



An-Najah National University

Faculty of Graduate Studies

**EVALUATING TOXOPLASMOSIS GENO-
PREVALENCE IN SLAUGHTERED SHEEP
USING PCR METHOD IN NORTHERN
PALESTINE**

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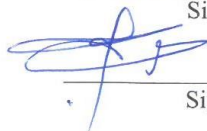
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Dedication

I dedicate this work to God Almighty, who is my creator, my steadfast supporter, and my wellspring of insight, understanding, and wisdom. My late father who taught me to be an independent and determined person, my mother (Khaznih), who has been a consistent source of support and encouragement throughout my academic career and facing the hardships that life has thrown at me, has been the source of my strength.

Thanks to the committee for keeping me on track and my academic advisor for guiding me through this process.

My cherished brothers and sisters, who support me when things seem hopeless, and my friends.

I dedicate this research to everyone who has touched my heart throughout my life.

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Finally, I would like to extend my sincere gratitude to the general research laboratory of the faculty of medicine at An-Najah National University for their technical assistance.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

**EVALUATING TOXOPLASMOSIS GENO-PREVALENCE IN
SLAUGHTERED SHEEP USING PCR METHOD IN NORTHERN PALESTINE**

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: _____

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Date: _____

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Abstract

Background: Toxoplasmosis is a worldwide zoonotic disease due to *Toxoplasma gondii*; it affects warm-blooded animals, including sheep and humans. Ingesting free-range undercooked meat of livestock, specifically sheep, is highly associated with human toxoplasmosis. At least 30% of people in most developing countries are positive for IgG antibodies to *T. gondii*. This parasite affects women and increases the chance of abortion due to damage to the fetus's central nervous system via causing dangerous defects in newborns.

Methodology: 1062 sheep's tissues (252 liver, 74 lungs, 280 heart, 254 brain, and 202 tongue) were randomly obtained from 346 sheep (ram and ewe) and they were slaughtered in the abattoirs of Nablus and Jenin. These tissues were examined for the presence of deoxynucleic acid (DNA) for *T.gondii* DNA extracted from the lung, liver, brain, tongue, and heart of sheep using the phenol-chloroform-isoamyl alcohol (PCI) method. Extracted DNA was examined for the presence of TOX4/TOX5 genes (non-coding fragment, repeated 200-300-fold). Polymerase chain reaction (PCR) was used to visualize and analyze the forward (5' CGCTGCAGGGAGAAGTTG3') and reverse (5' CGCTGCAGACACAGTGCATCTGGATT3') amplicons.

Results: Geno-positive PCR results were found in 25.72% (89/346) of sheep. The total infection rate within Jenin was 19.27% (28/162), whereas it was 29.44% (53/180) in Nablus. The highest was found in Nablus. Data analysis showed that the overall infection rates in heart, brain, liver, tongue, and lung samples were 7.86% (22/280), 2.36% (6/254), 4.76% (12/252), 20.79% (44/202), and 21.78% (8/37), respectively. Among the samples successfully genotyped for *T.gondii* (89 sheep), 16 of them had two infected tissues, and one of these tissues was the tongue. The results demonstrated the

presence of *T.gondii* DNA in tissues of slaughtered sheep from the northern area of the west bank. The highest percentage rate was recorded in the tongue, so that it is mostly causative of a high risk of toxoplasmosis. Ewes have a higher infection rate compared with rams.

Conclusions : Due to the high rate of toxoplasmosis among the slaughtered animals as well as meat products specially in tongue, consuming under-cooked meat obtained from infected sheep can be one of the main risk factors of transmission of the parasite to humans. It is necessary to avoid eating the raw and undercooked tissues of animals.

Keywords: PCR; Palestine; *T.gondii*; Toxoplasmosis; TOXO4/TOXO5; Genoprevalence.

Chapter One

Introduction and Theoretical Background

1. Introduction and literature review

The state of Palestine is located in the Middle East in the Mediterranean. It has a strategic location that connects Asia to Africa. The total population in Palestine including both genders and all ages is (4,854,013), its population density is 780 people per Km² (2,021 people per m²) (PCBS, 2018). According to the Archaeological studies, a shred of evidence indicates that domestic animals existed in Palestine and the Middle East region for more than 8000 years ago. Palestinians depended on animal farming for their livelihood. They used primary resources for feeding 730.000 sheep and 215 .000 goats were raised according to the Palestinian ministry of agriculture in the year 2015 (Salama, 2017).

In Palestine, food consumption patterns vary depending on the economic status, so that consumption of animal products in wealthy families is higher than in poor ones. The former relies on meat and dairy products, while the latter uses lentis as an alternative for red meat. Contaminated and undercooked food causes a variety of health issues, including parasitic infections, specifically Toxoplasmosis disease (Dardona *et al.*, 2021).

The apicomplexan parasite *T. gondii* is obligately intracellular, a unicellular eukaryote that relies on host cells for survival, and it is also a zoonotic parasite that can spread from animal to human hosts (Stelzer *et al.*, 2019). Cats and their relatives are the only known infected hosts for *T. gondii* (Hill & Dubey, 2002). One of the principal viruses responsible for food-borne illnesses that are spread to people by a variety of means is *T. gondii* (Dubey, 2010). Among 32 infectious disorders, toxoplasmosis came in fifth place and was first among 14 food-borne illnesses (Nissen *et al.*, 2017). The infective stage comes in two different forms: oocysts and tissue cysts. *T. gondii* can spread from an unhealthy host (a cat) to a different organ through the consumption of oocysts that are found in contaminated water or from the cat itself (Lindsay, 1997). It can also spread from vegetables that are washed in dirty water that has been contaminated by *T. gondii* oocysts to plants that have been irrigated by contaminated water, and ingesting it can cause toxoplasmosis (Jones & Dubey, 2012).

In a prior investigation for genotyping *T. gondii* on plants, it was discovered that mint stood at (18.00%), watercress (14.00%), dill (13.00%), parsley (10.00%), thyme (10.00%), and lettuce (6.00%) (Dardona *et al.*, 2021).

T. gondii is one of the most effective protozoan parasites on earth because of its extensive intermediate host range, which allows it to infect nearly every cell type within a broad host range, including those of humans, livestock, pets, and wildlife (Dubey, 2010). Most of the time, *T. gondii* remains latent in numerous tissues for a very long period in healthy individuals, including skeletal muscle, cardiac muscle, and the central nervous system (CNS), which includes the brain, spinal cord, and retina (Mendez & Koshy, 2017). By infecting the central nervous system, *T. gondii* causes life-threatening adverse effects in people with HIV, congenital immunodeficiency disease, blood malignant tumor patients, particularly during chemotherapy that inhibits immunity, newborns, and people with suppressed T-cell immunity (Robert-Gangneux & Dardé, 2012). Additionally, *T. gondii* has been linked to a decline in memory function in people over 65 (Gajewski *et al.*, 2014).

Human toxoplasmosis can be acquired after birth or is congenital. Raw and partially cooked meats are to blame for 50% of congenital toxoplasmosis cases. Toxoplasmosis can be spread to the human community by the consumption of raw or undercooked meat from slaughtered animals (Rahdar *et al.*, 2012).

Bradyzoites and oocysts are the principal sources of infection for both humans and animals. After ingestion, both bradyzoites and sporozoites transform into tachyzoites within tissues (Bohne *et al.*, 1997). In contrast, *T. gondii* can cause severe neurologic damage and even the death of a fetus if acquired during pregnancy, which is why the majority of infected people are unaware of their condition because they exhibit no symptoms at all. However, if left untreated, they may develop symptoms later in life. People with compromised immune systems, such as immunodeficient patients, newborns, the elderly, those undergoing certain types of chemotherapy, and those who have recently received a contaminated organ transplant (heart), are also susceptible to harm to the brain, eyes, and other organs (Bohne *et al.*, 1997). It is unknown which component of a sheep, such as the tongue, heart, or liver, is primarily responsible for a

high risk of toxoplasmosis because there is a lack of data on this subject in northern Palestinian territories.

1.2 Pathogenesis and pathology

Toxoplasmosis is caused by the protozoan parasite *T. gondii*, which can infect nearly all warm-blooded animal species, including humans. *T. gondii* has a complicated life cycle, with several infectious forms and phases that are both sexual and asexual. The first one only affects cats, making them the "definitive" hosts for *T. gondii*. The second one affects humans as well as other warm-blooded creatures like terrestrial and aquatic mammals as well as birds (Dubey, 1998).

The sexual life cycle is only present in the definitive host family Felidae (domestic cats and their relatives), and it starts when a member of the feline family ingests tissues containing bradyzoite cysts or oocysts. When cats eat cysts found in the tissue cysts of intermediate hosts, the cyst wall may be dissolved by digestive enzymes. When cats eat cysts found in the tissue cysts of intermediate hosts, the cyst wall may be dissolved by digestive enzymes. The bradyzoites settle into enterocytes, where they undergo self-stopping asexual multiplications that are characterized by the growth of merozoites inside schizonts. Following that, a female and a male gamete form, initiating the sexual development (gametogony). Oocysts that were originally contained within enterocytes are then released through cell distribution and expelled in cat feces in an unsporulated form (Robert-Gangneux & Dardé, 2012).

The immature oocysts (un-sporulated oocysts) discharged at the end of this cycle took one to five days in the environment to mature into oocysts (sporulated oocysts). They might last months or even years in the environment (Black and Boothroyd, 2000).

Any warm-blooded animal that consumes infectious oocysts contaminated in food, water, or soil becomes affected, including a number of species of birds and animals. Then *T. gondii* multiplies asexually and divides into tissue cysts that contain the bradyzoite stage (Ong *et al.*, 2011).

Tachyzoites should change into bradyzoites at the start of the asexual cycle. After ingestion, the sporozoite released from the oocyst infects the intestinal epithelium and differentiates into a tachyzoite stage. Tissue cysts then form as a result of differentiation to the bradyzoite stage after an acute infection, which is characterized by the spread of tachyzoites throughout the body. Following consumption of these tissue cysts in raw or partially cooked meat from a chronically infected host, the subsequent susceptible host may contract the infection after the bradyzoites infect their intestinal epithelium cells, at which point the bradyzoites reverse their differentiation to the tachyzoite stage to complete the asexual cycle (Black and Boothroyd, 2000). The *Toxoplasma gondii* life cycle and transmission methods is depicted in Figure 1; figure (a) show both sexual and asexual life cycles for *Toxoplasma gondii* meanwhile figure (b) show the transmission pathways for *Toxoplasma gondii*.

Figure 1

Toxoplasma gondii life-cycle and methods of transmission

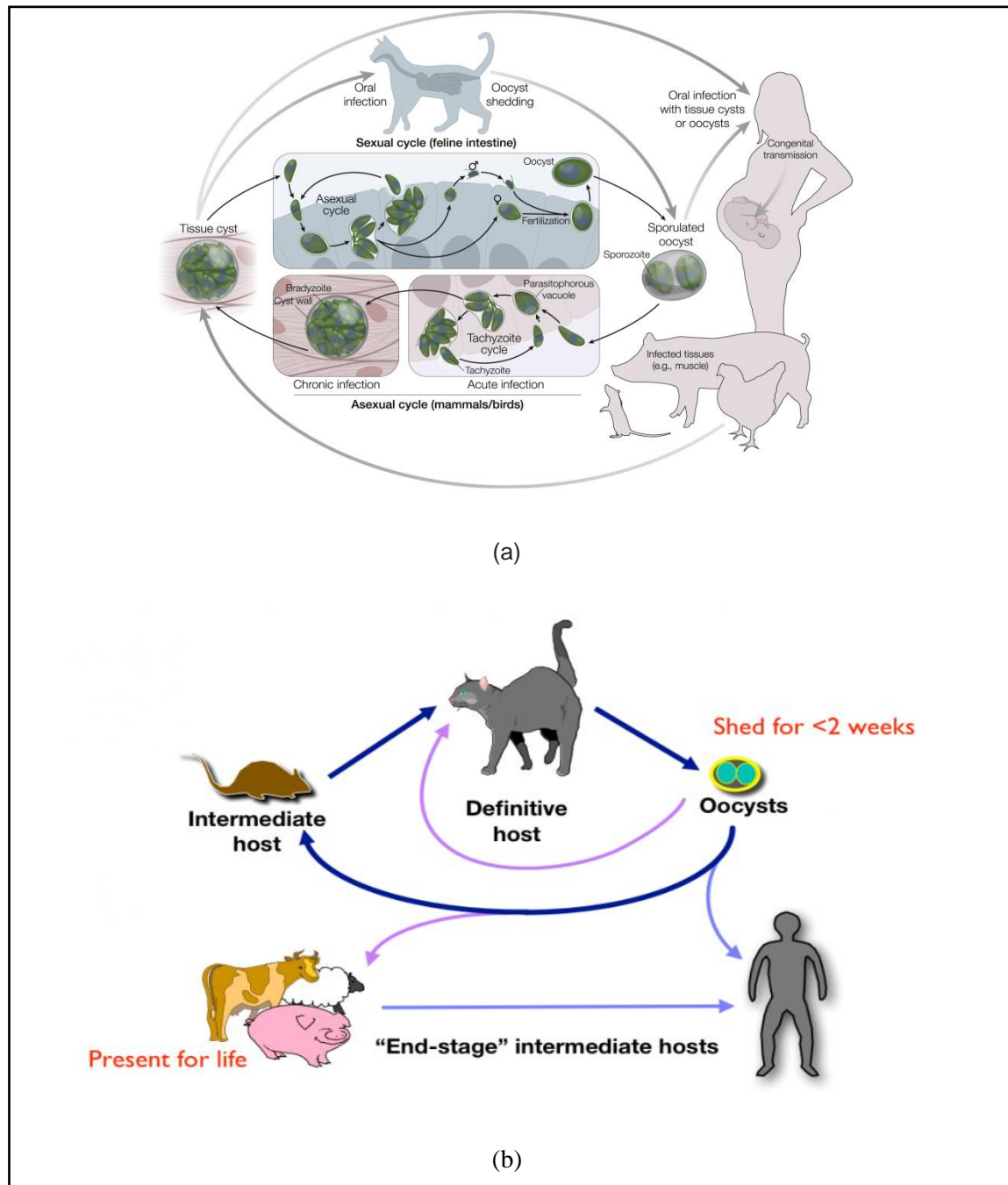


Figure (a) *Toxoplasma gondii* life-cycle (Lourido, 2019).

Figure (b) Transmission pathways for *Toxoplasma gondii* (Chan, 2022).

The definitive hosts of *T. gondii* expel oocysts 3–10 days following ingestion of tissue cysts or bradyzoites, 18 days following ingestion of oocysts, and 13 days following ingestion of tachyzoites (Dubey, 1998). The three contagious stages of *T. gondii* are the tachyzoite, bradyzoite, and sporozoite. Tachyzoite is a rapidly reproducing organism that multiplies in any cell of the intermediate host as well as in non-intestinal epithelial cells of the definitive host. It then collects in large numbers to form clones, terminal colonies, or groups. Immune response is typically focused on levels of this parasite infection (Dubey *et al.*, 1998). Bradyzoites are slow-replicating organisms that cause cysts to form. Depending on their age or diameter, tissue cysts can range in size from two bradyzoites in a young cyst up to hundreds of organisms in an older cyst. They also differ in shape depending on the tissue they are located in; for instance, a cyst in the brain has a spherical shape, but an intramuscular cyst is elongated. The liver, lungs, and kidneys are examples of visceral organs where tissue cysts can form, although they are more common in neural and muscular tissues like the brain, eyes, skeletal, and cardiac muscles. A tissue cyst doesn't injure the host and persists for their entire lives without inciting an inflammatory reaction (Dubey, 1998).

Congenital infection is when a woman has recently contracted *T. gondii* during her pregnancy or right before it. Although the woman may not exhibit any symptoms, the fetus may suffer severe effects such as eye and nervous system disorders (Bohne *et al.*, 1997). Tachyzoites that pass the placenta to the fetus can cause severe health problems include mortality, seizures, blindness, hearing loss, microcephaly, too much brain fluid, and mental retardation (Jones *et al.*, 2003). The incidence of congenital toxoplasmosis varies with the trimester during which maternal infection was acquired, if pregnant women have toxoplasmosis baby's risk to infected was 30% in second trimester, but in third trimester it was 60%. For untreated women, the transmission rate is approximately 25% in the first trimester, 54% in the second trimester, and 65% in the third trimester (Robbins *et al.*, 2012).

Pediatric Toxoplasmosis can be chronic or acute, acquired postnatally or congenital. Congenital toxoplasmosis is the most serious form of infection, caused by vertical transmission of *T. gondii* trans-placentally from mother to offspring (Hill *et al.*, 2005).

Nearly 75% of newborns with toxoplasmosis are asymptomatic at birth. In some cases, irregularities in a baby's brain and eyes can be detected alongside congenital toxoplasmosis. However, sometimes toxoplasmosis can be lethal for the baby after birth (Kota and Shabbir, 2019). In rare cases, some signs and symptoms include: whites of the eyes and yellowing of the skin; jaundice; skin rash; enlarged spleen or liver; low numbers of red blood cells which lead to anemia; poor feeding; easy bruising due to low platelet count; bleeding, or purple dots on the skin, repetitive movement of the eyes, crossed eyes, eye damage; an involuntary seizure; low birth weight; cerebral calcifications, signs that *T. gondii* has caused damage to the brain, Hydrocephaly, in which fluid accumulates in the skull; macrocephaly, in which the head is larger than normal; or microcephaly, in which the head is smaller than normal (Moncada & Montoya, 2012).

To reduce the possibility of toxoplasmosis, pregnant women should not handle cat litter. If this is not possible, the cat litter box should be changed every day because the toxoplasmosis eggs need 24 hours after shed to become infectious. Women should also carefully wash their hands and wear gloves to reduce the chance of infection. Infected pregnant women may be given spiramycin to reduce the risk of toxoplasmosis transmission from mother to fetus. However, this drug doesn't treat the fetus (Jones *et al.*, 2001).

Toxoplasmic encephalitis is an opportunistic infection which developed speedily and threatens the patient's life. It resulting from pass *T. gondii* across blood brain barrier use three different mechanisms paracellular entry (direct transmigration), transcellular entry (parasite pass across endothelium to the brain parenchyma) or via infected immune cell via "Trojan horse" mechanism, neuronal and non-neuronal brain cell well be affected due to parasitic ability infect any nucleated cell. In immunocompromised patients it considered as the most common manifestation of acquired toxoplasmosis and if not treated is fatal (Georgiev, 1994). It affects the central nervous system (CNS), specifically white matter, basal ganglia, periventricular region, and corticomedullary

junction, causing severe damage depending on the site. Common symptoms of cerebral toxoplasmosis are altered mental status, fever, and headache (Porter & Sande, 1992). Patients may also present with cranial nerve abnormalities, seizures, sensory disturbances, and visual disturbances. Common neurological signs include speech disturbance and motor weakness (Lee *et al.*, 2009). There is a relationship between toxoplasmosis and behavioral changes. In India, 1.33–3.33% of HIV-infected patients have CNS toxoplasmosis (Ramachandran *et al.*, 2014). Brain biopsy is the definitive diagnostic procedure for cerebral toxoplasmosis, but it is risky; serological tests and CT scans are safer. This type of toxoplasmosis is treatable, which means most patients will have a full recovery, but it can become fatal if left untreated (Madi *et al.*, 2012).

Ocular toxoplasmosis is defined as an infection in the eye by *T. gondii*. Toxoplasmosis is the most common cause of eye inflammation all over the world. A previous infection attacked the retina and generally resolved without symptoms. However, the parasite may later become active and cause blurred vision, eye pain, and possibly permanent damage, including blindness (Commodaro *et al.*, 2009). This disease affects the posterior pole of a single eye, and the lesion can be multiple, solitary, or satellite to a pigmented retinal scar. Active lesions show up as grey-white focuses of retinal necrosis with vasculitis, hemorrhage, adjacent choroiditis, and vitreitis (Commodaro *et al.*, 2009). *T.gondii* primarily affects the retina in the eye, but the vitreous, choroid, and anterior chamber are also involved. In the absence of retinal infection, choroidal lesions don't occur. Although the optic nerve head can be involved in ocular toxoplasmosis (Holland, 2004). Immunosuppressed patients or the elderly may show more aggressive bilateral or multifocal disease, retinal vascular occlusions. Other atypical presentations include retinal vasculitis, punctate outer retinal toxoplasmosis, rhegmatogenous with serous retinal detachments, peripheral retinal necrosis, neuroretinitis, and additional forms of optic neuropathy (Smith & Cunningham, 2002).

1.3 Clinical manifestations of toxoplasmosis

T. gondii infection is usually asymptomatic in animals and humans and may result in a self-limiting illness, mild in immunocompetent individuals. The only evidence of disease is the detection of antibody against toxoplasma. In many cases, symptoms of toxoplasmosis were flu-like as well as fever, pain in the head, muscle aches and pains (myalgia), sore throat, and enlarged lymph nodes, especially in the neck. Toxoplasmosis is exclusively serious for people who have extremely depleted immune systems, possibly leading to complications including the eyes, lungs, brain, or other organs. People with HIV, people who treat cancer by taking chemotherapy, and organ transplant recipients are all at risk for serious infection (Onduru, & Aboud, 2021). Those patients have severe toxoplasmosis, which occurs as a result of reactivation of latent infection. Also, if acute acquired infection may occur, the most dangerous outcome of toxoplasmosis is Toxoplasmic encephalitis, substantial brain tissue damage and inflammation that occurs due to a recurrence of toxoplasmosis from parasites encysted in the central nervous system (Blanchard *et al.*, 2015). This happens when a woman becomes infected during pregnancy and the parasite passes to her fetus via placenta and causes neurological, ocular, or systemic damage with variable severity. This severity depends on the gestational age. The most severe manifestations of toxoplasmosis occur at the first-trimester of maternal infection (Dunn *et al.*, 1999). The most important symptoms for the newborn include mental retardation, epilepsy, hydrocephalus, and blindness, although some of these can also happen later in life (McAuley, 2014). Table One summarizes the possible symptoms of congenital toxoplasmosis in infancy and later life.

Table 1

Possible Signs and Symptoms of Congenital Toxoplasmosis in Infancy and Later in Life

Abnormal spinal fluid	Hepatomegaly	Mental retardation
Anemia	Hydrocephalus	Microcephaly
Chorioretinitis	Intracranial calcifications	Spasticity and palsies
Convulsions	Jaundice	Splenomegaly
Deafness	Learning disabilities	Thrombocytopenia
Fever	Lymphadenopathy	Visual impairment
Growth retardation	Maculopapular rash	

(Jones, et al., 2003).

Cardiac toxoplasmosis is rare and limited in immunocompromised patients; sudden death occurs due to heart failure. It's associated with other infections like aspergillosis and tuberculosis. This paper aims to systematically review all available information regarding the cardiovascular implications of toxoplasmosis. In 2021, researchers systematically reviewed all available information about the cardiovascular implications of toxoplasmosis using 48 articles and provided a summary of cardiac toxoplasmosis including an original algorithm facilitating diagnosis and treatment (Zhou *et al.*, 2021). Table 2 shows the summary of studies mentioning cardiac complications and clinical outcomes of toxoplasmosis for variable patients from different regions.

Table 2

Summary of studies mentioning cardiac complications and clinical outcomes of toxoplasmosis

Literature	Study design	No. of cases	Age	Sex	Immune status	Cardiovascular involvement	Clinical outcomes
Hermanns <i>et al.</i> , 2001	Case report	1	64	M	Immunocompromised (heart transplant)	Myocarditis, dilated ventricles, <i>T.gondii</i> microorganisms	Death due to toxoplasmosis & respiratory failure
Hofman <i>et al.</i> , 1993	Retrospective study	21	Mean: 33.6 (range: 25-52)	F:38% M:62%	Immunocompromised (AIDS)	Myocarditis (14), <i>T.gondii</i> cyst (4), cardiac lesions (3)	Death due to heart disease (6), nervous system lesions (12), pulmonary failure (2), gastrointestinal bleeding (1).
Holliman <i>et al.</i> , 1990	Case report	1	58	M	Immunocompromised (heart transplant)	<i>T.gondii</i> cysts, inflammatory infiltrate, thickened ventricles	Sudden death due to toxoplasmosis
Jautzke <i>et al.</i> , 1993	Retrospective study	12	N/A	N/A	Immunocompromised (AIDS)	<i>T.gondii</i> pseudocysts or tachyzoites (8), necrosis & mined infiltrate (4)	Death due to AIDS in all cases.
Lanjewar <i>et al.</i> , 2006	Case report	1	35	M	Immunocompromised (AIDS)	Myocarditis, inflammatory infiltrate, <i>T.gondii</i> pseudocysts	Sudden death due to cardiac arrest.
Levveque <i>et al.</i> , 2019	Case report	1	23	M	Immunocompetent	Myopericarditis, necrosis	Recovery without treatment.
Mariani <i>et al.</i> , 2006	Case report	1	19	M	Immunocompetent	Myocarditis, atrioventricular block	Recovery with co-trimoxazole + pacemaker implant.

(Zhou *et al.*, 2021).

1.4 Toxoplasma in Sheep and goats

Sheep play major role in the economy for many countries because they are a source of food for humans. In Palestine wealthy families consume 10.8 kg (red meat) -per year, 33g (Animal fat)-per day, 43g (animal protein)-per day (PCBS, 2018). Goats and sheep are immensely susceptible for toxoplasmosis and may play a main role in transmission of toxoplasmosis to humans (Tzanidakis *et al.*, 2012). Sheep are commonly infected with the protozoan parasite, *T. gondii* without clinical signs; however, immune-compromised sheep which acquired the infection during pregnancy perhaps developed a reproductive disorders, fetal death, stillbirth, abortion or birth of weak lambs. These parasites detected particularly in divers' tissues such as, liver, brain, heart and Tongue (Stene *et al.*, 1999). *T. gondii* considered as the fourth most important food-borne parasite in the world. Due to its wide host range the parasite is of importance not only in public health, but also in livestock industry and wildlife management (Authority, 2021). The above-mentioned parasite is contemplated to be a prime reason of reproductive losses in ruminants all over the world. Sheep and goats are defenseless against toxoplasmosis. Furthermore, toxoplasmosis is a pertinent zoonosis so that plays a main role in its transmission from animals to human (Almeria & Dubey, 2021). Cats contributes mainly in the spread of toxoplasmosis throughout shedding oocysts in its feces then transmit it to sheep, ewe or goats horizontally by the oral uptake of contaminated fodder or water, with environmental resistant oocysts this way considered as the most important rout of transmission (Buxton *et al.*, 2007). In human infections occurs via direct or indirect ways, first one arises when ingested Oocyst directly via eating contaminated food or ingested Oocyst from contaminated soil to hands, The second one happens when we eat animal tissue cyst. At least 30% of people in most developing countries are positive for IgG antibodies to *T. gondii* (Dubey, 2010).

Animals carrying the parasite are transmitting it to their offspring through the placenta, which causes severe damage to the fetus, such as seizures, mental retardation, and death. Generally, less than 2% of sheep become infected congenitally, and less than 4% of infected sheep pass the infection to their offspring. This route is known as endogenous trans-placental transmission. In sheep, it was more common than in goats (Dubey, 2016). Furthermore, the transmission of the infection to sheep and goats through semen has also been proven, both under mating with experimentally infected

rams. This was proven by a scientific experiment held in Brazil (Bezerra *et al.*, 2014). Similarly, DNA of *T. gondii* has been identified in milk samples which were taken from naturally infected ewes and goats. This means that it may also pose a risk of infection to lambs or goat kids (Saad *et al.*, 2018). *T. gondii* infection can be transmitted to humans through livestock (Stelzer *et al.*, 2019).

Toxoplasmosis is a major possible causal factor for abortion and reproductive failure in sheep. Abortion is reported as the most common sign in ewes (CDC, 2014). In Cobleskill, in 1985 and 1986, during the lambing season, 14.5% of ewes aborted. Specific anti-*T. gondii* antibodies increased with age; 89.2% of 2-year-old ewes had recorded antibodies vs. 40.2% of 1-year-old ewes (Dubey & Welcome, 1988). A similar study using PCR tests noted that congenital transmission toxoplasmosis occurs in sheep with a high frequency of 69%. Interestingly, 19.8% of toxoplasmosis is transmitted from mother to baby in humans (Hide *et al.*, 2007). Previous research suggested that raising lambs from sickly ewes increases the risk of subsequent abortion and infection (Hide *et al.*, 2009). In 2010, abortions on U.S. sheep operations were nearly 43.8%. Just 3.4% was due to toxoplasmosis, and 73.8% had unknown causes (Sidik *et al.*, 2018). Sheep which were previously aborted due to *T. gondii* develop protection against future abortion, but the protection is not complete (Edwards & Dubey, 2013). In Great Britain and Uruguay, annual costs of *T. gondii*'s infection in livestock production are about 5–15 million US \$ per country (Stelzer *et al.*, 2019). Transmission of disease between humans and animals is one of the major health problems in many countries. Sheep can transmit the parasite to humans but cannot pass it to another sheep. A juvenile cat contracts the disease after consuming an infected mouse or bird and spreads it to sheep by defecating in water, straw, food, or pasture. The infected cat gains lifetime immunity and stops transmitting toxoplasma after making a full recovery. Any aborted ewe must be dealt with in a number of steps. In particular, confine any aborted ewes and treat them as contagious unless proven otherwise. After examining the aborted ewes, remove the lambs, thoroughly clean the area, call your veterinarian for advice, gather samples of the fetus or lamb during the cleaning process for lab testing to determine the cause, properly dispose of the contaminated items and bedding, and then seek treatment from your veterinarian (Tenter *et al.*, 2000).

1.5 The epidemiology of *T. gondii*

1.5.1 Seroprevalences

The global seroprevalence rate was 25.7%, but the overall range of it was 0.5-87.7%. The highest average seroprevalence rate of 61.4% was reported in African countries, followed by Oceania, South America, Europe, USA/Canada, and Asia at 38.5%, 31.2%, 29.6%, 17.5%, and 16.4%, respectively (Molan *et al.*, 2019). From 1955 to 2020, the overall seroprevalence of *T. gondii* in southern Africa was 17%. Cattle accounted for 20%, small ruminants (goats and sheep) for 11%, humans for 14%, chickens and birds for 22%, domestic fields for 29%, and wild felids for 79% (Omonijo *et al.*, 2022). Surveys of unselected population groups with immunologic tests indicate that seroprevalence of *T. gondii* in sheep occurred in multiple countries, and we came up with the following conclusion: In Tunisia, *T. gondii* infection was found in 40.2% of sheep (Lahmar *et al.*, 2015). In China, it has been estimated to be 11.8%. In South Asian countries, the reported prevalence of sheep and goats was 21.1% (Khan *et al.*, 2017). In another study done from 2000 to 2017, *T. gondii* infection was tested in food animals (sheep, goats, swine, chickens, yaks, and cattle) and human reports. The overall seroprevalence was 23.7% in food animals and 8.2% in humans (Dong *et al.*, 2018). Another study found that the range of seroprevalence in sheep and goats was 3.98-43.02%, 0.72-23.41% in humans and 0.75%-30.34% in yaks and cattle (Su *et al.*, 2022). Urban and rural areas in the Amazon have 70.6% of anti-*T. gondii* antibodies. 62.6% were performed in rural areas while 81.9% were in urban areas (Morais *et al.*, 2021). In Hebron in 2005, *T. gondii* specific IgM and IgG in pregnant women were 17.6% and 27.9%, respectively. The seroprevalence increased with age. The percentage varies according to life area, so that 36.8% of women from rural areas had IgG antibodies whilst 21.4% of urban women had them. 37% of women had previous abortions (Al Amleh & Nijem, 2009). In 2014, toxoplasma IgG antibodies within goats in TulKarm and Jenin were 13.4%. The highest incidence was in Jenin, 17.44%, while it was 7.69% (Othman & Alzuheir, 2014). In Gaza, the overall prevalence of *T. gondii* antibodies, IgG and IgM, in women was found to be 12.8% and 17.9%, respectively (2021, Al-Hindi).

1.5.2 Geno-prevalence of *T. gondii*

The molecular prevalence and genetic characterization of *T. gondii* more specific and accurate some of these results took the following form: From 1995 to 2017 type II *T. gondii* was 58.0%, it reported as the most predominant genotyping profile, Systematic review records the prevalence as 64.3%, 62.1% and 41.7% in patient with AIDS, congenital and ocular toxoplasmosis, respectively (Hosseini *et al.*, 2019). In China, slaughtered goat's brain tissue DNA was 5.2% in central *et* China (Qian *al.*, 2020), whereas in eastern China the prevalence in sheep's meat was 9.84% Meat collected from supermarkets (6.84%) is notable because it is lower than that collected from rural markets (16.04%) (Ai *et al.*, 2020). *T. gondii* tissue cysts were found in 33.3% of cases in Northwest Tunisia (Amdouni *et al.*, 2017). 1.69% in slaughter sheep due to low food safety (Kalambhe *et al.*, 2017). In Iran (18.57%) slaughtered sheep brain samples (Gorji *et al.*, 2018), in southwest Iran sheep, 38% were infected. The infection rate in the age groups under 1 year, 1-2 years, and more than 2 years was 25%, 35.29%, and 52.94%, respectively. The infection rate in the femur muscle, brain, liver, and tongue was 28%, 32%, 30%, and 16%, respectively (Azizi *et al.*, 2014). *T. gondii* B1 gene was found in the diaphragm, heart, and brain of sheep in Eastern Iran (Kareshk *et al.*, 2017). In Southern Iran, genotyping found that the total prevalence of *Toxoplasma gondii* infection was found to be 33.3% (37.5%) There is a difference between the prevalence rate of infection among females (46%) and males 'animals (29.5%) A positive correlation was found between the age of animals and the rate of infection; animals greater than 2 years old showed a higher rate of infection (47%) in comparison with those less than 2 years old (25%) The most infected tissue was the tongue (21.8%), followed by the brain (19.2%) and femoral and intercostal muscles (17.9%) (Asgari *et al.*, 2011). In Bangladesh, the overall geno-prevalence of toxoplasmosis in the aborted fetus of goats was 35%. PCR tests showed that heart muscle, liver, brain, and placenta were positive (Hasan *et al.*, 2021). In 2014, Shwab *et al.* summarized the data about *T. gondii* samples that have been typed via PCR-RFLP genetic markers, which were reported until the end of 2012. 1457 samples were typed into 189 genotypes. PCR-RFLP genotypes 1, 2, and 3 are identified globally. In Europe, genotypes 1 and 2 are dominant, genotypes 2 and 3 are dominant in Africa, genotypes 1,2,3,4,5 are dominant in North America, and genotypes 9 and 10 are relevant in Asia (Shwab *et al.*, 2014).

Figure 2

Global geno- and sero-prevalence of *Toxoplasma gondii*

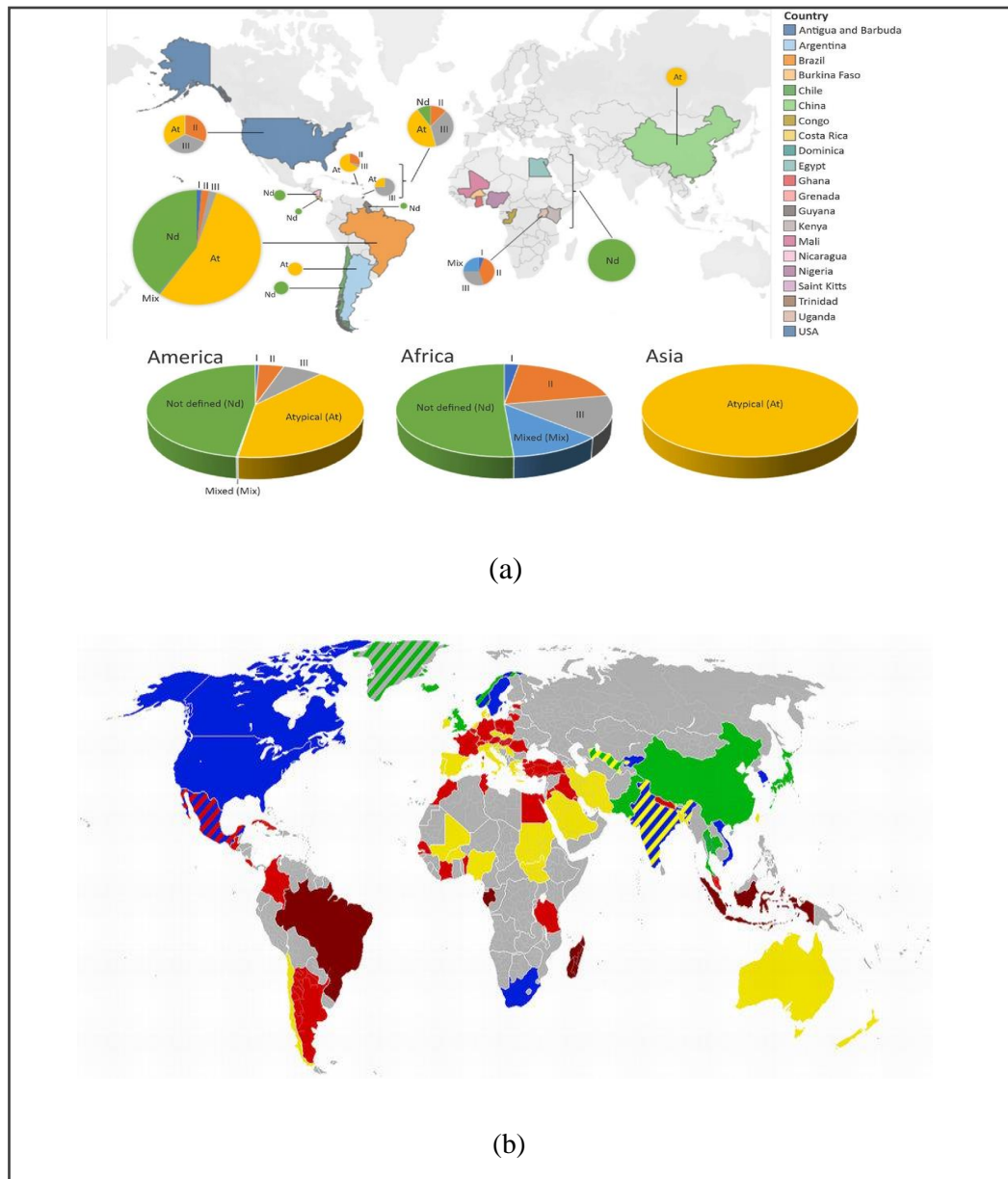


Figure (a) A systematic review of *Toxoplasma gondii* genotypes in *Gallus gallus domesticus* worldwide (Costa et al., 2021), figure (b) *Simplified world map for seroprevalence world distribution of Toxoplasma gondii*

Note: Color code: dark red: >60%; red: 40-60%; yellow: 20-40%; blue: 10-20%; green: <10%; grey: no data available; striated areas represent strong regional differences (Pleyer et al., 2014).

1.6 Diagnostic tests

PCR amplification of specific nucleic acid sequences (Remington, 2006), histologic examination of the parasite and/or its antigens, serologic testing, and organism isolation. Other uncommonly used methods involve demonstration of antigen and antigenemia in body fluids and serum, antigen-specific lymphocyte transformation, and a toxoplasmin skin test (Montoya, 2002).

The immune system plays an essential part in susceptibility and the clinical course of toxoplasmosis. This is due to infection with *T. gondii* commonly giving rise to general symptoms or none at all, so that it needs laboratory diagnosis. The main analytical procedures available are serological ones like ELISA and immunoblotting. In addition, *T. gondii*-specific antibodies (IgG and IgM) were found in animal or human blood. In acute infection, both IgG and IgM antibodies increase within one to two weeks of infection. The elevated IgG antibodies suggest that the infection happened but aren't determining the time of infection so that IgM is used to determine whenever the infection occurs. IgM positive means that the infection has recently occurred, whereas IgG positive and IgM negative suggest that infection has previously occurred (Jones *et al.*, 2003).

A main problem with *Toxoplasma*-specific IgM testing is the absence of specificity. Two cases happen frequently: individuals with positive IgM and negative IgG and individuals with positive IgG and IgM results. In the first case, a negative IgG with a positive IgM result in the same specimen should be observed with great misgiving; the patient's blood should be redrawn 14 days after the first and tested together with the first specimen. If IgG and IgM are highly positive in the second sample, it means that the first specimen was drawn very early after infection. If IgM is negative and IgG is negative in both specimens, the IgM results should be investigated to be a false positive and the patient should be investigated to be not infected. In the second case, a second specimen should be drawn and the two specimens should be introduced together to a reference lab which applies a different IgM testing procedure for affirmation (Oka *et al.*, 2019). An avidity test for IgG should be performed when a patient is pregnant and both IgM and IgG are positive. A high avidity result in the first 3 to 4 months of pregnancy (time depends upon the commercial test kit) importantly rules out an infection acquired during gestation. Some individuals have persistent low IgG avidity for several months

after infection, so a low avidity for IgG should be understood as indicating recent infection (Lefever-Pettazoni *et al.*, 2006).

Newborn infants suspected of having congenital toxoplasmosis should be tested for both IgA and IgM antibodies. Identification of Toxoplasma-specific IgA antibodies is more sensitive than IgM antibodies in congenitally infected babies (Herwadt, 2000). IgM positivity should be confirmed by other serologic profiles: IgM enzyme-linked immunosorbent assay [ELISA], IgA ELISA, and IgE ELISA (Liesenfeld *et al.*, 2001).

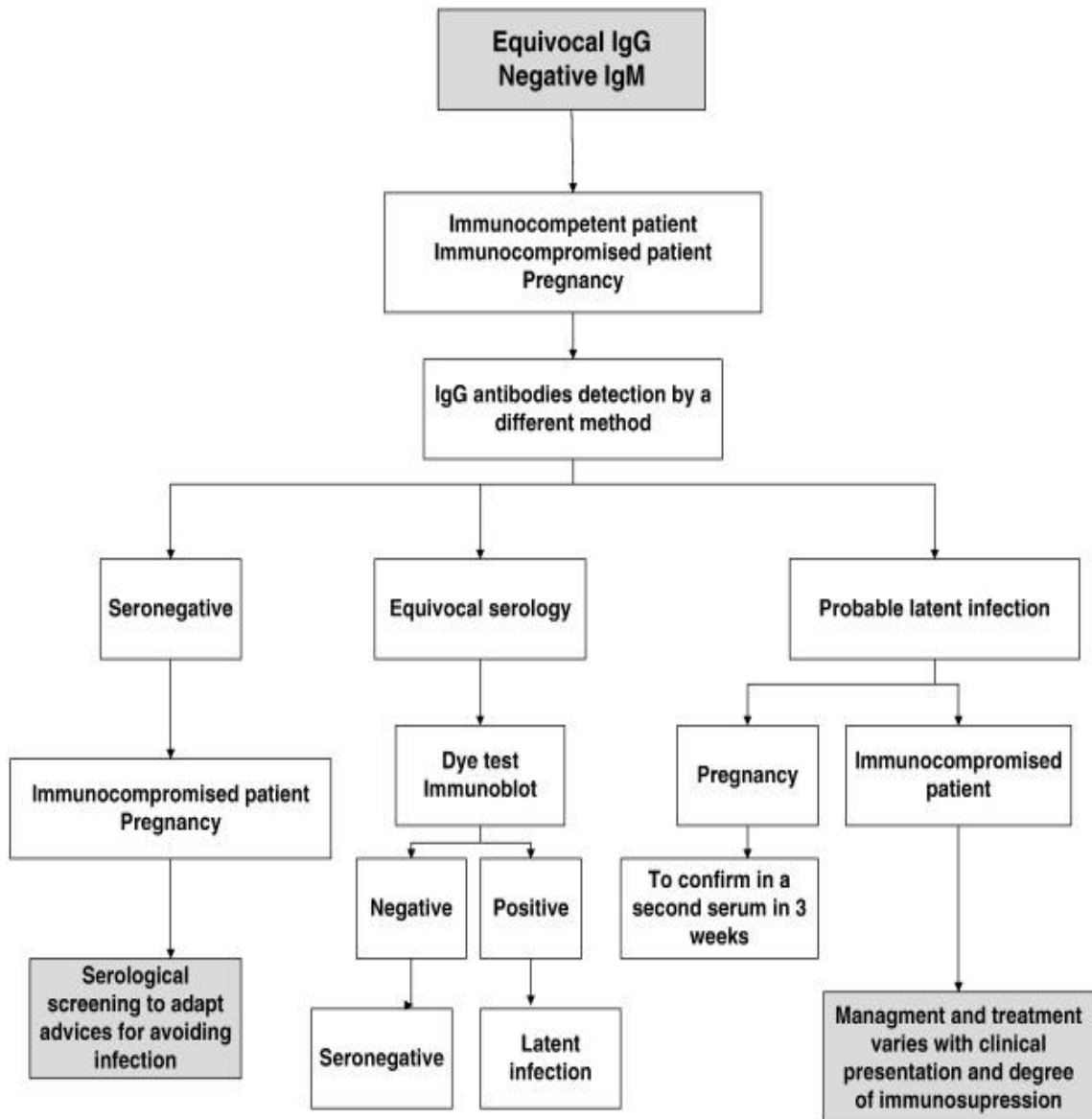
Diagnosis of toxoplasmosis can also be made via direct observation of *T. gondii* in stained tissue sections, cerebrospinal fluid (CSF), or other biopsy material. Previous techniques are used less frequently in order to reduce the difficulty of collecting these specimens. Molecular techniques such as PCR can identify the parasite's DNA in different body fluids like CSF, bronchoalveolar lavage (BAL) fluid, and blood in patients with AIDS. This procedure can be useful in cases of possible congenital transmission to emphasize the results when anti-*T. gondii* antibodies are estimated among pregnant women in serological tests, for safe and more sensitive to fetus PCR testing for amniotic fluid is used for amplification of specific nucleic acid sequences to diagnose *T. gondii*'s DNA in amniotic fluid surrounding the fetus (Foulon *et al.*, 1999). Amniocentesis: an amniotic fluid test for toxoplasmosis at 15 to 20 weeks of pregnancy is defined as amniocentesis. It can also be tested for birth defects or genetic problems. One in three babies with toxoplasmosis has a problem that can be observed on ultrasound (Gay-Andrieu *et al.*, 2003).

T. gondii is diagnosed in ocular toxoplasmosis based on the presence of lesions in the eye and usually serologic testing (Maenz *et al.*, 2014).

When patients suffering from vomiting, headache, neurological symptoms and their serological test positive also have a ring-enhancing mass lesion (s) by brain imaging, we went to take a brain biopsy, which is known as a definitive diagnosis to detect the organism in the specimens or detect their parasitic DNA via using molecular techniques, particularly PCR procedures (Ozaras *et al.*, 2016).

Figure 3

Advice algorithm for toxoplasma serology with equivocal IgG and negative IgM. Adapted from



(Villard *et al.*, 2011).

1.7 Protection from toxoplasmosis

A few procedures need to be done in order to secure and protect ourselves from toxoplasmosis and prevent it from spreading: Meat needs to be approximately cooked at a suitable heat (160 F) (71 C) to guarantee it is cooked and toxoplasmosis free, to reduce the chance of infection. Keeping meat frozen at sub-zero temperatures for several days before cooking, washing and beetling fruits and vegetables is highly recommended. Raw seafood should not be consumed, and unpasteurized goat and cow milk should not be consumed. Hunted or dead animals should be buried to prevent scavenging. Keep cats indoors to reduce the risk of infection from eating small animals suspected of being infected with toxoplasmosis. Protecting your cat leads to protecting yourself. Pregnant women should wear gloves when gardening or touching soil or sand. Finally, unfiltered water should be avoided, especially in developing countries, and children should be taught the importance of washing their hands to avoid toxoplasmosis. Do not touch your mouth, nose, or eyes when handling raw meat. Use soap and water to wash your hands after handling vegetables, fruits, shellfish, or poultry. If you have a cat, only feed your cat well-cooked food or canned dried cat food from the store; don't feed your cat raw or undercooked meat; don't touch stray cats or kittens (Jonea & Dubey, 2012). It is important to realize that there are no licensed human vaccines able to block human toxoplasmosis (Barros *et al.*, 2021), but in the last few years, researchers have progressed towards the development of a vaccine for toxoplasmosis. This vaccine is based on the live attenuated s48 strain developed for veterinary uses. However, this vaccine has a side effect, has a short half-life, and is expensive. Furthermore, this vaccine may revert to a pathogenic strain so that it is not suitable for human use (Kur *et al.*, 2009). The main prevention tips for toxoplasmosis are summarized in Figure 4.

Figure 4

Prevention of Toxoplasmosis

Toxoplasmosis Prevention Tips

- **Garden in gloves**

Wear gloves when handling soil, and wash with soap and water after you're finished.
- **Wash kitchen utensils and appliances**

Washing utensils and appliances with soap and water is especially important after preparing raw meat and produce.
- **Avoid undercooked or raw meat**

Undercooked and raw meat, including cured meat, can contain *T. gondii*.
- **Cover your sandbox**

Keep your outdoor sandbox covered so passing animals don't use it as a litter box.
- **Wash and peel fruits and vegetables**

Thoroughly wash and peel any fresh produce before eating.
- **Avoid unpasteurized dairy products**

Dairy products that haven't undergone the pasteurization process may occasionally contain *T. gondii*.
- **Avoid stray cats**

Most cats become infected with *T. gondii* through hunting and eating infected prey.

(Source : <https://aminoco.com/blogs/health/toxoplasmosis-humans-symptoms-watch>)

1.8 Objectives

The main objectives of this study were to estimate:

- The geno-prevalence of *T. gondii* within slaughtered sheep in the Northern Palestinian region.
- The geno-prevalence of *T. gondii* infection in brain, liver, heart, lung, and tongue tissues obtained from slaughtered sheep.
- The types of slaughtered sheep tissues have the highest incidence rate of toxoplasmosis.

Chapter Two

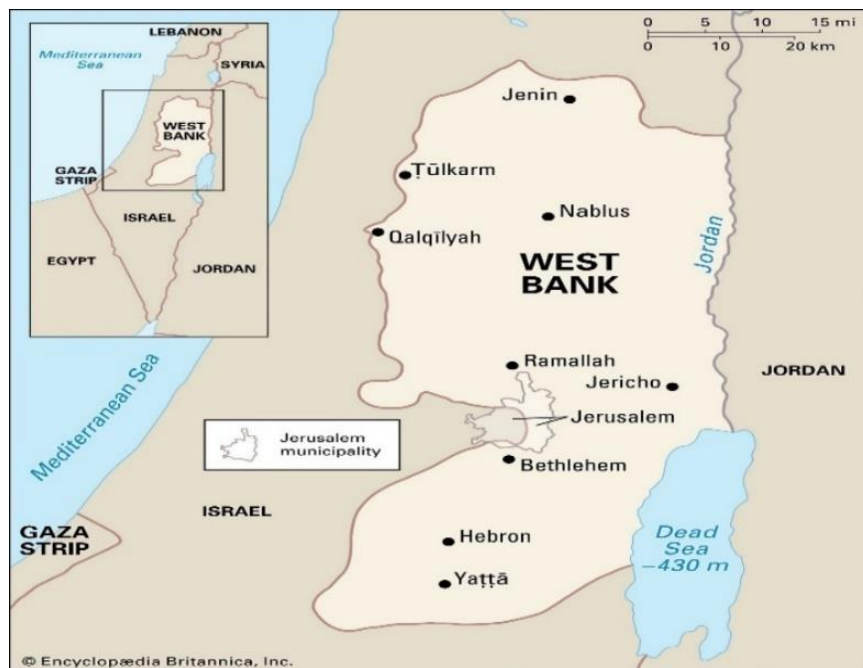
Material and Method

2.1 Study area

The research location was at the eastern Mediterranean region, specially the West Bank (west of the Jordan River) the Northern part of Palestine specifically Jenin and Nablus.

Figure 5

Study area; Northern Palestine (Jenin and Nablus)



(Source: <https://www.britannica.com/place/Nablus>)

2.2 Animals and samples

During the period from June 2021 to July 2022, Heart, liver, brain, lung and tongue biopsy (0.5-1 g) samples were obtained from sheep (n = 346) slaughtered in abattoirs from the Northern Palestinian cities (Jenin/ Nablus), we obtained 1062 tissues including 252 liver, 74 lung, 280 heart, 254 brain and 202 tongue, sheep randomly were chosen from different abattoirs. samples stored at -80 °C until the practical procedure took place.

2.3 DNA extractions

Tissue was minced by sterile scalpel in a sterile Eppendorf tube, and resuspended in 50 mM Tris-HCl (pH 8.0)-62.5 mM EDTA-2% sodium dodecyl sulfate, and the resulting lysates were then incubated with proteinase K (20 µg/ml) at 37°C overnight. DNA will be extracted by three consequence treatments of Phenol, a mixture of phenol and chloroform-isoamyl alcohol (CIA; 25:24:1), and CIA and precipitated with ethanol. *T. gondii* was examined with a PCR technique so that all DNA extracts were stored at -80 °C for use in PCR reactions.

2.3.1 Procedure of DNA extractions

At the beginning, we took biopsies, which are estimated at 0.5–1.0 g from the heart, liver, brain, lung, and tongue, and cut them down to tiny pieces. The second step was putting the product (cut tissue) in a 2 mL Eppendorf tube, which we named number 1. After that, we added 500 µl of lysis buffer to damage the cell membrane via changing the pH (50 mM Tris-HCl (pH 8.0)-62.5 mM EDTA-2% sodium dodecyl sulfate) to tube 1. At alkaline pH 8.0, lysis tris interacts with lipopolysaccharide (LPS) for abrupt structural changes, leaving DNA well desaturated. SDS is an anionic detergent that aids in the disruption of biological membrane proteins. EDTA (ethylene-diamine-tetraacetic acid) is used as a chelating agent in DNA extraction by sequestering divalent metal ions such as calcium and magnesium, which blocks the activity of the DNase enzyme. Third, for 1 hour, place tube 1 in the Thermo shaker AccuTherm instrument (temperature = 98 °C, shake 650 rpm). This step is essential to the lysis of the nuclear membrane, breaking open the cell and removing unwanted cellular components and precipitation. Heat is necessary for the death of any microorganism and the weakening of tissue cells.

After one hour, pick up tube 1 from the AccuTherm device and wait until it cools down, then add 20 µl of proteinase k to tube 1. After that, put it in an incubator at 37.0 °C and leave it overnight. Proteinase K is an enzyme significant in inactivating DNase and RNase, the demolition of proteins in cell tissue, and the release of nucleic acid. The next day, add 500 µl of phenol to tube 1, vortex for 1 minute, and centrifuge for 1 minute at 10,000 rpm. Generally, DNA is insoluble in phenol. Phenol bonds between amino acids and breaks them, so protein denaturants are also useful for breaking down superfluous cell materials that contaminate the nucleic acid sample. After centrifuging tube 1

components, they separate into two layers; supernatants and pellets. Pick up the supernatant, which contains protein, DNA, and organic compounds, and set it in a new tube that is labeled as tube 2.

Fill tube 2 with 500 μ l of a 25:24:1 mixture of phenol, chloroform, and isoamyl alcohol. Attention phenol: chloroform (25:24) is to separate aqueous and organic cellular components. Chloroform increases the efficiency of phenol to denature the protein, and adding to that, it allows proper separation of the organic phase and aqueous phase and keeps DNA protected in the aqueous phase. Isoamyl alcohol (1) is essential to prevent emulsification of solution via reducing foaming between interphases. For one minute, vortex tube 2 for one minute, then centrifuge for one minute at 10,000 rpm, which results in forming two layers; an upper aqueous layer that contains DNA, and a bottom organic layer that contains protein and organic compounds. Remove the aqueous layer and lay it in a fresh tube named tube (3). Finally, pour 800 μ l of 100% cold alcohol and 50 μ l of 5 molar NaCl into tube 3 and leave it overnight at -20 °C to extract as much DNA as possible. 100% cold ethanol helps to promote DNA aggregation precipitate more quickly, whereas 5 molar NaCl helps to remove proteins that are bound to the DNA. The next day, centrifuge tube 3 for 15 minutes at 14000 rpm, discard the supernatant, and let the pellet air dry for 10 minutes. Pellets consist of DNA.

At the end of DNA extraction, the product should be washed to purify DNA via protein or any other unwanted product except DNA. First, we inserted 300 μ l of 70% ethanol (to reduce DNA solubility, remove protein and contaminants, and allow the salts to dissolve), vortexed for 1 minute, centrifuged at 14000 rpm for 15 minutes, removed the supernatant, and repeated the process twice. After the final wash, discard the alcohol and leave the tube for 10 minutes to air dry. Finally, add 50 μ l of distilled water to the dried pellet in tube 3 to resuscitate the DNA. We obtained a human histopathological hematoxylin and eosin stain slide that had previously been diagnosed as positive for toxoplasmosis. Extracted DNA from formalin-fixed paraffin embedded (FFPE) tissue blocks First, the cover slip must be removed, and the slide placed in an autoclave at 100 degrees Celsius for 1 hour to soften the paraffin wax. Finally, we removed the tissues from the slide and placed them in an Eppendorf tube to extract DNA using the PCIA methods described above.

After DNA extraction, we checked the quantity using a spectrophotometer, observed the absorption at 260nm, found that the concentration was 1000 ng/L, ran the product in a 2% agarose gel, and used UV light to observe running gel electrophoresis to ensure that the extracted sample contains DNA. We used a PCR technique to test *T. gondii*'s DNA by observing the bands in an agarose gel at the end of electrophoresis. We used products as a positive control for our test sample.

Image 1

Manual DNA extraction using PCI procedure for slaughter sheep



Image (a) Slaughter ram and ewe collected from Jenin and Nablus territories, image (b) Steps of manual DNA extraction using PCI procedure. *Note:* for more details for image (b) view appendix 1.

2.3.2 Check extracted DNA

At the end of the extraction, it is important to know the concentration and quality of the DNA. The most popular technique to identify DNA yield and purity is the measurement of absorbance. For the absorbance method, we need a spectrophotometer equipped with a UV lamp, UV-transparent cuvettes, and a solution of purified DNA. Optical density readings taken by a spectrophotometer can be used to determine the concentration and purity of DNA in a sample. The concentration was then calculated as the total amount of extracted DNA using the formula (absorbance*dilution factor*50 g/ml).

DNA purity is evaluated via measuring the absorbance from 230 nm to 320 nm in order to detect other possible contaminations. The most commonly used method for purity calculation is the ration of the absorbance at 260nm divided by the reading at 280nm. If the A260/A280 ratio is equal to 1.7-2.0, it means we have good-quality DNA. But low ratios demonstrate more contaminants are present.

Gel electrophoresis can be used to show the presence of DNA. These fragments are separated according to their size. For the production of an agarose gel for gel electrophoresis, use 0.35 g of agarose powder with 50ml (1X) Tris-Acetate-EDTA buffer (TAE), boiling it in the microwave for 1 min. After that, empty it into the mold and leave it until it becomes solid.

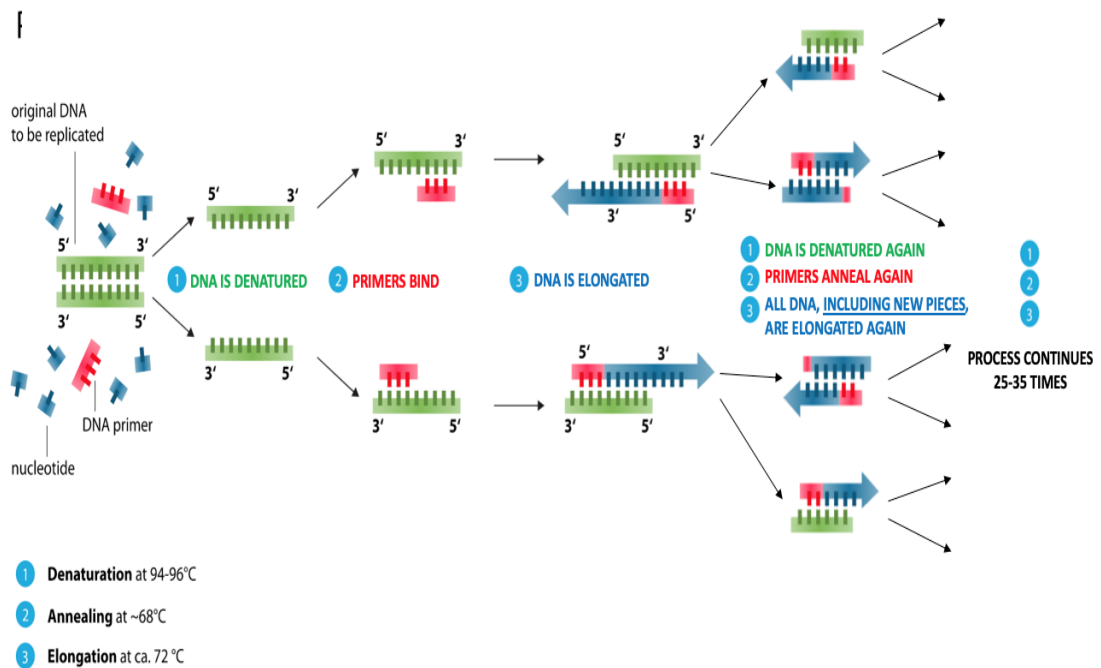
A solution of DNA molecules is placed in a gel by adding 10 microliters with 2 µl of stain, then running in an agarose gel for 30 minutes. Use UV light for observing gel electrophoresis to ensure that the extracted sample contains DNA. The result is a series of "bands". Small DNA molecules move more quickly through the gel than larger DNA molecules. The bands closest to the start of the gel contain the largest DNA fragments.

2.4 Polymerase Chain Reaction (PCR)

For PCR, primers were selected to amplify 529 bp (base per) fragment which are TOX4/TOX5 Forward sequence (5'CGCTGCAGGGAGGAAGACGAAAGTTG3') and Reverse sequence (5'CGCTGCAGACACAGTGCATCTGGATT3') (Homan *et al.*, 2000). The reaction conditions as follows: one initial denaturation cycle for 7 min at 94 °C, 40 cycles of denaturation at 94 °C for 45 sec, annealing at 55 °C for 45 sec, and extension at 72 °C for 45 sec, with a final cycle extension for 7 min. The PCR products analyzed on 2% agarose gel and stained using ethidium bromide and visualized in the UV illuminator.

Figure 6

PCR cycle (denaturation, annealing and elongation)



(Hochstetter, 2020).

2.4.1 Procedure for PCR

Prepare a PCR mixture for work use 750 microgram ready mix (contains Taq DNA polymerase, dNTPs, MgCl₂, reaction buffer, tracking dye, density, PCR stabilizer and enhancer), 640 µl water negative DNA, 5 microgram forward primers, 5 µl reverse primer.

Add 23 µl of PCR mixture to a 0.5 ml Eppendorf emptied tube, then add 2 µl of extracted DNA sample, mix it, and insert the tube into the PCR machine. Using the gradient PCR procedure, the target fragment of 529 bp could be amplified from this sample. After initial denaturation of the DNA at 94°C for 4 min, 35 cycles were run, as follows: 94°C for 35 sec, 50°C for 1 min, and 72°C for 1 min. 35 sec, 52°C for 1 min, and 72°C for 1 min. 35 sec, 54°C for 1 min, and 72°C for 1 min. The final extension step continued for an additional 10 min at 72°C. Aliquots of PCR products were electrophoresed on a 2% agarose gel and observed for the presence of the specific fragment (529 bp for *T. gondii*) under UV light.

Table 3*Program of gradient PCR procedure.*

	Temperature	Time	
1	95.0°C	0:04:00	
2	95.0°C	0:00:35	
3	52.0°C	0:01:00	10 cycles
4	72.0°C	0:01:00	
5	95.0°C	0:00:35	
6	54.0°C	0:01:00	10 cycles
7	72.0°C	0:01:00	
8	95.0°C	0:00:35	
9	56.0°C	0:01:00	15 cycles
10	72.0°C	0:01:00	
11	72.0°C	0:10:00	

Analysis of PCR Products: Distilled water is supplied as the negative control. The polymerase chain reaction products were run at 90V for 30 minutes on a 2% agarose gel in 1X TBE buffer, stained with ethidium bromide, and the images were visualized in UV. The PCR products (529 bp) were identified by a 100 bp DNA size marker (Fermentas, Germany). Agarose gel is prepared by thawing 0.7g agarose powder with 50ml TAE buffer, mixing well, boiling in the autoclave, cooling the shed in the mold, and waiting for solidification.

Chapter Three

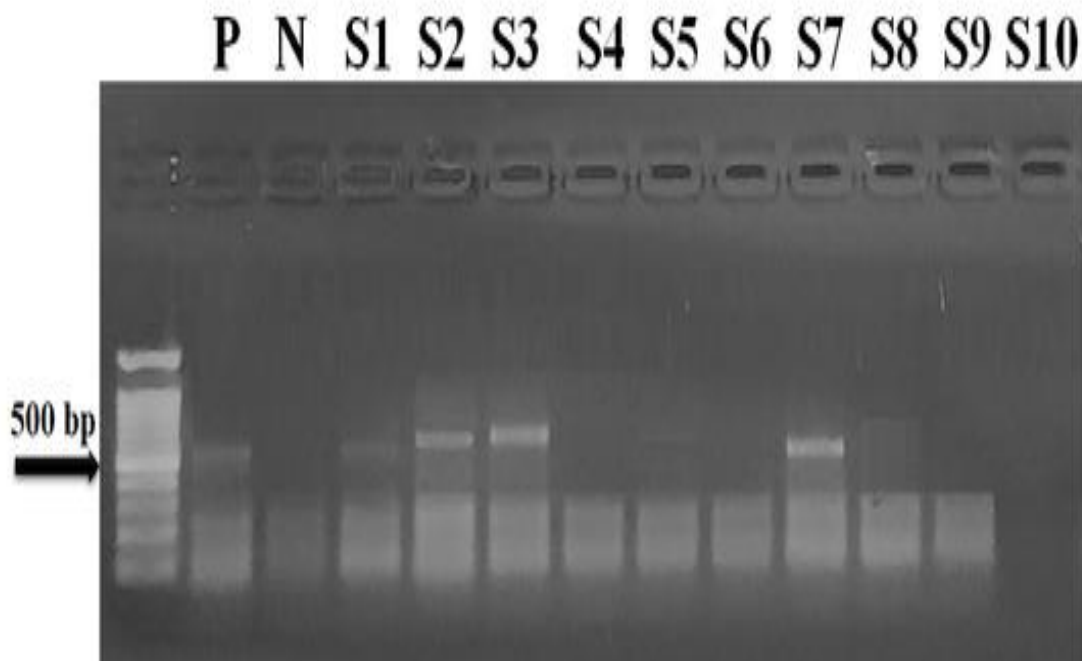
Results and Discussions

3.1 Results

1062 sheep's tissues, including 252 livers, 74 lungs, 280 hearts, 254 brains, and 202 tongues, were obtained from 346 (ram and ewe) and they were slaughtered in the abattoirs of Nablus and Jenin. These tissues were examined for the presence of deoxynucleic acid (DNA) for *T. gondii*. DNA was extracted from the lungs, liver, brain, tongue, and heart of sheep. These tissues were examined for the presence of TOX4/TOX5 genes (non-coding fragment, repeated 200-300-fold) and were amplified using polymerase chain reaction (PCR). The amplicons were visualized and the results were analyzed (figure 7).

Figure 7

PCR analysis for T.gondii



Note: Detection of *T. gondii* in sheep tissues samples by PCR amplification of the B1 gene. Amplicons were loaded onto a 2% agarose gel, visualization of PCR end product under UV light, P (positive control), N, (Negative control). S1-10 (samples 1-10) S1,2,3,5 and 7 shows positive.

In this study, a total of 346 sheep (ram = 296, ewe = 50) were randomly selected as samples from the two provinces. The overall geno-prevalence of *T. gondii* in sheep was 25.72% (89/346) of sheep, while the overall prevalence of *T. gondii* was 18.21% (63/346) of slaughtered rams, but it was 7.51% (26/346) of slaughtered ewes. In Nablus, the total infection rate of slaughtered ewes was 12.22% (22/180) whereas it was 2.41% (4/166) but the total infection rate within slaughtered rams was 19.44% (35/180) in Nablus whereas it was 16.87% (28/166) figure 8a shows the number of infected sheep within slaughtered sheep, ewes, and rams in northern Palestine, Nablus, and Jenin, but figure 8b summarizes the percentages of the total infection rate of Toxoplasmosis within slaughtered sheep, rams, and ewes in northern Palestine, Nablus, and Jenin.

Figure 8

The overall infection rate of toxoplasmosis within slaughtered sheep

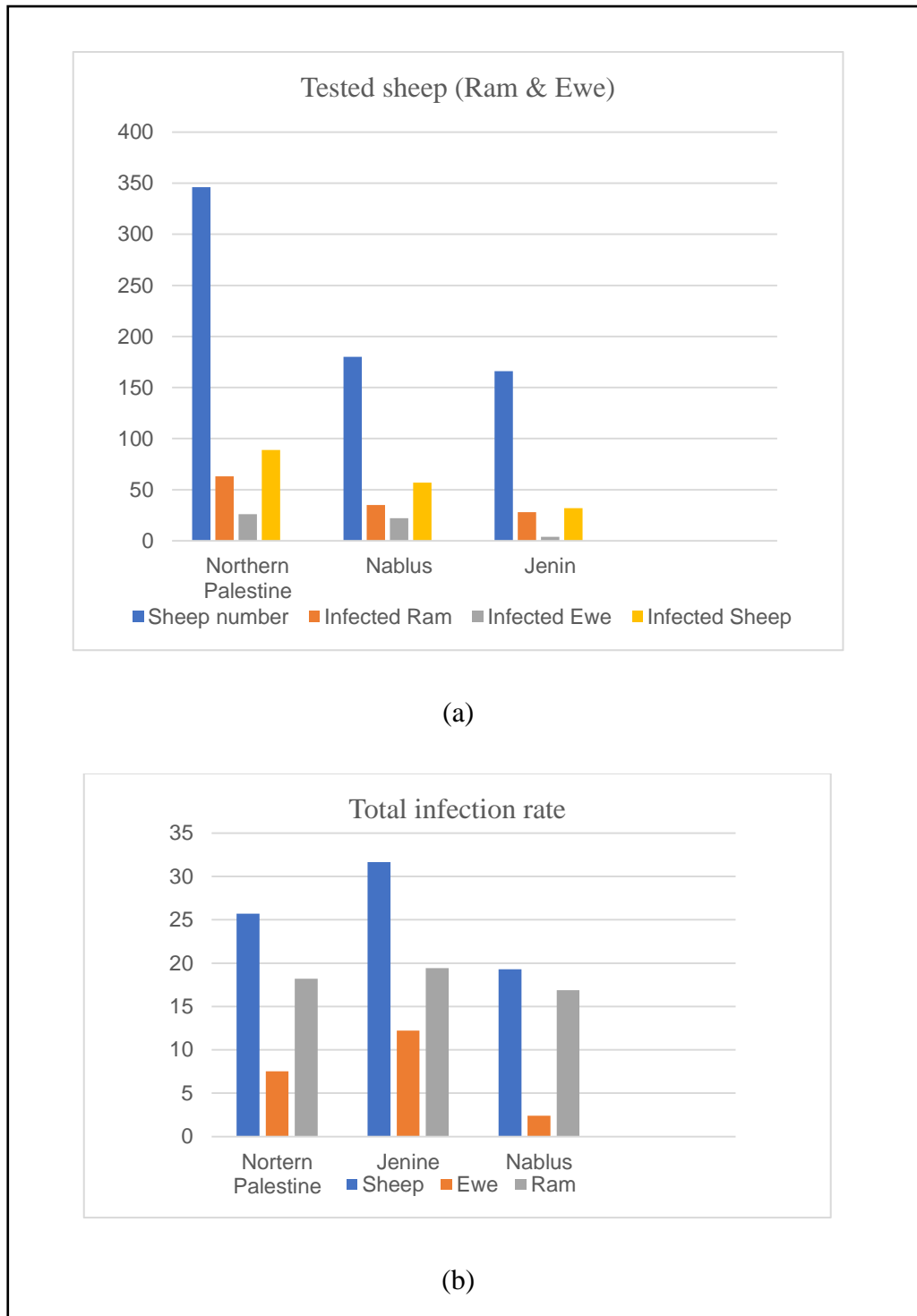


Figure (a) the total infection within slaughtered sheep, ewes, and rams in northern Palestine, Nablus, and Jenin, figure (b) Total infection rate of toxoplasmosis within slaughtered sheep, ram and ewe in northern Palestine, Nablus and Jenin.

The total infection rate of toxoplasmosis in Nablus was 29.44% (53/180), whereas it was 19.27% (28/162) in Jenin. The overall infection rates in all tested samples were 7.86% (22/280) in the heart, 2.36% (6/254) in the brain, 4.76% (12/252) in the liver, 21.78% (44/202) in the tongue, and 21.6% (16/74) in lung samples. The highest percentage was noticed in the tongue. The second one was in the lung. The third was the heart. The fourth was the liver. Finally, the lowest percentage of infection was recorded in the brain. The total infection rate for rams was 21.82% (63/296), whereas it was 52% (26/50) in ewes.

Total infection rates in rams' liver, lung, heart, brain and tongue were demonstrated as 3.77% (8/212), 21.6% (16/74), 3.42% (8/234), 0.96% (2/208) and 17.72% (158), respectively. Although it was 10% (4/40), 30.43% (14/46), 8.69% (4/46) and 36.36% (16/44) in ewes' liver, heart, brain, and tongue, respectively. Figure 9a summarize the Total infection rate of toxoplasmosis within slaughtered sheep (ewe and ram) in Northern Palestine, Nablus and Jenin.

In Nablus, *T. gondii* TOX4/TOX5 genes were reported in slaughtered tissues as 7.35% (10/136) liver, 10.71% (18/168) heart, 1.25% (2/160) brain, and 22.89% (38/160) tongue. Including 4.76% heart (6/126), 0.00% brain (0/118), 6.0% liver (6/100), and 18.97% tongue (22/116) of rams, the infection rate in the heart, brain, liver, and tongue of ewes was 28.57% (12/42), 4.76% (2/42), 11.11% (4/36), and 36.36% (16/44) respectively. The table summarizes the prevalence of the *Toxoplasma gondii* TOX4/TOX5 gene on slaughtered sheep tissues (tongue, liver, heart, brain, and tongue) in Northern Palestine (Nablus and Jenin).

In Jenin, comprehensive toxoplasma infection was found to be 17.28% in slaughtered rams (28/162) but 100% (4/4) in slaughtered ewes. The *T. gondii* TOX4 and TOX5 genes were reported in tissue samples as the following: The infection rates in the ram's heart, brain, liver, tongue, and lung were 1.82% (2/110), 0.00% (0/92), 1.72% liver (2/116), 14.29% tongue (6/42), and 21.62% lung (16/74) respectively, whereas the infection rates in the ewe's heart, brain, liver, tongue, and lung were 50% (2/4), 50% (2/2), 0% (0/2), and 0% (0/4). Figure 9a summarizes the total infection rate of toxoplasmosis within slaughtered sheep tissues (tongue, liver, brain, heart, and lung). Meanwhile, figure 9b summarized the total infection rate of toxoplasmosis within

slaughtered rams, ewes, and sheep tissues (tongue, liver, brain, heart, and lung) in Northern Palestine, Nablus, and Jenin. Table 4 shows the overall prevalence of the *Toxoplasma gondii* TOX4/TOX5 gene on slaughtered sheep's tissues (tongue, liver, heart, brain, and tongue) in Northern Palestine, as determined with PCR technique, but Table 5 Comparison between Jenin and Nablus prevalence of *Toxoplasma gondii* TOX4/TOX5 gene within slaughtered sheep tissues (liver, lung, heart, brain, and tongue). Meanwhile, Table 6 compares the prevalence of the TOX4/TOX4/TOX4/TOX4/TOX5 gene in Jenin and Nablus based on gender in liver, lung, heart, brain, and tongue tissues.

Figure 9

The overall infection rate of toxoplasmosis within slaughtered sheep tissues



Figure (a) the overall infection rate of toxoplasmosis within slaughtered sheep tissues, figure (b) the total infection rate within slaughtered sheep, ram and ewe in Northern Palestine, Nablus and Jenin.

Table 4*Geno- prevalence of TOX4/TOX5 genes on slaughtered sheep's tissues.*

Factor	Category	Sample size	Number with positive TOX4/TOX5	Geno prevalence %	
Species	Ram And Ewes 346	Tissue 1062	Liver (252)	12	4.76%
			Lung (74)	16	21.6%
			Heart (280)	22	7.86%
			Brain (254)	6	2.36%
			Tongue (202)	44	21.78%
			Liver (212)	8	3.77%
			Lung (74)	16	21.6%
			Heart (234)	8	3.42%
			Brain (208)	2	0.00%
			Tongue (158)	28	17.72
	Ewe 50	Tissue 176	Liver (40)	4	10.0%
			Heart (46)	14	30.43%
			Brain (46)	4	8.69%
			Tongue (44)	16	36.36%

Table 5*Prevalence's comparison of studied areas for TOX4/TOX5 genes of slaughtered sheep tissues*

Factor	Category	Sample size	Number with positive TOX4/TOX5	Geno prevalence %	
Area	Nablus 180	Tissue 624	Liver (136)	10	7.35%
			Heart (168)	18	10.71%
			Brain (160)	2	1.25%
			Tongue (160)	38	22.89%
	Jenin 166	438	Liver (116)	4	3.45%
			Heart (112)	4	3.57%
			Brain (94)	2	2.13%
			Tongue (42)	6	14.29%
			Lung (74)	16	21.62%

Table 6*Gender comparisons among the prevalence of TOX4/TOX5 genes in studied area.*

Factor	Category	Sample size	Number with positive TOX4/TOX5	Geno prevalence %			
Gender			Liver (116)	2	1.72%		
			Lung (74)	16	21.62%		
			Jenin 162 Tissue 434	Heart (110)	2	1.82%	
				Brain (92)	0	0.00%	
			Ram 296		Tongue (42)	6	14.29%
					Liver (100)	6	6.00%
				Nablus 134 Tissue 460	Tongue (116)	22	18.97%
					Heart (126)	6	4.76%
					Brain (118)	0	0.00%
					Liver (36)	4	11.11%
					Tongue (44)	16	36.36%
				Nablus 46 Ewes 122	Heart (42)	12	28.57%
			Female 50		Brain (42).	2	4.76%
				Jenin 4 Ewes 8	Brain (2)	2	100%
		Heart (4)	2	50%			

Among the samples successfully genotyped for *T. gondii* (89 sheep), 16 of them had double infected tissue. One of these tissues is the tongue. Their percentages were 17.98% (16/89), including 62.5% (10/16) in ewe and 37.5% (6/16) in ram. 50% (8/16) have *T. gondii* DNA located in both their tongue and liver, 25% (4/16) in both their tongue and brain, and 25% (4/16) in both their tongue and heart. On the other hand, 82.02% (73/89) have only one infected tissue. Figure 10a summarized the healthy and infected sheep, double and single infected sheep, including ram and ewe, whereas figure 10b shows the co-infected tissues within slaughtered sheep in Northern Palestine.

Figure 10

Distribution of infection in animal and tissues

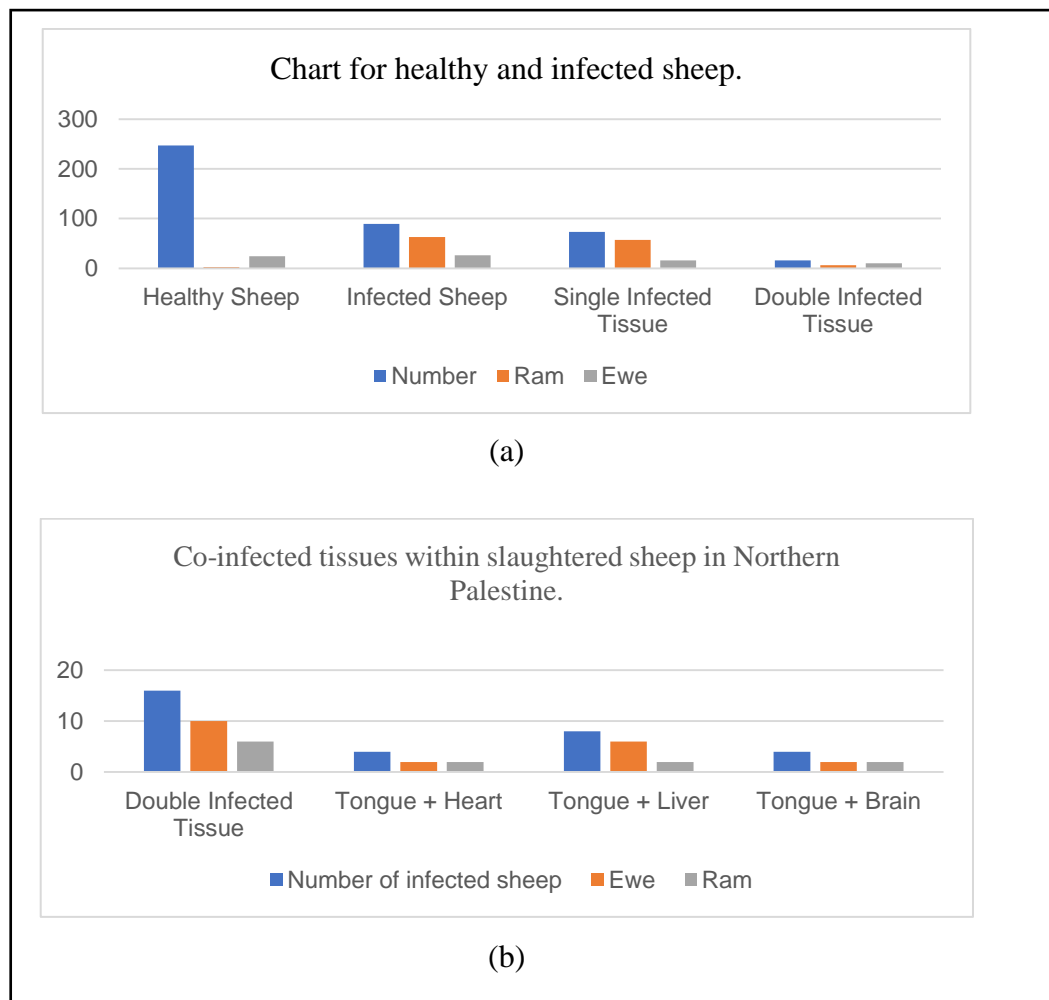


Figure (a) Summarized the healthy and infected sheep, double and single infected sheep including ram and ewe, figure (b) Co-infected tissues within slaughtered sheep in Northern Palestine.

Chapter Four

Discussions and Conclusions

4.1 Discussions

Toxoplasmosis is expressed as one of the most important food-borne diseases. The disease is transmitted to humans via eating infected tissues of sheep (Santos et al., 2010). This study was conducted to determine the molecular detection of the *T. gondii* TOX4/TOX5 gene in sheep in the northern Palestinian areas, particularly in Nablus and Jenin. Sheep are the most important livestock in societies that deal with agriculture and animal husbandry, such as Palestine, and their products are considered the main nutritional sources for humans because they are rich in high-quality protein and many vitamins and minerals. In spite of some advances in the diagnosis and treatment of toxoplasmosis, the illness persists as an extensive zoonotic disease in many parts of the world, and it is responsible for compelling public health and economic losses. For example, in 1992, in the US alone, the treatment costs of toxoplasmic encephalitis in HIV patients were \$ 23 to 106 million (WHO, 1996).

This is the first time that this study is being applied to slaughtered sheep tissues that have aged less than one year, using the polymerase chain reaction (PCR) method. We reported the results of our background with the use of PCR for the identification of *T. gondii* TOX4/TOX5 genes in liver, tongue, heart, brain, and lung tissues that were previously randomly collected from slaughtered sheep.

In our study, we used a PCR test, which is a rapid and reliable test used to confirm and support the seropositivity of toxoplasmosis by detecting *T. gondii*'s DNA. It is performed to detect and measure the parasitic DNA in blood samples, amniotic fluid, cerebrospinal fluid, milk, placental tissues, or any type of tissue. A positive PCR result indicates that the disease exists. We tested 346 sheep, including 296 rams and 50 ewes. We found that 63 rams and 26 ewes have a *T. gondii*'s gene in their tissues in order to report that the geno-prevalence within slaughtered sheep in the northern regions of Palestine was 25.72% (89 from 346) and the total infection rate within slaughtered rams was found to be 21.82% (63/296). Meanwhile, it was 52% (26/50) among slaughtered ewes. These Sheep haven't been previously tested for *T. gondii*, having no signs or symptoms of any disease, and there were no abortion cases. When we applied this study

to each region separately, we discovered that the overall infection rate within Jenin was 19.27% (32/166) sheep. Figures will vary according to gender; it becomes 17.28% (28/162) in a slaughtered ram and 100% (4/4) in a slaughtered ewe.

On the other hand, the overall infection rate within Nablus was 29.44% (53/180). Depending on gender, the prevalence of toxoplasmosis is going to be 47.83% (22/46) in slaughtered ewes and 23.13% (31/134) in slaughtered rams. Depending on the previous results, we concluded that the highest incidence rate was in Nablus at 29.44%, followed by Jenin at 19.27%. Regarding gender, the prevalence of toxoplasmosis among male animals (rams) was less than the prevalence of toxoplasmosis among female animals (ewes). There is a positive correlation between the prevalence of toxoplasmosis and females. Ewes have the highest incidence rate of *T. gondii* compared to rams in both Nablus and Jenin. These values differ from two previous studies that were performed by Othman in 2011 and some are similar to a study that was done in 2017. The first study (2011) tested 151 goat blood samples and demonstrated that the seroprevalence of *T. gondii* in female goats aged between 2 and 4 years from Tulkarem and Jenin using ELISA was 13.4%. The highest incidence was in Jenin (17.44%), but it was 7.69 % in Tulkarm. In the second study, which was done in 2017, researchers used an indirect ELISA technique to test for toxoplasmosis in 2479 sheep blood samples that were collected from three different regions in Palestine. The results of these samples showed that the percentage of infected sheep was 20.5% of the total samples. The infection rate reached its highest point in Jenin, which was 30.2%, followed by Nablus (24%) (Othman *et al.*, 2017). A difference in results occurred due to Othman's usage of the serological methods to observe toxoplasmosis in living sheep. This method discovered anti-*T. gondii* antibodies IgM and IgG. Toxoplasma IgM is considered evidence of an acute infection. IgM increases in the blood circulation 1-2 weeks after the infection. However, IgM can persist for several months or years after acute infection. When IgM is negative, this suggests that the infection has previously occurred. A positive toxoplasma IgG indicates a current or past infection with *T. gondii*. IgG positivity indicates that infection has occurred at some point in the infected individual's life but cannot specify when. Studies which depend on serological tests to find the percentage of infection rate will measure both recent and previous infections, raising the possibility of a high infection percentage.

By comparing our essay with previous studies from different parts of the world, we detected more or fewer similarities and differences. Different studies using similar testing methods were applied to live sheep and goats. In our study, the DNA of *Toxoplasma gondii* was recorded within slaughtered sheep's tongue, lungs, hearts, livers, and brain. We found that the prevalence of parasitic DNA was 21.78% (44 from 202), 21.6% (16 from 74), 7.86% (22 from 280), 4.76% (12 from 252), and 2.36% (6 from 254), respectively. The tongue has the highest infection rate, whereas the lowest infection rate was in the brain. This result was contraindicated by a study that was applied to sheep tissues (n = 6). It found that the brain was the most frequently affected organ among other organs. The results for internal organs such as the liver and spleen were negative. Heart results were positive in half of the sheep. A third of the sheep's skeletal muscles were positive, while only one positive result was found in the kidney. The difference in population size between our study (346 sheep) and the previous study (6 sheep) is a major factor in the disagreement between these results (Juránková et al., 2015).

Another study conducted in Northeastern Iran collected blood samples, heart, and diaphragm tissue from 296 animals (168 sheep and 128 goats) and tested them for toxoplasmosis using a modified agglutination test and nested PCR to estimate toxoplasmosis prevalence. It found that seroprevalence was 27.4% (46 from 168) of sheep and 23.4% (30 from 128) of goats. Furthermore, the B1 gene was found in 40% (12 of 30) of the diaphragm and 23.3% (7 of 30) of the heart tissues in goats (Firouzeh et al., 2021). For example, consumption of raw meat containing *T. gondii* cysts is a main route of transmitting the parasite from sheep to humans, so vaccination of food-animals will reduce the risk of toxoplasmosis and enhance the safety of meat for human consumption. To support this statement in 2014, Katzer's research tested the influence of immunizing lambs with the incomplete S48 strain of *Toxoplasma gondii*. *T. gondii* DNA was found in muscle or lymph nodes. Non-vaccinated sheep show parasitic DNA in their muscles and nervous systems more than vaccinated animals. The vaccinated group had a (0% in heart and 5.9% in skeletal muscles) when compared to the non-vaccinated animals (75% heart and 87.9% skeletal muscle) (Katzer et al., 2014).

The global prevalence of *Toxoplasma gondii* infection in aborted fetuses and ruminants that had abortions was 56% and 50%, respectively. And the prevalence of *T. gondii* infection in aborted fetuses was 42% in sheep and 31% in goats using molecular methods (Omonijo *et al.*, 2022). In 2014, in Wasit Province, 300 blood samples and 5 placentas were collected from suspected sheep. Amplified B1 toxoplasma genes showed that 4% of blood samples and 80% of placental tissue samples were positive in sheep (Khlaty & Aá, 2015). In 2015, in the East Azerbaijan province of Iran, the prevalence rate of *T. gondii* in sheep was 24.8% and in goats it was 10.6%. Testing of sample serum for antibodies against *T. gondii* found that it was 18.3%. It was also noticed that the prevalence of toxoplasmosis antibodies was significantly higher in adult sheep and goats than in younger animals. In the same study, researchers used a PCR test for more specificity. The DNA of *T. gondii* in blood serum, fetal brain, and cotyledons was 14.7%, 69%, and 23%. According to Kavari *et al.* (2013), in Mymensingh, Bangladesh, 15.52% of tissue samples from aborted fetuses of sheep and goats were positive for the *T. gondii* parasite (Sah *et al.*, 2019). Egyptian sheep have a 17.65% infection rate (Ibrahim *et al.*, 2017).

In Jordan, *T. gondii* was observed in 41.8% of aborted goats. Meanwhile, in the Mafraq region of Jordan, researchers found that the overall toxoplasma-specific prevalence rate was 35.1% within goat and sheep flocks that had cases of abortion (Abu-Dalbouh *et al.*, 2012). The incidence and prevalence of toxoplasma infection within animals has a positive correlation with the incidence of abortion, as we showed in the studies above, which means that there is an increase in economic loss due to the loss of animals as a result of abortion, which leads to a decrease in the chances of reproduction. *T. gondii* forms tissue cysts in different types of tissue. It is most predominantly found in the myocardium, skeletal muscle, and the eye; these cysts are able to stay throughout the life of the host. In our study in the northern Palestine region, we found that the prevalence rate of *T. gondii* DNA within meat sheep muscles (liver, heart, tongue, and brain) was 25.72%, including 19.27% in Jenin and 29.44% in Nablus. On the other hand, the prevalence in Turkey was 40.8%, including 20.8% obtained from Ankara and 22.4% obtained from Kırkkale. A *T. gondii* B1 gene in sheep was 10.73% in goats, according to a study conducted in three cities in Shandong province, eastern China.

In Turkey, it was 40.8%. Depending on these results, Turkey has the highest incidence rate, followed by Northern Palestine, with the lowest rate found in China (78.79%). Another study in China detected the prevalence of *T. gondii* DNA in brain samples was 5.2%. Eating semi-cooked meat increases the risk of human toxoplasmosis. In Poland, 65.0% of milk samples that have been taken from goats are positive for *T. gondii*'s DNA. The lactation process from the mother to her offspring increases the risk of transmitting the parasite (Dubey *et al.*, 2020).

Various studies using different testing methods were applied to sheep, goats, and humans. Serologically, tests in Egypt determined that the true prevalence of antibodies to *T. gondii* in both sheep and goats was high. Toxoplasma in Egyptian goats was 62% higher than in sheep. Sheep slaughtered at the Cairo abattoir had the lowest true prevalence (4.1%), while the true prevalence in Dakahlia, Giza, and Sharkia governorates (26%, 23%, and 12%, respectively) was substantially higher (Al-Kappany *et al.*, 2018). In El-Gadarif state, the overall sero-prevalence of *T.gondii* within sheep and goats was 52.0%. This includes 45.7% in sheep and 27.2% in goats (Atil, 2017). In North Lebanon, 42% of sheep and 34% of goats have anti-*T. gondii* IgG antibodies (El Safadi *et al.*, 2019). 17.8% of tested serum, which was obtained from aborted ewes from different sheep flocks, was positive for anti-*T. gondii* antibody (Alazemi, 2014).

In Lebanon in 2017, researchers identified the prevalence of *T. gondii* specific antibodies IgG and IgM among pregnant women was 82.6% and 1.8%, respectively. The prevalence of IgG seropositivity among women aged 35–44 years was 87.81%. and in the governorate of "Mount Lebanon" (82.95%) (Nahouli *et al.*, 2017). In Egypt, 90 aborted women were tested for specific *T. gondii* antibodies, IgM and IgG. It was found that 35.6% (32 from 90) were positive for the test; 27 represented IgG; two had both IgG and IgM; and three had IgM.

Toxoplasma gondii was recognized in 32 blood samples tested by nested PCR. *T. gondii* was found in four of thirty-two aborted women; one in aborted women with IgM and IgG, one in a woman with IgG, and two women with IgM. Also, tested placental tissues for *T. gondii* found that the prevalence of *T. gondii* was 78.1% (25 out of 32). All of these 32 aborted women were seropositive for toxoplasma; of them, one in aborted women with both IgG and IgM, and 24 in aborted women with IgG and IgM. There was

no evidence of this in any aborted mother. 45.5% of *T. gondii* seropositive cases were recorded in women aged >35 years, and aborted women lived in rural communities more susceptible to infection (43.5%) than those living in urban areas (9.5%). The second trimester of pregnancy was recorded with the highest infection with *T. gondii*; it was 43.44%. For the second (41.7%) and third (43.8%) times, aborted women had higher infection rates than those aborted for the first time (19.2%) (Abdel-Aziz et al., 2020).

4.2 Conclusions

Depending on the high rate of *T. gondii* infection in sheep's tissues, proper and active preventive measures should be done in order to eliminate and prevent the transmission of this parasite to humans. It is essential to prevent eating the raw or under-cooked tissues of infected sheep. Furthermore, standardization of meat preparation methods and meat products is very important.

This research aimed to detect the prevalence of toxoplasmosis within slaughtered sheep. Based on the results we can be concluded that due to the high rate of toxoplasmosis among the slaughtered animals as well as meat products specially in tongue, consume under-cooked meat that obtained from infected sheep can be one of the main risk factors of transmission of the parasite to humans. The results indicate that it is necessary to avoid eating the raw and undercooked tissues of infected animals.

4.3 Recommendations

- Based on these conclusions, we recommend washing hands and knives after preparing raw meat, avoiding eating raw or undercooked meat, and cooking meat at temperatures high enough to kill *T.gondii* in order to prevent a risk of toxoplasmosis from food.
- Depending on the results, we note that the tongue was demonstrated as the most infected tissue, perhaps due to taking parasitic oocysts from grass or from livestock feed. We recommend testing animal foods and green feeds before feeding them to ensure that they are free from toxoplasmosis.

- To reduce the prevalence of toxoplasmosis, it is recommended to vaccinate sheep and humans to induce protective immunity and reduce the risk of infection with toxoplasmosis.

4.4 Summary

Toxoplasmosis is an intracellular parasitic infection that causes toxoplasmosis, a serious zoonotic disease that affects people all over the world. Cats are the only definitive hosts; it can affect nearly all warm-blooded animals. Today, people become infected with toxoplasmosis via contact with infected animal feces, especially cat feces. It usually occurs due to eating semi-cooked contaminated meat, exposure to infected cat feces, or mother-to-child transmission during pregnancy. This increases the risk of stillbirth, birth defects, and sometimes abortion. Most people with a healthy immune system never develop signs or symptoms, and they don't need to be treated for toxoplasmosis because symptoms are mild and usually go away within a few weeks. However, people with weakened immune systems, such as HIV patients, infants, the elderly, or chemotherapy patients, can experience serious complications such as blurred vision, loss of coordination, which requires immediate medical attention, confusion, blindness, or severe nephropathy.

Accidental ingestion of toxoplasma oocyst picked up from feed, water, or grass causes toxoplasmosis in sheep. Infected ewes can pass parasites to their fetus through the placenta, causing stillbirth or severe defects. Parasites can live for a long time with no symptoms, forming tissue cysts in various tissues such as muscles, the heart, lungs, brain, and liver. Ingestion of parasitic oocyst-containing tissue from infected animals transmits toxoplasmosis from animal to human. In sheep, abortion usually occurs in the last four weeks of pregnancy. Lambs may be born dead or alive but weak and die, usually occurring within the first 2 weeks of life. This causes an economic loss for farmers.

There is a live vaccine for use in sheep. On the other hand, ingestion or accidental self-administration of vaccines may disrupt normal fetal development, so they should not be handled by women of childbearing age or pregnant women.

List of Abbreviations

Abbreviation	Meanings
Bp	base pair
°C	Celsius <i>degrees</i>
CIA	Chloroform Isoamyl Alcohol
CNS	Central Nervous System
DNA	deoxyribonucleic acid
EDTA	Ethelene Diamino Tetra Acetic Acid
g	Gramm
HIV	Human Immunodeficiency virus
hr	Hour
IgM/ IgG	Immunoglobulin M / Immunoglobulin G
LPS	lipopolysaccharide
µg	Microgram
µl	Microliter
Min	Minutes
PCI	Phenol Chloroform Isoamyl Alcohol
PCR	Polymerase chain reaction
PH	Potential of hydrogen
RNA	ribonucleic acid
Rpm	Round per Minute
Sec	Second
TAE	Tris-Acetate-EDTA buffer
UV	Ultraviolet

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Appendices

Appendix A

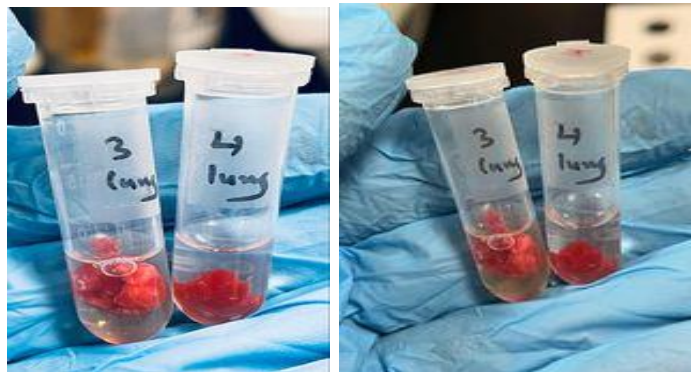
Manual DNA extraction using PCI (25;24;1)



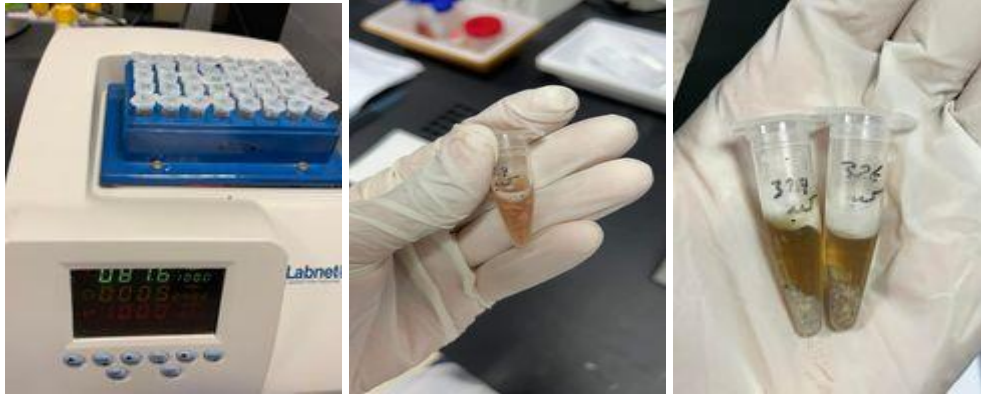
1. Slaughtered Sheep from Nablus & Jenin.



2. Cut tissues (tongue, lung, liver, brain and heart).



3. Add 500 μ l lysis buffer to each tube.



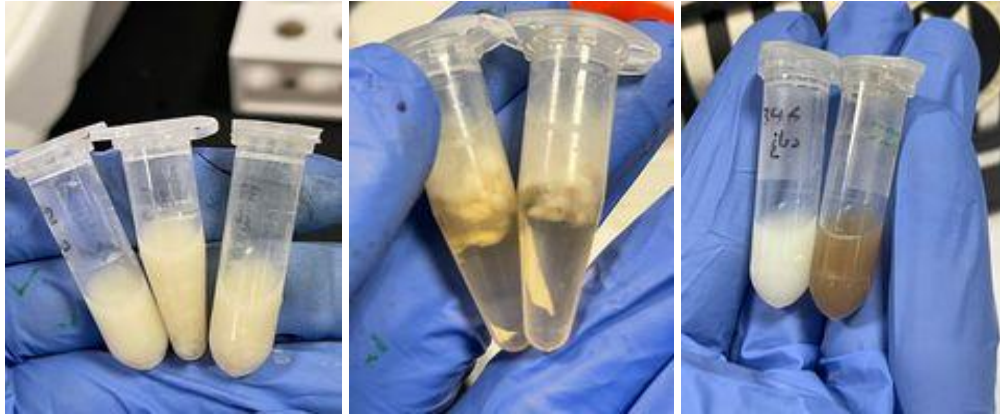
4. Boiling tubes that contain tissues and lysis buffer via place tube 1 in the Thermo shaker AccuTherm instrument (temperature = 98 °C, shake 650 rpm).



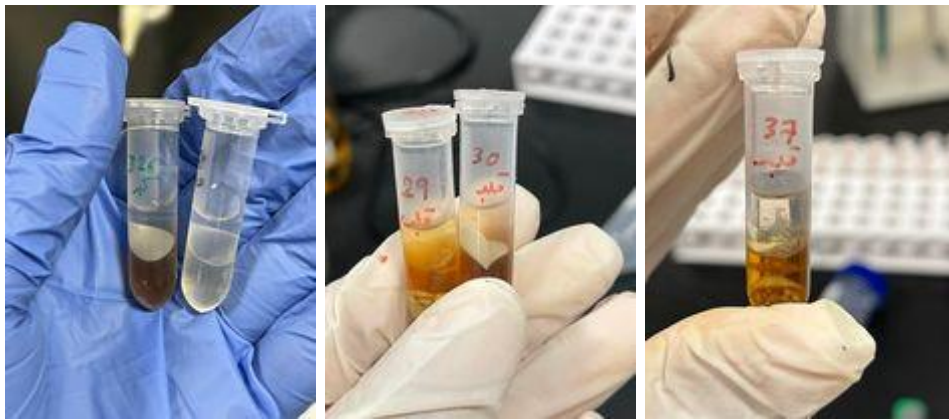
5. Add 20 μ l proteinase k to each tube after that tubes will incubate over-night at 37 Celsius degrees.



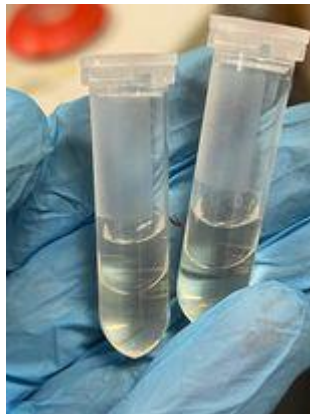
6. After overnight incubation.



7. Add 500 μ l phenol then vortex for 1 minutes.



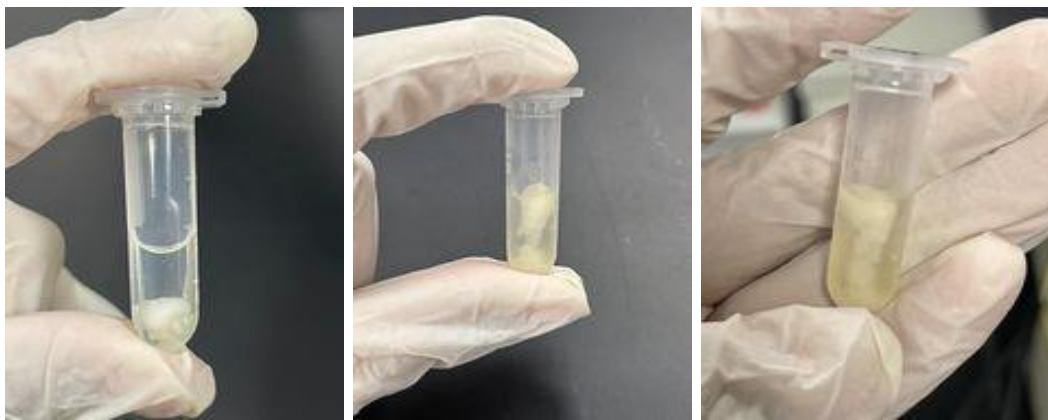
8. After centrifugation at 10000 rpm for 1 min .



9. Separate supernatant from previous tube then add 500 microliter phenol; chloroform; isoamyl alcohol (25;24;1) , centrifuge at 10000 rpm for 1 min.



10. Separate supernatant from previous tube add 800 μ l 100% cold ethanol + 50 μ l from 5 molar NaCl.



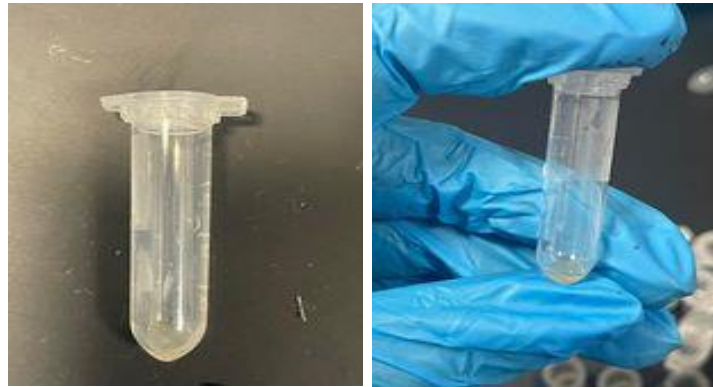
11. After put tubes over night at -4 celcius.



12. Precipitated DNA via centrifugation at 14000 rpm for 15 min, remover supernatant saves pellets which consist of DNA.



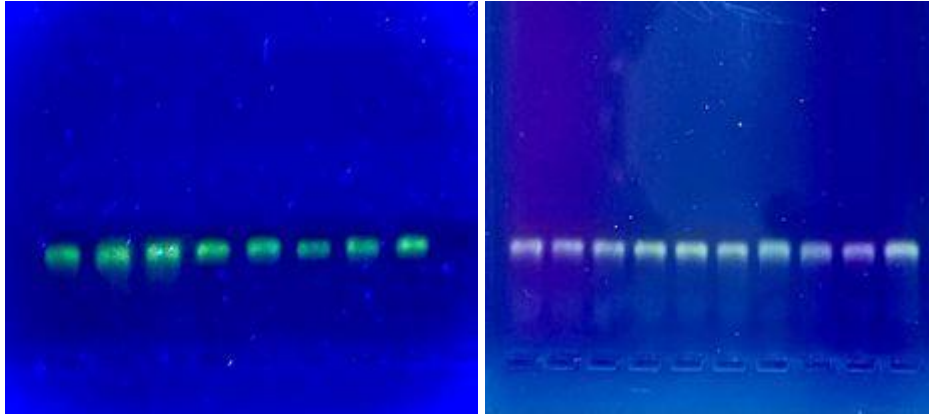
13. Add 300 μ l from 70% ethanol for DNA washing then leave tubes open for 10 min allow air-drying.



14. Add 50 μ l of distilled water to the dried pellet in tube to resuscitate the DNA.



15. Reading the absorbance via spectrophotometer at 260nm to calculate DNA concentration.



16. Running gel electrophoresis to ensure that extracted sample containe DNA.



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قدمت هذه الرسالة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية ، من كلية الدراسات
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الملخص

خلفية الدراسة : داء المقوسات هو مرض حيواني المنشأ في جميع أنحاء العالم يحدث بسبب التوكسوبلازما جوندي. يصيب الحيوانات من ذوات الدم الحار بما في ذلك الأغنام والبشر. يرتبط تناول لحوم الماشية غير المطبوخة جيدًا ، وخاصة لحوم الأغنام ، ارتباطًا وثيقًا بداء المقوسات البشري . 30% على الأقل من البشر في معظم البلدان النامية ايجابيون للأجسام المضادة من النوع جيم لداء المقوسات . يؤثر هذا الطفيل على النساء ويؤدي إلى زيادة فرصة الإجهاض بسبب تلف الجهاز العصبي المركزي للجنين من خلال التسبب في عيوب خطيرة عند الأطفال حديثي الولادة. تم الحصول على 1062 نسيج (252 كبد و 74 رئة و 280 قلب و 254 دماغ و 202 لسان) من 346 خاروف نُبِحت في مسالخ نابلس و جنين.

منهجية الدراسة: تم فحص هذه الأنسجة بحثًا عن وجود الحمض النووي الرايبوزي منزوع الأكسجين (المادة الوراثية) للتوكسوبلازما جوندي المستخرجة من الرئة والكبد والدماغ واللسان و القلب في الأغنام باستخدام طريقة فينول-كلوروفورم-ايزواميل ، تم فحص الحمض النووي المستخرج لوجود جين TOX5 /TOX4 (جزء غير مشفر ، متكرر 200-300 ضعف) التسلسل الأمامي (5' CGCTGCAGGGAGAAGTTG3') والتسلسل العكسي (5' CGCTGCAGACACAGTGCATCTGGATT3') تم تضخيمه باستخدام تفاعل البلمرة المتسلسل (PCR) ، تم تصوير الأمبليكونات وتم تحليل النتائج.

نتائج الدراسة: عثرنا على نتائج إيجابية جينياً في 25.72% (346/89) من الأغنام. وبلغت نسبة الإصابة الإجمالية في محافظة جنين 19.27% (162/28) بينما كانت 29.44% (180/53) في نابلس. أعلى نسبة وجدت في نابلس. أظهر تحليل البيانات أن معدلات الإصابة الإجمالية في عينات القلب والدماغ والكبد واللسان والرئة كانت 7.86% (280/22) ، 2.36% (254/6) ، 4.76% (252/12) ، 20.79% (44/202) و 21.78% (37/8) على التوالي. النعاج لديها معدل إصابة أعلى مقارنة مع الخراف. من بين 89 من العينات التي تم تمطيها وراثياً بنجاح ل داء المقوسات ، كان هناك 16 عينة منها مصابة بنسجين مصابين و أحد هذه الأنسجة هو اللسان. أظهرت النتائج وجود الحمض النووي للتكسوبلازما جوندي في أنسجة الأغنام المذبوحة من المنطقة الشمالية للضفة الغربية . سجلت أعلى نسبة مئوية في اللسان بحيث يكون في الغالب سبباً لخطر الإصابة بداء المقوسات.

الخلاصة: نظراً لارتفاع معدل الإصابة بداء المقوسات بين الحيوانات المذبوحة وكذلك منتجات اللحوم خاصة اللسان ، فإن تناول اللحوم غير المطبوخة جيداً والتي يتم الحصول عليها من الأغنام المصابة يمكن أن تكون أحد عوامل الخطر الرئيسية لانتقال الطفيلي إلى الإنسان. من الضروري تجنب تناول أنسجة الحيوانات النيئة وغير المطبوخة جيداً.

الكلمات المفتاحية: تفاعل البلمرة التسلسلي ؛ فلسطين ؛ TOX4 / TOX5 ؛ انتشار جيني.