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College of Veterinary Medicine

Parasitic and molecular study of toxoplasmosis in human, camels & goats in Al_Qadisiyah province

A Thesis
Submitted to the Council of the College of Veterinary Medicine at University of AL-Qadisiyah in Partial fulfillment of the Requirements for the Degree of Master Sciences \ Parasitology

By

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بِسمِ اللهِ الرَّحمَنِ الرَحِيمِ

﴿فَتَعَلَى اللهُ المَلِكُ الحَقُ ولا تَعجَل بِالقُرآنِ مِن قَبلِ
أن يُقضى إلَيكَ وَحيُهُ وَقُل رَّبِ زِدني عِلمَاً﴾

صَدَقَ اللهُ العلي العَظِيم

سورة طه الاية (114)

Certification of supervisor

I certify that this thesis (**Parasitic and molecular study of toxoplasmosis in human , camels & goat in AL-Qadisiyah province**) has been prepared under my supervision at the College of Veterinary Medicine – University of AL- Qadisiyah, in partial fulfillment of the requirements for the degree of Master Sciences / Parasitology .

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2018/ /

In view of the available recommendation, I forward this thesis to debate by the examination committee.

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2018/ /

Examination committee

We, the examining committee , after reading this thesis entitled (Parasitic and molecular study of toxoplasmosis in human , camels & goats in Al-Qadisiyah province) and examining the student (RASHA IMAD AYYAL) in its content , and that our opinion it is adequate as a thesis for the Degree of master sciences / Parasitology .

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Dedication

To the memory of my lovely brother…Ja'affer

To my believing father and mother, for their endless

Love and sacrifice

Tomy husband my partner Ali for his efforts in helping and supporting me

To my lovely son…..Abdullah

To my lovely brother and sister

Ramy,Ruqia

I dedicate the fruit of

my effort.

rasha

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Rasha

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List of abbreviations

Abbreviation	Meaning
Abs	antibodies
AF	Amniotic fluid
CHEN	chennei
CMI	Cell mediated immunity
CSF	complement fixation test
DAT	Direct agglutination test
DDIA	Dipstick Dye immunoasssay
DNA	Deoxy nucleic acid
DT	Sabin Feldman Dye test
ELFA	Enzyme linked fluorescent assay
ELISA	Linked immunosorbent assay
HSPGs	Heparin sulfate proteglycans
IFAT	Indirect fluorescent antibodies test
IgA	Immunoglobulin Class A
IgE	Immunoglobulin Class E
IgG	Immunoglobulin Class G
IgM	Immunoglobulin Class M
IgM-ISAGA	IgM-immunesorbent agglutination assay
IHAT	Indirect Hemagglutination test
IZN	IZatnagar
LAT	Latex agglutination test
NPCR	Nested Polymerase chain reaction
ORE	Open reading frame
pAS	Periodic acid shift
PCR	Polymerase chain reaction
TgPLP1	A protein secreted by the parasite

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Summary

The current study included the detection of *Toxoplasma gondii* in camels, goats from slaughter house in AL- Qadisiyah province , and also from aborted women from Maternity and Childhood Teaching Hospital. It pointed out the rate of infection in the humans , and the organs of animals, in order to detect the differentiation between them by phylogenic with SAG3 gene. The samples were collected during the period from November 2017 to February 2018 .

A total of 200 samples were distributed as males and females of camels and goats, and from aborted women . The samples were divided into, (70) sample from camels, (liver, meat and uterus), 70 samples of goats' meat, uterus, placenta and fetus and 60 samples were collected randomly from aborted women who received to the Maternity and Childhood Teaching Hospital.

The first method of diagnosis was an impression method for all samples. The result of camls shows that was 48/ 70 positive (68.57%), while the result of goats were 45/70 (64.28%) and the aborted women show 20/60 positive (33.33%) , there was a statistically significant among the species in $p < 0.05$. to suspected *Toxoplasma gondii* bradyzoite.

The present study included a molecular method to detect small subunit ribosomal RNA (ssRNA) using Nested Polymerase Chain Reaction (nPCR) ,the results of the nPCR for ssRNA gene showed that 18/32 (56.25%) were positive in the camels , while the result of the goats were 17/33 (51.51%) were positive, the rate of toxoplasmosis in the aborted

women was Positive 60%. There is no a statistically significant between species in $p < 0.05$.

The present study also detected for Surface Antigen (SAG3) gene to conform the nPCR result and to send the samples for sequences.

The SAGA gene showed 100% positive results according to the ssRNA gene .

There was a statistically difference $p < 0.05$between the organs in the camels, while in the other species , there were no statistically difference $p \geq 0.05$.

There was Homology sequence identity between local *T.gondii* (Human ,goat and camel) isolates surface Ag SAG3 gene with NCBI BLAST *Toxoplasma gondii* isolates, and the DNA nucleotides sequencing analysis of surface Ag SAG3 complete gene showed a clear genetic variation between isolates from different host.

ChapterOne

Introduction

1. Introduction

Toxoplasmosis is a classic zoonosis, A wide range of vertebrate animals serve as hosts and while human infections are common, serious complications occur primarily in immunocompromised hosts, Human infections are caused by accidental ingestion of oocysts, shed into the environment by cats, or tissue cysts contained in undercooked meat, Infections in healthy adults are generally benign, although toxoplasmic retinitis is frequently a cause of serious eye disease in otherwise healthy adults (Gilbert *et al.*, 1999). More profound disease occurs in immunocompromised hosts or as the result of congenital infections (McLeod *et al.*, 2000)

Toxoplasmosis intracellular parasite has a two stage asexual cycle in warm-blooded animals and a sexual cycle in felidae, The parasite comprises three clonal lineages (I, II and III) in the main, with type II and III being associated with disease in animals while type II is the predominant form identified in human disease (Howe and Sibley, 1995, Khan *et al.*, 2006). In the asexual cycle, the two developmental stages are the rapidly multiplying tachyzoite and the slowly multiplying bradyzoite, in acute infection, tachyzoites actively penetrate host cells where they multiply causing the cell to rupture and release organisms locally and into the blood stream As the host develops immunity, the parasite retains its overall size and shape but transforms it into the bradyzoite stage and multiplies more slowly within tissue-cysts to establish a persistent infection, These microscopic tissue cysts are present most frequently in brain and skeletal muscle and represent the quiescent stage of the parasite within the host, Viable tissue cysts within muscle (meat) are a significant source of human infection, In animals that succumb to acute infection tachyzoites may be demonstrated in ascitic fluid or in lung impression smears as well as in tissue sections of the liver and other affected organs (Luft *et al.*, 1993).

The livestock are susceptible to various parasitic diseases, including toxoplasmosis (Othman and ALzuheir, 2013). Which cause various reproductive problems such as the death of newborn animals or the giving birth of a dead fetus or abortion (Edwards and Dubey, 2013). Serological surveys indicate that the infection of *Toxoplasma gondii* is found largely among animals used to produce meat such as pigs, sheep, goats (Tenter *et al.*, 2000). The nested polymerase chain reaction technique was used for the first time in the diagnosis of DNA *Toxoplasma gondii* by using the B1 gene, This method has proved successful in the diagnosis of clinical Toxoplasmosis (Petersen and Dubey, 2001) .

Aims of the study

1-To detection Toxoplasmosis in human, camels and goat by impression method from the tissue

2-To conduct a Molecular detection to Toxoplasmosis species by PCR (nested-PCR) .

3- To compare the phylogenetic analysis of parasite in different hosts, by using SAG3 gene to find out the differences.

ChapterTwo Literatures Review

2.Literature Review

2.1. History of toxoplasmosis

Charles Nicolle observed the presence of a crescent-shaped organism in the tissues during studied the infectious diseases of the desert rodent (Nicolle, 1907). In 1907, at the Institute Pasteur in Tunis, the initial description of this discovery was published by Nicolle and his collaborator, Louis Manceaux (Nicolle, 1908). In Brazil, identified the same microbe as a parasite of rabbits (Tait and Hunter , 2009)

A year later, they provided a more complete report and named the organism *Toxoplasma gondii*, referencing the parasite's characteristic morphology and the source of the original isolate (Nicole and Manceaux, 1909). At the time, the discovery of *T. gondii* perhaps seemed to be just of academic interest; while *T. gondii* was recognized as a cause of congenital disease discovered the parasite in infants with encephalomyelitis (Wolf *et al.*, 1939).

Since *T. gondii*'s discovery, its clinical importance has influenced the research groups investigating the immune biology of toxoplasmosis, Today, *T. gondii* has been recognized as an important opportunistic parasite of fetuses, newborns and patients with a variety of primary genetic and acquired immune deficiencies (Petersen and Dubey, 2001).The majority of immunocompromised patients that develop clinical disease have defects in T cell function, highlighting the importance of lymphocytes in controlling this persistent infection, Consequently, there has been a focus on understanding how T cells provide protection against disease and how cytokines modulate T cell responses ,This theory was provided an overview of the events, consider how the study of *T. gondii* has had a significant impact on the field of immunology and discuss future studies that may provide new insights into the mechanisms necessary for the control of this pathogen(Lieberman and Hunter, 2002)

2.2.Taxonomy

Toxoplasma gondii, is classified according to (Jomaa, 2017) as follows:

Kingdom	Animalia
Sub Kingdom	Protozoa
Phylum	Apicomplexa
Class	Sporozoea
Subclass	Coccidia
Order	Eucoccidea
Suborder	Eimeriina
Family	Sarcocystidae
Genus	*Toxoplasma*
Species	*gondii*

2.3. Morphology

There are three different morphological stages, they are:

1. Tachyzoites (Rapid multiplication stage)

The term ’tachyzoite‘‘(tachos= speed in Greek) was coined by Frenkel (1973). Also called trophozoites proliferating stage or endozoites, this stage can be observed during acute stage, Measure about (4-8 μm) in length and (2-3 μm) in width and generally crescent shape, Tachyzoite contains a single central nucleus having a central nucleolus with apical complex at the blunt end of the parasite (Dubey, 2004).It rabidly multiplies in stage found in the Macrophages and other host cell of the human and domestic animals as (intermediate host)

and the non intestinal epithelial cells of the definitive host (feline) (Garcia and Bruckner, 2001),

and a rounded posterior end, It requires an intracellular habitat to survive and multiply, despite having its own Golgi apparatus, ribosomes and mitochondria, it multiply asexually within the host cell by repeated binaryfision to produce tachyzoites which can enter almost any type of host cells including muscles, brain, eyes and can be found free in blood in acut stage (Montoya and Rosso, 2005) .

Tachyzoites are obligate intracellular forms, which can invade, replicate and multiply in the host cell of all mammalian cells except erythrocytes (Lopes *et al.*, 2010). They appear rounded enclosed by a host cell membrane called pseudocyst and intracellular multiplication continues until the host cell lyses or a tissue cyst is formed (Kim *et al.*, 2008).

2. Bradyzoites:(slow multiplication stag)

The term "bradyzoite" brady= (slow in Greek) was also coined by Frenkel (1973). It is used to describe the bradyzoites organism multiplying slowly within a tissues cyst , The deactivated bradyzoites or cystizoites that inhabit in infected tissue, Bradyzoite found during chronic stage of the disease(Tobin *et al.*, 2010). The spheroidal cyst, had very resistance membrane contain as few as 50 and up to as several thousand (bradyzoites) which has only come into contact with host cells, if the cyst exposure to the stress will give ruptures(Jomaa, 2017).

Tissue cysts are formed most commonly in the brain, liver and muscles(Hakan, 2010). The parasite remains viable within cysts as long as immune system is intact, but if reduce , the cyst wall will open and active tachyzoites will start to invade other cells and multiply(Gazzinelli *et al.*, 1996)
Its morphology is similar to that of tachyzoite except the former contains higher

amount of polysaccharide and it can give rise positive reaction with periodic acid shift (PAS) (John and Petri, 2013).

They are more slender than tachyzoites and their nucleus located more to the posterior end(Jones and Dubey, 2010) .

The tissue cysts are less resistant to environmental conditions than oocysts ,they are relatively resistant to changes in temperature and remain infectious in refrigerated meat (1- 4 °C) or minced flash for up to 3 weeks, i.e. probably as long as the meat remains suitable for human consumption(Tenter *et al.*, 2000). Although most tissue cysts are killed at temperatures of -12°C or lower, occasionally some tissue cysts may survive in deep freezing ,and it has even been suggested that some strains of *Toxoplasma* may be resistant to freezing , heating to 67°C or higher is considered sufficient to immediately kill tissue cysts , Survival of tissue cysts at lower temperatures depends on the duration of cooking, For example, under laboratory conditions tissue cysts remained viable at 60°C for about 4 min. and at 50°C for about 10 min, It is important to note that cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *Toxoplasma* in all parts of the meat (Hill and Dubey, 2018).

3- Oocysts :

It is ovoid in shape and measures approximately 10 – 20 μm , This stage is found in intestine of feline, the definitive host when *Toxoplasma* to produce the infection which pass in the feces of the infected cat, sporulation occurs outside the body in environment, especially on soil, vegetables, and house thresholds (Frenkel, 1991).

Oocyst develop sexual reproduction (gametogony) in the epithelial cell of the cat intestine ,The mature one contains two sporocysts, each of them contain four sporozoites it(Jones and Dubey, 2010).

The cat are shed of oocyst in feces in a single day after (3-24) days of infection(Pavesio and Lightman, 1996). Sporulated oocysts are resistant,

survive in soil for several months resist the stomach juices(Brooks *et al.*, 2006). Can remain infectious for as long 18 months in water or warm moist soils(Johnsen, 2009, Jones and Dubey, 2010).

The sporozoite is similar to the tachyzoite, except that there is an abundance of micronemes, rhoptries, and amylopectin granules in the former, Sporozoites are (2 by 6-8 μm) in size with a sub terminal nucleus(Tenter, 2009).

2.4. Life-cycle of the *toxoplasma* gondii

T.gondii is a single-celled protozoan parasite of which felids are the definitive host (Dubey *et al.*, 1970).

Dubey and Beattie (1988) explained that the *T.gondii* has three infectious stages, including sexually produced oocyst, rapidly dividing tachyzoites and slow multiplying bradyzoites in tissue cysts.

Cats shed oocysts after ingesting any of the three infectious stages of *T.gondii*, i.e. tachyzoites, bradyzoites and sporulated oocyst(Frenkel *et al.*, 1970). Pre-patent periods (time to the shedding of oocysts after initial infection), frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Pre-patent periods are 3–10 days after ingesting tissue cysts, 18 days or longer after ingesting oocysts, irrespective of the dose(Dubey and Frenkel, 1972, Petersen and Dubey, 2001). fewer than 50% of cats shed oocysts after ingesting tachyzoites or oocysts,where as nearly all cats shed oocysts after ingesting tissue cysts(Petersen and Dubey, 2001).

After the ingestion of tissue cysts by cats, the tissue cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine, The released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *T. gondii*. Five morphologically distinct types of *T. gondii* develop in intestinal epithelial cells before gametogony begins. These are asexual stages in the feline intestine are structurally distinct from tachyzoites that also develop in the lamina propria , They replicate inside a cell with a generation time of 6 to 8 h (in vitro) until they exit the cell to infect neighboring cells, usually after 64 to 128

parasites have accumulated per cell(Radke and White, 1998). When the host cell can no longer support growth of the tachyzoite it ruptures. The release of the parasite is mediated by TgPLP1, a protein secreted by the parasite(Kafsack *et al.*, 2009).

Gamonts are found throughout the small intestine but most commonly in the ileum, 3–15 days after inoculation. After fertilization, an oocyst wall is formed around the parasite. Infected epithelial cells rupture and discharge oocysts into the intestinal lumen, Intermediate hosts are infected by sporulated oocyst, which is an oocyst excreted by a cat that has sporulated to contain sporozoites, Unsporulated oocysts require only 24 hours outside the host to sporulate and become infectious, and the sporozoite is extremely resilient(Ortega *et al.*, 2007).

T.gondii tachyzoites are disseminated throughout the body of the intermediate host in macrophages and lymphocytes as well as free in the plasma, Tachyzoites continue to divide within the host cell by endodyogeny (internal division into two) until the host cell is filled with parasites, At a given time the dividing tachyzoites cannot be contained within the host cell, which bursts, The tachyzoites are released and seek new host cells to repeat the process, Depending on the strain of *T. gondii* and the host resistance, tachyzoites may be found for days or even months after acute infection, For example, tachyzoites persist in fetal membranes for weeks after infection of the mother or the dam, and are nearly always present in placenta of mother at the time of parturition, if the fetus was infected in utero, At some time after infection the tachyzoites transform to bradyzoites in tissue cysts, The signals responsible for the transformation are not known, and the debate continues as to whether signals from the host immune system are needed. Bradyzoites also divide by endodyogey. Bradyzoites are enclosed in a thin cyst wall, Tissue cysts may be found as early as 3 days after infection but are usually not numerous until 7 weeks after infection(Dubey and Frenkel, 1976, Derouin and Garin, 1991). Figure (1-2).

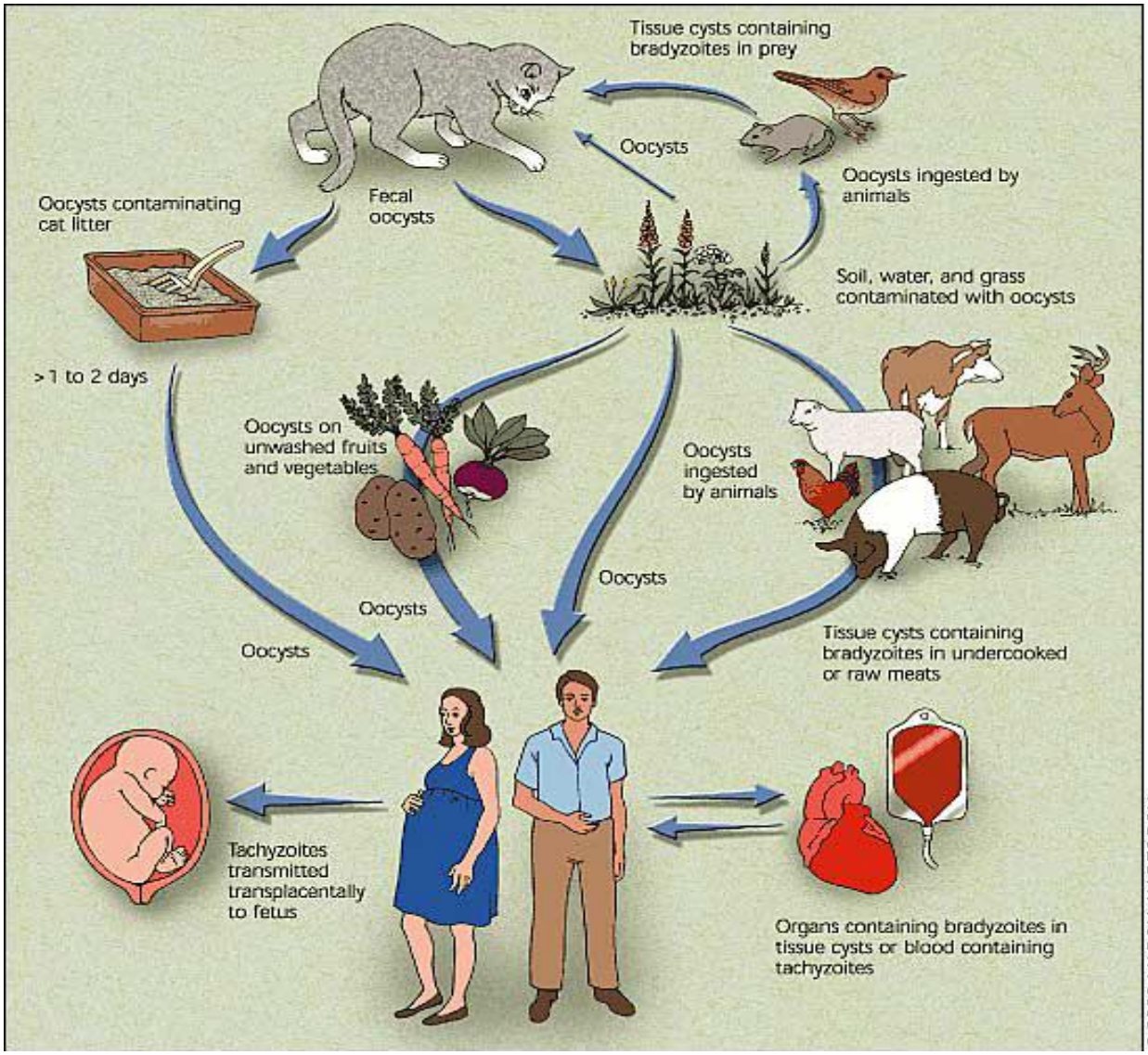


Figure (1-2): Life Cycle of *T. gondii* (Alcamo, 1997)

2.5.The clinical presentation of toxoplasmosis

The clinical signs of toxoplasmosis is asymptomatic in both final and intermediate host, Most human infections with *T. gondii* are asymptomatic, but infection may result in severe clinical diseases and on occasion be fatal, Infection in humans may be acquired postnatally or *in uterus* and may result in fetal death, congenital toxoplasmosis, toxoplasmic encephalitis, ocular toxoplasmosis or less severe acute self-limiting disease(Montoya and Liesenfeld, 2004)

In healthy adults and children the majority of postnatally acquired infections are asymptomatic with only 10–20% of individuals developing a self-limiting

and non-specific illness(Montoya and Liesenfeld, 2004, Pereira *et al.*, 2010). Symptoms of disease may include mild, flu-like illness with low grade fever, muscular pain, swollen lymph nodes, lethargy and headache(Abu-Madi *et al.*, 2010). Enlarged lymph nodes are the most commonly observed clinical manifestation of human toxoplasmosis , the onset of illness is 3–25 days (mean of 11 days)(Hill and Dubey, 2018).

Toxoplasmic retinochoroiditis (inflammation of the retina and choroid) can be associated with congenital or postnatally acquired disease as a result of acute infection or reactivation of a latent infection(Montoya and Liesenfeld, 2004). In humans, the parasite multiplies in the retina causing inflammation in the choroid; the parasite does not multiply in the choroid(Dubey *et al.*, 2012). Typical findings of both postnatally acquired and congenital retinochoroiditis include white-appearing lesions with overlying severe inflammation of the viscous fluid at the back of the eye(Montoya and Liesenfeld, 2004, Delair *et al.*, 2011).

These symptoms occur as a consequence of active retinal lesions, leading to retinal scarring, Toxoplasmic retinochoroiditis is a significant cause of vision loss, The natural course of ocular toxoplasmosis and the long term impact on vision depends on the frequency of recurrences, with retina destruction minimized if active disease is treated early, Recurrence of retinochoroiditis can occur for both postnatally acquired and congenital toxoplasmosis. Severe complications associated with ocular toxoplasmosis may include fibrous bands, retinal detachment, cataracts and inflammation and damage to the optic nerve(Delair *et al.*, 2011). Ocular disease is one of the most important clinical manifestations of acute, postnatally acquired toxoplasmosis, particularly in countries such as Brazil. The majority of cases of ocular toxoplasmosis are postnatally acquired (Dubey *et al.*, 2012).

Congenital toxoplasmosis occurs when a human and animales becomes infected with *T. gondii* during pregnancy, Tachyzoites circulating in the

mother's bloodstream can invade and multiply in the placenta and subsequently infect the fetus, Transmission of the parasite *in utero* can cause congenital defects or spontaneous abortion, These congenital defects can include ocular toxoplasmosis, hydrocephalus (big head), mental retardation and intracranial calcifications(Zhou *et al.*, 2011, Hill and Dubey, 2018). Although the risk of transmission is less common in the first trimester, congenital infections acquired during the first trimester are more severe than those acquired in the second or third trimester of pregnancy(Montoya and Liesenfeld, 2004, Ayi *et al.*, 2009, Hill and Dubey, 2018).

In infected immune compromised individuals, the parasite may be uncontrollably released due to the rupture of tissue cysts in the brain(Feustel *et al.*, 2012). This leads to symptoms that affect the central nervous system, including headache, altered mental status, seizures, hemiparesis muscle weakness on one side of the body, ataxia and/or facial weakness. If left untreated; the infection may progress to fatal toxoplasmic encephalitis (Snydman *et al.*, 2005, Feustel *et al.*, 2012). Immunocompromised individuals are susceptible to toxoplasmic encephalitis from either acquired infection or reactivation of a latent infection, however it is believed the majority of toxoplasmic encephalitis cases are due to the latter (Montoya and Liesenfeld 2004). Reactivation occurs if the bradyzoites are released from the cysts and convert into tachyzoites due to the suppression of the host immune response that previously inhibited parasite activity, This rupture of the cysts in immunocompromised individuals generally occurs in the brain(Feustel *et al.*, 2012).

2.6.Transmission

The principal modes of *T. gondii* transmission are ingestion of faecal oocysts or tissue cysts, and the transplacental transmission of tachyzoites from mother to unborn child, Infection with fecal oocysts may occur by accidentally

ingesting contaminated soil (e.g. not washing hands after gardening or eating unwashed fresh produce), drinking untreated contaminated water, eating shellfish grown in contaminated water, or contact with cat faeces (e.g. a cat litter box). Infection from tissue cysts may occur by consuming raw or undercooked meat, by accidentally consuming tissue cysts after handling raw meat and not washing hands thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat (Abu-Madi *et al.*, 2010, Pereira *et al.*, 2010). Oocyst-acquired infections in humans are clinically more severe than tissue cyst-acquired infections(Dubey, 2004). It is also possible that parasite transmission could occur as the result of blood transfusion or haematopoietic stem cell transplantation, The chance of either of these occurring is very low and could only occur if the donor was recently infected with *T. gondii* and so had tachyzoites present in their blood and bone marrow (Derouin *et al.*, 2008).

Infection of the feline definitive host occurs when a cat consumes an intermediate host (such as a mouse or bird) infected with tissue cysts, Upon ingestion of a tissue cyst by a susceptible cat, the walls of the cyst are digested by proteolytic enzymes and bradyzoites are released, The bradyzoites undergo asexual reproduction followed by sexual reproduction in intestinal epithelial cells to produce microgametocytes and macrogametocytes, The microgametocytes fertilize the macrogametocytes, leading to the production of zygotes. The zygotes differentiate into unsporulated oocysts and are shed in the faces of the definitive host (Lorenzo *et al.*, 2018, Jones and Dubey, 2010). After a prep tent period of up to 10 days following primary infection with tissue cysts, a cat may shed more than 100 million oocysts into the environment over a 2-3 week period(Tenter *et al.*, 2000).

2.7.Epidemiology.

2.7.1. Epidemiology in Human

Toxoplasmosis has a worldwide distribution, but its prevalence greatly, and also differs within the same countries .*T.gondia* is zoonotic parasite affecting large number of population around the world, with infection rate 12% - 90%. This percentage may increase with age, sanitary habits, education, crowding, consumption of inadequately cooked meat and contact with animals(Esch and Petersen, 2013).

The weather conditions has an important role in spread of infection, and surviving of oocyst occurred in warm and moist soil rather than hot and dry soil(Pergola *et al.*, 2010). Higher prevalence is found in areas with hot and humidity climate, i.e. tropical countries, while those with lower prevalence occurred mostly in country with colder climate, However there is risk factors effects on sero prevalence variations in world population, such as, dietary habits, social factors, sanitation and health education(Petersen and Schmidt, 2003).. Also higher sero prevalence was found more in older age group, but this varies in different country and in different social level. In children whom lived in bad sanitary conditions like water contamination; the sero prevalence may reach maximum levels (Robert-Gangneux and Dardé, 2012).

Many countries have high sero prevalence rate approximately reach 75% such as France and Germany,while the rate of toxoplasmosis in women of childbearing age may reach greater than 50% in Africa, America and western Europe (Saeij *et al.*, 2005).

During pregnancy the rate of *T. gondii* infection ranges from about 1-310 cases per 10,000 pregnancies in Europe, Australia, Asia and Americas. While prenatal *T.gondii* infection in these countries 1-120 cases per 10,000 births (Gómez-Marín *et al.*, 2012).

Recent sero prevalence of *T.gondii* is 0.79-12.9% in Korea recorded by (Shin *et al.*, 2009), and 36% In Turkey (Ali *et al.*, 2007). While the sero prevalence

rate of toxoplasmosis among the general population in Iran was 39.3% (Daryani *et al.*, 2014). Another study in India suggests a prevalence rate 20–40% in India(Sreekumar *et al.*, 2003). And around 40 % in Egypt (El-Massry *et al.*, 2000). While in Jordan, the rate of toxoplasmosis was 31, 6% among pregnant women reported by (Jumaian, 2005).

2.7.2. Epidemiology in animals

Disease does not exist in the areas that leave them(Takeet *et al.*, 2009). Toxoplasmosis is zoonosis occurs naturally in human , domestic and wild animals and birds(Radostits *et al.*, 2007). Among the farm animals, pigs, sheep and goats are more likely to be affected by *Toxoplasma gondii* parasites compared to other animals , Cats have a major role in the epidemiology of toxoplasmosis and the rate of infection in humans and animals is different in different regions depending on the weather conditions such as temperature, humidity and ventilation play an important role and animal species are among the factors that determine the normal degree of prevalence of *Toxoplasma gondii*(Radostits *et al.*, 2007). The rate of infection in domestic birds and rodents which are considered the food source for cats, significantly determines the rate of infection, for example, the Oocyst of the toxoplasma paracite are 23.2% of the 237 cats in Costa Rica, where infection in local rodents and birds is high, The incidence of toxoplasma among wild cats is very high compared to domestic cats (Dubey *et al.*, 2002). Moreover in Saudi Arabia rate of 13.6% have been detected in 2012(Al-Anazi, 2012). Furthermore 4% infection rate with Toxoplasmosis were registered in Iran in 2006(Sadrebazzaz *et al.*, 2006).

2.7.3.Toxoplasmosis in Iraq:

the prevalence in aborted women was 15.62 % in Kurdistan area in 1992(Niazi *et al.*, 1992) .while the prevalence of infection was 9.4% in age 16-20 years (Al- Dageli, 1998). In Basrah showed that the prevalence of

toxoplasmosis was 41.1-52.1%(Yacoub *et al.*, 2006). In Baghdad province revealed the infectious rate was 32.1% (in aborted women) using ELISA (Karem, 2007). While rate of toxoplasma infection was 4.16% and 15.83% in aborted women in Baghdad by using both of ELISA and PCR(Al-Rawi, 2009). in Baghdad found the females with age ranging from (15-19) and (20-24) years showed 30.63% prevalence rate of infection with *Toxoplasma* by ELISA IgG (AL-Shikhly, 2010). In Kut found that the rate of Toxoplasma infection province was 66.35 % IgM , 44.71% IgG in women using ELISA technique(AL-Mayahi, 2011). in males ageing between (18-57) years found that the ratio of infection was 30.25% IgG by using ELISA technique (Al-Saadii, 2013).

prevalence of Toxoplasmosis among camels, in Iraq(Latif, 1988, Al-Mudhfer, 2012). with different infection rate 6.04%,16.35% and 20.34%. among different years 1998,2006 and 2012 respectively.

2.8.The pathogenesis of toxoplasmosis

The pathogenicity are major factors; the strains virulence and dose of the infection, the immunological status and heredity composition of the animals ,Toxoplasmosis is one of the most common parasitic disease of mammalian host including man , acute or chronic inflammatory reactions may have occurred by actively replicating asexual stages(Frenkel, 1973).

the most serious manifestation of the disease found in pregnant females. Abortion is a common sequel in severe infections acquired early in pregnancy and if a child is born alive he may suffer from serious mental retardation within a few weeks after birth, Necrotizing retinochoroiditis is reported as the commonest clinical manifestation of congenital toxoplasmosis in human,

Toxoplasmosis is a major cause of abortion in sheep in Newzealand and England(Hartley and Marshall, 1957). *Toxoplasma* infection is an important contributor to ovine infertility and perinatal mortality(Hartley and Boyes, 1964).

It causes abortion in goats(Dubey, 1981) recorded an outbreak of toxoplasmosis in a herd of Brown Swiss cattle. The herd showed dyspnoea, coughing, sneezing, nasal discharges, trembling and shaking of head, In dromedary, a case report of natural infection of toxoplasmosis was first reported by (Hagemoser *et al.*, 1990).

2.9.Diagnosis

symptoms of toxoplasmosis are non specific and cannot be depended on for a definite diagnosis; Toxoplasmosis clinically mimics several other infectious diseases (Gutierrez *et al.*, 1991).

Many different methods are known, these methods can be classified as follows:-

2.9.1.Direct Methods

- impression methods

Diagnosis can be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy, This procedure is particularly useful in immuno- suppressed patients with AIDS, in whom antibodies (Abs) synthesis may be delayed and low. *T. gondii* infection can be rapidly diagnosed by making impression smears of lesions on glass slides, After drying they fixed in methyl alcohol and stained with Giemsa stain(Prince *et al.*, 1990). Tachyzoites are not usually detectable in biopsy specimens from lymph nodes, although characteristic histopathologic features can support the diagnosis(Frenkel, 1991). Also *T. gondii* can be detected in sediment of cerebrospinal fluid (CSF), brain aspirate or impression smears of biopsy tissue(Montoya and Rosso, 2005).

-.Isolation of *T.gondii*

Inoculation of blood or body fluids such as CSF, amniotic fluid (AF), brain aspirate and retina into tissue culture or intra peritoneally or intra cerebrally, inoculation into mice, then after (8-10) days, the exudates examined for tachyzoites (Boyer *et al.*, 2005).

In neonate isolation of the organism from the placenta is a usually diagnostic tool for congenital toxoplasmosis(Steven *et al.*, 2008).

2.9.2 .Indirect Methods

- Skin test (delayed hypersensitivity test)

This test is used to measure the cell mediated immunity (CMI). It is one of the useful population surveys in epidemiological studies as a screening test(Frenkel, 1991). Frenkel employed it for the first time in 1948, which includes the intradermal injection of soluble antigen (toxoplasmin) from a pathogen. The positive test demonstrate past infection with that pathogen, but the negative result does not always indicate the absence of antibodies or infection (Roitt *et al.*, 2001).

- Serological tests

The use of serological tests to show specific antibodies (Abs) to *T. gondii* is the primary method of diagnosis, The problem with serologic diagnosis is that Abs to *T. gondii* is present in relatively high numbers of individuals in most human populations. These Abs titers may persist at high levels for years in healthy people(Wilson *et al.*, 1997). The clinical diagnosis of toxoplasmosis is difficult, because it can remain latent asymptomatic for unpredictable length of time, therefore, laboratory tests including serolo- gical tests are essential to detect Abs to *T. gondii* (Liesenfeld *et al.*, 2001).

-Sabin Feldman Dye test (DT):

It was the first test system able to detect specific Abs to *T.gondii* at low levels and to differentiate acute and latent infection. Sabin and Feldman (1948) described the test, The test is based on complement mediated cytolysis of Ab-coated live *T. gondii* tachyzoites, which is indicated by their inability to take up methylene blue. It is still the reference method for the serodiagnosis of

toxoplasmosis (Reiter-Owona *et al.*, 1999). It is the "gold standard" test, which is a highly specific and sensitive test, but its main disadvantages are its high cost and the human hazard of using live organisms(Dubey, 2002).

-Indirect Fluorescent antibodies test (IFAT):

Goldman first described this test in 1957 for detection of *T. gondii.* The test is based on the use of antiglobulins labeled with fluorescent dyes, the fluorochrome emit visible light after excitation by ultra violate light (Gutierrez *et al.*, 1991).The Abs that are detected by IFAT act mainly against the cell wall of the parasite similar to the Dye test Abs, but do not need live parasites (Garcia, 2009).The test can detect both IgM and IgG in serum .When monoclonal Abs are used, this test is simple and easy to apply in comparison with the Dye test and the results are parallel to those of the Dye test(Willis *et al.*, 2002).

- Direct agglutination test (DAT):

Formalin-preserved whole parasite which is used to detect Abs, and act against the cell wall of the parasite, This test is rapid, simple, accurate, inexpensive, excellent for screening sera of pregnant women and safe because no living organisms are handled after the stock antigen is prepared (Suzuki *et al.*, 1988)

- Indirect Hemagglutination test (IHAT):

Jacolos and Lunds (1957) first used it for *Toxoplasma*. This test is based on the capacity of Abs to agglutinate different kind of red blood cells sensitized with the homologous antigen and it may develop at a later stage of infection(Elliot *et al.*, 1985). Thus, the test should not be used in infants with suspected congenital infection or in screening to determine if the infection has been acquired during pregnancy, since the test may be negative for too long in early infection(Prince *et al.*, 1990).

- Latex agglutination test (LAT):

It is a modified agglutination test in which antigen coated latex particles have been used as a carrier of the antigen. This test is used normally for the diagnosis of *Toxoplasma* IgM and IgG Abs, Titers rise rapidly during the acute phase. The reaction allows visual observation of the antigen-antibody complex (El-Gamal *et al.*, 1989). It is easy to perform, inexpensive and sensitive to be used as a screening test (Holliman, 2003).

- Dipstick Dye immunoassay (DDIA):

This test was developed to detect IgM and IgG Abs against toxoplas- mosis in human sera, The assay employs blue dye particles conjugated to sheep antihuman IgG and rabbit antihuman IgM and soluble antigen of tachyzoites (TSA) of *T. gondii* RH strain as the detective antigens, The TSA in mixture can capture human specific anti toxoplasmosis Abs onto nitro cellulose membrane dipstick by means of immunochromatography, The test required only 15minutes, and it agrees with ELISA test(Jin *et al.*, 2005).

- Enzyme Linked immunosorbent assay (ELISA):

The test is highly sensitive and based on the use of enzymes conjugated with the antibody or with antigen to measures the antigens and antibodies, respectively. It is a simple, rapid and accurate method for the detection of IgM antibodies in individuals with acute acquired toxoplasmosis (Camargo *et al.*, 1978), and to determine whether pregnant woman has been infected during gestation or before conception, IgM typically appear within the first week of infection, rapidly rise, and thereafter decrease at variable rates, to disappear after a few months, The IgM antibodies may persist for years after the acute infection and that the reliability of commercially available assay varies considerably(Wilson *et al.*, 1997). The most widely used method for the

demonstration of IgG Abs is the IgG ELISA. IgG antibodies usually appear within 1 to 2 weeks of acquisition of infection, peak within 1 to 2 months, then fall at variable rates, and likely persist for demonstration of IgG antibodies. One cannot use a single IgG titer, to predict whether the infection was recently acquired or acquired in the distant past (Montoya and Rosso, 2005).

- Enzyme linked fluorescent assay (ELFA):

It is a simple, rapid and accurate technique for measurement of anti-*Toxoplasma* IgM or IgG in patients with acute or chronic toxoplasmosis, The VIDAS machine performs all of the steps automatically(Thulliez *et al.*, 1992). The assay principle combines a two-step,enzyme immunoassay sandwich method with a final fluorescent detection, This method has a high specificity for detection of IgM and IgG (Berrebi *et al.*, 1994).

- IgM-Immunesorbent agglutination assay (IgM-ISAGA):

This assay, which binds the patient's IgM to a solid surface and uses intact, killed tachyzoites to detect IgM antibodies, is sensitive and specific. The test is simple to perform, does not require the use of enzyme conjugate, and is read in the same manner as the agglutination test. It avoids false-positive results related to the presence of rheumatoid factor and or antinuclear antibodies. Due to its sensitivity, is frequently used for the diagnosis of congenital infection. The ISAGA method also has been used to detect IgA and IgE antibodies((Remington *et al.*, 1983).).

- IgG Avidity test:

Many tests for avidity of *Toxoplasma* IgG Abs has been introduced to help differentiate between recently acquired and distant infection, this method is based on the observation that during acute *T. gondii* infection, IgG Abs bind Ag weakly (i.e. have low avidity), whereas chronically infected patients have more

strongly binding (i.e. high avidity). Low or equivocal avidity test results can persist for months to years after the primary infection, thus a low or equivocal avidity test result must not be used to determine whether the infection was acquired recently (Hedman *et al.*, 1989).

2.9.3.Moecular Diagnosis:-

Polymerase chain reaction(PCR) method was developed by using as a target a (35) fold repetitive-sequence the *B1*, gene (Burg *et al.*, 1989), or *p30* gene (Savva *et al.*, 1990).

This method is capable of detecting *T. gondii* DNA in either an aqueous samples including urine, CSF, amniotic fluid (AF), blood, vitreous sample (ocular fluid) ,tissue samples including brain tissues and bronchiolar lavage fluid, It has been successfully used to diagnosis congenital, ocular, cerebral and disseminated toxoplasmosis (Burg *et al.*, 1989, Filisetti and Candolfi, 2004).
PCR methods are highly sensitive for detecting the organism in AF in congenital infection(Hohlfeld *et al.*, 1994). The test could be performed after (18) weeks of gestation, It has specificity of (100%)(Pinard *et al.*, 2003).

Invasive techniques are usually reserved for difficult cases such as patients who are immune compromise, A single organism can be detected directly from a crude cell lysate or as few as to parasites in the presence of (100,000) leucocytes (Savva *et al.*, 1990)

2.10.Molecular and sequence phylogenetic analysis of (SAG3) Gene of *Toxoplasma gondii*

The surface antigens of *T. gondii* are the major targets as key molecules for immune diagnosis as well as immune prophylaxis because of their initial presentation to the host immune system. Surface antigen (SAG3), an under-reported 43kDa glycoaminoglycan-binding protein associated with binding of

host heparin sulfate proteoglycans (HSPGs)(Jacquet *et al.*, 2001), involved primary structure similarity with another proven Surface antigen 1 (SAG1)

(Cesbron- Delauw *etal* .,1994) protein. It was considered interesting to carry out the primer-directed amplification of the open reading frame (ORF) of surface antigen 3 (SAG3) gene of Indian isolates of *T. gondii viz.* Chennei (CHEN) and Izatnagar (IZN) isolates, maintaining them at the IVRI and cloning them in a heterologous prokaryotic system. Moreover, the two Indian isolates used in the study are known to vary between themselves as far as homologies related to other gene loci like GRA 5(Singh *et al.,* 2011). MIC 3 (Praveen *et al.,* 2012). and SAG 2are concerned, but there is no literature available as far as SAG3 homologies are concerned. In the study of (Singh *et al.,* 2011) the cloned genes were custom sequenced and the information was compared with the available sequences of the same gene in the GenBank in order to establish the phylogenetic identity of the SAG3 gene among the various isolates.

Chapter Three

Materials and Methods

3- Materials and Methods

3-1- Materials

3-1-1- Equipments and Instruments

No.	Equipment & instrument	Company
2	Containers	AFCO(Jordan)
3	Digital camera	Samsung/ china
5	Disposable syringe 10 ml, 5ml and 3ml	Sterile EO. / China
6	Eppendorf tubes	Bioneer/ Korea
7	Exispin centrifuge	Bioneer/ Korea
8	Gel electrophoresis	Shandod Scientific/ UK
9	High Speed Cold centrifuge	Eppendorf /Germany
10	Incubator	Mammert/Germany
11	Microscope	Olympus(Japan)
12	Micropipettes 5-50, 0.5-10, 100-1000µl	CYAN/ Belgium
13	Refrigerator	Concord /Lebanon
14	scalpel	John Bolton(England)
15	Sensitive Balance	Sartorius/Germany
16	slides	Superstar(India)
17	Sterile test tube	Superestar/ India
18	Thermocycler PCR	BioRad/ USA
19	UV Transilluminator	ATTA/ Korea
20	Vortex	CYAN/ Belgium
21	Water Bath	Mammert/Germany

3-1-2- Chemicals

No.	Chemical	Company and Origin
	Absolute Ethanol	BDH (England)
	Agarose	BioBasic (Canada)
	DN A marker ladder (100bp)	Promgam / USA
	Ehidium Bromide	BioBasic (Canada)
	Free nuclease water	Biolab/ USA
	Giemsa stain	CDH/ India
	methanol ALCOHOL	Panreac/EU
	normil salin	BDH\Uk
	TBE buffer	BioBasic (Canada)

3-1-3- kits

Table (3-2): The kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	**gSYAN DNA Extraction Kit**	**Geneaid**	**USA**
	GST buffer		
	GSB buffer		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	**Maxime PCR PreMix**	**iNtRON**	**Korea**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris-HCl pH 9.0		
	KCl		
	$MgCl_2$		
	Stabilizer and Tracking dye		

3-1-4- Primers

The Nested PCR primers for detection small subunit ribosomal RNA gene in *Toxoplasma gondii* were design by pervious study (Vitale et al., 2008). These primers was provided from Macrogen company, Korea as following table:

Primer	Sequence		Amplicon
18SrRNA gene First round PCR Primers	F	TGCGGAAGGATCATTCACACG	530bp
	R	CCGTTACTAAGGGAATCATAGTT	
18SrRNA gene Second round Nested PCR Primers	F	GATTTGCATTCAAGAAGCTGATAGTAT	313bp
	R	AGTTAGGAAGCAATCTGAAAGCACATC	
DNA sequence Toxo-SAG3primers	F	ATGCAGCTGTGGCGGCGCAG	1158bp
	R	TTAGGCAGCCACATGCACAAG	

3-2- Methods

3-2-1- Samples collection

The study was carried out during the beginning of November 2017 until the end of febrewary2018 in AL-Qadisya province, total 200 samples were examined in the current study divided as 70 samples of different camel organs (liver,meat,utrues) in the slaughter house , 70 samples of goat also taken from slaughter house (uterus,meat, Placenta and fetus).

Also the present study include 60 human samples from aborted women (placenta and fetus) who attended to the Maternity and Childhood Teaching Hospital .The samples were collected randomly and using two clean

Container containing 1st one containing normal saline and kept cool and transferred to the laboratory to make the imppsresion method and the 2nd one then put in deep freezeunder for DNA extraction as a target for PCR amplification.

3-2-2-Microscopic Examination (by stamp method or immersion method)

Stamp the tissue on the glass slid and left in air to dray and methanol alcohol was added for (3-5)minute then was stained with Giemsa stain for 30 mints and was washed by tab water then left to dry, after drying they were examined under the oil immersion lens of light microscope (Zafar *etal*., 2006).

3-2-3- Tissue Genomic DNA Extraction

Tissue Genomic DNA from tissue samples were extracted by using DNA extraction kit (Tissue DNA protocol) Geneaid. USA and the extraction was done according to company instructions as following steps:

1. Weight of 100mg Human tissue samples was transferred to sterile 1.5ml microcentrifuge tube.
2. Volume of 200µl GST 20µl and proteinase K were added and mixed by vortex. And incubated at 60℃ for 1 hour.
3. Volume of 200µl of GSB was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 70℃ for 15 minutes, and inverted every 3 minutes through incubation periods.
4. Volume of 200µl absolute ethanol were added to lysate and immediately mixed by shaking vigorously.
5. DNA filter column was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to column. Then centrifuged at 10000rpm for 5 minutes. And the 2 ml collection tubes containing the

flow-through were discarded and placed the column in a new 2 ml collection tube.

6. Volume of 400µl W1 buffer were added to the DNA filter column, then centrifuge at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.
7. 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flow-through was discarded and placed the column back in the 2 ml collection tube.
8. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.
9. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of pre-heated elution buffer were added to the center of the column matrix.
10. The tubes were let stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA

3-2-4- Nested Polymerase chain reaction (nPCR)

The nested PCR technique was performed for detection *Toxoplasma gondii* based small subunit ribosomal RNA gene from all tissue samples of all specise. This method was carried out according to method described by (Vitale *et al*., 2008) as following steps:

3-2-5- Genomic DNA estimation

The extracted genomic DNA was by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, DNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1µl of DNA was added to measurement.

3-2-6- Primary PCR master mix preparation

Primary PCR master mix was prepared by using (**Maxime PCR PreMix Kit**) and this master mix done according to company instructions as following table:

PCR Master mix	Volume
DNA template	5µL
18SrRNA primary Forward primer (10pmol)	1µL
18SrRNA primary Reverse primer (10pmol)	1µL
PCR water	13µL
Total volume	20µL

After that, these PCR master mix component that mentioned in table above placed in standard **PCR PreMix Kit** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (T100 Thermal cycler. BioRad USA).

3-2-7- PCR Thermocycler Conditions

PCR thermocycler conditions by using convential PCR thermocycler system as following table:

PCR step	Temp.	Time	repeat
Initial Denaturation	95C	5min	1
Denaturation	95C	30sec.	40 cycle
Annealing	58C	30sec	
Extension	72C	30sec	
Final extension	72C	5min	1
Hold	4C	Forever	-

3-2-8- Secondary PCR master mix preparation

Secondary PCR master mix was prepared by using (**Maxime PCR PreMix Kit**) and this master mix done according to company instructions as following table:

Nested PCR Master mix	Volume
PCR product	2µL
18SrRNA secondary Forward primer (10pmol)	1µL
18SrRNA secondary Reverse primer (10pmol)	1µL
PCR water	16 µL
Total volume	20µL

After that, these PCR master mix component that is mentioned in table above placed in standard **AccuPower® PCR PreMix Kit** that containing all other components which needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye).

Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (T100 Thermal cycler. BioRad USA).

3-2-9- PCR Thermocycler Conditions

PCR thermocycler conditions by using convential PCR thermocycler system as following table:

PCR step	Temp.	Time	repeat
Initial Denaturation	95C	5min	1
Denaturation	95C	30sec.	40 cycle
Annealing	58C	30sec	
Extension	72C	30sec	
Final extension	72C	5min	1
Hold	4C	Forever	-

3-2-10- PCR product analysis

The Nested PCR products was analyzed by agarose gel electrophoresis following steps:

1-A percent of 1% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Volume of 3µL of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 5ul of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt and 80 AM for 1hour.

5- Nested PCR products were visualized by using UV Transilluminator.

3.3.DNA sequence method

DNA sequencing method was performed for study of genetic relationship between *Toxoplasma gondii* isolates from different hosts The genetic analysis done by phylogenetic tree analysis between local *Toxoplasma gondii* isolates and NCBI-Blast submission *Toxoplasma gondii*. Then the identification *Toxoplasma gondii* isolates were submitted into of NCBI-GenBank. The PCR SAG3 genes positive products were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

The DNA sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis of the partial SAG3 gene based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

3.4. Statistical Analysis:

The Statistical Analysis System- SAS (2012) program has used to effect of difference factors in study parameters . Chi-square test has used to significant compare between percentage and Least significant difference –LSD test is used to significant compare between means. P value 0.05or 0.01 consider as significant value of variation. Estimate of correlation coefficient between variables. Estimate of Sensitivity and Specificity in this study.

Chapter Four

Results And Discussion

4.1 Detection of *Toxoplasma gondii* bradyzoite using impression method .

A. Detection of Toxoplasmosis in camel

1- Incidence of suspected bradyzoites Toxoplasmosis according to organ

The present study was used impression method for detect suspected toxoplasma bradyzoites in different organs of camel, 70 samples were examined (9 liver, 25 meat and 36 uterus).

The results shows the percent of the infection in general 48/70 (68.57%), while the rate of the infection according to the organs were 3/9 (33.33%), 12/25 (48%) and 33/36 (91.66%) in the liver, meat and uterus respectively table (1-4), figure (1-4).

Table (1-4) the percentage according to organs suspected of bradyzoites of toxoplasmosis in camel

Organ	No.	(+ve)	%
Liver	9	3	33.33^{B}
Meat	25	12	48^{B}
Uterus	36	33	91.66^{A}
Total	70	48	68.57

Similar litter represent no statistically significant at p > 0.05

Different letter represent statistically significant at p < 0.05

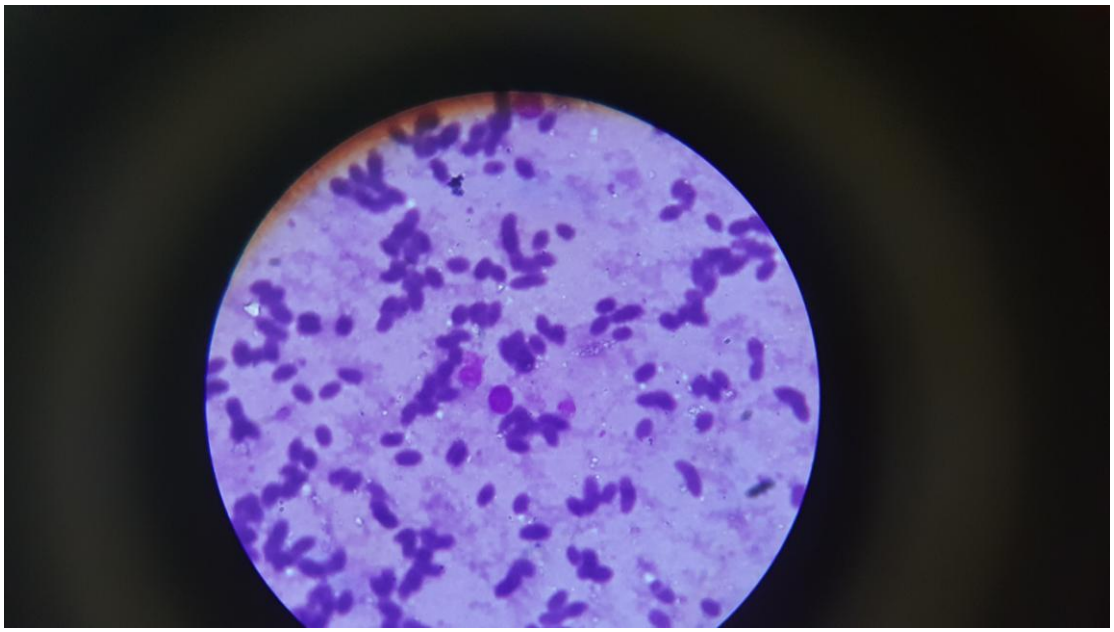


Figure (1-4) the bradyzoite in camel meat using impression method , staining with Gemisa (X 100).

2- Incidence of suspected bradyzoites according to gender

The study divided the camel samples based on gender into 28 male and 42 female, the infectious rate of toxoplasmosis according to the gender based on impression method were 9/28 (68.57%) and 39/42(92.85%) in male and female respectively table (2-4).

Table (2-4) the percentage according to gender suspected to bradyzoites of toxoplasmosis in camel

Sex	N0.	Number (+ve)	%
Male	28	9	32.14^{B}
Female	42	39	92.85^{A}
Total	70	48	68.57

Different letter represent statistically significant at $p < 0.05$

In this study we found the infectious rate of toxoplasmosis was 68.57%,this result agree with other study using the LAT as a serological test in the Sudan 67% (Elamin etal.,1992), also another prevalence study for *Toxoplasma gondii* seropositivity detection in Sudan using the LAT was (61.7%)(Manal ,2003). In Egypt 1990 using IHAT as a serological test, the study obtained 21%, and latter prevalence reached 49% by the impression method technique (EL Ridi *et al*., 1990; Ibrahim *et al*., 1997).

In this study we see the rate in female 92.85% more high than male 32.14% , in other hand using LAT in Al-Najaf Province see the result in female 30.4% near to male 21.9%(Mahmoud *etal*.,2014)

the variation of the results may be due to using different technique ,time of study environmental condition and different region.

In other species the prevalence of *T. gondii* in slaughter sheep were studied in many countries, the percent were range between 3% in Pakistan using LAT (Zaki, 1995) to highest percent in Indonesia (60%) by using IHAT (Iskandar, 1998). In Norway using ELISA recorded 18% (Skjerve et al., 1996). In Saudi Arabia, Egypt, and Djibouti where using IHAT, the prevalence 39% in Saudi Arabia (Amin and Morsy, 1997), in Egypt 29% (EL Ridi et al., 1990), and 10% in Djibouti (Chantal et al., 1994). This variation of infection among different species may be according to immunity of different species.

B. Detection of Toxoplasmosis in goat.

1- Incidence of suspected bradyzoites Toxoplasmosis in the goat according to the organs.

The current study use impression method to detected suspected toxoplasma bradyzoite in the uterus, meat, placenta and fetus samples from slaughter house.

The result of the present study was 45/70 (64.28%) was positive result in the method above., while according to the organs were 46.66 %, 73.33%, 73.33% and 64% in the uterus ,placenta , fetus and meat respectively table(3-4) figure (2-4).

Table(3–4) show the percentage according to organs suspected to bradyzoites to toxoplasma in goat

Organ	N0	(+ve)	%
Uterus	15	7	46.66A
Placenta	15	11	73.33B
Fetus	15	11	73.33B
Meat	25	16	64A
Total	70	45	64.28

Similar litter represent no statistically significant at p > 0.05

Different letter represent statistically significant at p < 0.05

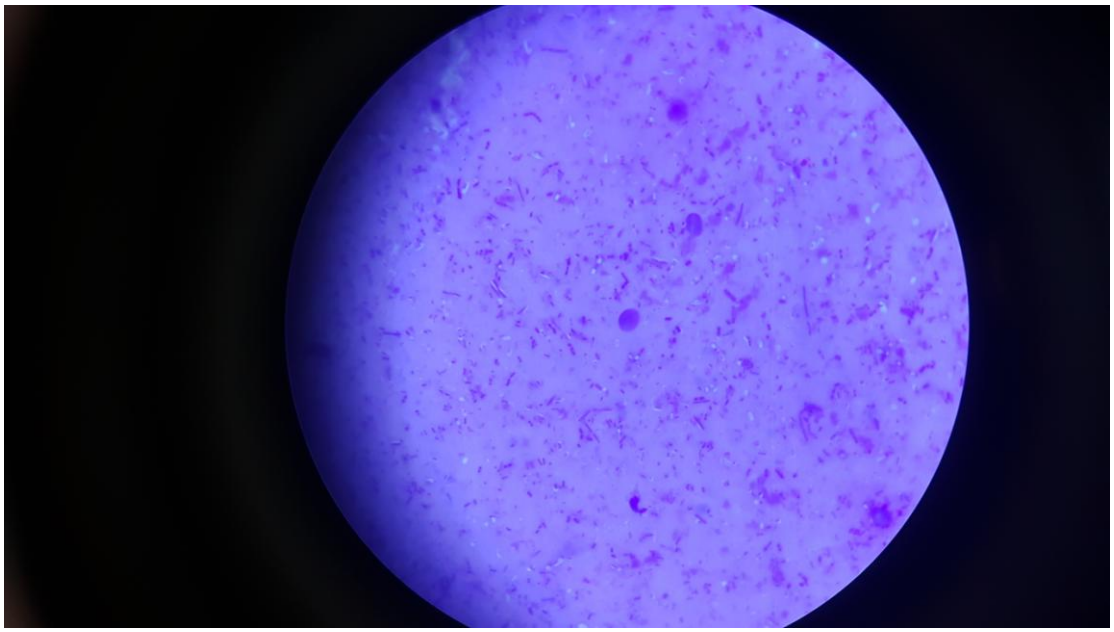


Figure (2-4) the bradyzoite in goat meat using impression method and staining with Gemisa (X 100).

2- **Incidence of bradyzoites Toxoplasmosis according to gender**

The preset study divided the goat samples based on gender into 25 male and 45 female, the infectious rate of toxoplasmosis according to the gender based on impression method were 16/25 (64%) and 29/45(64.44%) in male and female respectively table (4-4).

Table (4-4) the percentage according to gender suspected to bradyzoites of toxoplasmosis in goat

Sex	N0.	Number (+ve)	%
Male	25	16	64^{B}
Female	45	29	64.44^{A}
Total	70	45	64.28

Different letter represent statistically significant at p < 0.05

The present study found 64.28% in the goat, On the other hand the result of another study which were nearly to the result of the present study in Bangladesh reported 61% by used latex method (Rahman etal.,2014) , in Egypt reported 59.4% by used indirect hemaaglutination assay, (Barakat etal.,2009).

on the other study (Abdulkadir ,2014) in Somalia reported 26.7% by used latex method ,while in south of Tunisia reported 34.5% by used Modified agglutination test (Lahmar etal., 2015).

Other study use PCR dis agree with present study reported in Poland (3%) by (Cisak et al., 2017). A lower percentage (6%) was obtained in Brazil (Bezerra et al., 2015) and Italy (13%) (Mancianti et al., 2013). A relatively high prevalence (33%) was recorded by (Spišák et al.,2010) in Slovakia.

In the present study, the female(64.44%) nearly than male (64%) ,in other hand found the female (12.4%) higher than male (9.9%) reported by (zou *etal* ., 2015).

this different rate results may be due to the difference in the species, strain are resistant ,the sensitive to infection, the age and sex of the animal and the nature of the environmental surrounding it, and the different technical type use.

2 – Comparative of infectious rate of toxopasmosis between camel and goat based on impression method

The current study used the impression method to detect the bradyzoite in the uterus and meat in both camels and goat, the uterus result showed 33/36 (91.66%) , 7/15 (46.66%) in the camel and goat respectively, there was a statistically significant between the species table (5-4).

Table (5 –4) comparison between camels, goat according to infected uterus

Species	No.	Positive result	%
Goat	15	7	46.66 a
Camel	36	33	91.66 b
Total	51	40	78.43%

Different letter represent statistically significant at p < 0.05

The present study shows that the uterus infectious rate of toxoplasmosis in the camel was higher than goat and this result may be due to the samples of camel was higher than goat.

On the other hand the current study used the impression method to detect the bradyzoite in the meat and in both camels and goat, the result

showed 12/25 (48%) , 16/25 (64%) in the camel and goat respectively, there was a statistically significant between the species table (6-4).

Table (6 –4) comparison between camels, goat according to meat

Species	No.	Positive result	%
Goat	25	16	64A
Camel	25	12	48A
Total	50	28	56%

Similar litter represent no statistically significant at p > 0.05

The present study shows that there was no statistically a difference between the meat of camel and goat.

C- Detection of Toxoplasmosis in human

--Incidence of bradyzoites Toxoplasmosis according to organ

The current study used the impression method to detected toxopasmosis bradyzoite in the placenta and fetus from the same aborted woman who attended to the maternity and childhood hospital in Al Diawanyia province .

A total number of placenta and fetus samples were (60 each sample to the single patient) samples exam by impression method, and the result of this study found 20 /60 (33.33 %) positive

That mean the placenta source of transmission of infection to the fetus. table (7-4) figure (3-4) .

Table (7–4) the percentage of toxoplasmosis according to organs suspected to bradyzoites to toxoplasmosis in human

Organ	N0	(+ve)	%
Placenta	60	20	33.33[A]
Fetus	60	20	33.33[A]
Total	60	20	33.3%

Similar litter represent no statistically significant at p > 0.05

The present study found 33.33% in the human, In other hand by ues ELISA test the sero prevalence in women was 50% in USA (Stagno, 1980), 54% in Kenya (Griffin and Williams, 1983), 7.5% in Scotland (Jackson and Hutchinson, 1987), 37.5% in Libya (Kassem and Morsy, 1991), 47% in Nigeria (Onadeko *et al.*, 1992), Brazil and Argentina was 11.0%, 7.3–77.5% respectively (Pappas *et al*., 2009).

In other study use LAT in male sex in many Iraqi provinces, the rate that recorded by Hasson, (2004) and Dargham, (2011), which was 19.7% and 26.8% in Al-Najaf province respectively. While Al-Dalawi (2007), recorded 29.2% in Baghdad and Al- Kaise and Ali (2010) who they have 22% in blood donors, These differences may be related to several other factors, including cultural level, nutritional habits, age and rural or urban area (Etheredge and Frenkel,1995).

or may due to different manufacture origins of the kits used

The studies in neighboring countries also recorded different result contained 24 - 34% in Tehran, Iran (Noorbakhsh *et al*., 2002) and the seropositivity of *T. gondii* in a Turkish study was 1.34 % for IgM and 24.6 % for IgG (Harma *et al*, 2004), the seroprevalence in women was 95.5% in Kuwait (Be-hbehani and Al-Karmi, 1980), 37% in Jordan (Morsy and Michael, 1980), and 37.4% in Saudi Arabia (Abbas *et al.*, 1986).The variation in above results may be attributed to climate, cultural

differences regarding hygienic, feeding habits and/or may be due to different manufacture origins of the used kits.

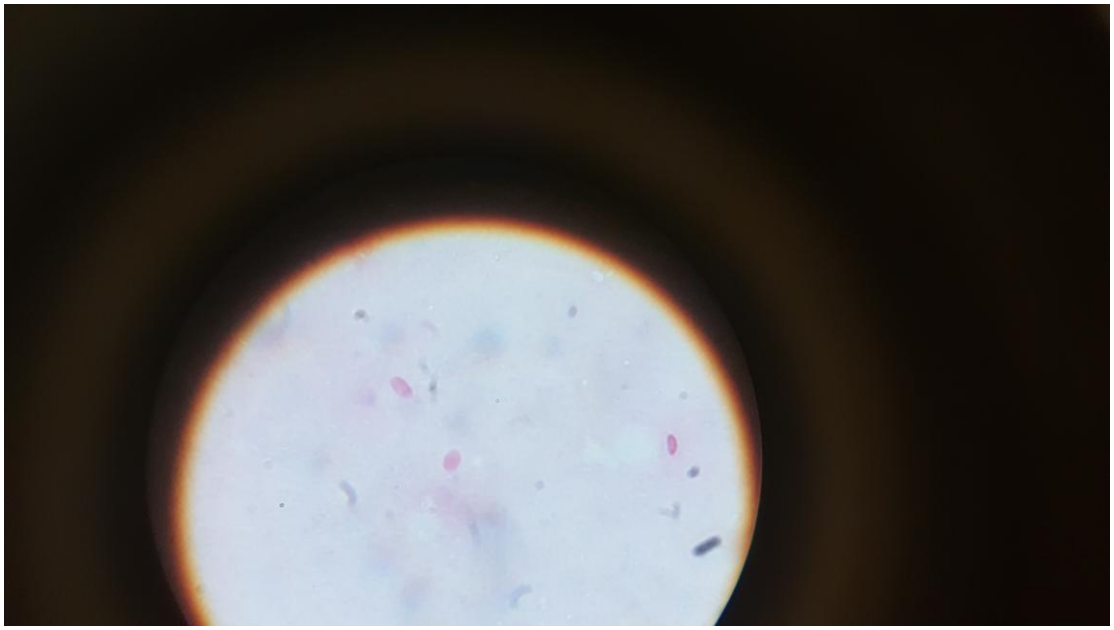


Figure (3-4) the bradyzoite in human using impression method and staining with Gemisa stain (X 100).

4.2 Detection of *Toxoplasma gondii* using Molecular methods.

1- DNA extraction.

The DNA of positive samples to impression method which are (95) was extracted and predicated by using genome DNA purification kit. The results were detected by nano drop and the result were excellent .

2- **Nested polymerase chain reaction nPCR to detect small sub unit ribosomal RNA gene.**

The results of PCR amplification which was performed on the DNA extracted of small subunit ribosomal RNA gene of *Toxopalasma gondii*, the samples were taken from 95 samples which were positive result with impression method as (32,33and 30) camel ,goat and human respectively the studied isolates confirmed by electrophoresis analysis. By this analysis the strands of DNA which are resulted from the successful binding between specific primers and extracted DNA of

isolates, These successful binding appear as single band together with the 313 pb band product size .

The result of nPCR in present study of camel was 18/32 (54.45%) from positive samples to **impression method**, while the result of goat in the same method was 17/33% figure (4-4) .

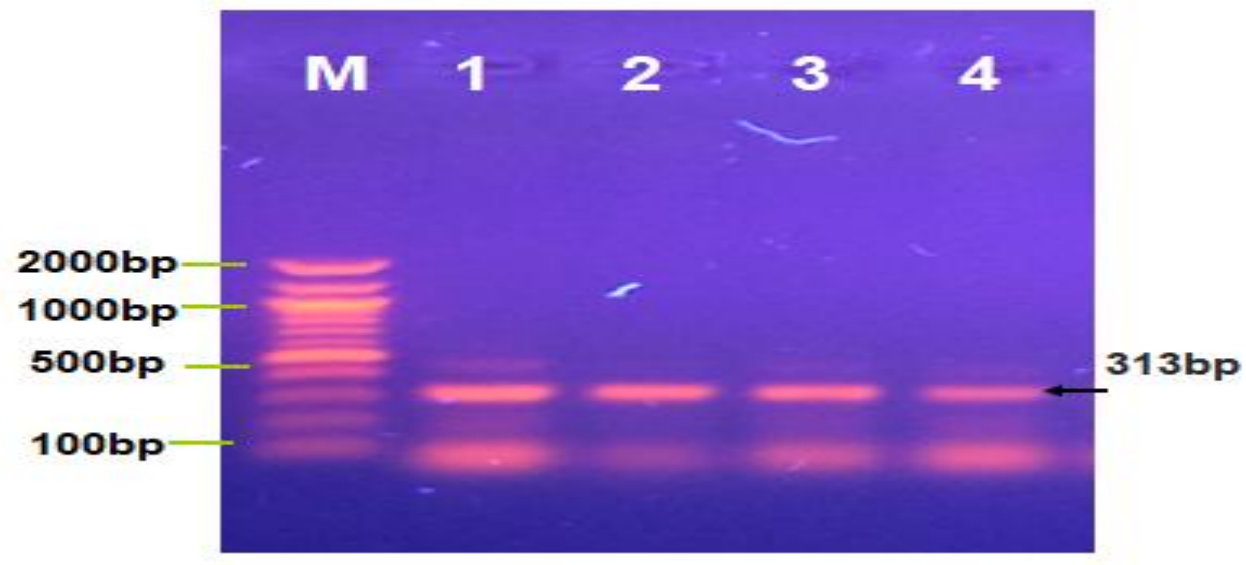


Figure ((4-4): Agarose gel electrophoresis image that show the Nested PCR product analysis of small subunit ribosomal RNA gene in *Toxoplasma gondii*. Where Marker ladder (2000-100bp), (1-4) some positive *Toxoplasma gondii* from camel samples at 313bp PCR product size.

The electrophoresis also used to estimate DNA weight depending on DNA marker (2000 bp DNA ladder) and the result of this estimation revealed that the amplified DNA figure(5-4)

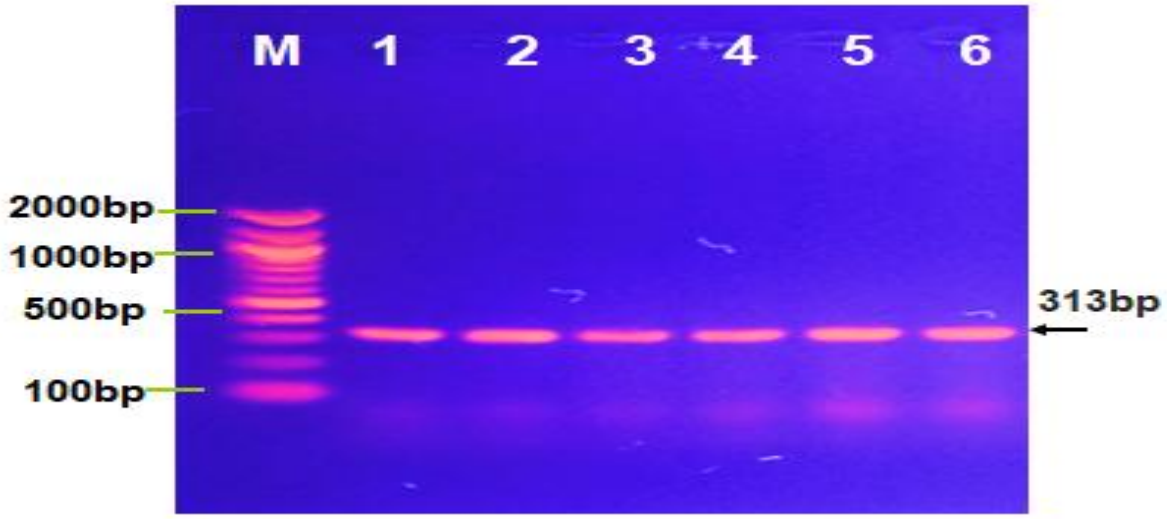


Figure (5-4): Agarose gel electrophoresis image that show the Nested PCR product analysis of small subunit ribosomal RNA gene in *Toxoplasma gondii.* Where Marker ladder (2000-100bp), (1-6) some positive *Toxoplasma gondii* from goat samples at 313bp PCR product size.

Out of 95 were taken from **impression method** positive cases, and examined by nested PCR found (53) positive result with a percent (55.20 %).

In human the number of samples were 18/30 from positive **impression method** table(8-4) .

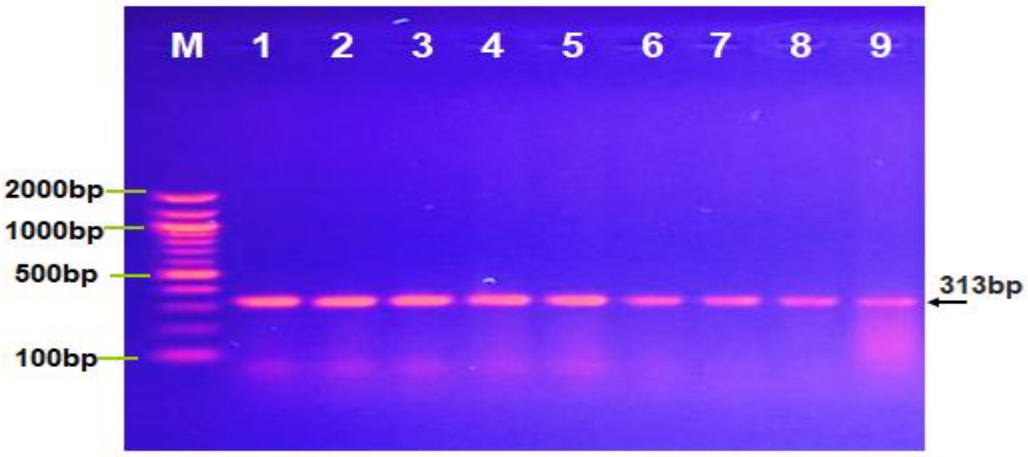


Figure (6-4): Agarose gel electrophoresis image that show the Nested PCR product analysis of small subunit ribosomal RNA gene in *Toxoplasma gondii*. Where Marker ladder (2000-100bp), (1-9) some positive *Toxoplasma gondii* from Human samples at 313bp PCR product size.

Table(8-4) Compression of the result of nPCR according to the different species

Species	nPCR	(+ ve)	%
Camel	32	18	56.25^{A}
Goat	33	17	51.51% ^{A}
Human	30	18	60$^{\% A}$
Total	95	53	55.78%

Similar litter represent no significant difference at p > 0.05

The current study found 56.25% in camel, other study use LAT of Al-Qadisiya province reported 16.34%(Al-Hindawe,2006) , Hilali *etal.* (1998) detect aprevelance of 17.4%. The differences among percentages that, recorded in the current study and other studies may be due to method of diagnosis or the time of collected samples.

The current study found 51.51% in goat ,this result compare with other result use the blood sample for an indirect antibody test in the slaughterhouse in Iran (Sharif *etal*., 2007) which reported 30% .

In Italy use pcr reported 12.98% by (Mancianti *etal*.,2013) , in alslimanieh (41.67 %) by (Al-taie and shadan,2008) , and in the south of Iran (17.9%) by (Asgari *etal*., 2008).

the Differences of percentages in the correct study and other studies may be due to method of diagnosis, number of sample and time of study.

The present study found 60% in the human, while the result of Al-Kalaby, (2008) who recorded that 83.3% of tested samples from Iraq women was positive by PCR technique using *B1* gene, on the other hand the results of Okay *et al.,* (2009) who reported that 17.65%, the result of present study agreed with the results of Al-Addlan, (2007) who reported that 63.49% . The differences among percentages that, recorded in the current study and other studies, may be attributed to the different origins of used samples (blood, amniotic fluid, , etc.) and to immune competent or immune compromised status of patients.

The sero prevalence of *T gondii* varies greatly within as well as between regions. The sero prevalence in women of child-bearing age in United States of America, Brazil, Argentina and Colombia was 11.0%, 7.3–77.5%, 48.7–53.4%, and 47.0-63.5% respectively while in Europe it varied between 8.2% (in Switzerland) and 63.2% (in Western Pomerania, Germany). In Asia and Oceania, the sero prevalence ranged from 0.8% (Suwon region, South Korea) to 63.9% (Babol, Iran) and in Africa it was between 25.3% (Burkina Faso) and 75.2% (Sao Tome and Principe) (Pappas etal.,2009).

4.3.gene SAG3

Detection of SAG3 gene in the present study was done for to select the samples sequences, it was selected randomly 53 (18,17 and 18) from purified DNA of camel, goat and human and with positive result in nPCR.

All of samples which were selected positive in both nPCR and SAG3 gene positive result (100%) table(9-4).

Table(9-4) the number and percentage of SAG3 gene from different species

Species	Sag3	%
Camel	18	33.96^{A}
Goat	17	32^{A}
Human	18	33.96^{A}
Total	53	100

Similar litter represent no significant difference at p>0.05

In the present study the result of SAG3 gene was 100% in all samples showed highly transitional substitutions between (G) nucleotide that substituted by (A) nucleotide at (24.3)% from total SAG3 gene nucleotides. Whereas highly nucleotide variations Substitution at transversionsal substitutions were show at (7.24%) between (G) nucleotide that substituted by (T) and (C) nucleotide, but in other hand the Adenine and Thymine (A+T) content of the SAG3 gene of both the Indian isolates was found to be 42.57%, whereas the Guanine and Cytosine (G+C) content was 57.43%. The nucleotide homology was found to be 99.9% by(Sudan *et al*., 2015).

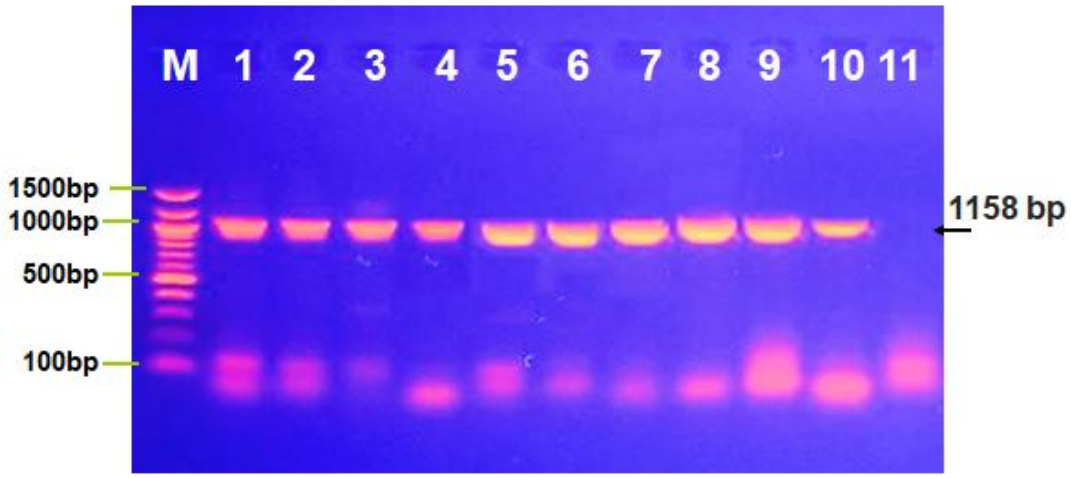


Figure (7-4): Agarose gel electrophoresis image that show the PCR product analysis of SAG3gene in *Toxoplasma gondii*. Where Marker ladder (2000-100bp), (1-10)SAG3 positive *Toxoplasma gondii* from camel samples at 1158bp PCR product size.

4.4 Comparison among the nPCR Method , SAG3 gene and impression method according to the organ in study animals

A - In the camel

Microscope: the present study showed that from 36 uterus samples there were 33 positive with percentage (91.66 %), while the meat showed 12/25 (48%) table(10-4) .

The result of liver was reported 33.33%

Nested Polymerase Chain Reaction nPCR: the present study showed that from 20 uterus samples there were 14 positive with percentage (70 %), while the meat showed 4/9 (44.44%) , in the liver the infectious rate was 0% table(10-4).

SAG3 gene: SAG3 gene showed the same result of n PCR table(10-4)

Table (10-4) the number of positive and negative samples in microscope, nPCR and SAG3 gene in camel from differente organ

Organ	Microscope			Nested pcr			SAGA		
	No.	+	%	No.	+	%	No.	+	%
Uterus	36	33	91.66[a]	20	14	70[a]	20	14	70[a]
Meat	25	12	48[a]	9	4	44.44[a]	9	4	44.44[a]
Liver	9	3	33.33[a]	3	-----	0[b]	3	-------	0[b]

- **Similar litter represent no significant difference at p > 0.05 whereas different letter represent significant difference at p < 0.05**
- **Capital letter represent vertical statistical reading whereas small letter represent horizontal statistical reading**

The present study showed higher percent in uterus in all method than other organ due to the uterus target of parasite, where were lower percentage in liver reported all method that was may be due to little samples .

B- In the goat

Microscope:

The present study showed that from 15 uterus, placenta and fetus samples there were 7,11 and 11 positive respectively with percentage 46.66, 73.33, and 73.33 while the meat showed 16/25 (64.28) table (11-4)

Nested Polymerase Chain Reaction nPCR:

the present study showed that from 7 uterus, placenta and fetus samples there were positive only 2 in the uterus where are 5 positive to the placenta, fetus and meat with percentage (71.42,71.42 and 41.66) respectively table (11-4)

SAG3 gene: SAG3 gene showed the same result of n PCR table (11-4)

Table (11-4) the number of positive and negative samples in microscope, nPCR and SAG3 gene in goat from different organs

Organ	Microscope			Nested pcr			SAG3		
	No.	+	%	No.	+	%	No.	+	%
Uterus	15	7	46.66 a	7	2	28.57a	7	2	28.57a
Placenta	15	11	73.33 a	7	5	71.42a	7	5	44.44a
Fetus	15	11	73.33 a	7	5	71.42a	7	5	71.42a
Meat	25	16	64 b	12	5	41.66b	12	5	41.66b

- **Similar litter represent no significant difference at p > 0.05 whereas different letter represent significant difference at p < 0.05**

Capital letter represents vertical statistical reading whereas small letter represent horizontal statistical reading

C- In the human

Microscope:

In placenta collect (60) found (40) negative and (20) positive (33.33%)

Nested pcr:

found (12) negative and (18) positive (60%) from (30)sample positive

SAG3 gene:

found (18) positive (60%) from (18)sample positive case in nPCR

Table (12-4) the number of positive and negative samples in microscope, nPCR and SAG3 gene in human from different organs

Organ	Microscope			Nested pcr			SAG3		
	No.	+	%	No.	+	%	No.	+	%
Placenta	60	20	33.33[a]	15	9	60[b]	15	9	60[b]
Fetus	60	20	33.33[a]	15	9	60[b]	15	9	60[b]

Similar litter represent no significant difference at p > 0.05

Different letter represent significant difference at p < 0.05

The present study showed that molecular method more sensitive than microscopic method and that may be due to some parasite like Neospora and sarcoscyste was microscopically similar to Toxoplama bradyzosite which can infect all organ.

4-5-DNA Sequence results:

The DNA sequencing analysis of *Toxoplasma gondii* surface antigen (Sag3) complete gene was showed a clear genetic variation between *Toxoplasma gondii* isolates from different hosts according to phylogenetic tree analysis that analyzed local *Toxoplasma gondii* Human goat, and camels isolates with Standard NCBI-BLAST *Toxoplasma gondii* isolates. As show in figure (7-4) and figure (8-4).

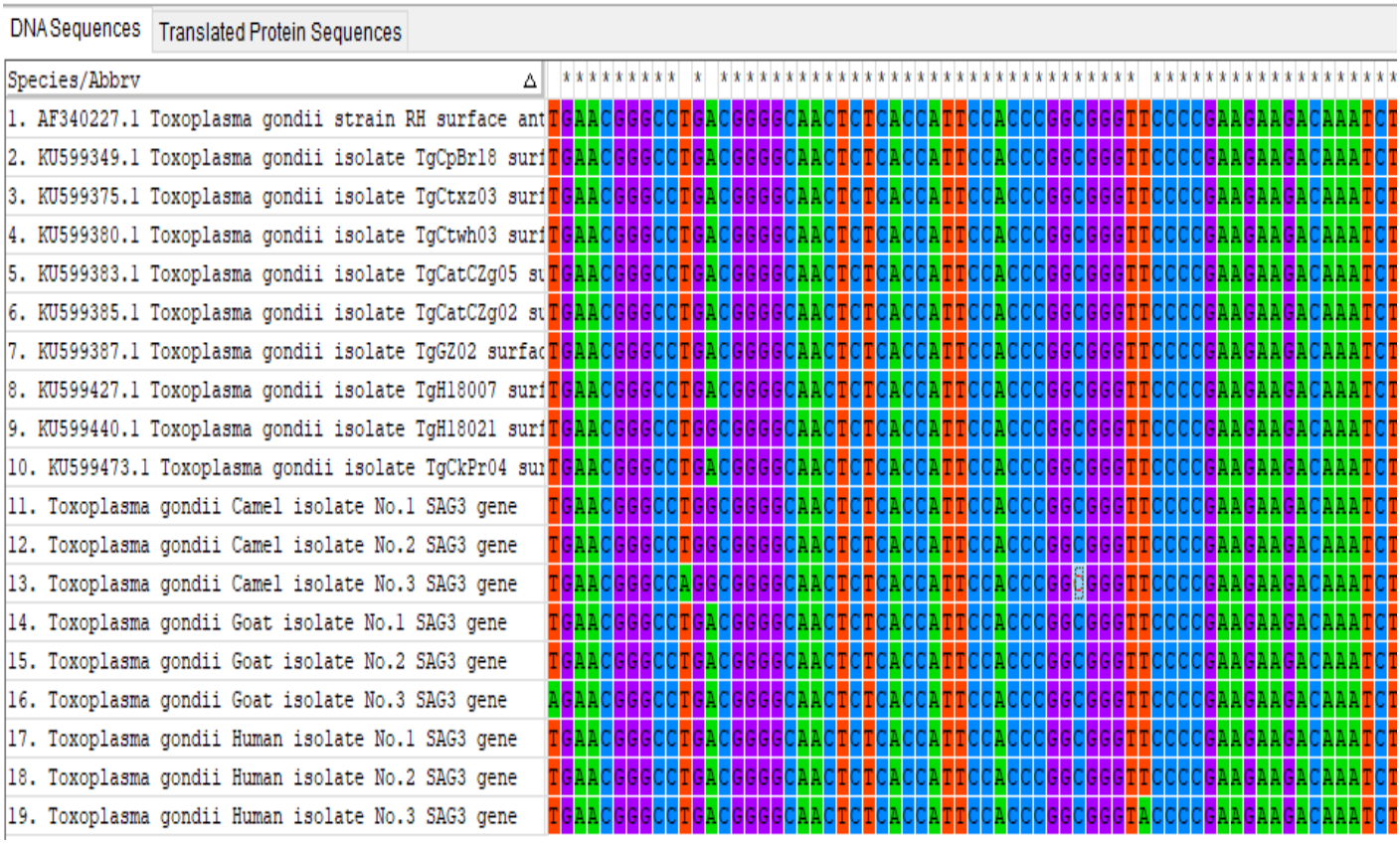
The local *Toxoplasma gondii* Human isolates (No.1 – No3) showed difference from other host isolates with closed related to NCBI-Blast *Toxoplasma gondii* (KU599349.1), The local *Toxoplasma gondii* Goat isolates (No.1 – No3) were show different from other host isolates with closed related to NCBI-Blast *Toxoplasma gondii* (KU599385.1 and KU599387.1), Whereas, The local *Toxoplasma gondii* Camel isolates (No.1 – No3) were show different from other host isolates with closed related to NCBI-Blast *Toxoplasma gondii* (KU599440.1).

The Homology sequence identity between local *Toxoplasma gondii* (Human, Goat, and Camel) isolates surface antigen (SAG3) gene and NCBI BLAST *Toxoplasma gondii* isolates. The local *Toxoplasma gondii* Human isolates (No.1 – No3) were show (99-100%) homology identity to NCBI-BLAST Human isolate (KU599349.1), The local *Toxoplasma gondii* Goat isolates (No.1 – No3) were show(99-100%) homology identity to NCBI-BLAST Human isolate (KU599385.1 and KU599387.1), and The local *Toxoplasma gondii* Camel isolates (No.1 – No3) were show (100%) homology identity to NCBI-BLAST Human isolate

(KU599440.1). and all isolates were submitted in NCBI-Genbank for accession numbers as show in table (13-4)

The Nucleotide variations Substitution analysis between local *Toxoplasma gondii* (Human, Goat, and Camel) isolates surface antigen (SAG3) gene and NCBI BLAST *Toxoplasma gondii* isolates were show highly transitional substitutions between (G) nucleotide that substituted by (A) nucleotide at (24.3)% from total sag3 gene nucleotides. Whereas highly nucleotide variations Substitution at transversionsal substitutions were show at (7.24%) between (G) nucleotide that substituted by (T) and (C) nucleotide. As show in table (14-4).

Our results show that molecular characterization of the SAG3 gene has genetic variation in *Toxoplasma gondii* isolates from different host. This finding was agreement with previously study by **(**Sudan *et al*., 2015). Who revealed that that SAG3 gene in *Toxoplasma gondii* is considered significant homologous variations between the different loci of various strains of parasites. In present study**,** the genetic variation in SAG3 gene from Human, goat, and camels it may be related to pathogenesis of parasite in different host. The Surface antigen (SAG3) gene is encoded for glycoproteinduring in the developmental stages of (tachyzoites and bradyzoites) *Toxoplasma gondii* (Kazemi *et al*.,2007). This SAG3 protein associated with binding of host heparin sulfate proteoglycans (HSPGs), their interaction facilitates the parasite's attachment to target cells **(**Jacquet *et al*.,2001).



Figure(8-4): Multiple sequence alignment analysis of surface antigen (SAG3) gene partial sequence in local *Toxoplasma gondii* (Human, Goat, and Camel) isolates and different NCBI-Genbank *Toxoplasma gondii* based ClustalW alignment analysis by using (MEGA 6.0, multiple alignment analysis tool). The multiple alignment analysis similarity (*) and differences in surface antigen (SAG) gene nucleotide sequences.

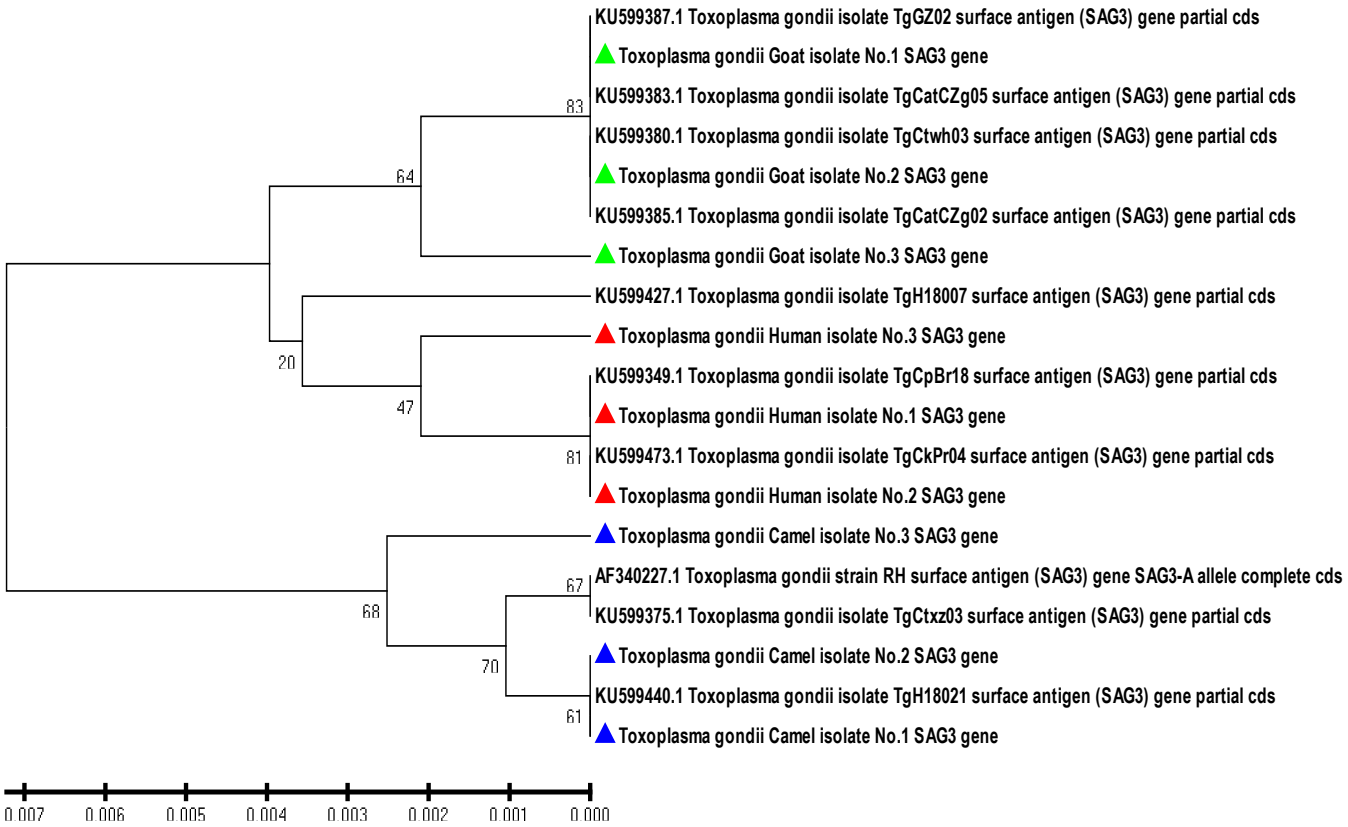


Figure (9-4): Phylogenetic tree analysis based on the partial sequence of surface antigen (SAG3) gene in local *Toxoplasma gondii* (Human, goat, and Camel) isolates that used genetic relationship analysis. The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (MEGA 6.0 version)

Table (13-4) Homology sequence identity between local *Toxoplasma gondii* (Human, Goat, and Camel) isolates surface antigen (SAG3) gene and NCBI BLAST *Toxoplasma gondii* isolates:

Local *Toxoplasma gondii* No.	Genbank accession number	Homology sequence identity	
		NCBI BLAST *Toxoplasma gondii*	Identity %
Toxoplasma gondii Human No.1	Mk293955	KU599349.1	100%
Toxoplasma gondii Human No.2	Mk293956	KU599349.1	100%
Toxoplasma gondii Human No.3	Mk293957	KU599349.1	99%
Toxoplasma gondii Goat No.1	Mk293985	KU599385.1	100%
Toxoplasma gondii Goat No.2	Mk293959	KU599385.1	100%
Toxoplasma gondii Goat No.3	Mk293960	KU599387.1	99%
Toxoplasma gondii Camel No.1	Mk293961	KU599440.1	100%
Toxoplasma gondii Camel No.2	Mk293962	KU599440.1	100%
Toxoplasma gondii Camel No.3	Mk293963	KU599440.1	100%

Table (14-4): Nucleotide variations Substitution analysis between local *Toxoplasma gondii* (Human, Goat, and Camel) isolates surface antigen (SAG3) gene and NCBI BLAST *Toxoplasma gondii* isolates

	A	T	C	G
A	-	*4.53*	*6.39*	**24.3**
T	*5.07*	-	**7.16**	*7.24*
C	*5.07*	**5.08**	-	*7.24*
G	**17.02**	*4.53*	*6.39*	-

The table shows the probability of substitution from one base (row) to another base (column). For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in *italics*. Evolutionary analyses were conducted in MEGA6.

Chapter Five

Conclusions And Recommendations

5.1.Conclusion

The current study show :

1 - The proportion of *Toxoplasma gondi* parasite in the organs of slaughter animals and in humans by using the different technique was determined . It also showed that the presence of parasite in a high rate in the camel

2- There is a variation in the incidence of infection among different animals and human

3- There is a variations in the incidence of the infection of *toxoplasma gondi* parasite between different organs of the camel depending on the stage of infection and the importance of the organ relative to the parasite

4- The uterus is considered the best organs as a target of the *toxoplasma gondi* when compared to the others organs.

5- Meat of slaughter animals is an important source of toxoplasmosis and a threat to consumer health.

6- The isolated samples from the study animals belonging to the *toxoplasma gondi* parasite were 100% identical according to SAG3 gene.

7.Homology sequence identity between local Toxoplasma (Human , Goat and Camel) isolates surface antigen SAG3 gene and NCBI-BLAST *Toxoplasma gondii* isolates.

8-The DNA nucleotides sequencing analysis of *T.gondii* sureace Ag SAG3 complete gene was show clear genetic variation between isolates from different host.

5.2. Recommendations

1- Studying once of *toxoplasma gondii* in male
2- Attempting to prepare a vaccine against the *toxoplasma gondii* parasite to weaken the parasite in different ways and choose the most suitable ones
3- studying the effect of plant extracts on infected animals and their importance in the treatment or reduction of infection
4- Conducting further molecular studies to determine the genetic patterns of parasite species found in the province.
5- Investigating local strain in different host and bond that with severity of pathogenesis to the parasite.

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وعند تحليل تتابع نيوكلوتيدات DNA لنفس الجين بين الحيوانات والانسان وجد ان هناك اختلاف في التطابق الجيني بين العزلات . في تتابع النيوكلوتيدات G,C,T,A قد يكون هذا الاختلاف له علاقة بأمراضية الطفيلي في الحيوانات والأنسان.

الخلاصة

هدفت الدراسه الى البحث في الاختلاف في الشجره الوراثيه لانواع مختلفه من المضائف التي تصاب بطفيلي المقوسه الكونديه (الانسان ، الإبل ، الماعز) من خلال جمع العينات من المجزرة في محافظة القادسية ، وجمع أيضا من النساء المجهضات من مستشفى النسائية والاطفال التعليمي. لمعرفة معدل الاصابه فيها ومدى اصابة الأعضاء المختلفة الموجودة في الحيوانات، ولمعرفة الاختلاف الجيني للطفيلي في المضائف المختلفة عند تحليل الشجرة الوراثية ، خلال الفترة من نوفمبر ٢٠١٧ إلى فبراير ٢٠١٨.

تم جمع٢٠٠ عينة من الذكور والإناث من الجمال والماعز ، ومن النساء المجهضات ، تم تقسيم العينات إلى (٧٠) عينة من الجمال من (الكبد واللحم والرحم) ، ٧٠ عينة من لحم الماعز والرحم والمشيمة والجنين و ٦٠ عينة عشوائياً من النساء اللواتي تعرضن للإجهاض في مستشفى النسائية والاطفال التعليمي في الديوانية.

لغرض التشخيص تم استخدام طريقة الطبعة ، أظهرت نتائج العينات الماخوذة من الجمال أن ٧٠/٤٨ عينة موجبه وبنسبة (٦٨.٥٧٪) ، بينما كانت نتيجة العينات الماخوذة من الماعز ٤٥/ ٧٠وبنسبة (٦٤.٢٨٪). وأن النساء المجهضات يظهرن ٦٠/٢٠ موجبة (٣٣.٣٣٪) ، كان هناك دلالة إحصائية بين الأنواع في.$P < 0.05$ للاصابه بالطور البطيء للمقوسة الكونديه المحتملة .

كذلك شملت الدراسة الحالية على أتباع الطرق الجزيئية للكشف عن الحمض النووي الريبوزي subunit ribosomal (ssRNA)باستخدام تفاعل متسلسل Polymerase المتسلسل (nPCR) ، أظهرت نتائج nPCR للجين ssRNA أن ٣٢/١٨ (٥٦.٢٥٪) كانت إيجابية في الإبل ، بينما كانت النتيجة من الماعز ٣٣/١٧ (٥١.٥١ ٪) كانت إيجابية ، وكان معدل داء المقوسات في النساء المجهضات 30/18 إيجابي ٦٠ ٪. هناك دلالة إحصائية بين الأنواع في.$P < 0.05$

كما كشفت الدراسة الحالية عن جين Antigen 3 (SAG3) لمطابقة نتائج nPCP وإرسال العينات للتسلسلات .

وكانت النتاءيج لجين (SAG3) بنسبة ١٠٠% اعتماد على ssRNA جين .

كانت هناك فرق معنوي وإحصاءي بين اصابة اعضاء الجمال وكذلك لايوجد فرق معنوي في الانواع الاخرى.

ووجد تطابق جيني بين *T.gondii* الموجوده في الانسان والماعز والجمال بين جين SAG3 وعزلات الجيني حسب NCBI-BLAST.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسيه- كلية الطب البيطري

دراسة طفيليه وجزيئيه لداء المقوسات في الانسان والجمال والماعز في محافظة القادسيه

رسالة مقدمة الى
مجلس كلية الطب البيطري / جامعة القادسية
كجزء من متطلبات نيل درجة الماجستير علوم
/ طفيليات

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تتقدم بها
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