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Parasitology

Entitled

Evaluation of the risk of toxoplasmosis infection from chicken in the region of Relizane

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"*In the name of Allah, the Most Gracious, the Most Merciful All praise belongs to Him the One who places light on our paths when all seems uncertain, who gifts us the strength to persist, and who blesses our efforts with fulfillmen It is by His grace alone that this humble thesis has seen the light.*

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From the depths of our hearts thank you."

Nora and chaima

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2-ME: 2 mercaptoethanol

DNA: Deoxyribonucleic Acid

ELISA: Enzyme-Linked Immunosorbent Assey

HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

IFAT: Indirect fluorescence antibody test

IgG: Immunoglobulin G

IgM: Immunoglobulin M

MAT: Modified Agglutination Test

MRI: Magnetic Resonance Imaging

PBS: phosphat-buffered saline

PCR: Polymerase Chain Reaction

***T.gondii*:** *Toxoplasma gondii*

µm: micro mol

Theoretical Section

Chapter I

General introduction

Toxoplasmosis is a globally significant zoonotic disease caused by the intracellular protozoan *Toxoplasma gondii*. This parasite is widely distributed in nature and infects a broad range of warm-blooded hosts, including humans, domestic animals, and wildlife (Dubey, 2020; Tenter et al., 2000). The infection is of major concern due to its potential impact on human health, food safety, and the livestock industry. While many infections are asymptomatic, toxoplasmosis can cause severe complications in immunocompromised individuals and pregnant women, particularly in cases of congenital transmission (Montoya & Liesenfeld, 2004).

The primary routes of infection include ingestion of food or water contaminated with oocysts from feline feces, consumption of raw or undercooked meat containing tissue cysts, and transplacental transmission from an infected mother to her fetus (Robert-Gangneux & Dardé, 2012). Given its global prevalence and diverse transmission pathways, toxoplasmosis remains a significant challenge in both public health and veterinary medicine (CDC, 2020).

In humans, toxoplasmosis is often asymptomatic in immunocompetent individuals, but it can cause severe clinical manifestations in immunosuppressed patients, such as those with HIV/AIDS, organ transplant recipients, or individuals undergoing chemotherapy. In these cases, toxoplasmosis may lead to life-threatening conditions such as encephalitis, myocarditis, and chorioretinitis, which can result in permanent neurological damage or blindness (Weiss & Dubey, 2009). One of the most critical forms of the disease is congenital toxoplasmosis, which occurs when a pregnant woman acquires a primary infection and transmits it to the fetus through the placenta. The severity of fetal infection depends on the gestational age at which transmission occurs. Early gestational infections often result in spontaneous abortion or severe neurological damage, while later infections may lead to milder, but still significant, symptoms such as hydrocephalus, intracranial calcifications, and vision impairments (Pappas et al., 2009). Preventative measures such as serological screening of pregnant women, proper hand hygiene, and avoiding the consumption of undercooked or raw meat play crucial roles in reducing the burden of congenital toxoplasmosis (Montoya & Liesenfeld, 2004).

Toxoplasmosis in livestock represents a major concern due to its implications for food safety and economic losses in animal production. *T. gondii* can infect a variety of food-producing animals, including pigs, sheep, goats, and cattle. Among these, sheep and goats are particularly susceptible, often experiencing reproductive issues such as abortion, stillbirths, and weak offspring, which result in significant economic losses for farmers (Dubey, 2009).

Free-range and pasture-raised livestock are at higher risk of infection due to their increased exposure to contaminated environments, whereas intensive farming practices can help mitigate transmission risks. Strategies to control toxoplasmosis in livestock include improved biosecurity measures, vaccination development, and consumer education on the importance of thoroughly cooking meat before consumption (Tenter et al., 2000).

Avian species play a significant role in the epidemiology of *T. gondii*, serving as intermediate hosts that contribute to the transmission of the parasite within ecosystems. Birds can become infected through the ingestion of oocysts from contaminated soil, water, or feed. While some bird species remain asymptomatic carriers, others exhibit severe clinical manifestations, including neurological disorders, respiratory distress, and reduced reproductive success (Dubey et al., 2010). Free-ranging poultry, scavengers, and birds of prey are particularly vulnerable to infection due to their feeding habits and environmental exposure. Studies have shown that *T. gondii* prevalence is higher in free-ranging chickens compared to industrially farmed poultry, reinforcing the importance of controlling environmental contamination to limit transmission (Liu et al., 2017).

Additionally, wild birds can act as reservoirs for *T. gondii*, facilitating its spread to other animals and contributing to its persistence in the environment. Understanding the role of birds in the epidemiology of toxoplasmosis is essential for developing targeted prevention and control measures to reduce its impact on both domestic and wildlife populations (Meerburg et al., 2012).

As toxoplasmosis continues to pose significant health and economic challenges worldwide, ongoing research and improved surveillance are necessary to better understand its epidemiology and develop effective intervention strategies. Addressing this parasitic disease requires a multidisciplinary approach, integrating public health, veterinary medicine, and environmental management to reduce transmission risks and mitigate its impact on human and animal populations.

In light of these concerns, this study focuses on avian toxoplasmosis in the Relizane region of Algeria, specifically targeting chickens as potential intermediate hosts. By determining the seroprevalence using the Modified Agglutination Test (MAT) and comparing

the results with data from other species, particularly bovines, this research aims to contribute to a better understanding of the epidemiology of *T. gondii* in the region. The following chapters will present the methodology, results, and implications of this study.

Objectives of the Study :

- To determine the seroprevalence of toxoplasmosis in chickens from Relizane using the Modified Agglutination Test (MAT).
- To compare the findings with existing avian prevalence data, and in other species, especially in sheep and cattle, in order to evaluate interspecies differences and potential risk factors.
- To contribute to the understanding of environmental contamination and public health risks related to toxoplasmosis in the studied region.

Chapter II

Literature review

II.1 Definition of Toxoplasmosis:

Toxoplasmosis is an infectious disease caused by the protozoan parasite *Toxoplasma gondii*, an obligate intracellular organism that infects a wide range of warm-blooded animals, including humans and birds (Dubey, 2009; Tenter et al., 2000). The parasite completes its sexual reproductive cycle in felids, which are the definitive hosts, while other animals act as intermediate hosts where the parasite replicates asexually (Ferguson, 2004).

Infection is often asymptomatic in healthy individuals but can cause serious health problems in immunocompromised patients and during pregnancy, where it may result in congenital infection with severe consequences (Montoya & Liesenfeld, 2004; Weiss & Dubey, 2009). The clinical expression of the disease varies widely depending on the immune status of the host and the timing and route of infection (Robert-Gangneux & Dardé, 2012).

II.2 Causative Agent: *Toxoplasma gondii*

Toxoplasma gondii is a single-celled, obligate intracellular protozoan parasite belonging to the phylum *Apicomplexa*. It is the causative agent of toxoplasmosis, a disease that affects a wide range of warm-blooded animals**,** including humans (*Dubey, 2009*).

The parasite has a complex life cyclewith two main phases: sexual reproduction occurs exclusively in the intestines of felids (cats), which are the definitive hosts, while asexual reproduction takes place in various intermediate hosts such as birds, livestock, and humans (*Tenter et al., 2000*).

During infection, *T. gondii* exists in two main forms: rapidly multiplying tachyzoites**,** which cause acute infection**,** and slow-growing bradyzoitesenclosed in tissue cysts, which allow the parasite to persist chronicallyin host tissues (*Montoya & Liesenfeld, 2004*; *Weiss & Dubey, 2009*).

II.2.1 History:

The history of *Toxoplasma gondii* spans over a century of scientific discovery. From its initial identification to its current relevance in human and animal health, this parasite has captured the attention of researchers worldwide due to its complex life cycle and wide host range.

➢ **1908:***Toxoplasma gondii* was first discovered independently by Nicolle and Manceaux in Tunisia and by Splendore in Brazil.

➢ **1909:** The organism was officially named *Toxoplasma gondii*, with "*Toxoplasma*" referring to its arc-like shape and "*gondii*" after the rodent *Ctenodactylus gundi* in which it was first found.

➢ **1923–1937:** Observations of similar parasites were made in various animal species, but the full understanding of its life cycle was still missing.

➢ **1939:** The first confirmed case of congenital toxoplasmosis in humans was reported.

➢ **1957–1959:** Researchers identified the domestic cat as the definitive host capable of shedding oocysts into the environment.

➢ **1970s–1980s:** The parasite gained attention for its role in opportunistic infections, particularly in immunocompromised individuals such as those with HIV/AIDS.

➢ **1990s–Present:** Advancements in molecular biology led to deeper insights into the genetics, epidemiology, and pathogenesis of *T. gondii*. Studies also began to investigate its possible links to neuropsychiatric disorders in humans.

II.2.2 Taxonomy and Classification

Toxoplasma gondii is a unicellular, eukaryotic protozoan parasite that belongs to the phylum Apicomplexa. This phylum includes many important parasitic organisms that possess an apical complex used for host cell invasion. The classification of *T. gondii* is as follows:

Kingdom:	Animalia
Subkingdom:	Protozoa
Phylum:	Apicomplexa
Class:	Sporozoea
Subclass:	Coccidiar
Order:	Eucoccidiid
Suborder:	Eimeriidea
Family:	Sarcocystidae
Subfamily:	Toxoplasmatinae
Genus:	*Toxoplasma*
Species:	*Toxoplasma gondii*

The genus *Toxoplasma* contains only one recognized species: *Toxoplasma gondii*.

II.2.3 Morphology:

II.2.3.1 Tachyzoite Form:

The tachyzoite is the rapidly multiplying stage of *Toxoplasma gondii***,** primarily responsible for the acute phase of infection. It has a crescent or banana-like shape, typically measuring about 6–8 µm in length and 2–4 µm in width. The anterior (apical) end is tapered, while the posterior is more rounded. It contains a single, centrally located nucleus and is equipped with specialized organelles grouped in the apical complex, including micronemes, rhoptries, and dense granules. These organelles are crucial for host cell invasion and survival. Tachyzoites move by gliding motility, facilitated by a complex cytoskeletal system that includes subpellicular microtubules and an inner membrane complex. Once inside a host cell, tachyzoites reside within a parasitophorous vacuole, where they replicate by endodyogeny, an asexual process forming two daughter cells within the mother cell(Dubey, 2010; Black & Boothroyd, 2000).

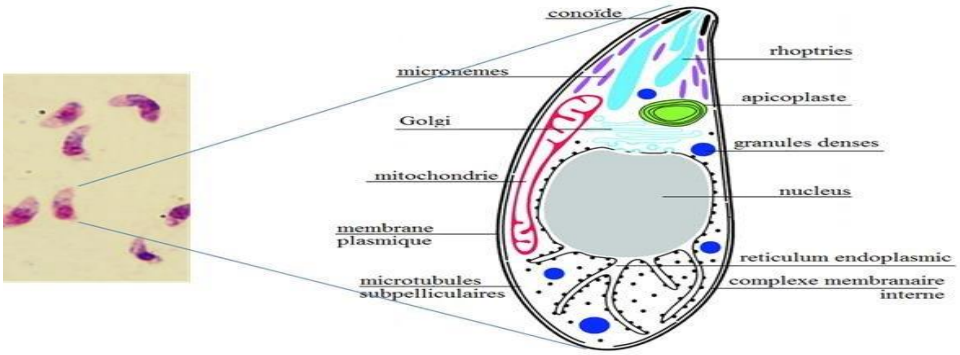

Figure 1: Ultrastructure of T. gondii (tachyzoite).

Source: Image adapted from Joiner and Roos, 2002.

II.2.3.2 Bradyzoite Form:

Bradyzoites are the slow-replicating stage and represent the chronic phase of infection. They are found encysted within tissue cysts in organs like the brain, retina, and skeletal muscle. Bradyzoites are slightly smaller than tachyzoites and share similar structural features, including the apical complex and a single nucleus.

However, they exhibit a denser cytoplasm and more tightly packed organelles, adaptations for long-term survival within host tissues. The tissue cysts, which can range in size from 20 to 100 µm or more, are enclosed by a resilient cyst wall that protects the parasite from immune attack and environmental damage. Bradyzoites remain dormant for extended periods, but under certain conditions, such as immunosuppression, they can convert back into tachyzoites and reactivate the infection (Dubey, 1998; Weiss & Kim, 2000; Montoya & Liesenfeld, 2004).

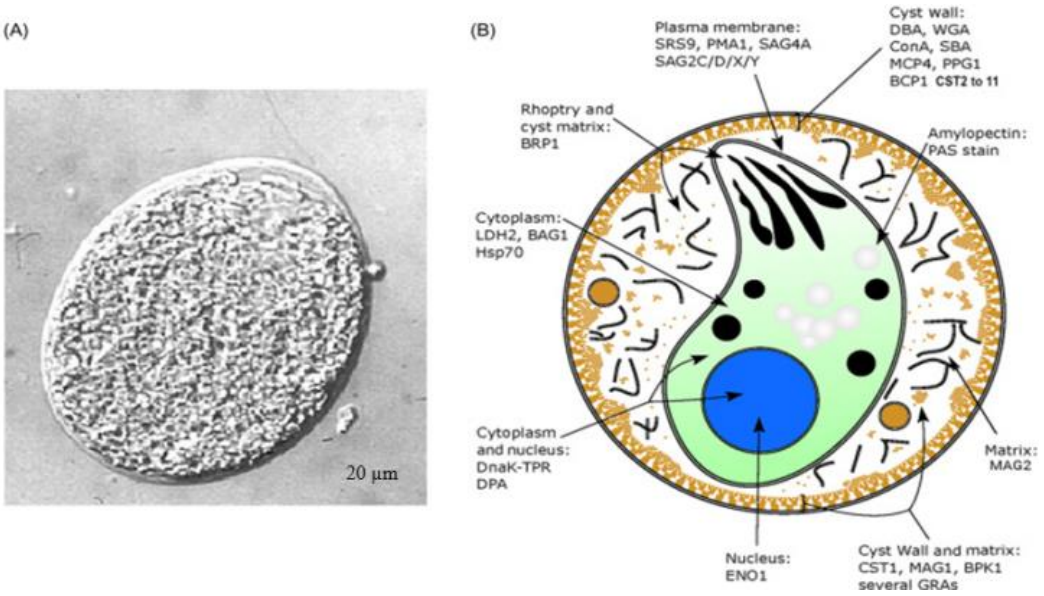

Figure 2: Ultrastructural Features of the Toxoplasma gondii Bradyzoite.

Toxoplasma gondii (Third Edition) .The Model Apicomplexan - Perspectives and Methods.2020, Pages 1-19. J.P.Duby

II.2.3.3 Sporozoite Form:

Sporozoites are the environmentally resistant, infectious forms contained within oocysts shed in the feces of infected felines, the definitive hosts. After sporulation in the environment, each oocyst (approximately 10–12 µm in diameter) contains two sporocysts, each holding four sporozoites.

These elongated, motile cells possess a fully functional apical complex, enabling them to invade intestinal epithelial cells upon ingestion by an intermediate host. Sporozoites are structurally similar to tachyzoites but are adapted to survive in harsh environmental conditions and initiate infection through the oral route. Once inside the host, they differentiate

into tachyzoites and begin the asexual reproductive cycle(Dubey, 1998; Tenter et al., 2000; Montoya & Liesenfeld, 2004).

II.2.4 The life Cycle of *Toxoplasma gondii:*

Toxoplasma gondii exhibits a complex heteroxenous life cycle involving both sexual and asexual reproduction. The sexual cycle occurs exclusively in definitive hosts (members of the Felidae family), while the asexual cycle takes place in a wide range of intermediate hosts, including humans. Understanding this cycle is crucial for comprehending the transmission dynamics and pathogenesis of toxoplasmosis.

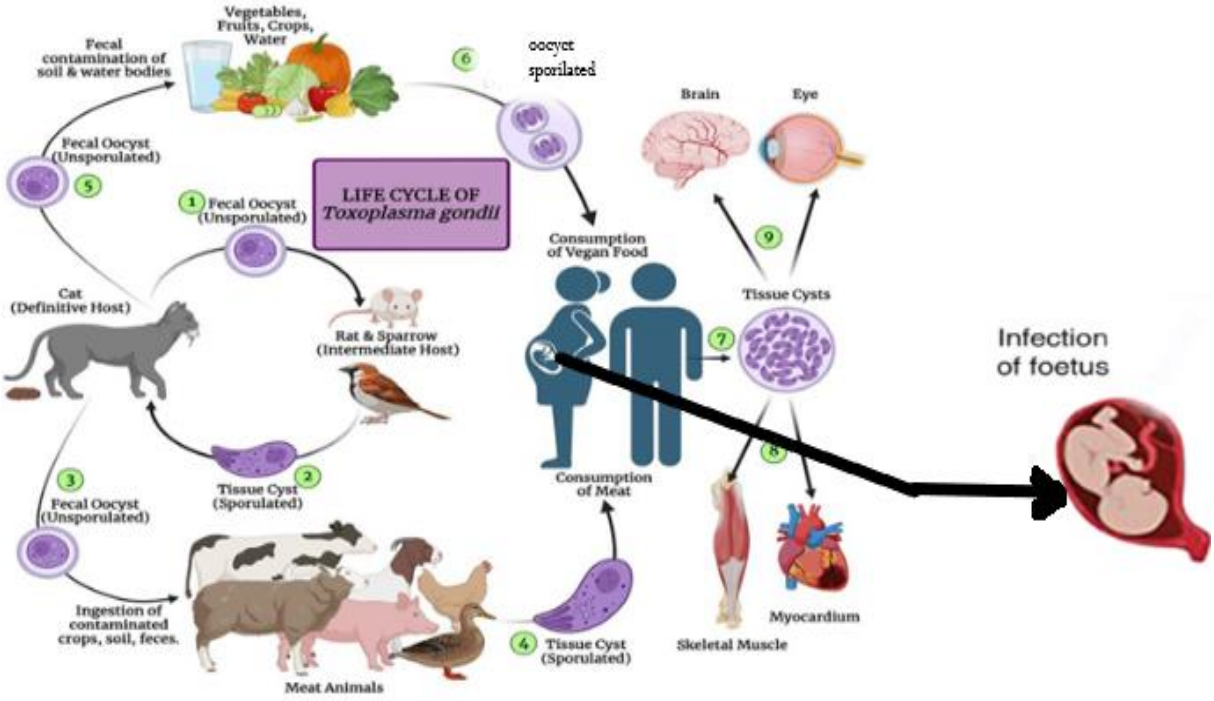

Figure 3: Life Cycle of Toxoplasma gondii in Humans and Animals.

(Adapted from CDC, 2020, Toxoplasmosis Parasite Biology and Transmission)

II.2.4.1 Sexual Reproduction in Definitive Hosts (Felidae):

The sexual phase of *Toxoplasma gondii* occurs exclusively within the intestinal epithelium of felids. Cats become infected by ingesting tissue cysts containing bradyzoites from infected prey. Once inside the feline intestine, bradyzoites invade epithelial cells and undergo a series of asexual divisions before differentiating into male (microgametes) and female (macrogametes) gametes. Fertilization results in the formation of zygotes that develop

into unsporulated oocysts. These oocysts are then shed in the cat's feces over a period of 1–3 weeks. Although oocyst shedding is typically transient, a single cat can excrete millions of oocysts during this period, significantly contributing to environmental contamination (Dubey, 1995; Dubey, 2010).

II.2.4.2 Sporulation and Environmental Persistence of Oocysts:

Unsporulated oocysts excreted by cats are not immediately infectious. In the environment, these oocysts undergo sporulation over 1–5 days under favorable conditions, such as adequate oxygen levels and moderate temperatures. Sporulated oocysts contain two sporocysts, each housing four sporozoites, rendering them infectious to intermediate hosts.

These oocysts are remarkably resilient, capable of surviving in soil, water, and on food surfaces for extended periods. Their resistance to environmental stressors, including temperature fluctuations and chemical disinfectants, poses significant challenges for public health (Dubey, 1998; Dubey, 2004; Dubey, 2010).

II.2.4.3 Asexual Multiplication in Intermediate Hosts:

Intermediate hosts, encompassing all warm-blooded animals, acquire *Toxoplasma gondii* infection through ingestion of sporulated oocysts from contaminated sources or consumption of undercooked meat containing tissue cysts. Upon ingestion, sporozoites (from oocysts) or bradyzoites (from tissue cysts) are released in the small intestine, invade epithelial cells, and differentiate into tachyzoites. Tachyzoites are the rapidly dividing form that disseminate through the bloodstream and lymphatic system to various organs, leading to acute infection.

As the host's immune response intensifies, tachyzoites convert into bradyzoites, which encyst within tissues, particularly in the brain, muscle and eyes. These tissue cysts can remaindormant for the host's lifetime, representing a chronic phase of infection (Dubey et al., 1998; Tenter et al., 2000; Weiss & Dubey, 2009).

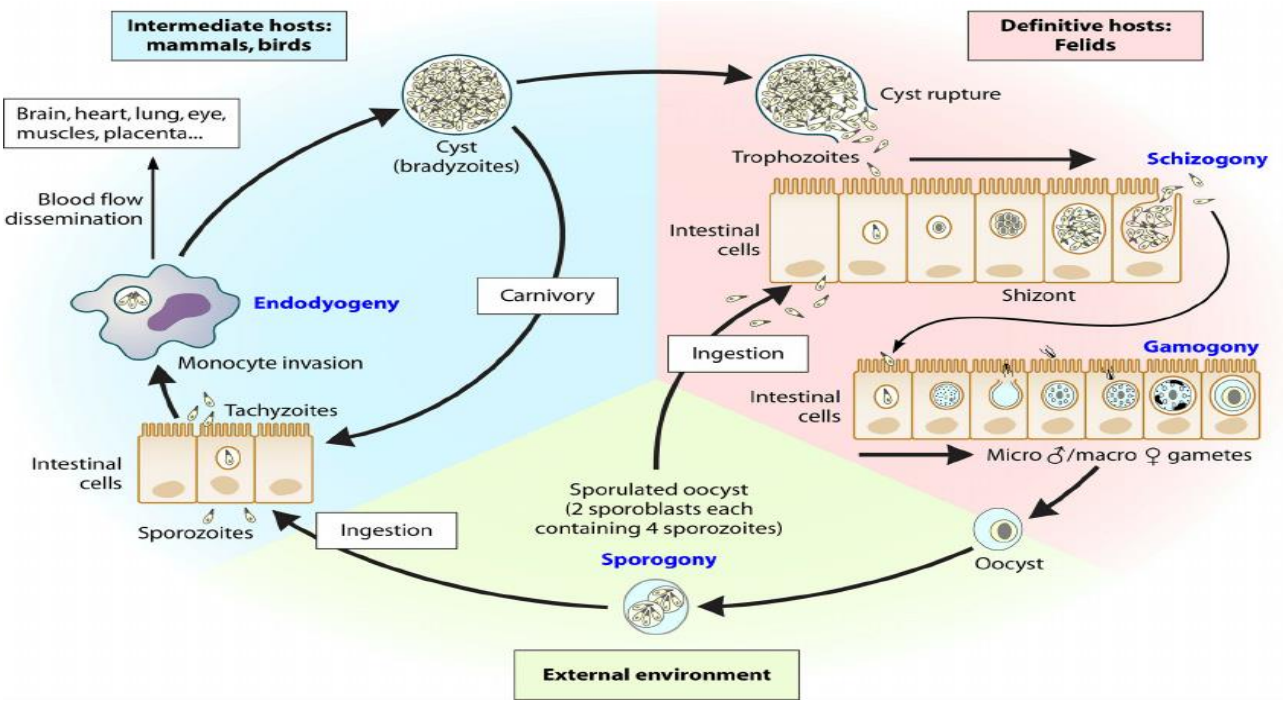

Figure 4: Cellular and Reproductive Stages of Toxoplasma gondii in Definitive and Intermediate Hosts.

(Adapted from Dubey, J.P., Lindsay, D.S., & Speer, C.A., 1998. Structures of *Toxoplasma gondii*: Asexual and Sexual Cycles in Hosts. *Veterinary Parasitology*, 79(1), 1–16.

II.2.4.4 Congenital Transmission:

Congenital toxoplasmosis occurs when a previously uninfected woman acquires primary infection during pregnancy. Tachyzoites can cross the placental barrier, infecting the fetus. The severity of fetal involvement depends on the gestational age at the time of transmission. Early gestation is associated with more severe outcomes, including miscarriage, hydrocephalus, and intracranial calcifications. This route of transmission is one of the most clinically significant and is a major focus of public health interventions (Montoya & Liesenfeld, 2004; Robert-Gangneux & Dardé, 2012; Weiss & Dubey, 2009).

II.2.4.5 Transmission via Tissue Cysts in Meat:

Tissue cysts containing bradyzoites can be found in undercooked or raw meat of infected intermediate hosts, such as pigs, sheep, and goats. When consumed by either humans or felines, the bradyzoites are released in the gastrointestinal tract, initiating infection. In felines, this can complete the sexual cycle if the cat is a naïve host, leading to new oocyst shedding. In humans, this represents one of the major routes of infection, especially in regions

with poor meat-cooking practices (Dubey, 2009; Tenter et al., 2000; Robert-Gangneux & Dardé, 2012).

In summary, *T. gondii* can be transmitted via ingestion of sporulated oocysts from contaminated food, water, or soil; consumption of undercooked meat containing bradyzoite-filled tissue cysts; transplacental transmission during pregnancy; and, although rarely, through organ transplantation and blood transfusion (Tenter et al., 2000; Montoya & Liesenfeld, 2004; Robert-Gangneux & Dardé, 2012).

II.2.5 Clinical Aspects:

Toxoplasmosis presents a wide clinical spectrum depending on the immune status of the host, the route of infection, and the timing of infection during pregnancy. This section elaborates on the different clinical forms, manifestations, and diagnostic challenges of the disease.

II.2.5.1 Clinical Presentation in Immunocompetent Individuals:

In most immunocompetent persons, infection with *Toxoplasma gondii* is asymptomatic or causes only mild, nonspecific symptoms. When symptomatic, patients may present with a self-limiting, mononucleosis-like illness characterized by low-grade fever, malaise, headache, myalgia, and cervical or generalized lymphadenopathy (Montoya & Liesenfeld, 2004). The incubation period ranges from 5 to 23 days post-exposure. Lymphadenopathy is usually painless and most commonly affects the cervical lymph nodes (Robert-Gangneux & Dardé, 2012). Rarely, complications such as myocarditis, pneumonitis, or polymyositis may develop in otherwise healthy individuals (Remington et al., 2011).

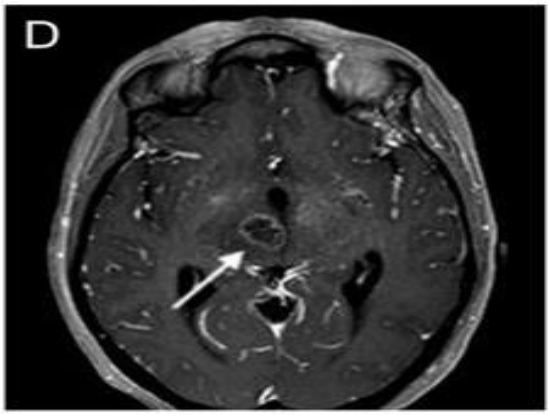

Figure 5: MRI of brain toxoplasmosis in Immunocompromised Patient.

II.2.5.2 Clinical Manifestations in Immunocompromised Patients:

In individuals with compromised immunity—such as HIV/AIDS patients, organ transplant recipients, or those receiving immunosuppressive therapies—*T. gondii* can cause severe, potentially fatal disease. Reactivation of latent infection commonly leads to toxoplasmic encephalitis, characterized by focal neurological deficits, headache, seizures, confusion, and altered consciousness (Weiss & Dubey, 2009). Cerebral lesions are typically multiple and ring-enhancing on neuroimaging. Pulmonary toxoplasmosis, presenting as interstitial pneumonitis, and disseminated toxoplasmosis affecting multiple organs can also occur in this population (Portela et al., 2017).The figure 05 shows a brain MRI illustrating the intracranial classificationof cerebral toxoplasmosis in an immunocompromised patient.

II.2.5.3 Congenital Toxoplasmosis:

Congenital infection results from primary maternal infection during pregnancy and transplacental transmission of tachyzoites to the fetus. The severity of disease in the fetus depends on the gestational age at the time of maternal infection. Early infections can result in miscarriage, stillbirth, or severe neurological abnormalities such as hydrocephalus, intracranial calcifications, and microcephaly (Montoya & Liesenfeld, 2004; Robert-Gangneux & Dardé, 2012). Late gestational infections may lead to subclinical disease at birth but present later with chorioretinitis, developmental delays, or hearing loss. Prenatal diagnosis can be achieved by PCR analysis of amniotic fluid (Foulon et al., 1999).

II.2.5.4 Ocular Toxoplasmosis:

Ocular toxoplasmosis is a common cause of posterior uveitis worldwide. It is characterized by necrotizing retinochoroiditis, often leading to vision loss if untreated (Holland, 2003). The disease may be congenital or acquired, and patients often report floaters, decreased visual acuity, and eye pain. Recurrences are frequent and may cause cumulative retinal damage. Diagnosis is based on clinical examination supported by serology and ocular imaging (Mackensen et al., 2017).

II.2.5.5 Other Clinical Manifestations:

Although rare, toxoplasmosis can involve other organ systems, resulting in myocarditis, hepatitis, pneumonitis, or lymphadenitis. These presentations are more commonly reported in immunocompromised individuals but may occasionally occur in immunocompetent hosts (Remington et al., 2011).

II.2.5.6 Clinical Aspects in Avian Species and Domestic Animals:

Toxoplasmosis in avian species, particularly chickens, and other domestic animals manifests differently from human infection due to species-specific immune responses and parasite-host interactions. Chickens are considered important intermediate hosts in the epidemiology of *T. gondii*, often serving as reservoirs that facilitate zoonotic transmission to humans through the consumption of undercooked meat or contact with contaminated environments (Dubey, 2010).

a- Clinical Presentation in Chickens:

Infection in chickens is frequently subclinical, with most individuals showing no overt signs of disease despite harboring tissue cysts (Dubey et al., 2002). However, in some cases, particularly in young or immunocompromised birds, clinical signs such as lethargy, anorexia, reduced weight gain, ruffled feathers, diarrhea, and even sudden death have been reported (Dubey & Jones, 2008). Post-mortem examination may reveal multifocal necrotic lesions in various organs including the liver, heart, lungs, and brain, where tachyzoites and tissue cysts are identified histologically (Miller et al., 1972). The low-grade pathology in chickens suggests a degree of resistance to severe clinical toxoplasmosis compared to mammals.

b- Clinical Presentation in Other Domestic Animals:

In other farm animals such as sheep, goats, and pigs, toxoplasmosis is an important cause of reproductive failure, with abortions, stillbirths, and neonatal mortality being common clinical outcomes (Tenter et al., 2000). Clinical signs in these animals vary depending on the stage of gestation during infection, immune status, and parasite burden. For instance, sheep infected during early pregnancy often abort or deliver weak lambs, while late gestational infections may result in stillbirth or asymptomatic infection of offspring (Buxton & Innes, 1995).

In contrast, cattle are generally considered poor hosts for *T. gondii*, with rare clinical disease and low susceptibility to infection, which reduces their epidemiological significance in toxoplasmosis transmission (Dubey et al., 1995).

c- Diagnostic Challenges in Avian and Animal Hosts:

Diagnosis of toxoplasmosis in chickens and other animals remains challenging due to the frequent absence of clinical signs and the limitations of available diagnostic tools. Serological assays such as the Modified Agglutination Test (MAT), indirect fluorescent

antibody test (IFAT), and ELISA are commonly used to detect antibodies against *T. gondii* but cannot distinguish active infection from past exposure (Dubey et al., 2005).

Molecular detection by PCR targeting parasite DNA in tissues provides a more definitive diagnosis but requires invasive sampling and may have variable sensitivity depending on parasite load and tissue distribution (Robert-Gangneux & Dardé, 2012).

II.2.6 Epidemiology:

Toxoplasma gondii infection is a widespread parasitic zoonosis that affects a wide range of warm-blooded hosts, including humans, livestock, and avian species. Its ubiquitous distribution makes it a significant public health and veterinary concern. Understanding the epidemiological patterns of *T. gondii* is essential for establishing effective control strategies, especially in endemic regions where the parasite presents notable health and economic threats. On a global scale, it is estimated that up to one-third of the human population has been exposed to *T. gondii* (Montoya & Liesenfeld, 2004).

However, the prevalence varies significantly by region, influenced by environmental, cultural, and socio-economic factors. Climate plays a key role, as oocysts survive longer in warm and humid conditions, promoting transmission in tropical and subtropical regions. Additionally, cultural practices such as consumption of undercooked meat, hygiene levels, and the density of felid populations—the only known definitive hosts responsible for shedding oocysts—further shape the epidemiological landscape (Tenter et al., 2000).

In animals, the infection poses serious implications for both health and food safety. Livestock species, particularly sheep and goats, are highly susceptible to *T. gondii* and often suffer from reproductive issues such as abortion and stillbirth. Although cattle and pigs may show lower clinical susceptibility, they remain important reservoirs that can transmit the parasite to humans through the food chain (Dubey, 2010). The main route of infection in animals is via ingestion of sporulated oocysts present in contaminated water, feed, or the environment.

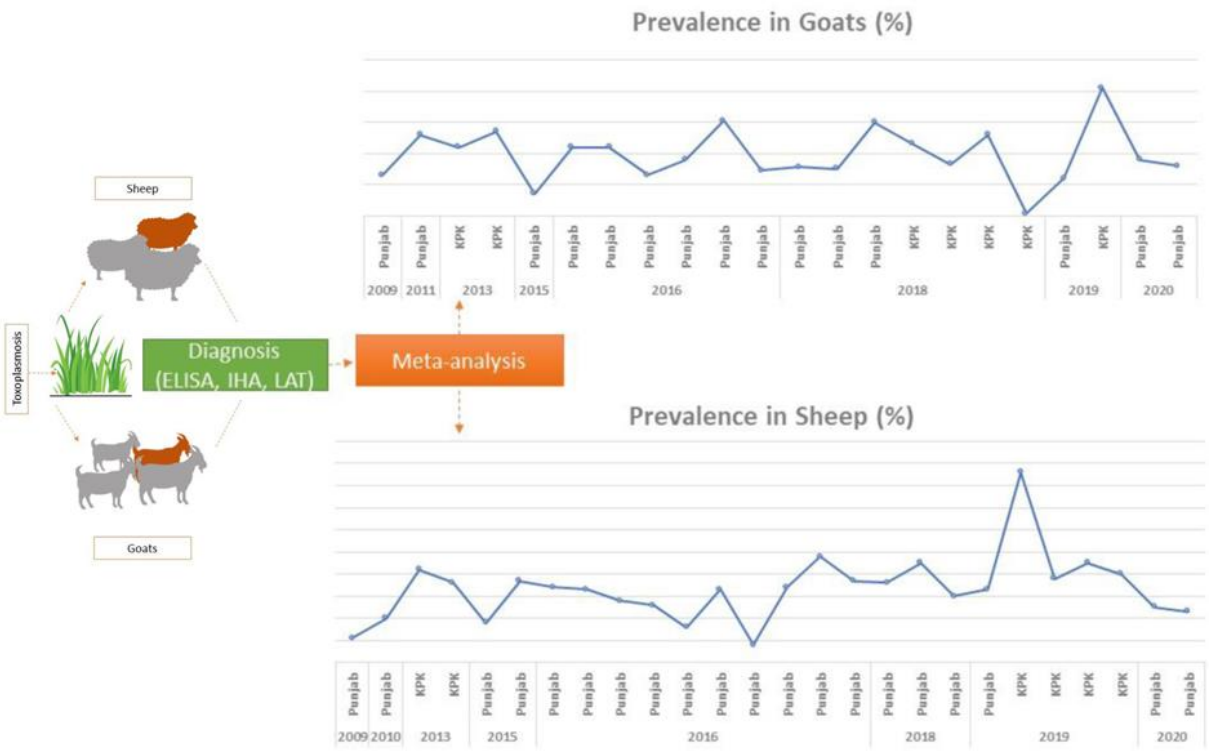

Figure 6: Graphs of the prevalence of toxoplasmosis among sheep and goats.

Taken from Prevalence of Toxoplasmosis in Sheep and Goats in Pakistan: A Systematic Review and Meta-Analysis. 2022

Table 1: Seroprevalence of Toxoplasma gondii infection in domestic cattle, sheep, and goats from Algeria.

Seroprevalence of *Toxoplasma gondii* infection in domestic cattle, sheep, and goats from Algeria.
Taken from Cross-Sectional Survey on *Toxoplasma gondii* Infection in Cattle, Sheep, and Goats in Algeria: Seroprevalence and Risk Factors 2019

Species	Animal Level				Herd Level			
	n	No. of positive	Percentage (%)	95% CI	*n**	No. of positive	Percentage (%)	95% CI
Cattle	1452	418	28.7	26.5–31.1	95	93	97.8	95–100
Sheep	2144	549	25.6	23.8–27.4	70	70	100	100–100
Goat	478	57	11.9	9–14.8	47	41	87.2	77.7–96.8
Total	4074	1024	25.1	23.8–26.5	212	204	96.2	93.7–98.8

Table 2: Prevalence of T. gondii infection among sheep and goats by sex.

Taken from Prevalence of Toxoplasmosis in Sheep and Goats in Pakistan: A Systematic Review and Meta-Analysis. 2022

Reference	Province	City	Sheep							Goats						
			Total	Male	NI	%	Female	NI	%	Total	Male	NI	%	Female	NI	%
Ahmad et al. [59]	Northern Punjab	Pothwar Region	413	156	20	12.82	257	55	21.4	419	153	16	10.46	266	44	16.54
Lashari and Tasawar [58]	Southern Punjab	Dera Ghazi Khan, Multan and Khanewal	518	63	19	30.15	455	84	18.4							
Ramzan et al. [57]	Southern Punjab	Rahim Yar Khan	90	44	2	4.5	46	8	17.3	110	62	10	16.1	48	18	37.5
Ahmed et al. [62]	Central Punjab	Bhalwal, Kotmomin, Sahiwal, Shahpur, Silanwali, and Sargodha	470	72	16	22.2	398	107	26.9	530	150	35	23.3	380	192	50.5
Shah et al. [61]	Khyber Pakhtunkhwa	Mardan	290	120	55	45.83	170	73	42.94	350	150	39	26	200	109	54.5
Tasawar et al. [54]	Southern Punjab	Multan								200	20	5	25	180	99	55
Hanif and Tasawar [60]	Southern Punjab	Multan, Khanewal	500 LAT	51	16	31.37	449	152	33.85							
			500 ELISA	51	10	19.6	449	127	28.28							
Ullah et al. [47]	Southern Punjab	Multan	125	63	16	25.39	62	40	64.52	125	63	15	23.81	62	36	58.06
Khan et al. [64]	Khyber Pakhtunkhwa	Charsadda								149	56	18	32.14	93	44	47.31
Shah et al. [61]	Khyber Pakhtunkhwa	Mohmand agency	100	52	16	30.76	48	20	41.6	104	52	20	38.46	52	36	69.23
Lashari et al. [68]	Southern Punjab	D.G. Khan district	103 LAT	15	5	33.3	88	21	23.86	101 LAT	8	3	37.5	93	33	35.48
			103 ELISA	15	5	33.3	88	19	21.59	101 ELISA	8	2	25	93	31	33.3
Hussain and Zahid [66]	Khyber Pakhtunkhwa	Charsadda	103	33	25	84.78	70	64	91.42	121	29	21	72.4	92	78	84.78
Kamal et al. [67]	Khyber Pakhtunkhwa	Charsadda	143	78	26	33.3	65	32	49.23							
Ahmad and Tasawar [55]	Southern Punjab	Cholistan	335	169	52	30.7	166	73	43.9							

Among avian hosts, chickens—especially those raised in extensive, free-range, or backyard systems—are important indicators of environmental contamination. Their foraging behavior and constant exposure to soil make them particularly prone to ingesting oocysts shed by infected felines. As a result, seroprevalence studies in poultry populations provide valuable insight into the environmental burden of *T. gondii*. These rates vary widely across countries and production systems, with higher prevalence noted in rural areas with poor biosecurity (Dubey, 2010; Lopes et al., 2013). In Algeria, although research is still limited, recent evidence suggests increasing detection of *T. gondii* in poultry flocks, with the Modified Agglutination Test (MAT) emerging as a standard diagnostic tool.

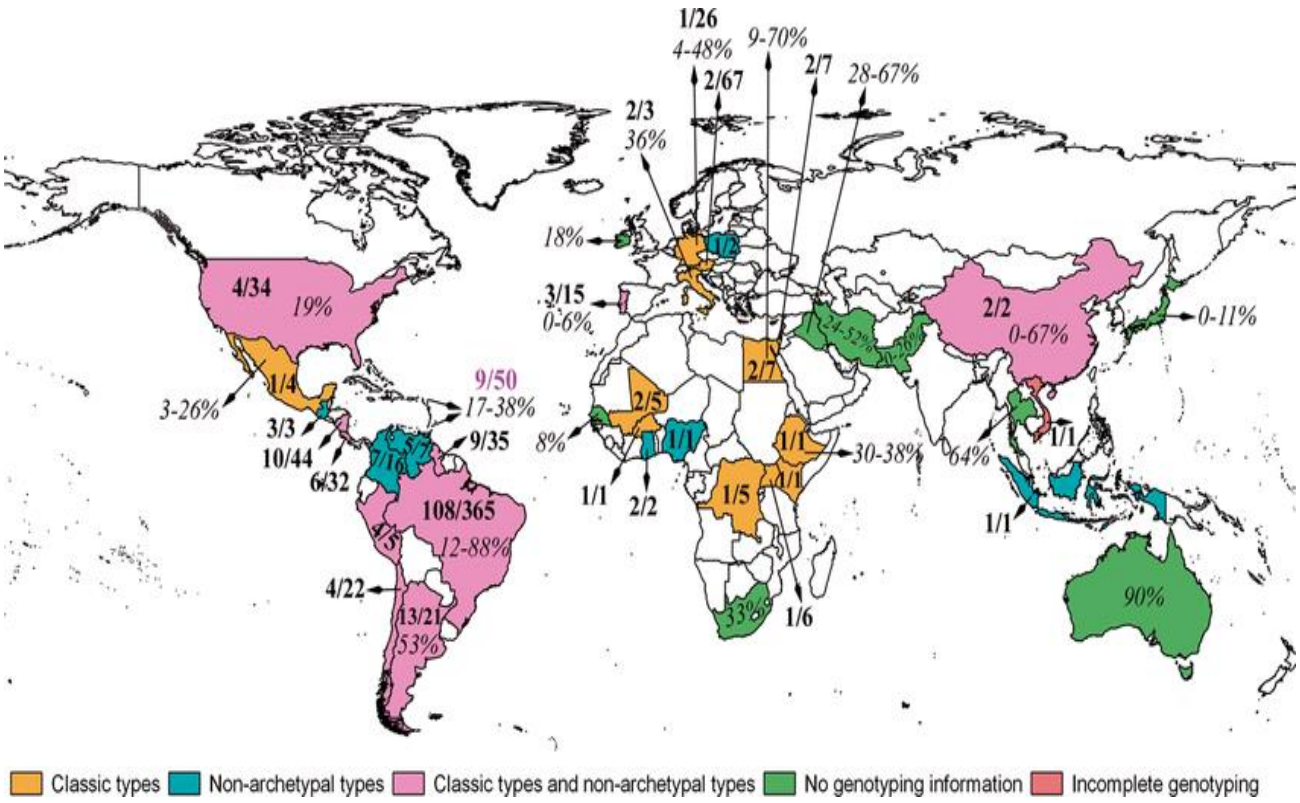

Figure 7: Worldwide distribution of T. gondii infections in chickens.

Numbers in bold are the number of *T. gondii* genotypes/number of viable isolates.
Seroprevalences are given as %

Table 3: Seroprevalence of T. gondii in free-range chickens, ducks, and geese in different regions of china using MAT.
(Adapted from Wang et al., 2013)

Species	No. tested	No. positive (%)	No. of positive sera with MAT titers of:						Central region		East region		West region		Statistics
			1:20	1:40	1:80	1:160	1:320	≥640	No.	No. positive (%)	No.	No. positive (%)	No.	No. positive (%)	
Free-range chicken	243	127 (52.3)	13	21	14	23	16	40	67	41 (61.2)	89	36 (40.4)	87	50 (57.5)	$P=0.018^*$ $X^2=8.06$ df = 2
Ducks	87	40 (46)	–	8	7	3	4	18	63	32 (50.8)	11	3 (27.3)	13	5 (38.5)	$P=0.29$ $X^2=2.43$ df = 2
Geese	5	5 (100)	–	–	–	1	1	3	5	5 (100)	–	–	–	–	–
Total	335	172 (51.3)	13	29	21	27	21	61							

In North Africa, and particularly Algeria, *T. gondii* is recognized as a major zoonotic threat. Human seroprevalence rates in Algeria have been reported to range from 35% to 60%, with pregnant women being a particularly vulnerable group due to the risk of congenital transmission (Bouzid et al., 2020). Agricultural practices, including the use of open pasture and the high number of stray cats in urban and rural environments, facilitate widespread oocyst contamination. The role of poultry in transmitting the parasite to humans is also gaining recognition, especially in traditional farming settings where biosecurity measures are minimal or absent.

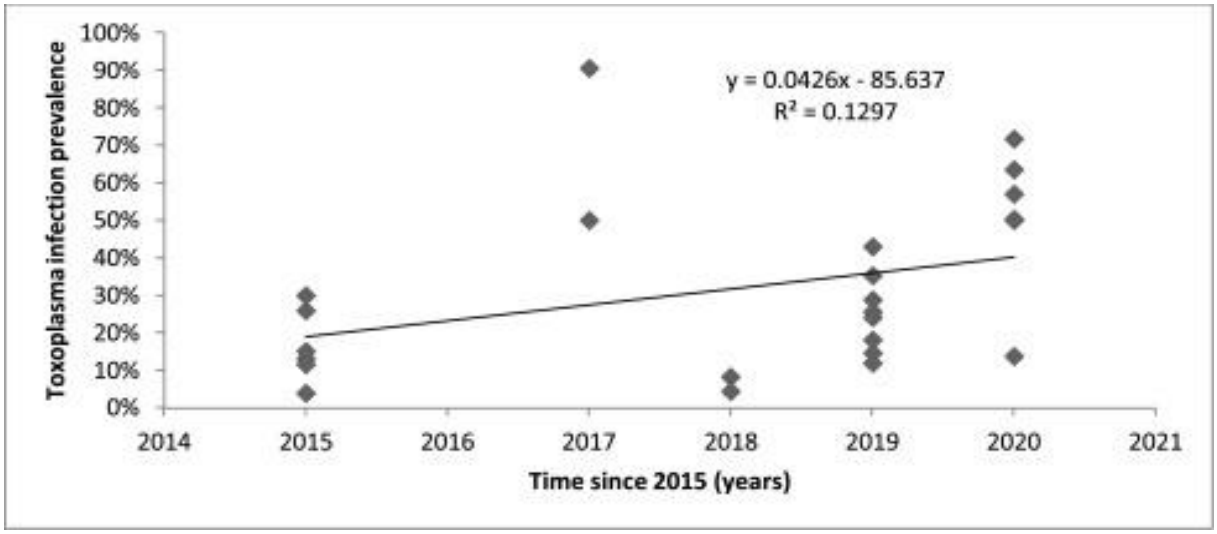

Figure 8: Prevalence of Toxoplasma gondii infection among animals in Algeria: A systematic review and meta-analysis.

Transmission of *T. gondii* in both humans and animals primarily occurs through three main routes. The first is ingestion of tissue cysts through consumption of undercooked or raw meat, particularly from infected intermediate hosts. The second route involves accidental ingestion of oocysts from contaminated water, fruits, vegetables, or soil. The third and most concerning mode, especially from a clinical perspective, is vertical transmission—whereby a primary maternal infection during pregnancy leads to congenital toxoplasmosis. Risk factors for infection include poor sanitation, lack of veterinary regulation, inadequate meat inspection, and frequent contact with cats or contaminated environments (Robert-Gangneux & Dardé, 2012).

The implications of toxoplasmosis extend beyond public health and into the economic domain. Congenital toxoplasmosis is a major cause of birth defects and long-term disabilities in infants. In immunocompromised individuals, such as HIV/AIDS patients or organ transplant recipients, reactivation of latent infection can lead to life-threatening complications.

From an agricultural standpoint, reproductive losses in livestock due to *T. gondii* can severely affect productivity and profitability, while the contamination of poultry meat presents challenges for food safety and export regulations.

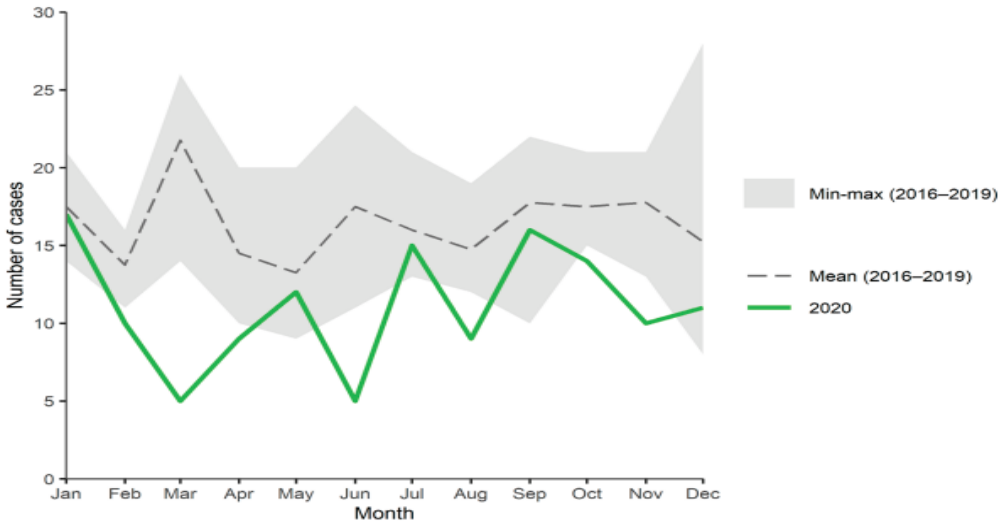

Figure 9: Number of confirmed cases of congenital toxoplasmosis by month, EU/EEA, 2020 and 2016–2019.

Source: Country reports from Cyprus, Czechia, Estonia, Finland, France, Germany, Hungary, Iceland, Ireland, Latvia, Lithuania, Luxembourg, Malta, Poland, Romania, Slovakia, Slovenia, and Spain

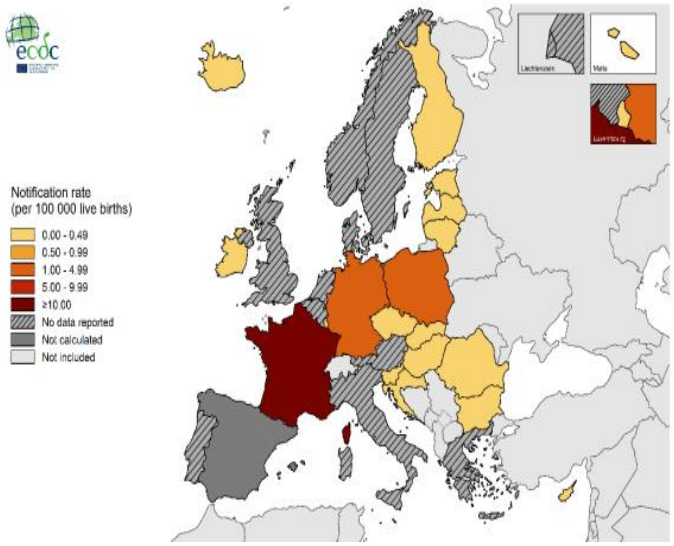

Figure 10: Number of confirmed cases of congenital toxoplasmosis per 100 000 live births by country, EU/EEA.

Taken from Congenital toxoplasmosis Annual Epidemiological Report for 2020

Experimental Section

Chapter III

Materials andmethods

Objectives

The primary objectives of this study are as follows:

- ✓ To detect the presence of *Toxoplasma gondii* antibodies in chicken serum samples collected from Relizan farm and Ramka slaughterhouse using the Modified Agglutination Test (MAT).
- ✓ To estimate the seroprevalence of *T. gondii* infection in poultry within the study areas.
- ✓ To identify potential risk factors associated with *T. gondii* infection in chickens, considering environmental and management practices.
- ✓ To evaluate the reliability of the MAT in comparison to other diagnostic techniques such as PCR in the detection of *T. gondii*.
- ✓ To provide baseline data that may assist in future control strategies and risk assessments for toxoplasmosis in poultry and humans in the region.

III.1 Study Area

This sampling was obtained fromRamka slaughterhouse. Chicken were from a farm situated in Ami Moussa, Wilaya of Relizane.

Ami MoussaFarm is characterized by a semi-arid mediterranean climate, with hot, dry summers and mild, wet winters. This climate influences local agricultural practices, particularly poultry farming, which is mostly conducted under intensive systems.

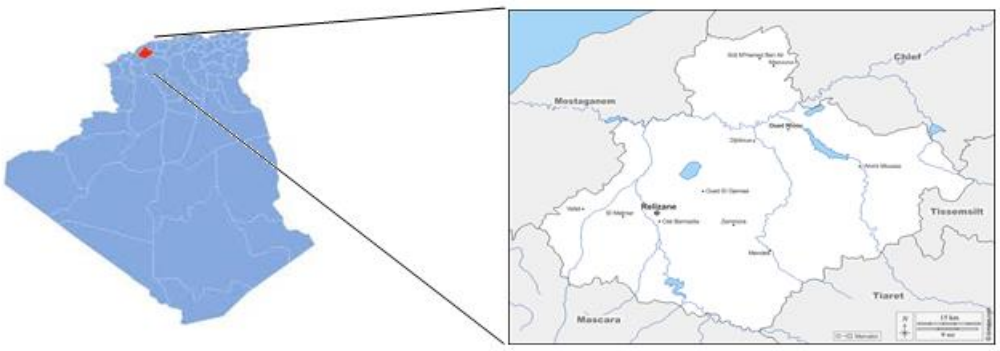

Figure 11: Study area indicating sampling localization in Ramka, Wilaya of Relizane

Ramka Slaughterhouse serves as a regional processing center, handling poultry from various farms in the surrounding areas. The slaughterhouse operates under controlled hygienic conditions, though the diversity of animal sources and transportation practices pose potential

risks for pathogen transmission. The study's sampling period spanned from April to May 2025, coinciding with the transition from spring to summer.

III.2 Study Population:

The study population consisted of 97 broiler chickens including both males and females, reflecting the typical commercial poultry demographic within the region. The birds were around 50 days old, with individual body weights ranging between 2.8 and 3.3 kilograms. These chickens were raised under intensive farming conditions at Ami Moussa, characterized by well-structured poultry houses designed to optimize environmental control such as temperature, humidity, and ventilation.

Furthermore, the flock was subjected to a comprehensive vaccination program targeting common avian diseases, including infectious bronchitis, and avian influenza, thereby minimizing the risk of concurrent infections and ensuring the overall health status of the population.

Throughout the sampling period, the chickens appeared clinically healthy, exhibiting no signs of illness, which supports the reliability of the serological and molecular results in reflecting the true epidemiological status of *Toxoplasma gondii* infection in this controlled production environment. The intensive husbandry practices, combined with proper biosecurity measures, aimed to reduce external contamination, yet the presence of *T. gondii* infection in this context highlights the potential for environmental exposure despite rigorous management

III.3 Sample Collection:

Samples were obtained from chickens raised in the Ami Moussa region and subsequently slaughtered at Ramka slaughterhouse, ensuring traceability of the animals' origin and rearing conditions.

A total of 97 individual chicken samples were collected, including blood and tissue specimens such as brain, heart, and muscle, selected to maximize detection sensitivity for *Toxoplasma gondii*.

Figure 12: A: Sera obtained after blood samples centrifuge ; B: blood sampling.

Systematic random sampling was employed to minimize selection bias and ensure representative sampling across different batches of slaughtered chickens.

Each sample was aseptically collected using sterile instruments to avoid cross-contamination, then properly labeled with unique identifiers for accurate tracking and data management.

Samples were immediately stored in sterile containers and transported in cooled conditions (4°C) to maintain sample integrity until laboratory processing.

All procedures complied with ethical guidelines for animal handling and were approved by relevant local veterinary and research authorities.

Exclusion Criteria :

- ✓ Poor quality or hemolyzed serum samples.
- ✓ Free range or from outside the study region samples

III.5 Diagnostic Test: Modified Agglutination Test (MAT)

III.5.1 Principle:

The Modified Agglutination Test (MAT) is a serological assay widely employed for the detection of IgG antibodies specific to *Toxoplasma gondii* in serum samples from various animal species and humans. This test exploits the agglutination reaction between formalin-fixed whole tachyzoites of *T. gondii* and specific anti-*T. gondii* antibodies present in the tested serum (Desmonts & Remington, 1980).

Upon mixing the serum with the standardized antigen suspension of fixed tachyzoites, IgG antibodies bind to surface antigens of the parasite. This antibody-antigen interaction results in cross-linking of tachyzoites, causing visible agglutination that appears as a diffuse mat or film at the bottom of the well. Positive samples show this characteristic agglutination pattern, while negative samples form a compact sediment or button due to the absence of specific antibodies (Dubey & Desmonts, 1987).

MAT is a direct agglutination test that does not require species-specific secondary antibodies or conjugates, making it particularly useful in veterinary epidemiology where multiple species may be tested (Dubey et al., 1995). The test primarily detects IgG antibodies, which reflect exposure or chronic infection rather than acute infection, thus allowing the identification of both recent and past infections (Montoya & Liesenfeld, 2004).

III.5.2 Sensitivity and Specificity:

MAT is highly sensitive and specific for detecting *T. gondii* antibodies and can identify both acute and chronic infections. It correlates well with other serological methods such as ELISA and less with the Sabin-Feldman dye testwhich is considered as the gold standard in toxoplasmosis diagnosis in humans (Dubey & Desmonts, 1987; Lopes et al., 2013).

III.5.3 Advantages and Limitations:

The test is cost-effective, easy to perform, and does not require sophisticated equipment or species-specific reagents, making it practical for large-scale epidemiological surveys (Lopes et al., 2013). However, MAT cannot distinguish between recent and past infections due to its inability to differentiate antibody isotypes IgG and IgM. Subjectivity in interpreting agglutination at low titers and occasional nonspecific reactions are notable limitations (Dubey, 2010).

III.5.4 Cut-offDetermination

A serum dilution was chosen as Cut-offDetermination for positivity based on published validations in poultry (Dubey et al., 2005; Lopes et al., 2013). Samples with agglutination at or above this dilution were considered positive for *T. gondii* antibodies.

III.5.5 Procedural Overview:

Starting dilutions began at 1:10;1:20 ;1:40;1:100 prepared in phosphate-buffered saline (PBS). Equal volumes of antigen suspension were added to microtiter wells, mixed gently, and incubated overnight at room temperature. Positive results showed diffuse mat formation, while negative results exhibited a compact sedimented button (Dubey & Desmonts, 1987).

III.5.6 Relevance to This Study:

MAT was selected due to its suitability for high-throughput screening and robustness in detecting *T. gondii* antibodies in poultry. This facilitated reliable seroprevalence assessment among broiler chickens in the studied region (Dubey, 2010).

III.5.7 Laboratory Materials and Reagents:

A. Materials:

- 96-well round-bottom microtiter plates (Greiner Labortechnik)
- Magnifying mirror
- Adhesive sealing sheets
- Adjustable micropipettes (2–20 µL, 20–200 µL, 200–1000 µL)
- Plate shaker (or manual agitation)

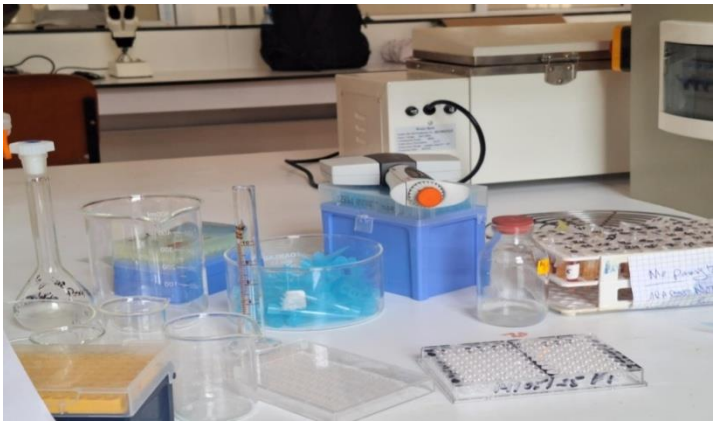

Figure 13: Materials used in MAT

B. preparing samples: Centrifugation

Before performing the Modified Agglutination Test (MAT), serum samples must be clarified to eliminate cellular debris that may interfere with test specificity. This is done by centrifugation to reduce the risk of nonspecific agglutination reactions.

Figure 14: Centrifuge tube stock

Each serum sample is transferred into a clean, labeled microcentrifuge tube and centrifuged at 3000 rpm for 10 minutes at room temperature (20–25°C). This protocol is adequate for separating cellular elements from the fluid phase without compromising antibody integrity.

After centrifugation, the supernatant—containing the antibodies of interest—is gently aspirated using a micropipette, making sure not to disturb the pellet formed at the bottom of the tube.

The recovered supernatant is then aliquoted into sterile, labeled microtubes, typically in volumes of 100 to 200 µL. The aliquots are stored at –20°C until needed for analysis.

To preserve antibody activity, it is essential to avoid multiple freeze–thaw cycles. Each aliquot should be thawed only once, at room temperature, and gently inverted to ensure homogeneity before testing.

This centrifugation step is crucial because the presence of cell debris or other particulates may result in nonspecific agglutination, leading to false-positive interpretations. Thus, the procedure directly contributes to the reliability and diagnostic accuracy of the MAT technique.

C. Reagents:

(All reagents are stored at 4°C in a rack unless otherwise noted)

1. **Antigen Suspension**

- Concentration: 3 × 10^5 tachyzoites/µL in PBS with 0.1% sodium azideStorage: Vertically at 2–8°C; stable for at least 6 months

- Preparation: Dilute 1:30 in BABS buffer (pH 8.95; Biomérieux, 125 mL,) to a finalconcentration of 15,000 tachyzoites/µL

- Shelf life of diluted antigen: 3–4 weeks at 4°C
- Mix thoroughly before use

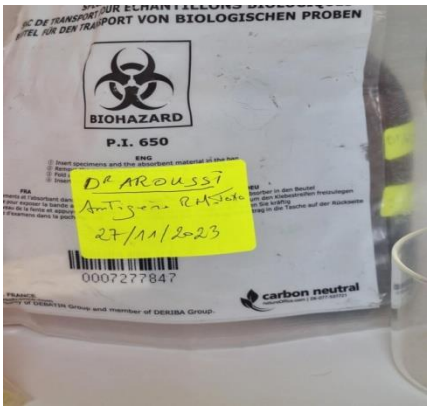

Figure 15: Antigen used in MAT

Phosphate Buffered Saline (PBS)

pH 7.2; Biomérieux, 150 mmol/L, Stability: 2 months at 4°C (avoid contamination)

2-Mercaptoethanol (2-ME)

Stock solution diluted 1:2 in PBS and stored at 4°C
Shelf life: 2–3 weeks
Working solution (0.2 mol/L) preparation:
0.35 mL of 2-ME stock + PBS to 25 mL
Storage: up to 4 weeks at 4°C in amber glass bottle, protected from light

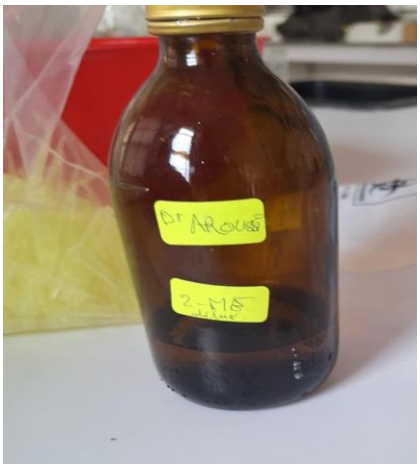

Figure 16: 2-Mercaptoethanol (2-ME)

C. Sample Dilutions :

	1:10	1 :20	1 :40	1 :100
2-ME (µL)	90	50	50	75
Serum (µL)	10	50	50	50

Discard Discard Discard 50(µL)

After each transfer, mix gently to ensure homogeneity.After the last dilution (1:100), discard 75 µL to maintain volume integrity.

D. Test Procedure

1. Dilute antigen at 1:17 in PBS.
2. Add 50 µL of diluted antigen suspension to each well (final volume 100 µL).
3. Briefly shake plates and seal with adhesive film.
4. Incubate for at least 5 hours at room temperature, protected from light and vibrations.

E. Interpretation of Results

- **Positive reaction**: Formation of a diffuse agglutination veil covering more than 50% of the well's bottom.
- **Negative reaction**: Sedimentation of parasites at the bottom forming a distinct white button (veil covering <50%).

Chapter IV

Results and discussion

IV.1 Results:

A total of 97 chicken serum samples were subjected to serological screening for *Toxoplasma gondii* antibodies using the Modified Agglutination Test (MAT)**,** a method well-suited for avian species due to its high sensitivity and specificity. The chickens sampled were of the *Gallus gallus domesticus* species, raised under intensive conditions on the Ami Moussa farm (Relizan) and subsequently slaughtered at the Ramka abattoir (Relizane)**.** Sampling was carried out during the spring season of 2025, and blood was collected post-slaughter. The serum was separated and stored at -20°C until analysis.

Each serum sample was tested at four serial dilution levels: 1:10, 1:20, 1:40, and 1:100, following standardized MAT procedures. The objective was to detect specific IgG antibodies against *T. gondii*, which would indicate previous exposure or infection.

According to MAT interpretation criteria, a positive reaction is characterized by the formation of a diffuse, veil-like agglutination layer across the well bottom. This agglutination results from antigen-antibody interactions indicating the presence of specific anti-*T. gondii* antibodies. In contrast, a negative result is marked by a sharp, compact button of sediment at the well's center, denoting the absence of agglutination and, consequently, no detectable antibodies.

Figure 17: Microplate of MAT showing negative results for T. gondii antibodies in chicken sera

Representative microplate from the MAT assay displaying negative serological results across multiple chicken serum samples. Each well containing test sera exhibited a distinct, compact, and sharply defined button of sediment at the bottom, indicating the absence of agglutination and thus the absence of *T. gondii*-specific IgG antibodies. Positive and negative controls were included in the assay to validate test performance. The negative control wells showed compact sedimentation, while the positive control wells displayed veil-like agglutination, confirming the test's reliability. These results are consistent with a 0% seroprevalence in the analyzed poultry population.

Across all 97 samples and all dilution levels, no agglutination was observed. Each well presented a clearly defined, compact sedimented button at the bottom, signifying that all samples were seronegative for *T. gondii* antibodies.

Each microplate included both positive and negative controlwells, which functioned as expected. The positive controls consistently exhibited veil-like agglutination, while the negative controls displayed sedimented buttons. This confirmed the validity and integrity of the assay throughout the testing process.

Based on the results obtained, the seroprevalence of *Toxoplasma gondii* in the tested chicken population was calculated to be 0% (0/97). These findings strongly suggest that the sampled birds had no prior exposure or infection with *T. gondii*.

This could be attributed to effective biosecurity and hygiene measures implemented at the Ami Moussa farm, as well as limited environmental contamination and minimal exposure to potential sources of oocysts, such as feline feces.

The detailed individual serological test results for each chicken, including the outcomes at all four dilution levels and the final interpretation, are presentedin:

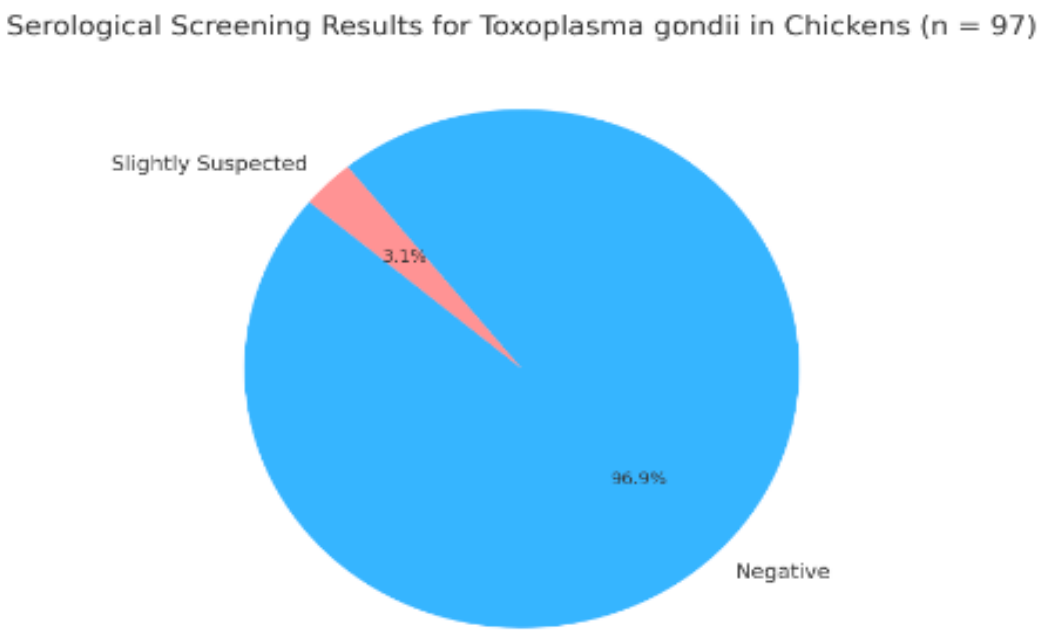

Figure 18: Distribution of Modified Agglutination Test (MAT) results.

96.9% of samples tested negative for Toxoplasma gondii antibodies, 3.1% showed weak non-confirmatory reactions that warrant cautious interpretation, though not considered seropositive.

IV.2 Discussion

The complete absence of detectable *Toxoplasma gondii* antibodies in the 97 chicken serum samples tested by MAT strongly suggests that the chicken population studied was not exposed to the parasite. This result aligns with several studies reporting low seroprevalence rates of *T. gondii* in poultry raised under controlled or intensive farming systems. Chickens are considered good indicators of environmental contamination due to their feeding habits, so the negative results may reflect minimal environmental presence of *T. gondii* oocysts in the Ami Moussa area.

One possible explanation for these results is the implementation of strict biosecurity measures at the Ami Moussa farm, including controlled feed, limited contact with felines, and general hygiene practices. Cats are the definitive host of *T. gondii* and shed oocysts in their feces; limiting feline access to poultry areas significantly reduces infection risk.Another factor could be the age of the chickens at slaughter. If the birds were relatively young, they might have had less environmental exposure time to acquire the infection. Unfortunately, age data was not recorded, which limits deeper analysis.

Although MAT is a highly sensitive method, no diagnostic test is 100% flawless. Thus, while the seroprevalence is reported as 0%, it is scientifically appropriate to consider a minimal residual risk of infection that might have been undetected due to early infection stages or low antibody titers below the test's detection threshold. Future investigations could complement MAT with molecular techniques such as PCR, which detect parasite DNA and can reveal early or active infections.

From an epidemiological stand point, these results are significant. The lack of seropositivity suggests low environmental contamination with *T. gondii* oocysts in this geographic area. This is important for both animal and public health, particularly since chickens serve as intermediate hosts and potential sources of infection for humans through the consumption of undercooked poultry meat. Comparative studies in other regions of Algeria have reported varying seroprevalence rates in chickens, depending on local farm practices, presence of cats, and overall environmental sanitation. For example, surveys in more peri-urban or traditional backyard systems often show higher prevalence, up to 10–15% in some rural settings. Our seroprevalence supports the idea that controlled rearing conditions substantially reduce the risk of *T. gondii* transmission.

Algerian Backyard Poultry Study – Tahri et al. 2020:

A serological survey conducted in 2020 examined **121 chickens**, **14 geese**, and **7 ducks** from traditional, free-range (backyard) farms in north-central Algeria, including Blida province. Using MAT with cut-offs starting at 1:20, the overall seroprevalence was **51%**: 50% in chickens, 57% in ducks, and 50% in geese.

Table 4: Seroprevalence of T. gondii infection among chickens, geese and ducks in north central Algeria by MAT.
(2020) taken from "report on seroprevalence and risk factors of Toxoplasma gondii on some traditional poultry farms in north central Algeria".

Animal species	No. tested	No. of positive with anti-*T. gondii*				Total positive
		1:20	1:40	1:80	1:160	
Chicken	121	5	4	1	51	61(50%)
Goose	14	3	2	0	2	7 (50%)
Duck	7	2	0	0	2	4 (57%)
Total	142	10	6	1	55	72 (51%)

Table 5: Multivariate logistic regression analysis of risk factors related to T. gondii infection.
(2020). from "report on seroprevalence and risk factors of *Toxoplasma gondii* on some traditional poultry farms in north central Algeria".

Variables	Classes	Prevalence (95% CI)	OR	Confidence interval (95%)	*p*
Gender	Male	39.47 (22.58 - 58.32)		Reference	
	Female	47.14 (37.02 - 57.73)	2.52	(1.02 - 6.46)	0.047
Species	Ducks and Geese	53.86 (30.36 - 74.97)		Reference	
	Chicken	51.79 (42.14 - 61.44)	1.28	(0.46 - 3.53)	0.62
Region	Algiers	50.77 (39.66 - 61.87)		Reference	
	Blida	80.95 (63.15 - 93.50)	4.42	(1.72 - 12.59)	**0.003**
	Médéa	9.81 (0.73 - 31.96)	0.10	(0.01 - 0.42)	**0.005**

The study of seroprevalence and risk factors of Toxoplasma gondii on some traditional poultry farms in north central Algeria shows that Female birds had 2.5 times higher odds of seropositivity than males (Odds Ratio [OR]=2.52, p = 0.047), and birds from Blida province were 4.4 times more likely to be seropositive than those from Algiers or Médéa (*OR*= 4.42, p = 0.003)

Production System Differences

Birds forage freely, often ingesting soil, insects, and vegetation contaminated with *T. gondii* oocysts shed by cats. While Ami Moussa intensive birds likely had restricted outdoor access, controlled feed/water, reduced exposure to contaminated ground—leading to significantly lower infection risk**.**

Regional Environmental Factors

Blida lies in a landscape conducive to oocyst survival: wetter soils and high stray cat density facilitate environmental contamination. While Ami Moussa region (Relizane) may have less favorable conditions (drier climate, fewer cats), reducing oocyst survival and exposure risk.

Farm Management and Cat Control

Backyard farms rarely control feline access. Ami Moussa's facility likely maintained strict biosecurity, including limited cat access and improved hygiene, resulting in near-zero exposure rates. These findings also raise questions about the regional variation of *T. gondii* epidemiology. In areas where felines are abundant and veterinary oversight is limited, chickens may serve as sentinel species to estimate environmental contamination. However, in settings like Ami Moussa, where management appears more structured, chickens reflect a low-exposure context. This contrast should inform risk-based surveillance strategies across Algeria.

And from a public health perspective, these findings are highly relevant. Chickens serve as a potential source of human infection through consumption of undercooked meat. The absence of antibodies in chickens from Ami Moussa suggests that poultry meat from this area poses minimal zoonotic risk for *T. gondii*, contributing to safer food supply chains.

IV.3 General Summary and Preventive Measures:

The present study investigated the seroprevalence of *Toxoplasma gondii* in 97 chickens raised in an intensive poultry farm in Ami Moussa (Relizane) and slaughtered at the Ramka abattoir. Using the Modified Agglutination Test (MAT) across multiple serum

dilutions (1:10 to 1:100), all samples tested negative for specific IgG antibodies, indicating an absence of prior exposure or infection. These findings suggest a low to negligible zoonotic risk associated with poultry meat originating from this production system.

The zero seroprevalence observed contrasts with reports from more traditional backyard systems, where birds have higher environmental exposure to *T. gondii* oocysts. The controlled conditions in semi-intensive farming—such as regulated feed, reduced contact with soil, and biosecurity measures limiting feline access—are likely key contributors to the absence of infection.

To maintain or improve these favorable conditions, the following preventive measures are recommended:

- One of the most critical measures is the strict control of cat populations in and around farms and slaughterhouses. Since felines are the definitive hosts of *T. gondii*, their presence near poultry production sites poses a substantial risk of environmental contamination through the shedding of oocysts in feces.
- Equally important is the enforcement of rigorous biosecurity protocols. These include secure storage of poultry feed to prevent contamination, access to clean and uncontaminated water, and regular sanitation of poultry housing facilities. Such measures limit the potential for indirect transmission via contaminated surfaces or feed.
- Public health awareness campaigns also play a vital role. Educating farmers, consumers, and slaughterhouse personnel on the zoonotic risks associated with *T. gondii*, as well as on the importance of proper meat handling and cooking practices, can significantly reduce the risk of human infection.
- In addition, routine surveillance programs should be established to monitor the prevalence of *T. gondii* in both livestock and poultry. These programs should combine serological screening methods, such as the MAT, with molecular diagnostic tools to enhance detection accuracy and enable early identification of outbreaks.
- Finally, educational initiatives targeting poultry producers are essential. These initiatives should promote best practices in animal husbandry and hygiene, thereby fostering a culture of prevention. Raising awareness about parasite transmission pathways and encouraging the adoption of protective measures can collectively contribute to a reduction in the public health burden posed by *T. gondii*.

Chapter V

Conclusion

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, remains a globally significant zoonosis due to its broad host range, environmental persistence, and potential risk to human health through foodborne transmission. Among potential sources of infection, poultry has received increasing attention as both a sentinel species for environmental contamination and a possible vector in human exposure pathways.

This thesis focused on evaluating the presence of *T. gondii* antibodies in domestic chickens using the Modified Agglutination Test (MAT), a reliable and widely recognized serological method. A total of 97 chickens, originating from an intensive poultry farm in Ami Moussa (Relizane) and slaughtered at the Ramka abattoir, were tested at serial dilutions, and all results returned seronegative, indicating the absence of detectable exposure to the parasite among the sampled population.

The negative seroprevalence observed in this study is epidemiologically meaningful as it reflects the success of biosecurity measures, controlled feed and water supply, and restricted access to potential definitive hosts such as felines. When compared to findings from other regions in Algeria—particularly from traditional backyard systems where *T. gondii* seroprevalence can reach 50%—the data from this research strongly underscore the protective effect of modern, intensive farming practices. This work not only provides baseline epidemiological data for the region but also supports the implementation of continued surveillance programs and stricter biosecurity enforcement.

It reinforces the importance of public health education regarding safe food handling, cat population management, and the necessity of integrating both serological and molecular diagnostic methods in future research. While the results are reassuring for the study site, they do not preclude the risk in other regions or under different conditions. Therefore, ongoing research across varied geographical zones, production systems, and host species remains essential to build a more complete understanding of *T. gondii* transmission dynamics in Algeria.and finally our study has confirmed that chicken raised in intensive and restricted conditions is safe to be consumed.

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