Our approach was to extend our study to the level where different factors were also taken in account in order to know the risk factors associated with the transmission and the spread of the parasite.

Objectives:

- Serological detection of anti-*Toxoplasma gondii* IgM antibodies in pregnant and non-pregnant women from the districts of Khyber Pakhtunkhwa, Pakistan.
- To evaluate the risk factors associated with prevalence of *Toxoplasma gondii* in women of childbearing age.

MATERIALS AND METHODS

2.1 Sampling Area

A planned study was designed in which blood samples were collected between February 2020 to March 2021 from women visiting different health centers and diagnostic laboratories of four districts of Hazara division and one district of Peshawar Division, Khyber Pakhtunkhwa. Districts are Abbottabad, Battagram, Haripur, Mansehra and Peshawar (Fig 2.1).

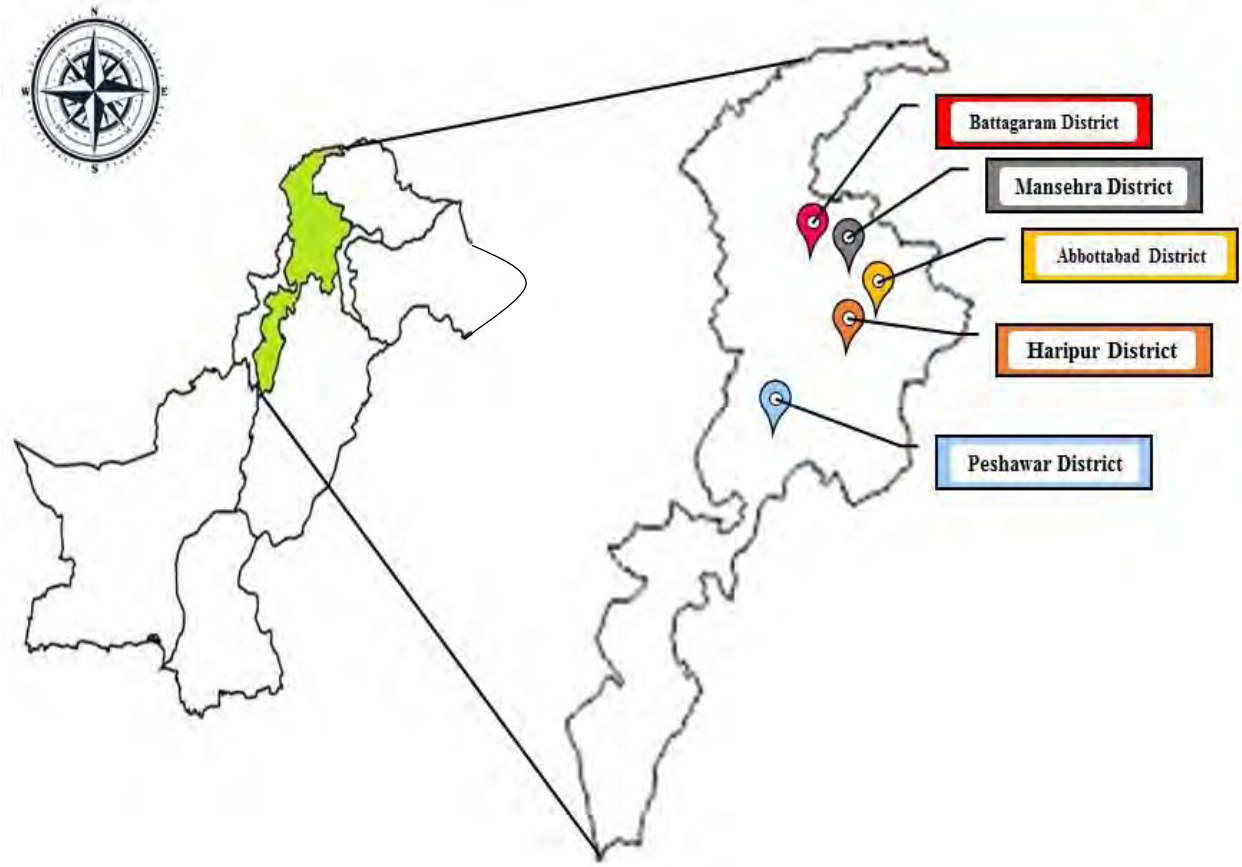


Figure 2. 1: Map of Pakistan where Khyber Pakhtunkhwa province is highlighted in green, and further, the districts from where the samples were taken are shown via navigation icon.

2.1.1 Abbottabad district

This district occupies an area of 1969 km^2, with the city of Abbottabad being its main hometown. It is Pakistan's 40^{th} largest city and by population it ranks 6^{th} in Khyber Pakhtunkhwa. At an altitude of 1,256m, it is about 120km north of Capital and Rawalpindi. Climate is humid with moderate to warm temperatures during spring and autumn seasons, snowfall occurs periodically in December and January, but it is scarce, heavy rainfall occurs

during monsoon sometimes causing flooding in the lower parts of the region. On average Abbottabad has a literacy rate of around 56 percent. Abbottabad, with a combination of historical and contemporary cultures, is a well cultured city. Islamic practices are very common in rural areas. People work in fields and consume vegetables but eating habits also vary with the locality. People belonging to this area love eating out as it is the only source of entertainment for them. Majority of people keep domestic animals and consume fresh dairy products, poultry products, and meat.

2.1.2 Battagram district

District with fertile soil and rich in mining reservoir is located at latitude of 34.41 and longitude 37.1 in Khyber Pakhtunkhwa province. To its north lies Kohistan district. Many of the immigrants reside in this district with lots of diversity in their cultural norms. Temperature in summer is quite high and in winters it lows to various degrees.

2.1.3 Haripur district

This district of Hazara division is rich in natural resources, having two dams Tarbela dam and Khanpur dam. Territorially it is a gateway to Hazara division and the federal capital of Islamabad. Its urban areas are occupied by 12.0% of people while 88.0% people reside in rural areas. Agriculture is the main livelihood of the population. The literacy rate is 53.7%, significantly greater than the literacy rate in the Hazara zone which is 35.2%. Male literacy rate is higher as compared to the females. Summers are extreme hot and winters are mild cold.

2.1.4 Mansehra district

This district is blessed with much natural beauty surrounded by many beautiful valleys which are the tourist destinations. Hindus and Sikhs were dominant population before indo Pak separation now Muslims dominate this region. There is lots of divergence in mode of living, traditions and occupation among people. Summers are very hot and winters are extreme cold.

2.1.5 Peshawar district

Peshawar district is the capital of Khyber Pakhtunkhwa. Geographically it is located about 160km west of Islamabad. Climate is semi-arid with very hot summers and mild winter .Many civilizations had a rise and fall in this district, so it has an enriched history. Literacy wise it is quite better but the education of girls is still a problem in this district. According to 2017 census its population is 4269079. Mostly females stay at home. Males work in divergent fields.

Table 2.1: Demographic features of study areas.

Study area (Districts)	**Location** (Longitude and Latitude)	**Altitude** (masl)	**Annual mean rainfall** (mm)	**Mean temperature**
Abbottabad	34.1688° N, 73.2215° E	1256	1262	18.0 °C
Battagram	34°41'N 73°1'E	1038	1218	18.5 °C
Haripur	33.9946° N, 72.9106° E	520	1267.5	28 °C
Mansehra	34.3313° N, 73.1980° E	1088	376	25.6 °C
Peshawar	34.0151° N, 71.5249° E	331	384	25 °C

2.2 Ethical considerations

Institutional Review Board of Quaid-i- Azam University, Islamabad gave approval for the conduction of the study; much cooperation was shown many health centers and laboratories.

2.3 Research design

A population based cross-sectional study was structured to find out the seroprevalence of antibodies IgM of *Toxoplasma gondii.* Inclusion and exclusion criteria are adopted in the step wise procedure of the study summarized in Fig 2.3. A total of 500 patients gave blood samples willingly, among them 423 samples were selected for immunodiagnostic assay and 77 samples were rejected due to several reasons.1. Blood sample was not sufficient to be considered for further processing 2. Some samples were not properly labelled. 3. Some samples were in duplicates. Summary of the respondents in our study were given in figure 2.2

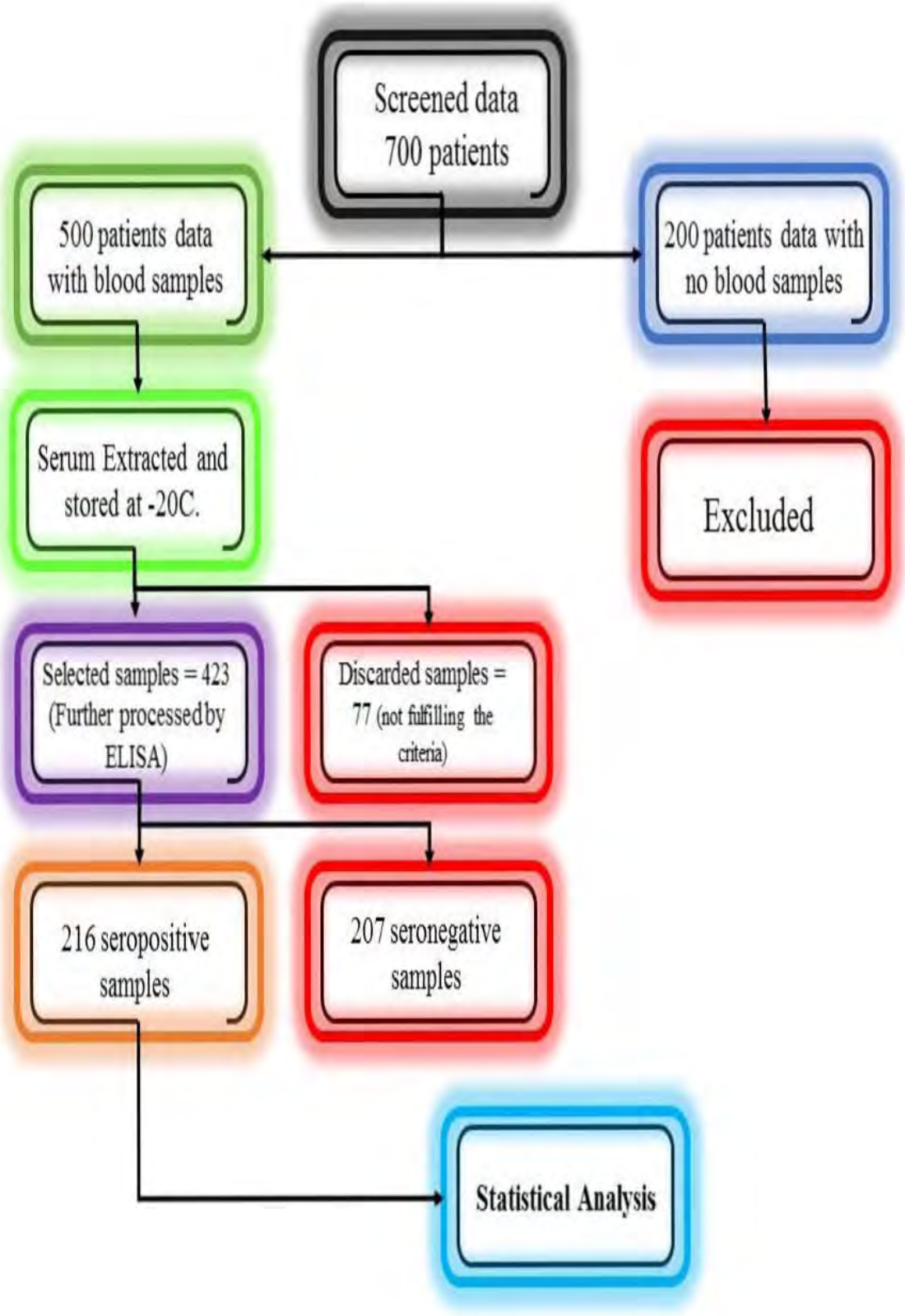


Figure 2. 2: Flow chart diagrammatic representation of studied population and diagnostic assay.

2.3.1 Study subjects

The study was planned and carried out only on women of child bearing age reporting to local health centers and diagnostic laboratories. These women aged between 18-47 years. All subjects with complaints of fever, headache, sore throat, muscle aches and pain were included in the study.

2.3.2 Sample size determination

Sample size was determined using single population proportion formula. Confidence level was taken as 95% (z=1.96) and 5% (d=0.05) as marginal error and the initial sample size was calculated as 202. Formula used for sample size determination was $n=Z^2P\ (1-P)/d^2$, here n=Sample size, Z is level of confidence, P is expected prevalence, and d is the precision.

2.3.3 Sample collection and processing

5ml of venous blood sample each from 423 patients were taken in non EDTA vacutainers. The collected blood samples were carried to the diagnostic parasitology laboratory in the ice boxes. Sera were than separated and collected in Eppendorf tubes from the blood samples by centrifugation process (3000 rpm for 10 mints) and stored at -20^0C until used for serological (ELISA) and biochemical assays.

2.4 Serological diagnostic assay

By using immunodiagnostic technique total anti *T. gondii* antibodies IgM were detected. All the stages were performed at room temperature and sera were tested at 1:100 dilution. The cut off was set by the mean optical density (OD) of the negative reference serum, addition to three times standard deviation. Serum samples with OD =0.80 were taken as positive.

2.4.1 Anti-*Toxoplasma gondii* IgM detection assay

Commercial Enzyme-Linked immunosorbent assay (ELISA) kit (ab108778- Anti-*Toxoplasma gondii* IgM Human ELISA kit) was used to detect anti-*Toxoplasma gondii* IgM antibodies from serum samples collected from women of childbearing age. Whole kit was used without any breakage. The first well was left for substrate blank, in second well 100µL of negative control was added followed by the addition of 100µL positive control in third well and then the 100µL diluted samples were then added with much care in rest of the wells. Then all the loaded wells were covered by the foil supplied with the kit and the plate was then incubated for 60 minutes at 37ºC.After 1 hour all the contents of the wells were removed, and wells were washed three times with 300µL of 1X Washing Solution. Each well was decanted after the last wash to remove the remaining 1X Washing Solution. Excess liquid was removed by inverting and blotting the plate against the paper towel many times. Then 100µL

of *Toxoplasma gondii* HRP Conjugate was added in all the wells except the first blank well and covered with the foil and incubated for 1 hour at room temperature away from direct sunlight. After an hour the wells were again washed three times with 300µL of 1X Washing Solution and the plate was decanted. 100µL of TMB Substrate Solution was then added into all the wells followed by incubation for sharp 15 minutes at room temperature in the dark. After 15 minutes of incubation on observation blue color appears in the wells and then 100µL of Stop Solution was added into all the wells with the same rate and order in which the TMB Substrate solution was added, yellow color develops immediately in all the wells in which blue color was seen. Highly positive samples developed dark precipitates of the chromogen. The plate was then placed in ELISA (AMP Platos R 496 AMEDA Labordiagnostik) reader for OD reading at 450 nm within 30 minutes of addition of the Stop Solution. Results were interpreted following the manufacturer's instructions. Samples with an absorbance value of less than 10% above or below the Cut-off control value were taken as inconclusive (GREY ZONE) i.e., neither positive nor negative. Samples were marked negative if the absorbance value was lower than 10% below the cut off. Samples with OD=0.80 were considered as positive.

2.5 Data Management and Statistical Analysis

All data generated from this study was maintained in Microsoft Excel (2013) and statistical analysis was carried out in SPSS version 20.0. Statistical methods included were percentages for categorical variables, while mean $\pm$ Standard Deviation for numerical variables. Chi-square test and the odds ratio were computed to measure the strength of association. The OD values of anti- *Toxoplasma gondii* IgM for each sample were presented in graphs by using GraphPadPrismV9.1.0.

RESULTS

3.1 Attributes of study respondents

Participants of the study belonged to five districts of Khyber Pakhtunkhwa, Pakistan. Total 423 women of child bearing age were included in our study. These women from each districts were then divided into two categories as pregnant 265 (62.64%) and non-pregnant women 158 (37.35). Pregnant ladies were then further classified on the basis of their gestational periods as 1st trimester (10.94%), 2nd trimester (28.30%) and 3rd trimester (60.75%) respectively. Other parameters such as age group of the suspected ladies, and their profession was also taken in account in our study. Further four age groups were made and the average (SD±) age of subjects was 29(±7.51), and most of the respondents in our study were housewives.

3.2 Overall seroprevalence of anti T. gondii IgM antibodies

A total of 423 serum sample of suspected cases were examined by ELISA. To assess the diagnostic efficiency of human specific *T. gondii* IgM ELISA sensitivity was recorded 95.8% and its specificity was 98%. Diagnostic performance of the assay is given in (Table 3.1). The antibody titer exceeded the cut off value in all the infected women (OD>0.80). The overall percentage of seropositivity of *T. gondii* IgM antibodies of women of childbearing age was 56.26% (238) and seronegative was 43.73% (185).

Serological results obtained from *T. gondii* suspected as well as control samples showed least difference in optical density (OD) between *T. gondii* positive and negative sera and the mean (±SD) OD values of pregnant and non-pregnant women are shown in (Fig. 3.1)

The percentage of seropositive pregnant women was 34.70% (147), while seropositive non pregnant women were 21.51% (91) (Fig. 3.2ab). The results indicated that the pregnant women are more infected than non-pregnant women and the significant association was observed (P= 0.001, χ^2=27.06) and (Table 3.2).

Table 3.1: Diagnostic performance of the *Toxoplasma gondii* IgM ELISA test.

ELISA	*Toxoplasma* suspected N (%)	Negative control N (%)	Positive control N (%)*
+	238 (56.26)	0 (0)	6 (100)
-	185 (43.73)	6 (100)	0 (0)
Total Number	423	6	6

*Parentheses represent percentages

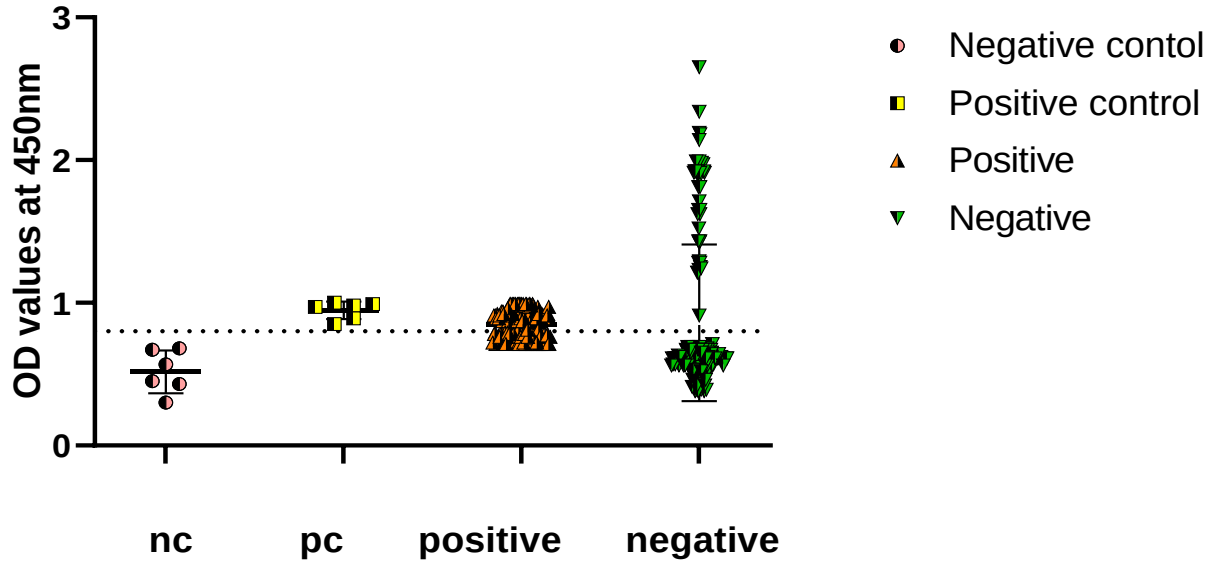


Figure. 3.1. Mean OD at 450 nm obtained with the *Toxoplasma gondii* IgM ELISA test in women of childbearing age population with control sera (Positive control (pc); (Negative control (nc), *Toxoplasma* positive and negative. A sample is considered positive when the absorbance value is above 10% of Cut-off. The results were negative if OD <0.70, positive if OD >0.80.

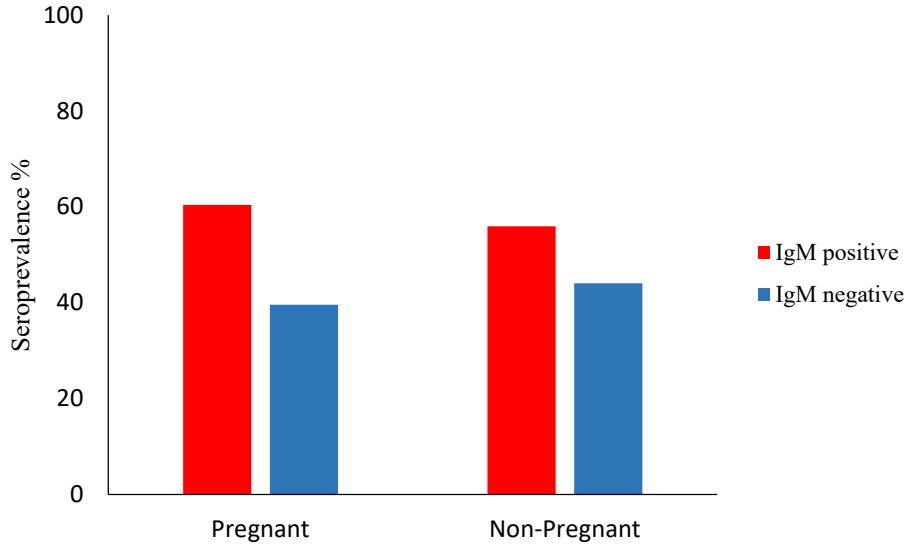


Figure 3.2a: Total percentage of seropositive and seronegative anti-*T. gondii* IgM antibodies in in pregnant and non- pregnant women

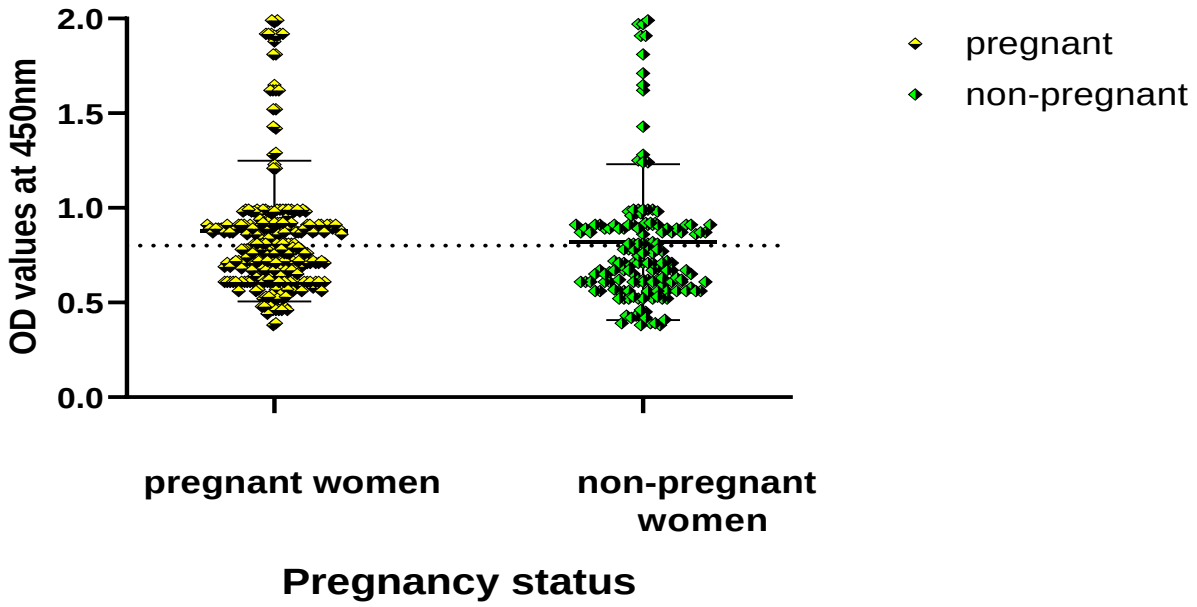


Figure: 3.2b: Scatter plots illustrating the mean SD and ranges of OD (450nm) obtained from *T. gondii* IgM ELISA test according to two categories of women of childbearing age.

Table: 3.2 Results of logistic regression model to identify seroprevalence of *Toxoplasma gondii* IgM antibodies according to pregnancy status.

Characteristics	Positive n%	Negative n%	χ^2	Odd ratio	95% CI		p-value
Pregnancy status					Lower	Upper	
Pregnant	147(34.75)	116 (27.4)	27.06	0.32	0.292	0.410	0.001**
Non-pregnant	91 (21.51)	69 (16.31)		Reference			

** Significant association

3.3 Seroprevalence of *T. gondii* according to age groups

The results indicated highest percentage of seropositive anti-*T. gondii* IgM antibodies in pregnant women 42% (n=58) observed at age 25-29 years followed by 20-24 years 36.9% (n=51), 30-34 years age group 10.86% (n=15), 35-39 years,15-19 7.97% (n=11) years and the lowest total percentage observed was 2% (n=3) at age group of >40 years (Fig. 3.3a)

The highest prevalence of seropositive anti-*T. gondii* IgM antibodies in non-pregnant women were found in age group >40 years 41.4% (n=29) followed by age group 25-29 18.57% (n=13), 30-34 14.28% (n=10), 20-24 12.85% (n=9), 35-39 10% (n=7) while lowest prevalence was 2.85% (n=2) at age group of 15-19 years (Fig. 3.3a). In our results the age factor was found statistically non-significant (p=0.842, χ^2=2.099) among suspected women of childbearing age (Fig. 3.3b and Table 3.3).

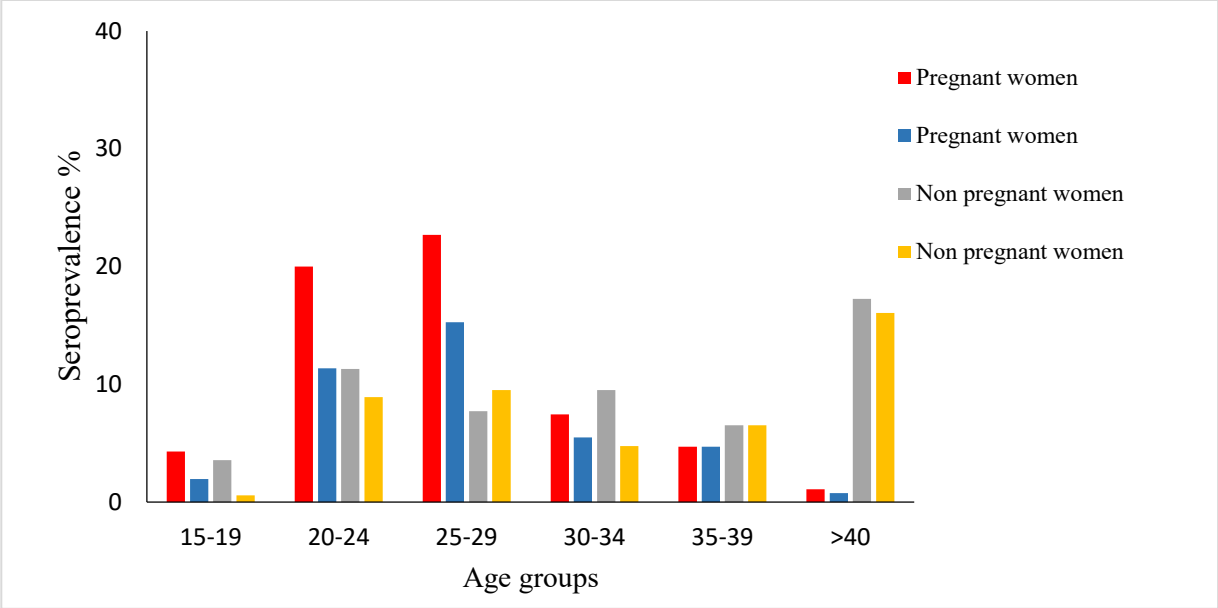


Figure 3.3a: Seroprevalence of anti-*T.gondii* IgM antibody among different age groups.

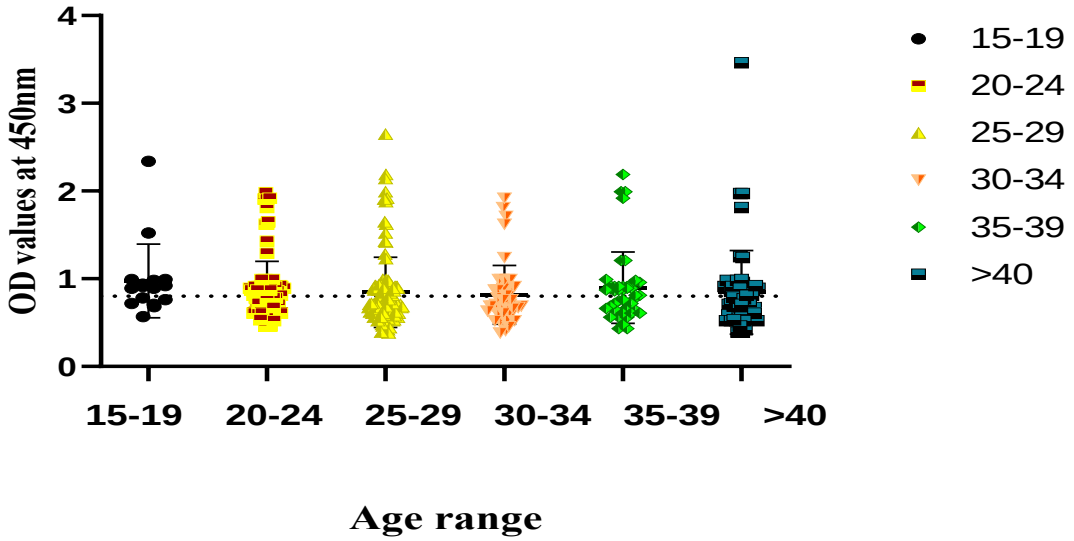


Figure 3.3b: Scatter plots showing the mean, SD and ranges of OD (450nm) obtained by *T. gondii* IgM ELISA test according to different age group.

Table 3.3: Results of logistic regression model to identify seroprevalence of *Toxoplasma gondii* IgM antibodies, according to different age groups in women of child bearing age

Characteristics	Positive n%	Negative n%	χ^2	Odd ratio	95% CI		p-value
Age groups in years					Lower	Upper	
15-19	13 (3.07)	6 (1.41)	2.099	Reference			
20-24	60 (14.18)	48 (11.34)		1.882	0.643	5.507	0.249^{NS}
25-29	71 (16.78)	62 (14.65)		1.104	0.605	2.012	0.748^{NS}
30-34	25 (5.91)	30 (7.09)		1.008	0.566	1.796	0.977^{NS}
35-39	32 (7.56)	19 (4.49)		0.903	0.439	1.856	0.782^{NS}
>40	37(8.74)	20 (4.72)		0.914	0.418	1.998	0.822^{NS}

^{NS} non-significant

3.4 Seroprevalence status in different trimesters

As determined by ELISA the total percentages of seropositive anti-*T. gondii* in pregnant women according to trimesters are 4.90% (n=13) in 1st trimester, 17.7% (n=47) in 2nd trimester and 33.20% (n=88) in 3rd trimester (Fig 3.4a). Higher number of expecting ladies was observed in the third trimester.

Present study showed that, seropositivity of *T. gondii* infection had statistically no significant association with stage of gestation (p=0.185, χ^2=3.432) as the level of significance was found p>0.05 (Table 3.4). OD (450nm) SD, mean values computed for *Toxoplasma gondii* IgM level between stages of gestation is given in Fig. 3.4b.

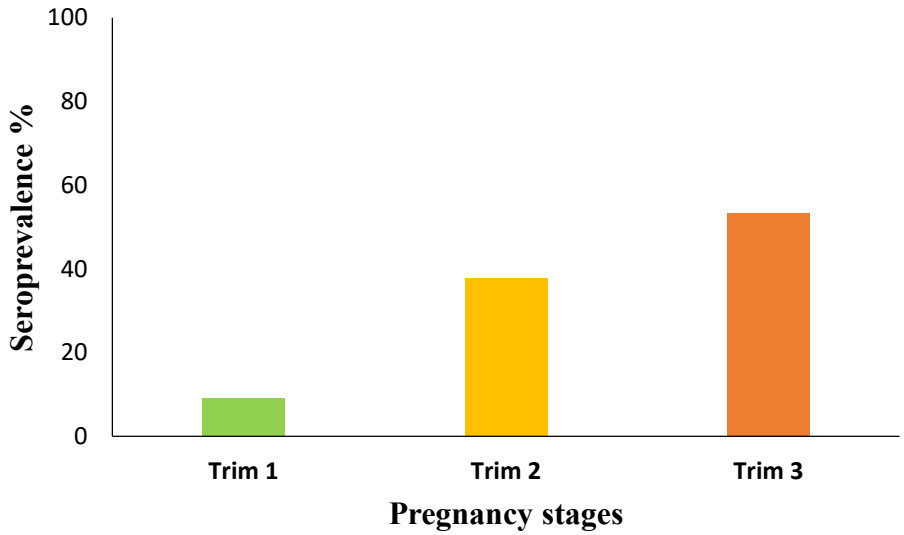


Figure: 3.4a Seroprevalence positivity status at different stage of pregnancy predicted via ELISA.

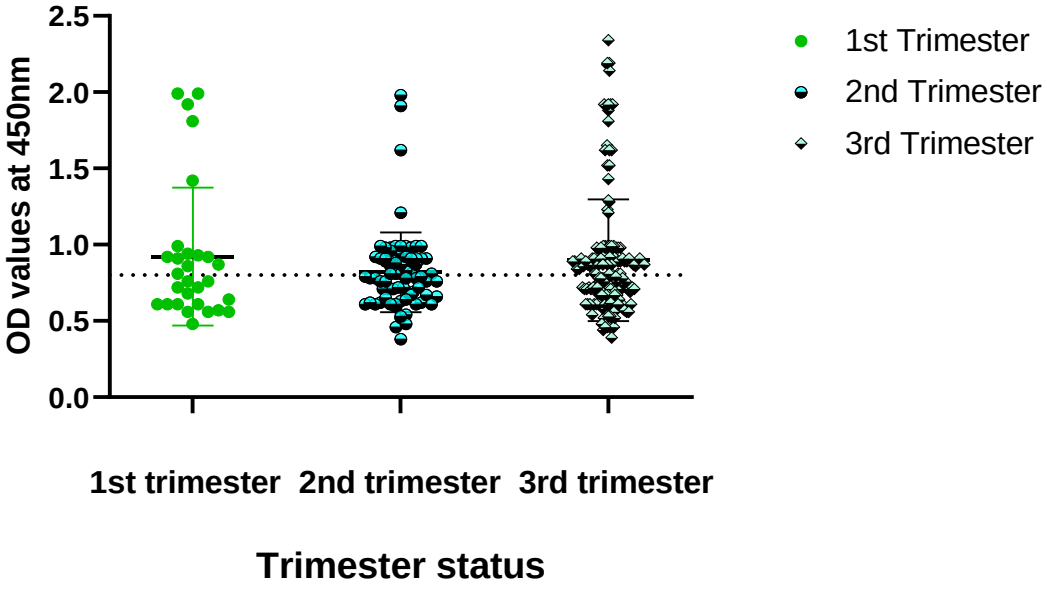


Figure: 3.4b Seroprevalence status in trimesters obtained through *T. gondii* IgM ELISA represented by scatter plots showing mean, SD and ranges of OD values (450 nm).

Table 3.4: Results of logistic regression model to identify the seroprevalence status of *Toxoplasma gondii* IgM antibodies, according to stages of pregnancy.

Characteristics	Positive n%	Negative n%	χ^2	Odd ratio	95% CI		p-value
Pregnancy stage					Lower	Upper	
Trimester 1st	13 (4.90)	16 (6.03)	3.432	Reference			
Trimester 2nd	47 (17.7)	28 (10.56)		0.657	0.297	1.456	0.301^{NS}
Trimester 3rd	88 (33.20)	73 (27.52)		1.438	0.818	2.530	0.207^{NS}

^{NS} non-significant

3.5 Seroprevalence according to professions

According to profession the highest percentage of seropositive women of childbearing age was found to be housewives 29.53% then 16.54% teachers, 8.4% student followed by and 3.87% nurses and least percent of seropositive women was banker 2.42% (Fig. 3.5a).

Results showed statistical non-significant association among professions (p=0.227, χ^2=6.10) as level of significance was found p>0.05 (Table 3.5) The OD (450nm) mean and SD computed for *T. gondii* IgM level among professions is given in (Fig. 3.5b)

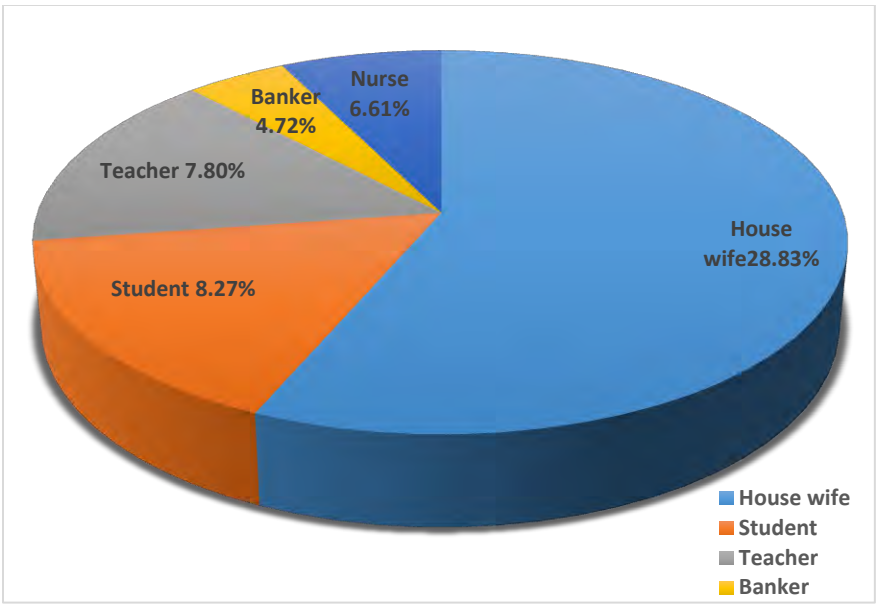


Figure 3.5a Seropositivity status shown by a pie chart diagram.

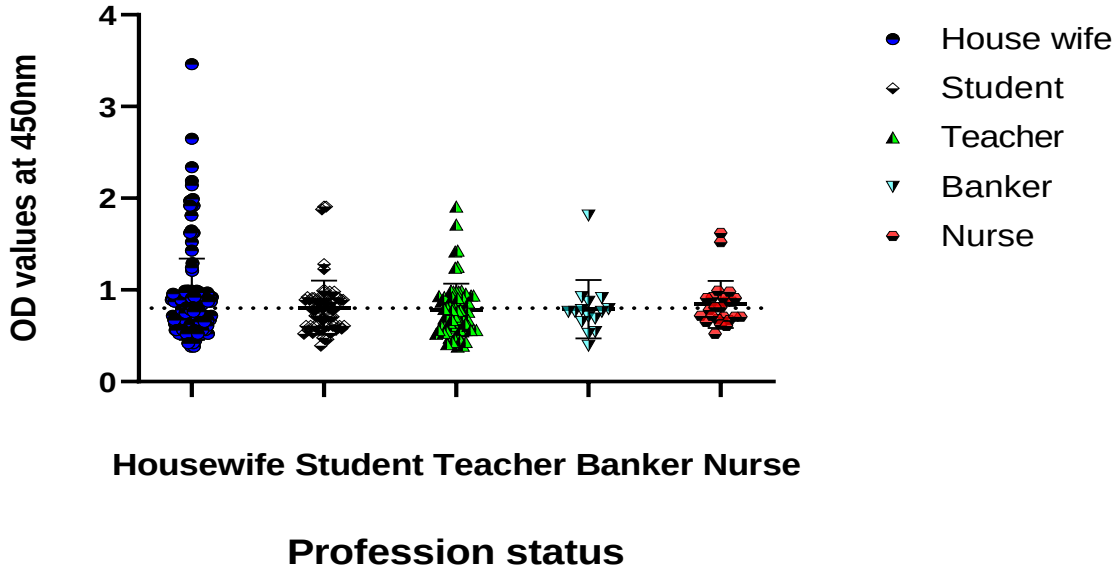


Figure 3.5b Scatter plots illustrating the mean SD and ranges of OD (450nm) obtained from *T. gondii* IgM ELISA test according to profession.

Table 3.5: Results of logistic regression model to identify seroprevalence of *Toxoplasma gondii* IgM antibodies, according to profession.

Characteristics	Positive n%	Negative n%	χ^2	Odd ratio	95% CI		p-value
Profession					Lower	Upper	
Housewife	122 (28.8)	124 (29.3)	6.10	Reference			
Student	35 (8.27)	21 (4.96)		0.492	0.203	1.192	0.116^{NS}
Teacher	33 (7.80)	26 (6.14)		0.581	0.219	1.540	0.275^{NS}
Banker	20 (4.72)	6 (1.41)		0.459	0.175	1.210	0.115^{NS}
Nurse	28 (6.61)	8 (1.89)		1.375	0.331	5.716	0.661^{NS}

^{NS} non-significant

3.6 Seroprevalence of anti- *T. gondii* IgM antibody among different districts

According to ELISA findings district Mansehra had the highest seropositive cases 13% (n=55) equally out of total 238 positive cases, followed by 12.05% (n=51) Abbottabad, Battagram 12.76% (n=54) and Peshawar 10.16 % (n=43) and least number of cases were observed in 8.27% (n=35) Haripur (Fig 3.6a).

Present study estimated that seropositivity of *T. gondii* infection in women of childbearing age had statistically significant association among five districts of Khyber Pakhtunkhwa (p=0.045, χ^2=9.98) as significance level is less than 0.05 (Table.3.6). The OD computed for *Toxoplasma gondii* IgM level between districts is given in Figure 3.6b.

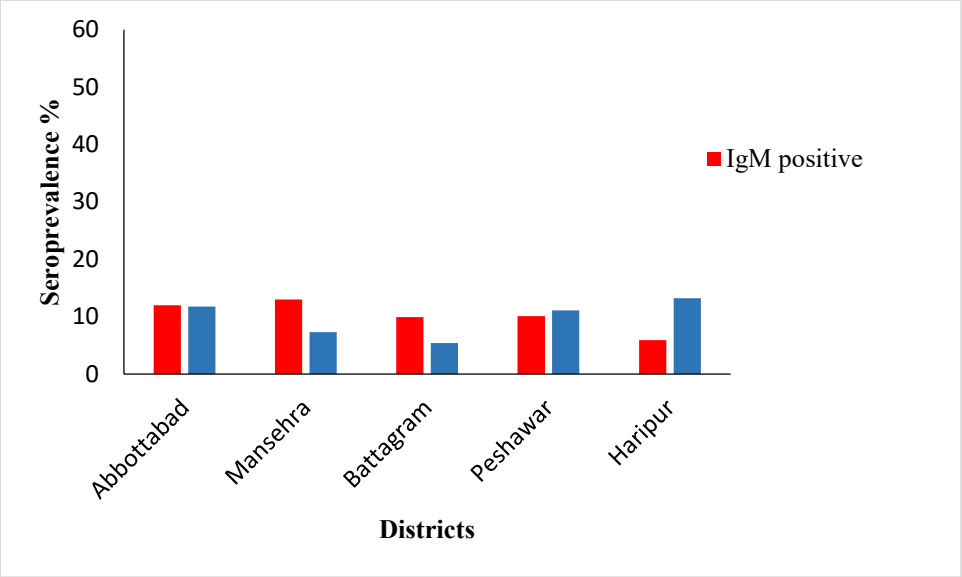


Figure 3.6a: Seroprevalence of anti-*T.gondii* IgM antibody among different districts predicted via ELISA in KP.

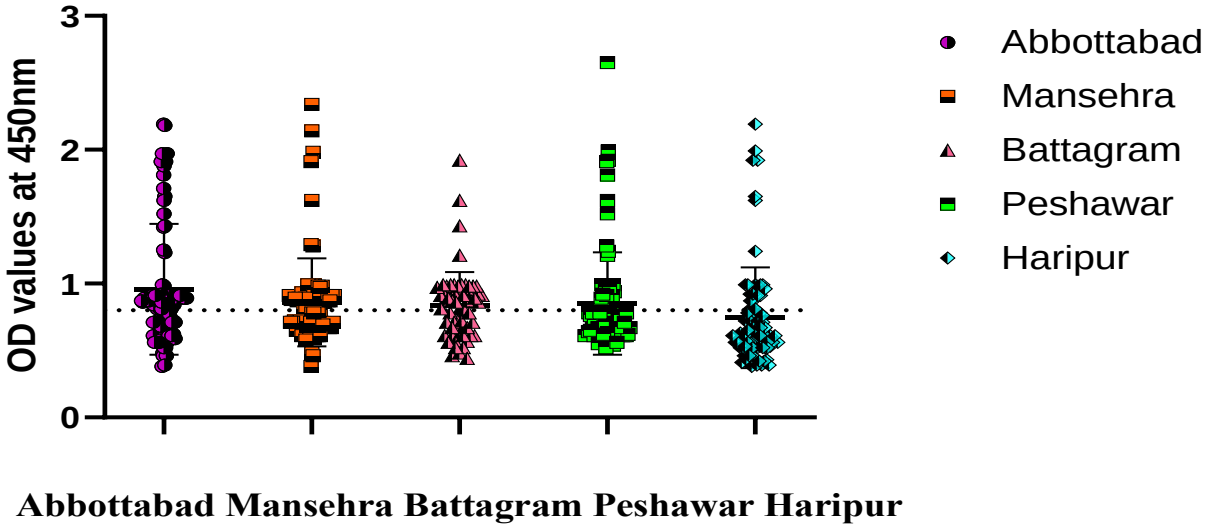


Figure 3.6b Scatter plots illustrating the mean SD and ranges of OD (450nm) obtained from *T. gondii* IgM ELISA test according to the districts.

Table 3.6: Results of logistic regression model to identify seroprevalence of *Toxoplasma gondii* IgM antibodies, according to districts in KP.

Characteristics	Positive n%	Negative n%	χ^2	Odd ratio	95% CI		p-value
Districts					Lower	Upper	
Abbottabad	51 (12.0)	50 (11.8)	9.982	Reference			
Mansehra	55 (13)	31 (7.3)		1.190	0.658	2.151	0.565^{NS}
Battagram	54 (12.76)	22 (5.2)		2.005	1.076	3.739	0.029^{*}
Peshawar	43 (10.1)	47 (11.1)		2.181	1.111	4.280	0.023^{*}
Haripur	35 (8.27)	46 (10.87)		1.092	0.596	2.002	0.211^{NS}

^{NS} non-significant

*significant association

Discussion

The suspected subjects of our study were pregnant women and women of child bearing age from Khyber Pakhtunkhwa province, Pakistan. Studies found that *Toxoplasma gondii* causes moderate infections in non-pregnant ladies, while in pregnant women have drastic impacts, especially on the fetus (Liu *et al.,* 2015). Congenital defects, deficits in the intra uterine development and foetal death are the results of congenital intra-uterine infections which contribute to both economic and social burdens (Wam *et al.,* 2016). Identification of these infections in both the mother and fetus is the most crucial component of prenatal care. Routine prenatal diagnosis of certain infections including *T. gondii* is advised during first trimester likewise we have conducted during our study, since primary infections can develop in the patients who are seronegative, which has the risk of vertical transmission to the fetus (Kuo Zhang *et al.,* 2016).

Present study recorded seroprevalence of *T. gondii* IgM antibodies were 56.26% from five study units which is significant to the reported global seroprevalence trends, as seroprevalence of *Toxoplasma gondii* infections in women of child bearing age ranges between 7.7%-76.7% in different countries of the world, India 45% (Sigh *et al,* 2004)., Nigeria 75.4% (Onadeko *et al.,* 1996), Brazil 50-76 % (Ricciardi *et al*., 1978), France 71% (Jeannel *et al.,* 1988), Iran 51.9% (Assmar *et al.,* 1997), Turkey 43-85% (Tamer *et al.,* 2009) and non-significant to the reported seroprevalence in United kingdom 7.7-9.1% (Allain *et al.,* 1998, Nah *et al.,* 2009), Spain 18.8% (Gutierrez-Zufiaurre *et al.,* 2000), Norway 10.9% (Jenum *et al.,* 1998), Sweden 14-25.7% (Peterson *et al.,* 2000).

The results showed higher prevalence rate for *Toxoplasma gondii* in pregnant ladies 34.70% as compared to the non-pregnant women 18.6% and the difference was statistically significant in our study. This could be attributed to the fact that lifestyle such as frequent contact with the soil, inhabitation, and eating habits such as consumption of undercooked meat and raw vegetables could be the possible

associated factors (Shahid *et al.,* 2011). One possible reason for such a high number of seropositivity in pregnant ladies could be lack of awareness about the parasite, its transmission, prevention and control (Gebremedhin *et al.,* 2013). Due to weak immunity during early pregnancy possess a high risk factor of getting infection (Gebremedhin *et al.,* 2013).

According to age factor more pregnant women of 20-29 years of age group (42%) were seropositive for *Toxoplasma gondii* anti- IgM antibodies from all districts in the present study followed by 20-24 years of age group (36.9%) and the lowest number was observed in >40 years of age group (2%). Similar findings were reported in which same age group women were more infected in their study which can be attributed to the fact that young ladies of 20-29 years of age group in these districts get marry in this age have active lifestyle which increases their exposure to the environment with high density of contamination or to the fact that during pregnancy might be the consumption of more fresh but unhygienic fruits and vegetables can be the factor of high seropositivity, this factor need to be studied (Susann *et al.,* 2010). Study carried out in Iraq is also in agreement with our study in which the pregnant women of same age were highly seropositive which can be ascribed to the similarity in consumption similar kind of food (Abdullah *et al.,* 2017). Our study results differs from the study carried out in Turkey where more pregnant women of >40 years of age group were seropositive (Tamer *et al.,* 2009). This aspect can be assigned to the varying degree of exposure to the oocyst of *Toxoplasma gondii* among different age groups.

In non- pregnant women the highest prevalence was observed in women of >40 years of age group (41.4%), which is in agreement with the study carried out in Togo and the reason for such high seropositivity with increase in age is referred to the certainty that women of older age group might have frequent cumulative exposure with the contaminated environment and to the level of immunity which decreases with the age (Amivi *et al.,* 2018).

According to trimester higher percentage of seropositivity was observed in women at 3rd trimester of gestation period followed by 2nd trimester and 1st trimester,

which is in agreement with other studies in China (Wong *et al.,* 1994). On the other hand our results are contrary to the research carried out in Iraq where more pregnant women at 1st trimester were seropositive (Abdullah *et al.,* 2017).One possible reason of this varied results can be the unequal level of exposure with the contaminated environment, our results also shows that there are less chances of drastic outcomes as less women in 1st trimester are infected but still this factor need further investigation in order to minimize the number of infected ladies during their gestation period. Our results were statistically non-significant among gestational stages which is in agreement with the studies carried out in Iraq, Yemen, Ethiopia (Pappas *et al.,* 2009))(Endris *et al.,* 2014).

Among profession housewives showed increased seropositivity, which could relate with more contact with soil, consumption of raw or improperly cooked meat or vegetables or who do not follow basic hygienic practices in daily routine (Tenter *et al.,* 2000).Statistically non-significant association was found among the professions in our study. The results of our study were when compared with the already evaluated studies carried out by Kadhim and Mohammed, 2013 has similar findings. Zargar *et al.,* 1996 reported the findings about the seroprevalence of anti *T.gondii* IgM antibodies in relation with the profession and found that more women were housewives which correlate with our study. Socio demographic profile of the study conducted in Saudi Arabia matched with our findings more women were housewives and other were working ladies by profession (Mona *et al.,* 2012)

When a comparative analysis was carried out more women from Mansehra district were seropositive followed by Abbottabad, Battagram, Peshawar and Haripur. Seroprevalence of infection with *Toxoplasma gondii* is governed by the traditional, cleaning and eating habits and by the environmental and the climatic conditions of the region (Susann *et al.,* 2010). High seroprevalence in women of child bearing age reported from central and southern America has similar climatic conditions when compared to the environmental surroundings of the districts of our study (Remington *et al.,* 2001). This can be the possible reason that the similar eating habits and environmental conditions which aid in

the maturation and survival of the oocyst and as a result contribute to the high seropositivity of the anti- *T. gondii* antibodies in human population.

Conclusion

The results of our study showed a high seroprevalence 56.26% of anti-*Toxoplasma gondii* antibodies in women of child bearing age and this indicate that toxoplasmosis is a public health concern especially in women population of Khyber Pakhtunkhwa. Although *Toxoplasma gondii* is considered harmless in non-pregnant women whereas in pregnant ladies it possess serious health problems on the foetus which as a result increases the social economic burden of the society. The findings of this study should encourage the country's health officials to develop sensitization and prevention programs for women of all age groups and to conduct awareness programs at all health centers so that women which are unaware of the parasite and its propagation should at least have some knowledge in order to escape the drastic outcomes. Further investigations are needed in order to know the exact risk factors associated in the propagation of Toxoplasmosis in Khyber Pakhtunkhwa.

Future recommendations

Toxoplasmosis is potentially a dangerous disease, the deep understanding of *Toxoplasma gondii* infection may provide many new research areas for its prevention and control. Understanding the application of advance molecular markers one can achieve the timely detection especially in the pregnant females which are at higher risk and fetus can be prevented from serious outcomes. Early detection and inactivation or destruction of oocysts in intermediate host, definitive hosts and external environment can be the potential mean in the control of transmission of the disease. There is currently no reported data from Pakistan on cat role and oocyst inactivation as well as role of eating habits as most people preferably consume meat in northern areas Khyber Pakhtunkhwa to keep themselves warm. Veterinarians can play the most important role by working on the blood and fecal samples of the cats and by conducting proper research on the examination the litter boxes and the role of external environment.

We can reduce the infection by educating people about practicing the good hygiene i.e., washing hand after contact with the soil, use of gloves while gardening, washing vegetables and fruits before consumption, heat treatment of meat and freezing the meat -12^{o}C for 24 hours, drinking only the treated water. Pet cats should be kept at home and their litter boxes should be cleaned properly on daily basis, litter sand should be either washed or be kept under sun so that it can be heat treated.

Future potential initiatives should strive to strengthen the awareness for doctors, veterinarians, clinicians, cat owners, physicians, researchers, teachers, students and public for prevention and associated risk factors. To minimize the effects of toxoplasmosis in expecting ladies and newborn routine serological testing before and during pregnancy is advised.

References

Abbottabad. (2021). Retrieved 21 January 2021, from https://en.wikipedia.org/wiki/Abbottabad

Abdel-Rahman, M.A.M., 2017. Toxoplasmosis in man and animals. *Egypt J Chem Environ Health*, *3*, pp.54-73.

Abdullah, H.M. and Mahmood, M.A., 2017. Seroprevalence of Toxoplasma gondii among Pregnant Women in Erbil City/Kurdistan Region/Iraq. *Polytechnic Journal*, *7*, pp.54-63.

Alford Jr, C.A., Stagno, S.E.R.G.I.O. and Reynolds, D.W., 1974. Congenital toxoplasmosis clinical, laboratory, and therapeutic considerations, with special reference to subclinical disease. Bulletin of the New York Academy of Medicine, *50*(2), p.160.

Allain, J.P., Palmer, C.R. and Pearson, G., 1998. Epidemiological study of latent and recent infection by Toxoplasma gondii in pregnant women from a regional population in the UK. *Journal of infection*, *36*(2), pp.189-196.

Assmar, M., Amirkhani, A., Piazak, N., Hovanesian, A., Kooloobandi, A. and Etessami, R., 1997. Toxoplasmosis in Iran. Results of a seroepidemiological study. *Bulletin de la Societe de pathologie exotique (1990)*, *90*(1), pp.19-21.

Bastien, P., 2002. Molecular diagnosis of toxoplasmosis. *Transactions of the Royal society of Tropical medicine and hygiene*, *96*(Supplement_1), pp.S205-S215.

Bahia-Oliveira, L.M.G., Jones, J.L., Azevedo-Silva, J., Alves, C.C., Oréfice, F. and Addiss, D.G., 2003. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerging infectious diseases*, *9*(1), p.55.

Battagram. (2021). Retrieved 21 January 2021, from https://en.wikipedia.org/wiki/Battagram

Benenson, M.W., Takafuji, E.T., Lemon, S.M., Greenup, R.L. and Sulzer, A.J., 1982. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. *New England Journal of Medicine*, *307*(11), pp.666-669.

Carabin, H., Budke, C.M., Cowan, L.D., Willingham III, A.L. and Torgerson, P.R., 2005. Methods for assessing the burden of parasitic zoonoses: echinococcosis and cysticercosis. *TRENDS in Parasitology*, *21*(7), pp.327-333.

Chomel, B.B., 2008. Control and prevention of emerging parasitic zoonoses. *International journal for parasitology*, *38*(11), pp.1211-1217.

Cook, A.J.C., Holliman, R., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E. and Dunn, D.T., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control studyCommentary: Congenital toxoplasmosis—further thought for food. *Bmj*, *321*(7254), pp.142-147.

Couvreur, J., Desmonts, G., Tournier, G. and Szusterkac, M., 1985. Study of a homogeneous series of 210 cases of congenital toxoplasmosis in infants aged 0 to 11 months and screened prospectively. *Paris Hospitals Week* , *61* (43), pp. 3015-3019.

Dawson, D., 2005. Foodborne protozoan parasites. *International journal of food microbiology*, *103*(2), pp.207-227.

Deepak, S. and Singla, L.D., 2016. Immunodiagnosis Tools for Parasitic Diseases. *J. Microb. Biochem. Technol*, *8*, pp.514-8.

Desmonts, G.E.O.R.G.E.S. and Couvreur, J.A.C.Q.U.E.S., 1974. Toxoplasmosis in pregnancy and its transmission to the fetus. *Bulletin of the New York Academy of Medicine*, *50*(2), p.146.

Dubey, J.P., Lindsay, D.S. and Speer, C.A., 1998. Structures of Toxoplasma gondii tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clinical microbiology reviews*, *11*(2), pp.267-299..

Dubey, J.P., 1998. Advances in the life cycle of Toxoplasma gondii. *International journal for parasitology*, *28*(7), pp.1019-1024.

Dubey, J.P., 1998. Toxoplasmosis, sarcocystosis, isosporosis, and cyclosporosis. *Zoonoses*.

Dubey, J.P., 2016. Transmission of Toxoplasma gondii—From land to sea, a personal perspective. *A Century of Parasitology*, pp.148-164.

Dubey, J.P., 2020. The history and life cycle of Toxoplasma gondii. In *Toxoplasma gondii* (pp. 1-19). Academic Press.

Dubey, J.P., Murata, F.H.A., Cerqueira-Cézar, C.K. and Kwok, O.C.H., 2020. Public health and economic importance of Toxoplasma gondii infections in goats: the last decade. *Research in Veterinary Science*, *132*, pp.292-307.

Efstratiou, A., Ongerth, J.E. and Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks-an update 2011–2016. *Water research*, *114*, pp.14-22.

Endris, M., Belyhun, Y., Moges, F., Adefiris, M., Tekeste, Z., Mulu, A. and Kassu, A., 2014. Seroprevalence and associated risk factors of Toxoplasma gondii in pregnant women attending in Northwest Ethiopia. *Iranian Journal of Parasitology*, *9*(3), p.407.

Fallah, M., Rabiee, S., Matini, M. and Taherkhani, H., 2008. Seroepidemiology of toxoplasmosis in primigravida women in Hamadan, Islamic Republic of Iran, 2004. *EMHJ-Eastern Mediterranean Health Journal, 14 (1), 163-171, 2008*.

Faisal, I.A., Khan, A.U., Waqar, M., Ahmad, T., Shah, T., Khan, M.I., Ali, N., Faisal, S., Saif, I., Ahmad, W. and Javid, U., 2014. Distribution of Toxoplasma gondii in the pregnant women of district Swabi Khyber Pakhtunkhwa Pakistan. *World Appl Sci J*, *29*(1), pp.77-9.

Frenkel, J.K., 1973. Toxoplasma in and around us. *Bioscience*, *23*(6), pp.343-352.

Frenkel, J.K. and Lunde, M.N., 1966. Effects of Corticosteroids on Antibody and Immunity in" Besnoitia" Infection of Hamsters. *The Journal of infectious diseases*, pp.414-424.

Frenkel, J.K. and Taylor, D.W., 1982. Toxoplasmosis in immunoglobulin M-suppressed mice. *Infection and immunity*, *38*(1), pp.360-367.

Frenkel, J.K., 2000. Biology of Toxoplasma gondii. In *Congenital toxoplasmosis* (pp. 9-25). Springer, Paris.

Frenkel, J.K., 1974. Pathology and pathogenesis of congenital toxoplasmosis. *Bulletin of the New York Academy of Medicine*, *50*(2), p.182.

Freppel, W., Ferguson, D.J., Shapiro, K., Dubey, J.P., Puech, P.H. and Dumètre, A., 2019. Structure, composition, and roles of the Toxoplasma gondii oocyst and sporocyst walls. *The Cell Surface*, *5*, p.100016.

Gao, X.J., Zhao, Z.J., He, Z.H., Wang, T., Yang, T.B., Chen, X.G., Shen, J.L., Wang, Y., Lv, F.L., Hide, G. and Lun, Z.R., 2012. Toxoplasma gondii infection in pregnant women in China. *Parasitology*, *139*(2), pp.139-147.

Gebremedhin, E.Z., Abebe, A.H., Tessema, T.S., Tullu, K.D., Medhin, G., Vitale, M., Di Marco, V., Cox, E. and Dorny, P., 2013. Seroepidemiology of Toxoplasma gondii infection in women of child-bearing age in central Ethiopia. *BMC Infectious Diseases*, *13*(1), pp.1-9.

Gross, U. and Pohl, F., 1996. Influence of antimicrobial agents on replication and stage conversion of Toxoplasma gondii. *Toxoplasma gondii*, pp.235-245.

Gutiérrez-Zufiaurre, N., Sánchez-Hernández, J., Muñoz, S., Marín, R., Delgado, N., Sáenz, M.C., Muñoz-Bellido, J.L. and García-Rodríguez, J.A., 2004. Seroprevalence of antibodies against Treponema pallidum, Toxoplasma gondii, rubella virus, hepatitis B and C virus, and HIV in pregnant women. *Enfermedades infecciosas y microbiologia clinica*, *22*(9), pp.512-516.

Haripur, Pakistan. (2021). Retrieved 21 January 2021, from https://en.wikipedia.org/wiki/Haripur,_Pakistan

Hill, D.E., Chirukandoth, S. and Dubey, J.P., 2005. Biology and epidemiology of Toxoplasma gondii in man and animals. *Animal health research reviews*, *6*(1), pp.41-61.

Jansen, F., Dorny, P., Trevisan, C., Dermauw, V., Laranjo-González, M., Allepuz, A., Dupuy, C., Krit, M., Gabriël, S. and Devleesschauwer, B., 2018. Economic impact of bovine cysticercosis and taeniosis caused by Taenia saginata in Belgium. *Parasites & vectors*, *11*(1), pp.1-10.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. and Daszak, P., 2008. Global trends in emerging infectious diseases. *Nature*, *451*(7181), pp.990-993.

Joynson, D.H. and Wreghitt, T.G. eds., 2005. *Toxoplasmosis: a comprehensive clinical guide*. Cambridge University Press.

Jeannel, D., Niel, G., Costagliola, D., Danis, M., Traore, B.M. and Gentilini, M., 1988. Epidemiology of toxoplasmosis among pregnant women in the Paris area. *International Journal of Epidemiology*, *17*(3), pp.595-602.

Jenum, P.A., Stray-Pedersen, B., Melby, K.K., Kapperud, G., Whitelaw, A., Eskild, A. and Eng, J., 1998. Incidence of Toxoplasma gondii infection in 35,940 pregnant women in Norway and pregnancy outcome for infected women. *Journal of Clinical Microbiology*, *36*(10), pp.2900-2906.

John, D.T. and Petri Jr, W.A., 2006. Markell and Voge's medical parasitology. 9th. *Ed. St. Louis, Missouri: Saunders Elsevier*, pp.178-81.

Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. and Daszak, P., 2008. Global trends in emerging infectious diseases. *Nature*, *451*(7181), pp.990-993.

Kalantari, M., Asgari, Q., Rostami, K., Naderi, S., Mohammadpour, I., Yousefi, M., Davami, M.H. and Azizi, K., 2020. Molecular and serological evaluation of Toxoplasma gondii among female university students in Mamasani district, Fars province, southern Iran. *Journal of Health Sciences & Surveillance System*, *8*(2), pp.75-80.

Kijlstra, A. and Jongert, E., 2009. Toxoplasma-safe meat: close to reality?. *Trends in parasitology*, *25*(1), pp.18-22.

Kortbeek, L.M., Hofhuis, A., Nijhuis, C.D.M. and Havelaar, A.H., 2009. Congenital toxoplasmosis and DALYs in the Netherlands. *Memorias do Instituto Oswaldo Cruz*, *104*, pp.370-373.

Khademvatan, S., Saki, J., Yousefi, E. and Abdizadeh, R., 2013. Detection and genotyping of Toxoplasma gondii strains isolated from birds in the southwest of Iran. *British poultry science*, *54*(1), pp.76-80.

Levine, N.D., 1961. Protozoan parasites of domestic animals and of man. *Protozoan Parasites of Domestic Animals and of Man.*

Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T. and Murray, C.J., 2006. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *The lancet*, *367*(9524), pp.1747-1757.

Mansehra. (2021). Retrieved 21 January 2021, from https://en.wikipedia.org/wiki/Mansehra Maryland: U.S.A. CRC Press; 2010.

Macpherson, C.N., 2005. Human behaviour and the epidemiology of parasitic zoonoses. *International journal for parasitology*, *35*(11-12), pp.1319-1331.

Martínez-Ocampo, F., 2018. Genomics of Apicomplexa. *Farm Animals Diseases, Recent Omic Trends and New Strategies of Treatment*, p.101.

McLeod, R., Boyer, K., Roizen, N., Stein, L., Swisher, C., Holfels, E., Hopkins, J., Mack, D., Karrison, T., Patel, D. and Pfiffner, L., 2000. The child with

congenital toxoplasmosis. *Current clinical topics in infectious diseases*, *20*, pp.189-208.

Muñoz-Zanzi, C.A., Fry, P., Lesina, B. and Hill, D., 2010. Toxoplasma gondii oocyst–specific antibodies and source of infection. *Emerging infectious diseases*, *16*(10), p.1591.

Nash, J.Q., Chissel, S., Jones, J., Warburton, F. and Verlander, N.Q., 2005. Risk factors for toxoplasmosis in pregnant women in Kent, United Kingdom. *Epidemiology & Infection*, *133*(3), pp.475-483.

Onadeko, M.O., Joynson, D.H., Payne, R.A. and Francis, J., 1996. The prevalence of toxoplasma antibodies in pregnant Nigerian women and the occurrence of stillbirth and congenital malformation. *African journal of medicine and medical sciences*, *25*(4), pp.331-334.

Overdulve, J.P., 1970. The identity of Toxoplasma Nicolle and Manceaux, 1909 with Isospora Schneider, 1881. *Reprinted from Proc. K. Ned. Akad. Wet., Ser. C*, *73*(1), pp.129-51.

Pal, R.A., Qayyum, M. and Yaseen, M., 1996. Seroprevalence of Antibodies to Toxoplasma Gondii with Particular Reference to Obstetric History of Patients in Rawalpindi-Islamabad,Pakistan. *JOURNAL-PAKISTANMEDICAL ASSOCIATION*, *46*, pp.56-57.

Paul, B.K., 2011. *Environmental hazards and disasters: contexts, perspectives and management*. John Wiley & Sons.

Pappas, G., Roussos, N. and Falagas, M.E., 2009. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. *International journal for parasitology*, *39*(12), pp.1385-1394.

Peshawar. (2021). Retrieved 21 January 2021, from https://en.wikipedia.org/wiki/Peshawar

Petersen, E., Vesco, G., Villari, S. and Buffolano, W., 2010. What do we know about risk factors for infection in humans with Toxoplasma gondii and how can we prevent infections?. *Zoonoses and public health*, *57*(1), pp.8-17.

Petersson, K., Stray-Pedersen, B., Malm, G., Forsgren, M. and Evengård, B., 2000. Seroprevalence of Toxoplasma gondii among pregnant women in Sweden. *Acta obstetricia et gynecologica Scandinavica*, *79*(10), pp.824-829.

Polomoshnov, A.P., 1979. Definitive hosts of Toxoplasma. *Problems of Natural Nidality of Diseases. Institute of Zoology, Kazakh Academy of Sciences, Alma Ata*, *10*, pp.68-72.

Ricciardi, I.D., Sabroza, P.C., Sandoval, E.D. and Mayrink, W., 1978. Seroepidemiological study on the prevalence of human toxoplasmosis in Brazil. *Revista Microbiologica*, *9*(4), pp.181-187.

Robert-Gangneux, F. and Dardé, M.L., 2012. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical microbiology reviews*, *25*(2), pp.264-296.

Sadaruddin, A., Agha, F., Anwar, F. and Ghafoor, A., 1991. Seroepidemiology of Toxoplasma gondii infection in young school children in Islamabad. *J Pak Med Assoc*, *41*(6), pp.131-134.

Sharbatkhori, M., Moghaddam, Y.D., Pagheh, A.S., Mohammadi, R., Mofidi, H.H. and Shojaee, S., 2014. Seroprevalence of Toxoplasma gondii infections in pregnant women in Gorgan city, Golestan province, Northern Iran-2012. *Iranian Journal of Parasitology*, *9*(2), p.181.

Stillwaggon, E., Carrier, C.S., Sautter, M. and McLeod, R., 2011. Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLoS neglected tropical diseases*, *5*(9), p.e1333.

Singh, S. and Pandit, A.J., 2004. Incidence and prevalence of toxoplasmosis in Indian pregnant women: a prospective study. *American Journal of Reproductive Immunology*, *52*(4), pp.276-283.

Sroka, S., Bartelheimer, N., Winter, A., Heukelbach, J., Ariza, L., Ribeiro, H., Oliveira, F.A., Queiroz, A.J.N., Alencar Jr, C. and Liesenfeld, O., 2010. Prevalence and risk factors of toxoplasmosis among pregnant women in Fortaleza, Northeastern Brazil. *The American journal of tropical medicine and hygiene*, *83*(3), p.528.

Tamer, G.S., Dundar, D. and Caliskan, E., 2009. Seroprevalence of Toxoplasma gondii, rubella and cytomegalovirus among pregnant women in western region of Turkey. *Clinical and investigative medicine*, pp.E43-E47.

Tasawar, Z., Aziz, F., Lashari, M.H., Shafi, S., Ahmad, M., Lal, V. and Hayat, C.S., 2012. Seroprevalence of Human toxoplasmosis in southern Punjab, Pakistan. *Pak J Life Soc Sci*, *10*(1), pp.48-52.

Tanyuksel, M. and Petri Jr, W.A., 2003. Laboratory diagnosis of amebiasis. *Clinical microbiology reviews*, *16*(4), pp.713-729.

Tenter, A.M., Heckeroth, A.R. and Weiss, L.M., 2000. Toxoplasma gondii: from animals to humans. *International journal for parasitology*, *30*(12-13), pp.1217-1258.

Torgerson, P.R. and Macpherson, C.N., 2011. The socioeconomic burden of parasitic zoonoses: global trends. *Veterinary parasitology*, *182*(1), pp.79-95.

Todjom, F.G., Tsapi, E.M., Gamago, G.A., Vignoles, P., Pone, J.W. and Teukeng, F.D., 2019. Seroprevalence of toxoplasmosis and associated risk factors in pregnant women at the Protestant Hospital, Mbouo-Bandjoun, Cameroon. *African Journal of Clinical and Experimental Microbiology*, *20*(3), pp.221-230.

Torrey, E.F., Bartko, J.J., Lun, Z.R. and Yolken, R.H., 2007. Antibodies to Toxoplasma gondii in patients with schizophrenia: a meta-analysis. *Schizophrenia bulletin*, *33*(3), pp.729-736.

Villard, O., Cimon, B., L'ollivier, C., Fricker-Hidalgo, H., Godineau, N., Houze, S., Paris, L., Pelloux, H., Villena, I. and Candolfi, E., 2016. Serological diagnosis of Toxoplasma gondii infection: recommendations from the French National

Reference Center for Toxoplasmosis. *Diagnostic Microbiology and Infectious Disease*, *84*(1), pp.22-33.

Wade, T.J., Converse, R.R., Hilborn, E.D. and Krueger, W.S., 2014. Drinking water source and human Toxoplasma gondii infection in the United States: a cross-sectional analysis of NHANES data.

Wam, E.C., Sama, L.F., Ali, I.M., Ebile, W.A., Aghangu, L.A. and Tume, C.B., 2016. Seroprevalence of Toxoplasma gondii IgG and IgM antibodies and associated risk factors in women of child-bearing age in Njinikom, NW Cameroon. *BMC research notes*, *9*(1), pp.1-8.

Webster, J.P., 2010. Dubey, JP Toxoplasmosis of animals and humans.

World Health Organization, 2002. Foodborne diseases, emerging. *World Health Organization*.

Woolhouse, M.E., 2002. Population biology of emerging and re-emerging pathogens. *Trends in microbiology*, *10*(10), pp.s3-s7.

Wong, S.Y. and Remington, J.S., 1994. Toxoplasmosis in pregnancy. *Clinical infectious diseases*, pp.853-861.

Zargar, A.H., Wani, A.I., Bashir, M.I., Laway, B.A., Rashid, S. and Masoodi, F.A.S., Frequency of various factors associated with spontaneous abortions. *Blood*, *6*(9), p.84.