Kurdistan Regional Government
Ministry of Higher Education and Scientific Research
Erbil Polytechnic University
Erbil Medical and Health Technical College
Medical Laboratory Technology (MLT)

2023-2024

A Report Submitted to the Medical Laboratory Technology Department Erbil Medical and Health Technical University/ Erbil Polytechnic University of complete requirements for the Bachelors/degree in Medical Laboratory Technology

B. Sc. graduation project about:

Seroprevalence of *Toxoplasma gondii* In Back yard Chicken in Erbil City

Prepared by:

Hardy Mahdi Mustafa
Awat Qadir Mustafa

Supervised by:

Assist Prof. Dr. Hemdad Hawez Mawlood

MARCH **KHAKALEW (NISAN)** **SHAWWAL**
2024 AD **2724 K** **1445 AH**

In the name of Allah

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَّرَهُ مَنَازِلَ
لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابَ ۚ مَا خَلَقَ اللَّهُ ذَٰلِكَ إِلَّا بِالْحَقِّ
يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُونَ ﴿٥﴾

سورة يونس الآية 5

I certify that this Bachelor's thesis titled "Seroprevalence of *Toxoplasma gondii* In Back yard Chicken in Erbil City" was prepared by Hardy Mahdi Mustafa, Awat Qadir Mustafa, Farhang khattab Othman, Asma Sharif Ali under my supervision at the Faculty of Medical Laboratory Technology, Erbil Health and Medical Technical College, Erbil Polytechnic University. This thesis was completed in partial fulfillment of the requirements for the Bachelor of Science degree in Medical Laboratory Technology.

Signature:

Supervisor: **Assist Prof. Dr. Hemdad Hawez Mawlood**

Date: 18 / 04 / 2024

In view of the available recommendations, I forwarded this project for debate by examining committee.

Signature:

Name: **Assist. Professor: Najat J. Ahmad**

Chairman of MLT Dept.

Date: 18 / 04 /2024

MLT Department
Erbil Medical and Health Technical College
Erbil Polytechnic University

18 April 2023

We certify that we have read this project and as an examining committee examined the students in its contents and that in our opinion it is adequate with ( ) standing as research for the degree of Bachelor in Medical Laboratory Technology.

Signature:

Name: **Assist Prof. Dr. Hemdad Hawez Mawlood**

Scientific grade: **Assist. Professor**

Date: 18 / 04 / 2024

(Supervisor)

Signature:

Name: **Assist. Prof. Dr. Zuber Ismahil Hassan**

Scientific grade: **Assist. Professor**

Date: 18 / 04 / 2024

(Member)

Signature:

Supervisor: **Assist. Prof. Dr. Karwan Najm Salo**

Scientific grade: **Assist. Professor**

Date: 18 / 04 / 2024

(Member)

Dedication

We dedicate this significant accomplishment to Almighty Allah and Prophet Muhammad, acknowledging their divine guidance and blessings throughout our journey. And thanks to our fathers and merciful mothers.

Acknowledgement

At first many thanks for Allah give us the ability and helping us to succeed in prepared this Research. we try best to prepare this research about Seroprevalence of Toxoplasma gondii In Back yard Chicken in Erbil City we appreciating our research with all trying of the works at the beginning till the end to be perfect. we thanksgiving to our family because of everything they do for us till now and give us very good life and help us to be success and happiness in our life, we can't find the right words to describe and tell our love and thanks to them. we would express our respectful to Assist Prof. Dr. Hemdad Hawez Mawlood. we appreciate his attempt to give us important information in our graduation project, we learn a lot with him and we have a great respect and appreciate his efforts. We extend our heartfelt gratitude to all those who contributed to the completion of this thesis. Our deepest thanks go to our supervisor, Dr. Hemdad Hawez Mawlood, Assistant Professor. His invaluable guidance, encouragement, and insightful suggestions have been instrumental in shaping the trajectory and success of this project. We also gratefully acknowledge the constructive feedback and advice from other supervisors and panel members. Their insights have significantly enriched our presentation skills and enhanced the overall quality of our work. Finally, our sincere and profound appreciation is extended to our families. Their constant love, patience, and understanding have been the foundation of our academic pursuits and successes. Their enduring support has been a source of strength and inspiration, for which we are eternally grateful.

ABSTRACT

Background: One third of population causes toxoplasmosis among the word. All birds and animals without exception infected by *Toxoplasma gondii* during our research we conducted that beside human also back yard chicken causes *T. gondii* in Erbil city. It is nonsignificant relations between age group and Toxoplasmosis among back yard chicken especially age group > 3.5 years it was (7%). In another hand collections of samples during different months also find out nonsignificant correlation between monthly distribution with *T. gondii* infection among back yard chicken especially in December the rate of infection it was (8%). Farther study it is necessary for doing in future to find out pathogenic strain for *Toxoplasma gondii* among back yard chicken by DNA sequencing or RFLP-PCR and the best choose to that goal using NGS Techniques.

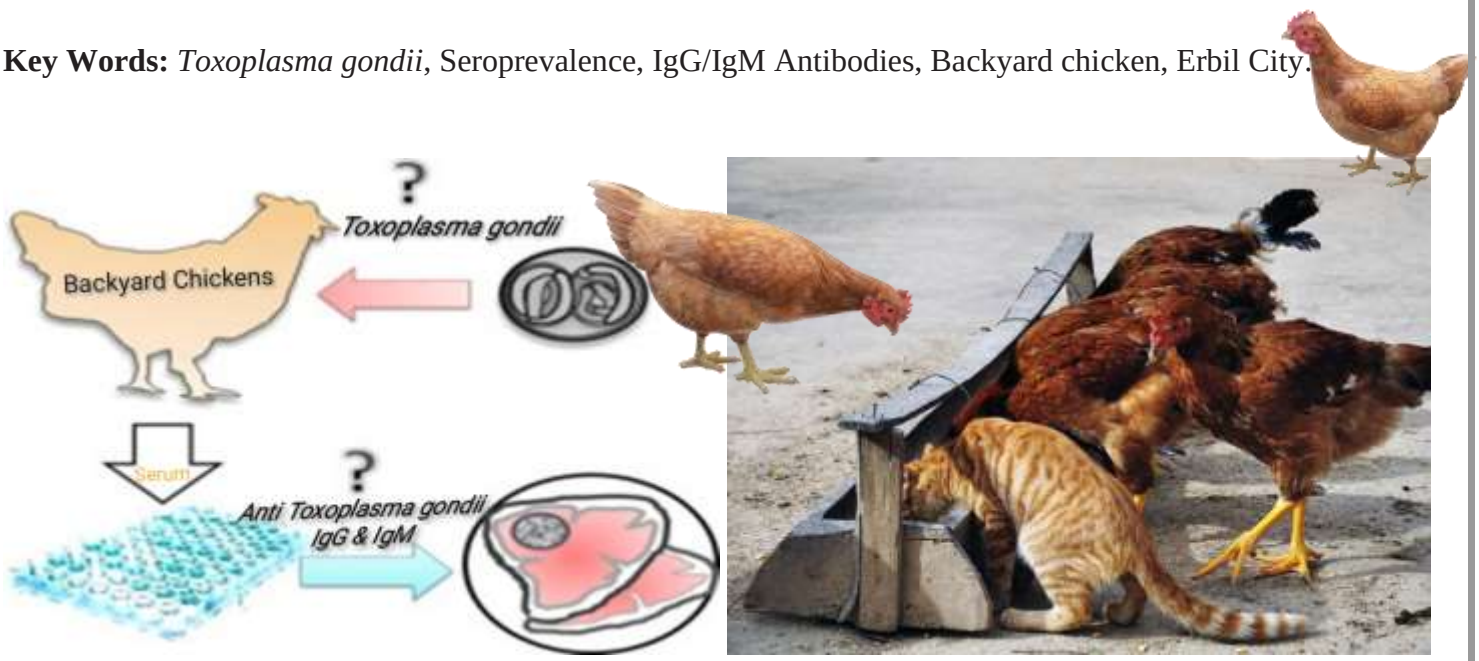
Methodology: For this aim we were used, updating techniques for measuring Anti-Toxoplasma IgG & IgM by using Cobas 6000.

Results: In this study the outcome of infection for toxoplasmosis among back yard chicken (Total positivity was 18%). While highest infection it was (7%) among age group (> 3.5 Years of back yard chicken). The total positivity of *T. gondii* according to Monthly distributions, was (18%). While highest infection was (8%) in December.

Conclusion: We concluded that our researchers should be study *T. gondii* in birds beside human in our region because in this research we find out positive cases of *T. gondii* among back yard chicken.

Recommendation: We recommended to our population do not eat fresh back yard chicken only after 2 Ours of freezing. And this kind of study it need budget from government to supporting that kind of research also using Molecular technique to find out mutation gene and pathogens strains.

Key Words: *Toxoplasma gondii*, Seroprevalence, IgG/IgM Antibodies, Backyard chicken, Erbil City.



List of Abbreviations:

1- IgG - Immunoglobulin G.

2- IgM - Immunoglobulin M.

3- *T. gondii* - *Toxoplasma gondii*.

4- PCR - Polymerase Chain Reaction.

5- RFLP - Restriction Fragment Length Polymorphism.

6- ISE - ion selective electrode.

7- ECL – Electrochemiluminescence.

8- NGS – Next Generating Sequencing.

Outline:

Chapter One

Chapter Two

Chapter Three

Chapter four

Chapter Five

LIST OF FIGURES

List of tables

List of charts

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction

Toxoplasmosis, which is induced by the parasite *Toxoplasma gondii*, is prevalent in humans and animals worldwide. *T. gondii* is classified as a protozoan parasite that requires living inside the cells of warm-blooded vertebrates. The prevalence of this infection is extensive among human and animal communities across the globe. (1) Both humans and animals, serving as intermediate hosts, acquire the infection by ingesting food or water that is contaminated with durable oocysts excreted by cats, which are the definitive hosts. Furthermore, humans can also become infected with Toxoplasma gondii by consuming undercooked or raw meat that contains tissue cysts. (2) Cats acquire *T. gondii* through the consumption of infected tissues from intermediate hosts, such as birds and rodents. Backyard chickens, also known as domestic free-range chickens, are effective indicators of environmental contamination with *T. gondii* oocysts because of their ground foraging habits. These chickens, especially those kept in backyard settings, play a significant role as intermediate hosts in the life cycle of *Toxoplasma gondii.* Therefore, studying the impact of *T. gondii* on backyard chickens is essential for considerations related to animal welfare and public health. (2) Cats possess the capability to release a vast number of oocysts in their feces, which can persist for several months, even when subjected to outdoor elements. These oocysts undergo sporulation and transform into a contagious state within a span of 1 to 5 days, a process that is influenced by prevailing climatic conditions. (3) Birds and mammals, such as humans, have the potential to acquire the infection by consuming sporulated oocysts that are present in water or unwashed food that has been contaminated with cat feces. An alternative mode of infection is through the consumption of tissue cysts that are found in raw or undercooked meat. (3) Additionally, in mammals, there is a possibility of vertical transplacental transmission of the infection. (4). Chickens, particularly those granted the freedom to wander, have been suggested as noteworthy intermediary carriers in the study of toxoplasmosis. (5) Owing to their inclination for an outdoor existence, inclination to forage on the ground, and vulnerability to infection, chickens hold the potential to serve as indicators of environmental pollution caused by *T. gondii.* Additionally, they can act as sentinels in areas where there is a high occurrence of *T. gondii* infection among humans. (6) Free-range chickens have been commonly employed in research to examine the occurrence and genetic diversity of *T. gondii* on a global scale. (7)

Aim of the study

Given the substantial global consumption of poultry meat and the potential risk of human infection through this pathway, it is crucial to understand the factors contributing to the spread and risk of *T. gondii* infection in poultry. To address this concern and ensure the production of safe poultry products in Erbil city, a seroprevalence survey was conducted among backyard chickens. The aim was to determine the prevalence of *T. gondii* infections and identify potential risk factors for chicken infection. The Cobas 6000 system, utilizing the same kit used for human sera, was employed to detect Anti-*Toxoplasma gondii* IgG and IgM antibodies. Additionally, the study aimed to investigate the pathogenicity of consuming chicken meat within the Kurdish community. Moreover, if admitted to a master's degree program following graduation, there is a plan to conduct further research involving genotyping *T. gondii* in tissues from backyard chickens. This would involve utilizing PCR sequencing, microsatellite analysis, and PCR-RFLP genotyping techniques to identify pathogenic genes.

Literature Review

1.1 Epidemiology of Toxoplasmosis and Free-range Chicken

The release of oocysts into the environment by cats has been linked to multiple instances of Toxoplasmosis outbreaks in humans. However, it is believed that direct interaction with cats has minimal impact on the spread of the disease on an epidemic scale. (8) Detecting *T. gondii* oocysts in the environment poses challenges due to cats typically burying their feces in soft, damp soil. Nonetheless, free-range chickens have emerged as effective indicators of soil contamination with *T. gondii* oocysts. This is because they forage on the ground, and the tissues of infected chickens are viewed as significant sources of infection for cats. (9) Cat fed with infected chicken tissue has been demonstrated to shed large number of oocysts. (10) Ingestion of infected chicken meat can also be a source of infection for T. gondii infection in humans and other animals. (11) Chicken has been proposed as a more effective intermediate host for *T. gondii* and may play a more significant role in the parasite's epidemiology compared to rodents. This is because chickens exhibit clinical resistance to *T. gondii* and have longer lifespans than rodents. (12) Despite their importance in the transmission dynamics of the parasite, research on the seroprevalence of *Toxoplasma gondii* in chickens, particularly in Erbil, has been limited. However, international research has yielded positive results regarding the seroprevalence of *Toxoplasma gondii* in backyard chickens across various countries. Some example of Seroprevalence of *T. gondii* in chickens shown in the figures below.

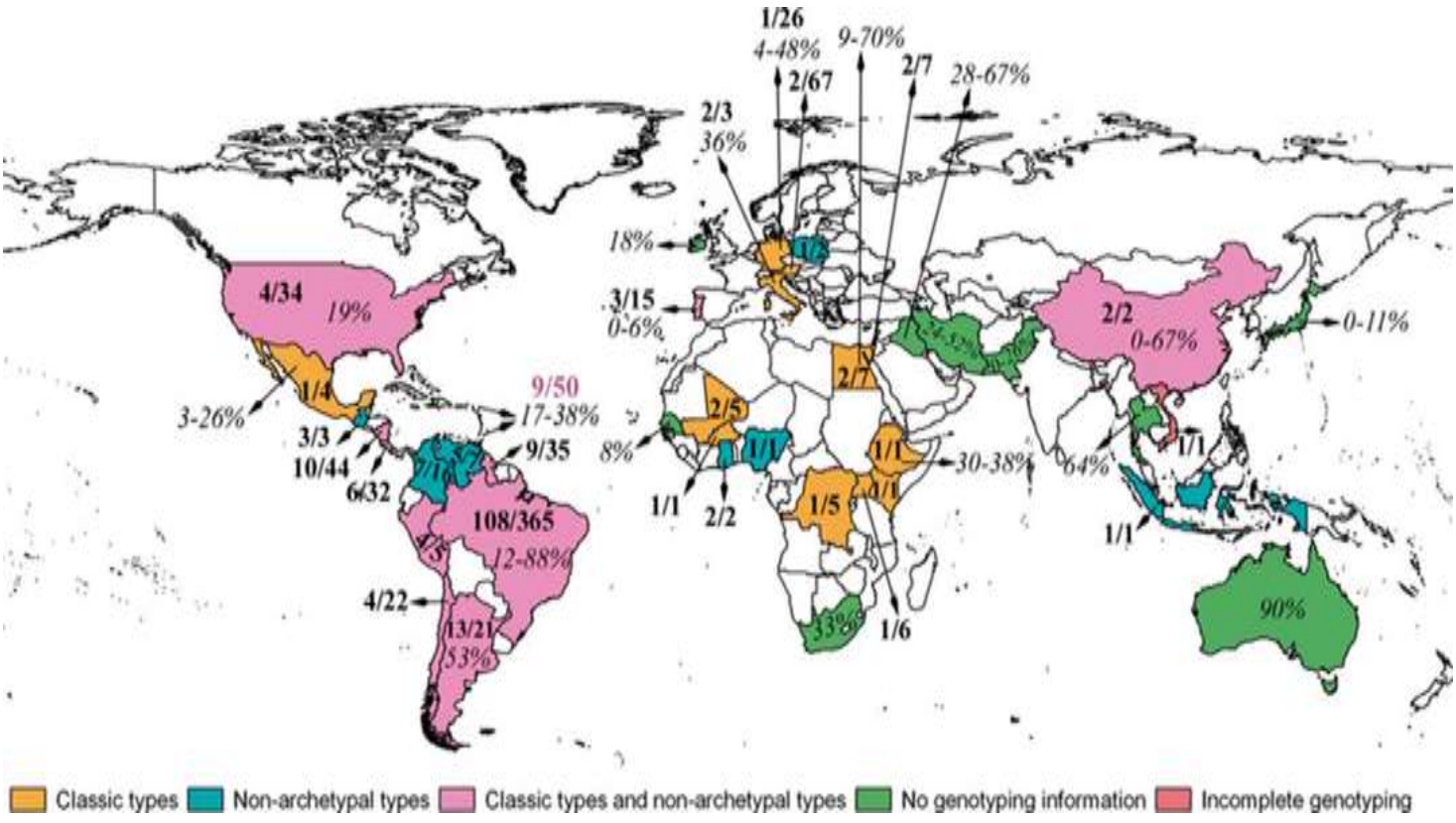


Figure 1: Epidemiologic significance of *Toxoplasma gondii* infections in chickens.

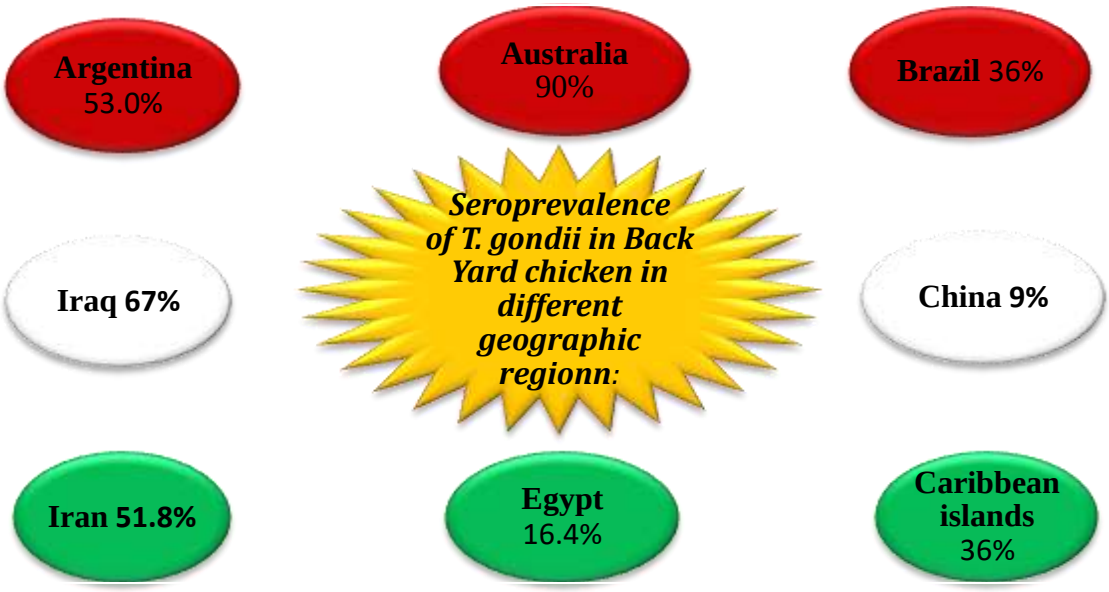


Figure 2: Seroprevalence of *T. gondii* in Back Yard chicken in different geographic region.

1.2 Chickens as Source of T. gondii Infection for Humans

Consuming chicken meat contaminated with *T. gondii* can serve as a potential source of infection for both humans and other animals. (13) There are lots of report indicating that both chickens raised in backyard and commercial free-range systems harbors viable *T. gondii* (14) In many developing countries, a significant number of chickens raised in these settings are slaughtered at home without supervision. This practice can potentially lead to the transmission of *T. gondii* infection to humans if proper precautions are not taken, such as thorough handwashing after handling and cooking the meat. Additionally, the disposal of chicken viscera is often inadequate, leaving them accessible to scavengers and thus facilitating the transmission of *T. gondii* to reservoirs like rodents or even cats, the final host of the parasite. While the likelihood of humans becoming infected by consuming eggs from infected chickens has not been fully established, studies have shown that *T. gondii* can survive in egg yolk and albumin when boiled for three minutes, and even when the egg yolk is fried. However, there is ample evidence to suggest that raw eggs are unlikely to be a source of infection for humans. (13) To minimize the risk of *T. gondii* infection, it is crucial for individuals to avoid consuming raw eggs. (13)

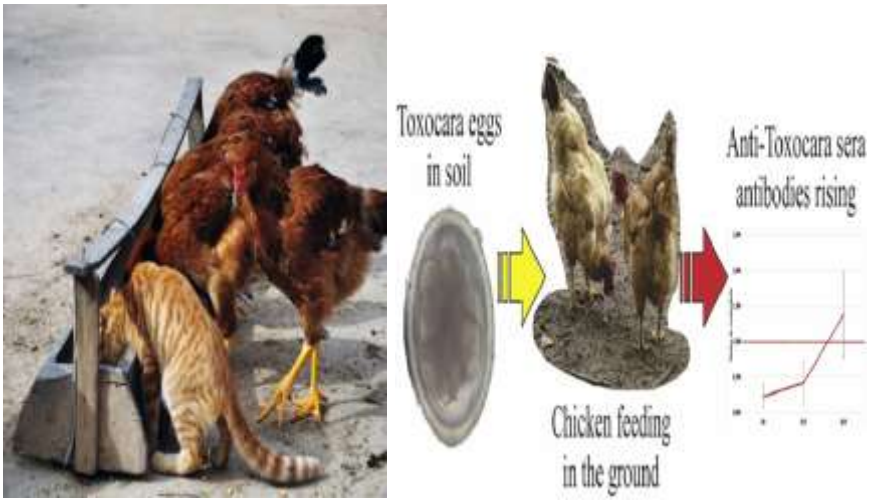


Figure 3: Chickens play a significant role as intermediate hosts for Toxoplasma gondii and can potentially transmit the infection to humans. This is because chickens often coexist with cats, occasionally come into contact with them, and typically feed on the ground. The tissues of infected chickens. are believed to be a crucial source of infection for both cats and humans.

1.3 Backyard chicken

Chickens serve as significant intermediate hosts for *Toxoplasma gondii* and can potentially transmit the infection to both humans and carnivores. (15). The infection of chickens with *T. gondii* is regarded as significant from an epidemiological perspective. Ground-foraging birds, including chickens, play a crucial role as indicators of environmental contamination with oocysts due to their feeding habits. Additionally, the tissues of infected chickens are believed to be a key source of infection for both felids and humans. (16). In Kurdistan, chickens are raised for multiple purposes, including income generation, fulfilling socio-cultural roles, and serving as a source of animal protein. They often have close contact with humans, especially in rural areas and among impoverished urban communities. The unsanitary conditions in which they are raised may directly or indirectly influence the presence of *T. gondii* in chickens, particularly in backyard settings where chickens scratch the ground in search of food, often from waste material. (17). Dead chickens from trading sites and households are frequently disposed of without proper biosafety measures, becoming potential sources of infection for cats and other felids. Moreover, backyard chickens often share the same environment, including water sources, with people. Combined with poor hygiene practices, this increases the potential risk of *T. gondii* infection.

Figure 4: Three different adult chicken breeds. (a) Commercial layer hen (HL); (b) Hybrid red jungle fowl with native chicken breed (Kai-Tor) and (c) White tail yellow chicken (WTYC).

1.4 Toxoplasma gondii infection: Kurdistan situation

Numerous research on the seroprevalence of *toxoplasmosis* in cattle in Kurdistan have primarily focused on ruminants, with limited reports available on canids. These studies show that scavenging on trash dumps, which are a common place for stray cats to congregate, is a primary source of livestock infection, which could subsequently pose a risk of infection to humans. However, despite increased investigation into the role of livestock and other domestic animals in the epidemiology of *Toxoplasma gondii* infection, the significance of free-range chickens has been overlooked. Free-range chicken is highly prized in Kurdistan due to its superior taste compared to commercial chicken, as well as its perceived health benefits from being reared without drugs and artificial feed additives. Despite the abundance of free-range chickens in Kurdistan, little attention has been paid to their management system, and most are slaughtered in backyards without inspection. In contrast to developed countries, keeping cats as household pets is not widespread in Kurdistan due to varying socio-cultural values across different regions. However, recent studies have revealed a high seroprevalence of *Toxoplasma gondii* antibodies among the Kurdistan population that does not keep pets. Although these studies were unable to pinpoint the exact sources of infection, the role of free-range chicken cannot be underestimated as a potential transmission source of *T. gondii* infection to humans. This is especially significant since most households in Kurdistan have free-range chickens. There is currently limited information available on the role of free-range chicken in assessing the level of environmental contamination by *T. Gondii* and its role in transmitting the infection to humans, cats, and other livestock in Kurdistan. (17)

CHAPTER TWO

METHODOLOGY

2.1 Material and Devices

2.1.1 The Materials that have been used in our research are listed below:

1. Cotton
2. Alcohol 70%
3. Syringe 3cc or 2.5ml
4. Needles 25 gauge for small chickens
5. Needles 23 gauge for larger chickens
6. Gel tube (Yellow tube)
7. Bandage
8. Micropipette
9. Tips
10. Cup
11. Anti Toxoplasma IgG Kit
12. Anti Toxoplasma IgM Kit
13. Labels or marking pen to label each syringe and tubes.

2.1.2 The Devices and Machines used and their Manufacturers:

1) **Centrifuge**
2) **Refrigerator (-20)**
3) **Micropipette**
4) **Cobas 6000 (Roche)**
5) **Autoclave**

2.2 Method of Sample Collection

2.2.1 Selection Criteria:

Chickens from various regions of Erbil city were selected randomly for checking positive sample for Toxoplasmosis.

2.2.2 Blood Collection:

Blood samples was collected during aseptic area, blood was drawn from usable site which include (Brachial wing vein, Medial metatarsal vein, and Jugular vein location), focusing to using merciful technique with the Chicken.

Figure 5: Sites of blood collection from Back Yard Chickens.

2.2.3 Storage:

Serum was separated by centrifugation of blood by 3000rpm during 10 minutes, then stored at -20°C refrigerator.

2.2.4 Procedure of Sample Collection

1) Instruct an assistant to hold the chicken horizontally on its back, supporting the legs with one hand and placing the other hand under the back for support.
2) Extend one wing of the chicken towards you.
3) Identify the wing vein, which is clearly visible between the biceps and triceps muscles, forming a V shape. Take note of the pronator muscle tendon running across the V.
4) Remove any small feathers that may obscure the vein.
5) Clean the area around the bleeding site by swabbing with 70 percent alcohol.
6) Insert the needle under the tendon and direct it into the wing vein following the direction of blood flow. Take care not to insert the needle too deeply and avoid the ulnar nerve.
7) Once the needle tip is in the vein, gently pull the syringe plunger to draw blood. If blood flow is not observed, release the plunger and make slight adjustments to reposition the needle.
8) Use gentle suction to withdraw the blood as chicken veins collapse easily.

9) If a hematoma develops, attempt bleeding from the other wing.
10) After removing the needle, apply pressure to the vein for a few seconds to prevent further bleeding.
11) Ideally, discard the needle into a needle disposal container and cap the syringe to prevent serum leakage. However, if disposal containers are unavailable, cap the needle end of the syringe carefully to avoid needle stick injuries.
12) Pull the syringe plunger back approximately 1 cm and place the syringe at an angle with the needle end up in a rack to facilitate clotting. (17)

Procedure of Sample Collection from Back Yard Chickens:

Figure 6: Procedure of Sample Collection from Back Yard Chickens.

2.3 Diagnostic Techniques

Serological test by using Back yard chickens' serum sample in Sample cup: 2.5 mL to detect Anti *Toxoplasma gondii IgG &* Anti *Toxoplasma gondii IgM test by* Cobas 6000.

Serological Test, Includes:

3 *Anti Toxoplasma gondii* IgG.

4 **Anti *Toxoplasma gondii* IgM**.

Figure 7: Cobas 6000 and Anti *Toxoplasma gondii* IgM and Anti *Toxoplasma gondii* IgG Kits.

2.3.1 Cobas 6000 *Toxoplasma gondii* IgG antibody immunoassay

A 10-microliter sample containing a biotinylated recombinant *T. gondii*-specific antigen and a *T. gondii*-specific recombinant antigen labeled with a ruthenium complex form a sandwich complex. Once streptavidin-coated microparticles are added, the complex binds to the solid phase through the interaction of biotin and streptavidin. The reaction mixture is then transferred into a measuring cell, where the microparticles are magnetically captured onto the electrode surface. Any unbound substances are removed using ProCell. Application of voltage to the electrode induces chemiluminescent emission, which is measured by a photomultiplier. Results are quantified as international units per milliliter in the IgG assay. Interpretation of IgG results follows the manufacturer's criteria: IgG antibodies are considered positive if ≥3.0 IU/mL, equivocal if ≥1.0 to <3.0 IU/mL, and negative if <1.0 IU/ml. (18)

2.3.2 Cobas 6000 *Toxoplasma gondii* IgM antibody immunoassay

To evaluate the serological status of *T. gondii* infection, the detection of Toxoplasma IgG antibodies is utilized, serving as an indicator of either latent or acute infection. In cases of acute acquired infection during pregnancy, diagnosis relies on either seroconversion or a notable increase in antibody titers (IgG and/or IgM) across consecutive samples. The µ Capture test operates with an assay duration of 18 minutes and follows these steps:

First Incubation: A 10 µL sample is automatically diluted 1:20 with Diluent Universal. *T. gondii*-specific recombinant antigen labeled with a ruthenium complex is introduced. Any anti-Toxoplasma IgM antibodies present in the sample react with the ruthenium-labeled *T. gondii*-specific recombinant antigen.

Second Incubation: Biotinylated monoclonal IgM-specific antibodies and streptavidin-coated microparticles are added. The resulting complex binds to the solid phase through the interaction of biotin and streptavidin.

The reaction mixture is transferred into the measuring cell, where the microparticles are magnetically captured onto the electrode surface. Unbound substances are then removed using ProCell/ProCell M.

Application of voltage to the electrode induces chemiluminescent emission, which is detected by a photomultiplier. Results are automatically determined by the software, comparing the electrochemiluminescence signal of the sample reaction product with the previously calibrated cutoff value. (19)

2.3.3 The Principle of Cobas 6000

The Cobas 6000 analyzer series represents a robust solution for comprehensive diagnostic laboratory automation. Designed to handle high throughput workloads efficiently, it combines ion selective electrode (ISE) and photometric analysis in the (c 501 module), along with an immunoassay analysis module in the (E 601 module). The Cobas E 601 module utilizes Electrochemiluminescence (ECL) technology for precise measurement. (20)

2.4 Data Analysis

All results have been collected and recorded, and the data has been analyzed using GraphPad. A total of 100 samples were collected and analyzed based on age group and monthly collection.

CHAPTER THREE

3.1 RESULTS

Table 1: Seropositivity of *Toxoplasma gondii* among backyard chickens by using Cobas 6000 according to Age Group			
Age Group	Total No. of Case	Negative (%)	Positive (%)
<1.5 Y	18	17	1
(1.5 - 2.5) Y	24	19	5
(2.5 - 3.5) Y	32	27	5
>3.5 Y	26	19	7

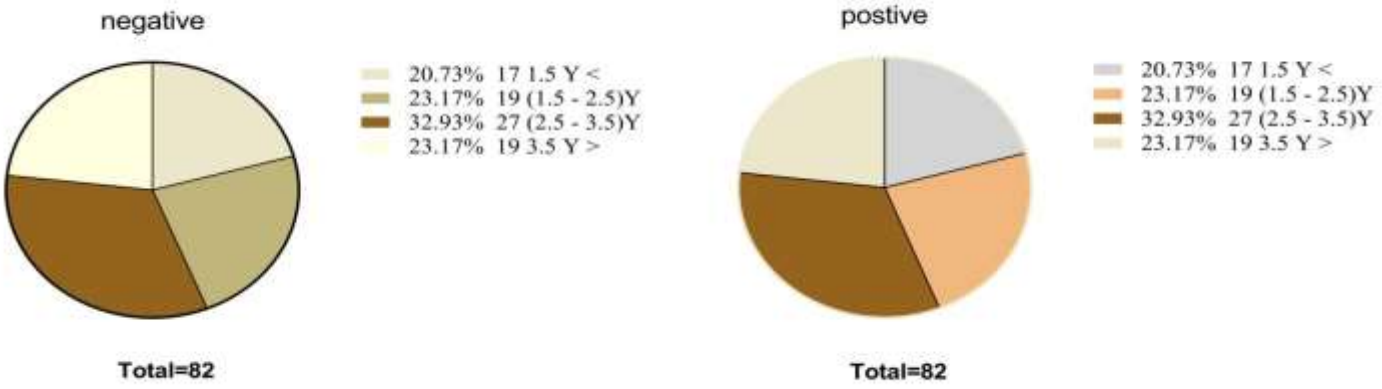


Figure 8: Seropositivity of *Toxoplasma gondii* among backyard chickens by using Cobas 6000 according to Age Group

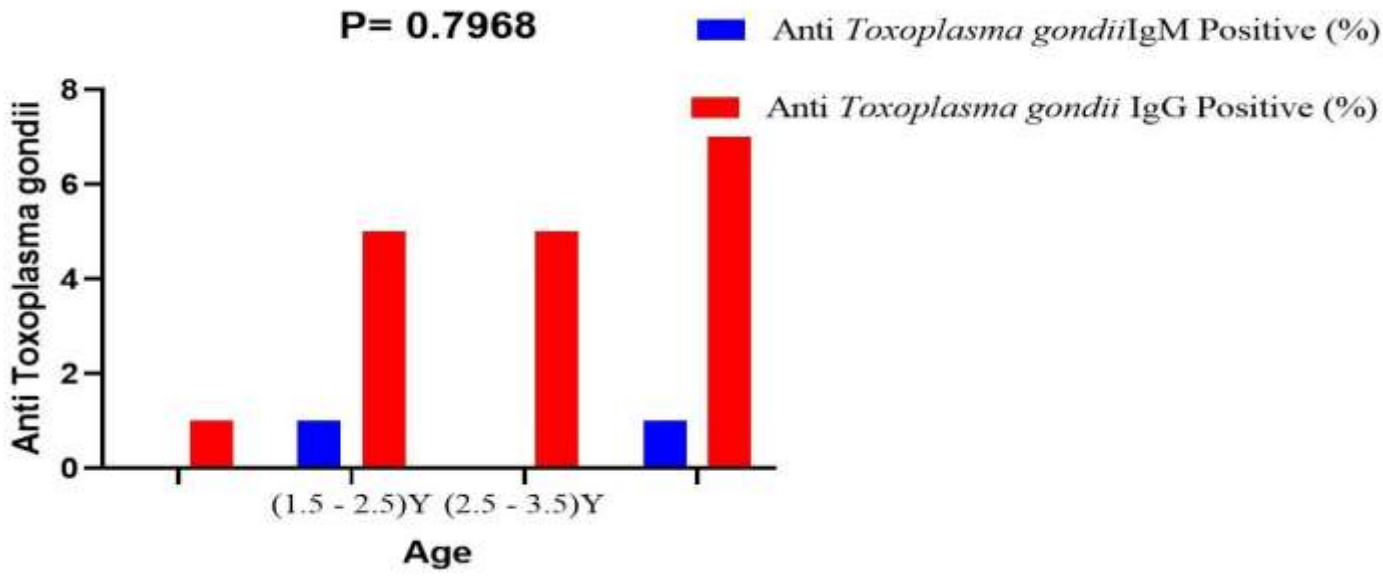


Chart 1: Seropositivity of Toxoplasma gondii among backyard chickens by using Cobas 6000 according to Age Group

Table 2: Seropositivity of T*oxoplasma gondii* among backyard chickens by using Cobas 6000 according to Monthly Distribution			
Collected sample according to Monthly distribution	Total No. of Case	Negative (%)	Positive (%)
October (2023)	2	2	0
November (2023)	14	13	1
December (2023)	31	23	8
January (2024)	30	27	3
February (2024)	14	10	4
March (2024)	9	6	3

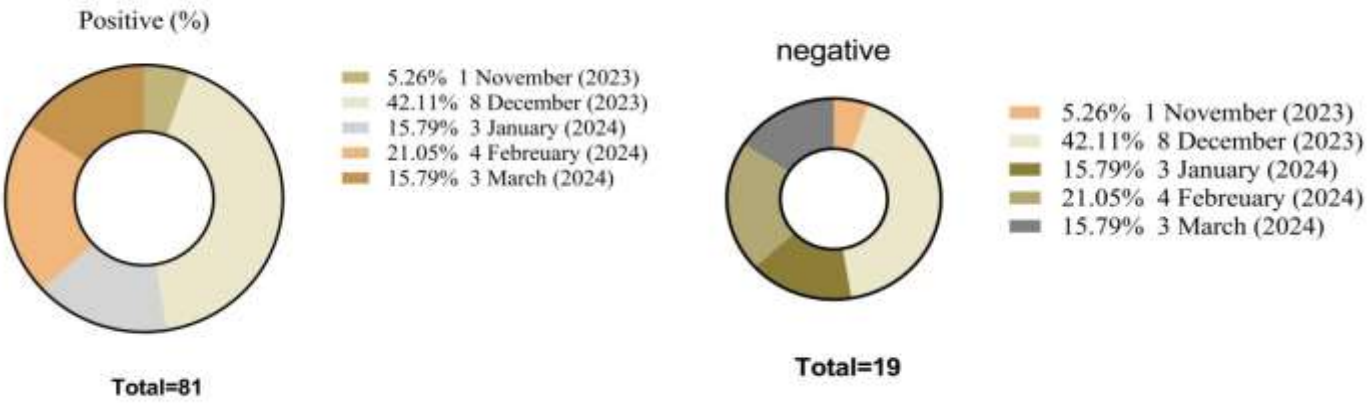


Figure 9: Seropositivity of *Toxoplasma gondii* among backyard chickens by using Cobas 6000 according to Monthly Distribution

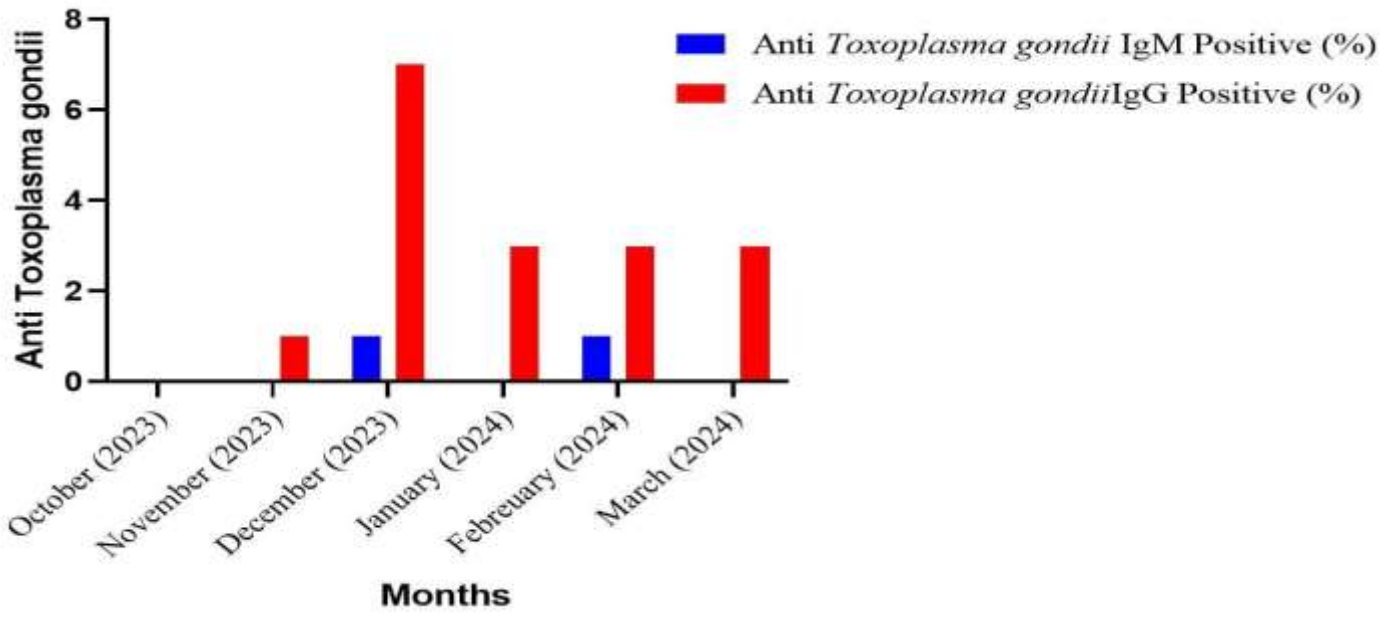


Chart 2: Seropositivity of Toxoplasma gondii among backyard chickens by using Cobas 6000 according to Monthly Distribution

Table 3: Seropositivity of Anti *Toxoplasma gondii* IgG & Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Age Group					
Age group	Total No. of Case	Anti *Toxoplasma gondii* IgM Negative (%)	Anti Toxoplasma gondii IgM Positive (%)	Anti Toxoplasma gondii IgG Negative (%)	Anti Toxoplasma gondii IgG Positive (%)
<1.5 Y	18	18	0	17	1
(1.5 - 2.5) Y	24	23	1	19	5
(2.5 - 3.5) Y	32	32	0	27	5
>3.5 Y	26	25	1	19	7

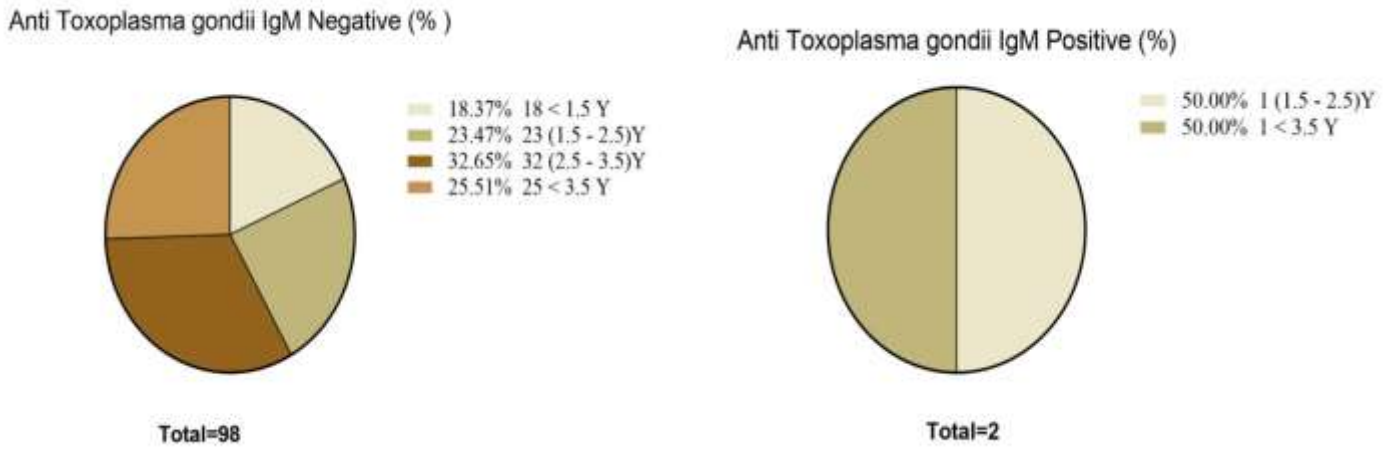


Figure 10: Seropositivity of Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Age Group

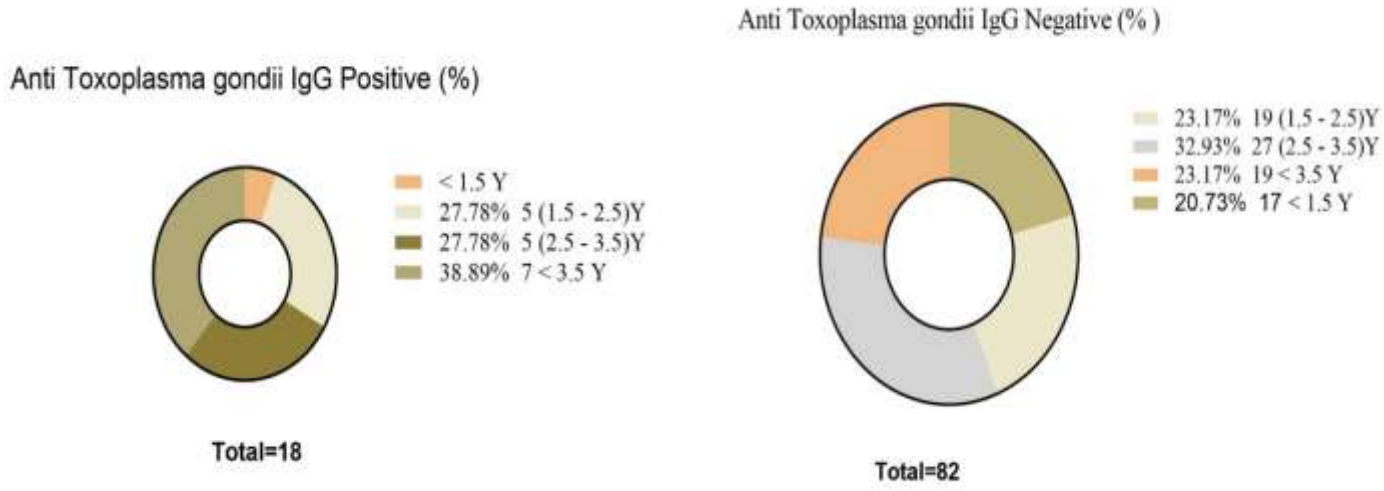


Figure 11: Seropositivity of Anti *Toxoplasma gondii* IgG among backyard chickens by using Cobas 6000 according to the Age Group

Table 4: Seropositivity of Anti *Toxoplasma gondii* IgG & Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Monthly Distribution					
Monthly distribution	Total No. of Case	Anti Toxoplasma IgM Negative (%)	Anti Toxoplasma IgM Positive (%)	Anti Toxoplasma IgG Negative (%)	Anti Toxoplasma IgG Positive (%)
October (2023)	2	2	0	2	0
November (2023)	14	14	0	13	1
December (2023)	31	30	1	25	7
January (2024)	30	30	0	27	3
Febreuary (2024)	14	13	1	10	3
March (2024)	9	9	0	6	3

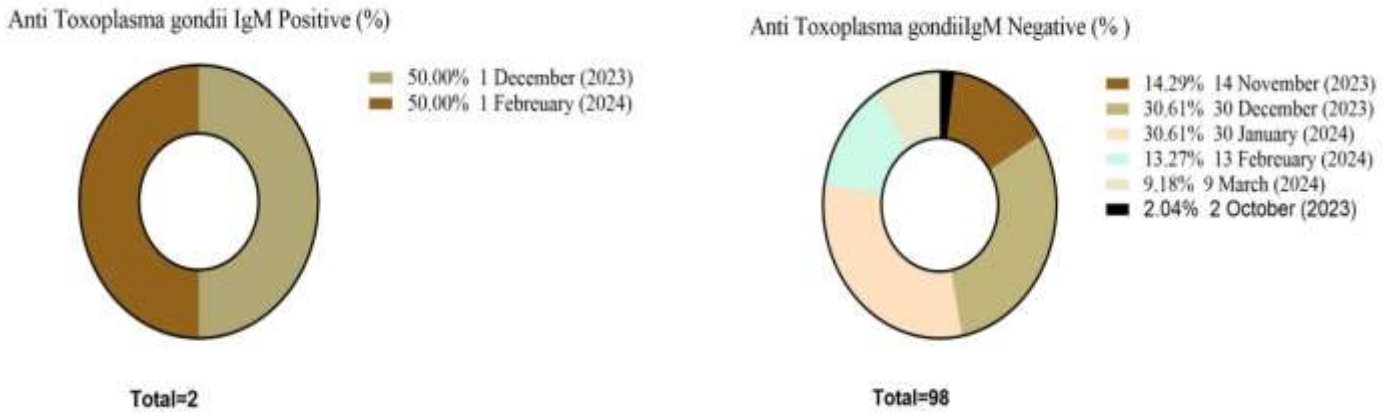


Figure 12: Seropositivity of Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Monthly Distribution

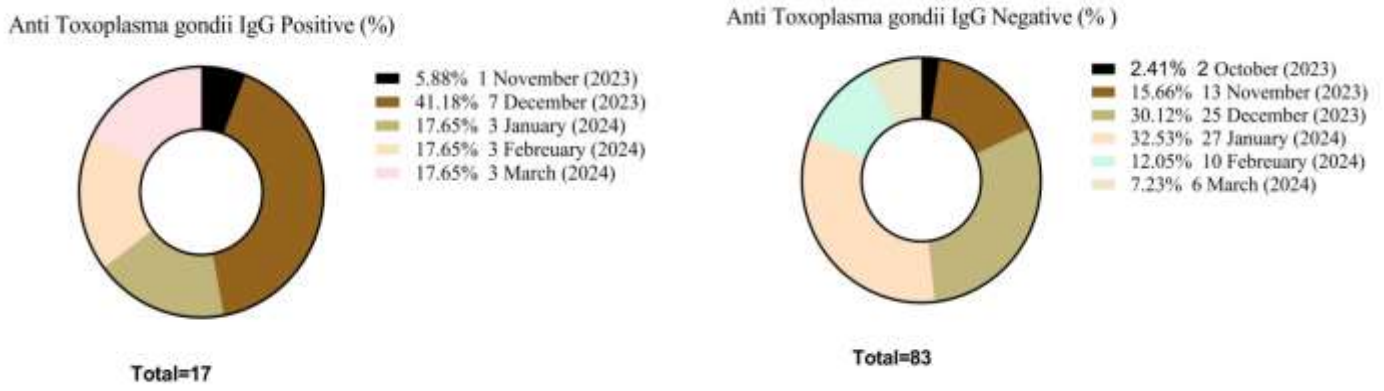


Figure 13: Seropositivity of Anti *Toxoplasma gondii* IgG among backyard chickens by using Cobas 6000 according to the Monthly Distribution

Table 5: Seropositivity of Anti *Toxoplasma gondii* IgG & Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Back Yard Chicken live with Cat					
Back Yard Chicken	Total No. of Case	Anti Toxoplasma IgM Negative (%)	Anti Toxoplasma IgM Positive (%)	Anti Toxoplasma IgG Negative (%)	Anti Toxoplasma IgG Positive (%)
Live With Cat	66	64	2	46	20
Do not live with Cat	34	34	0	32	2

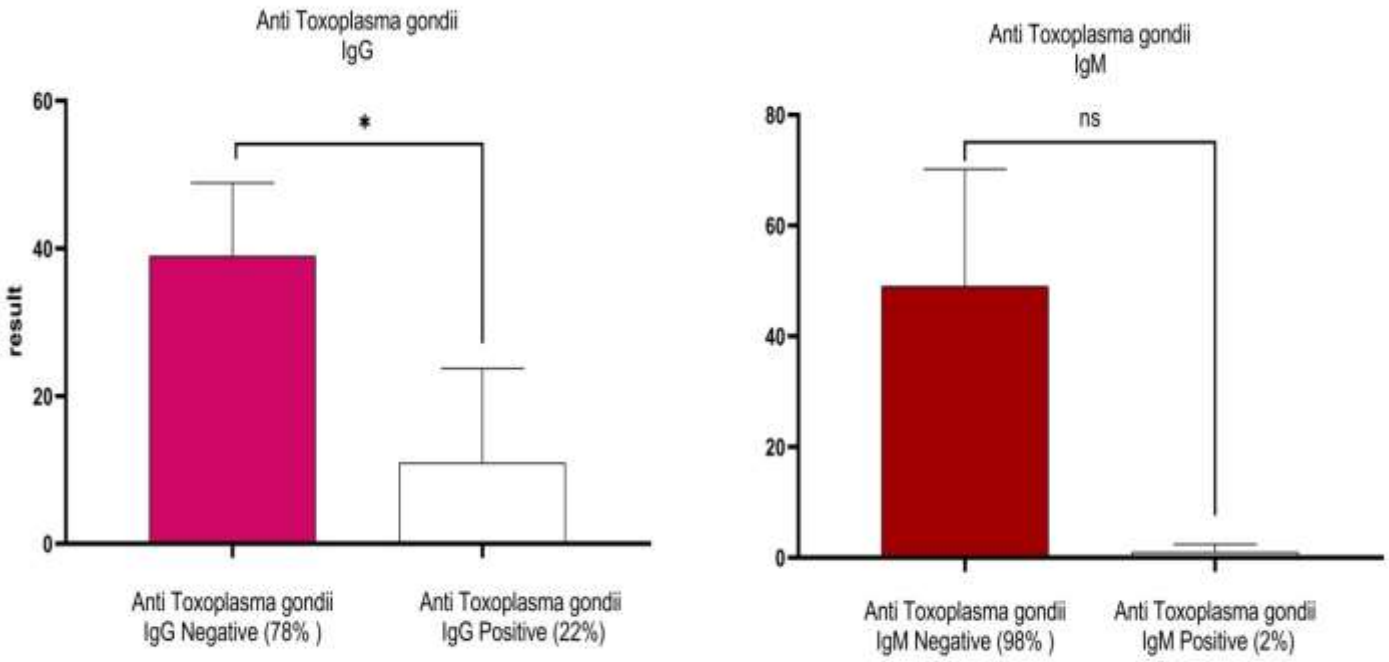


Chart 3: Seropositivity of Anti *Toxoplasma gondii* IgG & Anti *Toxoplasma gondii* IgM among backyard chickens by using Cobas 6000 according to the Back Yard Chicken live with Cat

CHAPTER FOUR

DISCUSSION

4.1 Discussion

Toxoplasmosis is recognized as a significant zoonotic illness, and there is growing interest in identifying the primary sources of human toxoplasmosis (21). Studies have shown that humans can acquire T. gondii infection by ingesting contaminated food and water containing infectious oocysts, or by consuming undercooked meat containing tissue cysts, tachyzoites, and/or bradyzoites. (22) With the rising popularity of consuming backyard chicken in Erbil province and the heightened concern over human toxoplasmosis, particularly in pregnant women, the current study was undertaken to assess the prevalence of T. gondii among backyard chickens from various areas in Erbil city.

In this study, the Cobas 6000 anti T. gondii IgG and IgM antibodies were utilized for the first time to detect T. gondii infection rates in backyard chickens in Erbil city. The findings revealed an overall infection rate of 18%. While there is limited literature available on T. gondii infection in backyard chickens in Iraq using Cobas 6000 and PCR for comparison, a previous study reported a higher infection rate of 60% in domestic chickens in Sulaimani city. Discrepancies between the two studies could arise from differences in the techniques employed or the number of samples tested; this study analyzed 100 samples using Cobas 6000 IgG and IgM, whereas the previous study tested 65 sera using latex agglutination test (LAT). (23) The escalating infection rates of T. gondii in free-range local chickens are attributed to the birds' feeding habits, particularly their tendency to forage directly from the ground. (24) This exposes them to food contamination, especially T. gondii oocysts, making them significant indicators of environmental contamination and potential sources of human infection. Additionally, it has been noted that cats can exacerbate chickens' toxoplasmosis by defecating on grass, which serves as a feeding ground for chickens. (25) This study observed variations in infection rates from month to month, with the highest rate recorded in December and the lowest rate in November. This study noted fluctuations in infection rates from month to month, with the highest rate observed in December and the lowest in November. These differences are presumed to be influenced by various factors, including variations in the densities of stray and domestic cats in the area during different months, the number of backyard chickens, and their feeding patterns. (26)

Previous research has indicated higher infection rates of T. gondii in backyard chickens in areas with greater cat densities and overcrowded chicken populations per farm. Moreover, evidence suggests that infection rates of T. gondii in backyard chickens vary widely across different regions of the world, ranging from 2% to 100%. (27) The significant variations observed in infection rates can be attributed to several factors, including differences in climate changes, which greatly impact the lifespan of oocysts, variations in diagnostic techniques used, the types of samples analyzed, and seasonality.

Previous studies have highlighted the influence of these factors on infection rates of T. gondii in various populations. Additionally, this study examined the infection rates of T. gondii among different age groups of backyard chickens. The data revealed that older chickens exhibited higher infection rates compared to younger ones. This variation is likely directly linked to increased exposure times with age, as older animals have had more opportunities for exposure to the parasite. Previous research has also demonstrated an increase in T. gondii infection with age. (28)

CHAPTER FIVE

CONCLUSION AND FUTURE WORKS

5.1 Conclusion

In this study, it is highlighted that consuming infected chicken meat poses a risk of *T. gondii* infection for both humans and other animals. Numerous reports indicate that chickens raised in both backyard and commercial free-range systems can harbor viable *T. gondii*. Particularly in developing countries, where many chickens are slaughtered at home without supervision, there is a potential for *T. gondii* transmission to humans if proper hygiene practices are not followed during meat handling and cooking. Serological tests conducted using serum samples from backyard chickens reveal the presence of Anti-*Toxoplasma gondii* IgG and Anti-*Toxoplasma gondii* IgM antibodies using the Cobas 6000 system, confirming *T. gondii* infection in the chickens.

5.2 Future Work

Moreover, upon acceptance into a master's degree program following graduation, we aim to pursue further research involving the genotyping of Toxoplasma gondii in tissues obtained from backyard chickens. Our objective is to identify pathogenic genes using a combination of PCR Sequencing, Microsatellite analysis, and PCR-RFLP genotyping techniques.

REFERENCES

1. Zhang XX, Zhang NZ, Tian WP, Zhou DH, Xu YT, Zhu XQ. First report of Toxoplasma gondii seroprevalence in pet parrots in China. Vector Borne Zoonotic Dis. 2014;14(6):394-8.
2. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. Int J Parasitol. 2009;39(8):895-901.
3. Webster J. Review of "Toxoplasmosis of Animals and Humans (Second Edition)" by J.P. Dubey. Parasites & Vectors - Parasites Vectors. 2010;3:1-2.
4. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol. 2000;30(12-13):1217-58.
5. Tassi P. Toxoplasma gondii infection in horses. A review. Parassitologia. 2007;49(1-2):7-15.
6. Paphitis K, Metcalf D, Weese JS. Backyard chickens - A cross-sectional survey of current and prospective backyard chicken owners in Ontario (2019-2021). Can Vet J. 2023;64(1):54-62.
7. Ouologuem Toure D, Djimde A, Diallo N, Doumbo O, Roos D. Toxoplasma gondii Seroprevalence in Mali. The Journal of parasitology. 2012;99.
8. Mustafa KM, Mohammed AB, Mero WMS. Seroprevalence of Toxoplasma gondii Antibodies and Associated Risk Factors Among Women in Zakho City, Iraq. Cureus. 2024;16(3):e56328.
9. Mace JL, Knight A. From the Backyard to Our Beds: The Spectrum of Care, Attitudes, Relationship Types, and Welfare in Non-Commercial Chicken Care. Animals (Basel). 2024;14(2).
10. Kamani J, Mani AU, Egwu GO. Seroprevalence of Toxoplasma gondii infection in domestic sheep and goats in Borno state, Nigeria. Trop Anim Health Prod. 2010;42(4):793-7.
11. Hiob L, Koethe M, Schares G, Goroll T, Daugschies A, Bangoura B. Experimental Toxoplasma gondii and Eimeria tenella co-infection in chickens. Parasitol Res. 2017;116(11):3189-203.
12. Geuthner AC, Koethe M, Ludewig M, Pott S, Schares G, Maksimov P, et al. Development of an in vivo model for Toxoplasma gondii infections in chickens and turkeys simulating natural routes of infection. Vet Parasitol. 2019;276:108956.
13. Fernández-Escobar M, Schares G, Maksimov P, Joeres M, Ortega-Mora LM, Calero-Bernal R. Toxoplasma gondii Genotyping: A Closer Look Into Europe. Front Cell Infect Microbiol. 2022;12:842595.
14. Elbez-Rubinstein A, Ajzenberg D, Dardé ML, Cohen R, Dumètre A, Yera H, et al. Congenital toxoplasmosis and reinfection during pregnancy: case report, strain characterization, experimental model of reinfection, and review. J Infect Dis. 2009;199(2):280-5.
15. Dubey JP, Pena HFJ, Cerqueira-Cézar CK, Murata FHA, Kwok OCH, Yang YR, et al. Epidemiologic significance of Toxoplasma gondii infections in chickens (Gallus domesticus): the past decade. Parasitology. 2020;147(12):1263-89.
16. Dubey JP. Toxoplasma gondii infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis and public health significance. Zoonoses Public Health. 2010;57(1):60-73.
17. Dubey JP. History of the discovery of the life cycle of Toxoplasma gondii. Int J Parasitol. 2009;39(8):877-82.
18. Dubey JP. Refinement of pepsin digestion method for isolation of Toxoplasma gondii from infected tissues. Vet Parasitol. 1998;74(1):75-7.
19. Donahoe SL, Lindsay SA, Krockenberger M, Phalen D, Šlapeta J. A review of neosporosis and pathologic findings of Neospora caninum infection in wildlife. Int J Parasitol Parasites Wildl. 2015;4(2):216-38.
20. Chumpolbanchorn K, Lymbery AJ, Pallant LJ, Pan S, Sukthana Y, Thompson RC. A high prevalence of Toxoplasma in Australian chickens. Vet Parasitol. 2013;196(1-2):209-11.
21. Chikweto A, Sharma RN, Tiwari KP, Verma SK, Calero-Bernal R, Jiang T, et al. Isolation and RFLP Genotyping of Toxoplasma gondii in Free-Range Chickens (Gallus domesticus) in Grenada, West Indies, Revealed Widespread and Dominance of Clonal Type III Parasites. J Parasitol. 2017;103(1):52-5.
22. Chikweto A, Kumthekar S, Tiwari K, Nyack B, Deokar MS, Stratton G, et al. Seroprevalence of Toxoplasma gondii in pigs, sheep, goats, and cattle from Grenada and Carriacou, West Indies. J Parasitol. 2011;97(5):950-.1

23. Chaklu M, Tarekegn ZS, Birhan G, Dagnachew S. Toxoplasma gondii infection in backyard chickens (Gallus domesticus): Seroprevalence and associated risk factors in Northwest Ethiopia. Vet Parasitol Reg Stud Reports. 2020;21:100425.
24. Basso W, Sollberger E, Schares G, Küker S, Ardüser F, Moore-Jones G, et al. Toxoplasma gondii and Neospora caninum infections in South American camelids in Switzerland and assessment of serological tests for diagnosis. Parasit Vectors. 2020;13(1):256.
25. Andreopoulou M, Chaligiannis I, Sotiraki S, Daugschies A, Bangoura B. Prevalence and molecular detection of Eimeria species in different types of poultry in Greece and associated risk factors. Parasitol Res. 2022;121(7):2051-63.
26. Alvarado-Esquivel C, González-Salazar AM, Alvarado-Esquivel D, Ontiveros-Vázquez F, Vitela-Corrales J, Villena I, et al. Seroprevalence of Toxoplasma gondii infection in chickens in Durango State, Mexico. J Parasitol. 2012;98(2):431-2.
27. Al-Rawazq H. Review of Seroprevalence of Toxoplasmosis in Iraq Introduction. 2020;5:40-56.
28. Al-Kappany YM, Rajendran C, Abu-Elwafa SA, Hilali M, Su C, Dubey JP. GENETIC DIVERSITY OF TOXOPLASMA GONDII ISOLATES IN EGYPTIAN FERAL CATS REVEALS NEW GENOTYPES. The Journal of Parasitology. 2010;96(6):1112-4.