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Serological Study of Toxoplasmosis in Women and Cats in Al-Nasiriyah City/ Iraq

A Thesis

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(وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

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Dedication

To my kind father, who taught me how to ride the waves of life and overcome difficulties; to my role model and ideal, the symbol of kindness and tenderness. To my dear mother, my friend, the symbol of love, sacrifice, and warm heart, I still see in your eyes the anguish of losing my brother (Haidar). How I wish my soul could become a handkerchief to wipe away your tears.

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I dedicate the fruits of my humble efforts...

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Summery

Toxoplasmosis is a common protozoan parasitic disease. Both humans and animals are susceptible to this disease, which is caused by the parasitic infection *Toxoplasma gondii* (*T.gondii*). The current research project aims to conduct serological and molecular diagnostic analyses and to determine the prevalence of *T. gondii*, which is thought to be a common zoonotic protozoan among women and cats in Al-Nasiriyah City in Iraq.

In this study, 91 serum samples were aseptically collected from pregnant and non-pregnant women, aborted and non-aborted women, and women from urban and rural areas. The samples were obtained from the obstetrics and gynecology department of Al-Haboubi Teaching Hospital and private clinics, during the period from early November 2024 to the end of March 2025. All samples were subjected to serological analysis to meet the objectives of the study. The samples were analyzed using the indirect ELISA test to detect anti-*Toxoplasma* IgM and IgG antibodies.

The results indicate the effectiveness of immunological tests in detecting infection with the parasite *T. gondii* through the detection of IgM and IgG antibodies using the Enzyme-Linked Immunosorbent Assay (ELISA). The findings showed a clear variation in infection rates between women living in rural and urban areas. Regarding IgM antibodies, the highest positive rate was recorded among the group of pregnant women using the ELISA test, reaching (91.6%), while the lowest rate was recorded among the group of non-aborted women at (75%). The results also showed that the infection rate was higher among women living in rural areas compared with those in urban areas, reaching (100%) versus (72.5%) for IgM antibodies.

As for IgG antibodies, the highest positive rate was recorded in the group of non-aborted women at (37.5%), whereas the lowest rate was observed among aborted women at (25.4%). The results also demonstrated that the infection

rate was higher among women living in urban areas compared with those in rural areas, reaching (32.5%) versus (18.2%) for IgG antibodies.

The microscopic examination results of 100 fecal samples collected from veterinary clinics and cat shelters in the city of Nasiriyah showed that the overall infection rate was 30%. When the results were analyzed according to months, the highest infection rate was recorded in February (35.71%), followed by November (35%), with no statistically significant difference among the studied months.

The results also indicated a difference in infection rates between sexes. Female cats showed a higher infection rate (33.96%) compared to male cats (25.53%), although this difference was not statistically significant. Regarding the source of the cats, those living in cat shelters (Cats Sanctuary) showed a higher infection rate of 32.5%, while domestic cats recorded 28.33%, with no significant difference between the two groups. These findings suggest that the highest infection rates occurred during February, whereas lower infection rates were observed in male and domestic cats, which may reflect the influence of environmental factors and housing conditions on the spread of the parasite among cats in the study area.

The study results also demonstrated that traditional diagnostic methods for detecting *T. gondii* in cats were effective in identifying the infection. Additionally, it was observed that the prevalence of infection increased during moderate and colder months compared to other periods, which may be related to environmental conditions that allow the parasite's oocysts to survive longer in the environment.

Additionally, 92 serum sample from cats serological examination results using the ELISA test indicate a clear difference in the seropositivity rate of IgG antibodies against *T. gondii* among the studied age groups of cats. The age group of 2–11 months recorded the highest seroprevalence rate at 53.57%

suggesting that relatively young cats are more susceptible to exposure to the parasite during this stage of life. This may be explained by the fact that cats at this age are in a phase of increased activity and environmental exploration, which raises their chances of encountering sources of infection such as contaminated soil or prey carrying tissue cysts of the parasite.

In contrast, cats older than 11 months (adult cats) showed a lower seropositivity rate of 43.75% which is lower than that recorded in the younger age group. This may be attributed to the development of acquired immunity in adult cats as a result of previous exposure to the parasite, or due to reduced exposure opportunities compared with younger cats.

Based on these results, it can be concluded that both traditional and immunological methods for diagnosing *T. gondii* infection in cats were effective, and that the infection rate of toxoplasmosis in cats increases during the mild and cold months, with a higher prevalence observed in young cats aged 2–11 months compared to older cats.

Additionally, Molecular screening was performed using conventional polymerase chain reaction (PCR) and real-time polymerase chain reaction (real-time PCR), targeting the GRA6 and B1 genes, respectively on cat fecal samples. However, no positive results were detected in any of the applied molecular assay.

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List of Abbreviations

Abbreviations	Meaning
%	Percentage
ADCC	Antibody- dependent cell mediated cytotoxic
APCs	Antigen-Presenting Cells
CD4	Cluster Differentiation 4
CD8	Cluster Differentiation 8
CMI	Cell-Mediated Immunity
DCs	Dendritic Cells
ddH ₂ O	double distilled water
DNA	Deoxy Nucleic Acid
DRG	Diagnosis –Related Group
ELISA	Enzyme-linked immune sorbent assay
LAT	Latex Agglutination Test
<i>et al</i>	And Others
IFN- γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IgA	Immunoglobulin Alpha
IL	Interleukin
IU	International Unit
MHC	Major Histocompatibility Complex
NK	Natural Killer Cells
PCR	Polymerase Chain Reaction
RNA	Ribo Nucleic Acid

RPM	Revolutions Per Minute
RT-PCR	Real Time Polymerase Chain Reaction
SPSS	Statistical Package for the Social sciences
<i>T.gondii</i>	<i>Toxoplasma gondii</i>
TBE	Tris –Borate-EDTA
Ths	T helper Cells
UV	Ultraviolet
UVP	Unique Value Proposition
μl	Microliter
GIT	Gastrointestinal Tract
IMC	Inner Membran Complex
TE	Toxoplasmic Encephlitis
CNS	Central Nervous System
GRA6	Dense Granule Antigen 6
AIDS	Acquired Immunodeficiency Syndrome
TNF	Tumor Necrosis Factor

Chapter One

Introduction

Chapter One.....Introduction

1.1. Introduction

Toxoplasmosis is a globally prevalent zoonotic disease caused by the single-celled parasite *T. gondii*. This obligate intracellular parasite forms oocysts that can infect warm-blooded animals, including humans (Ahmed *et al.*, 2021). Cats play a crucial role in the epidemiology of toxoplasmosis because they are the only hosts capable of shedding environmentally tolerant oocysts. Multiple studies have demonstrated the widespread presence of *T. gondii* infection in cat populations globally (Montazeri *et al.*, 2020). Tissue cysts (bradyzoites) of *Toxoplasma* can be acquired through consumption of undercooked or raw meat and oocysts in water, food and soil contaminated with cat feces (Pinto Ferreira *et al.*, 2019).

In the majority of cases, infection with *T. gondii* is asymptomatic, sometimes, fever, malaise and lymphadenopathy may occur, but this infection in immunocompromised individuals expresses itself severely as disseminated toxoplasmosis or Toxoplasmic Encephalitis (TE) or both and it often causes death (Grant *et al.*, 1990). The parasite is opportunistic and primarily affects immunocompromised individuals, such as Acquired Immunodeficiency (AIDS-human) (Passos *et al.*, 2000). When immunocompetent mothers contract an infection before pregnancy their immune systems prevent the parasite from spreading to their infants (Szymanska, 2010).

The probability of transmission increases with the age of pregnancy, but fetal disease severity decreases. Indeed, the placental barrier is more effective during the first trimester of pregnancy and less than 10% of pregnant women infected with *Toxoplasma* parasites transmit the parasite from mother to fetus (Bollani *et al.*, 2022). Resulting in all kinds of pathological problems, and the fetus has various congenital malformations or spontaneous abortion infection is chiefly due to immune function decrease in both cases (Halonen and Weiss, 2013).

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Acute infection during the first trimester Furthermore, pregnancy-related infections can result in congenital toxoplasmosis in the fetus, which can cause spontaneous abortion, stillbirth, hydrocephalus, microcephaly, and neurological symptoms that can be detected in the uterus or at birth (Verteramo *et al.*, 2022). whereas late trimester infections are more common but usually subclinical at birth (Cook, 1990). Nonetheless, most patients (70% of cases) are asymptomatic at birth but show evidence on clinical examination during later life. According to the Walker *et al.*, (1992), extensive exposure to *T. gondii* has been demonstrated in seroprevalence studies, particularly in unsanitary conditions where the risk of infection and oocyst exposure from infected cats the definitive host for this pathogen increases (Ghenciu *et al.*, 2024).

T. gondii was detected by several serological examination tools, such as enzyme-linked immune sorbent assay (Pittman and Knoll, 2015). Due to its simplicity, low-cost sensitivity and specificity, Enzyme Linked Immune Sorbent Assay (ELISA) for antibodies of *T. gondii* has been modified for use in humans and in most domestic animals. Modified antigen detection techniques for *Toxoplasma* in body fluids have supplanted older tests in most laboratories (Mohamed *et al.*, 2012).

IgG antibodies against *T. gondii* can serve as a trustworthy serological biomarker for the detection of prior exposure to this parasite since they last for years, unlike IgM antibodies (Kataki *et al.*, 2022). In pregnant women, toxoplasmosis can be diagnosed by serology and PCR (Al-Yami *et al.*, 2021). The overall prevalence of toxoplasmosis in the world population is estimated to be around 30%, although country-to-country variation and other factors such as socioeconomic status and health condition, have been reported (Doghish *et al.*, 2023). The parasite has a sexual phase that occurs in small intestinal epithelial cells of cats. They also excrete infectious oocysts in their

Chapter One.....Introduction

feces, playing an important role in the epidemiology of toxoplasmosis (Tenter *et al.*, 2000).

Fever, inappetence, depression, conjunctivitis, Gastrointestinal tract (GIT) signs and neurologic abnormalities are common signs of the disease due to *Toxoplasma* infection in kittens (Dubey, 2010). *T. gondii* has only one definitive host, the domestic cat and other members of the family Felidae. The parasite reproduces sexually in the epithelial cells of the small intestine. Rates of seropositive results generally rise with age and this points to the possibility of postnatal transmission for toxoplasmosis. Most cats have detectable *T. gondii* antibodies 6 to 10 weeks after weaning. Low antibody titers in some kittens may suggest that maternal antibodies were transferred (Dubey *et al.*, 2020).

The primary immune protection against *T. gondii* is cell-mediated immunity, and certain immunosuppressive treatments may increase the risk of clinical disease in certain cats (Barrs *et al.*, 2006). Age, sex, and lifestyle all affect *T. gondii* prevalence rates (Dubey *et al.*, 2020). IgM antibodies are typically found initially after a cat is exposed to *T. gondii*, and in healthy cats, this is followed by seroconversion to IgG (Lappin, 2010). Due to their brief duration, IgM assay results are frequently false negatives, while indirect antibody ELISA results can be false positives (Dabritz *et al.*, 2007). Most cats continue to have tissue infections after developing IgG antibodies. Therefore, real infection rates are typically estimated using the presence of IgG antibodies (Honarchian *et al.*, 2024). One of the closest human companions is a household cat, and regular contact with cats can raise a person's chance of contracting *T. gondii*. More significantly, stray cats are more likely to move around and contribute significantly to the spread of toxoplasmosis to humans and other animals (Elmore *et al.*, 2010).

Chapter One.....Introduction

1.2. Aim of the Study

This study aimed to diagnose toxoplasmosis in women and cats using serological, microscopic, and molecular techniques. Through the following objective:

1. The study aim is to detect of *T. gondii* infections in aborted women, cats by serologic and molecular techniques.
2. Detection of anti -*Toxoplasma* IgG antibody in the serum of infected cats using serological test (enzyme linked immunosorbent assay .
3. Estimation of the incidence of toxoplasmosis in different regions of Al-Nasiriyah.
4. To determine the prevalence, risk factors, and potential transmission routes of *T. gondii* among women and cats in the study regions.
5. To assess the prevalence of *T.gondii* infection in domestic cats, particularly those in proximity to households with pregnant women.
- 6.To evaluate the applicability of PCR for detection of *T.gondii* in cats targeting B1 gene and GRA6.

Chapter Two

Literatures Review

Chapter Two.....Literature Review

2. Literatures Review

2.1. History of *Toxoplasma gondii*

Toxoplasma gondii, one of the most common parasites, infects a significant portion of the global population causes toxoplasmosis, an infectious disease that can lead to a variety of clinical symptoms in humans (Nidhal and Safaa, 2010). According to global serological studies, in some countries, nearly one-third of the population has antibodies against *Toxoplasma. T. gondii* (Tenter *et al.*, 2000). *T.gondii*. The name *Toxoplasma* originates from the mouse species *Ctenodactylus gundi*, first isolated in 1908; the genus name derives from the Greek words toxon (bow) and plasma (form) (Weiss and Dubey, 2009). Ferguson, (2009) demonstrated that *T.gondii* could be isolated from brain lesions in mice, rabbits, and rats by intracranial injection of brain and spinal cord samples, which resulted in encephalitis. Weiss and Dubey (2009) noted that the first identification of *T. gondii*.

In 1939, Wolff, Cowen, and Page first identified this human pathogen in a full-term female infant born via cesarean section. The infant developed epilepsy and bilateral chorioretinitis three days after birth, which subsequently progressed to encephalomyelitis, and she died at one month of age. Since then, numerous researchers have identified the pathogen. *Toxoplasma* forms, including tissue cysts (Dubey, 2020). First documented discovery of *T. gondii* DNA from a single tachyzoite using PCR amplification of the B1 gene (Coupe *et al.*, 2019).

2.2. Description and Classification

According to Graindorge *et al.*, (2016) *T. gondii* has a crescent shape, and resides inside host cells. The nucleus is located close to the end of the spiral, and the conoid is in charge of destroying the host cell wall. According to Ullah *et al.*, (2020), *T. gondii* is a coccidian parasite that has warm-blooded species as intermediate hosts and cats as its definitive host.

Chapter Two.....Literature Review

The following is the classification of *T. gondii* according to (Roberts and Janovy, 2000).

Phylum: Apicomplexa

Class: Sporozoasidae

Subclass: Coccidiasina

Order: Eimeriorina

Family: Cyclosporinae

Subfamily: Toxoplasmatidae

Genus: *Toxoplasma*

Species: *Toxoplasma gondii*

2.3. Morphology Structure

Dubey *et al.* (1998), explained that the *T. gondii* infection process consists of three stages: oocysts containing sporozoites produced through sexual reproduction, rapidly dividing tachyzoites, and slowly proliferating bradyzoites within tissue cysts. The infection stages of *T. gondii* include: tachyzoites as the invasive stage, bradyzoites as the persistent stage (present in tissue cysts), and sporozoites protected by the oocyst wall also present in the oocysts (Alvarez Garcia *et al.*, 2021).

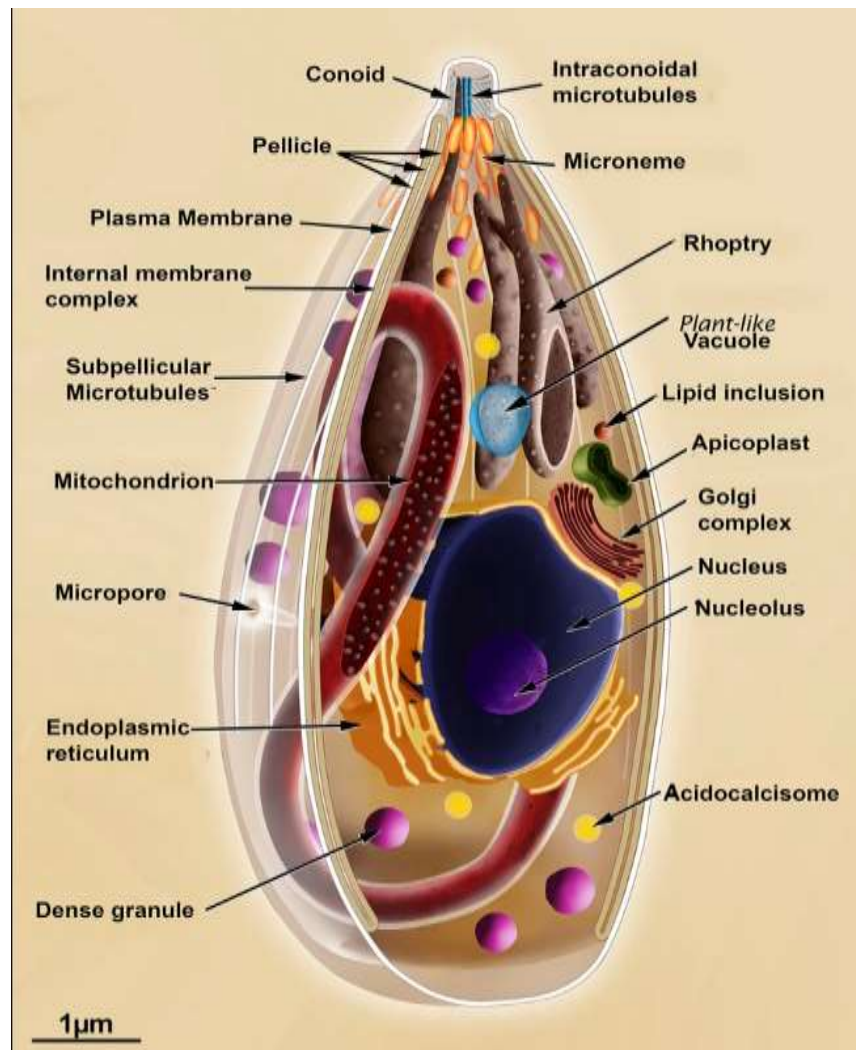
2.3.1. Tachyzoites (Rapid Multiplication Stage)

The tachyzoite is often crescent-shaped and is approximately the size are 4–6 µm long and 2–3 µm wide. They have a crescent nucleus with a central nucleolus (Delgado *et al.*, 2022). According to Gangneux *et al.* (2012), The anterior end of the tachyzoite is pointed, and the posterior end is round. It has a pellicle (outer covering), several organelles including subpellicular microtubules, mitochondrion, smooth and rough endoplasmic reticulum, a Golgi apparatus, apicoplast, ribosomes, a micropore, and a well-defined nucleus. The nucleus is usually situated toward the central area of the cell.

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The Golgi complex lies at the center of the spherical nucleus and exhibits a mild furrow toward its apex (Attias *et al.*, 2020). The nuclear envelope and its contiguous ER are an integral unit (Ferguson *et al.*, 2014). Mitochondria are themselves branched and folded within the cell, extending to lengths of 10 μm (Melo *et al.*, 2000).

The centrosome is tightly positioned to the nuclear membrane and essential for cell division and replication of organelles (Chen *et al.*, 2013). Other *T. gondii* structures such as the acromere, secretory organelles, Inner membrane complex (IMC), and apical and basal complexes are also be extremely specialized.



Figure(2-1): Tachyzoite form *T. gondii* Sanchez and Besteiro,(2021).

Chapter Two.....Literature Review

2.3.2. Bradyzoites (Slow Multiplication Stage)

This is what *T. gondii* looks like in chronic infection (Fig.2-2), which typically occurs in the brain and muscle tissue. cyst are spherical, measuring 10-200 μm across, and filled with hundreds of bradyzoites (tubercle-like, slow-moving structures) (Beamann *et al.*, 1995). The cyst wall is elastic and thin ($<0.5 \mu\text{m}$) and can contain hundreds of bradyzoites (elanoid, crescent shaped stage of *T. gondii* within, according to (Hill and Dubey, 2018). Since tissue cysts are highly resistant to digestive juices and can persist for life in tissues, humans may become infected by ingesting undercooked or raw meat. Yet they can be inactivated by radiation or being frozen at -20°C for 24 hours and then thawed (Beamann *et al.*, 1995).

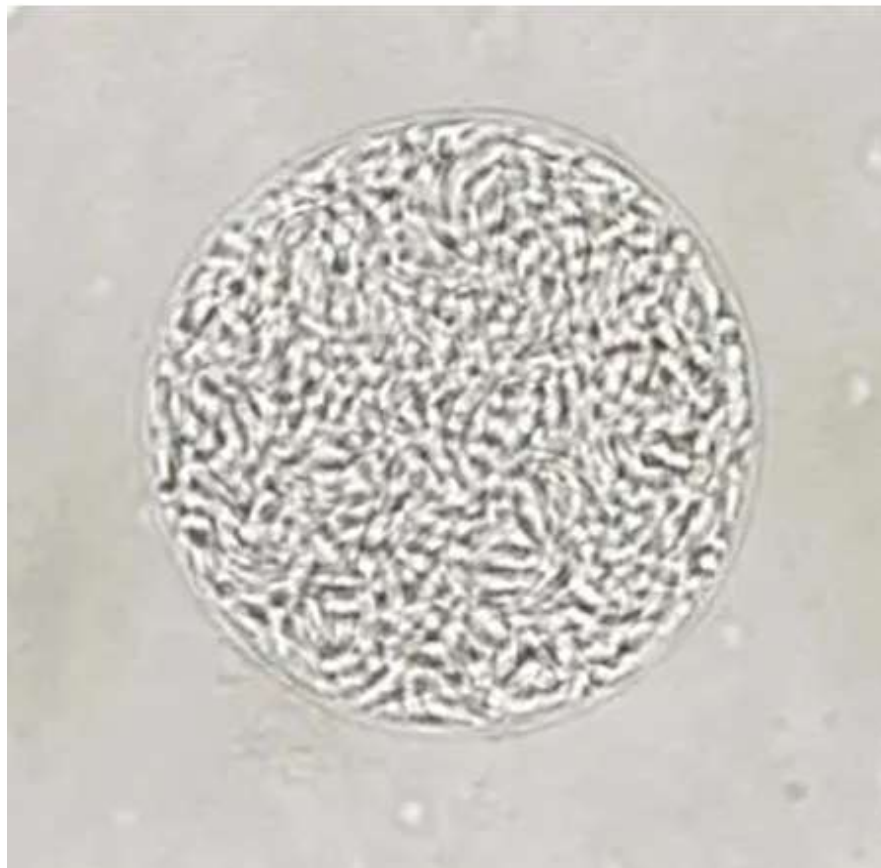


Figure (2-2): Bradyzoites cysts(Kanaan and Raiaan, 2019).

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2.3.3. Oocysts (Resistant form in the External Environment)

Oocysts (fig.2- 3) are small (10-12 μm) and round but become oval (11-13 μm); they can survive in the environment for months and are very resistant to disinfectants, freezing, and drying, but are killed by heating to 70°C for 10 minutes, or boiling of fluids and strong solutions of ammonia and iodine (Sibley *et al.*, 1995). The contents of the two-layered wall of the unsporulated oocyst transform into two sporocysts each with four sporozoites (Dubey *et al.*, 1998).



Figure (2-3): sporozoite oocyst AL-Tameemi and Kabakli ,(2019)

2.4. Life Cycle of *Toxoplasma gondii*

T. gondii has a complicated life cycle (fig. 2-4) with two hosts: the definitive host (cats), where the sexual stage takes place, and the intermediate host (mammals and birds), where asexual stages take place (Borrelli *et al.*, 2020). The *T. gondii*-genome is a haploid, except during sexual division in cats (Behnke *et al.*, 2020).

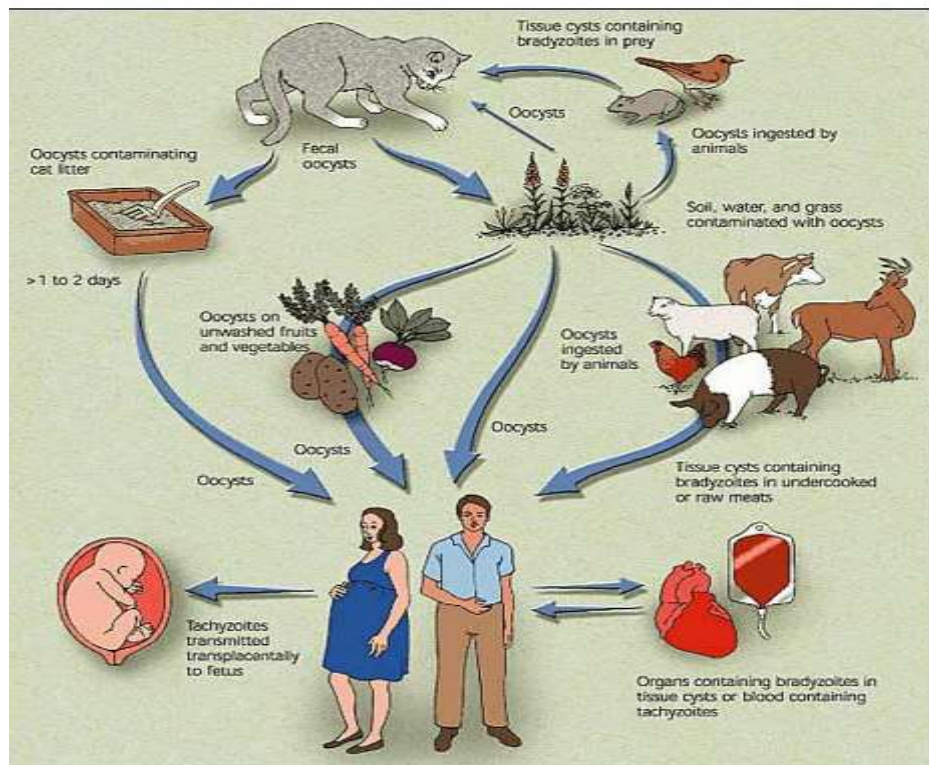


Figure (2-4): Life cycle of *T. gondii* (Al-Saadii *et al.*, 2013).

2.4.1. Sexual Cycle

The infection in cats (fig.2-5) occurs orally through ingestion of an infected intermediate host (e.g., birds or rodents) containing cystic bradyzoites. The bradyzoites are released from the cysts into small intestine, where they encounter digestion by gastric acid and intestinal enzymes enabling invasion of enterocytes. Only exceptionally species of parasites multiply in the definitive host with accompanying signs of disease (Attias *et al.*, 2020).

When the intestinal cell ruptures, oocysts are released in the intestines. These oocysts are shed in the feces Peterson and Dubey, (2001). According to AL-Tameemi and Kabakli (2019), merozoites develop into microgametes and macrogametes after several asexual cycles of replication. Following fertilization of the macrogamete a wall is produced, and the oocyst then has four asporogenous mother-cells, each with four spores. Oocysts can be

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periodically excreted by wild cats during their entire life, while they are shed only in a period of 1–3 weeks post-infection within domestic cats Zhu *et al.*, (2022). Infected cats produce millions of oocysts that are released into the environment and Contamination of plants, water sources, soil and food. *T.gondii* oocysts are very hardy and can survive for long period of time in the environment leading to their dispersion on soil and water (Shapiro *et al.*, 2019). In cool and arid areas oocyst of *T. gondii* is able to survive for months, which is dependent on factors such as soil type, temperature or texture (Aguirre *et al.*, 2019).

2.4.2. Asexual Cycle

The outer walls of tissue cysts / oocysts are broken down by enzymatic digestion, releasing bradyzoites or sporozoites into the intestinal lumen, which invade adjacent cells motile tachyzoites that adapt after invasion to non-intercalary producing: nucleated forms of uninfected tissues mitochondria become thickening they transform into tachyzoites (Triana *et al.*, 2018). The two developmental stages for the asexual cycle are invasive tachyzoites that reproduce quickly and that invade the host, and bradyzoites, which multiply at a slow rate while residing within the host (Ahmed *et al.*, 2024). High jacking immune cells from the host allows spread throughout the organisms Barrett *et al.*, (2019). Using this route as well as paracellular entry and transcellular migration (Jeffers *et al.*, 2018).

Tachyzoites can cross non-permissive biological barriers, including the blood–brain barrier to immune privileged organs such as the brain. Immunocompetent hosts will of course effectively contain this early stage of infection which however, coincidentally with the maturation of the host immune response led to a shuttling of intracellular rapidly replicating tachyzoites towards slowly growing cyst-forming stages that lie essentially sequestered from the immune system (Sanchez and Besteiro, 2021). These persistent parasite forms are predominantly located within the CNS and

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muscle (Mendez and Koshy, 2017). Tissue cyst is found in the viscera such as lung, liver kidney etc., and more frequently present in cerebral neural tissues including the brain, eye and limb or heart muscle. A life-long persistence of tissue cysts would seem possible with the non-disruptive type and, hence, *T.gondii* normal intact tissue cysts are harmless over at least a host's lifetime (Dubey *et al.*, 1998). The cyst wall is digested by proteolytic enzymes in the feline stomach and small intestine upon ingestion of bradyzoite contaminated meat, releasing bradyzoites into the gut of the host (Ransom, 2000).

In the lamina propria of the gut there occurs an invasion and replication in some bradyzoites of *T.gondi* within hours. A portion of invading bradyzoites will traverse the wall of the epithelial cell in a small intestine and undergo several rounds of asexual multiplication; although parasite can spread to extra intestinal sites. This provides the opportunity for felids to feed on the intermediate host, particularly rodents, which guarantees that parasites are transmitted back into a definitive host where they can develop in their life cycle, these parasites can still be spilled to additional intermediate hosts by carnivorism even if they do not fit the typical felid prey, allowing parasite circulation without sexual reproduction (Sanchez and Besteiro, 2021). The main ways of transmission of *T. gondii* to humans include: ingestion of tissue cysts (bradyzoites) in undercooked meat; consumption of food or water contaminated with oocytes that are excreted by cats; and vertical transmission from parent to offspring. Non-pathogenic in immunocompetent hosts phase of infection (Wilson *et al.*, 2020).

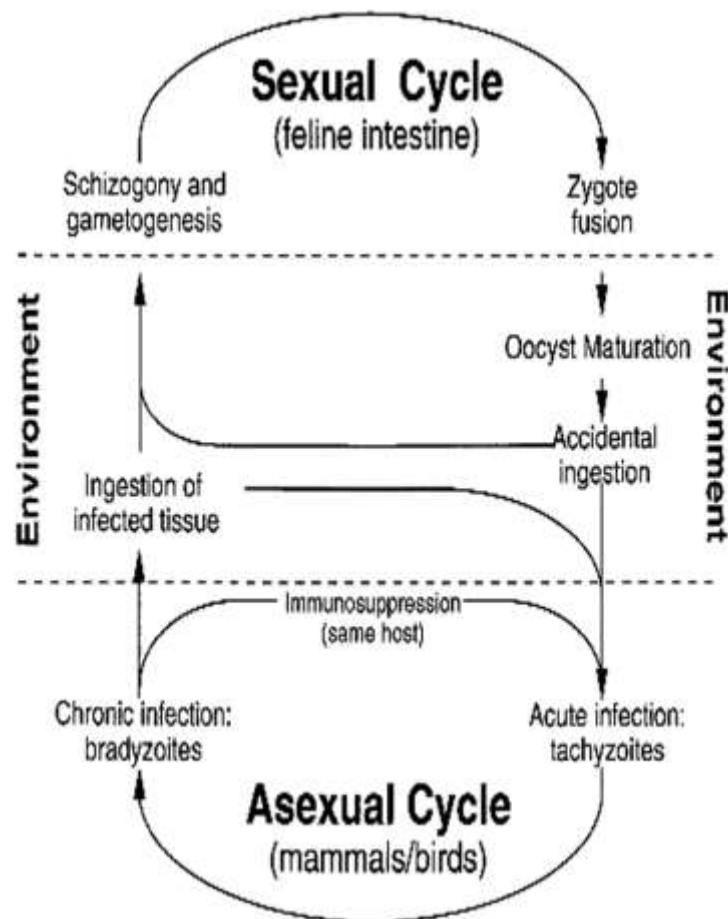


Figure (2- 5): Sexual and asexual stages of life cycle(Michael *et al.*, 2000).

2.5. Epidemiological of *Toxoplasma gondii*

Humans and many other warm-blooded species Fig. (2-6) are susceptible to toxoplasmosis. Although there is significant geographic heterogeneity in the prevalence of latent *T. gondii* infection, the parasite is thought to infect approximately one billion people globally (Flegr, 2014). Approximately one third of the population might be infected, and toxoplasmosis has been reported in humans from almost every country worldwide (Khairullah *et al.*, 2024). Internationally, there are significant variations in the prevalence of disease (Montazeri *et al.*, 2020). The incidence of toxoplasmosis is known to vary greatly, with rates as high as 60% in some European populations and closer to 90% among certain demographic subpopulations residing in hyperendemic regions like parts of Africa (Gangneux *et al.*, 2012).

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Lopes *et al.*, (2021) this condition is described in almost all animals, mammals and several bird species alike.(Vidal ,2019) Africa, the Middle East, Southeast Asia, Central Europe, Latin America, and Eastern Europe are regions with highest recorded rates of toxoplasmosis. The *T. gondii* strain was identified as type III similar by Mawlood *et al.*, (2019) in stray cats in Iraq, Kurdistan. Seroprevalence differs greatly between nations, with estimates indicating that 33% of blood donors globally are infected with *T. gondii* (Lupu *et al.*, 2022). Regional variations in toxoplasmosis incidence rates in Iraq are influenced by climate and hygiene practices at both personal and community (Ismael, 2023).

In October, November, and December of 2009 and January of 2010, Al-Se'adawy and Hemza, (2010) found a significant prevalence rate of toxoplasmosis in pregnant women in Al-Muthanna province. The seroprevalence of toxoplasmosis among women in Duhok City was 28% and 0.46% according to anti-toxoplasma IgG antibody and IgM antibody, respectively (Mikaeel and Al-Saeed, 2020). As was 31.8%, in Dohk (Murad and Eassa, 2023). *T. gondii* IgG was most highly recorded (42.1%) in a study performed another study from Erbil Province, Iraq by (Jaber and Noori, 2021).

The incidence of toxoplasmosis among women was 17% (n=89) in Wasit province Al-Sray *et al.*, (2019) and 13.6% (n=110) in Baghdad Al-Rawazq, (2017). Infection appears to be more prevalent at lower altitudes and in warmer climates (Borkens and Wildlife, 2021).

2.6.Transmission

Sporozoites, bradyzoites and tachyzoites are the three parasitic forms of *T. gondii* that infect other cells to establish infection horizontally or vertically (Delgado *et at.*, 2022). There are two methods that allow Human to be infected with this toxoplasmosis:

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2.6.1. Horizontal Transmission

One of the most important sources for propagation of toxoplasmosis is eating fruit, vegetables or taking anything which carries an infection with oocysts (Hussain *et al.*, 2017). The presence of toxoplasmosis is also driven by the genetic factors, immune status of individual and favorable environmental conditions such as warm weather in which to enable the survival of oocysts that are passed by cats in their faces (Carter, 2013). Recent research has shown that toxoplasmosis is a potential risk factor for mental and neurological health issues (Fallahi *et al.*, 2017). Type of farm, water quality, access to pasture (with animals not entering barn), and the presence of cats, rats and birds were risk factors for exposure of animals on farms to *T. gondii* (Shapiro, 2019).

Cultural practices food and eating habits significantly contribute to current transmission mechanism (Al-Adhroey *et al.*, 2019). Animals, mainly domestic species such as dogs, cats and birds are reported to carry of this parasite (Stelzer *et al.*, 2019). According to Gangneux *et al.*, (2012) infection may also be acquired by ingestion of milk, excreta, mucus and saliva from an infected host by mucous membranes. Transmission occurs predominantly via ruminants and many environmental factors can impact oocyst survival (Stelzer *et al.*, 2019). Infection also occurs by using animal milk with *T. gondii* tachyzoites (Almeria and Dubey, 2021). In addition, organ transplantations and blood transfusions are other potential sources of infection (Stopis *et al.*, 2022).

2.6.2. Vertical Transmission

Pregnant women who acquire primary toxoplasmosis during pregnancy are at a significantly increased risk of transmitting the infection to the fetus compared to those infected before they become pregnant (Ismael, 2023). 10–15 % of pregnant women are infected in the first trimester, while 45 and

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occasionally up to 60% become infected in the second and third (Mohammed *et al.*, 2019). A pregnant woman infected with *Toxoplasma* will transmit the infection trans placentally to her fetus, although she may remain clinically asymptomatic; however the manifestations of the disease emerge post-partum (Peyron *et al.*, 2019).

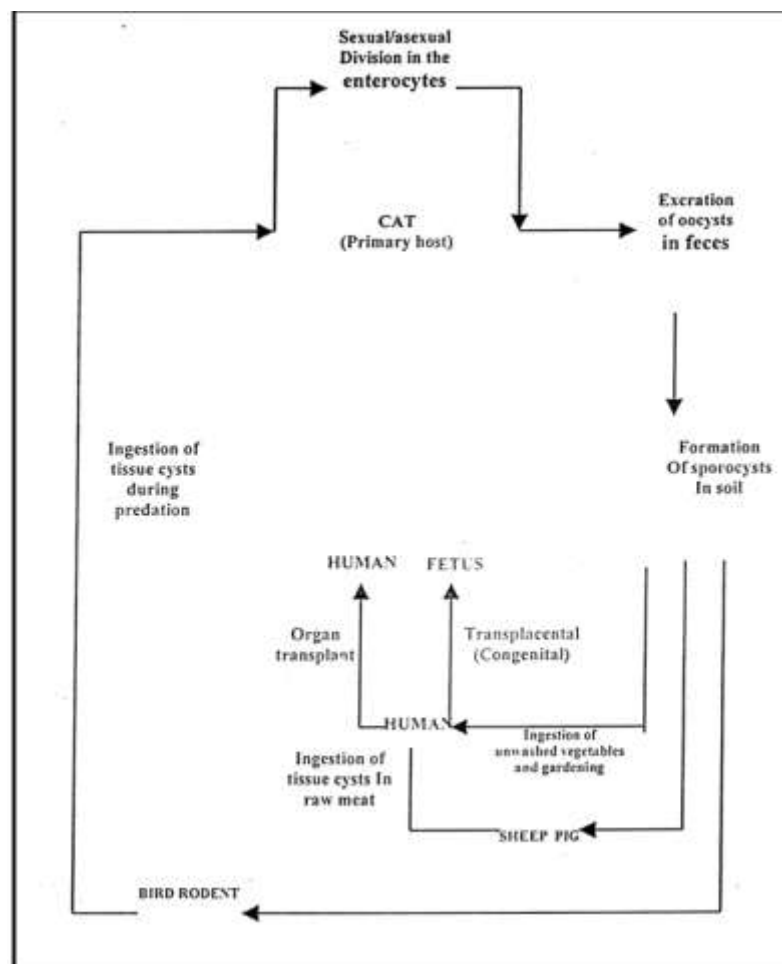


Figure (2- 6): Mode of transmission of *T. gondii* (Alcamo, 1997)

2.7. Pathological and Clinical Signs of Toxoplasmosis

The progression of infection in humans and animal models of toxoplasmosis appears to be influenced by inoculum size, virulence of the organism, genetic background, sex, individual host and host species susceptibility, and immune status (Montoya and Liesenfeld, 2004). When a host ingests a bradyzoite-containing tissue cyst or a sporozoite-containing oocyst, the parasite is released from the cyst during digestion (Triana *et al.*,

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2018). Bradyzoites are resistant to pepsin and can invade the host's gastrointestinal tract. The tachyzoite reproduces asexually within the host cells through repeated endodyogeny (Dubey *et al.*, 1998). After rupturing cells and infecting nearby cells, they spread hematogenous throughout the body through the lymphatic system (McLeod and Remington, 2000). Tachyzoites spread via the blood or lymphatic stream to infect any nucleated host cell after *Toxoplasma* are released from cysts in the gastrointestinal tract and multiply in the macrophage. The multiplication of the tachyzoites does not seem to disrupt host cell function until the dividing organisms cause the cell to rupture, as adjacent cells become infected and rupture themselves, causing progressive tissue necrosis and an inflammatory response (Joiner, 1991).

Clinical toxoplasmosis is un common because most infections (over 80%) are asymptomatic or present mild symptoms, Clinical infections can be classified as acute, subacute, and chronic. If immunity develops slowly, the illness can persist for a longer period; this condition is called subacute infection. During this stage, tachyzoites continue to damage cells and cause widespread lesions in the lungs, liver, heart, brain, and eyes (Robert and Jr,1996). According to Marquardt *et al.*, (2000) chronic infection occurs when the body develops immunity to tachyzoite growth. This is related to the formation of sarcocysts (or cysts) resistant to the immune response.

Therefore, the infection can remain latent for months or even years. Another important medical concern is pregnant women recently infected with acute toxoplasmosis. In this case, the pregnant woman lacks acquired immunity to the parasite, and tachyzoites can infect the fetus through the placenta. On the other hand, mothers with chronic infections rarely transmit the infection to their unborn children, presumably because maternal immunity keeps tachyzoite proliferation at a subclinical level (Denkers *et al.*, 1997). The acquired infection is typically subclinical and asymptomatic, and in 10–20%

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of cases that do show symptoms, in immunocompetent individuals, the illness is benign and self-limiting (Ismael, 2023). Clinical indicators in certain hosts: Clinical signs including congenital and ocular toxoplasmosis are frequently seen in cats, frequently accompanied by severe neurological and respiratory symptoms (Dubey, 2009). Can differentiate between three categories of clinical illness forms:

Acquired form: the parasite is caught by consuming oocyte-infected meat or contaminated vegetables. Flu-like symptoms include headache, fatigue, enlarged lymph nodes, mild fever, and muscle soreness. The most frequent clinical sign of human toxoplasmosis is enlarged lymph nodes (Hill, 2002). the acquired infection is typically subclinical and asymptomatic, and in 10–20% of cases that do show symptoms, in immunocompetent individuals, the illness is benign and self-limiting (Ismael, 2023).

Congenital toxoplasmosis: Congenital toxoplasmosis is caused by the transmission of *T. gondii* from the pregnant woman to the fetus across the placental barrier. This usually occurs when an immunocompetent pregnant woman develops a primary infection during pregnancy (Hegab and Al-Mutawa, 2003). The risk of acute infection is highest in early pregnancy and can lead to serious illness, even miscarriage, stillbirth, or fetal death. Infection is more common in late pregnancy but is usually asymptomatic at birth (Cook, 1990). The rate of transmission from *T. gondii* to the fetus is approximately 15% in early pregnancy, 30% in mid-pregnancy, and 60% in late pregnancy (Gangneuxa, 2012). Congenital malformations may include ocular toxoplasmosis, hydrocephalus or microcephaly, deafness, motor disorders, retinal and brain damage, as well as signs of intellectual disability and intracranial calcification (Chaudhry *et al.*, 2014).

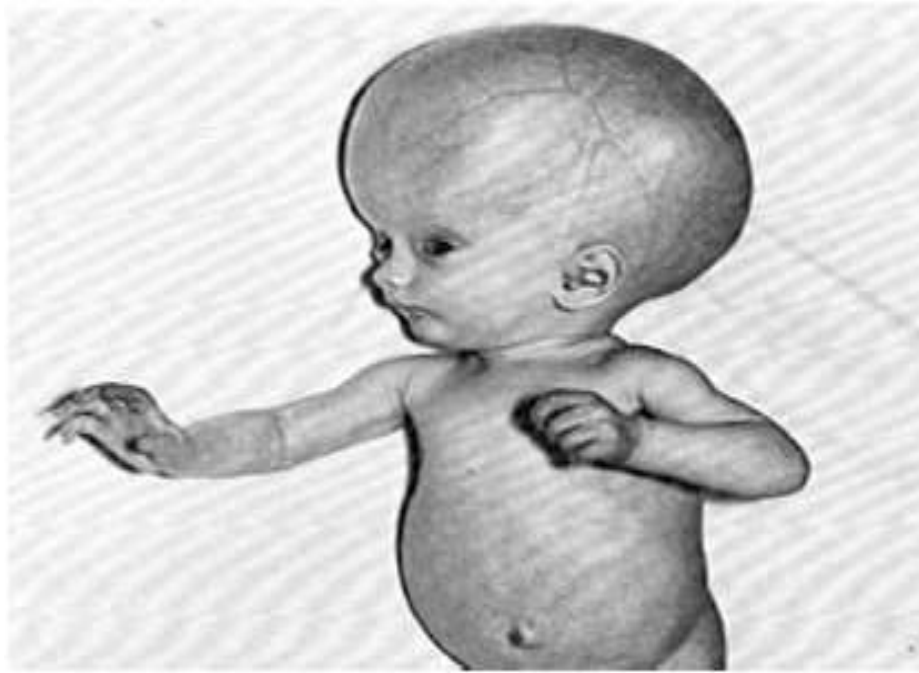


Figure (2-7): Congenital toxoplasmosis (hydrocephaly) (AL-Maamuri, 2014).

Cats typically have asymptomatic *T. gondii* infections (Dubey and Prowell, 2013). Fever, sadness, anorexia, neurologic symptoms (such as altered behavior, seizures, ataxia, and polyneuropathy) and uveitis Cucos *et al.*, (2015) are all present in cats with toxoplasmosis (Mari *et al.*, 2016). weight loss, dyspnea, diarrhea, myopathy, icterus, lameness, and muscle hyperesthesia (Guyen and Ceylan, 2020). Congenital infections are generally more severe than adult cat infections (Dubey and Lappin, 2006). Kittens acutely infected by a lactogenic or transplacental route suffer from more severe forms and often die from liver and pulmonary disease (Atmaca *et al.*, 2013). Brownlee and Sellon (2001) indicated that the hallmark of disseminated toxoplasmosis is pneumonia. (which can be complicated by septic shock and acute respiratory distress syndrome (Evans *et al.*, 2017).

2.8. Immunity Response of Toxoplasmosis

T.gondii is a member of Apicomplexa, which can infect any nucleated cell of the warm-blooded animals. During infection *T. gondii* widely spreads in

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circulatory system and can cause persistent infection in several organs such as the brain and heart (Harker *et al.*, 2015). Around 30% of the world's population has a persistent asymptomatic infection (Pappas *et al.*, 2009). The parasite also induces a cellular immunity (directed against intracellular parasites) and humoral immunity (against blood parasites) as well (Shatha, 2005). The immune response includes innate immunity, which represents adaptive immunity, which is divided into cellular and humoral immunity (Ivanova *et al.*, 2019).

2.8.1. Innate Immunity

The most important factors in the innate immune response are macrophages, dendritic cells (DCs), natural killer cells (NK cells), and cytokines (Negishi *et al.*, 2018). The inflammatory human innate immune response begins early after infection, and starts to be shut off approximately two weeks after a primary *T. gondii* infection (Denis and mutual households) (Ermanno, 2004). As described by Yarovsky, (2014) innate immunity is considered the first defense mechanism against invasive microbial infection until adaptive immunity join becomes relevant. IL-12. This developmental process marks the maturation of the immune response during *T. gondii* infection, ultimately leading to the production of protective interferon (IFN) and the killing of toxic tachyzoites (Mahmoudzadeh *et al.*, 2021; Hamid *et al.*, 2021).

IL-12 stimulates NK cells to secrete interferon- γ (IFN- γ). IFN- γ is known to induce the killing of phagocytic tachyzoites in both the acute and chronic phases of *T. gondii* infection and is a key mediator of host resistance (Hou *et al.*, 2022). NK cells are involved in the early production of IFN- γ in *T. gondii* infection. This cytokine is thought to be primarily produced by CD4 and CD8 T cells (Ribot *et al.*, 2021). Myeloid cells, in addition to producing IL-12, also produce TNF, which promotes the production of more IFN- γ by lymphocytes and acts as a cofactor to induce the microbicidal mechanism of macrophages

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(Denkers and Microbiology, 2003). In fact, killing and cytokine production are among the functions of NK cells (Poznanski and Ashkar, 2019). Pro-inflammatory cytokines from dendritic cells (DCs), neutrophils, and macrophages can enhance NK cell production of IFN- γ , thereby promoting early resistance to the parasite in the stages of *T.gondii* infection. NK cells help control the number of *T. gondii* in infected mice by killing intracellular and extracellular parasites and promoting cytotoxicity(Briukhovetska *et al.*, 2020).

2.8.2. Adaptive Immunity

Adaptive immunity is divided into cellular and humoral immunity (Ivanova *et al.*, 2019). According to Abdulbaset *et al.*, (2020) humoral and cellular immunity, which includes antibodies and T cells (CD4+ and CD8+ T lymphocytes), is crucial for offering protection against *T. gondii* infection.

2.8.2.1. Cell Mediated Immunity Responses

Cellular immunity is one of the most important components of the host's immune response to *T. gondii* infection (Gao *et al.*, 2018). It not only protects the host from acute toxoplasmosis but also helps prevent chronic infection (Khan *et al.*, 2019). Since *T.gondii* is an obligate intracellular parasite, cellular immunity (CMI) is crucial for defense against toxoplasmosis. Secondary lymphocytes, macrophages, natural killer cells, and lymphokine-activated killer cells work synergistically to protect the host from toxoplasmosis. CD8+ T cells possess cytotoxic activity against infected host cells, forming another protective barrier (Hakim *et al.*, 1991).

Th1 cells produce IFN- γ and TNF- β and induce IL-2 production, while Th2 cells release IL-4, IL-5, IL-10, and IL-13 (Sen *et al.*, 2021). The Th1 immune response is associated with immunity to toxoplasmosis (Mercer *et al.*, 2020). *T. gondii* peptide antigens are presented by antigen-presenting cells (APCs) via their MHC class I molecules (Hammed-Akanmu *et al.*, 2022).

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After recognizing the *T.gondii* peptide antigens, naive CD4 T lymphocytes begin to differentiate and undergo clonal expansion. Following the secretion of IL-12 by macrophages and other APCs, this cytokine stimulates naive CD4 cells to form a Th1 effector cell subset (Groft *et al.*, 2020). Infected cells carrying tachyzoites are recognized and eliminated by CD8 cytotoxic T cells, thereby eliminating the source of infection in the acute phase (Smith *et al.*, 2021). This IFN- γ activity appears to be limited to MHC class I molecules and contributes to enhancing host resistance in the chronic phase of *Toxoplasma gondii* infection (Gazzinelli *et al.*, 1992).

2.8.2.2 Humoral Immunity Response

Humoral immunity the term refers not only to the antibody itself but also to other processes associated with its generation and lifespan (e.g., activation of type 2 helper T cells, cytokine production, germinal center formation, isotype conversion, affinity maturation, and memory cell formation). It also encompasses the role of antibodies in the host's defense mechanisms, such as neutralizing pathogens/toxins, activating classical pathways via the complement system, and opsonization (Janeway, 2001). Dziubek *et al.*, (2001) described a humoral immune response in which antibodies such as IgM, IgA, IgG, and IgE are secreted by B cells. In antibody-dependent cell-mediated cytotoxicity (ADCC), these antibodies are mobilized along with the complement system to clear parasites from the body fluid. Antibodies can clear parasites in two ways: 1) by agglutinating parasites, which are then phagocytosed by phagocytes; and 2) by activating the complement system, leading to parasite lysis (death) (Sah *et al.*, 2018).

Following infection, antibody titers increase, with IgM being the first antibody produced, indicating a primary acute infection. However, this immune response is short-lived, while post-infection IgG antibodies persist for life (Krahebuhl and Remington, 1982). IgM antibodies are expressed earliest and can be detected in mouse peritoneal fluid as early as the second

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day after infection, attached to the surface of *T. gondii*, while specific serum IgM antibodies are not detectable until the end of the seventh day after infection (Clark, 1983). These immunoglobulins are highly cytotoxic due to their structural characteristics and effectively activate the complement system. They are also very effective at agglutination (Filisetti and Candolfi, 2004). IgG antibodies typically appear 1–2 weeks after infection, peak within 1–2 months, and then decline slowly, but remain detectable for life. On the other hand, IgM antibodies may appear earlier and decline more rapidly than IgG antibodies (Abdelbaset *et al.*, 2020). Filisetti and Candolfi, (2004) noted that IgG is the second most abundant immunoglobulin in toxoplasmosis, existing in four subclasses: IgG1, IgG2, IgG3, and IgG4. They are described as predominant and completely dominant in toxoplasmosis as part of the host's immune response strategy. Their primary target antigens are the surface antigens of the parasite.

Antibody titers rise after infection (Krahebuhl and Remington, 1982). IgM is the primary antibody, indicating an acute, transient infection, while IgG is detectable lifelong after infection. The presence of antibodies (elevated IgG and IgM antibodies against *T. gondii*) is evidence of toxoplasmosis infection in patients (Gasior and Solowinska, 2022). IgA exists in two forms: serum type and mucosal type, the latter present in mucus secretions. In toxoplasmosis, both types of IgA are present in the gastrointestinal tract and serum of both animals and humans. In congenital toxoplasmosis, IgA detection is particularly important because IgA can be detected even in the absence of IgM. IgA (like IgM) cannot cross the placenta and plays a crucial role in the diagnosis of congenital toxoplasmosis (Pinon *et al.*, 1990). The immune mechanism against toxoplasmosis in cats is not fully elucidated. According to research by Sanchez *et al.*, (2010) cell-mediated effector immune responses are essential in both humans and mice. All infected cats produce IgG and IgA antibodies, and over 80% also produce IgM antibodies.

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IgG antibody titers peak two to three weeks after their initial appearance, while full manifestation of IgG antibodies can take four to six weeks (Dubey and Lappin, 2006).

2.9. Diagnosis of Toxoplasmosis

Toxoplasmosis can be identified in a variety of ways, there are many methods for identifying *T. gondii* infections, which are separated into serological and non-serological assays, because there are no clear and recognizable clinical toxoplasmosis symptoms (Abdelbaset *et al.*, 2020).

2.9.1. Microscopical Examination

T. gondii infection is diagnosed using several methods, such as polymerase chain reaction and microscopic detection, have been used in laboratory toxoplasmosis diagnosis (Salant, 2007). By using direct microscopy to find tachyzoites or tissue cysts, *T. gondii* can be diagnosed directly (Xue *et al.*, 2021). Its conventional method for detecting *T. gondii* infection in fecal, water, and tissue samples is less sensitive and cannot be relied upon alone. Filtration or centrifugation techniques, as well as the impression method for examining tissue cysts, can detect oocysts from large amounts of samples in fecal material and water. Cats are diagnosed by looking for oocysts in their feces (Elmore *et al.*, 2010).

2.9.2. Serological Tests

Serologic testing for *Toxoplasma*-specific immunoglobulin G (IgG) antibody is the most used technique for identifying toxoplasmosis (Hughes *et al.*, 2000). IgG titers rise within 1 to 2 months after infection and are raised for life, but *Toxoplasma* immunoglobulin M (IgM) antibody titers rise shortly after infection but usually fall quickly and become undetectable within weeks or months. (Subauste *et al.*, 2004). The primary method for diagnosing *Toxoplasma* infection is the identification of antibodies specific to *Toxoplasma* (Molaei *et al.*, 2022). *Toxoplasma* IgM detection is a sensitive

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indicator of primary toxoplasmosis, but its specificity is restricted since natural IgM antibodies can sometimes bind to *Toxoplasma* antigens even when there is no infection. *T. gondii* was identified using a variety of serological diagnostic techniques, such as the enzyme-linked immune sorbent assay (ELISA) (Pittman and Knoll, 2015). Mahalakshima *et al.*, (2007) found that the technique for amplifying the B1 repetitive sequence was more sensitive than the traditional method (enzyme-linked immunosorbent assay, or ELISA) when applied to the analysis of lens aspirates from congenitally infected human cataract patients. IgA, IgM, IgG, and IgE are antibodies that have distinct patterns of rising and falling over time following infection and are frequently measured using various immunological techniques (Zhang *et al.*, 2016). The latex agglutination test, enzyme linked immunosorbent assay, and other various serological tests are among the many ways that humoral antibodies can be found (Al-Ani *et al.*, 2020).

2.9.2.1. Enzyme Linked Immuno Sorbent Assay (ELISA)

An essential method for primarily detecting IgG and IgM through a series of stages performed on the patient's serum is the enzyme-linked immune sorbent assay (ELISA) (Iddawela *et al.*, 2015). *Toxoplasma* RH strain tachyzoites are used to make a soluble antigen preparation for the original ELISA (Voller *et al.*, 1976). The presence or absence of anti-*T. gondii* specific antibodies in the patient's serum sample was the primary determinant of whether toxoplasmosis was detected (Tekkesin *et al.*, 2011). Is an easy, quick, and precise way to show if a person has acute or chronic toxoplasmosis IgM or IgG antibodies (Pinon *et al.*, 1990). It makes use of an enzyme, antibody, and antigen mixture that is adsorbed onto a test well. IgM and IgG can be detected with great specificity using the ELISA approach (Pinon *et al.*, 1995). According to Kamal *et al.*, (2014), the ELISA approach is crucial for identifying *T. gondii* in pregnant women during the first few months of

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pregnancy and is useful for diagnosing infected women within the period of four to five months.

2.9.2.2. Latex Agglutination Test (LAT)

Toxoplasma IgM and IgG are typically diagnosed with this modified agglutination test, which uses antigen-coated latex particles as a carrier of the antigen. The antigen-antibody complex can be seen visually thanks to the response, and Abs and titers increase quickly during the acute phase (Ye *et al.*, 2011).

2.9.3. Polymerase Chain Reaction (PCR)

PCR is frequently employed in biological and medical research for a variety of purposes, including fingerprinting, infectious illness detection, and the identification of genetic diseases (Schonian *et al.*, 2011). Recognition of genetic *T. gondii* can be quickly detected in a variety of clinical samples using real-time PCR, an additional diagnostic technique (Su *et al.*, 2010). According to Al-Ani *et al.*, (2020) PCR is a nucleic acid-based recognition method for DNA extraction from *T. gondii*. The following are some of the several PCR principles that are employed to detect *T. gondii* DNA: The purpose of a PCR is to produce a large number of copies of a gene, which is required to have an adequate starting template for sequencing (Roth *et al.*, 2001).

2.9.3.1. Conventional PCR

The four nucleotides that make up DNA are symbolized by the letters A (adenine), C (cytosine), G (guanine), and T (thymine). The PCR is based on the same mechanisms for copying DNA as those found in nature (Campos and Quesada, 2017).

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2.9.3.2. Nested PCR

Nested PCR, which uses two sets of primers in two consecutive PCRs to detect *T. gondii*, is thought to be more sensitive than conventional PCR and is also based on B1 gene and ITS-1 sequences (Jones *et al.*, 2000). The product of the first reaction serves as a template for the second PCR reaction. According to Rostami *et al.*, (2018) nested PCR is utilized to both improve the specificity of DNA amplification and identify diseases that are present in extremely small quantities.

2.9.3.3. Real -Time PCR

Recently, RT-PCR-based assays have been developed that enable the assessment of parasite load and have a high level of complex-specific diagnostic accuracy for pathogen diagnosis in clinical samples (Rostami *et al.*, 2018).

RT-PCR is a more sensitive method than other molecular and biological techniques, according to most of these research (Santos *et al.*, 2014). PCR, real-time PCR, and nested-PCR tests have emerged as crucial instruments for *T. gondii* molecular diagnostics in recent years (Xue *et al.*, 2021). According to Rostami *et al.*, (2018) real-time PCR with probe hybridization is the most sensitive test among these PCR-based amplification methods, which have demonstrated good sensitivity and specificity.

2.10. Control and Prevention and Treatment

Health education initiatives to increase knowledge about appropriate food hygiene, cooking techniques, and limiting contact with potentially contaminated sources are among the preventive interventions and methods for toxoplasmosis control in Iraq (Ismael, 2023). When handling meat, one should wash their hands with soap and water to avoid the human colonic parasite *T. gondii*. Additionally, all meat-processing equipment should be

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cleansed to remove the parasite's infectious stages (AL-Tameemi *et al.*, 2019). To do this, it is advised to freeze meat at -12°C for 24 hours and to practice proper hygiene (e.g., washing hands after coming into contact with soil, washing raw fruits and vegetables) (Elmore *et al.*, 2010).

It is best to avoid tasting food while it is cooking and to cook animal meat until it reaches 64°C. (Al-Tameemi *et al.*, 2019). According to Elmore *et al.*, (2010) cat owners can lower the exposure risk for their pets by keeping all cats indoors and avoiding giving them raw meat. Since there is currently no effective vaccination for humans, the best way to combat toxoplasmosis may be to avoid zoonotic transmission, which requires restricting exposure to tissue cysts or oocysts (Elmore *et al.*, 2010). Khairullah *et al.*, (2024) recommend the use of dihydropteroate synthetase and dihydrofolate reductase inhibitors as treatment for toxoplasmosis. Spiramycin is used to prevent *T. gondii* congenital defects in expectant mothers (Thiebaut *et al.*, 2007).

Spiramycin is only effective in preventing the infection from spreading to the fetus if the parasite hasn't yet reached him. If the infection has reached the fetus, the woman can treat it with pyrimethamine and sulfadiazine combined with folic acid, but keep in mind that pyrimethamine has an antifolate effect, so the treatment should be given after the first trimester. We also know that a lack of folic acid will interfere with the development of the fetus's brain and may result in thrombocytopenia (Jones *et al.*, 2003).

Sulfadiazine and pyrimethamine are used to prevent fetal damage in the event that the fetus is found to be infected (if the amniotic fluid is PCR positive) (Kishore *et al.*, 2003). Pyrimethamine found synergistic with sulfonamides against dividing tachyzoites (Eyles and Coleman, 1953). Pyrimethamine found synergistic with sulfonamides against dividing tachyzoites (Eyles and Coleman, 1953). According to Verma and Khanna,

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(2013) there is presently no approved human vaccination against *T. gondii*, and research into human vaccines is still in progress. As of 2016, no approved human vaccine exists against *T. gondii*.

Chapter Three

Materials and Methods

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3. Materials and Methods

3.1. Materials

3.1.1. Instruments and Equipment

Instruments and Equipment used in this study are listed in the table (3-1).

Table (3-1). Instruments and Equipment have been used in this study

No.	Instruments	Company/ origin
1	Autoclave	Wisd / USA
2	Balance	Kern / China
3	Beaker, Conical Flask, Cylinder	Kern / China
4	Beakers 100, 500ml	Memmert / Germany
5	Centrifuge (Micro and Head)	Mikro Hettich / Taiwan
6	Cover Slides	Memmert/ Germany
7	Digital Camera	Samsung / Korea
8	Disposable pipettes	Deans Gale / China
9	Disposable Syringes	Sterile EO / China
10	Dry microtubes incubator	Ae / UK
11	EDTA Tube	Biozek / Netherlands
12	Electrophoresis system	Cleaver / UK
13	Eppendorf Tube (1.5, 2.0ml)	Eppendorf/ Germany
14	Gauze	Kardelen / Turkey
15	Gel Tube	Biozek / Netherlands
16	Glass Slides	Sail brand / China
17	HERATHERM IMC 18	Thermo Scientific /UK
18	Ice Pack	Kern /China

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19	Ice crash	Jouan/ China
20	Incubator	Memmert / China
21	Kern PFB balance	Kern & Sohn / Germany
22	Latex gloves	Suzkou dukanging medical /China
23	Light Microscope	Nova/ China
24	Micrometer (Ocular)	Japan / optical
25	Micropipettes difference in size 0.5-10, 5-50, 100-1000 μ l	Sterilin/ UK
26	Microplate Reader	Paramedical PKL /Italy
27	Microplate washer	Biotech /USA
28	Microfuge IB Centrifuge	Beckman Coulter/Germany
29	Optimus 96G thermal Cyclor	QLS / UK
30	Pipette Tip	Eppendorf/ Germany
31	Pipettes	DARWELL/China
32	Rack	Alpha/ India
33	Sterilized cotton swabs	Falcon/ USA
34	Stratagene Real-Time thermal cyclor MX3005P	Stratagene / USA
35	Disposable Syringe 3ml	Suzkou dukanging medical / China
36	Disposable Syringe 5ml	Suzkou dukanging medical / China
37	Timer	Ker / China
38	UVP	Analytik Jena / UK
39	Water bath	GFL/China

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3.1.2. Laboratory kits

The kits used in the present study are listed in table (3-2).

Table (3-2): Device and Kits used in this study

No	Kits	Company / Origin
1	DRG Instruments GmbH IgG	Elecsys / Germany
2	DRG Instruments GmbH IgM	Elecsys / Germany
3	Sunlong Biotech. Co.	LTD/ China

3.1.3. Solutions

The solutions employed in this study are displayed in the table (3-3).

Table (3-3). Solutions used in this study

NO.	Solutions	Company/ origin
1	Distilled water	SDI/ Iraq
4	Ethanol 70%,96%	Alpha / India
2	Normal saline	SDI/ Iraq
3	Potassium dichromate 2.5%	BHD/ UK

Table (3-4). Materials used in PCR through the Study

No.	Material	Cat. No.	Company
1	AddPrep Genomic DNA Extraction Kit	10023	Addbio / Korea
2	Agarose LE	32034-50	Intron / Korea
3	GoTaq® G2 Green Master Mix	M7822	Promega / USA
4	GoTaq® Probe Qpcr	A6101	Promega/USA
5	Primers	---	MacroGen/Korea
6	RedSafe nucleic acid staining solution	21141	Intron / Korea
7	Safe-Green 100bp Opti-DNA Marker	G473	Abm / Canada
8	10X TAE (Tris Acetate EDTA) buffer	V4271	Promega / USA

3.2. Methods:

3.2.1. Study Design

This study was designed to detect *T.gondii* in samples of women and cats in Al-Nasiriyah City. The study design was as follows:

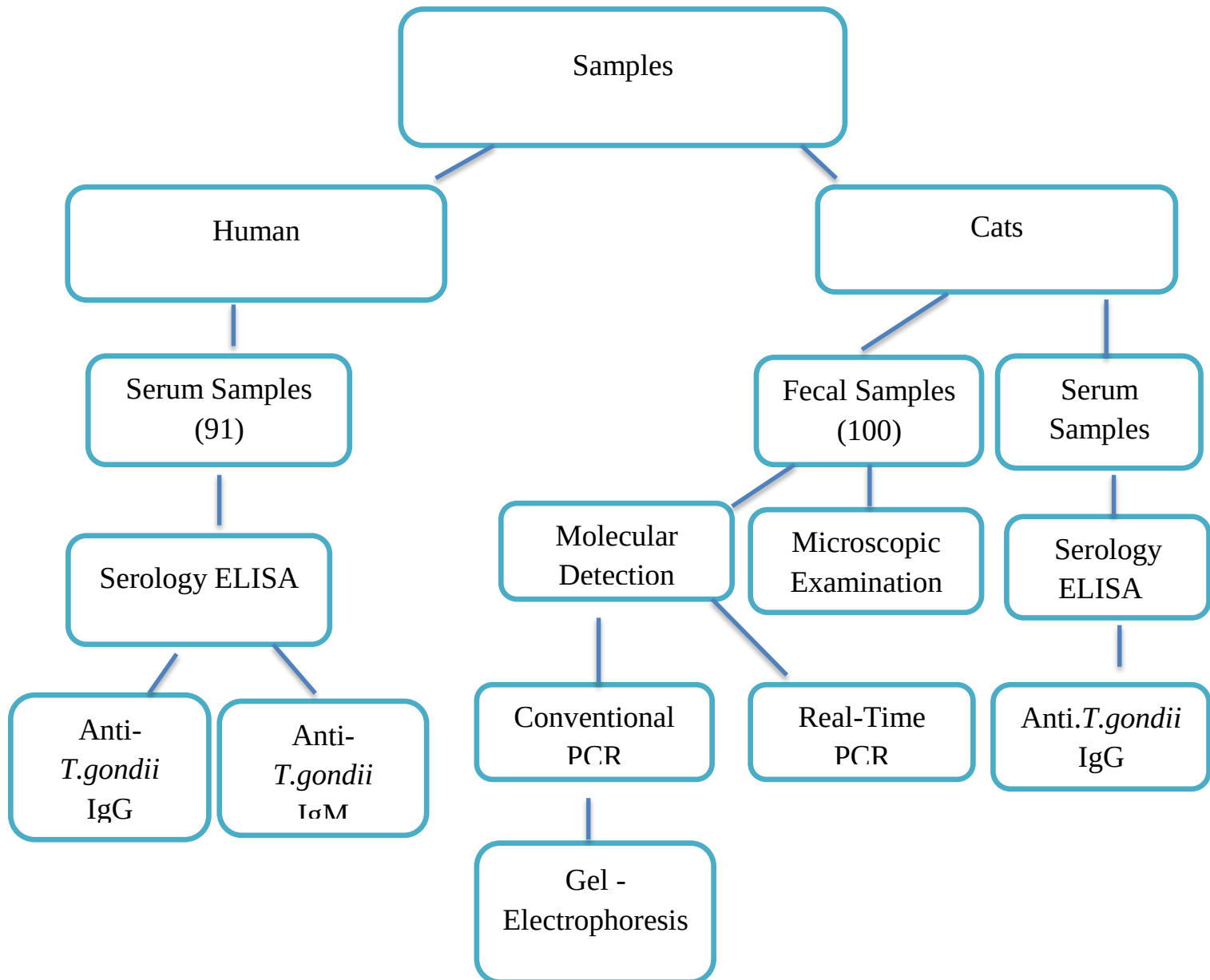


Figure (3-1): Study Design

3.2.2. Study Area, Samples Collection and Preparation

Investigation of *T.gondii* in serum samples of women by ELISA

This study was conducted in the city of Nasiriyah from November 2024 to March 2025. Samples were collected sterile from pregnant and non-pregnant women, those who had miscarriages, and those who had not randomly at Al-

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Haboubi Teaching Hospital, Obstetrics and Gynecology Department and private clinics. The required information was gathered by administering a questionnaire and creating an information sheet including (age, address, dietary habits, contact with cats, breed of the reared cat.

Using disposable syringes, five milliliters of venous blood were extracted from each woman's antecubital fossa or median cephalic vein after the area was cleaned with an alcohol swab for thirty seconds were put in an inactivated tube (gel tube) and allowed to coagulate at room temperature. The serum was then separated using a centrifuge set to 3000 rpm for roughly ten minutes.

3.2.2.1. Serological Tests for Investigation of *T.gondii* in Women by ELISA

T.gondii was determined based on indirect ELISA to evaluate IgG and IgM antibodies was carried out as for the manufacturer's protocol (DRG Instruments GmbH, Germany). In addition to IgG and IgM antibodies for pregnant and non-pregnant women, abortive and non-abortive in rural and urban areas in Al-Nasiriyah city. The manufacturer company claimed a diagnostic specificity of 98.3% for the kit for all three DRG production batches. For all three DRG production lots, the diagnostic sensitivity was 100%. The IgG cut-off values: 50 IU/mL interpretation of quantitative results are Positive: >55 IU/mL, Negative: 28 IU/mL, Negative < 23 IU/mL and Grey zone (equivocal): 23-28 IU/mL. ELISA's were used by indirect method as described by the manufacturer company.

A. Procedure (IgM) Antibody

- . Half μL of diluent was added and mixed with 10 μL of serum to prepare mixture Part 1.
- . Sixty μL of sorbent was added and mixed with 60 μL of mixture Part 1 to prepare mixture Part 2.

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- . One hundred μL of mixture Part 2 was taken and incubated for 60 minutes at 37°C .
- . The wells were washed five times with 300 μL of wash buffer each time.
- . One hundred μL of conjugate was added.
- . The plate was incubated for 30 minutes at room temperature in a dark place.
- . The wells were washed five times with 300 μL of wash buffer.
- . One hundred μL of substrate was added.
- . The plate was incubated for 15 minutes at room temperature in a dark place.
- . One hundred μL of stop solution were added to each well to stop the enzymatic reaction.
- . The optical density was read at 450/620 nm using a microtiter plate reader within 30 minutes following the addition of the stop solution.

B. Procedure (IgG) Antibody

- . One μL of diluent was added and mixed with 10 μL of serum.
- . One hundred μL of the mixture was taken and incubated for 60 minutes at 37°C .
- . The wells were washed five times with 400 μL of wash buffer each time
- . One hundred μL of conjugate was added.
- . The plate was incubated for 30 minutes at room temperature.
- . The wells were washed five times with 400 μL of wash buffer.
- . One hundred μL of substrate was added in a dark place.

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. The plate was incubated for 15 minutes at room temperature.

. One hundred μL of stop solution were added to each well to stop the enzymatic reaction.

After adding the stop solution, we measured the optical density (OD) of the solution in each well at 450 nm for the primary reading, and using a microtiter plate reader, we also subtracted the background at 620 to 630 nm. We make sure to read the wells within 30 minutes of stopping the reaction.

3.2.2.2. The Study Area, Sample Collection and Preparation for investigation of *T. gondii* in Cats Feces by Microscopic Examination

This study was conducted in Nasiriyah from October 2024 to February 2025. Researchers collected 100 cat fecal samples from various locations in Nasiriyah, including those from domestic cats (both male and female) and cats from animal shelters. Information such as age, address, breed, and sex of the cats was collected using questionnaires. Samples were stored in clean plastic containers, with 1 gram of each sample placed in sterile Eppendorf tubes and frozen for DNA extraction. Some samples were sent to the Parasitology Laboratory of the Faculty of Veterinary Medicine at Shatrath University for routine examination. Laboratory diagnosis and oocyst morphological identification were performed using standard laboratory procedures, including the direct wet mount method and the flotation method. After determining morphological characteristics, samples were treated with potassium dichromate for 1–3 days, followed by microscopic examination.

3.2.2.1. Detection of Parasite

3.2.2.1.1. Direct Wet Smear

One gram of feces was mixed with 10 ml of physiological saline (0.9%). The granule suspension was scraped off with gauze and placed on a sterilized glass slide, which was then covered with a coverslip. The slide was observed

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under a microscope at low magnification (100x) and high magnification (400x) to detect *T.gondii* oocysts (Duszynski and Wilber, 1997).

3.2.2.1.2. Flotation Technique

Mix approximately 1 gram of feces with 10 ml of water, and filter the mixture through gauze. Centrifuge and decant the clear supernatant. Mix the precipitate thoroughly with a saturated sodium chloride solution, centrifuge at 1500 rpm for 10 minutes, and pour the supernatant directly into a centrifuge tube without removing the precipitate. Placed the oocysts on a coverslip, allowing them to float to the surface and adhere. Invert the coverslip onto a slide and observed the *T. gondii* oocysts under a microscope (100x and 400x magnification) (Charles and Robinson, 2006).

3.2.2.1.3. Sporulation of Oocysts

Positive samples were treated with 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$), allowed to sporulate oocysts at room temperature in stool collection containers., and then utilized for morphological analysis (Duszynski and Wilber, 1997).

3.2.3. Serological Tests

3.2.3.1. Study Area , Samples Collection and Preparation for Investigation of *T.gondii* by ELISA Technique

This study was conducted in AL-Nasiriyah City from October 2024 to February 2025. Researchers collected 100 blood samples from stray cats in the Nasiriyah regions , including both domestic cats (male and female) and cats from animal shelters. Researchers used questionnaires and information sheets to collect necessary information about the cats, including their age, address, breed, and sex. Blood was collected using disposable syringes. 3 ml of blood was drawn from the jugular vein, transferred to an inactivated (gel)

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tube, and allowed to coagulate at room temperature. The serum was then separated by centrifugation at 3000 rpm for approximately 10 minutes. Finally, the serum samples were tested for IgG antibodies using an enzyme-linked immunosorbent assay (ELISA).

3.2.3.1.1. Procedure of ELISA to Determine IgG Antibodies in Serum Samples of Cats

The procedure was performed as recommended by the manufacturer (Senlong Biotechnology Co., Ltd., China): the same advanced working method for detecting IgG antibodies in human female serum samples was also applied to cat serum samples, except for the standard solution step, since the standard solution for cats is based on positive and negative standard solutions.

3.2.4. Molecular Studies in Cats

3.2.4.1 Extraction of Genomic DNA (Modified Specifically for the Extraction of Parasite DNA)

To isolate genomic DNA from whole fecal samples collected in stool collection containers. we used a commercially available purification method (Add bioPrep Genomic DNA Extraction Kit, Korea) Ahmed *et al.*, (2024). Additionally, we prepared fecal samples for DNA extraction using the QIAamp Fast DNA Fecal Mini Kit (Germany) according to the manufacturer's instructions.

3.2.4.1. Conventional PCR

3.2.4.1.1. Primers preparation

Dissolve the (initially lyophilized) primers in double-distilled water to a final concentration of 100 $\mu\text{M}/\mu\text{l}$. Store this stock solution at -20 °C according to the primer synthesizer manufacturer's instructions. Prepare working primers at a concentration of 10 $\mu\text{M}/\mu\text{l}$ using these stock primers.

Table (3-5). Amplification of A Specific GRA6 Gene Region (for genotyping of *Toxoplasma*)

Target gene		Sequence (5'-3')	Ta (°C)	Product size	Accession number	Reference
GRA6	F	GTAGCGTGCTTGTGGCGA C	56	773 bp	KX781158.1	Norouzi et al., 2016
	R	TACAAGACATAGAGTGCCC C				

3.2.4.1.2. Condition of Conventional PCR**Table (3-6). Conditions of Conventional PCR**

Phase	Tm (°C)	Time	Cycles
Initial denaturation	94°C	5 min	1X
Denaturation	94°C	30 sec.	35X
Annealing	56°C	30 sec.	
Extension	72°C	1 min	
Final extension	72°C	5 min	1X

3.2.4.1.3. DNA Electrophoresis on an Agarose Gel

After the PCR reaction is complete, gel electrophoresis is used to observe the size of the PCR products and assess the quality of the DNA extract. The gel concentration depends on the type of product. Typically, the concentration of DNA in an agarose gel is 0.7%, while the concentration of typical PCR products ranges from 1% to 2%.

3.2.4.2.1. Agarose Gel Preparation

Agarose gels were prepared using the method described by Sambrook *et al.*, (1989). Briefly, 1 gram of agarose was dissolved in 100 mL of 1×TBE buffer and then heated in a microwave oven. After the gel cooled to 45–50 °C, 5 µL of RedSafe nucleic acid staining solution was added, and the gel was poured into an electrophoresis tank and allowed to solidify for approximately half an hour. The electrophoresis tank was then placed inside the tank, and the

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gel surface was completely covered with 1×TBE buffer. After sample loading, Safe-Green 100 bp Opti-DNA Marker was added, and the electrophoresis apparatus was set as follows:90 V, constant current, 45 min. Finally, the gel was transferred to a UVP system, and the PCR products were observed under a 320 nm UV light source.

3.2.4.2.2. PCR Product Loading

Add 5 μ L of PCR product to the center of each well on a 1% gel. To determine the size of the PCR product, add approximately 5 μ L of Safe-Green 100bp Opti-DNA Marker to the first well of the gel lane.

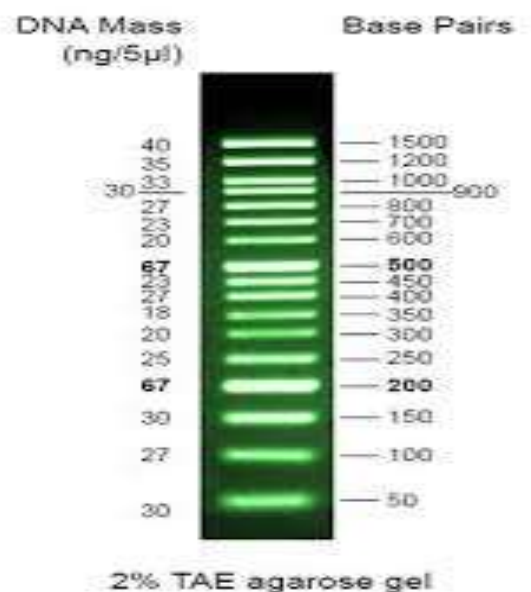


Figure (3- 2): Safe-Green 100bp Marker Opti-DNA

3.2.4.2. Real -Time PCR Assay

3.2.4.2.1. Primers Utilized in this Investigation

Table (3-7): Real –Time qPCR primers and probe

Target gene	Primer name	Sequence (5'-3')	Ta (°C)	Product size	Reference	Accession number
B1	F	CTAGTATCGTGCGGCAATG TG	56	62 bp	Kompali c-Cristo et al., 2007	AF17987 1
	R	GGCAGCGTCTCTTCCTCTT TT				
	Probe	FAM-5'- CCACCTCGCCTCTTGG-3'- BHQ1	60			

3.2.4.3. Real-Time PCR Assay

All suspected samples were subjected to probe-based real-time quantitative PCR. Conserved regions of the *T. gondii* B1 gene were amplified using specific primer pairs and probes. The reaction volume was 20 µL, with 0.2 µL per well containing 2 ng of genomic DNA and GoTaq® Probe qPCR Master Mix (Promega, USA). The reaction wells were placed in a PCR instrument (Stratagene, USA) and pre-denatured at 95 °C for 10 min. This was followed by 40 cycles, each consisting of 15 seconds of denaturation at 95 °C and 1 minute of annealing/extension at 60 °C. The error rate, calculated based on PCR repeat experiments, was less than 1%, and the amplification success rate exceeded 95%.

Table (3-8). PCR Solution Preparation

Components	Concentration	Volume (20 µl)
ddH ₂ O	-	5 µl
GoTaq® Probe qPCR Master Mix	1X	10 µl
Forward primer	10 µM/µl	1 µl
Reverse primer	10 µM/µl	1 µl
Probe 1	10 µM/µl	1 µl
DNA	40 ng	2 µl

3.2.4.3. Conditions Real -Time PCR

Table (3-9). Conditions of Real -Time PCR

Phase	T_m (°C)	Time	Cycles
Initial denaturation	95°C	10 min	1X
Denaturation	95°C	15 sec.	40X
Annealing	60°C	1 min	
Final Extension	72°C	30 sec	

3.2.4.4. Statistical Analysis

For statistical analysis, a Chi-square test (X²) was utilized to assess the independence of the variables. The analysis was conducted using IBM SPSS Statistics software, Version 27. A p-value of less than or equal to 0.05 was defined as the threshold for statistical significance (Mousa, 2005).

Chapter Four

Results

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4. Results

4.1. Results of Seroprevalence Studies of Toxoplasmosis in Women

The level of IgM in aborted and non-aborted women was studied using the ELISA test, and the results showed that women who had aborted were superior (out of 59 women who had aborted, 45 women gave positive results at with a precentage of 76.2%) compared to 24 women who gave positive results at a rate of 75% out of 32 who had not aborted, although there were no statistically significant differences between the two groups Table (4-1).

Table (4-1): Distribution of Anti-*Toxoplasma gondii* IgM Seropositivity in Aborted and Non aborted Women.

Women	Total No.	Positive		Negative	
		N.	%	N.	%
Aborted	59	45	76.2%	14	23.7%
Non aborted	32	24	75%	8	25%
Total	91	69	75.8%	22	24.1%
X2	0.018				
P value	0.892(No significant difference)				

The level of IgM was studied in pregnant and non-pregnant women using the ELISA test and the results showed superiority for non-pregnant women (out of 79 non pregnant women, 58 women gave positive results, at a rate of 73.4%), compared to 11 women who gave positive results, with percentage of 91.6%, out of 12 pregnant women. Despite the absence statistically significant differences, clear statistical differences were observed between the two groups Table (4-2).

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Table (4-2): Distribution of Anti-*Toxoplasma gondii* IgM Seropositivity in Pregnant and Non pregnant Women.

Women	Total No.	Positive		Negative	
		N.	%	N.	%
Pregnant	12	11	91.6%	1	8.3%
Non-pregnant	79	58	73.4%	21	26.5%
Total	91	69	75.8%	22	24.1%
X2		1.89			
P value	0.169(No significant difference)				

The level of IgG was studied in aborted and non-aborted women using the ELISA test, and the results showed that women who had aborted had a higher rate (out of 59 women who had aborted, 15 women gave positive results, at a rate of 25.4%), compared to 12 women who gave positive results, at a rate of 37.5%, out of 32 women who had not aborted. Despite the lack of significant differences, clear statistical differences were observed between the two groups Table (4-3).

Table(4-3): Distribution of Anti-*Toxoplasma gondii* IgG Seropositivity in Aborted and Non aborted Women.

Women	Total No.	Positive		Negative	
		N.	%	N.	%
Aborted	59	15	25.4%	44	74.5%
Non aborted	32	12	37.5%	20	62.5%
Total	91	27	29.6%	64	70.3%
X2		1.45			
P value	0.229(No significant difference)				

Chapter Four..... Results

The level of IgG in pregnant and non-pregnant women was studied using the ELISA test and the results showed superiority for non-pregnant women. Out of 79 non-pregnant women, 24 women gave positive results (30.3%) compared to 3 women who gave a positive result (25%) out of 12 pregnant women. Although there were no significant differences, clear statistical differences were observed between the two groups Table (4-4).

Table (4-4): Distribution of Anti-*Toxoplasma gondii* IgG Seropositivity in Pregnant and Non pregnant Women.

Women	Total . No	No. Positive		No.Negative	
		N.	%	N.	%
Pregnant	12	3	25%	9	75%
Non-pregnant	79	24	30.3%	55	69.6%
Total	91	27	29.6%	64	70.3%
X2		0.144			
P value	0.704(No significant difference)				

The level of IgM was studied in urban and the rural areas using the ELISA test and the results showed that women in the city had a higher rate (out of 80 women, 58 gave positive results, at a rate of 72.5%) compared to 11 women from the rural areas who gave positive results, at a rate of 100% out of 11. Despite the of statistical differences, a significant difference was observed between the two groups Table (4-5).

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Table (4-5): Distribution of Anti-*Toxoplasma gondii* IgM Seropositivity in Women of Different Regions of Al-Nasiriyah.

Region	No.Total	No. Positive		No.Negative	
		N	%	N	%
Urban	80	58	72.5%	22	27.7%
Rural	11	11	100%	0	0
Total	91	69	75.82%	22	24.1%
X2		3.98			
P value	0.046(Significant difference)				

The level of IgG was studied in the city and the countryside by ELISA test and the results showed that women in the city were higher (out of 80 women in the city, 26 gave positive results, at a rate of 32.5%) compared to 2 women who show positive results, at a rate of 18.18%, out of 11 women in the countryside. Despite the lack of differences, there is a possibility of statistical differences between the two groups Table (4-6).

Table (4-6): The Distribution of anti-*Toxoplasma gondii* IgG Seropositivity in women of different regions of Al-Nasiriyah.

Region	Total	No. Positive		No . Negative	
		N	%	N	%
Urban	80	26	32.5%	54	67.5%
Rural	11	2	18.2%	9	81.8%
Total	91	27	29.6%	64	70.3%
X2		0.931			
P value	0.335(No significant difference)				

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4.2. Results of Microscopic diagnosis of *T. gondii* in Cats

The study recorded an overall infection rate of 30% from a total of 100 samples. 100 samples, indicating a prevalence of toxoplasmosis of 30%. Microscopic examination was performed for the laboratory diagnosis of *T. gondii* in feline fecal samples using the wet direct smear and the flotation technique. Initially, wet direct smears were examined directly under the microscope to detect oocysts by identifying their characteristic features of *T. gondii* oocysts (figure 4-1).

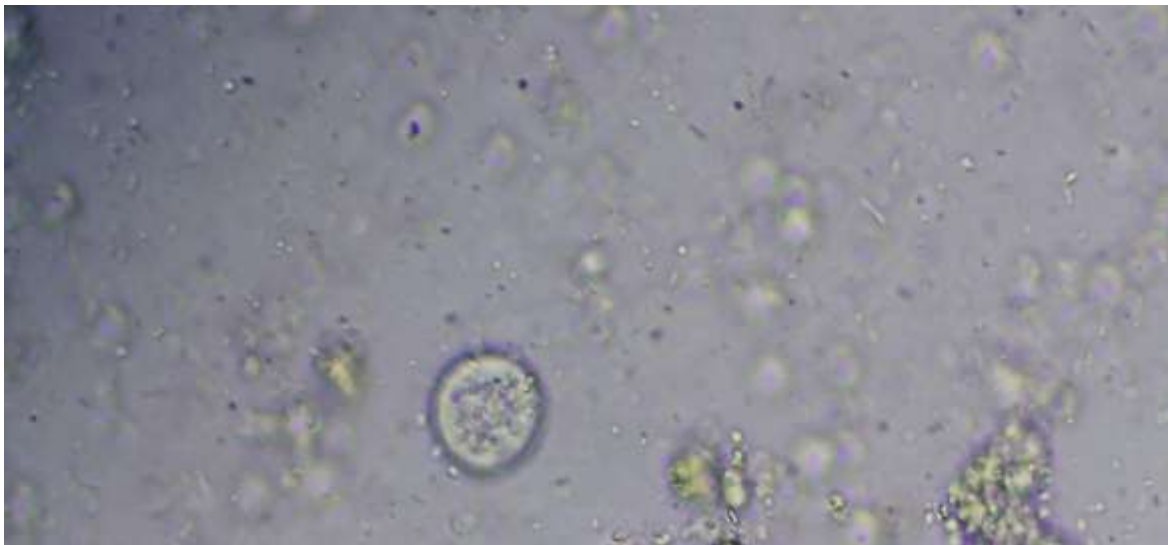


Figure (4-1): Non-sporulated oocyst of *T. gondii* 40X (direct wet smear).

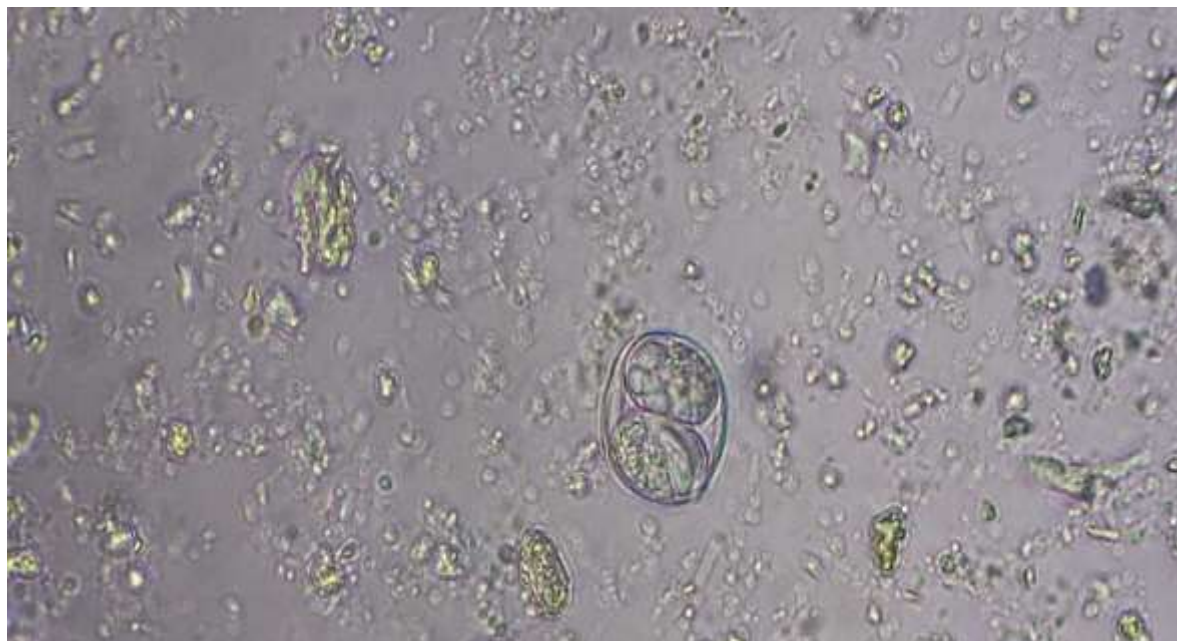


Figure (4-2): Sporulated oocyst of *T. gondii* 40X (direct wet smear), After the addition of potassium dichromate (2.5%).

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All slides were examined under various magnifications to confirm the diagnosis and record the findings. The results of *T. gondii* infection rates in cats showed an increase in infections during the months of February and November compared to other months of the study, with rates of 35.71% and 35% respectively, although no significant differences were noticed Table (4-7).

Table (4-7): Rate of Infection of Cats by *T. gondii* According to the Months of Year.

Month	No. of Examined samples	No. of infected samples	Rats
October 2024	15	3	20 %
November	20	7	35 %
December	15	3	20 %
January 2025	22	7	31.81 %
February	28	10	35.71%
Total	100	30	30 %
X ²		2.13	
P value	0.711(NS)		

NS: No significant difference at (P<0.05)

Regarding the rates of *T. gondii* infection in cats in the current study areas based on the sex of the cats, a higher infection rate was observed in female cats compared to males, although there were no significant differences between the two sexes. Female cats recorded an infection rate of 33.96% compared to 25.53% in males Table (4-8).

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Table(4-8): Rate of Infection of Cats by *T. gondii* According to Animal Sex

Sex of cats	No. of Examining samples	No. of infected samples	Rats
Female	53	18	33.96 %
Male	47	12	25.53 %
Total	100	30	30%
X ²		0.843	
P value	0.359(NS)		

NS: No significant difference at (P<0.05)

On the other hand, a higher rate of *T. gondii* infection was observed in exotic (pet) cat breeds compared to local breeds in different regions Al-Nasiriyah, with rates of 32.5% and 28.33%, respectively. However, there were no significant differences between the two cat breeds under study Table (4-9).

Table (4-9): Rate of Infection of Cats by *T. gondii* According to Breed of Cats.

Breed of cats	No. of Examiting samples	No. of infected samples	Rats
Locally	60	17	28.33
Cats Sanctuary	40	13	32.5
Total	100	30	30%
X ²		0.198	
P value	0.656(NS)		

NS: No significant difference at P<0.05)

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4.3. Results of Serological Detect of *T.gondii* in Cats

Regarding the results of the anti-*Toxoplasma gondii* IgG seropositivity test in cats in some areas of Al- Nasiriyah according to the sex of the cats under study , the results showed similar infection rates between the two sexes, with no significant difference, at 45.09% and 46.78% in males and females, respectively Table (4-10).

Table (4-10): Distribution of anti-*T. gondii* IgG Seropositivity in Cats According to Sex.

Sex	No. of Examined Samples	No. of Positive Samples		No.of Negative samples	
		N	%	N	%
Male	51	23	45.09A	28	54.90
Female	41	20	48.78A	21	51.21
Total	92	43	46.73	49	53.26
Calculated X^2	0.124				
Calculated P	0.725(No significant)				

Similar letters denote no significant difference at $P < 0.05$

While the results, according to the ages of the cats under study, showed a clear significant higher rate for the ages between 2-11 months compared to adult cats that were older than this age in the same serological test for anti-*Toxoplasma gondii* IgG seropositivity, with a rate of 53.57% and 43.75% in the two age groups mentioned, respectively Table (4.11).

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Table (4.11): Distribution of anti-*Toxoplasma gondii* IgG Seropositivity in Cats According to Age

Age	No. of Examined Samples	No. of Positive Samples		No. of Negative Samples	
		N	%	N	%
2-11 months	28	15	53.57A	13	46.42
Adult	64	28	43.75B	36	56.25
Total	92	43	46.73	49	53.26
Calculated X2		0.755			
Calculated P	0.385(No Significant difference				

Similar letters denote no significant difference at $P < 0.05$

While no significant differences were recorded between the cat breeds under study, the percentages were very close, at 40.9% in domestic cats compared to 48.57% in sanctuary cats Table (4-12).

Table (4-12): Distribution of anti-*Toxoplasma gondii* IgG Seropositivity in Cat according to the Breed Type

Breed Type	No. of Examiting Samples	No. of positive Samples		No. of Negative Samples	
		N	%	N	%
Domestic cats	22	9	40.9A	13	59.09
Cat sanctuaries	70	34	48.57A	36	51.42
Total	92	43	46.73	49	53.26
Calculated X2		0.395			
Calculated P	0.530(No significant difference)				

Similar letters denote no significant difference at $P < 0.05$.

4.4. Results of Molecular Analysis

The molecular assays performed on the cat fecal samples results of PCR conventional and Real -Time PCR to diagnose *T.gondii* were negative, indicating the absence of detectable *T. gondii* DNA within the diagnostic sensitivity limits of the methods employed.

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Discussion

5. Discussion

5.1. Seroprevalence Studies of Women

Women who own or do not own cats may contract toxoplasmosis, which is linked to a number of factors such as living in a rural area or consuming food and water that have been tainted with *Toxoplasma* oocysts, as well as cats, whether they are stray or owned and direct contact with cats may increase the risk of *T. gondii* infection (Alkubaisi, and Al-Zubaidy , 2023). The present study recorded an IgM and IgG rate of 75.8% and 29.7% respectively in women in Al-Nasiriyah region. Results were also different to results recorded by Mustafa *et al.*,(2024) in Zakho ,Iraq who recorded a ratio of IgG (42.1%) with no level of IgM recorded.

The results of the present study also differed from that of Jaber and Noori (2021) who discovered that the seroprevalence of Toxoplasmosis among women in Duhok city /Iraq using the ELISA technique was 28% for anti-*Toxoplasma* IgG antibody and 0.46% for IgM antibody, and results stated by Mikaeel and AL-Saeed (2011) in that *T. gondii* IgG was shown to be highly prevalent (42.1%) in another study conducted in Duhok Province, Iraq.

The differences in results of different mentioned studies may be due to environmental variation of cities of studies which tend to cold and tropical in the north of Iraq in comparison to the Nasiriyah in the south of Iraq which characterized by high temperatures in addition to the variation in the style of life in those cities which tend to be rural with poor health care in Al-Nasiriyah and Southern Iraq in compare to that of Northern Iraqi cities. Another cause of disparity in results may be the status of women (aborted and non-aborted, pregnant and non-pregnant...etc) as it was considered in our study compared to the other studies. Also, the high ratio of *Toxoplasma* IgM antibody in our study may be due to high number of acute infections especially in the aborted

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women, which may explain the elevated IgM titer among rural women in the current study compared to those urban women in Al-Nasiriyah regions (100% vs. 72.5%) (Murad and Eassa ,2023), also recorded a same elevated ratio within the rural women in compare to those urban women when they found that pregnant women from villages

of Akre City / Iraq had greater percentages of IgG (57.14%) and IgM (4.08%) based on the residency factor than those who resided in urban areas had IgM and IgG levels of 0% and 48.48%, respectively. Regional variations in toxoplasmosis incidence rates in Iraq are influenced by climate and personal and communal cleanliness practices (Ismael, 2023). The results of the current study also revealed higher rate of infection in comparison to results of Alkubaisi and Al-Zubaidy, (2023) in Al-Anbar provance / Iraq when they record that (27.5%) of blood samples of female human were positive by latex test (IgM, IgG).

Toxoplasmosis seroprevalence rates, however, have been shown to be significantly higher in a few Iraqi cities, with rates of 55.26%, 52.6%, and 42.6% (Saadi and Ahmed, 2020 ; Hadi *et al.*, 2016), found a significant prevalence rate of toxoplasmosis in pregnant women in Al-Muthanna province, which agreed with the present study in respect to *Toxoplasma* IgG ratio (16.5% vs. 13.2%) in non-pregnant and pregnant women respectively, and contradicted with our result which recorded a high ratio of *Toxoplasma* IgM in non-pregnant women in compare to that of pregnant (12. 9% vis 63.7%). Al-Seadawy and Hemza, (2010) refer to that the abortion was significantly correlated with IgM positivity, and the positive IgM ELISA results were significantly correlated with having domestic animals, consuming raw or undercooked meat, and coming into touch with cats and soil.

5.2. Microscopic Diagnosis of *T.gondii* in Cats

A study recorded a total of 100 samples collected to reveal the prevalence of *T.gondii* in cats' fecal samples in some regions in Al-Nasiriyah total infection rate reached (30%). The rate of infection recorded in our study is less than that of Jalil and Alwan, (2014) in stray cats in Baghdad (66%) and also less than result of Ubaid *et al.*, (2025) in India (55.2%). On the other hand, the rate of infected cats in our study was higher than that of Tan *et al.*, (2020) in Malaysia, and higher than the results of (Khan and Ibrar, 2024) in district Buner in Pakistan, who recorded a total ratio of (23.86%).

Results also higher than those of (Wallace, 1971; Dubey *et al.*, 1977), who found a ratio of (0.59%) and (1.3%) respectively. According to one meta-analysis study, around one in fifty cats worldwide are actively shedding *T. gondii* or oocysts that resemble it, and approximately one-third of both domestic and non-domestic cats have been exposed to *T. gondii* (Hatam-Nahavandi *et al.*, 2021).

The differences in infection rate may be explained by the number of samples and the prevalence of infection in study region. Dubey *et al.*, (2020) felids are essential to the epidemiology of *T. gondii*, because a single cat can release millions of oocysts, which can infect numerous additional vulnerable hosts. The parasite was detected by microscopy and identified by morphological characteristics. The results of *T. gondii* infection rate showed an increase in infections during the months of February and November compared to other months of the study, with rates of 35.71% and 35% respectively, ($P < 0.05$) without significant differences.

This result is exactly similar to the results of Khan and Ibrar (2024) in district Buner in Pakistan, who recorded an increased ratio in the same mentioned months compared to the other months, their results were (25.9% and 25.7%) in February and November respectively. This may be attributed to

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the cold climatic conditions during these months, which are favorable for the growth and development of oocysts, in comparison to the moderate or warmer environmental conditions in other months (Herman *et al.*, 2010; Khan and Ibrar ,2024). Sporozoites develop in Oocysts after (1 – 5) days of exposure to oxygen and appropriate environmental temperature and humidity, sporulated oocysts can survive in the environment for months to years and are resistant to most disinfectants (Powell *et al.*, 2001 ; Lappin, 2010).

Regarding the rates of *T. gondii* infection in cats in the current study regions based on the sex of the cats, a higher infection rate was observed in female cats compared to males, although there were no significant differences between the two sexes. Female cats recorded an infection rate of 33.96% compared to 25.53% in males with no significant difference. It may be due to the number of samples or the health status of the females during the pregnancy and lactation season, The increased rate of female cat infection with *T. gondii* in comparison to male cats. This agrees with recorded Jalil and Alwan (2014) in Baghdad. On the other hand, a higher rate of *T. gondii* infection was observed in exotic (pet) cat breeds compared to local breeds in regions of Al-Nasiriyah with rates of 32.5% and 28.33%, respectively. However, there were no significant differences between the two cat breeds under study. This may be due to the increasing popularity of raising exotic cats in Iraq and the lack of proper health care provided by some households, making them more susceptible to infection compared to local breeds (Ubaid *et al.*, 2025). Differences in lifestyle and diet play an important role in explaining shedding variation between free-ranging unowned domestic cats, owned domestic cats and wild felids. Domestic cats are probably the major source of contamination since oocyst formation is greatest in domestic cats (Hill and Dubey, 2002).

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Result was in contrast with that of Tan *et al.*, (2020) who noticed that the rate of infected stray cats in Malaysia was higher than of pet ones, and also higher than that mentioned by (Hatam-Nahavandi *et al.*, 2021). who refer that there was an estimated 2.6% worldwide pooled prevalence of oocysts in the fecal investigated specimens from domestic cats and 2.4% in the fecal samples from wild felids. The difference in results between studies may be due to the difference in the care programs, especially for pet cats, depending on country of study.

5.3. Seropositivity in Cats

On the other hand, the results of *anti-Toxoplasma gondii* seropositivity IgG showed similar infection rates between the two sexes of cats under study, with no significant difference, at 45.09% and 48.78% in males and females, respectively. This result is in contrast with that of Kim *et al.*, (2008) in Korea, who found that female stray cats had a higher infection rate (29.2%) than male cats (24.0%). Sroka *et al.*, (2018) also referred to increase anti-*Toxoplasma gondii* seropositivity in females (74.1%) compared to males (58.8%). The global estimated seroprevalence of *T. gondii* in domestic cats is between 30 and 40 percent (Elmore, *et al.*, 2010). Jalil and Alwan, (2014) in Baghdad also found that the females had more seropositive anti-*Toxoplasma* IgG antibodies (75%) than males (30%), this may be attributed to hormonal factors affecting the immune response, namely progesterone in female which lead to reduce immunity in different phases of reproductive status, which in turn explain the elevation the ratio of infection in female versus the male.

According to the ages of the cats under study, there was a clear significant higher rate for the ages between 2-11 months compared to adult cats that were older than this age in the same serological test for anti-*Toxoplasma gondii* IgG seropositivity, with a rate of 53.57% and 43.75% in the two age groups mentioned, respectively. This result is in agreement with that found by Jalil

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and Alwan (2014) in Baghdad, who ensured that the young cats had a higher seropositive ELISA-IgG percentage (100%) than adult cats (63.82%). These findings corroborated the theory put forth by Jittapalapong *et al.*, (2005) who discovered that antibody prevalence varied with age and that *T. gondii* seroprevalence was often higher in older animals than in younger ones. Sroka *et al.*, (2018) also found that anti-*Toxoplasma* antibody frequency was considerably higher in older cats (>1 year) (83.5%) compared to younger cats (48.3%). conversely, the results of this study contradicted with the results of Gyorko *et al.*, (2011) the refer of this study that the seroprevalence was considerably greater in adult and rural cats in Romania.

The differences in results in different studies may be due to the health systems adopted for raising cats vary according to the country in which the study was conducted, in addition to the differences in the environment for raising cats accordingly. No significant differences were recorded between the cat breeds under study, the percentages were very close, at 40.9% in domestic cats compared to 48.57% in sanctuary cats. Results are relatively agreed with the results of several previous studies. According to an analysis of 337 reports by (Hatam-Nahavandi *et al.*, 2021).

The estimated seroprevalence of *T. gondii* in domestic cats was 37.5%, while the estimated seroprevalence in wild felids was 64%. Honarchian *et al.*, (2024) in Los Angeles, refer that the urban cats had lower ratio of infection with the *T.gondii* compared to the rural breeds. While it was in contrast with the results of Sroka *et al.*, (2018), who recorded an increase anti-*Toxoplasma gondii* IgG seropositivity in cats housed outside compared to indoors (69.7% vs. 16.7%).

T. gondii oocysts can be shed by infected felids for a little period (10–15 days) following primoinfection, after which they become permanently seropositive, which explains the significant discrepancy between the

incidence of oocysts in feces and positive blood antibodies (Hatam-Nahavandi *et al.*, 2021). The results may be impacted by the various diagnostic techniques used to find *T. gondii* oocysts and antibodies. Although the various methods for detecting anti-*T. gondii* antibodies shown comparatively strong diagnostic performance (Hatam-Nahavandi *et al.*, 2021).

5.4. Molecular Study

All trials to diagnose *T. gondii* by Conventional and Real-Time PCR methods failed in this study, cats' fecal samples. Montoya *et al.*, (2009), reported that the serological and coprological techniques are typically used to diagnose toxoplasmosis in cats and also indicate that a quick method for identifying toxoplasmosis in cats could be nested-PCR. The aqueous humor if the infection is low grade, limited to the posterior region, or the animal has received anti-Toxoplasma medication in the past, PCR findings may be negative (Fioretti, 2004). Sroka *et al.*, (2018), found that only two (4.9%) of the 41 cat fecal samples that were tested had structures that resembled *T. gondii* oocysts, and Real -Time PCR was used to confirm the results for one of these samples (2.4% of the total).

Allu, (2025) also found that as little as 12 ng/ μ L of *T. gondii* might be found using the developed PCR approach. All the above studies showed that the results of PCR in diagnosing *T. gondii* are limited by several conditions and limitations which may cause the results to be negative.

Conclusions and Recommendations

1. Conclusions

In the light of the current study, It has been concluded:

1. Rural women are at a significantly higher risk of *T. gondii* infection due to environmental factors soil / animals, poor hygiene / water safety, and a lack of health awareness, which increases the danger of toxoplasmosis during pregnancy.
2. Women who had contact with cats had a higher seropositivity of anti- *T. gondii* antibodies as compared to those who had no contact with cats.
3. The findings of the present study highlight the importance of serological screening for antibodies against *T. gondii* among pregnant women, as it represents a valuable tool for early detection of infection. A higher prevalence was observed among rural women compared with urban women.
4. The results also indicate that age plays a significant role in the epidemiology of *T. gondii* infection in cats, with younger cats showing greater susceptibility to infection than older cats.
5. A relatively high prevalence of *T. gondii* infection was recorded among cats in Nasiriyah, particularly among female pet cats and during moderate and cold months, suggesting that environmental and host-related factors may influence the transmission of the parasite.
6. Furthermore, serological methods were found to be more practical and reliable than molecular detection using PCR for identifying *T. gondii* in cat fecal samples.
7. There was a greater difficulty in diagnose *T. gondii* using molecular methods (PCR) in cat fecal samples.

2. Recommendations

1. Conduct serological studies to diagnose *T.gondii* in different ages of cats and different provinces of Iraq.
2. There is a high necessity to screen for Toxoplasmosis in women before and during pregnancy.
3. community awareness programs on safe handling of domestic cats, proper cleaning of cat feces, and the importance of hand hygiene, especially in households with pregnant women or children.
4. Analyze risk factors in women (such as owning domestic cats, hygiene practices, and consumption of undercooked meat) to link them with infection rates and identify the most vulnerable groups.
5. Conduct molecular studies to diagnose *T.gondii* in placenta and the tissues from aborted women.
6. Control of reservoir hosts (rodents), proper disposal of slaughterhouse waste, and management of blood drainage.
7. Emphasis on the prevention and proper care of young cats to avoid infection, as they may act as a potential source of infection transmission.

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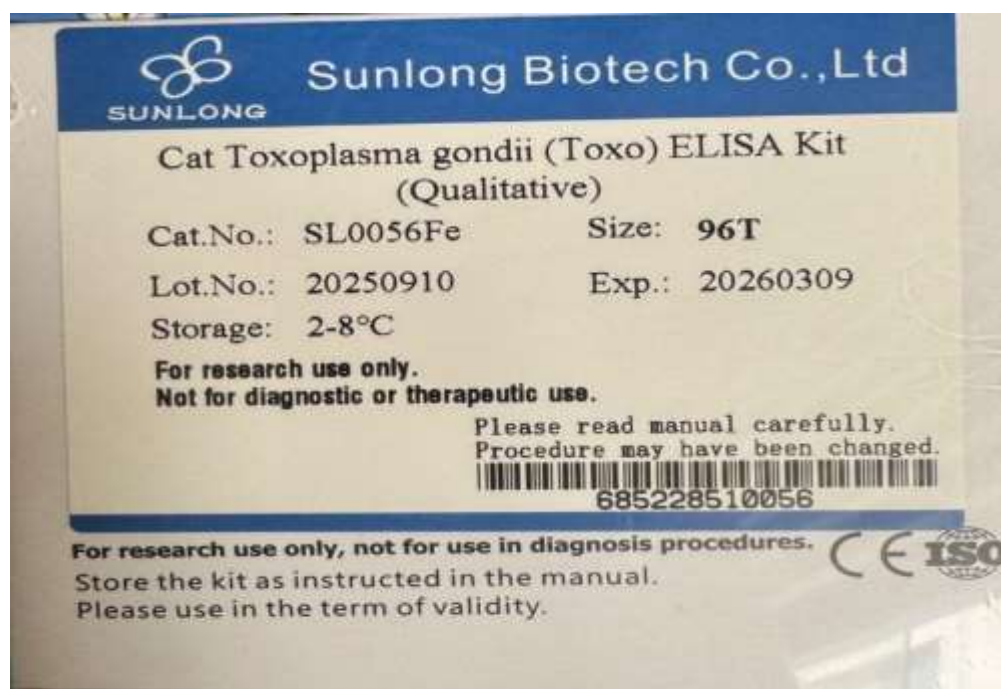
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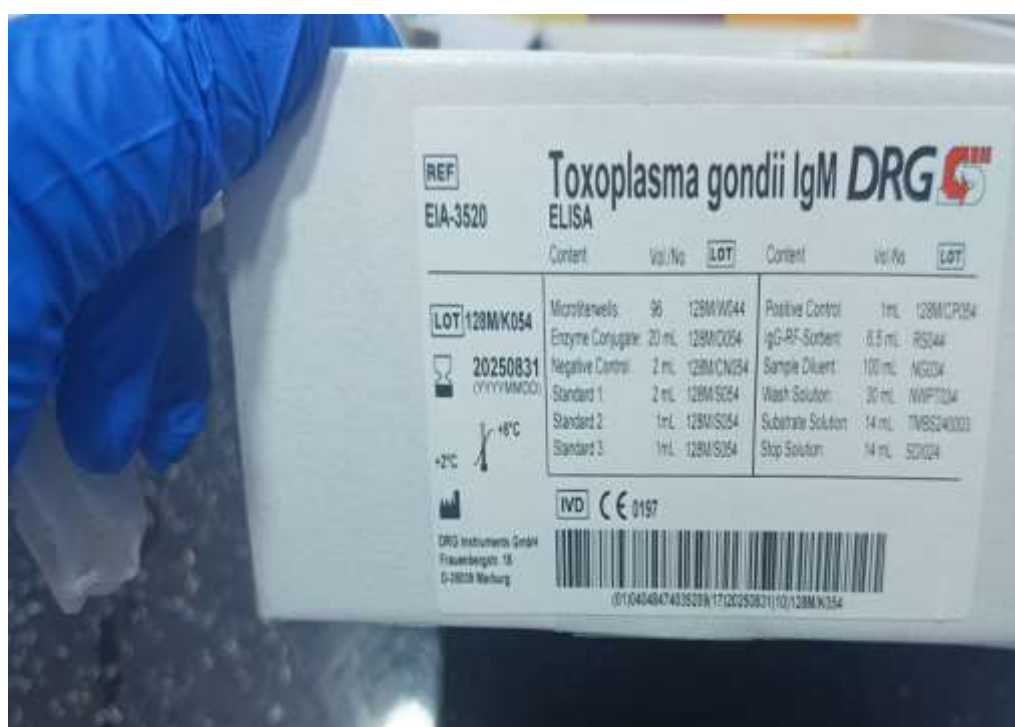
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Appendices



Appendic (1):Diagnostic Kits used for diagnosis of *T. gondii* in cats



Appendic (2):Diagnostic Kits used for diagnosis of *T. gondii* in women



Appendic (3):Samples of women



Appendic(4): Samples of Cats

The questionnaire sheet of study cases

The questionnaire

-Hospital :		Date :
-Name :		Age :
-Address:	rural	urban
-Sex:	Male	Female
-Occupation:		
-Marital status:	Married	single
- Pregnancy time :		
-Number of children:		
-Number of abortion		
-Time of abortion :		
Other informations:		

The questionnaire sheet of study cases

The questionnaire:

Veterinary Clinic:

Date:

Age:

Type:

Sex:

Ddress:

Appendic (6): The questionnaire sheet of study cases in cats



Appendixc (6) : Microplate Washer

Appendic (7)

MINISTRY OF HEALTH
THI-QAR HEALTH DIRECTORATE
Training and Human Development center

وزارة الصحة العراقية
المديرية الصحية في ذي قار
مركز التدريب والتنمية البشرية

التاريخ: ٢٠٢٤ / ١١ / ٤

No.:
Date: / /

الى / مستشفى بنت الهدى التعليمي - مستشفى الحبوبى التعليمي

تحية طيبة..

م/ تسهيل مهمة

اشارة الى جامعة الشعثرة / كلية الطب البيطري ذي العدد ٩٤١ بتاريخ ٢٠٢٤/١٠/٢١ وبناء على موافقتكم
الممنوعة على استمارة مشروع البحث المقدمة من قبل الباحثين، تم عرض الاستمارة على لجنة البحوث
في دائرتنا خلال جلسته الأسبوعية وكان قرار اللجنة:
(الموافقة على اجراء مشروع البحث بصيغته المقدمة ولا مانع من تنفيذه في مؤسسات الدائرة)
لذلك ربطا قرار لجنة البحوث ذي العدد (٢٠٢٤/٢٤٩) لا مانع لدينا من تسهيل مهمة اجراء البحث على
ان لا تتحمل دائرتنا اي تبعات مالية او قانونية وان يلتزم الباحث بالاعتبارات الأخلاقية اثناء اجراء البحث.
علما تم استيفاء اجور تسهيل المهمة بموجب الوصل المرقم (٠٩٧٤١٣٦) بتاريخ ٢٠٢٤/١١/٤.

... للتفضل بالاطلاع مع الاحترام...

الطبيب الاختصاص
مشعل زوري جبار الغوري
مدير مركز التدريب والتنمية البشرية
٢٠٢٤/١١/٤

شعبة إدارة البحوث والمعرفة.... للحفاظ مع الاوليات

الموقع: Habbobist. , Nasiriya , ThiQar
mail : thiqr_health@yahoo.com
web site : www.thiqrhealth.gov.iq
telephone: 0096442230113

الموقع: ذي قار
thiqr_health@yahoo.com
www.thiqrhealth.gov.iq
٠٠٩٦٤٤٢٢٣٠١١٣

Appendic (8)

Ministry of Higher Education
& Scientific Research
AL-Shatrah University
College of VET-MED
Dean Office

الجمهورية العراقية - جامعة الشطرة
2014 2015

وزارة التعليم العالي والبحث العلمي
جامعة الشطرة
كلية الطب البيطري
مكتب العميد

العدد: ٩٤٠
التاريخ: ٢٠٢٤ / ١٠ / ١١

الى / دائرة صحة ذي قار / مستشفى بنت الهدى
م/تسهيل مهمة

تحية طيبة:

● جى نفضلكم بالموافقة على تسهيل مهمة طالبة الماجستير (رباب احمد شعيب) لاكمال متطلبات التجربة والبحث .
...مع التقدير ...

العميد
الاستاذ المساعد الدكتور
نبيل مهدي عبد
٢٠٢٤ / ١٠ / ١١

خاتمة منه

- مكتب السيد معاون العميد للشؤون العلمية والدراسات العليا المحترم للتفضل بالاطلاع مع التقدير
- فرع الاحياء المجهرية . للتفضل بالاطلاع...
- وحدة الدراسات العليا
- المعلقة الشخصية للطلاب .
- الصادرة....

Email: university.of.alshatrah@utq.edu.iq

العنوان : محافظة ذي قار - قضاء الشطرة

Appendic (9)

Ministry of Higher Education
& Scientific Research
AL- Shatrah University
Office of the dean assistant for
scientific affairs & postgraduate

وزارة التعليم العالي والبحث العلمي
مكتب معاون الشؤون العلمية
و الدراسات العليا

العدد: ٥٥٢ / ج
التاريخ: ٢٠٢١/٢/١٤

الى /المستشفى البيطري التعليمي ذي قار

م/تسهيل مهمة

نية طيبة...

يرجى التفضل بالموافقة على تسهيل مهمة طالبة الدراسات العليا السيدة رباب احمد شعيبث للعمل في
المختبر لغرض اكمال متطلبات بحثها.

شاكرون تعاونكم معنا مع التندر...

الاستاذ المساعد الدكتور
فخرنام علي الحسن
معاون العميد للشؤون العلمية والدراسات العليا
٢٠٢١/٢/١٤

نسخة منه الى:

- مكتب السيد العميد المحترم... للتفضل بالاطلاع مع التقدير.
- الصغرة للحفظ

العنوان : محافظة ذي قار - قضاء الشطرة
Shatrah university of shatrahgk@edu.iq

Appendic (10)

وزارة الصحة
دائرة صحة ذي قار
مركز التدريب والتنمية البشرية
لجنة البحوث

رقم القرار: ٢٠٢٤/٢٤٩
تاريخ القرار: ٢٠٢٤/١١/٤

قرار لجنة البحوث
درست لجنة البحوث في دائرة صحة ذي قار مشروع البحث ذي الرقم (٢٠٢٤/٢٤٩) المعنون:
(Molecular study of toxoplasmosis infection in women and cats at thiqar province)
مقدم من الباحث/ين (مألية دراسات عليا /ماجستير) (رباب احمد شعيب) لوحدة إدارة البحوث والمعرفة في
مركز التدريب والتنمية البشرية في دائرة صحة ذي قار بتاريخ ٢٠٢٤/١٠/٤ وقررت

"الموافقة على تنفيذ مشروع البحث بصيغته المقدمة ولأمانه من تنفيذه في مؤسسات الدائرة"

الطبيب الاختصاصي
راشد نجم جاسم
رئيس لجنة البحوث

٢٤/١١/٢٠٢٤
الرجيم نايف ناصر
مدير قسم التنظيم

المرفقات:
تعديلات وملاحظات لجنة البحوث
ملاحظات:
تم تحويل رئيس لجنة البحوث او مقرر اللجنة للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لاجراء بحث والمعتمدة في وزارة الصحة. اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

الخلاصة

يُعدّ داء المقوسات (Toxoplasmosis) من الأمراض الطفيلية الأولية الشائعة التي تصيب الإنسان والحيوان، وينتج عن الإصابة بطفيلي المقوسة الغوندية (*Toxoplasma gondii*). هدفت هذه الدراسة إلى إجراء الفحوصات المصلية والجزيئية وتحديد معدل انتشار طفيلي *T. gondii* بوصفه أحد أهم الأمراض المشتركة الشائعة بين النساء والقطط في مدينة الناصرية – العراق. شملت الدراسة جمع 91 عينة مصل بطريقة معقمة من النساء الحوامل وغير الحوامل، والنساء اللواتي أجهضن وغير اللواتي أجهضن في المناطق الحضرية والريفية. تم الحصول على العينات من قسم النساء والتوليد في مستشفى الحبيب التعليمي والعيادات الخاصة، خلال الفترة من أوائل نوفمبر 2024 حتى نهاية مارس 2025. أُخضعت جميع العينات للفحص المصلي باستخدام اختبار الإليزا غير المباشر (Indirect ELISA) للكشف عن الأجسام المضادة IgM و IgG الخاصة بطفيلي المقوسة الغوندية.

تشير النتائج إلى فعالية الاختبارات المناعية في الكشف عن العدوى بالطفيلي *Toxoplasma gondii* من خلال الكشف عن الأجسام المضادة IgM و IgG باستخدام اختبار ELISA غير المباشر. وأظهرت النتائج تبايناً واضحاً في معدلات العدوى بين النساء اللواتي يعشن في المناطق الريفية والحضرية. فيما يتعلق بالأجسام المضادة IgM، تم تسجيل أعلى معدل إيجابي بين مجموعة النساء الحوامل باستخدام اختبار ELISA غير المباشر، حيث بلغ 91.6%، بينما تم تسجيل أدنى معدل بين مجموعة النساء غير المجهضات بنسبة 75%. كما أظهرت النتائج أن معدل العدوى كان أعلى بين النساء اللواتي يعشن في المناطق الريفية مقارنة بالمدن، حيث بلغ 100% مقابل 72.5% للأجسام المضادة IgM. أما بالنسبة للأجسام المضادة IgG، فقد تم تسجيل أعلى معدل إيجابي في مجموعة النساء غير المجهضات بنسبة 37.5%، في حين لوحظ أدنى معدل بين النساء المجهضات بنسبة 25.4%. وأظهرت النتائج أيضاً أن معدل العدوى كان أعلى بين النساء اللواتي يعشن في المناطق الحضرية مقارنة بالريفية، حيث بلغ 32.5% مقابل 18.2% للأجسام المضادة IgG.

أظهرت نتائج الفحص المجهرى لـ 100 عينة براز تم جمعها من العيادات البيطرية ومحميات القطط في مدينة الناصرية أن معدل الإصابة الكلي كان 30%. وعند تحليل النتائج حسب الأشهر، تم تسجيل أعلى معدل إصابة في شهر شباط (35.71%)، يليه شهر تشرين الثاني (35%)، مع عدم وجود فروق ذات دلالة إحصائية بين الأشهر المدروسة. كما أشارت النتائج إلى وجود فرق في معدلات الإصابة بين الجنسين. فقد أظهرت الإناث معدل إصابة أعلى (33.96%) مقارنة بالذكور (25.53%)، على الرغم من أن هذا الفرق لم يكن ذا دلالة إحصائية.

أما فيما يتعلق بمصدر القطط، فقد أظهرت النتائج أن القطط المقيمة في محميات القطط معدل إصابة أعلى بنسبة 32.5%، في حين سجلت القطط المنزلية 28.33%، مع عدم وجود فرق معنوي بين المجموعتين. تشير هذه النتائج إلى أن أعلى معدلات الإصابة حدثت خلال شهر شباط، بينما لوحظت معدلات أقل في القطط الذكور والمنزلية، وهو ما قد يعكس تأثير العوامل البيئية وظروف السكن على انتشار الطفيلي بين القطط في منطقة الدراسة. ظهرت نتائج الدراسة أيضًا أن الطرق التشخيصية التقليدية للكشف عن *T. gondii* في القطط كانت فعّالة في تحديد العدوى. بالإضافة إلى ذلك، لوحظ أن معدل انتشار العدوى زاد خلال الأشهر المعتدلة والباردة مقارنة بالفترات الأخرى، وقد يكون ذلك مرتبطًا بالظروف البيئية التي تسمح لبويضات الطفيلي بالبقاء لفترة أطول في البيئة.

تشير نتائج فحص 92 عينة مصلية من القطط باستخدام اختبار ELISA غير المباشر إلى وجود فرق واضح في معدل الإيجابية المصلية لأجسام IgG المضادة لطفيلي *Toxoplasma gondii* بين الفئات العمرية المدروسة للقطط. سجلت الفئة العمرية من 2-11 شهرًا أعلى معدل انتشار مصلي بنسبة 53.57%، مما يشير إلى أن القطط الصغيرة نسبيًا أكثر عرضة للتعرض للطفيلي خلال هذه المرحلة من حياتها. ويمكن تفسير ذلك بحقيقة أن القطط في هذا العمر تكون في مرحلة نشاط واستكشاف بيئي متزايد، مما يزيد من فرصها في مواجهة مصادر العدوى مثل التربة الملوثة أو الفرائس التي تحمل أكياس نسيجية من الطفيلي. في المقابل، أظهرت القطط الأكبر من 11 شهرًا (القطط البالغة) معدل إيجابية مصلية أقل بنسبة 43.75%، وهو أقل من المعدل المسجل في الفئة العمرية الأصغر. ويمكن عزو ذلك إلى تطور المناعة المكتسبة لدى القطط البالغة نتيجة التعرض السابق للطفيلي، أو بسبب انخفاض فرص التعرض مقارنة بالقطط الأصغر سنًا.

استنادًا إلى هذه النتائج، يمكن الاستنتاج أن الطرق التقليدية والمناعية لتشخيص الإصابة بطفيلي *T. gondii* في القطط كانت فعّالة، وأن معدل الإصابة بداء المقوسات في القطط يزداد خلال الأشهر المعتدلة والباردة، مع ارتفاع نسبة الإصابة في القطط الصغيرة بعمر 2-11 شهرًا مقارنة بالقطط الأكبر عمرًا.

بالإضافة إلى ذلك، تم إجراء التحري الجزيئي باستخدام تفاعل البوليميراز المتسلسل التقليدي (PCR) وتفاعل البوليميراز المتسلسل الآني (Real-Time PCR)، باستهداف جيني GRA6 و B1 على التوالي في عينات براز القطط. ومع ذلك، لم تُسجَل أي نتائج إيجابية في أي من الفحوصات الجزيئية المطبّقة.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة الشطرة
كلية الطب البيطري
فرع الاحياء المجهرية



دراسة مصلية لداء المقوسات في النساء والقطط في مدينة الناصرية \ العراق

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري - جامعة الشطرة وهي جزء من متطلبات
الحصول على درجة الماجستير في اختصاص علوم الطب البيطري (الاحياء المجهرية)

قدمت من قبل:

رباب أحمد شعيب

بكالوريوس طب وجراحة بيطرية

كلية الطب البيطري \ جامعة البصرة (2011)

بإشراف

أ.م.د. حكمت كاظم عطية

م.د. منتظر محمد فليح