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TÍTULO:

Immunology, diagnosis and prevention Toxoplasma gondii during pregnancy and newborns

Trabajo de integración curricular presentado como requisito para la obtención
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Dedicatory

This dedication goes to my parents.

They are my motivation to improve daily and fulfill my curiosity in the sciences.

Thanks for your support in me in all this academic journey.

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Andrea Carolina Albán Cadena

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Resumen

La Toxoplasmosis es una de las infecciones más comunes a nivel mundial producida por el parásito protozoario intracelular *Toxoplasma gondii*, el cual pertenece al género Toxoplasma del filo Apicomplexa. Dicho parásito infecta animales mamíferos y de sangre caliente, y por tanto a los humanos. Esta enfermedad es transmitida principalmente por los felinos, los cuales son el huésped definitivo, al igual que por medio de comida/agua contaminados. Cuando la enfermedad es adquirida durante el embarazo se puede dar una infección transplacentaria y afectar al feto, trayendo consigo varias afecciones a nivel neurológico y visual, aborto y muerte fetal intrauterina. Por lo tanto, el correcto monitoreo y diagnóstico del *T. gondii* durante el embarazo es un excelente mecanismo de prevención de infecciones, disminución de la prevalencia de la enfermedad y una aplicación de tratamiento efectivo. Por consiguiente, el objetivo de este trabajo es revisar los diferentes aspectos de la Toxoplasmosis durante el embarazo como son el diagnóstico, prevención y la respuesta inmunológica. De igual manera, el impacto y las consecuencias en la salud humana. Este proyecto está basado en una revisión bibliográfica para determinar la respuesta inmunológica de las mujeres embarazadas frente a la infección por *T. gondii*, los métodos de diagnóstico y mecanismos de prevención que pueden ser aplicados como control de la enfermedad durante el monitoreo del embarazo. Finalmente, el reporte de análisis estadísticos determinará cuales son las condiciones que propician la infección.

Palabras clave: *Toxoplasma gondii*, parásito, embarazo, diagnóstico, prevención y respuesta inmune

Abstract

Toxoplasmosis is one of the most common infections worldwide produced by the intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*), which belongs to the Toxoplasma genus and the Apicomplexa phylum. This parasite infects mammalian and warm-blooded animals, including humans. Toxoplasmosis is transmitted principally by felines, the definitive host, and food/water contamination. When the disease is acquired during pregnancy can produce a transplacental infection, which affects the fetus carrying affections in the neurologic and visual fields, abortion, and intrauterine fetus death. Hence, the accurate monitoring and diagnosis of *T. gondii* during pregnancy is an excellent mechanism to prevent infections, decrease the Toxoplasmosis prevalence and apply effective treatments. Therefore, the aim of this work was a review the differents Toxoplasmosis aspects during pregnancy, including diagnosis, prevention, and immune response. Similarly, the impact and consequences on human health, especially in pregnant women, can produce miscarriages. This project will conduct a bibliographic review to determine pregnant women's immunologic response against *T. gondii*, the diagnostic methods, and prevention mechanisms that can be applied for infection control during pregnancy monitoring to decrease the Toxoplasmosis prevalence in the population. Finally, the statistical analysis reports will determine which conditions propitiate the infection and which are the most aggressive parasite strains.

Keywords: *Toxoplasma gondii*, pregnancy, diagnosis, prevention, and immunology response

Content

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Abreviations

Bak: Bcl-2 Antagonist Killer protein.

Bax: Bcl-2 Associated X protein.

Bcl-2: B-cell Lymphoma 2 protein.

BIM: Bcl-2-like protein 11.

CD4+: Cluster of Differentiation 4+ cells.

CD8+: Cluster of Differentiation 8+ cells.

CI: Confidence Interval.

CNS: Central Nervous System.

CT: Congenital Toxoplasmosis.

CCR5: chemokine receptor type 5.

DCs: Dendritic Cells.

DHPS: Dihydropteroate Synthetase.

DISC: death-inducing signaling complex.

ELISA: Enzyme Linked Immunoassay.

FA: Folic Acid.

Fas/CD95: Prototype death receptor.

GRA16: Dense Granule Protein 16.

GRA18: Dense Granule Protein 18

HIV: Human Immunodeficiency Virus.

HPP: High Pressure Processing.

HSP70: Heat-Shock Chaperone 70.

IDO: indoleamine 2,3-dioxygenase.

IFN-γ: Interferon γ.

IgA: Immunoglobulin A.

IgG: Immunoglobulin G.

IgM: Immunoglobulin M.

IL-1β: Interleukin 1β.

IL-2: Interleukin 2.

IL-4: Interleukin 4.

IL-6: Interleukin 6.

IL-10: Interleukin 10.

IL-12: Interleukin 12.

ILCs: Innate Lymphoid Cells.

iNOS: Inducible Nitric Oxide Synthase.

JAK1: Janus Kinase 1.

JAK2: Janus Kinase 2.

JNK: NH2-terminal kinase.

MEKK1: Mitogen-activated Protein 4Kinase 1.

MEK4: Mitogen-activated Protein Kinase 4.

MEK7: Mitogen-activated Protein Kinase 7.

Mi-2/NuRD: Nucleosome Remodeling Deacetylase.

miR-17-92: Polycistronic Micro RNA Cluster.

mRNA: Messenger Ribonucleic Acid.

MYD88: Myeloid Differentiation Primary Response 88.

NADPH: Nicotinamide Adenine Dinucleotide.

NKs: Natural Killers.

NLRP1: NLR family pyrin domain 1.

NLRP3: NLR family pyrin domain 3.

Nox4: NADPH oxidase 4.

PABPs: Poly-adenosine-binding proteins.

PCR: Polymerase Chain Reaction.

PRRs: Pattern Recognition Receptors.

PYR: Pyrimethamine.

PYR-SDZ: Pyrimethamine-sulfadiazine.

ROP16: Toxoplasma Rhoptry Protein 16.

ROS: Reactive Oxygen Species.

SPI: Spiramycin.

STAT3: Signal Transducer and Activation of Transcription 3.

STAT6: Signal Transducer and Activation of Transcription 6.

TgCyp18: *T. gondii*-derived cyclophilin-18.

TgIST: *T. gondii* inhibitor of STAT1- dependent transcription.

TgPI-1: *T. gondii* Protease Inhibitor 1.

TLF: TATA-binding protein-like factor.

TMP: Trimethoprime.

TMP-SMX: Cotrimoxazole.

TLRs: Toll-like Receptors.

TNF-α: Tumor Necrosis Factor α.

Treg: Regulatory T cell.

UHRF1: E3 Ubiquitin Protein Ligase 1.

UV: Ultraviolet.

General and Specific Objectives

General Objective

Determine the principal aspects of Congenital Toxoplasmosis during pregnancy due to the review of statistical analysis and immune response for informing about the disease and decrease the prevalence.

Specific Objectives

Analyze the risk factors and seroprevalence of Congenital Toxoplasmosis in pregnant women.

Determine the methods used for Toxoplasmosis detection during pregnancy, the advantages, and the problems.

Determine the prevention protocols against Toxoplasmosis that pregnant women can use during pregnancy.

Overview

This work has six chapters. The first chapter corresponds to the Introduction, where principal definitions and features review of the parasite *Toxoplasma gondii* is presented. The second chapter describes the theoretical background of Congenital Toxoplasmosis, focusing on the symptomatology, level of aggression, immune response in pregnant women and immunocompetent patients, and treatments. The third section presents the methodology of search information and population study, including the parameters measured to accept the review about seroprevalence and risk factors. A similar process is applied to the diagnosis and prevention protocols data. The fourth chapter describes and analyzes the results of seroprevalence, risk factors, diagnostic methods, and prevention strategies. The information xviii is summarized into tables for a better understanding. In chapter fifth, the previous results are discussed, mentioning the mechanisms of prevention and monitoring of Congenital Toxoplasmosis. Finally, the conclusion and following searches are described in the sixth chapter.

Chapter 1

Introduction

Toxoplasmosis is one of the most distributed zoonotic diseases worldwide, which possesses a considerable time latent permanence in the host (1). This illness has a huge impact on human health, especially during pregnancy. It is produced by the obligatory intracellular parasite *Toxoplasma gondii*, which belongs to the Apicomplexa phylum (2). Moreover, this parasite has three morphologic stages: tachyzoites, cysts, and oocyst (3). The disease was first described in 1908 by Nicolle and Manceaux in North Africa. However, until 1960, the definitive life cycle of the parasite (4) (5). In 1970, Toxoplasmosis began to be seriously studied due to its relation with immunocompromised people, especially in positive HIV patients (6). Later, it was determined that the disease affects immunocompetent people and can also be transmitted from the mother to the fetus in congenital Toxoplasmosis, producing miscarriages and malformations (1) (7).

Usually, this zoonotic disease infects warm-blooded animals such as mammals and birds, which are involved in the epidemiological chain (8). Thus, human beings are part of the affected organisms by *T. gondii* and constitute a susceptible intermediate host (9). On the other hand, the domestic cat is the definitive host, but it also affects other felines. In the feline intestinal tract happens the sexual reproduction of the parasite by which oocytes are liberated to the environment (5). Then, the asexual cycle occurs in the human being (10). The infection can be produced by cysts in the animal meal or by oocysts in contaminated food consumption (11). Because the consequences during women's pregnancy are serious, this review aims to 2 analyze the immunology of pregnant women, the diagnostic methods employed, and the parasitic prevention protocols for achieving better knowledge and understanding of the actual infected population situation (12) (13).

1.1. Parasitic generalities.

1.1.1 Morphology and parasitic stages.

As mentioned before, Toxoplasmosis is produced by the obligatory intracellular parasite *Toxoplasma gondii*, which belongs to the protist kingdom, Sarcocystidae family, Apicomplexa phylum. *T. gondii* presents three strain types: I, II, and III. Each of them presents differences in their virulence level. The virulence levels are determined for rhoptry proteins modification as ROP18, which permits an easier entry of the parasite into the organism (14). Strains I and II are found in humans. The strain I is responsible for acute infections in humans. Strain II produces chronic infections, and it is the most virulent strain of Toxoplasma. Strain III is present in warm-blood animals different from humans (15). This parasite has three principal parasitic stages: oocysts, cysts (with bradyzoites inside), and tachyzoites (Figure 1) (16). These last two morphological forms are human trophozoite shapes (17). They have a "pear" shape between 2 and 5 µm in width and large, respectively. In addition, thay are surrounded by a complex membrane called pellicle (1) (17). This membrane is connected with the cytoskeleton and the conoid for better motility and cellular invasion (18). Moreover, trophozoites possess a nucleus, Golgi apparatus, endoplasmic reticulum (ER), mitochondrion, ribosomes, apicoplast, and a set of protein secretory organelles such as rhoptries, dense granules, and micronemes (Figure 2) (17) (19). The conoid, rhoptries, microneme, and dense granules constitute the apical complex, which gives the name to the Apicomplexa phylum.

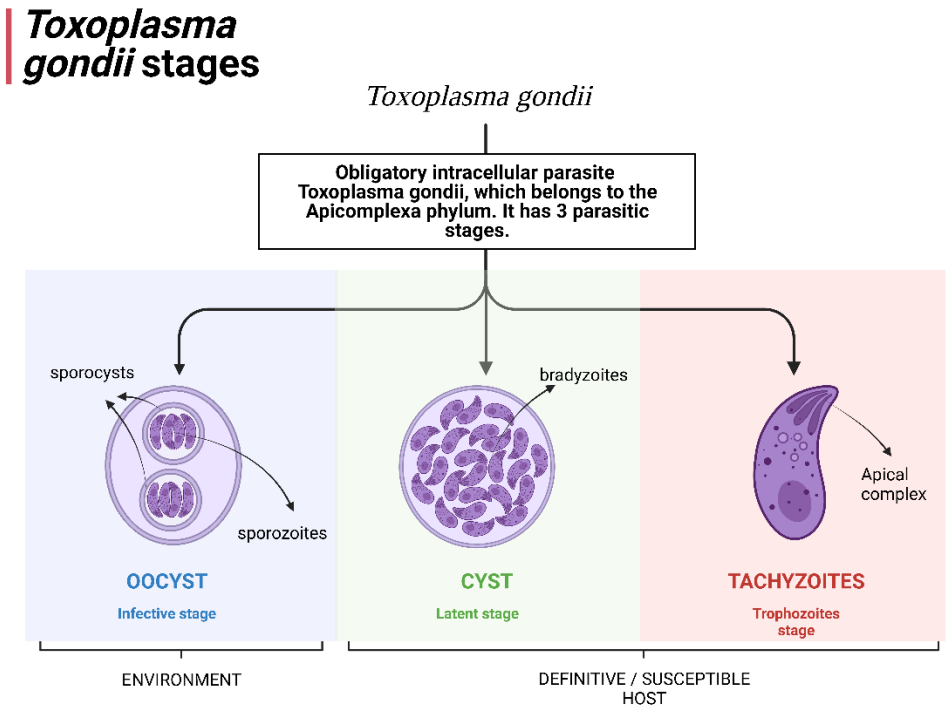

Figure 1: Parasitic stages of *T. gondii*

In the case of the morphology, oocysts are the infective stage of *T. gondii* which are eliminated due to the feline fesses and stay in the environment (5). They have an oval shape with 10-15 µm and 8-12 µm of large width,

respectively. Each of them contains two sporocysts with four sporozoites per sporulation (20). Structurally, they are conformed by a robust wall with several layers to protect the parasite in the external environment (21). On the other hand, tachyzoites vary between 3-7 µm in large per 2-4 µm width (17). They are characterized by their fast division and are the cellular dissemination stage of the parasite in susceptible hosts (22). Finally, the cysts (diameter: 12-100 um) are spherical structures located in animal tissue different from the intestinal ones and are considered the latent stage of the parasite (23). They contain bradyzoites (at least 50 individuals), the parasitic stage with slow division. Their shape and morphological structure are identical to the tachyzoites but in smaller dimensions.

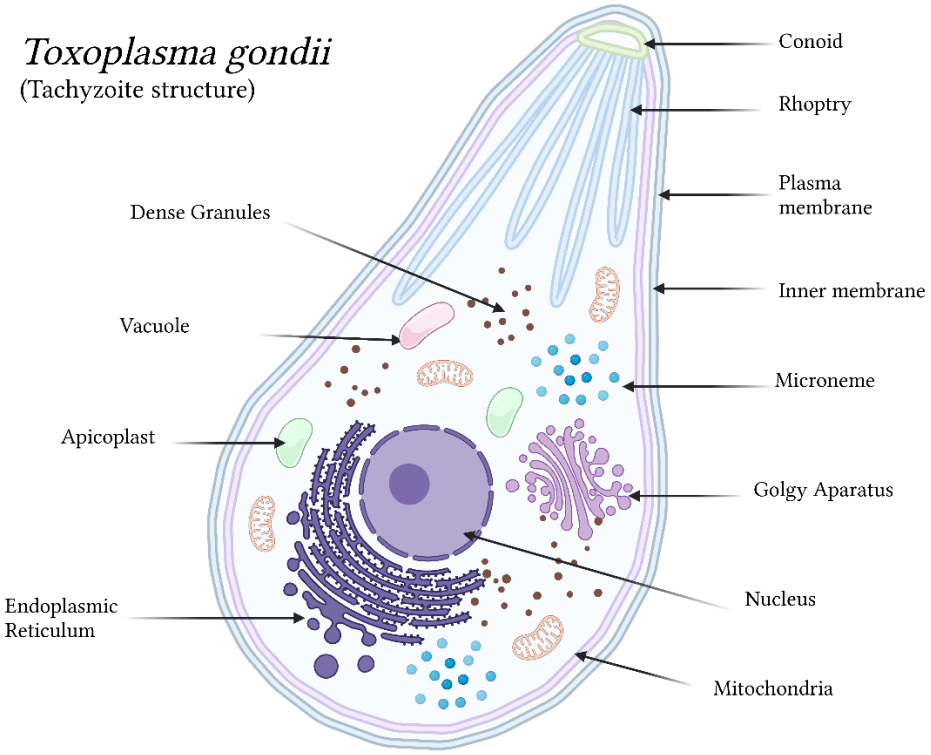

Figure 2: Longitudinal section view of the tachyzoite morphology of *Toxoplasma gondii:* structures and organelles.

1.1.2 Toxoplasma gondii life cycle

The life cycle of *T. gondii* can be divided into two stages: the sexual and the asexual cycles. The first one happens in the feline intestinal tract, and the second is inside the intermediary hosts (9). The complete parasite cycle is complex because it is transmitted by direct interaction between susceptible and definitive hosts and is influenced by a contaminated external environment (24). On the one hand, the sexual cycle of *T. gondii* begins when the feline consumes parasite cysts in infected tissue of other animals (25). This cyst passes through the feline digestive tract, where enzymes destroy it. As a consequence, bradyzoites are liberated (26). Then, they travel to the intestinal environment to colonize epithelial cells, where asexual reproduction happens 5 (27). In this stage, there is multiplication for producing merozoites which invade the epithelium again to form schizonts.

This stage starts a sexual development by gametogonia to form two structures: macrogamont (female) and microgametes (male) (28). After that, fecundation happens to form non-sporulated oocytes (25). Later on, two sporocysts are developed. At this point, oocysts are liberated to the external environment by feline fesses (17). After 72 hours, the oocysts are sporulated, containing four sporozoites per sporocyst. Consequently, the oocyst becomes infective and stays in the environment until 20 days (29). Then, they are consumed by intermediate hosts where tissue cysts are formed. Cats can eat infected mammals or birds to start the sexual cycle again (5). On the other hand, the asexual cycle of the parasite can be performed in two ways depending on if the oocyst or the cysts is consumed in contaminated and uncooked aliments (30). If oocytes produce the infection, it enters the gastric tract of the susceptible hosts to liberate sporozoites (17). They travel to the intestine and colonize epithelial cells to form tachyzoites. This stage replicates faster by endodyogeny and lyses the cell for reinfection (31). Several times, they pass the epithelium and travel by the blood flow to other tissues to form cysts with bradyzoites. Commonly, they are placed in the brain, eyes, and muscular tissue (32) (33). This process of migration happens ten days after infection. By contrast, if a cyst infects the host, they liberate bradyzoites into the gastric tract, colonizing epithelial cells and converting them into tachyzoites to repeat the asexual cycle (Figure 3) (34).

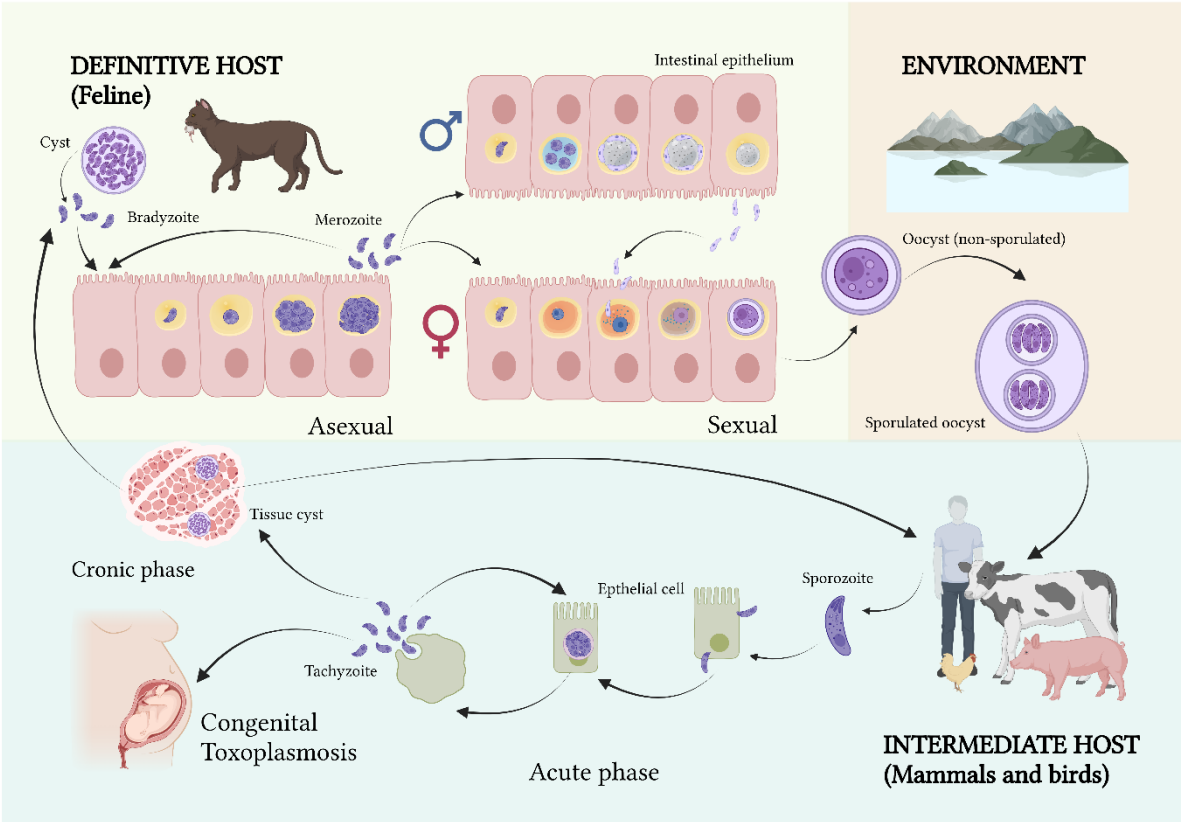

Figure 3: Diagrammatical representation of the *Toxoplasma gondii* life cycle

It shows the development of sexual and asexual parasite reproduction in the definitive and intermediate hosts, respectively. Also, acute and chronic disease phases and congenital transmission in pregnant women are referred.

Chapter 2

Congenital Toxoplasmosis

Congenital Toxoplasmosis is one of the most important studied fields in parasitic pathologies due to it produces several damages in fetal development, especially in the human being (35). This type of infection is produced during pregnancy when pregnant women acquire the infection for the first time (36). The parasite crosses the placenta and infects the fetus. Therefore, it is considered a vertical transmission (35). Similar effects happen in other mammals like sheep and goats. The first time that Congenital Toxoplasmosis was identified in humans was by three researchers, Wolf, Cowen, and Paige, in 1938. The detection was performed on a newborn girl who suffered convulsions and macular damage in both eyes. After her death, the autopsy revealed tachyzoites in the cerebral and ocular tissue, a signal of *Toxoplasma gondii* infection (33).

Therefore, with time, the transplacental movement of the parasite and its high impact during pregnancy was revealed (33). Consequently, congenital Toxoplasmosis is a public health problem nowadays, especially in developing countries, where the information, prevention, diagnosis, and treatments for this disease are unknown to the population and its high prevalence (37) (38).

2.1 Pathology and clinical manifestations.

Usually, congenital Toxoplasmosis generates several clinical manifestations depending on the pregnancy stage when the infection is acquired (36). While earlier is the infection acquired during pregnancy, more severe are the consequences for the fetus. The placenta plays an important function because it is the natural barrier that avoids passing the parasite to the fetus (39). It is very impenetrable at the beginning of the pregnancy, so during the first trimester, the placenta only permits the pass of the 10% of the parasite concentration (37). Over time, the placenta becomes more permeable until the third trimester. It allows access to 60-70% of the parasitic concentration, increasing the chance of Toxoplasmosis vertical transmission. Pregnant women usually are asymptomatic, by which they need to maintain a record of the Toxoplasmosis diagnosis to determine the antibody presence against the parasite. It helps treat the infection adequately and at a precise time (38).

In the case of the newborns, they present several clinical manifestations like hydrocephaly, microcephaly, cerebral calcifications, and chorioretinitis (Sabin's Tetrade syndrome) (39) (40). Most newborns with severe hurt die after birth or acquire permanent disabilities (36). When the infection starts during the first trimester of pregnancy, miscarriages and intrauterine fetal death happen. Also, this stage can produce severe inflammation in the ocular and cerebral tissue (41). Damages in the central nervous system, cerebrospinal fluid alterations, and convulsions can occur too. Second of all, when the infection happens during the second trimester, premature births are common, and encephalitis, convulsions, hydrocephaly, and retinal damage (42). Finally, when the infection is done in the last trimester, 80% of newborns are asymptomatic and the other 20% present pneumonia, myocarditis, hepatitis, thrombocytopenia, and retinochoroiditis (37).

The placenta structure and thickness determine these different consequences during the pregnancy. So, in the first stages of pregnancy, the placenta has several cell layers which function as natural barriers preventing the pass of microorganisms. Also, in the first stages of pregnancy, the placenta is in a formation process that is not connected directly to the maternal blood. As a result, the transmission of pathogens by the bloodstream to the embryo is reduced. In the following trimesters of pregnancy, these barriers decrease, and the blood connections increase, 9 diminishing the barriers and protection to the fetus. Consequently, the parasite can cross the placenta easier in the last trimesters of pregnancy (43).

2.2 Immunologic response in susceptible hosts.

Like other diseases, Toxoplasmosis produces several immunologic responses in the host's organism. These mechanisms include innate and acquired (cellular and humoral) responses. The base of the immune mechanism against *T. gondii* is the production of proinflammatory cytokine as Interferon-γ (IFN-γ) to activate other systems like GTPases, Natural Killers (NKs), macrophages, T and B cells, and ubiquitination (44). These mechanisms actuate at the first site of infection, which corresponds to the intestinal epithelium (44). They are so effective in the parasite's clearance, protection, and inhibition. As a result, the parasite is recognized basically by the damage produced inside of the host's cells by the infection (45).

In the case of the innate immune response, it is the first response to an antigen that enters the host. Several lymphoid cells and natural killer cells start the activation process against *T. gondii* (46). They lead to the

activation of Th1 and Th2 responses, which produce IFN-γ and TNF-α. Moreover, the innate immune response recognizes the parasite using the pattern recognition receptors (PRRs) in host immune cells (44). Some of the PRRs more used are Toll-like receptors (TLRs), Nod-like receptors, and C-type lectins (46). The activation of these receptors begins the production of proinflammatory cytokines, such as TNF-α, interleukin-6 (IL-6), and IL12 (44). Based on mouse models, there is shown that IL-12 accomplishes an important role in the activation of the acquired immune system. IL-12 production is related to the recognition of the parasite by the MyD88 adaptor molecule and CCR5 in the mouse. This chemokine receptor is present in dendritic cells, macrophages, and T cells (CD8+α subclass). The production of IL-12 is related to fetal lethality and miscarriages. So, pregnant women with low levels of this receptor are less susceptible to have consequences due of the infection (45). Usually, in humans, CCR5 detects TgCyp18 to stimulate IL-12 production (Figure 4) (44). The TLR families commonly used are TLR3, TLR7, TLR8 and TLR9 (44). In mice, TLR11 is the receptor responsible for parasite profilin recognition (46). Profilin is a protein responsible for the motility and multiplication of the 10 parasite. So, its quick detection and elimination can decrease the parasite invasion (45). A similar response occurs between human TLRs and parasitic profilin. Other mechanisms used in the innate immune response to avoid cell invasion are Inflammasomes, especially NLR family pyrin domain 1 (NLRP1) and NLR family pyrin domain 3 (NLRP3), which are intracellular sensor systems that detect parasite ligands (45) (46) (47). Finally, an important aspect of the production of IL-10 for the inflammatory suppression process (48). This mechanism starts with the production of CCL2 and CXCL2 by dendritic cells and macrophages. Consequently, these chemokines produce the displacement of monocytes and neutrophils to the infection site (49). At this moment, monocytes secret the IL-10. This interleukin will maintain the homeostasis in non-infected host cells and generate changes in the adaptative immune response (44).

In the case of the immune response at the infection site (intestinal mucosa), Innate lymphoid cells (ILCs) are responsible for detecting the parasite invasion. Natural killer cells (NKs), 1ILC, and 2ILC are examples of these lymphoid cells population (50). Th1 and Th2 immune responses are activated by 1ILC and 2ILC, respectively (44) . These mechanisms are the precursors for acquiring an immune response against *T. gondii*. This response is triggered by several cytokines produced by macrophages and dendritic cells, such as IL-1β and TNF-α (51). Consequently, other immune responses are activated using these cytokines as NKs proliferation and CD4+ /CD8+ activation(44). These new responses generate an IFN-γ massive production. Also, correct levels of IFN-γ are crucial for fighting against the parasite in acute infection (52). As a result of IFN-γ production, CD4+ and CD8+ cells detect it and activate other mechanisms such as Inducible Nitric Oxide Synthase (iNOS) induction and indoleamine 2,3-dioxygenase (IDO) production, respectively (44). iNOS is an IFN-inducible protein for the suppression of Toxoplasma infection. This protein, when activated, produces Nitric Oxide, which participates in the suppression of the infection in the brain (53). In the case of IDO production, it is crucial to avoid *T. gondii* growth in human fibroblasts, glioma cells, retinoblastoma cells, and macrophages (54). Finally, some mechanisms regulate inflammation during the acquired immune response (44). One of them is the decrease of IL2 levels in the host. IL-2 is released by dendritic cells. As a result, Th1 cells respond to IL-2 and are activated for a posterior cellular response induction. Another mechanism is the 11 inhibition of IL-12 by Th2 cytokines like IL-4 and IL-10. Consequently, hyper inflammation in infected tissue is avoided (48).

Failures in these mechanisms (innate and acquired immune response) can produce susceptibility to the infection in the host. DCS is important to initiate an appropriate T cell response against the parasite (55) (56) (45) (57).

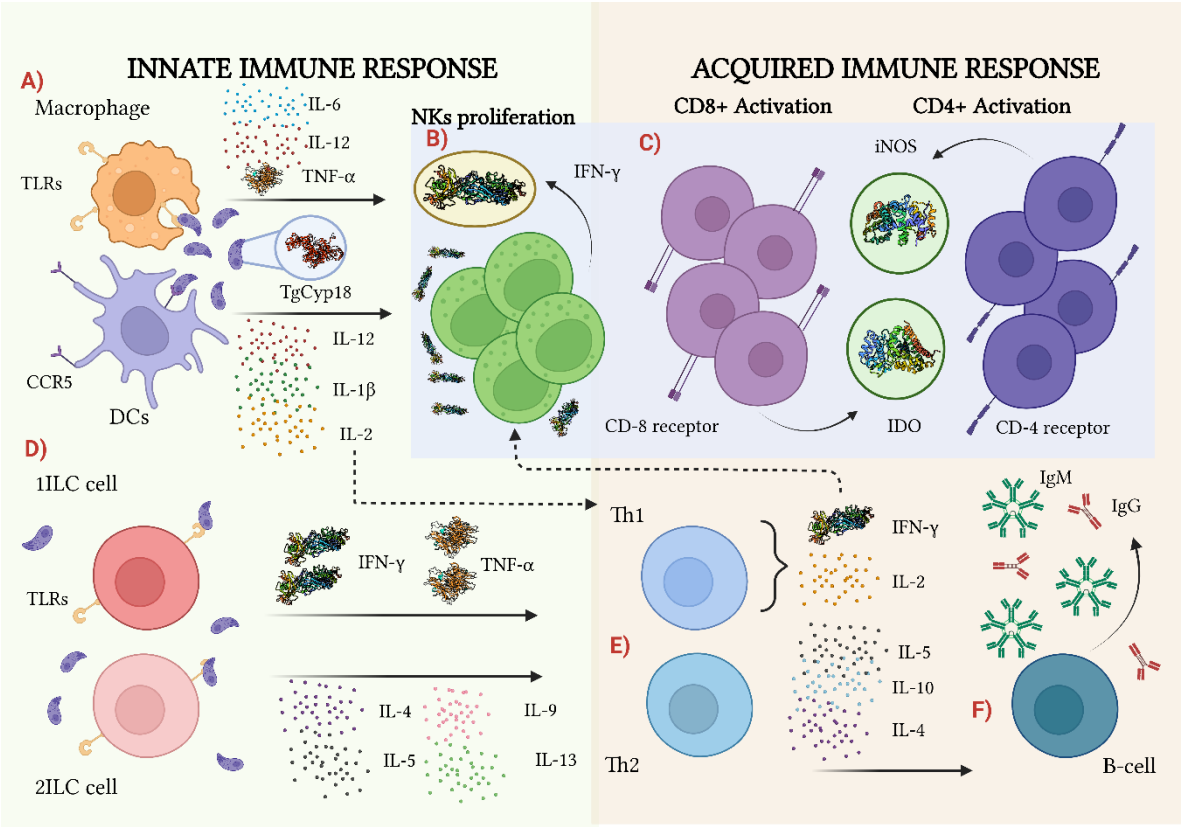

Figure 4: Immune response in human against *T. gondii*

This figure shows humans' innate and acquired immune response against the parasite. A) Macrophages and Dendritic cells are the primary defense against *T. gondii*. Using TLRs and CCR5, they detect the parasitic TgCysp18 protein to produce Interleukins. As a consequence, Nks proliferation and CD4+/CD8+ activation occur. B) When Nks proliferate, they produce IFN-γ for self-regulation and cell-immune response activation. C) CD4+ and CD8+ activation permit the production of iNOS and IDO, respectively. D) Also, 1ILC and 2ILC cells detect the parasite using TLRs. As a consequence, they produce interleukins, IFN-γ and TNF-α. E) As a result of this production, Th1 and Th2 responses are activated, respectively. Th1 response generates IL-2 and IFN-γ, which are used in the self-regulation of cell proliferation. Th2 response produces Il-10 and IL-4, responsible for inflammation reduction and B-cell proliferation. F) Th2 response generates B-cells, which will develop a maturation process for IgG and IgM production.

2.3 Immunologic response during pregnancy.

Like all parasitic infections, there are innate and adaptative immune responses in the mothers and newborns in Congenital Toxoplasmosis (58). In the case of pregnant women, a low percentage presents symptoms associated with the disease. However, all patients develop an immune response against *T. gondii* (59). They extend an innate immune response using macrophage cells and leukocytes, which recognize the parasite due to Toll-like receptors (TLRs) and C-type Lectins, which are parasite recognition receptors (PRRs) (44) (60). Commonly, pregnant women who transfer the infection to the fetus present stronger immune reactions, which are more

variable and aggressive (59). One important aspect is the immunosuppression of mothers to prevent miscarriages, and in consequence, they are susceptible to acquiring several infections.

As a consequence, the main interleukins produced are IL-6 and IL-12. These cytokines are necessary for the posterior activation of the adaptative immune response. Also, TNF-α is secreted by macrophages. DCS produces IL-1β and IL-2. NKs and Th1 cells secrete IFN-γ. Finally, IL-4 and IL-10 are secreted by Th2 cells (44). In the case of IFN-γ, in pregnancy, it activates a severe inflammatory process which can limit the infection in mothers. However, the offspring is the opposite. In vertical transmission, the Th1 response plays an important role in the secretion of proinflammatory molecules, damaging the fetus. That is why IFN-γ inhibition during pregnancy is necessary (59) (61).

On the other hand, the recognition of *T. gondii* -derived cyclophilin-18 (TgCyp18) by the CCR5 receptor in DCs also produces IL-12. The TgCyp18 recognition is important because it is associated with fetal death and miscarriages during the first trimester of pregnancy. In addition, it is related to severe encephalitis in the fetus. CCR5 is the precursor for miscarriage and fetal lethality in primary infections (44). By contrast, iNOS induction by CD4+ cells has a big impact on counteracting vertical infection transmission. This protein generates Nitric oxide, which is essential for preventing cerebral Toxoplasmosis in the fetus (44) (45). In the case of acquired immunity, pregnant women can produce cellular subpopulations like CD4+, CD8+, and CD19+ (61). T cells are important for the proliferation of cellular immune response against the parasite and B-lymphocyte induction. Also, CD4+ is the main cellular response 13 against the parasite (59). In the case of the humoral response, IgA, IgG1, and IgG4 immunoglobulins are present in mothers' serum. IgG2 and IgG3 are abundant in women who transfer the infection to the fetus. Also, these immunoglobulins give the mother a resistance against the parasite forever when they are present in high concentrations in blood (59).

2.4 Parasite immune evasion mechanisms

Based on the previous information, *Toxoplasma gondii* uses several mechanisms for invading host cells, producing acute and chronic infection phases depending on the invasion time. The mechanisms commonly used are the modulation of host signaling pathways, the inhibition of apoptosis, avoiding intracellular death, and establishing a replicative niche (62). Usually, the host immune response is sufficient to destroy the parasite and prevent the infection. However, the parasite can use the host biological systems to avoid the immune response (63) (64) (65).

Starting from the first mechanism, *Toxoplasma gondii* can control the host signaling pathways to decrease the amount of cytokine production and reduce the host immune response. To do this, *T. gondii* uses effector proteins produced by its secretor organelles (rhoptries and dense granules). For example, using the rhoptry kinase ROP16, the parasite can inactivate the Signal Transducer and Activation of Transcription 3 (STAT3) and Signal Transducer and Activation of Transcription 6 (STAT6) for down-regulating IL-12 production (66). Consequently, the number of IL-12 receptors decreases in target cells and produces a dimmish of the immune response (62) (67). By the same mechanism, STAT1 transcriptional activity can be inhibited by all Toxoplasma strains (68). As a result, IL-12 reduction decreases the activation of other immune mechanisms for killing the

parasite (63). Another signaling pathway that the parasite can dysregulate is IFN-γ production. The mechanism is achieved by blocking the transcription of IFN-γ, which is crucial in activating acquired immune response (62). The recognition of IFN-γ starts the inhibition of IFN-γ expression by its receptor, which generates a signal that activates the Janus Kinase 1 (JAK1) and Janus Kinase 2 (JAK2). These molecules activate STAT1. Consequently, this mechanism avoids the STAT1 dissociation from DNA in the host cell nucleus. The Nucleosome Remodeling Deacetylase (Mi-2/NuRD) protein helps the STAT1 binding process. As a result, the 14 transcription process of IFN-γ stops, and the STAT1 transducer protein cannot be recycled to repeat the process (63). This mechanism is mediated by *T. gondii* inhibitor of STAT1- dependent transcription (TgIST), which is a dense granule protein (62) (69). Other signaling pathways dysregulated in *T. gondii* infection are the inhibition of IL-1β production, which is responsible for the natural killer proliferation, and CD4+ and CD8+ activation. This mechanism starts with the inhibition of the NF-κB signaling pathway. As a result, inflammasome sensor NLRP3 transcription is reduced as well as IL-1β ones (70). Finally, the production of anti-inflammatory response is produced by *T. gondii* infection. This process is directed by GRA18, a dense granule protein that reprograms the inflammatory responses (Figure 5) (62).

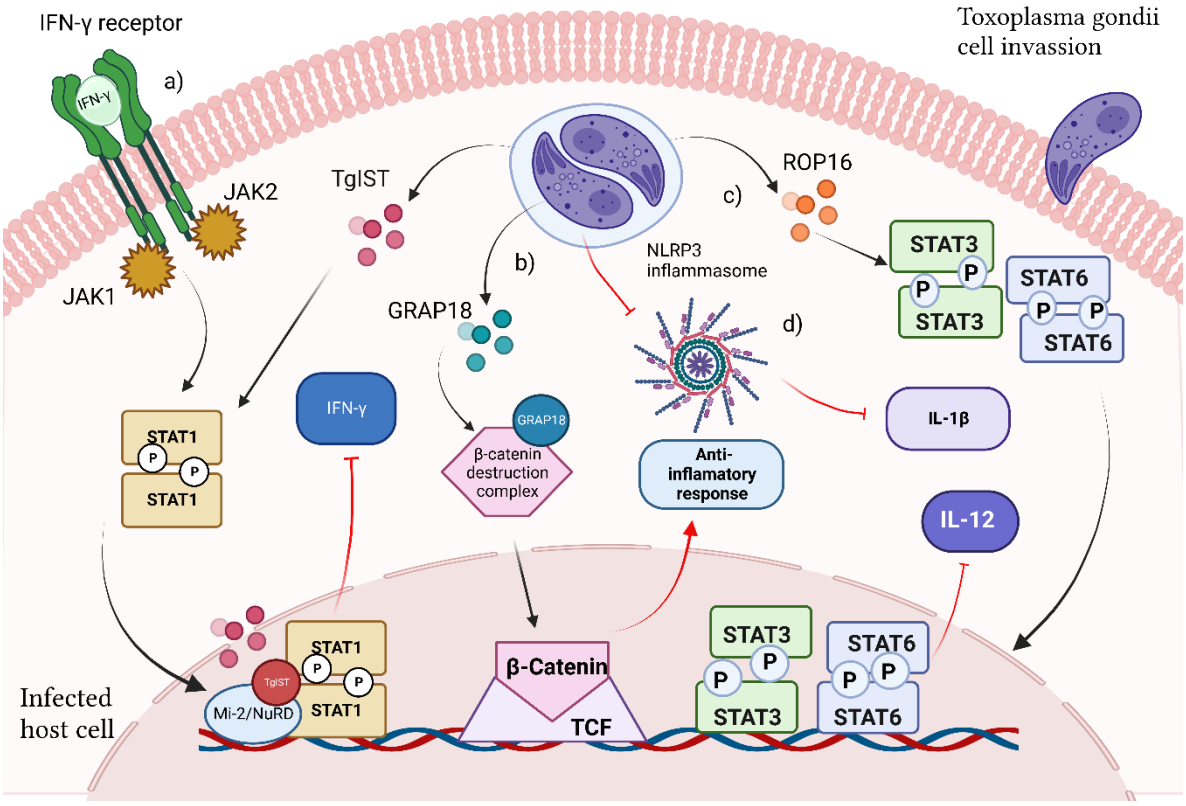

Figure 5: Signaling Pathways modified by *T. gondii* for survival

This figure shows how *T. gondii* regulates host signaling pathways for its survival. a) The first mechanism is the reduction of IFN-γ production. The process starts with the recognition of IFN-γ by its receptor, which activates JAK1 and JAK2 proteins. Then, they activate STAT3, which travels to the nucleus. At this point, TgIST parasitic protein binds STAT3 to

the DNA, avoiding its freedom for protein recycling. The binding is mediated by Mi-1. As a consequence, IFN-γ production is blocked. b) Another mechanism is producing an anti-inflammatory response by *T. gondii*. This process is mediated by the parasitic protein GRAP18, which binds to the β-catenin destruction complex to form β-catenin. This molecule binds to the TCF in the cell nucleus to produce anti-inflammatory proteins. Consequently, the normal inflammatory process is weakened, necessary for parasite destruction. c) Another mechanism is the reduction of IL-12 production. The ROP16 15 parasitic protein mediates this process. It activates STAT3 and STAT6, which travel to the nucleus and block the production of IL-12. As a result, the host immune response is stopped. d) Finally, Il-1β production is avoided by *T. gondii*. This molecule is needed for NKs, CD4+, and CD8+ activation. It is produced by inhibiting the NF-kB pathways, which commonly generate precursors for the formation of NLRP3 inflammasome. This structure is responsible for the activation of IL-1β production. So, blocking off pathways and inflammasome formation avoid Il production.

In the case of inhibition apoptosis, *T. gondii* can interfere in the cell-intrinsic and extrinsic apoptosis pathways by the inhibition of cytochrome c and apoptotic caspases (63) (71) (72). In the intrinsic pathway, the mechanism is commonly based on the decrease of the cytochrome c release by mitochondria and the posterior apoptotic caspase 3 and 9 cleavages (73). Also, UV-induced apoptosis is modified by *T. gondii* based on reducing cytochrome c levels and caspases activation. In this case, the final protein NH2-terminal kinase (JNK) inhibition happens, avoiding the infected host cell death (74). Moreover, other mechanisms modified by the parasite are the Bcl-2 family proteins production for the reduction of cytochrome c expression and caspase-3 activations. For example, the Bax and Bak proapoptotic proteins suffer conformational changes produced by the parasite infection (75). These changes avoid Bax/Bak translocation from the cytosol to the mitochondria. As a consequence, cytochrome c expression decreases. A similar effect happens with the overexpression of Bcl-2 and HSP70 (76). These mechanisms reduce the caspase-3 activation and cytochrome c release, which are the main components of the intrinsic apoptotic pathway (Figure 6) (62) (77).

By blocking the extrinsic apoptotic pathway, *T. gondii* avoids the activation of pro-caspase8. Consequently, the levels of caspase-8 decrease and its binding with the death-inducing signaling complex (DISC) diminishes effector caspase activation (78). Another mechanism altered in the extrinsic apoptotic pathway is the staurosporine-induced apoptosis blocking. This process is started by Fas/CD95-induced apoptosis blocking produced by the parasite. It is mediated by the expression of the miR-17-92 gene cluster. The promotor of this cluster is STAT3 (75).

In consequence, Bim protein expression decreases (75). This protein is responsible for pore formation in mitochondria and subsequent cytochrome c release. Therefore, low levels of Bim protein create a deficiency of next protein activation in the apoptosis process (Figure 6) (78).

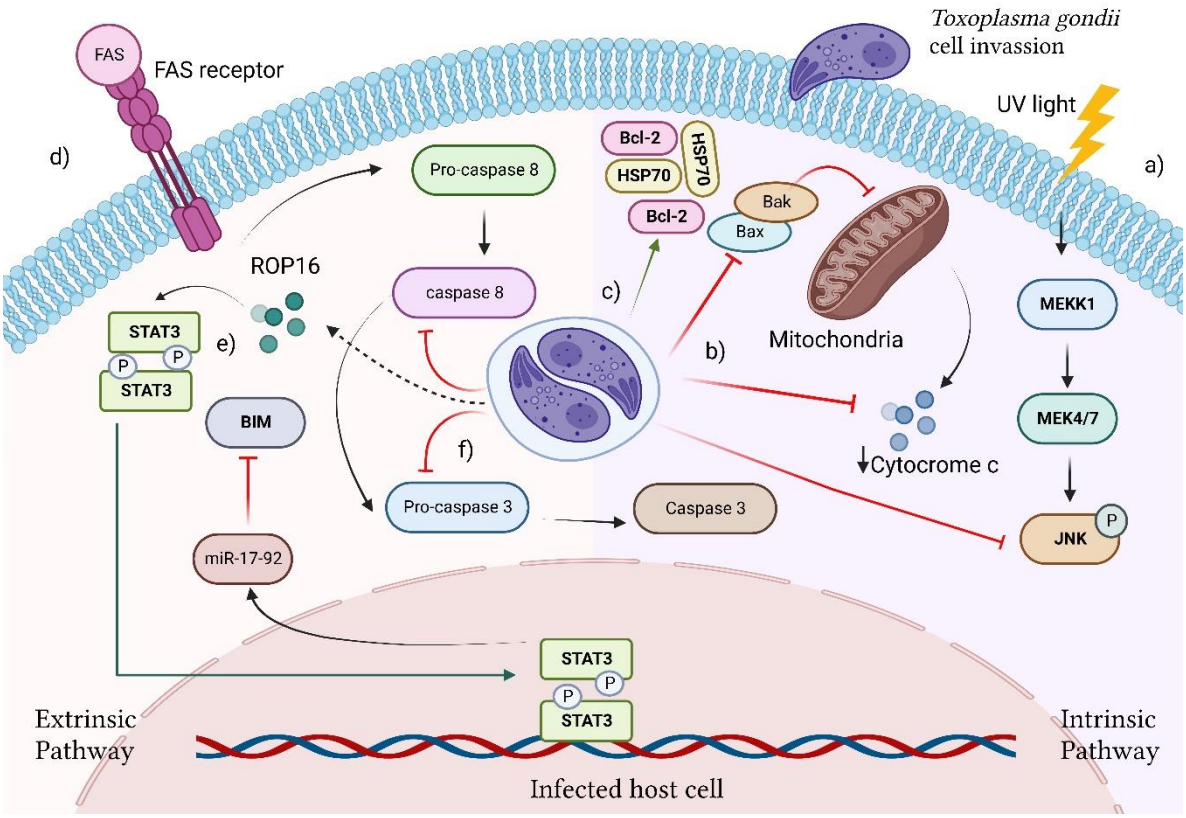

Figure 6: Apoptosis mechanisms dysregulated by *T. gondii* for survival

The figure shows the mechanisms used by *T. gondii* to regulate the intrinsic and extrinsic apoptosis pathways in infected host cells. a) The first mechanism is the blocking of UV-induced apoptosis. When the cell is exposed to UV light, it generates a chain of proteins like MEKK1 and MEK4/7 to produce JNK. This product is blocked by the parasite avoiding cell death. b) The modification of BAX/BAK protein conformation is another mechanism used by *T. gondii* to prevent cell apoptosis. BAK/BAX are proteins responsible for mitochondria stimulation for cytochrome c production. The conformational change produced in these proteins decreases cytochrome c production. c) Hyperproduction of Bcl-2 and HSP70 is another mechanism used by *T. gondii* to avoid apoptosis. Cytochrome c is released when Bcl-2 levels are low. So, overexpression of this protein reduced cytochrome c production. As a result, a high level of Bcl-2 decreases cytochrome c production. Chaperone's HSP70 high levels improve folding and displacement of Bali-2. d) Blocking *T. gondii* caspase-8 activations is another mechanism the parasite uses. In the normal pathway, it starts with the recognition of FAS by FASr. Then, this signal activates pro-caspase-8 to form caspase-8. Caspase-8 is the precursor for the activation of pro-caspase-3 in the apoptosis pathway. Inhibition of caspase-8 activation stops the apoptosis process. e) BIM inhibition is another mechanism used by the parasite. This process is mediated by the parasitic protein ROP16, which activates STAT3 to produce miR-17-92. This cluster avoids BIM production, which is responsible for pore creation and mitochondria stimulation for secreting cytochrome c. f). Consequently, the decrease in cytochrome-c and caspase-3 block the apoptosis pathway.

On the other hand, to avoid intracellular death, *T. gondii* decreases the production of ROS species, which is the principal mechanism of phagocytic cells for eliminating pathogens (62). Also, nonphagocytic cells can be

affected because they have a low production of intracellular ROS by decreasing Nox4 using NADPH oxidase (63) (79) (80). Another way to avoid intracellular death is the parasite's production of protease inhibitor 1 (TgPI-1) to inhibit 17 neutrophil activity (62). Finally, to establish a replicative niche, the host cell cycle can be altered by *T. gondii*. The mechanism is based on the progression from G0/G1 to the S phase and slows down the change to the G2/M phases (62). For this process, the parasite uses molecules E3 ubiquitin-protein ligase UHRF1. As a result, it down-regulates the cyclin B1 (73). Also, GRA16 protein is accumulated in the host cell nucleus to change the regulation of p53. In other processes, changes in metabolic pathways of mRNA are produced by Polyadenosine-binding proteins (PABPs) for changing sub-cellular distribution (62).

2.5 Congenital Toxoplasmosis treatments

Around the world, several treatments have been created to cure or stop the progression of diseases and infections. In the case of Toxoplasmosis, it is no exception. Commonly, due to the complexity of the parasite and the variety of stages of the parasite, the Toxoplasmosis treatments present a serious problem in their effectiveness. Toxoplasmosis treatments fight challenges such as lack of compliance, drug resistance, and distribution level to destroy cysts (81). As a result, different drugs have been tested in clinical trials, from chemotherapy to natural treatments (82). Therefore, drugs more used in congenital Toxoplasmosis infection are Pyrimethamine (PYR) and Trimethoprim (TMP), which are effective in the acute phase of the infection (81). These treatments bind to the dihydropteroate synthetase (DHPS) enzyme, the main target for anti-Toxoplasma drugs. DHPS is present in the parasite. However, because these drugs do not distinguish between parasites and human enzymes, several complications occur with time as myelotoxicity (81). New drugs are created to obtain the best result, especially for pregnant women and newborns. Consequently, some features searched in new treatments include low toxicity, the capacity to diffuse through the placenta, the hematoencephalic barrier, and eye tissue penetration (81).

Usually, two treatments are employed in congenital Toxoplasmosis: prenatal and postnatal treatments. Both mechanisms require specifications about the doses and the duration of the treatment. In the case of prenatal therapies, they were used to prevent parasite transmission during pregnancy and reduce fetal damage (81). The most common treatment is Spiramycin (SPI), a macrolide antibiotic. This drug can be concentrated in the placenta and attack the 18 parasite when it tries to cross this barrier (83). It can be applied from the first semester of gestation and show common adverse effects. However, the principal limitation happens when the infection has arrived in the fetus because the drug does not cross the placenta (81). Therefore, to increase the effectivity of the treatment, some combinations have been created like PYR – sulfadiazine combination (PYR-SDZ) (83). Even though the treatment decreases Toxoplasmosis dissemination, it is a teratogenic compound. So, a PYR-SDZ application is recommended after 14 weeks of pregnancy in both treatments. All those treatments diminish the severity of congenital Toxoplasmosis (81). Another treatment example is the Antepartum drug, but it presents miscarriage consequences (84).

Usually, postnatal treatments are recommended when the diagnosis of congenital Toxoplasmosis is positive in newborns to prevent eye damage. Postnatal therapies reduce clinical symptoms and prevent long-term sequels

in newborns (cerebral calcifications, retinal disease, microcephaly, and hydrocephaly) (81). Also, they prevent damage in asymptomatic patients. The postnatal treatment is based on a year-long PYR-SDZ treatment (85). It should be administered in newborns with congenital Toxoplasmosis in the first year of life. This drug avoids the DNA synthesis in the parasite. However, this treatment has several side effects, especially the inhibition of DNA synthesis in the host (81).

Consequently, the metabolic activity of bone marrow and skin can be affected (81). Folic acid (FA) administration is required during the treatment for prevention. Due to these side effects, new drugs have been proven as PYR-clindamycin, PYR-azithromycin, Atovaquone, and Cotrimoxazole (TMP-SMX). Nevertheless, they present less tolerability among pregnant women. So, it is only recommended if the patient does not show allergic reactions. Longterm exposition to these drug combinations can produce parasite resistance (86).

Chapter 3

Methods

3.1 Search strategy and study selection.

The literature search was managed using three electronic databases (Google Scholar, PubMed, and Clinical Trails) from 2017 until February 2022 to decrease the number of searching and provide precise information. Keywords used in all databases were related to Congenital Toxoplasmosis, including seroprevalence, immunology, and pregnancy. The studies must describe at least (1) the innate immune response of pregnant women; (2) the humoral immune response of pregnant women; (3) the immunologic response of newborns;(4) selected patients should suffer Toxoplasmosis; (5) the obtained results are independent of the use of pharmaceutical treatments. Papers must have full text and be written in English. Moreover, Journal papers were included in this review. Articles were excluded if they did not describe (1) statistical data about Toxoplasmosis seroprevalence; (2) if patients are not pregnant women or newborns; (3) if patients do not suffer Toxoplasmosis; (4) experiments in animals. The reference list was screened to avoid missing the information.

3.2 Prevention protocols.

Keywords used in searching were related to prevention protocols in food and domestic animal contact during pregnancy. The studies must describe (1) the description and features of the 20 protocols; (2) the advantages of the prevention search study; (3) be related to one of the risk factors for Toxoplasmosis obtained in the serological section; (4) the protocols must be applicable for pregnant women. Papers were excluded if they did not describe (1) the methodology of the prevention protocol; (2) if the effectiveness was not explained clearly. Articles must have full text and be written in English.

Chapter 4

Immunology, diagnosis and prevention.

4.1 Study design and population characteristics.

The bibliography was summarized in tables for a better understanding. In Table 1, all the information used shows the different seropositivity of cases worldwide and compares the diagnosis methods used for determining the infection presence. All the references used populations of pregnant women without any additional pathology, except HIV (87). Moreover, the authorities describe the age and the number of participants. However, few of them do not mention the pregnancy trimester of the participants.

Moreover, several articles used negative controls for comparing results. In the case of the participants who participated in trials, the number of women was between 77 to 2326 people (88) (89). Pregnant women were between 10-50 years and stayed in all pregnancy trimesters (90). Patients in the second and third trimesters were followed up during the rest of the pregnancy. In some references, patients detected with *Toxoplasma gondii* infection received complimentary treatment (91). Patients who were selected and suffered miscarriages before the experiment were excluded.

In the case of the study design, the information was summarized in Table 2, which shows different risk factors associated with the infection of *T. gondii* in pregnant women. Also, it compares the different seropositivity of each reference with the risk factor suggested in the bibliography. The researchers performed surveys of the study population to determine which risk factors are tightly associated with the infection acquisition. The serological analysis information was summarized in Table 3. It shows several serologic studies performed on congenital Toxoplasmosis. In all the studies, researchers perform ELISA tests to determine 22 IgM/IgG anti-Toxoplasma antibodies using blood samples (Table 1) (92) (93) (94) (95) (91) (90) (88). Recent past infections and reactivation are related to IgM+ /IgG- , IgM- /IgG+ , and IgM+ /IgG+ serological results, respectively (88). Some trials studied the avidity and specificity of antibodies, especially in recent infections. Other techniques were used, such as Electro chemiluminescence assays (87) (96). PCR was employed for determining parasite genes, especially the B1 gene (93) (94) (87). This gene is used because it presents several copies in the parasite genome, which becomes an excellent target for applying PCR for parasite detection (15). Finally, the numeric information presented in Tables 1 and 3 was acquired using statistical analysis such as Chi-square and Fisher tests. Moreover, bivariant and multivariant analyses were used to determine the correlation between risk factors and infection acquisition. These correlations used a 95% confidence interval (CI), a significance of 0.05. P-values inferior to the significant value show a correlation event between the infection acquirement and the risk factor (92) (93) (94) (95) (91) (90) (87) (88) (89).

4.2 Pregnant women seroprevalence, risk factors, and diagnostic methods.

On the one hand, based on the information analyzed and presented in all the tables, in all the references, there is a women group who were positive for the congenital Toxoplasmosis infection. In most of the trials (n=8), the negative seroprevalence of women overcame 50% (Table 1). The mean average for positive and negative seroprevalence in women is 42.57% and 57.43%, respectively. The highest positive seroprevalence was placed in Egypt with 68.7% and Iraq with 57.13% (95). In contrast, the less serologic positive value is placed in Saudi Arabia, Rafha City with 11.73% (93). In the case of antibody detection results, all references show IgM+ /IgG- , IgM- /IgG+ , and IgM+ /IgG+ values. The mean averages of IgM+ /IgG- , IgM- /IgG+ and IgM+ /IgG+ results were calculated based on the global population, being 9.07%, 30.12% and 4.13% respectively (Table 3) (92) (94) (95) (91) (90) (87) (88) .

Second of all, the risk factors described in the bibliography vary depending on the surveys performed by researchers (Table 2). Age, place of living, soil contact, contaminated food consumption, and pet presence are the factors that are mostly compared in the trials. The categories that are considered as main risk factors are the age of the pregnant woman, the 23 level of contact with soil, and the amount of uncooked meal consumption by the mother (92) (93) (94) (95) (91) (90) (87) (88). Seropositive results produced by pets' presence or living place present low cases (Table 2) (87). Other factors were the presence of HIV in the mothers showing a correlation between this pathology and positive Toxoplasmosis seroprevalence (92) (87). Record of miscarriages was an important factor related to positive *T. gondii* infection (92). Finally, surveys performed in trials determine that most women interviewed do not know about the parasite, and other percentages do not know how it is transmitted (89).

On the other hand, taking in mind the bibliography analyzed for this section, the principal diagnostic method was Enzyme-Linked Immuno Sorbent Assay (ELISA) for the detection of Anti-Toxoplasma antibodies, principally IgG and IgM isotypes (97) (98) (99) (100) (101) (102) (103) (104) (105) (106). Only in one case was IgA studied (Table 3) (98). The studies were performed on pregnant women in different pregnancy stages using blood samples, amniotic fluid, umbilical cord, and placenta. IgG seropositivity presents an average of 24.59% , IgM displays an average of 3.41% of the patients, and for both immunoglobulins, 15.96%. Another aspect evaluated was the avidity of IgG antibodies using an avidity test (n=5) (Table 3) (97) (98) (99) (103) (106). This parameter was classified into three categories: high, intermediate, and low avidity, giving positive results of 57.08%, 4.91%, and 38.75%, respectively. On the other hand, other techniques used in trials for the detection of *T. gondii* DNA were PCR and Nested-PCR (97) (99) (101) (102) (103) (104). Some genes used were the B1 and P30 genes of Toxoplasma (97). Other studies mentioned abortion, and fetuses were examined for detecting the protozoan genes. Comparisons between the different techniques and their challenges were described in summary tables. Finally, the statistical analysis was performed using the Chi-square method and Fisher exact test with a significance of p $p<0.05$ (100) (101) (104) (105).

Table 1: Population and study design results

Author	Participants' data	Region / Place	Method of diagnosis	Treatment after diagnosis	Measuring criteria	Clinical Events during test	Seroprevalence	
							Positive	Negative
Saad et al. (89)	N=250 Age=20-50 P. week=all trimesters	Saudi Arabia. Sakaka city	ELISA	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	Seropositive IgM revealed congenital anomaly in newborn	42.4% (106/250)	57.6% (144/250)
Ali et al. (90)	N=162 Age=16-45 P. week= no report	Saudi Arabia, Rafha City	ELISA PCR amplification	No	Screened for IgG and IgM anti *T. gondii* specific antibodies and B1/Re genes presence.	No	11.73% (19/162)	88.27% (143/162)
Paul et al. (91)	N=254 Age= >18 P. week= all weeks	Tanzania Moshi town, Kilimanjaro region	ELISA PCR amplification	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	No	44.49% (113/254)	55.51% (141/254)
Mandour et al. (92)	N=182 Age=17-43 P. week= no report	Assiut Governorate, Egypt	ELISA	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	Abortion and unexplained intrauterine fetal death	68.68% (125/182)	31.32% (57/182)

Silva et al. (88)	N=218 Age=25.5 (mean) P. week= no report	Lambayeque, Peru	ELISA	Yes	Screened for IgG, IgM and IgA anti *T. gondii* specific antibodies	Abortion.	35.78% (78/218)	64.22% (140/218)
Soltani at al. (87)	N=88 Age=10-50 P. week= no report	Southwest Iran, Abadan City	ELISA	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	Abortion.	34.09% (30/88)	65.91% (58/88)
Vueba et al. (84)	N=878 Age=15-47 P. week= all trimesters	Luanda city, Angola, sub-Saharan Africa	Electrochemilu minescence (ECL) Nested OCR	No	Screened for IgG and IgM anti *T. gondii* specific antibodies and avidity. Presence of B1 genes	Abortion.	39.64% (348/878)	60.36% (530/878)
Ahmed et al. (85)	N=77 Age= >15 P. week= all trimesters	Northern Iraq	ELISA	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	No	57.14% (44/77)	42.86% (33/77)
Flores et al. (86)	N=2326 Age=15-44 P. week= no report	Panama province	Elecsys Toxo IgG and IgM tests (Roche Diagnostics, Mannheim, Germany)	Yes	Screened for IgG and IgM anti *T. gondii* specific antibodies	Congenital toxoplasmosis in newborns diagnosis and hospital cares.g	44.41% (1033/2326)	55.59% (1293/2326)

Halıcı et al. (93)	N=752 Age= no report P. week= no report	Turkey	Chemiluminescent inmuno assay Enzyme-linked fluorescent assay	No	Screened for IgG and IgM anti *T. gondii* specific antibodies	No	47.34% (356/752)	52.66% (396/752)

N: number of participants

P. weeks: weeks of pregnancy.

Table 2: Risk factors and specific seroprevalence

Author	General Seropositive data			Factors influence seroprevalence					Result
	IgM+	IgG+	IgM+/IgG+	Age	Place living	Cat /dog pet	Soil expose	Raw food /liquid eating	
Saad et al. (89)	2.8% (7/250)	38.4% (96/250)	1.2% (3/106)	x					Association between toxoplasmosis and recurrent abortion. High toxoplasmosis seroprevalence in women between 41-50 years. High congenital transmission in the third trimester.
Ali et al. (90)	11.73% (19/162)	No report	No report	x			x		Seroprevalence was significantly high in women with more than 30 years. IgG seroprevalence cases was seen in women who ever had abortion.
Paul et al. (91)	4.33% (11/254)	35.43% (90/254)	4.72% (12/254)				x	x	Consumption of raw vegetables and frequent soil contact were associated with increased risk of *T. gondii* infection
Mandour et al. (92)	15.38% (28/182)	40.11% (73/182)	13.19% (24/182)				x	x	Consumption of raw vegetables and frequent soil contact were associated with increased risk of T. *gondii* infection. Geographical conditions increase favorable infections.
Silva et al. (88)	5.5% (12/218)	30.28% (66/218)	No report	x	x			x	High toxoplasmosis seroprevalence depending on the age, place of living and type of water consumption. Association between abortion and high levels of anti-Toxoplasma antibodies.
Soltani at al. (87)	1.14% (1/88)	32.95% (29/88)	No report					x	Uncooked food consumption and a history of abortion are the most significant risk factors for high toxoplasmosis seroprevalence
Vueba et al. (84)	0% (0/878)	39.41% (346/878)	0.22% (2/878)	x		x			Associated risk factors for high Toxoplasmosis seroprevalence are women age, gestational age, having children, spontaneous

									abortion, hepatitis B and HIV seropositive, history of miscarriage, and owning pets (dog, cat or both).
Ahmed et al. (85)	2.6% (2/77)	54.55% (42/77)	No report				x		Risk factors associated with toxoplasmosis were the farm working (soil contact) and miscarriages history.
Flores et al. (86)	0.21% (5/2326)	42.61% (991/2326)	1.33% (31/2326)	x					Presence of indeterminate cases (0.26%, IgG+/IgMi). High seroprevalence in women between 35-44 years.
Halıcı et al. (93)	46.94% (353/752)	0.4% (3/752)	No report	No report	No report	No report	No report	No report	Geographical factors increase and distribute the prevalence of toxoplasmosis.

X: risk factors described in the bibliography.

Table 3: Results of diagnostic methods in congenita toxoplasmosis

Author	Participants	ELISA										
		Measurement	Seropositivity	IgG+/ IgM-	IgG+ / IgM+	IgG- /IgM+	IgA	IgG avidity			Other methods	
								High	Intermediate	Low	PCR	Avidity test IgG
Berredjem et al. (94)	Individuals=143 Age=22-43 years WP= 1-24 weeks	IgG, IgM and IgA detection	57/143 (39.86%)	30/143 (20.98%)	27/143 (18.88%)	0/143 (0%)	7/143 (4.9%)	45/57 (78.95%)	3/57 (5.26%)	9/57 (15.79%)	Yes	Yes
Laboudi & Sadak (95)	Pw=128 Age= no report Trimester= 1-16 weeks	IgG and IgM detection	59/128 (46.09%)	54/128 (42.19%)	5/128 (3.91%)	0 (0%)	0 (0%)	50/54 (92.59%)	0 (0%)	4/54 (11.11%)	No	Yes
Saki et al (96).	Pw=200 Age=14-53 years Trimester=38-39 weeks	IgG and IgM detection	46/200 (23%)	42/200 (21%)	1/200 (0.5%)	3/200 (1.5%)	0 (0%)	38/46 (82.61%)	6/46 (13.04%)	2/46 (4.34%)	Yes	Yes
Shieh et al. (97)	Pw=261 Age=21-40 years Trimester=37-40 weeks	IgG and IgM detection	87/261 (33.33%)	85/261 (32.57%)	1/261 (0.38%)	1/261 (0.38%)	0 (0%)	No report	No report	No report	Yes	No
Arefkhah et al. (98)	Pw=100 Age=16-46 years Trimester=12.9 weeks	IgG and IgM detection	10/100 (10%)	7/100 (7%)	0 (0%)	3/100 (3%)	0 (0%)	No report	No report	No report	Yes	No
El-Sayad et al. (99)	Pw=100 Age=20-40 years	IgG and IgM detection	73/100 (73%)	27/100 (27%)	23/100 (23%)	23/100 (23%)	0 (0%)	No report	No report	No report	Yes	No

	Trimester= no report											
Pinto-Ferreira et al. (100)	Pw=5 Age=27-37 years Trimester=15-37 weeks	IgG, IgM and DNA detection	5/5 (100%)	0 (0%)	5/5 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	5/5 (100%)	Yes	Yes
Matin et al. (101)	Pw=200 Age=16-41 years Trimester= all trimesters	IgG, IgM and DNA detection	107/200 (53.5%)	86/200 (43%)	13/200 (6.5%)	8/200 (4%)	0 (0%)	No report	No report	No report	Yes	No
Naqid et al. (102)	Pw=500 Age=16-45 years Trimester=	IgG and IgM detection	152/500 (30.4%)	145/500 (29%)	0 (00%)	7/500 (1.4%)	0 (0%)	No report	No report	No report	No	No
Sharifi et al. (103)	Pw=250 Age=20-30 years Trimester=1-4 weeks	IgG and IgM detection	76/250 (30.4%)	58/250 (23.2%)	16/250 (6.4%)	2/250 (0.8%)	0 (0%)	5/16 (31.25%)	1/16 (6.25%)	10/16 (62.5%)	No	Yes

4.3 Toxoplasmosis Prevention

4.3.1 Food prevention

Due to the high risk of infection of *T. gondii* in pregnant women by contaminated food consumption, several methods exist for preventing the infection by cross-contamination (107). Three principal mechanisms can be applied in food for *T. gond*ii destruction: thermal methods, non-thermal methods, and chemical/biochemical methods (107) (108) (109). These methods can destroy tachyzoites, bradyzoites, and sporozoites, which are the three infectious stages of the parasite for humans (108). Also, destruction of the oocyst is one of the major approaches of alimentary prevention methods.

In the case of thermal methods, the main purpose is the inactivation of the parasite, especially cysts from the animal tissue and oocyst from environmental products (108) (109). Heating is one mechanism that can destroy pathogens such as microbes, viruses, and parasites, including heat-resistant (107) (108). *T. gondii* is susceptible to heat, especially oocysts, due to the core composition (90% cysteine- and tyrosine-rich protein) (109). The heating method is implemented for tissue cyst destruction (108). The principal requirement is long times and high temperatures for the inactivation of any parasitic stage (107). Another thermal method is cooking food before consumption (108). It is the easiest and most economical method and can be applied to pregnant women at home (107) (109). A cooking process of 60-70°C can destroy tissue cysts and inactivate tachyzoites (107). The last thermal method is freezing, which uses low temperatures to kill the parasite, especially tissue cysts (108) (109). The process is based on food freezing for some days at low temperatures. As a recommendation, freezing could be applied to meat for three days at -20 °C (107).

On the other hand, non-thermal methods can inactivate the parasite (107). Due to the materials needed and the high costs, meat industries can implement these methods to prevent product contamination (109) . This process uses physical processing that does not impact the properties and nutrients of food (107). One way to do this is the use of high-pressure processing (HPP), which uses high pressure for sterilizing products (340–550 MPa) (108) 32 (109). The advantages are low temperatures, loss of minerals/vitamins, and tissue cyst inactivation (107). Ionizing irradiation is another non-thermal method employed for killing *T. gondii*. Commonly, it is used in the pasteurization process. Moreover, it uses two rays: gamma and UV rays (108). The difference is based on the level of penetration for parasite destruction. Gamma rays are used for superficial sterilization and UV rays for dipper parasite destruction (107). Another non-thermal method is curing, which uses salt combinations, nitrates, and low-temperature smoking for product sterilization (108) (110) (111). The advantage is the destruction of tissue parasite cysts. In contrast, the disadvantage is the decrease in effectiveness depending on the cyst's maturation time (110). The recommendation is to use meat curing with salt and sugar for 64 h at 4°C or smoking saltinjected process at 50°C for 24–28 h (107).

Finally, chemical/biochemical methods can be an option for killing *T. gondii* (109). It is based on chemical agents like organic acids and salts for parasite destruction (109). Some agents used are oxidant compounds, formaldehyde, disinfectants, enzymes, food additives, and plant essential oils (107). For disinfection, chlorine and ozone are the most used methods in food treatment (109). In the other way, the use of oxidizing agents can cause the inhibition of the enzymatic activity of the parasite, producing cell death. Also, it can create damage

to DNA and alter the parasite membrane permeability. In the case of disinfectants, they are used for cross-contamination prevention in natural products (107) (109). Finally, essential oils are plant extracts used for killing parasites in food treatment. The main advantage is the low side effect in food when used in combination with pharmaceutical treatments (107).

4.3.2 Animal prevention

Toxoplasmosis infection is achieved in pregnant women mostly by parasite cysts in contaminated food. Therefore, there are some methods for preventing *T. gondii* in livestock and other animals (112). For example, the infection in pets, especially cats, can be a risk factor in pregnant women (113) (114) (115). Infected pets commonly present noise sensitivity, myositis, muscle wasting, stiffness, and some ocular problems like conjunctivitis, anterior uveitis, endophthalmitis, and chorioretinitis (113). So, in the case of cats that present 33 these symptoms, pregnant women must take precautions until they discard the pet infection (112). As a recommendation, cats at home should not have to stay outside for much time to avoid the consumption of rodents and birds contaminated with *T. gondii* (113).

In the case of livestock Toxoplasmosis prevention, farm biosecurity protocols are the best way to avoid environmental contamination with the parasite oocysts (112) (116). These protocols must be applied on all farms. As a result, oocysts can decrease in pasturelands and improve livestock quality (116). Some recommendations for the cat and dog are limited access to ruminant areas, food storage rooms, and water sources (112). Also, the proper removal of waste materials is crucial and the rodent control (116). Another way to avoid Toxoplasmosis in animals for consumption is through vaccines to prevent the disease (112). The main advantages are the low impact in the economic field and the prevention of vertical infections in animal pregnancy. One example is the use of Toxovax™, which is recommended for ovine to prevent abortion and fetal infection transmission (112). Also, vaccines can decrease the number of tissue cysts in the meat of animals, so the probability of acquiring the infection from tissue cyst consumption by pregnant women decreases. Moreover, some of the benefits of vaccines have been blocking all the stages of the parasite and stimulating the innate immune response (112). Finally, another way to treat livestock is the oral drug administration. These compounds are very effective against tachyzoite, bradyzoite, and sporozoites released from oocyst. These treatments can cross the blood barrier in the CNS, have low toxicity and arrive in all organs in animals like the brain. The most common treatment is the use of Spiramizine (112)

Chapter 5

Discussion

This review analyzes the congenital Toxoplasmosis seroprevalence of pregnant women and the risk factors for this pathology. Furthermore, this review looks to determine if the possibility of acquiring congenital Toxoplasmosis by the fetus is high depending on the mother's seroprevalence. Ten different trials were evaluated to determine the amount of pregnant women population affected with Toxoplasmosis previously and/or infected during pregnancy, the various risk factors that can affect the seroprevalence, and possible solutions for prevention. All the trials use collected blood samples from pregnant women in different places, especially in Medium East. The reason can be the high prevalence of the parasite in the population. Although South America is a tropical region, a smaller number of seroprevalence analyses are performed. Based on data, individual surveys analyzed where the possible risk factors were mentioned. In the following section, the discussion is related to the relation between seroprevalence of pregnant women and the risk factors consulted.

5.1 Pregnant women seroprevalence and risk factors.

Table 1 summarizes the information about congenital Toxoplasmosis seroprevalence in different places worldwide. The information compared in the table corresponds to the diagnostic method employed for infection detection and the percentage of seropositivity obtained. Also, the information about the treatment application, the assay evaluation criteria, and the events during the trials are included (Table 1). Based on the bibliography results analyzed, the positive seroprevalence of pregnant women means the average is 42.57%. It is a percentage less than 50%. However, it is closer to this value, which means that the prevalence is not so small to consider insignificant. The highest seropositive result is described by Mandour et al. in Assiut Governorate, Egypt, with 68.7% (95). Similar results are described by Ahmed et al. in Northern Iraq (88) and Halıcı et al. in Turkey (96) with 57.13% and 47.4%, respectively. Consequently, the possibilities of having a vertical fetal transmission of Toxoplasmosis and sick newborns are high (87). In contrast, the lowest seropositivity is described by Ali et al. (93) in Saudi Arabia, Rafha City, with 11.73%. Studies of seroprevalence are principally made in Medium East due to exposure to the pathogen and economic activities like farming (soil contact) that enhance the possibility of having a parasite contact (94). Other studies referred that the prevalence rate is different depending on geographical and climatic circumstances. The sporulation of oocysts is improved in warm climates, and a big population size can increment the number of people infected (117). For example, the high seroprevalence in the Sakaka area (Saudi Arabia) is higher because this region has an agricultural activity and a high presence of cats and goats, which are animals that can transmit the parasite like oocyst and cysts, respectively (92). In contrast, Rafha City, another place in Saudi Arabia, has a warm and dry climate that does not favor parasite survival because it needs humidity to develop in the environment (93). This factor is why the seroprevalence in this site is low (Table 1). Other examples of congenital Toxoplasmosis seroprevalence in pregnant women are 6.2% in Mexico (118); 4.5% to 5.8% in Vietnam (119); 44.5% in Tanzania (120); 80.3% in the Democratic Republic of Congo (121) and 85.3% in Ethiopia (122).

In Table 2, the comparison between risk factors and seroprevalence is analyzed. Each reference describes different risk factors based on surveys performed on the trial participants. In the case of risk factors, studied activities such as nutritional behaviors, hygiene practice, study population, sample size, age, the sensitivity of serological techniques used, and education level about *T. gondii* transmission are important to understand the seroprevalence of congenital Toxoplasmosis (123). his work shows that several risk factors in the infection are: age, place of living, soil contact, contaminated food consumption, and pet presence are the main risk factors for acquiring the parasite (Table 2). The reason is that these factors are events where the infectious phases of the parasite can be in contact with the pregnant women.

On the other hand, the detection of immunoglobulins isotypes is described in percentages. The principal isotype detected is the IgM+ because it shows the presence of recent infection in pregnant women. The highest rate of IgM+ is defined by Mandour et al. (95) at 15.4%. This value is related to soil contact and raw food consumption. In contrast, the lower percentages are described by Flores et al. (89) with 0.21% and Vueba et al. (87) with 0%. They are related to risk factors such as age and pet contact. So, the most important risk factors to consider are soil contact and raw food consumption. They are activities that pregnant women must avoid to prevent infection.

Following the trails analyzed, the detection of IgG+ and IgM+ /IgG+ isotypes are also performed to determine the presence of chronic infection or both infection stages in pregnant women, respectively.

Another factor-related is abortion record due to it has a direct correlation with positive IgM seroprevalence during pregnancy (124). About virus infection, the Hepatitis B, described in one study case, can contribute to the acquisition of *T. gondii* infection because it can decrease the liver function and, in consequence, avoid a correct immune response in pregnant women (125). HIV patients acquire the infection by their immunosuppression. The presented results vary due to the sensitivity and specificity depending on the type of serological test used and the quality of the kits (126). Finally, the results in Table 2 correspond to the findings of each reference (Table 2).

Using campaigns, lectures, pamphlets, and the guidance of medical personnel, the public health in general and pregnant women, can improve their knowledge about the parasite and their capacities to prevent the infection. A correct study of seroprevalence of Toxoplasmosis in pregnant women helps to prevent the infection. Also, the Toxoplasmosis study is important for the proper planning and educational programs for the population about the parasite, especially in pregnant women (127) (128). During pregnancy is recommended to avoid animal contact, potentially harmful contaminated materials, and raw or undercooked meat consumption (128).

5.2 Diagnostic methods in Toxoplasmosis.

Based on the studies' results, the ELISA test is the most common diagnostic method for Toxoplasmosis detection. IgM and IgG immunoglobulins are selected due to they can indicate the presence of an acute or a chronic phase of the disease, respectively (97) (98) (99) (100) (101) (102) (103) (104) (105) (106). IgA es took as a parameter of an acute phase of Toxoplasmosis in pregnant women. However, it is not common because it stays within the limit between the infection's chronic and the acute phase. So, IgA serological tests are not used (97). On the other side, the advantage of ELISA is based on the high sensitivity for antibody detection. Also, the resources and kits are present in the market worldwide. However, it cannot determine the specific disease stage and the infection time, so a precise diagnosis is not the best option (97) (102) (104). As evidence, Table 3 shows the comparison between the seropositivity of Toxoplasmosis in pregnant women and the ELISA test results. El-Sayad et al. (102) described the highest seropositivity percentages with 73% and PintoFerreira et al. (103) with 100%. In the case of Pinto-Ferreira et al., all participants show IgG+ isotype, determining a chronic infection. In contrast, El-Sayad et al.'s results show that 27% of the population study present the IgG isotype, so pregnant women present past infections. A similar percentage is described for the IgM+ isotype (23%), showing a recent infection. However, 23% of the population presents both immunoglobulin isotypes. As a result, determining the time of infection in pregnant women is uncertain (Table 3). This study is an example of the disadvantage of the ELISA test mentioned before.

On the other hand, according to the different serologic results in the studied pregnant women population, most of them only present IgG antibodies, showing a possible chronic phase of the disease (97) (102) (106). As a result, the infection could have been produced before the pregnancy began, and the risks for vertical transmission decreased. In contrast, patients with IgM seropositivity have more probability of letting the infection during the first weeks of pregnancy (98). So, they must guard to avoid fetal infection transmission

(98). Finally, patients who show both immunoglobulins need more studies to determine the infection stage. The presence of antibodies in the mother does not mean that the infection passes to the fetus (101).

On the other hand, the avidity test was used to determine the specific stage of the Toxoplasma infection. It determines the level of affinity that an antibody presents against a pathogen (98). This avidity is determined by a natural selection process of B-lymphocytes (97). As a result, a high avidity reflects a specific binding to a pathogen. So, the disease was acquired a time ago. In contrast, a low avidity shows a recent infection by a weak binding to the antigen (97). An intermediate avidity reflects a combination of both situations mentioned before. This method is recommended in the first trimester of pregnancy (97). In the Table 3, Berredjem et al. (97), Laboudi & Sadak (98), Saki et al (99)., Pinto-Ferreira et al. (103) and Sharifi et al. (106) used this method. The test was applied to the IgG isotype because the infection can be acute or chronic depending on the affinity level. When the IgG presents a low affinity to the parasite shows that the chronic phase of Toxoplasmosis is starting. Consequently, the treatment must be applied to prevent vertical transmission of the infection to the fetus. In the studies, low percentages of the population present this case. In contrast, the higher rates correspond to high and intermediate avidity. In the case of the disadvantage of this technique, it happens when the avidity results stay on the borderline of the previous categories. In this review, most patients have a high avidity so that mothers can be infected before pregnancy. Women with a low avidity test must require a Toxoplasma treatment to avoid congenital infection. Women who display an IgM + /IgG + with a low IgG avidity test must perform the test again to give a new result. Finally, to complement the avidity test and determine borderline results, PCR is an excellent tool because it presents a high sensitivity detection and *T. gondii* type (I or II) determination. It uses B1 and P30 gene primers for the detection of Toxoplasma DNA (97) (103) (104). B1 is the most common primer used, and it is based on a peripheral protozoa protein with several copies in the Toxoplasma genetic code. DNA detection helps to determine if the parasite crosses the placenta barrier (99). Positive results in samples of amniotic fluid, umbilical cord, and placenta can correspond to a possible vertical transmission (99). However, the confirmed diagnosis of congenital Toxoplasma is performed in newborns. Depending on the protozoa type, the pass of the parasite through the placenta and abortion possibility can increase. *Toxoplasma gondii* type II is the most common strain in congenital Toxoplasmosis (101). Authors that describe this method are Berredjem et al. (97), Saki et al (99)., Shieh et al. (100), Arefkhah et al. (101), El-Sayad et al. (102), Pinto-Ferreira et al. (103) and Matin et al. (104). Consequently, a high risk of vertical transmission during pregnancy depends on the detected strain (101). Therefore, giving a precise diagnosis permits starting treatments and reduces the risk of congenital Toxoplasmosis (105). Also, the combination of all these diagnosis methods improves the detection of the disease (97) (98) (104) (106).

5.3 Congenital Toxoplasmosis prevention protocols

Based on the information presented about prevention methods against *T. gondii*, hygiene protocols constitute the major way to avoid parasite infection (108). So, decreasing risk factors like food contamination and soil contact will reduce the probability of acquiring Toxoplasmosis during pregnancy. According to Table 1, most women who present this infection consume raw food, so methods to destroy the parasite at any stage are

essential for prevention (108) (109). Some features that the methods must accomplish are the easy procedure, low costs, and use of home materials.

On the one hand, thermal methods inactivate food parasites, especially environmental products and animal tissue (108) (109). So, methods like heating, cooking, and freezing can be used for pregnant women. The advantage of these processes is the use of home objects, so cooking food in the oven, boiling vegetables, or freezing meat for some days in the freezer are some solutions that common people can apply in general. Complete meat cooking must be achieved for parasite destruction. Also, pregnant women need to avoid meat terms like Blum or medium. In the case of raw vegetables, implementing disinfectant agents can be a solution for destroying oocyst contamination (109). Also, verifying the hygiene quality of the store where products are shopped can avoid cross-contamination. Other methods like radiation or chemical agents use can be applied by industry before production distribution (107). Also, preventing animal infection in the meat industry can reduce costs in the next disinfections.

On the other hand, preventing animal contamination in livestock is one of the best ways to avoid future human Toxoplasma infections (112). So, applying hygiene protocols and well storage of waste can decrease cross-contamination. Also, maintaining the healthiness of the pastureland can avoid animal oocyst infection (112) (116). Another important aspect is the application of vaccines for livestock. Vaccination prevents animals from miscarriages and decreases chronic infections. So, the development of cysts in animal tissue in low numbers will happen. Finally, cat infection prevention is another factor to consider (113). Despite pet infections being common and not representing a risk factor, cats' healthcare can decrease the possibility of women's infection, especially if the animal stays in a free environment. Therefore, veterinary revision during women's pregnancy can ensure pet contamination.

Chapter 6

Conclusion and Perspectives

6.1 Conclusion

Toxoplasmosis is a worldwide zoonotic disease produced by the obligatory intracellular parasite *Toxoplasma gondii*, which belongs to the Apicomplexa phylum. One of the principal impacts of this disease happens during pregnancy due to several miscarriages, fetal malformations, and mothers' infections. The percentage of prevalence of the infection varies depending on the geography location, the climate of the region, and the customs of the population. Also, several factors are associated with parasite infection, such as consuming uncooked vegetables and meat, contact with soil, and HIV suffering. Mothers must have cautious with these factors to prevent Toxoplasmosis. Depending on the pregnancy stage at the moment of infection, the fatal consequences can be serious. The vertical transmission of Toxoplasmosis in pregnant women is based on the overproduction of inflammatory cytokines such as IL-12, TGF-α, and IFN-γ. Also, the parasitic mechanisms for avoiding the host immune response are involved in congenital Toxoplasmosis, especially the decrease of inflammatory cytokines and apoptosis blocking. This transmission is less common in the first trimester, but malformations and abortion can result.

In contrast, infections in the last trimester are common, and the aftermath has less impact on newborns. So, the better methods for parasite control are the earlier diagnosis, prevention protocols, and effective treatments. In terms of the first one, using methods like ELISA to detect IgM and IgG antibody isotypes can indicate the presence of an acute or chronic infection, respectively. According to the diagnosis, the treatments and prenatal care can vary for great effectiveness. Another way for diagnosis is the PCR to determine the presence of the parasite. Also, avidity tests can be used to determine the time of infection. In terms of prevention, food contamination corresponds to the major factor for acquiring T. gondii during pregnancy. So, cooking at high temperatures, freezing, or using chemical/irradiation methods is best to contrast it. Also, having pet cares and low soil exposition contributes to Toxoplasmosis prevention. Finally, the employment of drugs when the infection is detected is a critical factor in avoiding fetal infection. The most common treatments are Spiramycin and Pyrimethamine-sulfadiazine. As a result, infection sequels can be prevented. For example, fetal death and miscarriages can be avoided in the first trimester, and fatal encephalitis and ocular damage in the second and third trimesters. Continuous controls and risk factors prevention are ways to prevent Toxoplasmosis during pregnancy. It is recommended that mothers have excellent hygienic measures to have a healthy pregnancy.

6.2 Future search.

This review analyzes the seroprevalence of congenital Toxoplasmosis in pregnant women as a control for the parasitology in tropical regions. As a result, new prevention protocols can be applied depending on the population traditions and decrease the vertical transmission of the Toxoplasma from mothers. The decrease in congenital Toxoplasmosis cases increases the health of newborns and mothers worldwide, avoiding

spontaneous miscarriages and syndromes like Torch ones. In other opportunities, the study of seroprevalence must be performed in Ecuador to consider the country's reality and verify the prevalence of Toxoplasmosis in this place. Similar study strategies can be applied, like surveys and ELISA tests.

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