Republic of Iraq

Ministry of Higher Education and Scientific Research

University of AL-Qadisiyah

College of Veterinary Medicine

Molecular Diagnosis of *Toxoplasma gondii* in Local And Imported Meat And Meat Products Of Cattle In AL-Diwaniyah Province

A Thesis Submitted to the Council of Veterinary Medicine College /Al- Qadisiyah University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Medicine / Parasitology

By

Farah Mohammed Sakban Al-Salhy

B. V .M.S. / 2013

Supervised by

Prof. Dr. Noaman Naji A'aiz

1440 ***2019***

بسم الله الرحمن الرحيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا)

صدق الله العلي العظيم

سورة طه : (آية **114**)

Supervisor Certification

I certify that this thesis entitle (Molecular diagnosis of *Toxoplasma gondii* in Local And Imported Meat And Meat Products Of Cattle In AL- Diwaniyah Province) has been prepared by the student under my supervision in Veterinary Medicine College / University of Al- Qadisiyah in partial fulfillment of requirements for the degree of master science in Veterinary Medicine /Parasitology.

Prof. Dr. Noaman Naji A'aiz

(Supervisor)

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Professor

Alaa Abdul Aziz Abed

Vice Dean of Scientific affairs and postgraduate Studies

College of Veterinary Medicine

University of AL- Qadisiyah

2019/ /

Certification of Examination Committee

We, the members of the examining committee, certify that after reading this thesis entitled (**Molecular Diagnosis of *Toxoplasma gondii* in Local And Imported Meat And Meat Products Of Cattle In AL- Diwaniyah Province**) and examining the student (**Farah Mohammed Sakban**) in its content, and that our opinion it is adequate as a thesis for the Degree of Master of Science Parasitology.

Professor

Dr.Hadi M.Hamza

Chairman

Assist. Professor

Dr. Azhar Ali Faraj

Member

Assist. Professor

Azhar Chafat Karawan

Member

Professor

Dr. Noaman Naji Aʾaiz

Member (Supervisor)

Approved by the Council of the College of Veterinary Medicine/ University of Al-Qadisiyah

Professor
Dr. Jabbar A.A. Al-Sa'aidi
Dean of the College of Veterinary Medicine
University of Al-Qadisiyah

Assist. Professor
Dr.Monyer Abd Al-ameer Al-fatlawi
Head of Department
of Microbiology

Date of examination: 28/ 3/ 2019

DEDICATION

To whom we imitate them … to seal of prophets and Mohammed… and prophet's family (Ahl elbait) peace be upon them.

To whom worried by thinking of my future…my parents .

To the safe way and source of my happiness and power my lovely daughter malak and my husband Ameer…

To all my best friends… my sisters and brothers…

I present my modest effort, deepest and sincere gratitude for their support.

All this work to my wounded country .

FARAH

ACKNOWLEDMENT

Foremost, I would like to thank merciful God for enabling me to perform this work.

I would like to thank professor Dr. Jabbar AL-Sa'aidi the dean of Veterinary Medicine College and Assistant professor Dr.Monyer abd-Ulameer the head of Microbiology and parasitology Dept. in Vet. Med. in AL-Qadisiyah University for their support to complete M.Sc.degree.

I'm deeply grateful to my supervisor professor Dr.Noaman Naji A'aiz for his guidance, patience and support, I consider myself very fortunate for being able to work with a very considerate and encouraging supervisor like him.

I do not forget to thank, Mr.Ali Mohammed Ghazi, Mr. Hassan Hachem in Vet. Med. / AL-Qadisiyah University I will never forget their help .

My sincere gratitude to Mr.Hussein J. Shoaibeth, Mr. Muta'a G., Mr.Hammeed A., and all Veterinarians who cooperated with me for getting samples in Al-Diwanyia abattoirs.

My sincere gratitude to my colleagues in study, Mrs.Rana saad,Marwa alfatlawi,Rash Emad,and Zainab H. and all people who help me in this study.

FARAH

Summary

This study was conducted during period from September 2017 to May 2018 in AL- Diwaniyah province for Molecular Diagnosis of *Toxoplasma gondii* in Local And Imported Meat And Meat Products Of Cattle In AL- Diwaniyah Province.

Three hundred samples were used in this study which collected from heart, tongue, muscles, of 100 slaughted local and imported cattle their ages ranged from months to four years, in addition to 90 samples of different local and imported meat products (beef burgers, minced meat).

meat samples subjected to microscopic examination for searching on suspected *T.gondii* bradyzoits, DNA extraction were done for all collected samples, and the extracted DNAs were examined by using conventional PCR technique to amplified *B1*gene to detect the infection of *T.gondii* .

The infection rate in local meat was 22% and its more than imported meat that rate of infection was 13.5% as for the local meat product infection rate was 16% and imported meat product was negative results, there is no significant difference at ($p< 0.05$) in case of present of the parasite in examined organs but with regard to seasonal variation Autumn recorded the highest period for infection with significant difference at ($p< 0.05$) , animals with age 2-4 years showed higher rate of infection .

Regarding to gene sequencer and phylogenetic tree analysis were done on ten samples of PCR product to determine the genotype of parasite, and the results revealed the presence of three strains of *T.gondii* ,Type I, II, and III, Type II was dominant type

LIST OF CONTENTS

LIST OF TABLIS

	according to phylogenic tree analysis and NCBI-BLAST alignment tool.	

LIST OF FIGURES

LIST OF ABBREVATION

Abbreviation	Full name
bp	base pair
°C	Centigrade
DNA	Deoxy nucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
g	Gram
HIV	HIV Human Immunodeficiency virus
IFN-γ	Interferon Gamma
IgA	Immunoglobulin Class A
IgE	Immunoglobulin Class E
IgG	Immunoglobulin Class G
IgM	Immunoglobulin Class M
MAT	Modified Agglutination Test

min	min Minute
mL	Milliliter
NK	Natural killer cells
PCR	Polymerase chain reaction
PV	parasitophorous vacuole
rpm	Round per minute
TBE	Tris borate EDTA
T- cells	Lymphocyte that differentiate in the thymus
TgPLP1	A protein secreted by the parasite
Th1	T- helper cells-1
Th2	T- helper cells-2
TBE	Tris borate EDTA
µl	microliter
X^2	Chi-square
Abs	Antibodies
dNTPs	Deoxy nucleotide Tri phosphates
ect.	extra
mg	Milligram
mm	Micron
W1	Wash Buffer1
W2	Wash Buffer2
Tris-HCL	Tris-Hydrochloric Acid
HCL	Hydrochloric Acid
NaCL	Sodium chloride
T- cell	T-lymphocyte
Sec.	second
ng/ µl	Nano gram per microliter
MgCl2	Magnesium Chloride

spp	species
LAT	Latex agglutination test
FIV	feline immunodeficiency virus
FLV	feline leukemia virus
ADCC	Antibody-dependent cell mediated cytotoxicity
IL-4	Interleukin -4
IL-5	Interleukin -5
IL-10	Interleukin -10
IL-12	Interleukin -12
IL-13	Interleukin -13
HE	Haematoxylin and Eosin
ITS-1	internal transcribed spacer

Chapter One
Introduction

1.1 Introduction

Toxoplasma gondii is protozoan an obligate intracellular parasite and cause Toxoplasmosis, its zoonosis disease worldwide distribution can infected all warm –blood animals which act as intermediate host and felids like domestic cats known as definitive hosts (Dubey,2000),this parasite consider from most successful parasite on the earth and can infect large number of vertebrates (Miller *et al.*,2004).

Pregnant women and immunocompromised individuals consider the targets of the parasite, and cause many pathological problems and different congenital defects in the fetus or lead to abortion, in addition illness in many features in case of immunocompromised individuals, in both cases the weakness of immune system the key of infection occurrence (Halonen and Weiss ,2013).

This protozoan required a complex life cycle consist of sexual part in which the cats spread millions of oocyst into environment and contaminated it, thus the parasite will transmitted to another hosts enable him to transmitted to human through ingestion of infected meat (Cook *et al.*, 2000).

The main ways of disease transmission are ingestion of oocyst that contain sporozoits by food , water, or any way lead to contact with cat feces , transmission via placenta , and consumption of raw or under cooked meat until dealing with it, this study focused on *T.gondii* infection in meat due to many peoples are get infection through this way, about 28% pregnant women get infection by this method (Kravetz and Federman, 2005).

But the role of beef in transmission of *T.gondii* infection to human is unclear yet because of the infection by it is reported as the one of the risk factors that cause acute infection, on the other hand the theory said that cattle poor

persistence of parasite and the tissue cyst are not very persistent in cattle ,so it is necessary make large scale screening of cattle for the present of *T.gondii* to clarify the role of cattle as a source of infection (Jones *et al*., 2009).

Dubey,(2008) mentioned that difficult to diagnosis of *T.gondii* infection in cattle because of there is high rate of subclinical infection and persistence of tissue cyst which complicated the diagnosis, however serological test can be used for diagnosis and its depend on detection of antibodies in agglutination tests,Burg *et al*., (1989) was the first how used polymerase chain reaction PCR for detection *T.gondii* infection and used *B1* gene after that several tests used another target genes, it is useful method for diagnosis of toxoplasmosis.

Due to the importance of toxoplasmosis, as a zoonotic disease and public health problem in the society, with the importance role of meat in transporting of *T.gondii* infection to human, also there is no previous studies in this field in AL- Diwaniyah so the aim of study was designed .

1.2 Aims of study

1-Evaluating the prevalence of *T.gondii* in meat and meat products of local and imported cattle and which is considered from main sources for *T.gondii* infection by using molecular methods.

2-Studying the relationship of some factors (organs , Ages, seasons) with *T.gondii* infection.

4-Determining the genotype in some isolated *T.gondii* samples .

-

Chapter two

Literatures Review

2.1 Historical Review:

Toxoplasma gondii is one of the most well-studied parasites because of it have medical and veterinary importance, and its Consider suitable example for cell biology and molecular studies with a unicellular organism, there are many thousands of references and studies to this parasite in the literature and it is not possible to give equal treatment to all authors and discoveries (Dubey, 2007).

In 1908 Charles Nicolle and Louis Manceaux were first described *T. gondii* in Tunisia and isolated it from North African rodent, the gundi (Ctenodactylus gundi) in the same year Splendore reported the protozoan in a rabbit and they publish their results in the same time , after that in 1909 Nicolle and Manceaux back and differentiated the protozoan from *Leishmania* and named it *gondii* depended on the curved shape of its infectious stage (Greek root 'toxon'= bow) (Weiss and Dubey, 2009).

The first reported of fatal toxoplasmosis in a domestic animal (a 4-month-old dog) that died from acute visceral toxoplasmosis and over the next 30year canine toxoplasmosis was reported in many countries (Dubey and Beattie 1988).

Sabin and Olitsky was the first who isolated *T.gondii* in1937 by using techniques previously developed for analyzing viruses, they using laboratory monkeys and mice and showed that *T.gondii* was an obligate intracellular parasite also explained that *T. gondii* as a pathogen contagious between animals (Ferguson, 2009).

Machattie in1938 who was the first recognize *T.gondii* infection in Iraq in smear from spleen and lung of two street dogs in Baghdad ,(Jasim, 1979).

Weiss and Dubey,(2009) mentioned that the first identification of *T. gondii* as a human pathogen was in 1939 by Wolf, Cowen and paige in an infant girl delivered full-term by caesarean section the infant developed seizures and chorioretinitis in both eyes at three days then developed encephalomyelitis and died at one month of age also they can isolated *T. gondii* from brain tissue lesions, this fact was ensured by Ferguson, (2009) by intracranial injection of brain and spinal cord samples into mice, rabbits and rats produced encephalitis in the animals.

In 1940 the first adult case of toxoplasmosis was reported by Pinkerton and Weinman in a 22-year-old man from peru who died from a subsequent bacterial infection and fever (Weiss and Dubey, 2009).

The Sabin Feldman Dye Test is the gold standard for identifying Toxoplasma infection this serological dye test based on the ability of the patient's antibodies to alter staining of toxoplasma this test was created by Sabin and Feldman in 1948

(Shaapan *et al.*,2008).

Frenkel and Friedlander,(1951) were recognized other forms of toxoplasma including tissue cysts the parasite was identified as a coccidian in 1960 years and 1970 years.

Desmonts *et al.,(*1965) was the First who demonstration about transmission of Toxoplasma by eating raw or undercooked meat they observed that the therapeutic consumption of raw beef or horse meat in a tuberculosis hospital was associated with a 50% per year increase in Toxoplasma antibodies this means that more *T. gondii* was being transmitted through the raw meat .

Frenkel *et al*., (1970) was one of several groups working on discover that cat was the definitive host, also there are many methods were developed for recognize the genetic differences among *T.gondii* that isolated from both animals and human during 1980 to 1990, the last research and studies reported *T.gondii* infection in many marine wild life mammals that refer to possibility of the contamination of sea water with *T.gondii* oocysts, (Dubey and Jones,2008).

Zhou *et al*., (2009) mention that molecular genotype of 90% isolated can be classified in to three lineage (Type I, II, III),the virulence of these types in laboratory mice was, type I is high virulent ,type II was relatively high virulent and type III was intermediate in virulence, (Peyron *et al*., 2006).

2.2 classification

The Taxonomy of *T.gondii* was placed in the phylum Apicomplexa class Sporozoasida, subclass Coccidiasina and family Sarcocystidae (Hill *et al*., 2007; Pereira *et al*., 2010).

until 1970 all coccidia were classified in the family Eimeriidae , *T.gondii* has been placed by different authorities in the different families like Eimeriidae, Sarcocystidae or Toxoplasmatidae this occur after the discovery of the life cycle of *T.gondii* in 1970.

phylum Apicomplexa which consists of intracellular parasites that have a characteristically polarized cell structure and a complex cytoskeletal and organellar arrangement at their apical end (Dubey *et al*., 1998)

The phylum Apicomplexa comprises important parasites, such as *Plasmodium* spp. the causative agents of malaria, *Babesia* spp.,

Cryptosporidium spp. and *T.gondii* which is widely spread in human populations of developed countries, Petersen and dubey ,(2005) classified *T.gondii* as the following:

Kingdom : Animalia

Sub Kingdom : Protozoa

Phylum: Apicomplexa

Class: Sporozoa

Subclass: Coccidiansina

Order: Eimeriorina

Family: Toxoplasmatidae

Genus: *Toxoplasma*

Species: *gondii*

2.3 Morphology

Dubey *et al*.,(1998)explained that *T.gondii* has three infectious stages including sexually produced oocyst that have sporozoits, rapidly dividing tachyzoites, and slow multiplying bradyzoites in tissue cysts.

2.3.1 Oocyst

The oocyst when be unsporulated found in the intestine of the final host (Felidae) and have subspherical to spherical shape, 10 x 12 µm in diameter the wall of it contains two colorless layers, Micropyle, polar granules are absent, also the sporont (which is a mass of cytoplasm and nucleoplasm)

almost fills the oocyst, then sporulation occurs outside the cat (Dubey and Thulliez ,1993).

During sporulation the sporont divides into two round masses called sporoblasts, then sporoblasts elongate and differentiate to form sporocysts each of the sporocysts measures 6 x 8 μm that divides by endodyogeny to four sporozoites (Dubey and Frenkel, 1972; Ferguson *et al*., 1979).

A study conducted by Lindsay *et al*., (2002) demonstrated that unsporulated oocysts can survive in the environment at 4°C and retain their ability to sporulate for at least 3 months, Sporulated oocysts more resilient than unsporulated oocysts, also Sporulated oocysts are resistant and can survive in soil for several months and can resist the stomach juices (Mims *et al*., 2004).

Jones and Dubey, (2010) mention that oocysts can remain infectious for as long 18 months in water or warm moist soils, the term oocyst may be confused with the terms tissue cyst, but should remember the tissue cyst develops as a result of nonsexual phases of endodyogeny in the extra intestinal tissues whereas the oocyst develops as a result of sexual processes in the feline intestine .

2.3.2 Tachyzoite

The term "tachyzoite" is derived from (tachos=speed in Greek) and it is the rapidly multiplying form of the parasite (Frenkel , 1973) also called trophozoites proliferating stage or endozoites, which can be seen during acute stage of toxoplasmosis, its Measure approximately (4-8 μm) in length and (2-3 μm) in width and it has a typical curved shape with a conoidal anterior end and a rounded posterior (Dubey, 2004).

Tachyzoites have no visible mode of motility (flagella, cilia or pseudopodia) but they can glide, rotate, undulate and flex, it can invade cells by phagocytosis or active penetration through the host cell plasma lemma (Chiappino *et al.*, 1984). Tachyzoite after entering the host cell, becomes ovoid in shape and surrounded by a parasitophorous vacuole (PV) then multiplies asexually within the host cell by repeated endodyogeny, its obligate intracellular forms and can invade, multiply within all mammalian cells except erythrocytes, also can be found free in blood in heavy infections (Lopez, 2010).

2.3.3 Tissue cyst and the Bradyzoites

As for with regard to the bradyzoites the term "bradyzoite" (brady = slow in Greek) was mention by Frenkel (1973) and it is used to characterize the organism multiplying slowly within a tissue cyst by repeated endodyogeny, it's also called a cystozoite, and can observed during chronic stage of the disease (Tobin *et al.*, 2010) .

Dubey and Frenkel,(1976) were the first who made in-depth study of the development of the tissue cysts and bradyzoites in meat of intermediate host . (1993) Dubey revealed to the presence of tissue cysts in beef and size of its, as well explain the numbers of bradyzoites dependent on type of host cell parasitized, cyst age, strain of *T.gondii*, and the cytological method used for measurement, as well refer to young tissue cysts contain two bradyzoites surrounded by a special cyst wall and measure about 5 µm in diameter in addition he explain that tissue cysts in myocytes are two to three times longer than those found in neural cells, in general tissue cysts size usually range between 5 and 50 µm in diameter.

Miller *et al*; (2009) they said that tissue cysts can be found in many organs, but are common in skeletal muscle, myocardium and the central nervous system (CNS).

Petersen and Dubey, (2001) describe the size of bradyzoite which approximately 7µm x 1.5 µm, and its differs only slightly from the tachyzoites, they are more slender than tachyzoites, their nucleus located extra to the posterior end compared with tachyzoites, also its contains higher amount of polysaccharide so it can give rise positive reaction with periodic acid shift (PAS) and its resistant to gastric digestion whilst tachyzoites are usually destroyed by the acid and proteolytic enzymes of the stomach (Tenter, 2009).

Dubey *et al*;(1990) show that tissue cysts are relatively resistant but this resistance is affected by many factors, and its stay infectious in refrigerated meat for more than 3 weeks, tissue cysts are usually killed directly by heating to 67°C and its can remain viable at 60°C for about 4 min and about 10 min at 50°C. (Dubey, 2004).

While El-Nawawi *et al*; (2008) refer to the Freezing meat at -10°C for 3 days or -20°C for 2 days and the treatment with gamma irradiation at a dose of 75 krad is sufficient to kill tissue cysts.

Lunden and Uggla, (1992) they said that the time is necessary to kill bradyzoites in the meat by heat will depend on the thickness of the meat and the homogeneity of the cooking method, microwave cooking in this aspect unreliable for killing *T. gondii* tissue cysts.

2.3.4 Sporozoites

This stage of the parasite residing within oocysts, When a human or other warm-blooded host consumes an oocyst, sporozoites are released from it and infecting epithelial cells before converting to the tachyzoite stage (Louis and Kami, 2011a).

sporozoite is similar to the tachyzoite, its size about 6-8 μm and different from tachyzoite in micronemes, rhoptries, and amylopectin granules that found as massive amount in the former which can facilitate the attachment with host cells, invasion and generation of the parasitophorous vacuole, (Tenter *et al*., 2001).

2.4 Life Cycle

Louis and Kami, (2011b) describe that the life cycle of *T. gondii* consists of two stages, sexual stage (Intestinal stage) that occurs only within felids wild or domestic which are considered as definitive host and an asexual stage (extra intestinal stage) that can occur within all warm-blooded animals, including humans, cats, and birds which are considered as intermediate host:

a-definitive host cycle

definitive hosts(cats) becomes infected with *T. gondii* in three way first ingestion oocyst that contaminated cat feces, second ingestion tissue cyst harbored by infected prey (mice, rats, rabbits, squirrels) and the third way ingestion tissue cyst in infected meat piece that feed it by their owner(parasitic disease).

Viable organisms (bradyzoites) infection units are release from rupture tissue cyst and invade epithelial cells of the small intestine where they undergo an asexual development (schizogony) schizonte formation then merozoite after that followed by a sexual development and reproduction (gametogony or sporogony) (Dubey, 2006).

After 3 to 15 days micro gametes and macro gametes will develop in the small intestine especially in the ileum, microgametes have 2 flagella for swimming to mature macrogametes and fertilize it to formation Zygote (Speer and Dubey, 2005), then an oocyst wall is formed and the Infected epithelial cells will eventually rupture and release millions of thick-walled zygote-containing cysts known as oocysts into the intestinal lumen ,which are excreted with the fecal mass of the cats to the environment and sporulation occur within 1 to 5 days depending on the temperature and surrounding aeration (Ruelhmann, 2010).

b-Intermediate host cycle

Intermediate hosts infected by sporulated oocyst which is contain sporozoites the wall of oocyst will dissolved by proteolytic enzymes in the stomach and small intestine freeing sporozoites that will invade cells in the intestinal epithelium and inside these cells, the sporozoites differentiate into tachyzoites, the motile and quickly multiplying cellular stage of *T.gondii,* in host cells the tachyzoites multiply inside specialized vacuoles (parasitophorous vacuoles),after that the host cell dies and ruptures releasing and spreading the tachyzoites via the bloodstream or lymph this leading to development of an acute disease (Parasitemia), tachyzoites making it way and reach to different tissues of the body but the normal immune response will force it to

transformation it into bradyziote in the tissue cyst, limit the acute stage ,and establish a chronic infection, over time the tissue cyst becomes bigger in size and rupture to infected another cells unless the immunity Curb it (Dubey *et al.*, 2011), in addition intermediate host can get infection from another intermediate host flesh contaminated with tissue cysts this way of transmission is common among carnivores and scavengers (Dubey *et al.*,2002)(Figure2.1)

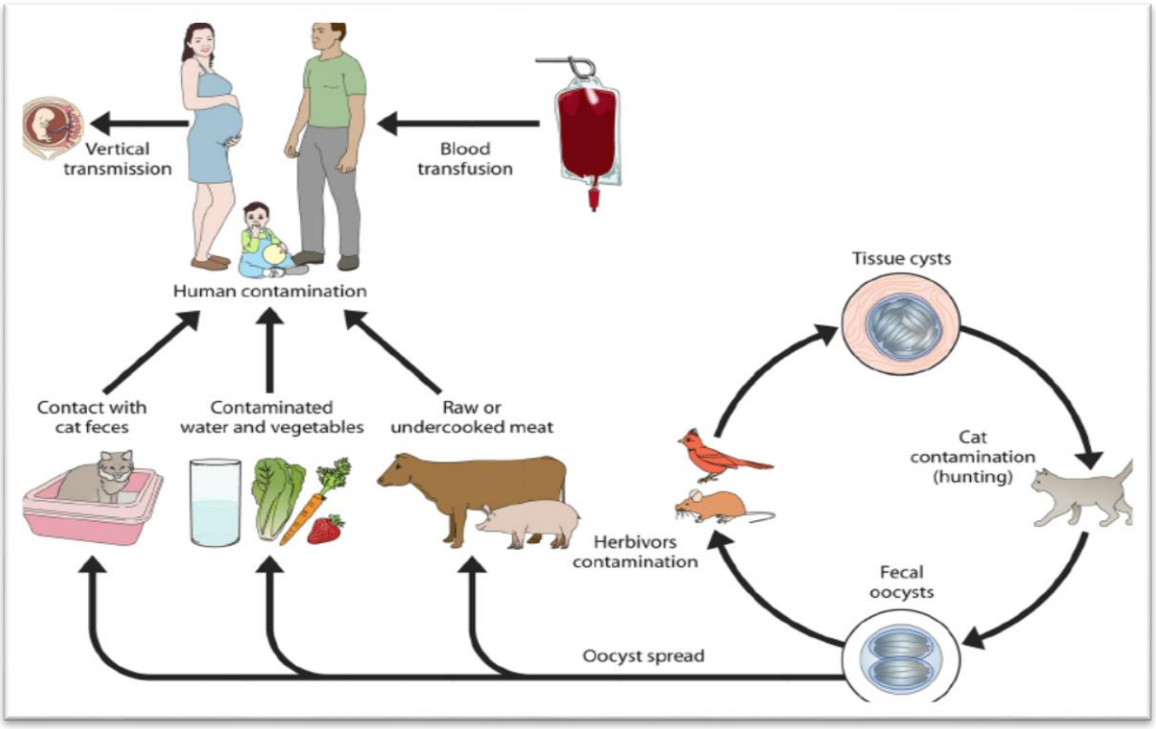


Figure (2.1) Life cycle of *Toxoplasma gondii*(Dubey *et al*.,2009).

2.5 Methods of transmission:

A-Infection with cat's faecal oocysts (Horizontal transmission routes) cats play an important role in the spread of toxoplasmosis, and it's fecal matter is particularly dangerous just one cyst consumed by a cat can result in thousands of oocysts and spread it in anywhere (Dubey *et al.*, 2005).

Infection occur by accidentally ingesting of this sporulated oocysts that contaminated environment , like eating unwashed fresh produce, drinking untreated contaminated water, eating shellfish grown in contaminated water or accidental ingestion of oocysts after touching or ingesting anything that has come into contact with a cat's feces that contain toxoplasma (Abu-Madi *et al*., 2010).

B- Infection from tissue cysts containing bradyzoites (Foodborne transmission) Infected meat plays a critical role in *T. gondii* transmission to humans and animals because of *T. gondii* can be present in meat animals ,this method occur by consuming raw or undercooked meat or by accidentally consuming tissue cysts after handling raw meat and not washing hands completely, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat(Mainar-Jaime and Barberan,2007).

C-Transplacental transmission from mother to fetus (the vertical method), a woman who is newly infected with *T.gondii* during pregnancy can pass the infection to her unborn child ,The woman may be not have symptoms but there can be severe consequences for the unborn child from this complications may result in prenatal mortality or birth defects such as diseases of the nervous system and eyes. (Tenter *et al*., 2000).

D-Organs transplant transmission(Rare instances of transmission) Derouin and Pelloux, (2012) demonstrate that recipients can infected with toxoplasmosis due to transmission of the parasite with the transplanted organ from a toxoplasma-seropositive donor to a toxoplasma-seronegative recipient and they mention the heart transplantation is the most common type of organ transplantation transmission the parasite as cysts form in the cardiac muscles ,

However its uncommon case, also they explain Possibility of the parasite transmission by blood transfusion and hematopoietic stem cell transplantation this occur only when the donor was recently infected with *T. gondii* and so had tachyzoites present in their blood and bone marrow.

2.6 Epidemiology

Gaetano, (2010) mention that toxoplasmosis is high incidence infection Infect 500 million person around the world with range about 12% - 90% and this ratio increasing with some factors like low education, crowding, age, sanitary habits, ethnic considerations, consumption of undercooked meat, socioeconomic and animal contacts, also climatic factors are very important for oocyst survive in moist, warm soil than dry, hot or arid regions (Petersen, 2007), in another side increasing this ration belongs to the presence of cats in homes and used in the biological struggle of rodents (Dabritz *et al*., 2008).

Montoya and Liesenfeld ,(2004) refered to that immunocompromised individuals and pregnant women have more effected for infection with *T.gondii* and it cause dangerous problems in both , a survey of 99 studies within 44 countries for women of childbearing age found that the areas of highest prevalence are within Latin America it's about 50–80%,parts of Southeast Asia about 20–60%, parts of Eastern and Central Europe about 20–60%, the Middle East about 30–50%, and parts of Africa about 20–55%. (Pappas *et al*.,2009).

Tenter *et al*., (2000) show that the infection with *T.gondii* in cattle ranges from 2 to 92% depend on the reports while Dubey and Jones, (2008) mention that seropositivity rates fall in older animals in spite of high rates of

seropositivity reported in some studies and the parasite has been detected very rarely in adult cows and in aborted fetus.

Opsteegh *et al*., (2011) they said that higher infection rates are seen in calves during their first grazing season so this indicating that calves become infected after exposure to Toxoplasma on pastures as well they refer to that the Serological studies in cattle are not easy due to the several tests that are routinely used for human diagnosis have a too low sensitivity and specificity for cattle sera so that will lead to a low discriminative power.

In case of present of *T.gondii* in meat Cook *et al*.,(2000) they said that any meat from warm-blooded animals or birds considered as major source of Toxoplasma infection and the risk associated with the type of meat (lamb, beef, and pork, etc.) this varies among countries according to local eating habits and the prevalence in meat-producing animals in this country (so that mean the rate of infection will vary from one country to another also type of meat).

As well Dubey, (2009) explained that eating raw or undercooked meat even viscera or dealing with fresh meat considered as a potential risk for transmission of *T.gondii* to humans and in many countries this way of transmission considered the major route of infection with *T.gondii*, in Europe a multicenter make study about this case; found meat consumption was responsible for 30 to 63% of cases of infection, while soil contact represented 6 to 17% of cases (Cook *et al*.,(2000).

Also study by Cook *et al*., (2001) in Norway show that raw or undercooked meat consumption responsible for toxoplasmosis in pregnant women and 50 % of congenital toxoplasmosis from this method of infection.

Jones *et al*., (2009) in a case-control study in the United States involving 148 infected adults found that consumption of ground beef and eating locally produced cured or smoked meat were all associated with *T. gondii* infection, as well in another study involving 131 mothers found that 50% of this mothers had given birth to children infected with *T. gondii* and they remember having eaten uncooked meat (Boyer *et al*., 2005).

in Chile Muñoz-Zanzi *et al*.,(2010) referred to57% of *T.gondii* infections in pregnant women it was because of consumption cyst-contained meat, while 43% of infections due to exposure to the oocysts in the environment, in another study by Guo *et al*.,(2015) they found the proportion of infections attributed to eating salami was 10–14% in Milan.

Dubey *et al*., (2005) they mention that the big problem in quantitative assessment of the risk of toxoplasma in meat for consumers It is in the lack data of the number of tissue cysts that can cause infection of humans, the distribution and the number of tissue cysts in the different sites of the body in and various hosts but they Confirms the major risk to consumers is come from products purchased in the form of fresh meat .

2.7 pathogenesis and clinical signs

The pathogenicity of *T.gondii* depend on many factors like the strain of it, age sensivity of the host, infected dose, degree of host acquired immunity, and virulence of the parasite (Dalgic,2008**),** The clinical signs in some hosts:

2.7.1 In cats

Most cats that infected with *T.gondii* show asymptomatic disease, usually when immunity system of the cat cannot stop spread of infection in the body

especially tachyzoite form, the disease more likely to appearance in cats with immune suppression, cats with feline immunodeficiency virus (FIV),and cats that have feline leukemia virus(FLV) in this case the sings of *T.gondii* infection are fever, loss of appetite, and lethargy the other signs will depend on the infection(acute, chronic), also the location of the parasite in the body will specifies the type of signs such as when found in lung the infection cause pneumonia lead to difficult breathing when infection occur in liver yellowish tinge appear in the skin and mucous membrane also *T.gondii* can infect the eyes and CNS and cause inflammation of uvea, pigmented retina and the space between lens and cornea abnormal pupil size, blindness and lack of coordination, another changes can occur like head pressing, circling, ear twitching(Taylor, 2007).

2.7.2 In human

Congenital toxoplasmosis occur when the infection transmitted from mother to her fetus during pregnancy 15% the chance of transmitted the parasite to the fetus in the first three month,30 % in the second three month and about 60% in last months of pregnancy and this infection will cause Miscarriage, Stillbirth, or congenital defect at birth like Trabismus, Chorioretinitis, blindness, Mental retardion, Hydrocephalus, Encephalitis (Many and Koren, 2006; Ross *et al* .,2006).

Ocular toxoplasmosis the infection in eye can cause permanent loss of vision in 25% from patients(Bhopale,2003), the ocular lesions can appear in any age in un treated cases also some treated cases, and the severity of the disease effected by trimester in which infection was acquired from mother(Lihleh *et al*., 2010).

Immunocompromised patients especially ADIS and HIV patients will suffer from inflammation of iris, Encephalitis, pneumonia ,inflammation in the intestine , sensory abnormalities, the infection usually came from reactivation of latent *T. gondii* acquired previously(Neves *et al.,* 2009).

2.7.3 In cattle

Calves are more susceptible to infection with *T.gondii* than adults the main clinical signs that appear when the infection occur orally are anorexia, diarrhea, poor weight gain, weakness, depression, fever and dyspnea in some cases show only lymphadenopathy, calves with congenital affected show cought, sneezing, some neurological signs, and fever, when the adults infected with *T.gondii*, the main symptoms are fever, dyspnea, nervous signs, and lethargy, Stillborn calves and neonatal deaths can be seen,(Dubey,1998).

2.8 Immunity against *T.gondii*

Filisetti and Candolfi,(2004) mention that immune system play important role to control on *T.gondii* infection in the body of host by innate immunity and adaptive immunity, the infection will activation of humoral and cell-mediate immune response.

Suzuki *et al*.,(1988) explain that cellular immunity is the main type for control to the infection because of *T.gondii* is intracellular parasite a combination with antibodies also they detected the Interferon gamma (INF -γ) is the main mediators to resistants toxoplasmosis.

Macrophage, Natural Killer cells (NK) , dendritic cell (DC) they release important cytokines against the parasite like Tumor necrosis factor (TNF-α

),Interferon gamma(INF -γ), Interleukin -12 (IL-12) they are play role in regulation of immune response (Alexander and Hunter,1998).

Suzuki *et al*.,(1989) refer to that the important role of (INF –γ) in activation of macrophages and cytotoxic T-cell to destroy *T.gondii* also they remember its role in recurrence of the disease by preventing cyst rupture.

Dziubek *et al*., (2001) they explain the humoral immunity response which represent by antibodies formation from B-cell such as IgM, IgG, IgE, IgA, which play important role with complement to remove the parasite from the body fluids, called Antibody-dependent cell mediated cytotoxicity (ADCC), IgA appear in the first stage of invasion which represent about 80% from immunity of intestine mucus membrane it is release from intestine cells (Villena *et al*.,1999).

Jenum and Stray-Pedresen, (1998) mention if the parasite access to distribution in the body of the host IgM will appear after 1-2 week from infection and reach to less level in three month, while IgG will appear after 2-3 week from appearance of IgM reach to top level in 1 -2month, it is stay along life with differ rates (Pelloux *et al*., 1998).

Because of *T.gondii* an obligate intracellular parasite ,cell-mediate immunity will play the main and important role to control and destroy the parasite (Miller *et al*., 2009), in this type of immunity lymphocyte cells (T- cell) will take the main role, IL-12that release by macrophage and dendritic cells will play important role in differentiation of T-cell to T-helper 1 (Th1) which release (INF –γ) ,(TNF-α) to activation eosinophil cytotoxicity through acute infection in addition inhibition of tachyzoite replication to convert it to bradyzoite form(Lambert *et al*., 2006), and Th2 cells will produce IL-4, IL-5,

IL-10, and IL-13 it have regulation role in inhibition of cytokines work.(Butcher *et al*., 2005).

2.9 Diagnosis

Because of the clinical signs of *T.gondii* infection are non-specific and its impossible diagnosis of the infection by their, thus the diagnosis made by laboratory methods such as serological, histological, and molecular methods or combination from them(Hill and Dubey, 2002).

2.9.1 Microscopic diagnosis

Its traditional method to detection of *T.gondii* infection in fecal material, water and tissue samples, however the diagnosis by light microscope is less sensitive and we can't depend on it alone, in fecal material and water the oocyst can be detected from large amounts of samples by filtration or centrifugation methods and impression method for examination of tissue cyst and can be stained by Giemsa and Haematoxylin and Eosin (HE) stain, it's simple and cost-effect and usually used for this purpose (da Silva PC *et al*., 2010).

These methods are may take along times and need high skill to give true results Electron microscope is can be used for diagnosis but it is difficult to be usable for routine use (Sims *et al*.,1989),

for detection tachyzoite in blood or CNS fluid by centrifugation and take The deposit fixed on slide then stained by Giemsa stain and examine it by oil lens (Paniker,2002).

2.9.2 serological methods

There are many serological tests for *T.gondii* diagnosis it is depend on detection of antibodies especially IgG, IgM amount ,due to the level of them will rise within 2 weeks from exposure to parasite ,increase level of IgG indicated that a patient has been exposure to parasite but don't distinguish between new or recent exposure and past exposure due to IgG Will found throughout the life but in low level (Jones *et al*., 2003).

Lopez *et al*., (2000) refered to that in case of increase IgM that indicated to an acute exposure and the degree of elevation can be used to recognize when the exposure occur:

A- enzyme-linked immunosorbent assay (ELISA)

This test used for first time in Holland by Van-Waeman and schurs in 1971 after that in Sweden by Engavell and Perlamann in 1972 this test consider famous, sensitive, and the most used test for *T.gondii* diagnosis used for detection of increase titers of the IgM, IgG, IgA, IgE, antibodies in individual (Hill *et al*., 2006), ELISA-IgM test that use IgM immunoglobulin in high sensitivity and specifity for Detection an acut phase of toxoplasmosis while ELISA-IgG for Detection a chronic phase of infection also detection of IgE in the serum of adults and new born in indicated to new acquired infection (Salman and Juma, 2009)

B- Sabin- Feldman test (Dye test)

In 1948 DT test first develop by Sabin and Feldman and it is consider as gold standard test to detection of anti- *T.gondii* antibodies in humans this test is specific and sensitive in human but not in cattle and avian specie, Ozkan *et al*.,

(2008)they explain that the test is need to a live parasite and healthy serum , also a high degree of technical expertise, as well incubation period with methylene blue dye, thus only performed in reference laboratories, it is hazard test for human ,methylene blue dye will detect *T. gondii* antibodies in serum, while the cytoplasm of the tachyzoites leaks out if the targeted antibody is present, which prevents tachyzoites from being stained, thus, a positive titer is recorded when 50% of the tachyzoites remain unstained.

C- Modified Agglutination test (MAT)

Fulton and Turk, (1959) was first discovered this test , used tachyzoite of *T.gondii* formalin-fixed, in U-shaped micro titer plates then add sera, the thin mat of agglutination indicator to positive serum samples while the compact pellet of precipitated tachyzoites at the bottom of the well indicator to negative serum samples .

this test is simple, easy, don't need to developed equipments, and the commercial Kit is available which preferred on ELISA to detection of *T.gondii* infection, it is also need less time and labor(Shaapan *et al*., (2008).

Seefeldt *et al*.,(1989) mention that MAT test detect only IgG antibodies against *T.gondii* because of using mercapethanol which destroy IgM antibodies, the results of this test depend on the keeper used to prepare the antigen In acute infection, MAT can detect IgG antibodies when used acetone instead of formalin which is very useful in diagnosis of toxoplasmosis in AIDS patients, and acute glandular toxoplasmosis (Montoya *et al*., 2007).

D- Latex agglutination test (LAT)

This test used for detection IgM, IgG antibodies that specific to *T.gondii* infection (Gamble *et al*., 2005), the latex particles that made from polystyrene and coated with antigen, the reaction allow visual observation of antigen-antibody complex It's inexpensive ,easy, and sensitive to use as screening test(Holliman, 2003).

2.9.3 polymerase chain reaction (PCR)

There are many polymerase chain reaction (PCR)-based assays have been developed to detection DNA of *T.gondii*, conventional PCR it is efficient enzymatic amplification method that make specific amplification of DNA from very small amount in short time, it is high sensivity and give several multicopy from the target genes (Saiki *et al*.,1988), the main target genes for *T.gondii* is *B1* gene which since 1989 used for detection *T.gondii* (Burg *et al*., 1989).

Another target genes are found like internal transcribed spacer (ITS-1) , 18S rDNA SAG1, SAG2, and GRA1 Savva *et al*., (1990),this method can be detect *T.gondii* DNA in different samples like blood, tissue ,fluid, It has been successfully used for diagnosis(Filiselti *et al*., 2004).

Nested PCR used widely to detect *T.gondii* in which are used two sets of primers in two successive PCRs the product of first reaction is used as template to the second reaction of PCR this test consider more sensitive than conventional PCR, also based on B1gene, and ITS-1 sequences (Jones *et al*.,2000).

2.10 Treatment

Most people those infected with toxoplasma and do not show any clinical signs they will treated their self (self-limiting) but the people who suffering from serious health problems must be treated immediately it is imported to stop replication of the parasite and prevent another damage in organs especially that occur in eyes which suffering from irreversible damage the retina and optic nerve and that can cause permanent blindness, the drug of choice to prevent toxoplasmosis is trimethoprim and sulfamethoxazole,and at 2012 a new study showed acute and latent infection of toxoplasma can treated by two endochin-like quinolones (Doggett *et al*., 2012).

A-Pyrumethamine : this drug interfere with purine synthesis pathway like sulfadiazine so we can used them in combination to give synergism effect against toxoplasmosis folic acid must be given with this combination to prevent incidence of thrombocytopenia.

B- spiramycin is effected for prevent of congenital defect of *T.gondii* in pregnant women(Thiebaut *et al.,* 2007).

C- Clindamycin is the drug of choice for treatment cats with toxoplasmosis, this drug is high absorbed and diffused well in CNS and it is rarely used in pregnant women due to it can enter fetal blood (Cox *et al.,* 2005).

If pregnant woman is infected with *T.gondii* amniocentesis should be done to ensure or determine whether the fetus has been infected or not, the chance of tachyzoite reaching to placental tissue is approximately a 30% and from their can reach to fetus and infected him at time of infection when gestational age increased the chance of fetus infected increase (Robert and Darde 2012).

Spiramycin is useful to prevent transmission of infection to fetus only in case of the parasite didn't reach to him yet, and in case of the infection reach to the fetus the woman can treated with pyrimethamine and sulfadiazine combination with folic acid but should remember that pyrimethamine has antifolate effect thus the treated given after first trimester and we know lack of folic acid will interfere with fetus brain formation and may cause thrombocytopenia (Jones *et al*., 2003).

2.11 Control and Prevention

Verma and Khanna, (2013) mention that because it is there is on effective vaccine for human against *T. gondii* so for prevention of transmition of disease should be decrease exposure to sources of infection (tissue cyst and oocyst), so the following points are recommended to prevent or reduce the infection chance with *T.gondii*.

A-From food

practices of basic food handling safety which can prevent or reduce the infection such as perfect washing of fruits and vegetables and must be avoid eating a raw and under cooking meat, as we mentioned earlier *T. gondii* is typically transmitted through cysts that found in the tissues of infected animals thus meat should be properly prepared on one hand cooking methods and on the other hand freezing temperature, freezing meat at (0°F or -18°C) for several days before cooking can destroy tissue cysts and during cooking the temperature have to (145°F,63°C) after cooking a rest period of 3 min should be allowed before consumption(Green and Aliza, 2005),in addition in case of dealing with meat should be washing hand with soap before done another tasks (Lopez *et al*.,2000).

B-From environment

Cat litter should be disposed daily this will reduce the chance of infection with oocyst significantly because of oocyst need days for sporulate and become infectious after shed, the infectious oocyst in cat's feces can spread and survive in the environment for many months so human should be wear gloves when dealing with or gardening with soil and have to wash their hands, also pregnant woman should be away from contact with cats and their litter ,food, and its prefer cats stay outside the house (Foulon *et al*.,2000).

In addition spreading health and cultural awareness about the risk of disease in areas that toxoplasma infection are spread (Yasodhara *et al*.,2004),precautions should be taken to reduce pasture contamination with cat's feces (Bowman *et al*., 2002).

Chapter Three
Materials and Methods

3.1 Materials

3.1.1 Equipments and instruments

Table(.31) Equipments and instruments used in study

NO.	Equipments and instruments	Manufacturer	Origin
1	Plastic Containers	Inc.	China
2	Surgical instruments	Anatomia	Pakistan
3	Sterile test tube	Superstar	India
4	Cylinder	BBL	USA
5	Glass Beaker and flask	BBL	USA
6	Incubator	Mammert	Germany
7	gauze	Kardelen	Turkey
8	Centrifuge	Universal 16A	Germany
9	Slides	Sail Brand	China
10	Cover slides	Citoglass	China
11	Gloves	Broche	Malaysia
12	Refrigerator / Deep freezer(20°C)	Concord	Lebanon
13	cotton	kardelen	Turkey
14	Disposable pipettes	Deans gale	China
15	Disposable Tips	Bio basic	Canada

16	Hot plate Stirrer	Witeg	Germany
17	Light Microscope	Olympus	Japan
18	Petri Dish	Plasti-lab	Lebanon
19	High Speed Cold centrifuge	Eppendorf	Germany
20	Disposable syringe 3ml	Sterile EO.	China
21	Water bath	Mammert	Germany
22	Vortex	CYAN	Belgium
23	Thermo cycler PCR	Bio-Rad	USA
24	UV Trans illuminator	ATTA	Korea
25	Gel electrophoresis	Shandod Scientific	UK
26	Digital camera	Samsung	china
27	Nano drop	Thermo	U.K
28	Sensitive Balance	Sartorius	Germany

3.1.2 Chemical Materials

Table(3.2) chemical Materials used in current study

NO.	chemical Materials	Manufacturer	Origin
1	Absolute Ethanol	BDH	England
2	TBE buffer	Bio Basic	Canada

3	Free nuclease water	Bio lab	USA
4	Agarose	Bio Basic	Canada
5	Ehidium Bromide	Bio Basic	Canada
6	DNA marker ladder (1500bp)	Promgam	USA
7	Proteinase-K	Bio Basic	Canada
8	Normal saline	Locally	Iraq
9	Hydrogen Chloride HCL	Gainland chemical company	UK
10	Pepsin	Gainland chemical company	UK
11	Sodium Chloride NaCL	Kedah, Sungai	Malaysia
12	Sodium bicarbonate	Kedah, Sungai	Malaysia

3.1.3 Primers

The PCR primer for detection *B1* gene of *T. gondii* were design by pervious study (Ortega-Pacheco *et al*., 2013).This primer was provided from (Macrogen company, Korea) as following table:

Table(3.3) The primer sequence that used in the study

Primer	Sequence		Amplicon
*B1*gene	F	AAAAATGTGGGAATGAAAGAG	469bp
	R	ACGAATCAACGGAACTGTAAT	

3.1.4 kits

Table (3.4) The kits used in current study

NO.	Kit	Company	Origin
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		

3.1.5 Solutions

Table(3.5) the solutions that used in study

NO.	Solutions	Chemical material	Quantity
1	Peptic digestion solution. For(500ml)	pepsin	2.6 g
		NaCl	5g
		HCL	7 ml
		Distilled water	485 ml
2	Sodium bicarbonate solution. For(500ml)	NaHCO3+distilled water	6g+500ml

3.2Methods

3.2.1 Samples collection

This study was conducted during period from September 2017 to May 2018 in AL- Diwaniyah province for evaluating the rate of *T.gondii* infection in local, imported meat and meat products in cattle, Three hundred samples were used in this study which collected from heart, tongue, muscles, of 100 slaughted local and imported cattle their ages ranged from months to four years, in addition to 90 samples of different local and imported meat products (beef burgers, minced meat).

3.2.2 Isolation of *T.gondii* bradyzoits

Firstly meat samples were subjected to a process called peptic digestion method to isolate toxoplasma bradyzoites from the examined tissue to prepare it for DNA extraction process, the idea of using this method is Provide suitable environment to lysis of tissue cyst for liberation of bradyzoite(and the method as follows:

A- Approximately 50 g of meat from each sample was Cut by scissors for a very small pieces then put in sterile test tube.

B- Ten ml from digestive solution was added to the tube and mixed well then put in the incubator at 40°C for 30 minutes.

C- The digestate was poured through two layers of gauze into a flask and then the filtrate was put in test tube and centrifuge, (5000 rpm for 10 min).

D- The supernatant was discarded and 5ml of sodium bicarbonate solution was added to precipitate then mixed well and re centrifuge, (5000 rpm for 10 min).

E- The supernatant was discarded again and the Precipitate was suspended in 600 µl of normal saline and mixed well.

F- kept at 4◦C until used in DNA extraction.

3.2.3 Genomic DNA Extraction

Genomic DNA were extracted from each prepared samples of meat and meat product samples, and extracted done by gSYAN DNA extraction Kit (Geneaid, USA), depending on the company's instructions As the following :

A- Two hundreds µl of the meat digestate suspension were transferred to a 1.5 ml epindorf tube, then 20 µl of proteinase K were added and mixed by pipetting after that incubate at 60◦C for 5 minutes.

in case of meat products samples 100 mg were taken from each sample and transferred directly to sterile 1.5ml epindorf tube then 200 µl of GST Buffer and 20 µl of Proteinase K were add to the samples with vortex ,after that incubated at 60℃ for 30 minute.

B- 200 µl of GSB Buffer were added to each sample mixed and shake vigorously by vortex, after that incubated at 60◦C for 5 minutes with inverting the tubes every 2 minutes.

In case of meat product samples, the incubated samples were centrifuge for 2 min. at 14000rpm, then the supernatant transfer carefully to a new 1.5 epindorf tube and 200 µl of GSB Buffer were added to each one and mixed by

vortex vigorously, incubated at 70◦C for 15 min, and inverted every 3 minutes through incubation periods.

C-200µl absolute ethanol were added to the samples lysate(meat and meat product) then mixed and shake vigorously by vortex immediately for 10 second GS columns(DNA filter column) was placed in a 2 ml collection tube and transferred all of the mixture (including any precipitate) to the GS column, after that 2ml collection tubes that containing the flow-through discard and transfer GS columns to a new 2ml collection tubes.

D- 400 µl of W1 Buffer were added to the GS columns and centrifuge at 14000 rpm for 30 second then the flow-through was discarded , the GS columns was back placed in 2ml collection tubes and 600 µl of Wash Buffer was added to it then centrifuge at 14000 rpm again for 30 second also the flow-through discard Finally dryness step the GS columns was back placed in 2ml collection tubes without any addition and make centrifuge at 14000 rpm again for 3 minutes.

E- Dried GS columns transfer to a clean 1.5 ml epindorf tube and 80µl of pre-heated elution buffer were added to center of columns matrix then let stand for 3mint for complete absorption of elution, after that last centrifuge at 14000rpm for 30 sec to get purified DNA.

3.2.4 Estimation of Genomic DNA

Genomic DNA that extracted was tested by using Nano drop spectrophotometer (THERMO.USA), Which works on measurement the concentration of DNA(ng/ µl) and check the DNA purity through reading the absorbance at (260 /280 nm).

3.2.5 Polymerase Chain Reaction (PCR)

Conventional PCR was performed for detection *T. gondii* based on amplify the *B1* gene, this procedure was applied according to technique explained by(Ortega-Pacheco *et al.*, 2013) as following steps:

A- The preparation of PCR Reaction

By using (Maxime PCR PreMix kit)PCR reaction was prepared and this master mix achieved depending on company instruction as following table:

Table (3.6) PCR reaction construction

Components of PCR reaction	volume
DNA template	5 µl
Forward primer (10pmol)	1.5 µl
Reverse primer(10pmol)	1.5 µl
Free nuclease water	12 µl
Total volume	20 µl

Then, all these ingredients mentioned above are placed in standard PCR PreMix Kit which contains all the necessary materials for the reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2 stabilizer, and tracking dye) after that the PCR tubes transferred into Exispin vortex centrifuge at 3000 rpm for 3 min. Then placed in PCR Thermo cycler.

B- PCR Thermo Cycler Conditions

The conditions of PCR Thermo cycler done by using conventional PCR thermo cycler system. Table (7) :

Table (3.7)The PCR Thermo cycler constructs reaction conditions of *B1* gene.

NO.	PCR	Temp.◦C	Time	Repeat
1	Initial Denaturation	95◦C	5 min.	
2	Denaturation	95◦C	30sec.	30 cycle
	Annealing	56◦C	30sec.	
	Extension	72◦C	30sec.	
3	Final extension	72◦C	5 min.	1
4	Hold	4◦C	Forever	-

C-PCR product analysis

The PCR products of *B1* gene was analyzed by agarose gel electro-phoresis as following steps:

1- 1% agarose gel was prepared by dissolved 1gram of Agarose in100 ml of 1X TBE in water bath at100°C for15 minutes, then left to cool at 50°C.

2- three µL 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 and left to solidified for 15 minutes at room temperature, after that the comb was removed gently from the tray and 10µl of PCR product were added in to each comb well and 10 µl of (100-1500bp 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- PCR products(469) for B1gene were visualized by using UV light Trans - illuminator.

3.2.6 Detecting of *T.gondii* strains

For detecting of *T.gondii* strains ,PCR products of Ten positive samples were sent for DNA sequencing by AB DNA sequencing system, to Bioneer Company in Korea .

3.2.7 Statistical Analysis

All data of this study were calculated and statistically tested by SPSS program Using Chi-square distribution (X^2) where the probability value was calculated as the following:

$P<0.05$ the data is statically significant

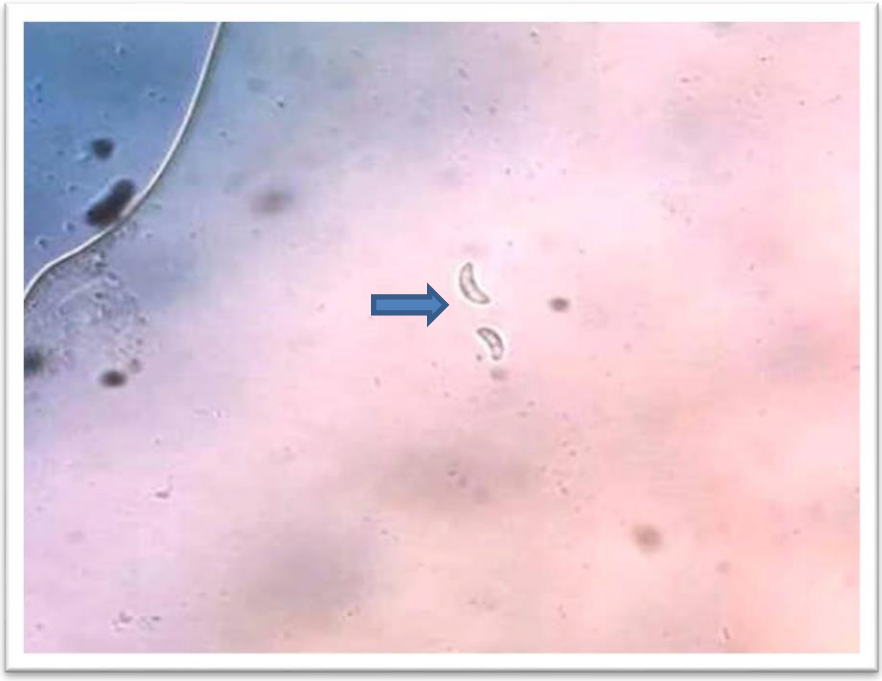
$P<0.01$ the data is statically high significant (Leech *et al*.,2011).

Chapter Four

Results

4.1 Microscopic Examination of *T.gondii* bradyzoites

T.gondii bradyzoites were seen when examined a drop of tissue digestate by peptic digestion method microscopically (Fig.4.1).



Figure(4.1): bradyzoite of *T.gondii* isolating from local meat sample (40X)

4.2 Molecular results of *T.gondii* infection in meat

4.2.1Molecular results of *T.gondii* infection in local meat

Out of 150 examined local meat samples taken from (50 slaughted cattle), 33 (22%) gave a positive result (Table 4.1, Figure 4.2).

Table (4.1) show molecular- positive result of *T.gondii* infection in local meat.

Examined No.	Positive No.	Percentage (%)
150	33	22

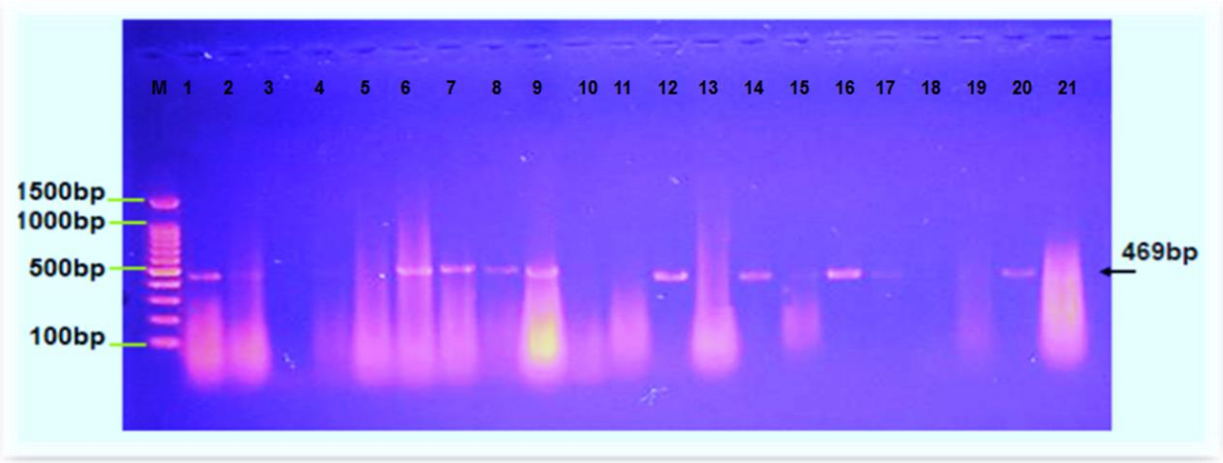


Figure (4.2): PCR analysis result of *B1*gene from *T.gondii* infected local meat,(M:marker,1,2,6,7,8,9,12,14,16,17,20, represent positive samples at bp469).

A-Molecular results of *T.gondii* infection in local meat according to organs.

The result of infection with *T.gondii* according to the organs revealed that muscle samples gave the highest positive result 15/48 (31.25%) while the heart samples recorded the lowest result 8/55(14.54%) Table (4.2).

Table (4.2): molecular results of *T.gondii* infection in local meat according to organs.

Organs	Examined No.	Positive No.	Percentage (%)
Muscle	48	15	31.25
Tongue	47	10	21.27
Heart	55	8	14.54
Total	150	33	22
X2	4.189*		
P value	0.123		

X2:chi-square

*No significant difference at p< 0.05

B-Molecular results of *T.gondii* infection in local meat according to seasons.

The highest rate of incidence was observed in Autumn (30%) with significant difference at p < 0.05, rather than other studied seasons Table (4.3).

Table (4.3) :molecular results of *T.gondii* infection in local meat according to seasons

Months	Examined No.	Positive No.	Percentage (%)
Autumn (Sept.- Nov.	70	21	30
Winter (Dec.- Fab.)	40	4	10
Spring (Mar.- May)	40	8	20
X^2	6.061*		
P value	0.048		

X2 : chi-square

* significant difference at p < 0.05

4.2.2 Molecular results of *T.gondii* infection in Imported meat.

The study discovered that 20/150 (13.3%) of examined imported meat were infected with *T.gondii* (Table 4.4, Fig. 4.3) .

Table (4.4): Molecular-Positive result of *T.gondii* infection in Imported meat.

Examined No.	Positive No.	Percentage (%)
150	20	13.3

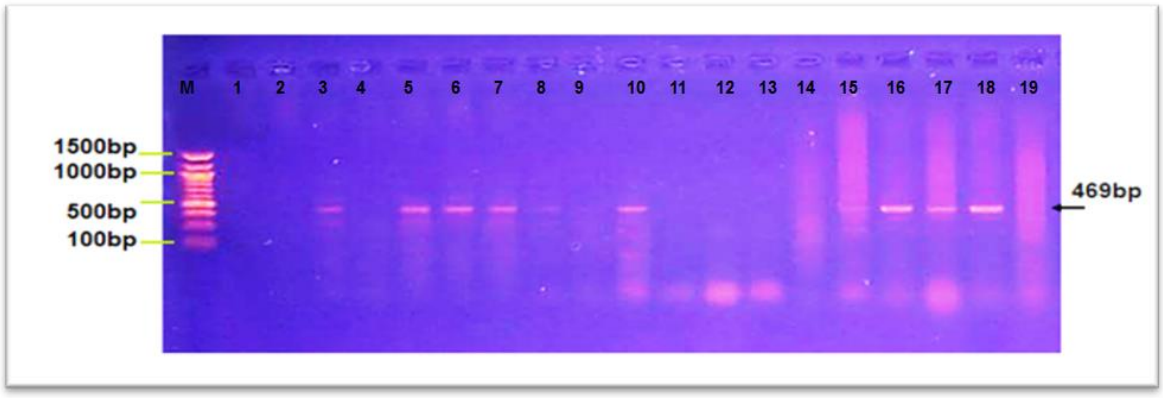


Figure (4.3): PCR analysis result of *B1*gene from *T.gondii* infected imported meat(M:marker,3,5,6,7,8,10,15,16,17,18,represent positive samples at 469bp.

A-Molecular results of *T.gondii* infection in Imported meat according to the organs.

The result of infection with *T.gondii* depended on the organs found that heart samples was the highest in infection with rate 9/50(18%) while the lowest rate was in Tongue samples with rate 4/46 (8.6%) Table(4.5).

Table (4.5): Molecular results of *T.gondii* infection in Imported meat according to the organs.

Organs	Examined No.	Positive No.	Percentage (%)
Heart	50	9	18
Muscle	54	7	12.9
Tongue	46	4	8.6
Total	150	20	13.5
X2	1.805*		
P value	0.406		

X2:chi-square

*No significant difference at p< 0.05

B- Molecular results of *T.gondii* infection in imported meat according to seasons.

In Autumn season the highest rate of infection was remarked (26.6%) whereas no infection was recorded during winter Table (4.6).

Table (4.6): show molecular results of *T.gondii* infection in imported meat according to seasons.

Months	Examined No.	Positive No.	Percentage (%)
Autumn(Sep. Nov.)	45	12	26.6
Winter(Dec.- Fab.)	66	0	0
Spring(Mar.- May.)	39	8	20.5
X^2	18.817*		
P value	0		

X2 : chi-square

* significant difference at $p < 0.05$

C-Molecular result of *T.gondii* infection in local and Imported meat according to the age.

The results demonstrate that majority positive samples were seen in ages 2-4 years(24,7),While the 6months-2 year ages show lowest rate of infection(5.25%) Table(4.7)

Age of animals	Examined No.	Positive No.	Percentage (%)
6month - 2 year	35	15	42.8
2-4 year	65	38	58.4
Total	100	53	53

4.3 Molecular results of *T.gondii* infection in local and imported meat products.

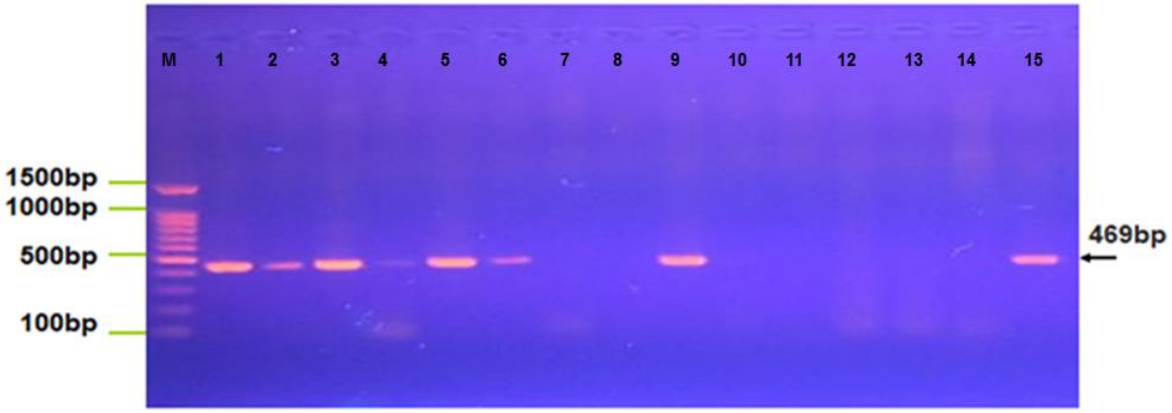
The positive results were recorded among different examined local meat products, where they revealed there were 3/20(15%) of hamburgers and 5/30 (16%) of minced meat samples had infection with *T.gondii*, while were no infection with *T.gondii* in imported meat products Table(4.8),Figure(4.4).

Table (4.8): molecular results of *T.gondii* infection in local and imported meat products.

Sample type	Examined No.	Positive No.	Percentage (%)
Local beef burgers	20	3	15
Local minced meat	30	5	16.6
Total	50	8	16
X2	0.25*		
P value	0.875		
Imported hamburgers	20	0	0
Imported minced meat	20	0	0
Total	40	0	0
X2	0*		
P value	1		

X2:chi-square

*No significant difference at p< 0.05



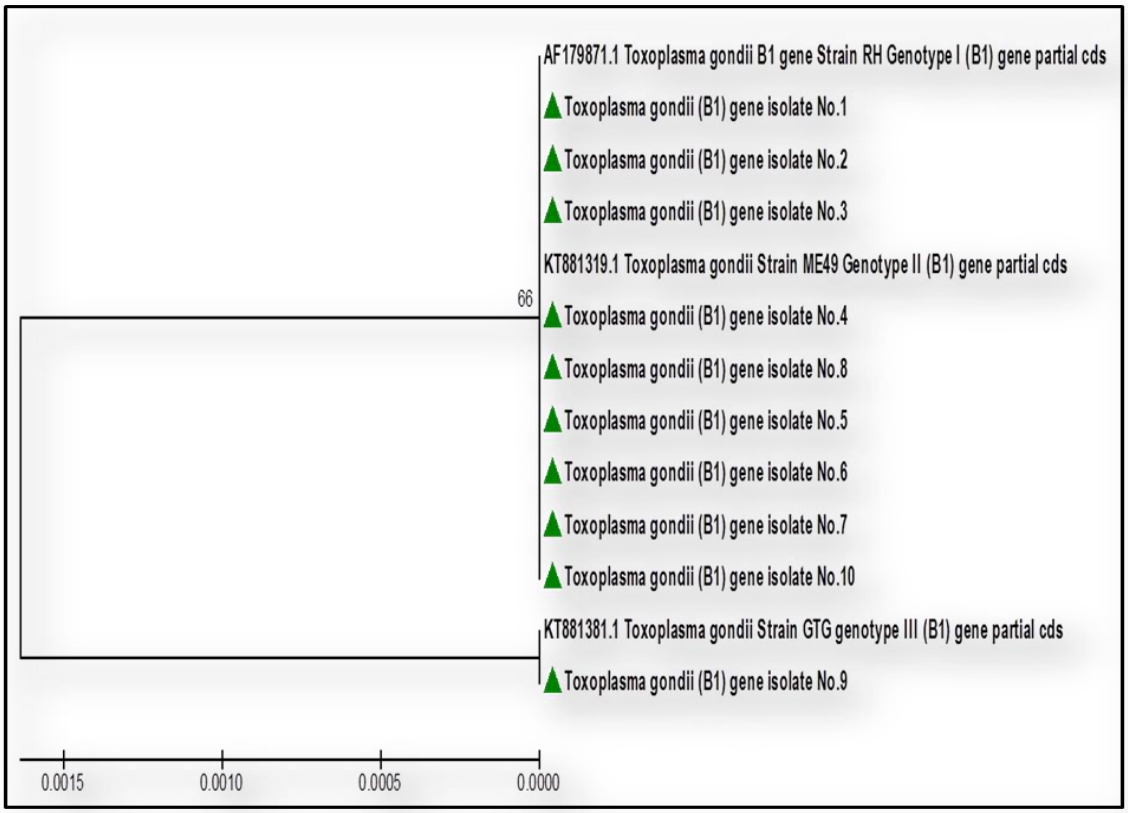
Figure(4.4) : Molecular results of *T.gondii* infection in local meat products (M: Marker, 1,2,3,4,5,6,9,15, represent positive samples at 469bp.

4.4 Phylogenetic Analysis results.

All three global strains RH (I), Me 49 (II), and GTG (III) were present in consumption meat(local, imported) in Iraq, where type (II) strain appeared in highest rate 6/10 (60%), whereas the type (III) recorded the lowest rate 1/10 (10%) Table (4.9), Figure (4.5).

Table (4.9) The genotype of *T.gondii* in meat (Local, Imported) using partial Sequence of *B1*gene according to phylogenic tree analysis and NCBI-BLAST alignment tool,(L:local. I:imported).

Local *T. gondii* isolates	**Source**	**Genbank accession numbers**	**NCBI BLAST Homology sequence identity**					
			Host	**Country**	**Source**	**Genotype**	**Genbank accession numbers**	**identity**
T. gondii No.1	Muscle L.	**MK012091**	human	USA	cerebrospinal fluid	**I**	**AF179871.1**	**99%**
T. gondii No.2	Muscle L.	**MK012092**	=	=	=	**I**	=	**99%**
T. gondii No.3	Muscle L.	**MK012093**	=	=	=	**I**	=	**99%**
T. gondii No4	Muscle I.	**MK012094**	Cat	Aust.	tissue	**II**	**KT881319.1**	**99%**
T. gondii No.5	Muscle I.	**MK012095**	=	=	=	**II**	=	**99%**
T. gondii No.6	Heart L.	**MK012096**	=	=	=	**II**	=	**99%**
T. gondii No.7	Heart L.	**MK012097**	=	=	=	**II**	=	**99%**
T. gondii No.8	Heart I.	**MK012098**	=	=	=	**II**	=	**99%**
T. gondii No.9	Tongue L.	**MK012099**	Marine mammals	USA	tissue	**III**	**KT881381.1**	**100%**
T. gondii No.10	Tongue I.	**MK012100**	Cat	Aust.	tissue	**II**	**KT881319.1**	**99%**



Figure(4.5):Phylogenetic tree analysis based on the partial sequence of *B1*gene in local *Toxoplasma gondii* beef isolates that used for genotyping identification analysis, The evolutionary distances were computed using the Maximum Composite Likelihood method by UPGMA phylogenetic tree (MEGA 6.0 version). The local *T. gondii* beef No.1-No.3 isolates were show closed related to NCBI-Blast *T. gondii* genotype I RH strain (AF179871.1), The local *T. gondii* beef No.4,5,6,7,8,and 10 isolates were show closed related to NCBI-Blast *Toxoplasma gondii* genotype II Me49 strain (KT881319.1), whereas, The local *T.gondii* beef No.9 isolates were show closed related to NCBI-Blast *T. gondii* genotype III GTG strain (KT881381.1) at total genetic changes (0.0005-0.0015)

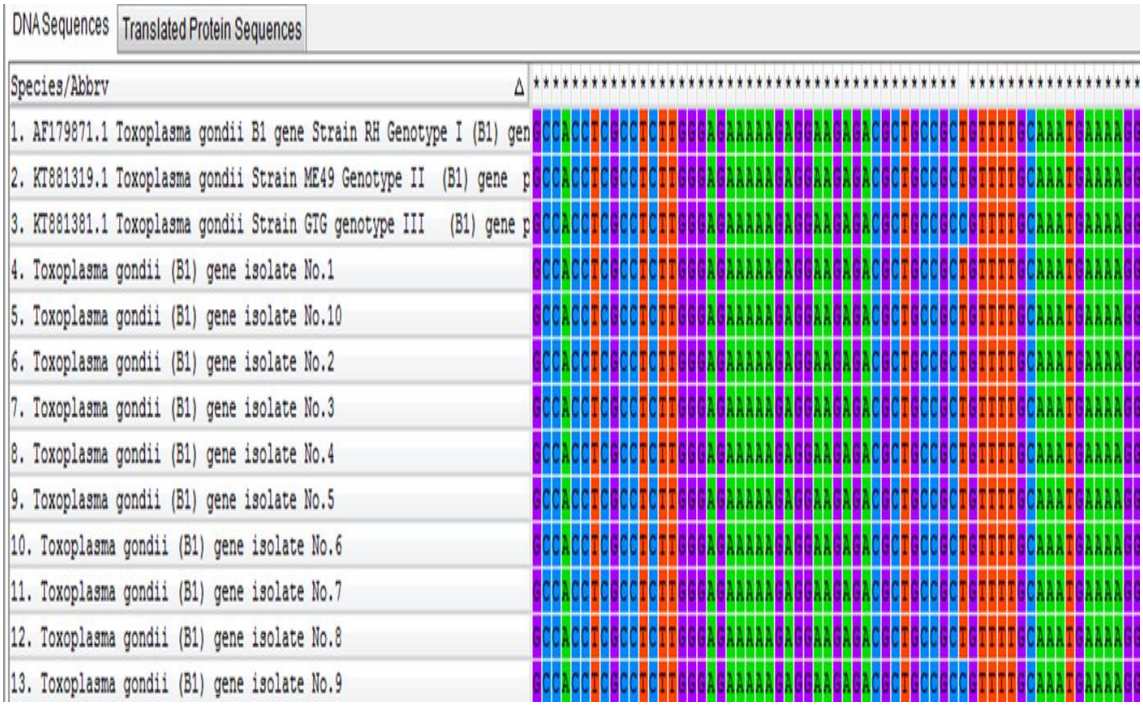


Figure (4.6) "Multiple sequence alignment analysis of the partial *B1* gene sequence in local *T. gondii* isolates and NCBI-Genbank *T.gondii* based Clustal Walignment analysis by using (MEGA 6.0, multiple alignment analysis tool. The multiple alignment analysis similarity was showed as (*) at nucleotide sequence.

Chapter Five
Discussion

5.1Prevalence of *T.gondii* in Cattle

Meat is an important source of human food, and among different kinds of meat, beef represent one of the most common consumption type (Dubey, 1986). *T.gondii* infects beef as whereas other meat and lead to many health problems like congenital defects and abortion in both humans and livestock (Saadatnia and Golkar 2012).

This is the first study that deals with the *T.gondii* infection in local and Imported beef in in AL- Diwaniyah /Iraq, in which molecular technique was used to detect the infection through amplification of *B1*gene, only one serological study associated with beef was done by Ehsan, (2011) in Iraq (Mosul) to detect *T.gondii* on different meat juices by LAT test, while another many Iraqi studies were on sheep ,aborted women, chicken, stray cats (Alkhaled *et al*.,2012; Switzer *et al*., 2013; Mohammed and Abdullah, 2013; Nazar *et al*., 2015; Aa'iz, 2016) and there are many researches on pregnant and aborted women in Iraq.

Out off 300 beef samples 33(22%) and 22(13.3%) for local and imported meat respectively were given a positive results. This results were resembling to the results of Ehsan, (2011,b) in Iraq (Mosul) who mentioned in serological study by used LAT test conducted on different meat juices including 100 sample for beef juices that were 17% of examined sample revealed *T. gondii* infection. With molecular study was done by Amdouni *et al*., (2017) from Tunisia showed that among 150 cattle meat samples that (29/150) 19.3% gave positive result.

The agreement between the results of the previous studies and the current study revealed the risky in consumption of infected beef with *T.gondii* .

The results of the present study was less than that values found in Northern Portugal (50%) (Lopes *et al.*, 2015), and the study in Colombia included 180 samples from chicken, swine, and beef ,targeted *B1* gene which recorded 37% rate of infection (Franco-Hernandez *et al.*, 2015). In another side the prevalence of *T.gondii* in present study was higher than those recorded in Brazil and Switzerland (2% and 3.8%, respectively) (Santos, Costa and Gondim, 2010; Berger-schoch *et al.*, 2011), also the study that where done in Iran by Rahdar *et al*., (2012) were recorded 4%

In comparing study between local and imported meat in Iran, Davood *et al.*, (2015), Show that the imported meat recorded higher infection rate 26% than local meat 6%, this results are conflict with present study which found the infection in local animals more than infection in imported, thus at the end all these differences among different studies may depend upon many factors like climate conditions, numbers of tested samples, rearing methods, false negative, and insufficient sample size ..etc

Regarding to the animal's age the current study mention that the highest prevalence of infection was recorded in age 2- 4years (58.4%), while lowest prevalence rate observed in ages 6months to 2year (42.8%), This results near to many another like the study did by Azizi (2014) and he referred to that infection in cattle and sheep in Iran were increase during ageing in rate 3.7% for animals less than 2 years while the rate of infection was 9.09% in animals with ages range from 2-4 years, also in Japan a serological study was done to detection the *T.gondii* infection in cattle showed that the rate of infection was 3.5% in animals with age less than 2years., while the infection rate was increase in animals with age more than 2years which was 13.7%., another molecular study was done by Amdouni *et al*., (2017), referred to that younger

animals less infection than the older animals. a serological study in Sudan conducted by Abdelghafar *et al.,*(2013) for detection of *T.gondii* in cattle, mentioned that the prevalence of *T.gondii* infection was significantly ($P < 0.05$) higher (36.4%) in animals less than one year old than those above two years (12.8%).

According to the effect of seasons on infection with *T.gondii,* Autumn was recorded the highest rate of infection(30%, 26.66%) in both local and imported meat with significant difference ($p < 0.05$), the present study agreement with the study in of Xin-Chao Liu *et al.,(*2017) in China where they revealed that *T.gondii* was found in 6 (3.33%) samples collected in Autumn and 1 (0.56%) collected in winter, this indicated that seasons might have important impacts on the presence of *T. gondii* ,while the study of seasonal variation of *T.gondii* infection of Logar *et al.,* (2005) on pregnant women recorded rising in infection rate during winter and spring than Autumn and summer and refered to that seasonal variation of *T.gondii* infection in human are remarkably different than animals, also seasonal variation study on pregnant women in France appeared that the maximal risk of infection between end of summer to end of Autumn (Morin *et al.,* 2o12). this may be related to the availability of favorable conditions for oocysts maturation and sporulation to become infective (Yan *et al.,* 2016).

Concerning to the infection of *T.gondii* in different examined organs no significant difference ($p< 0.05$) was recorded in the current study. This recording is in agreement with other previous study made by Burrells *et al.,* (2018) on calves when studied the probable variations of different organs infection and showed that there is no clear predilection site within bovine tissues.

In fact many factors impact on *T.gondii* infection incidence such as the type of livestock management and production, the hygiene standards of abattoirs, food processing, the density of cats in the environment, and the habits of human consumers, also the geographical location with respect to altitude, and the prevailing climatic conditions (Hall *et al.*,2001) .

5.2 prevalence of *T.gondii* in meat products

Totally 90 samples of local and imported human consumed meat products, the results showed that the local minced meat and beef burger were infected with *T.gondii* in rate 5/30 (16%), 3/20(15%) respectively in comparing to negative result in the imported meat products.

This results was agree with results recorded by Falah, (2011) in Iran when he found the infection in hamburger also 15% in addition to other products in his study like salami, sausages and kebab, the rates of infection were 16.66 %, 19.1 % and 56.6 % respectively. Tanya *et al*., (2002) in UK studied the *T.gondii* infection in 71 samples of pork, lamb and beef by using PCR technique and they showed rate of infection was 19%. Rahdar *et al*., (2012) disagreement with the present study where they saw there were no infection in 90 samples of hamburger salami, and sausages which examined by PCR method.

The low meat products *T.gondii* infection may be attributed to the processing of meat products with salting, freezing, commercial hot air drying, long fermentation times, hot smoking, and cooking, are able to reduce *T. gondii* levels in meat products (Guo *et al*., 2015).

5.3 Genotyping of *T.gondii*

Generally three strain were recorded under *T.gondii* species which were referring as RH (Type I strain), Me 49 (II) and GT1 (III).

The type I consider as highly virulence strain in mice and its mainly isolated from ADIS patients ,while type II strain virulent in mice and also seen in human whereas type III non-virulence in mice and virulence in animals (Dalimi and Abdoli, 2011).

The genotyping study revealed that all three types strain I, II and III were isolated from infected meat with rate 30% (3/10), 60% (6/10), and 10% respectively, so type II appear as a dominant type. This mentions are agree with other previous studies in Iraq like the studies on aborted women and sheep by Nazar *et al*.,(2015) and Aa'iz (2016) which revealed that also the three types are present and type II was more common, this was confirmed by Muhanned *et al.*, (2018) when they inoculated *T.gondii* taken from placenta of aborted women into rabbit as an experimental infection to increase the proliferation of parasite and according to molecular study they found that the three types were presenting and type II was the dominant type. In Egypt Hassan *et al.*,(2017) was also agree in their study with the results of the present study when they recorded all types strain in examined samples from human, cattle, sheep, goat, and camels and type II was the common type. also Dubey *et al.,* (2004) find all three types in study including animals in the wild life. While Mahmoud *et al.,* (2017) in Iran, disagree with results of the present study where they found type I is the common strain.

The predominance of type II strain in our study may be interpretation due to that this type is the more presence type in animals and this study is focusing the cattle meat and meat products**.**

Conclusions and Recommendations

Conclusions

1-The local cattle meat and meat products are more risky than the imported due to the higher rate of infection with *T.gondii.*

2- Processing meat products (Freezing, Salting…ect.) are more safe than fresh.

3- *T.gondii* infect all examined organs (Heart, Muscles, Tongue) in the same rates.

4-*T.gondii* infection rate cannot effected by age of animals, whereas the season play role in this rate.

5-Type II strain is predominance than other types.

Recommendations

1-Have to making health education for general population about this disease and methods of transmition of it and how to decrease from the risk of transmition by hygienic methods, women should be careful during dealing with meat they can use cloves in , perfect washing hands after ended from cutting of the meat and should be make perfect cleaning to the knife or any tools or containers that she use it, also farmer or owners of cattle should be eliminate stray cats (final host) that found near cattle houses or their foods, also in their pasture(farm management).

2-Good cooking of meat and have to away from some types of eat habits like smoking meat and avoid microwave cooking.

3-Further studies are needed for vaccination development against the parasite in cattle for preventing tissue cyst formation and more research including largest area in the country .

References

-**A'aiz,N.N., (2016).** Determination of *Toxoplasma gondii* lineages of sheep in Wasit, Iraq. Iraqi Journal of Veterinary Sciences, Vol. 30, No. 2, 2016 (23-26).

-**Abdelghafar M. Elfahal, Amira M. Elhassan, Mohammed O. Hussien, Khalid A. Enan, Azza B. Musa, and Abdelrahim M. ElHussein (2013).** Seroprevalence of *Toxoplasma gondii* in Dairy Cattle with Reproductive Problems in Sudan. ISRN Veterinary Science, Article ID 895165, 4 pages.

-Abu-Madi, M.A., Behnke ,J.M, and Dabritz, H.A (2010). *Toxoplasma gondii* Seropositivity and Co-Infection with TORCH Pathogens in High-Risk Patients from Qatar. Am. J. Trop. Med. Hyg., 82(4): 626–633.

-**Ahmad N., and Qayyum M. (2014).**Seroprevalence and risk factors for toxoplasmosis in large ruminants in northern Punjab, Pakistan. J. Infect Dev Ctries. 8(8):1022–8.

-**Alexander, J. and Hunter, C. (1998).** Immunoregulation during toxoplasmosis. Chem. Immunol. V.(70):81-102.

-**Alkhaled M. J. A., Yakoob A. Y., AL-hamadani A. H. U. (2012).** An investigation of Toxoplasmosis in Free Range chickens, Industrial chickens and Duck in mid Euphrates area of Iraq AL-Qadisiya Journal of Vet.Med.Sci. 11(2).

-**Amdouni, Y., Rjeibi, M. R., Rouatbi, M., Amairia, S., Awadi, S., and Gharbi, M. (2017).** Molecular detection of *Toxoplasma gondii* infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. Meat Science, 133, 180–184.

-**Anvari D., Dariush S., Reza N., and Majid Alipour. (2015).** Epidemiology and Molecular Prevalence of *Toxoplasma gondii* in Cattle Slaughtered in Zahedan and Zabol Districts, South East of Iran". Iranian Journal of Parasitology 13 (1); 114-19.

-**Azizi, H., Shiran, B., Boroujeni, A. B., and Jafari, M. (2014).** Molecular Survey of *Toxoplasma gondii* in Sheep, Cattle and Meat Products in Chaharmahal va Bakhtiari Province, Southwest of Iran. Iranian journal of parasitology, 9(3), 429-34.

-**Berger-Schoch, A. E., Herrmann, D. C., Schares, G., Müller, N., Bernet, D., Gottstein, B., and Frey, C. F. (2011).** Prevalence and genotypes of *Toxoplasma gondii* in feline faeces (oocysts) and meat from sheep, cattle and pigs in Switzerland. Veterinary Parasitology, 177(3-4):290–297.

-**Bhopale, G. M., (2003).** Pathogenesis of toxoplasmosis. Comparative immunology, microbiology and infectious diseases. 26 (4), 213-22.

-**Bowman, D., Hendrix, C., Lindsay, D., and Barr, S. (2002).** *Toxoplasma gondii* (Nicolle and Manceaux, 1908). In: (Feline Clinical Parasitology). Iowa State University Press, Iowa, 14-28.

-**Boyer, K.M., Holfels, E., Roizen, N., Swisher, C., Mack, D., Remington, J., Withers, S., Meier, P., and McLeod, R. (2005).** Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. Am. J. Obstet. Gynecol., 192: 564-571.

-**Burg, J.L., C.M Grover, P.Pouletty and J. Boothroyd. (1989).** Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbio., 27(8): 1787-1792.

-**Burrells, A., Taroda, A., Opsteegh, M., Schares, G., Benavides, J., Dam-Deisz, C., Bartley, P. M., Chianini, F., Villena, I., van der Giessen, J., Innes, E. A., Katzer, F. (2018).** Detection and dissemination of *Toxoplasma gondii* in experimentally infected calves, a single test does not tell the whole story. Parasites & vectors, 11(1), 45.

-**Butcher, B.A., Kim, L., Panopoulos, A.D., Watowich, S., and Denkers, E.Y., (2005).** IL-10-independent STAT3 activation by *Toxoplasma gondii* mediates suppression of IL-12 and TNF-α in host macrophages. J.Immunol. V. (174):3148-3152.

-**Chiappino, M.L., Nichols, B.A, and O'connor, G.R.,(1984).**Scanning eclectron Microscopy of *Toxoplasma gondii* torsion and host cell responses during invation.J.Protozoal.31:288-292.

-**Claudia A., Muñoz-Zanzi, Paulina F., Blaz Lesina, and Dolores H. (2010).** *Toxoplasma gondii* Oocyst–specific Antibodies and Source of Infection, Emerging Infectious Diseases , Vol. 16, No. 10: 1591-1593.

-**Cook A.J., Gilbert R.E., Buffolano W., Zufferey J., Petersen E., and Jenum P.A., Foulon W., Semprini A.E., and Dunn D.T., (2000).** Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ.321 (7254):142-7.

-Cook A, Gilbert R, Buffolano W, Zufferey J, Petersen E, Jenum P. (2001). Eating undercooked meat contributed to approximately 50% of cases of acute toxoplasma infection in pregnancy. Evid Based Obstetrics Gynecol.; 3 (2).

-Cox, R. M. and H.B. John-Alde . (2005). Testosterone has opposite effects on male growth in lizard (Sceloporus spp.) with opposite patterns of sexual size dimorphism. J. Exp. Biol.208 (24) : 4679-4687.

-Dabritz, H.A., Delauw, M.F., Ruffolo, B.B., Bugni, F.M. and Castro, M.V. (2008). Risk factors for *Toxoplasma gondii* infection in wild rodents from central coastal California and a review of *T. gondii* prevalence in rodents. J. Parasitol. V. (94): 675–683.

-Dalgic, N. (2008). Congenital *Toxoplasma gondii* infection. Marmora Med. J., 21(1): 175-177.

-Dalimi A. and Abdoli A. (2011) Latent Toxoplasmosis and Human. Iranian Journal of Parasitology. 7 (1): 1–17.

- da Silva P.C., Shiraishi C.S., Silva A.V., Goncalves G.F., Sant'Ana Dde M., Araujo E.J. (2010). *Toxoplasma gondii:* a morphometric analysis of the wall and epithelial cells of pigs intestine. Exp Parasitol.; 125:380–3.

-Derouin F., and Pelloux H., (2012). Prevention of toxoplasmosis in transplant patients. Clinical Microbiology and Infection 14:1089–1101.

-Desmonts, G., Couvreur, J., Alison, F., Baudelot, J., Gerbeaux, J. and Lelong, M. (1965). E ´ tude e´pide´miologique surla toxoplasmose: de l'influence de la cuisson des viandes de boucherie sur la fre´quence de l'infection humaine. Rev. Fr. E´tudes Clin. Biol., 10:952–958.

-**Doggett J.S., Nilsen A., Forquer I., Wegmann K.W., Jones-Brando L., Yolken R.H., Bordón C., Charman S.A., Katneni K., Schultz T., Burrows J.N., Hinrichs D.J., Meunier B., Carruthers V.B., and Riscoe M.K.,(2012).** "Endochin-like quinolones are highly efficacious against acute and latent experimental toxoplasmosis". Proceedings of the National Academy of Sciences of the United States of America. 109 (39): 15936–41.

-**Dubey, J. P. and Beattie, C. P. (1988).** Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, FL. p. 1–220.

-**Dubey, J. P. and Frenkel, J. F. (1972).** Cyst-induced toxoplasmosis in cats. J. Protozool. V. (19):155-177.

-**Dubey, J. P. and Frenkel, J. K. (1976).** Feline toxoplasmosis from acutely infected mice and the development of Toxoplasma cysts. J. Protozool., 23:537–546.

-**Dubey, J. P. (2007).** The history and life cycle of *Toxoplasma gondii*. In: Weiss,

-**Dubey J.P., Lindsay D.S., Lappin M.R. (2009).** Toxoplasmosis and other intestinal coccidial infections in cats and dogs. Vet. Clin. North Am. Small Anim. Pract 39:1009–1034.

-**Dubey, J. P., Kotula, A. W., Sharar, A., Andrews, C. D. and Lindsay, D. S. (1990).** Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. J. Parasitol., 76:201–204.

-**Dubey, J. P., (2004)**. Toxoplasmosis - a waterborne zoonosis. Vet Parasitol, 126 (1-2): 57-72.

-**Dubey, J.P. and Jones, J.L. (2008).** *T. gondii* infection in humans and animals in the United States. Int. J. Parasitol. V. (38): 1257-1278.

-**Dubey, J.P., Hill, D.E. and Jones, J.L. (2005).** Prevalence of viable *T. gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. J. Parasitol, V. (91): 1082-93.

-**Dubey, J.P., Lindsay, D.S. and Mevelec, M.N. (1998).** Structure of *Toxoplasma gondii* tachyzoites, bradyzoites and sporozoites and biology and development of tissue cyst. Clin. Microbiol Rev., 11(2): 267- 99.

-**Dubey J.P., and Jones J.L., (2008).** *Toxoplasma gondii* infection in humans and animals in the United States". International Journal for Parasitology. 38 (11): 1257–78.

-**Dubey J.P., Velmurugan G.V., Rajendran C., Yabsley M.J., Thomas N.J., Beckmen K.B., Sinnett D., Ruid D., Hart J., Fair P.A., McFee W.E., Shearn-Bochsler V., Kwok O.C., Ferreira L.R., Choudhary S., Faria E.B., Zhou H., and Felix T.A.,(2011).** Genetic characterisation of *Toxoplasma gondii* in wildlife from North America revealed widespread and high prevalence of the fourth clonal type. International Journal for Parasitology.41(11):1139–47.

-**Dubey, J. P., (1993).** Toxoplasma, Neospora, Sarcocystis, and other tissue cystforming coccidia of humans and animals. 1-158. in: Kreier. J. P. Parasitic Protozoa. Second Edition. V; 61993. Academic Press, London.

-**Dubey, J. P., (2009).** History of the discovery of the life cycle of *T.gondii*. Int. J. Parasitol.; 39:877-882.

-**Dubey, J.P., (2006).** Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gondii* for intermediate (mice) and definitive (cats) hosts. Vet. Parasitol., 140:69–75.

-**Dubey, J. P., (2002).** A review of toxoplasmosis in wild birds. Vet Parasitol.106 (2): 121-53.

-**Dubey, J.P., (2004).** Toxoplasmosis-a water borne zoonosis. Vet. Parasitol. 126:57–72.

-**Dubey J. P., Graham D. H., De Young R. W., Dahl E., Eberhard M. L., Nace E. K., Won K., Bishop H., Punkosdy G., Sreekumar C., Vianna M. C. B., Shen S.K., Kwok O. C. H., Sumners J. A., Demarais S., Humphreys J. G., and Lehmann T.(2004).** Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. J. Parasitol., 90(1):67–71.

-**Dubey, J. P. (1986)** A review of toxoplasmosis in cattle. Vet. Parasitol. 22:177–202.

-**Dubey, J. P. (2008).** The History of *Toxoplasma gondii*—The First 100 Years. J. Eukaryot. Microbiol., 55(6): 467–475.

-**Dubey J. P. (2000).** Sources of *Toxoplasma gondii* infection in pregnancy. Until rates of congenital toxoplasmosis fall, control measures are essential. *BMJ (Clinical research ed.)*, *321*(7254), 127-8.

-**Davood A., Dariush S., Reza N., and Majid AL-ipour E., (2015**). Epidemiology and Molecular Prevalence of *Toxoplasma gondii* in Cattle Slaughtered in Zahedan and Zabol Districts, South East of Iran Iran J Parasitol: Vol. 13, No. 1, Jan-Mar 2018, pp.114-119.

-**Dziubek, Z., Zarnowska, H., Basiak, W., orski, A., and Kajfasz, P. (2001).** Some aspects of immune response in toxoplasmosis, Przeglad Epidemiologiczny, vol. 55, (4):495-502.

-**Ehsan G. Zakaria (2011).** Detection of *Toxoplasma gondii* Antibodies in Different Meat Juices. Raf. J. Sci., Vol. 22, (4):17-25.

-**El-Nawawi F.A., Tawfik M.A., and Shaapan R.M., (2008).** Methods for inactivation of *Toxoplasma gondii* cysts in meat and tissues of experimentally infected sheep. Foodborne Pathogens and Disease 5(5):687–690.

-**Fallah E., Hajizadeh M., Farajnia S., and Khanmahammadi M. (2011).** SAG2 locus genotyping of *Toxoplasma gondii* in meat products of East Azerbaijan Province, North West of Iran During 2010-2011. Afr J Biotechnol., 10 (62):13631-5.

-**Ferguson, D. J.., Birch-Andersen, A., Siim, J. C. and Hutchison, W. M. (1979).** Ultrastructural studies on the sporulation of oocysts of *Toxoplasma gondii*. I. Development of the zygote and formation of sporoblasts. Acta Pathol. Microbiol. Scand. B, 87:171–181.

-**Filisetti, D. and Candolfi, E. (2004**). Immune Response to *Toxoplasma gondii*. Ann 1st. super sanita. 40 (1) : 71-80.

-**Foulon, W., Naessens, A. and Ho-Yen, D. (2000**). Prevention of congenital toxoplasmosis. J. Pernat. Med., V. (28): 337-345.

-**Franco-Hernandez, E. N., Acosta, A., Cortés-Vecino, J., and Gómez-Marín, J. E. (2015).** Survey for *Toxoplasma gondii* by PCR detection in meat for human consumption in Colombia. Parasitology Research, 115(2): 691–695.

-**Frenkel, J. K. and Friedlander, S., (1951).** Toxoplasmosis. Pathology of neonatal disease UNITED STATES PUBLIC HEALTH SERVICE PUBLICATION. 1951 No.141 pp.iv + 108 pp.

-**Frenkel, J. K., Dubey, J. P. and Miller, N. L. (1970).** *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. Science (New York, N.Y.), 167:893–896.

-**Frenkel, J. K. (1973).** *Toxoplasma* in and around us. Bio. Science; 23: 343 – 352.

-**Ferguson D.J.(2009).** *Toxoplasma gondii*: 1908–2008, homage to Nicolle, Manceaux and Splendore". Memórias do Instituto Oswaldo Cruz. 104 (2): 133–48.

-**Fulton, J. D. (1965).** Studies on agglutination of *Toxoplasma gondii.* Transactions of the Royal Society of Tropical Medicine and Hygiene, 59(6), 694–704.

-**Gaetano, P.C., Angelina R. and Michele (2010).** Acute pericarditis and myocarditis *Toxoplasma gondii* in an immune man. acase report. J. Infect. Med. 18(1): 48-52.

-**Gamble, R.; Dubey, J.P. and Lambillotte, D.N., (2005).** Comparison of acommercial ELISA with the modified agglutination test for detection of Toxoplasma infection in the domestic pig. Vet. Parasitol., 128 (3-4):Pp.177-181.

-**Green, Aliza (2005).** Field Guide to Meat. Philadelphia, PA: Quirk Books. pp. 294–295.

-**Guo M., Buchanan R.L., Dubey J.P., Hill D.E., Lambertini E., Ying Y., Gamble H.R., Jones J.L., and Pradhan A.K.(2015).** Qualitative assessment for *Toxoplasma gondii* exposure risk associated with meat products in the United States. Journal of Food Protection.78 (12):2207–2219.

-**Hall S., Ryan K. A., and Buxton D. (2001).**The epidemiology of toxoplasma infection. In Toxoplasmosis: a comprehensive clinical guide (eds Joynson D. H., Wreghitt T. J., editors.), pp. 58–124 Cambridge, UK: Cambridge University Press.

-**Halonen, S. K., and Weiss, L. M., (2013).** Toxoplasmosis. Handb Clin Neurol.114: 125-45.

-**Hassan A. Elfadaly, Nawal A. Hassanain, Raafat M. Shaapan, Mohey A. Hassanain, Ashraf M. Barakat and Khaled A. Abdelrahman,(2017).** Molecular Detection and Genotyping of *Toxoplasma gondii* from Egyptian Isolates. Asian Journal of Epidemiology, 10: 37-44.

-**Hill, D. and Dubey, J.P. (2002).** *Toxoplasma gondii* transmission, diagnosis and prevention. Clin. Microbiol Infect., V. (8): 634-640.

-**Hill, D.E., Chirukandoth, S., Dubey, J.P., Lunney, J.K., Gamble, H.R., (2006**). Comparison of detection methods for *Toxoplasma gondii* in naturally and experimentally infected swine. Vet. Parasitol. 141, 9–17.

-**Hill D.E., Sreekumar C., Jones J., and Dubey J.P. (2007).** *Toxoplasma gondii.* Ch. 12 In: Simjee S (ed) Foodborne diseases. Humana Press, Totowa, p. 337–353.

-**Holliman R. E. (2003).** Toxoplasmosis. pp: 1365-71. In: Cook G C (ed). Manson's Tropical Diseases. 24th ed. W. B. Saunders company Ltd.

-Jasim, A.N. (1979). Seroepidemiological studies on toxoplasmosis in Iraq, evaluation of serological tests used in diagnosis. M.Sc. Thesis. College of Medicine. University of Baghdad.pp 112.

-**Jenum, P.A. and Stray-Pederesn B.(1998).** Development of specific immunoglobulins G, M, and following primary *Toxoplasma gondii* infection in pregnant women. J. Clin. Microbiol. V. (36): 2907-2913.

-**Jones, C. D., Okhravi, N., Adamson, P., Tasker, S., and Lightman, S. (2000**). Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of *T. gondii* in aqueous humor. Invest. Ophthalmol. Vis. Sci.; 41, 3: 634-644.

-**Jones, J.L., and Dubey, J.P. (2010).**Water borne toxoplasmosis-recent developments. Exp. Parasitol., 124:10-25.

-**Jones J.L., Dargelas V., Roberts J., Press C., Remington J.S., Montoya J.G. (2009).** Risk factors for *Toxoplasma gondii* infection in the United States. Clin Infect Dis. 15; 49(6):878-84.

-**Jones J., Lopez A., and Wilson M. (2003).** Congenital toxoplasmosis. American Family Physician. 67 (10): 2131–8.

-**Kafsack, B. F., Pena, J. D., Coppens, I., Ravindran, S., Boothroyd, J. C., and Carruthers, V. B. (2009).** Rapid membrane disruption by a perforin-like protein facilitates parasite exit from host cells. Science; 323: 530-533.

-**Kim, K., Weiss, L. M., (2004).** *Toxoplasma gondii*: the model apicomplexan. International journal for parasitology, 34 (3), 423-32.

-Lambert, H., Hitziger, N., Dellacasa, I., and Barragan, A., (2006). Induction of dendritic cell migration upon *Toxoplasma gondii* infection potentates parasite dissemination. Cell Microbiol, V. (8): 1611-1623.

- **Leech, N. L., Barrett, K. C. and Morgan, G. (2011).** IBM SPSS for Intermediate statistics. 4th ed. Taylor and Francis Group . LLC. USA.

-Lihleh, W. U., Teodoro E., and Rafael A.G. (2010). Toxoplasmosis. Web MD. Professional.Industry spotlight (http://medicin medscape. com /article / 1204441-over view).

-Lindsay D.S., Blagburn B.L., and Dubey J.P. (2002). Survival of nonsporulated *Toxoplasma gondii* oocysts under refrigerator conditions. Veterinary Parasitology 103(4):309–313.

-Logar J., Soba B., Premru-Srsen T., and Novak-Antolic Z..(2005). Seasonal variations in acute toxoplasmosis in pregnant women in Slovenia. Clin Microbiol Infect.11(10):852-5

-Lopes A.P., Sargo R., Rodrigues M., and Cardoso L. (2010). High seroprevalence of antibodies to *Toxoplasma gondii* in wild animals from Portugal. Parasitol. Res. 108: 1163-1169.

-Lopes A.P., Vilares A., Neto F., Rodrigues A., Martins T., Ferreira I., Gargaté M.J., Rodrigues M., Cardoso L.(2015). Genotyping Characterization of *Toxoplasma gondii* in Cattle, Sheep, Goats and Swine from the North of Portugal. Iran J Parasitol. 10: 465– 472.

-Lopez, A., Dietz, V. J., Wilson, M., Navin, T. R., and Jones, J. L. (2000). In preventing congenital toxoplasmosis. CDC Recommendations Regarding

Selected Conditions Affecting Women's Health (49), Centers for Disease Control and Prevention MMWR.; 57–75.

-**Louis M. Weiss, and Kami Kim (2011)** p. 723.

-**Lundén A., Uggla A. (1992).** Infectivity of *Toxoplasma gondii* in mutton following curing, smoking, freezing or microwave cooking. Int J Food Microbiol (15): 357-363.

-**Machattie, C. (1938).** Notes on two cases of naturally occurring toxoplasmosis in Baghdad. Tran. Roy.Trop. Hyg., 23:373-376.

-**Mahmoud M., Mohammad M., Esmaeil F., Faezeh H., and Najibeh A. (2017).** Molecular Detection and Genotyping of *Toxoplasma gondii* in Chicken, Beef, and Lamb Meat Consumed in Northwestern Iran. Iran J. Parasitol.12 (1): 38–45.

-**Mainar-Jaime, R.C., and Barberán, M., (2007).** Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. Vet. Parasitol. 148:122–129.

-**Many, A. and Koren, G. (2006).** Toxoplasmosis during pregnancy. Can Fam Physician. V.(10) No. 52. Pp. 29-32.

-**Miao Guo, Jitender P., Dubey J. P., Hill D., Robert L., Buchanan, H. Raygamble, Jeffrey L., Jones, and Abanik. Pradhan, (2015).** Prevalence and Risk Factors for *Toxoplasma gondii* Infection in Meat Animals and Meat Products Destined for Human Consumption. Journal of Food Protection, Vol. 78, (2):457–476.

-**Miller, C.M., Boulter, N.R., Ikin, R.J., and Smith, N.C. (2009).** the immunobiology of the innate response to *Toxoplasma gondii*. International Journal for Parasitology. 39 (1): 23–39.

-**Miller, C.D., Nicola, R., Boulter, Rowan J., and Ikin, Nicholas (2009).** The immunobiology of the innate response to *Toxoplasma gondii*. International Journal for Parasitology, V. (39): 23-39.

-**Miller, M. A., Grigg, M. E., Kreuder, C., James, E. R., Melli, A. C., Crosbie, P. R., Jessup, D. A., Boothroyd, J. C., Brownstein, D., Conrad, P. A.,(2004).** genotype of *Toxoplasma gondii* is common in California sea otters (Enhydra lutris nereis) and is a cause of mortality. International journal for parasitology. 34 (3), 275-84.

-**Mims, C., H. M. Dockrell, R. V. Goering, I.Roitt, D.Wakelin, and M. Zuckerman.(2004).**Medical Microbiology.Updated 3rd ed., Elsevier Ltd, USA.pp50-55.

-**Mohammed A.A. and Abdullah Sh. H. (2013)** Diagnostic Study of Toxoplasmosis in Domestic Chickens in Sulaimani Province. AL-Qadisiya Journal of Vet.Med.Sci. 12 (2).

-**Montoya, A., Berry, F., Rosso, J.S. (2007).** Remington The differential agglutination test as a diagnostic aid in cases of toxoplasmic lymphadenitis. J Clin Microbiol, (45): 1463-1468.

-**Montoya, J.G. and Liedsenfeld, O. (2004).** Toxoplasmosis. Lancet, V. (363): 1965-1976.

- **Morin L., Lobry J. R., Peyron F. and Wallon M. (2012).** Seasonal variations in acute toxoplasmosis in pregnant women in the Rhoˆne-Alpes region (France) Clin Microbiol Infect ,18: E401–E403.

-**Muhannad S., Noorn Al-Hayani, Hatemm H., Thulfiqar F. M.,(2018).** Genotyping and DNA Sequencing Divergence of *Toxoplasma gondii* strains associated with Rabbit Toxoplasmsis, Iraq Int. J. Life Sci.; 8(3): 24-34.

-**Nazar Sh. Mohammed, Akeel H. A. Al-A'ssie, and Ihsan M. Al-saqur. (2015).**Genotyping of *Toxoplasma gondii* Isolated from Aborted Iraqi Women Diyala Journal of Medicine Vol. 9, Issue 1.

-**Neves, E., Bicudo, L., Curi, A., Carregal, E., Bueno, W., Ferreira, R., Amendoeira, M., Benchimol, E. and Fernandes, O. (2009).** Acute acquired toxoplasmosis: Clinical- labortorial aspect and ophthalmologic evolution in a cohort of immunocompotent. Mem. Inst.Oswaldo Cruz, Rio de janiero, 104(2): 393-396.

-**Nicolle, C., Manceaux, L. H., R., C., Surune,** infection a coyes de Leishman (ou organisms voisins) du gondi. Seance Acad Sci 1908, 147, 763-766.

-**Opsteegh, A., Swart, M., Fonville, L. Dekkers, J., and van der Giessen.(2011).** Age-related *Toxoplasma gondii* seroprevalence in Dutch wild boar inconsistent with lifelong persistence of antibodies ovine fetuses. J. of Vet. Diag. Investigation; 1: 124-127.

-**Ortega-Pacheco, A., Acosta Viana, K. Y., Guzmán-Marín, E., Segura-Correa, J. C., Alvarez-Fleites, M., & Jiménez-Coello, M. (2013).** Prevalence and risk factors of *Toxoplasma gondii* in fattening pigs farm from Yucatan, Mexico. BioMed research international, 2013, 231497.

-**Ozkan, A. T., Çelebi, B., Babur, C., Lucio-forster, A., Bowman, D.D., and Lindsay, D.S. (2008).** Investigation of anti-*T. gondii* antibodies in cats of the Ankara Region of Turkey using the sabin-feldman dye test and an indirect fluorescent antibody test. J. of Parasitol.; 94: 817-820.

-**Paniker, C. J. (2002).** Text book of medical parasitology. 5th. ed Jaypee Brothers Medical publishers, LTD, New Delhi., P: 89 - 95.

-**Pappas G., Roussos N., Falagas M.E. (2009).** Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol 39(12): 1385–94.

-**Pelloux, H., Brun, E., Vernet. G., Marcillat. S., and Ambroise-Thomas, P. (1998).** Determination of anti-*Toxoplasma gondii* immunoglobulin G avidity. Diagn. Microbiol. Infect.Dis. V. (32): 69-73.

-**Pereira K.S., Franco R.M., and Leal D.A. (2010).**Transmission of toxoplasmosis (*Toxoplasma gondii*) by foods. Advances in Food and Nutrition Research 60:1–19.

-**Petersen, E. and Dubey, J.P. (2001).**Biology of Toxoplasmosis. In ‘‘Toxoplasmosis’’, (D. H. M. Johnson and T. G. Wreghitt, eds), pp.1–42. Cambridge University Press, Cambridge.

-**Petersen, E. and Dubey, J.P., (2005).** Biology of Toxoplasmosis. In: Toxoplasmosis: A comprehensive clinical guide. Joynson, D.H. and Wreghitt, T.G. 1st. ed. Cambridge University Press, Pp. 1-43.

-**Petersen, E. (2007).**Toxoplasmosis. Semin Fetal Neonatal Med.12(3) : 214 – 223.

-Peyron, F., J.R. Lobry, K. Musset, B. Ferradiz, J. E. Gomez-Marin, E. Petersen, V. Meroni, B. Rausher, C. Mercier, S. Picot and M. F. Cesbron-Delauw. (2006). Serotyping of *Toxoplasma gondii* in chronically infected pregnant women: Predominance of type I in Europe and types II and III in Colombia (South America). Microbes Infect. 8(9-10): 2333-2340.

-Rahdar M., Samarbaf-Zadeh A., Arab L. (2012). Evaluating the Prevalence of *Toxoplasma gondii* in Meat and Meat Products in Ahvaz by PCR Method, Jundishapur J Microbiol; 5(4):570-573.

-Robert-Gangneux F., and Dardé M.L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. Clinical Microbiology Reviews. 25 (2): 264–96.

-Ross, D.S., Jones, J.L., and Lynch, M.F. (2006). Toxoplasmosis, Cytomegalovirus, Listeriosis, and Preconception Care. Matern. Child Health J. V. (10): 189-193.

-Ruelhmann, D. S. (2010). Myopathic disorders. pp 603. In August J. R. (ed.): Consultations in feline internal medicin.Elsever Saunders, Philadelphia.

- **Saadatnia G., and M. Golkar. (2012).** A review on human toxoplasmosis. Scand. J. Infect. Dis. 44:805–814.

-Saiki, R., Gelfand, D., Stoffel, S., Scharf, S., Higuchi, R., Horn, G., Erlich, H. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239(4839), 487–491.

-Salman, Shaimma L., Ameena, S. M., and Juma, (2009). Correlation between Apoptosis and Toxoplasma in Abortion Induction: Relevance of TUNEL Assay. European Journal of Scientific Research .37 (3): 406-425.

-Santos, S. L., Costa, K. D. S., and Gondim, L. Q. (2010). Investigation of *Neospora caninum*,*Hammondia* sp. and *Toxoplasma gondii* in tissues from slaughtered beef cattle in Bahia Brazil. Parasitology Research, 106(2), 457–461.

-Savva, D.; Morris, J. C.;Johnson, J. D., and Holliman, R. E. (1990). Polymerase chain reaction for detection of *T. gondii*. J. Med. Microbiol.; 32: 25–31.

-Seefeldt, S. L., Kirkbride, C. A., and Dubey, J. P. (1989). Comparison of enzyme-linked immunosorbent assay, indirect fluorescent antibody test, and direct agglutination test for detecting *Toxoplasma gondii* antibodies in naturally aborted ovine fetuses. J. Vet Diagn Invest. (2):124-7.

-Shaapan, R.M., El-Nawawi, F.A., Tawfik, M.A., (2008). Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. Vet. Parasitol. 153, 359–362.

-Sims T.A., Hay J., Talbot I.C., (1989). An electron microscope and immunohistochemical study of the intracellular location of Toxoplasma tissue cysts within the brains of mice with congenital toxoplasmosis. British J Exp Pathol. (70):317–25.

-Speer, C.A., and Dubey, J. P. (2005). Ultra structural differentiation of *Toxoplasma gondii* schizonts (types B to E) and gamonts in the intestines of cats fed bradyzoites. Int. J. Parasitol., 35:193–206.

-Suzuki, Y., M. A. Orellana, R.D. Schreiber and J. S. Remington. (1989). Interferon-γ the major mediator of resistance against *Toxoplasma gondii*. Science .240:516-518.

-**Switzer, A. D., McMillan-Cole, A. C., Kasten, R. W., Stuckey, M. J., Kass, P. H., and Chomel, B. B. (2013).** Bartonella and Toxoplasma infections in stray cats from Iraq. The American journal of tropical medicine and hygiene, 89(6), 1219-24.

-**Tanya V. Aspinall, Damian Marlee, John E. Hyde, Paul F.G. Sims (2002**). Prevalence of *Toxoplasma gondii* in commercial meat products as monitored by polymerase chain reaction – food for thought; International Journal for Parasitology 32:1193–1199.

-Taylor. A. ,Coop, R. L. AND Wall, R. L (2007). Veterinary Parasitology.3rd

(ed) Back well publishing LTD.

-**Tenter A.M., Heckeroth A.R., Weiss L.M. (2000).***Toxoplasma gondii:* from animals to humans. Int J Parasitol. 30 (12-13):1217-58.

-**Tenter A.M. (2009).** *Toxoplasma gondii* in animals used for human consumption. Mem Inst Oswaldo Cruz 104: 364-369.

-**Tenter, A. M., A. R. Heckeroth, and L. N. Weiss. (2001).** *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol., 31(2): 217-220.

-**Thiebaut, R., Leproust, S., Chene, G. and Gilbert, R. (2007).** Effectiveness of prenatal treatment for congenital toxoplasmosis: a metaanalysis of individual patients data. Lancet. V.(369):115-22.

-**Tobin C, Pollard A, Knoll L. (2010).***Toxoplasma gondii* cyst wall formation in activated bone marrow-derived macrophages and bradyzoite conditions. J. Vis. Exp. 2010: 2091

-**Verma R., and Khanna P. (2013).** Development of *Toxoplasma gondii* vaccine: A global challenge. Human Vaccines & Immunotherapeutics. US National Library of Medicine. 9 (2): 291–3.

-**Villena, I. D., Brodard, V., Queureux, C., Leroux, B., Chemla, C. and Pinon, J.M. (1999).** Detection of specific immunoglobulin E during maternal, fetal, and congenital toxoplasmosis. J. Clin. Microbiol. 37(11): 3487-3490.

-**Weiss L.M., Dubey J.P. (2009).** Toxoplasmosis: A history of clinical observations. Int. J. Parasitol. 1; 39(8):895-901.

Xin-Chao Liu, Yu He, Deng-Ge Han, Zhen-Chao Zhang, Ke Li, Shuai Wang, Li-Xin Xu, Ruo-Feng Yan and Xiang-Rui Li.(2017). Detection of *Toxoplasma gondii* in chicken and soil of chicken farms in Nanjing region, China Infectious Diseases of Poverty 6:62.

-**Yan, C., Liang, L. J., Zheng, K. Y., & Zhu, X. Q. (2016).** Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasites & vectors, 9, 137.

-**Yasodhara P., Ramalakshmi B.A., Lakshmi V., Krishna T.P.(2004).** Socioeconomic status and prevalence of toxoplasmosis during pregnancy. Indian J. Med. Microbiology. 22: 241-243.

-**Zhou, P., H. Zhang, R. Q. Lin, D. L. Zhang, H. Q. Song , C. Su and X. Q. Zhu. (2009).** Genetic characterization of *Toxoplasma gondii* isolates from China .In. J. Parasitol. 58:193-195.

الخلاصة

أجريت هذه الدراسة خلال الفترة من شهر أيلول 2017 الى أيار 2018 في محافظة الديوانية وذلك لتقيم انتشار داء المقوسات *Toxoplasma gondii* في عينات من لحوم الابقار ومنتجاتها المحلية والمستوردة

تم فحص ثلاثمئة وتسعون عينة 300 عينة كانت من اللحوم المحلية والمستوردة والتي جمعت من (القلب ,العضلات ,اللسان) ل100 عجل مذبوح تتراوح اعمارهم من 6 اشهر الى 4 سنوات و 90 عينة من منتجات لحوم مختلفة محلية ومستوردة (لحم مثروم ,هامبرغر) وخضعت عينات اللحوم للفحص المجهري وذلك للبحث عن طور bradyzoite المطمور بالأنسجة .

تم استخلاص ال DNA لجميع العينات وأخضعت الى تقنية تفاعل السلسلة المتبلمرة Conventional PCR للكشف عن الاصابة جزيئيا "من خلال تضخيم جين *B1* .

وأظهرت النتائج أن نسبة الاصابة باللحوم المحلية 22% أعلى منها في اللحوم المستوردة والتي سجلت نسية اصابة 13.5% أما بالنسبة لمنتجات اللحوم فقد سجلت المحلية منها نسبة اصابة 16% اما المستوردة فكانت النسبة للعينات المفحوصة Negative , وكشفت الدراسة بعدم وجود فرق معنوي بالنسبة لوجود الطفيلي بالأعضاء المفحوصة وكذلك وجدت الدراسة أنه على الرغم من تواجد الاصابة في جميع الاعمار لكن النسبة تزداد بتقدم الاعمار, ايضا سجلت الفترة من أيلول الى تشرين الثاني (الخريف) نسية اصابة اعلى من باقي مواسم الاخرى التي اجريت فيها الدراسة.

و تم اجراء فحص Gene sequencer لتحليل الشجرة الوراثية لمجموعة من العينات حيث وجدت الانواع الثلاثة(RH (Type I strain), Me 49 (II) and GT1 (III وكان (II) اكثر نسبة من بين الانواع الاخرى .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة القادسية

كلية الطب البيطري

التشخيص الجزيئي لطفيلي مقوسة كوندي في لحوم الابقار المحليه والمستوردة ومنتجاتها في محافظة الديوانية

رسالة مقدمة الى

عمادة كلية الطب البيطري/ جامعة القادسية

كجزء من متطلبات نيل شهادة الماجستير في علوم الطب البيطري /الطفيليات

من قبل

فرح محمد صكبان الصالحي

بكالوريوس طب وجراحة بيطرية /2013

بإشراف

أ.د. نعمان ناجي عايز

2019 **1440**