THESIS

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Overview of Congenital Toxoplasmosis in Pregnant Women; Mode of Infection, Diagnosis, Treatment and Prevention

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Abstract

Toxoplasma gondii, the agent of toxoplasmosis in all warm-blooded animals, undergoes sexual reproduction in cats and asexual reproduction in all other families. Through fecal excretion of oocysts, definitive host (cats) are responsible for the contamination of the environment, whereas intermediate hosts (livestock animals) upon infection, develop *T. gondii* in their muscle tissues depending on susceptibility, and become reservoirs for the disease. Humans can also get infected by *Toxoplasma gondii,* either by consuming oocyst from the environment or tissue cysts. Seronegative pregnant women infected with toxoplasmosis will in turn infect their infants through placental transmission, causing congenital toxoplasmosis, a serious disease leading to several neurological and ocular disorders and even death. Although congenital toxoplasmosis is considered rare, the global annual incidence is 190100 cases per year, with an average incident rate of 1.5 cases per 1000 live births, with a disease burden of 1.20 million DALYs (disability-adjusted life year). The primary modes of infection of pregnant women are through consumption of uncooked meat, uncooked seafood and contaminated water followed by contact with soil and lastly cat feces. Since congenital toxoplasmosis is not curable, prevention strategies have been developed: primary prevention measures (educating women of childbearing age about the risks of toxoplasmosis acquisition during pregnancy, developing vaccines), secondary prevention measures (prenatal diagnosis and treatment of congenital toxoplasmosis), and tertiary prevention measures (postnatal diagnosis and treatment of congenital toxoplasmosis). Even though secondary and tertiary prevention methods are useful, they face several challenges (the lack of specificity and sensitivity of screenings, contradicting data on drug therapy and lack of availably of prophylactic methods in many countries due to high costs), therefore education as primary prevention can be the most promising method of prophylaxis, especially since vaccines against congenital toxoplasmosis do not exist yet. Finally, multidisciplinary team approach between veterinarians and physicians can be the key to finding the most efficient way to fight against congenital toxoplasmosis in human infants.

Összefoglaló

A melegvérű állatokban előforduló toxoplazmózis parazitásfertőzés kórokozója *Toxoplasma gondii* protiszta, amely szexuális szaporodáson megy keresztül macskákban, illetve aszexuális szaporodáson a köztigazdákban. Míg a végleges gazdák (macskafélék) a környezet oociszta terheléséért felelősek, addig a köztigazdákban (haszonállatok) a megfertőződést követően, faji érzékenységüktől függően, *T. gondii* szöveti ciszta fejlődik az izomszövetekben, ezáltal a betegség hordozóivá (rezervoárrá) válva. Az emberek is megfertőződhetnek a *Toxoplasma gondii* protisztával, akár a környezetükben lévő oocisztákkal érintkezve vagy az köztigazdákban megjelenő szöveti ciszták elfogyasztása által. A toxoplazmózissal megfertőzött, szeronegatív várandós nők esetében a fertőzés placentális átvitel során anyáról magzatra terjed. Ez rendszerint veleszületett toxoplazmózist eredményez, amely komoly betegségnek számít, és számos idegrendszeri- illetve látási rendellenességhez, valamint akár a magzat halálához is vezethet. Bár a veleszületett toxoplazmózis kialakulását ritka jelenségként tartják számon, a globális évi gyakorisága összesen 190100 eset, ami átlagosan 1,5 esetet jelent minden 1000 élveszületésre számolva. Ennek 1,20 milliós betegségteher felel meg az Egészségkárosodással Korrigált Életévek indexét (DALY) tekintve. A várandós nők esetében az elsődleges fertőződési forrás a nem megfelelően átsütött húsfélék, tengergyümölcsei és szennyezett ivóvíz fogyasztása, továbbá a talajjal, illetve a macska ürülékével való érintkezés. Mivel a veleszületett toxoplazmózis gyógyíthatatlan, számos megelőzési stratégiát dolgoztak ki az évek során: elsődleges- (a szülőképes korú nők oktatása a terhesség alatti toxoplazmózissal való megfertőződés veszélyeiről, vakcinák kifejlesztése), másodlagos- (szülés előtti diagnózis és a veleszületett toxoplazmózis kezelése), valamint harmadlagos megelőzési eljárások (születés utáni diagnózis és a veleszületett toxoplazmózis kezelése). Bár a másodlagos és harmadlagos megelőzési módszerek hasznosak lehetnek, számos nehézségbe ütközhetnek (a különböző vizsgálatok pontosságának és érzékenységének hiánya, ellentmondásos adatok a gyógyszeres terápiát illetően, valamint sok országban a magas költségeknek köszönhető megelőző eljárások elérhetetlensége), ezért elsődleges megelőzésként az oktatás vezethet a legkedvezőbb eredményekhez, főként mivel még semmiféle vakcina nem létezik a veleszületett toxoplazmózis ellen. Végezetül pedig, az állatorvosok és orvosok összefogásából következő multidiszciplináris megközelítés kulcsfontosságú szerepet játszana a leghatásosabb módszer kidolgozásában, ami a csecsemőknél előforduló veleszületett toxoplazmózis elleni harc esetében.

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List of abbreviations

PV: Parasitophorus vacuole

MAT: Modified agglutination test

IFAT: Indirect fluorescent antibody test

CT: Congenital toxoplasmosis

AIDS: Acquired immunodeficiency syndrome

OT: Ocular toxoplasmosis

CI: Confidence interval

EFSA: European Food Safety Authority

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IgA: Immunoglobulin A

IgE: Immunoglobulin E

ELISA: Enzyme-lined immunoassay

DNA: Deoxyribonucleic acid

PCR: Polymerase chain reaction

ISAGA: Immunosorbent agglutination essay

PO: Per os

MHC 1: Major histocompatibility complex 1

HLA: Human leukocyte antigen

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1. Introduction

Toxoplasma gondii is a zoonotic obligate intracellular protozoan parasite [55], with worldwide distribution. It is the causative agent of toxoplasmosis, one of the most frequent parasitic infections of warm-blooded animals, including one-third of the human population [26]. Cats and other felids are a center of interest in the epidemiology of toxoplasmosis as they remain to this day, the only known hosts capable of excreting the highly resistant oocysts into the environment [28]. As such, toxoplasmosis is acquired horizontally either by ingesting these resilient oocysts from contaminated water or vegetables, or by consuming the tissue cysts of *Toxoplasma* infected animals. In immunocompetent individuals, toxoplasmosis is usually asymptomatic, or presents mild flu like symptoms such as fever and headache. However, immunocompromised patients will present more severe symptoms such as toxoplasmic encephalitis and retinochoroiditis [124]. In addition, rarer modes of transmission of *Toxoplasma gondii* include trans-placental transmission to the fetus or organ transplant. The vertical transmission occurs when a pregnant woman is infected with *Toxoplasma* during the gestation period, resulting in congenital toxoplasmosis. Congenital toxoplasmosis can harm the fetus to varying degrees. It can lead to miscarriage, stillbirth, malformation, as well as intellectual disability, blindness, and deafness in the infected infant [83]. Although rare, congenital toxoplasmosis should be considered a global burden, as the global estimated incidence rate is of approximately 1.5 cases per 1000 live births. [140]. Even though the disease is not curable, congenital toxoplasmosis upon diagnosis is managed through medication that prevents the placental infection. The importance of early and adequate diagnosis is therefore essential for the treatment of the infection. In addition, it has been proven that prenatal education about toxoplasmosis can improve a woman's behavior during pregnancy [84]. Unfortunately, thus far, a universal protocol for prophylaxis for congenital toxoplasmosis has not been put in place.

Based on literature, the aim of this thesis is to provide a general overview of congenital toxoplasmosis in pregnant women in consideration of life cycle, mode of infection, diagnosis, treatment, and prophylaxis. This thesis will also discuss the importance of inter-professional collaboration between health professionals and veterinarians to better supervise women who are susceptible to toxoplasmosis.

2. Methods of review

Theoretical work

This thesis is a literature report and presents the overview of congenital toxoplasmosis in pregnant women. Information about the lifecycle of the parasite *Toxoplasma gondii* was researched to understand the importance of cats and intermediate hosts in the propagation of the disease. To have a better understanding of the current relevance of congenital toxoplasmosis in medicine, statistics were collected from scientific material published in the last two decades. This included searching for updated information on the global prevalence of the disease. Data from several research on the mode of transmission of the parasite was collected, allowing the presentation of all habits which could be considered risks for toxoplasmosis infection in pregnant women. Moreover, diagnostic methods, treatment options and prophylaxis were given by scientific material and case studies. It is important to note that only a small number of scientific surveys have been conducted to evaluate the practical effectiveness of prenatal education regarding the prevention of congenital toxoplasmosis. The online database PubMed was used to get access to scientific papers, studies, and reviews.

3. *Toxoplasma gondii* parasite

Toxoplasma gondii is a ubiquitous obligate intracellular protozoan parasite that is part of the Phylum Apicomplexa. It has a wide range of warm-blooded intermediate hosts, including mammals, humans, and birds, but felids are the only existing definitive hosts. It is one of the most found parasites in animals and *T. gondii* remains the only known species. It is the causative agent of toxoplasmosis, a disease mostly asymptomatic in animals, but capable of causing severe ocular and neurological symptoms in immunosuppressed patients. With approximately 2 billion people infected, *T. gondii* is one of the most successful parasites. Evidence based on seroepidemiological studies has confirmed that approximately one third of the human population has been exposed to the parasite. Additionally, toxoplasmosis is the third leading infectious cause of food borne disease, following salmonellosis and listeriosis. Therefore, *Toxoplasma gondii* presents both medical and veterinary concern. [17, 26, 109].

3.1. Lifecycle of Toxoplasma gondii

Toxoplasma gondii is a parasite belonging to the subclass Coccidia. Like all coccidian parasites, it has a complex lifecycle undergoing both sexual and asexual reproduction. In Felidae (definitive hosts), sexual and asexual reproduction are both present, however *T. gondii* can only divide asexually in all remaining warm-blooded animals, including humans (intermediate hosts) (figure1). This parasite is peculiar because it can be transmitted by fecal-oral route, but also by carnivorism and through the placenta [26, 89]. *Toxoplasma gondii* has three morphological forms that correspond to three morphological stages: tachyzoites (free in exudates, or in groups in pseudocysts), bradyzoites (located in tissue and organ cysts), and sporozoites (in oocysts excreted in cat feces) [17]. *Toxoplasma gondii* tachyzoites, bradyzoites and sporozoites have a crescent shape, and range between 2-6 μm in width and 4-8 μm in length. They are equipped with a special organelle called apical complex which is used for invasion. The parasite is also motile and uses gliding motility to migrate through tissues and biological barriers (such as the blood placenta barrier) [55].

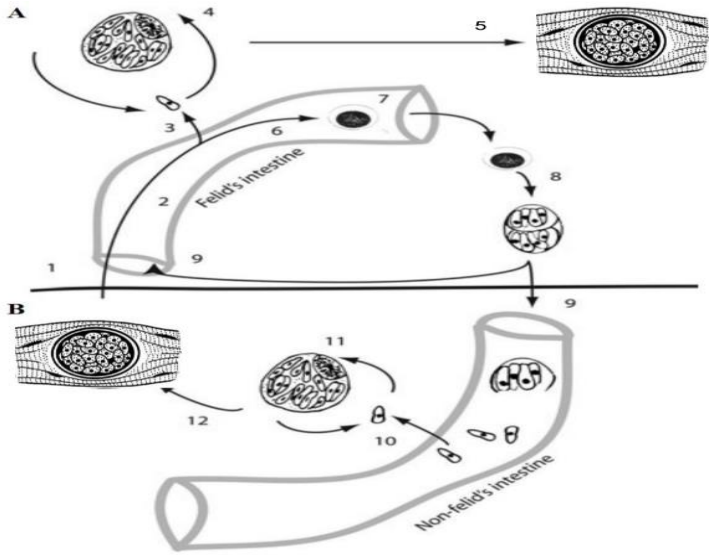

Figure 1: Life cycle of *Toxoplasma gondii*. A, in definitive host. B, in intermediate host Lifecycle based on literature.

(1) Cyst ingested by Felidae, (2) schizogony in the felid's intestinal mucosa, (3) diffusion of tachyzoites to the felid's body, (4) pseudocyst containing bradyzoites, (5) tissue cysts, (6) gametogony, (7) oocyst formation and expulsion in feces, (8) sporogony and maturation of oocysts that contain two sporocysts, each containing four sporozoites, (9) oocyst ingested by intermediate hosts, (10) diffusion and rapid multiplication by endodyogeny of tachyzoite, (11) pseudocyst – tachyzoite transform into bradyzoite, and (12) tissue cyst with slow multiplying bradyzoites.

The sexual stage (also known as gametogony) takes place in the intestinal epithelium of the cat. Cats usually become infected by consuming the flesh of intermediate hosts such as birds and rodents (which are infested with *Toxoplasma* cysts) or more rarely by ingesting oocysts found in the environment [109]. In case of cyst ingestion, the proteolytic enzymes in the gut break down the cyst wall and release bradyzoites. The bradyzoites enter the lamina propria of the intestine and multiply asexually as tachyzoites (schizogony/endodyogeny), giving rise to five morphologically distinct schizonts (A-E) [26]. In 3-15 days, the type E schizonts release merozoites and form male and female gametes, found in the small intestine but concentrated in the ileum [61]. The microgamete is equipped with two flagella, allowing it to swim towards the macrogamont and fertilize it. After fertilization, the zygote becomes an immature diploid oocyst surrounded by a very resistant wall. The oocyst is spherical in shape and has a diameter of 10 μm x 12 μm. Up to 10 million unsporulated oocysts [56] are then shed in the feces of the acutely infected cat, where they remain noninfectious until sporulation. Once exposed to air, sporulation of the oocysts occurs between 1-5 days. This duration depends on the ambient temperature of the environment. The sporulated oocyst thus becomes mature, and infectious. It comprises of two ellipsoid sporocysts and each sporocyst contains four sporozoites. Since the oocyte is surrounded by a hard outer wall, it can survive up to 18 months in temperate and moist environments [26, 62, 89].

Unlike the sexual reproduction, asexual reproduction occurs with no host specificity [17], such as felines (final hosts) but is also found in all mammals, birds, and humans. Since the sexual phase of *Toxoplasma gondii* is present only in cats, these other nonspecific hosts will be classified as intermediate hosts. Similarly to Felidae, intermediate hosts can be infected by *Toxoplasma gondii* by consuming oocysts from the environment or consuming the tissue cysts of infected animals. Following ingestion of oocysts, sporozoites excyst and invade the intestinal cells (enterocytes) and ileal epithelial cells. The sporozoites then convert into tachyzoites and begin multiplying asexually [61]. The asexual development is comprised of two phases [17]. In the first (or acute) phase, tachyzoites enter most nucleated host cells and form parasitophorous vacuole, where they multiply rapidly by endodyogeny [62]. The parasitophorus vacuole is a special adaptation which has given tachyzoites the ability to avoid fusing with the endolysosomal pathway of the host cell, a skill necessary for the intracellular growth of the parasite [55]. The tachyzoites continuously divide in the PV until the host cell ruptures, releasing the parasitic cells to invade and multiply in new host cells. At this

stage in the cycle, tachyzoites can cross the placental blood barrier and infect the growing fetus [89]. The migration of the parasite activates the host's immune system, which triggers the conversion of tachyzoites in host cells into bradyzoites. The latter hide from host's immune system by transforming the PV into a cyst wall. This marks the beginning of the second phase. The encysted bradyzoites divide very slowly by endodyogeny and initiate chronic toxoplasmosis, since they persist for a lifetime [62, 88]. The cyst then matures and is deposited in surrounding tissue [56], most commonly in the brain, eye, skeletal and cardiac muscle but can also be found in visceral organs (lungs, liver, kidneys). The tissue cysts have thin elastic walls and range between 70 to 100 μm in diameter. They contain thousands of slender bradyzoites, which are less susceptible to elimination by proteolytic enzymes of the host cell compared to tachyzoites [26, 55].The slow second phase is also known as the terminal life-cycle stage in the intermediate host because unlike oocysts shed in feces, the tissue cysts formed are immediately infectious [17]. - In the event of immunosuppression of the host, bradyzoites transform back into proliferating tachyzoites, and can cause latent acute infection in animals but can also trigger a new sexual cycle in the intestinal epithelium of cats [89].

3.2. Etiology and pathogenicity of *Toxoplasma gondii*

As previously mentioned, *Toxoplasma gondii* is not host specific. It will infect almost all warm-blooded animals and cause toxoplasmosis with varying degrees of severity. The incidence of toxoplasmosis differs globally. This variation in incidence can be explained by the different climates and population of cats and rodents in different regions. Additionally, seroprevalence of the disease differs from one species to another, as some species are more susceptible than the rest [17, 55, 66]. The pathogenicity of *T. gondii* depends on several factors including the age of the animal, the susceptibility of the host species, the virulence strain and morphological stage of the parasite. To maximize pathogenicity, *T. gondii* has adapted its mode of transmission to the host. Intermediate hosts (mostly herbivores) are infected through oocyst-oral cycle, whereas cats who are true carnivores are infected trough tissue-cyst oral cycle. The oocyst-oral cycle causes more severe clinical signs in intermediate hosts such as mice and pigs compared to definitive hosts (cats). To cause infection, it suffices for the intermediate host to ingest one live oocyst, whereas a cat would require over a hundred live oocysts to be infected with toxoplasmosis. In contrast, the tissue-cyst cycle is less infective in rodents than in definitive hosts. Ingesting a minute amount of bradyzoites causes the cat

to shed millions of oocysts into the environment, but a larger quantity of bradyzoites will not necessarily lead to an infection in the intermediate host [26].

3.2.1 *Toxoplasma gondii* in Felidae

Although toxoplasma gondii is present in all Felidae, domestic or wild (especially those in zoos), the cat (*Felis catus*) is the most crucial species in the spreading of the parasite, because cats are found in every corner of the world [28]. Additionally, according to literature, toxoplasmosis is rare in areas with no cats [26].

Serological testing such as MAT and IFAT are the main and most accurate way to detect *T. gondii* infections (either recent or old) in cats [28]. The differences in seroprevalence of toxoplasmosis in cats are determined by geographical location, lifestyle, and age [17].- The average global seroprevalence of *T. gondii* in domestic cats is between 30-40% and 51% in feral felids [89]. The prevalence is climate dependent. It increases in warmer countries with high humidity and precipitation, but decreases in drier, colder climates [28, 55]. Countries with better sanitation control and overall hygiene show a decreased rate of *Toxoplasma*--positivity. The number of positive cats also varies within the same areas of a country. This is explained by the lifestyle of the cat. Seropositivity is higher in feral, stray, and outdoor cats because they consume the tissue of potentially infected animals they have hunted. In contrast, seropositivity to *T. gondii* is much lower in domestic indoor cats, because they are fed preserved wet or dry cat food [28]. Infection rates of stray cats is also dependent on rate of infection of the avian and rodent species in the region [26]. Lastly, seropositivity increases with the age of the cat. In fact, antibodies of toxoplasmosis have mostly been detected in kittens after weaning, meaning that congenital toxoplasmosis in cats is not frequent [28].

Another less effective way to detect toxoplasmosis in felines is by coroscopy. Although elimination of the oocysts through the feces is the main way of *Toxoplasma gondii* propagation and cats are known for shedding millions of oocysts during an acute infection, oocysts are detected only in <1% in feces of cats [28]. This can be explained by several factors. The usual duration of shedding of T. gondii oocysts in the feces by cats is up to 3 weeks following acute infection, which can be considered short. Cats also develop a good immunity against toxoplasmosis once infected (lasting over 6 years), and therefore excrete oocysts only once in their lifetime. If they suffer from an immunosuppressive disease in parallel, such as viral infections (FIV, FeLV), or bacterial infections (*Bartonella* spp, *Leishmania* spp), re-shedding can occur [17, 28]. It is

important to note that some studies have concluded that cats might re-excrete oocysts even if they have antibodies against *T. gondii* and are in good health, but this is very rare [17]. The prepatent period is also influenced by which morphological stage the cat ingests. Ingestion of tissue cysts containing bradyzoites causes the shortest shedding period, 3-10 days, then ingestion of tachyzoites, 13 days, and lastly ingestion of oocysts containing sporozoites has the longest shedding period of 18 days [55]. In contrast, less than 50% of cats excrete oocysts after ingesting tachyzoites and oocysts, whereas almost all excrete oocysts after consuming tissue cysts [26].

The host specificity of *T. gondii* oocyst shedding in Felids is explained by the family's lipid metabolism. To undergo sexual reproduction, it was discovered that *T. gondii* require an excess of linoleic acid. Cats and their relatives are the only mammals that lack delta-desaturase activity in their intestines, which is essential for the metabolism of linoleic acid. The absence of this enzyme leads to an excess of linoleic acid in the guts, perfect for the sexual multiplication of the parasite. "The mechanism of species specificity is the first defined for a parasite sexual cycle" [28].

The diagnosis of this disease by clinical manifestation in cats is very challenging, as most cats infected with *T. gondii* are asymptomatic. Clinical infections can still occur in rare cases, to cats of any sex, age, or breed. The most frequent finding in clinical infection is pneumonia, and it is fatal. The most common clinical signs are anorexia and neurological signs. Since *T. gondii* can encyst in many organs, other lesions such as hepatitis, pancreatic necrosis, myocarditis, dermatitis, uveitis, and encephalitis can be seen. Although the rate of congenital toxoplasmosis is low in cats, kittens suffer from the worst lesions in case of congenital infection. Ocular and nervous system symptoms in cats can be a good opportunity for veterinarians to test for toxoplasmosis. Ocular signs include retinochoroiditis, uveitis, mydriasis, photophobia and even blindness. Veterinarians should also take note of any sensory depressions during physical examination. These include any changes in motor coordination, behavioral changes (fear and aggressiveness), or difficulty in mastication and urinary continence [17].

3.2.2. *Toxoplasma gondii* in food producing animals

Toxoplasmosis in intermediate hosts such as food producing animals was once thought to only be relevant in veterinary medicine as a public health concern. This was explained by fact that the prevalence of toxoplasmosis in farm producing animals was overall low but the risk of *Toxoplasma gondii* transmission to humans through consumption of meat

was high [17]. Nowadays however, with the rise in demand for free-range products, a considerable number of farm animals are being raised in nonconfinement systems which increases the risk of animals being infected with T. gondii through oocysts shed by cats. Some farms are also required to limit the number of veterinary visits to acquire the status "free-range" for their products [66]. In addition, *Toxoplasma gondii* can cause reproductive challenges in farm animals and is now also considered to have a great impact on the economy and welfare of livestock and farmers worldwide [61].

Sheep and goats are highly susceptible to *T. gondii*. The global seroprevalence of toxoplasmosis in sheep and goats ranges between 11.4-96.6%, with the highest seroprevalence found in Europe - In fact, it is an important source of congenital disease in sheep and goats around the world. Congenital toxoplasmosis is responsible for 10-23% of abortions in Europe and USA, 28% of ovine abortions in the UK, and 3-54% of ovine abortions in the Middle East. The outcome of CT depends on the period of gestation in which the infection occurs. In early pregnancy, fetal transmission results in fetal death in utero and reabsorption, whereas later in gestation, the infection causes abortion, stillbirth, or delivering weaker offspring. Otherwise, toxoplasmosis in adults has very limited evident clinical signs, such as a short episode of fever and lack of appetite for 4-5 days. In both sheep and goats, even after the horizontal or vertical infection of *T. gondii*, the parasitic cysts remain in the organs (liver, kidney, and brain) and muscle tissue of the animal or fetus [17, 62, 132].

Seroprevalence of *T. gondii* in pigs varies according two main factors in addition to age and geographical location: The management system on the farm and function of the pig. The seroprevalence of pigs raised in tighter controlled management systems with no access to the outside environment (all-in-all-out) is < 1%, compared to the higher prevalence of > 60% in pigs raised in free-range style farms. Additionally, fattening pigs have a higher rate of seropositivity for toxoplasma gondii than breeding sows (up to 64% and 31% respectively) [10]. During acute infection, pigs are usually asymptomatic. However, clinical manifestations are more frequent and higher in number in these species, especially in neonatal and weaned piglets. Unlike in ewes and dams, sows have also been described to show clinical signs. The clinical manifestations in pigs include anorexia, fever, lethargy, ocular and nasal discharge, dyspnea, cyanosis, pneumonia, weak limbs, neurological alteration (encephalitis) and finally death. CT also exists in pigs, causing similar reproductive failures seen in sheep, with the addition of fetal mummification [10, 17].

Toxoplasma gondii seroprevalence varies among different types of poultry. In chickens, the prevalence of the parasite depends on the management system of the farm. In indoor broiler chickens, seroprevalence is low, especially when the meat produced is injected with enhancing solutions. However, seropositivity in free-range farms is higher, with a recorded 17-100% in the United States. Prevalence of *T. gondii* in free-ranging chicken is considered an indicator for toxoplasma oocyst contamination in the environment. In the case of waterfowls, data collected indicates a global prevalence ranging between 1.7-55% in ducks, and 5.9-43% in geese. Since *T. gondii* oocysts are resistant in water, infection of waterfowls can be an indicator of water contamination. Finally, eggs are not considered infected with the parasite and therefore reproduction is unaffected. Clinical signs in poultry are rare. In one family farm, nervous system signs occurred in free-range chickens. [17, 27, 66].

From all food producing animals, cattle have the lowest global seroprevalence of *T. gondii*. They are therefore considered to be a clinically resistant species. These animals can clear the infection without having any clinical manifestations. Vertical transmission of *T. gondii* in cattle seems to be rare, as isolation of the parasites from aborted fetuses has been scarce [62, 132].

Lastly, seropositivity also exists in equids (horses, donkeys, and mules), with a prevalence ranging between 0-73% depending on the region. To date, only a few isolated cases in horses have manifested clinical signs, mainly revolving around ocular diseases [132].

3.2.3. *T. gondii* in humans

It is estimated that roughly one-third of the human population is currently infected with *Toxoplasma gondii*. The seroprevalence of the parasite is between 10-80%. Like in all other warm-blooded animals, the prevalence depends on the geographical area, the climate, but also the customs and culture of the regions in question. Humans are infected with *T. gondii* either horizontally or vertically. The parasite is horizontally transmitted by two routes: either ingestion of cyst infected tissue, or ingestion of oocytes found in vegetables or contaminated water. Vertical transmission of *T. gondii* occurs during pregnancy, congenitally infecting the fetus through the placenta. Toxoplasmosis in immunocompetent adults does not usually cause clinical symptoms, unless they are infected with a non-typical strain of the parasite (the atypical strain is highly virulent). The clinical aspect of toxoplasmosis in humans can be divided into four categories:

acquired toxoplasmosis, congenital toxoplasmosis, acquired or congenital ocular toxoplasmosis, and cerebral toxoplasmosis.

In immunocompetent individuals, acquired infection is asymptomatic 80% of the time [81]. In the remaining 20% of immunocompetent individuals, the most seen clinical manifestation of toxoplasmosis is lymphadenopathy. Lymphadenopathy is usually presented as enlarged lymph nodes in the head and neck area, although it can also be present in the mesenteric, retroperitoneal, inguinal, and axillary lymph nodes. Lymphadenopathy can be accompanied with discomfort, fever, tiredness, muscle pain, headache, and sore throat, which is why toxoplasmosis in humans can be often confused with mononucleosis. The symptoms disappear after 2-3 weeks, as acute infection is usually self-limiting. At this stage, the immune system of the host clears out the tachyzoites, and bradyzoites encyst in brain and muscle tissue to escape from the immune response. Once immunized, re-infection and re-activation of the disease does not typically occur. However, in individuals infected with a disease-causing immune deficiency (such as AIDS) chronically existing infection can be re-activated, causing latent toxoplasmosis. Necrotizing encephalitis is a consequence of re-activation of the disease, as bradyzoites in nervous tissue will convert to rapidly multiplying tachyzoites. Toxoplasmosis in AIDS patients is thus fatal [26, 55].

Congenital toxoplasmosis occurs when *T. gondii* is transmitted from the infected mother to the fetus during pregnancy. CT can be fatal in the fetus as it can cause spontaneous abortions, severe neurological problems, or can cause lifelong challenges for the infant such as mental retardation, epilepsy, and blindness. The disease's severity depends on which period of the gestation the parasite infects the pregnant women [142, 55]. Congenital toxoplasmosis will be further discussed in section 4.

Toxoplasma gondii can also cause ocular toxoplasmosis. It predominantly infects the retina but can also affect other parts of the eyes such as the choroid plexus, vitreous humor, and anterior chamber. The disease can occur either due to congenital toxoplasmosis, or postnatal infection, or reactivation caused by immunosuppression. The main clinical manifestation of OT is chorioretinitis, characterized by necrotizing retinopathy prompted by dormant cyst activation. OT can lead to blindness and is in fact one of the leading causes of visual loss in children in developing countries. Immunocompromised patients exhibit bilateral retinal necrosis and uveitis [18, 24].

Lastly, re-activation of latent *T. gondii* infection in immunosuppressed patients leads to cerebral toxoplasmosis. In both developed and underdeveloped regions of the

world, cerebral toxoplasmosis is “the most common opportunistic disease” in patients suffering with AIDS. The re-activation of bradyzoite cysts in the brain leads to unifocal or multifocal lesions, causing necrotizing encephalitis. The clinical signs of cerebral toxoplasmosis are headaches, fever, seizures, lethargy, visual abnormality, cognitive and motor dysfunction [110].

4. Congenital toxoplasmosis in humans

As previously mentioned, a woman infected with *T. gondii* during or shortly before pregnancy can transmit the parasite to their child through uterine transmission. This is especially true for women who have not been previously exposed to *T. gondii*, and thus are not immune. During primary infection, the parasite will multiply and enter the blood system of the seronegative mother as tachyzoites. *Toxoplasma gondii* tachyzoites are one of the rare parasites possessing the ability to cross the placenta and therefore can infect the fetus [95, 100]. In fact, the placenta is important in the trans-placental transmission of *T. gondii* because it is a target organ for parasite proliferation. In addition, the placenta acts as a protective barrier for the fetus against pathogens. It efficiently inhibits the transmission of tachyzoites at the beginning of the pregnancy but increases in permeability later in gestation. Therefore, the vertical transmission varies according to gestational age. The trans-placental transmission during the first trimester (12-14 weeks) and second trimester (14 to 26 weeks) is low and less than 10% and 30% respectively, whereas the transmission during the third trimester (26 to 40 weeks) increases to 80% and can reach a transmission rate of 90% by the end of pregnancy [121, 122].

In addition, the consequences of congenital toxoplasmosis also depend on the phase of pregnancy. The severity of the disease and the presence of sequelae decreases with increasing gestational age, starting with 60% risk of the fetus developing major clinical signs in the first trimester and dropping to 5% at the end of pregnancy [84]. *Toxoplasma gondii* infection in early pregnancy can lead to an increased chance of miscarriage and severe fetal damage, such as chorioretinitis, the development of hydrocephalus and intracranial calcification or microcephaly [35]. Conversely, infection that occurs later in pregnancy is subclinical and has a more favorable outcome for the fetus. Indeed, 80-85% of infants infected during the last trimester are usually asymptomatic at birth [46, 143], but rare clinical manifestations can occur. The classic triad of symptoms, also known as Sabin’s tetrad (chorioretinitis, hydrocephaly, intracranial calcification, and neurological abnormalities) exists in 5% of newborns

infected with CT [135]. In even rarer instances, the infection can lead to death of the newborn. From those infants asymptomatic at birth, 85-90% develop symptoms later in life, either during childhood or in early adulthood. The most common clinical signs developed are retinal diseases (chorioretinitis) with slight or severely diminished vision, neurological disorders, or hearing loss. Neurological issues include intellectual disability, motor deficits, seizures, microcephaly, hydrocephalus, and more rarely Alzheimer's disease and schizophrenia. 20 to 30% of ocular and intracranial lesions are seen in the first three years of an infected child's life. Systemic signs of CT infection may also occur in patients, and include fever, skin rash, enlarged spleen and liver, icterus, anemia, lymphadenopathy, and irregular spinal fluid [13, 45, 46, 70, 104].

It is thought that the likely cause of clinical signs in the fetus or infant following CT is the inflammation and damage caused by the multiplying parasite in the developing fetus [37]. In fact, the proliferation results in destruction of the white matter and aqueduct of Sylvius, causing the most common cephalic clinical manifestations seen in the fetus [121]. However, the immunological response of the placenta to *T. gondii* infection seems to also have a role in causing the symptoms. Although interleukin-10 IL-10 and T-helper 2 Th-2 are the main cytokines responsible for the immune reaction, T-helper 1 Th-1 can also take part in the response by secreting interferon gamma IFN-γ, tumor necrosis factor alpha TNF-α and interleukin-6 IL-6 which cause host resistance to toxoplasmosis. The IFN-γ produced during pregnancy can cause abortions in mice and preeclampsia in pregnant women [35, 96].

4.1. Global prevalence, incidence, and disease burden of congenital toxoplasmosis

Toxoplasma gondii was discovered in 1908 in rodents and became clinically significant for humans in the 1920s when it was recognized to congenitally infect children and cause severe neurological diseases [36, 81]. Toxoplasmosis is found in every country and seropositivity rates in women can range from less than 10% to more than 90% [140]. The seroprevalence is influenced by geographical location and warmer humid climates, socio-economic status of the region, stray cat population, cultural practices regarding food consumption habits and prenatal education [3, 34, 56, 148]. Additionally, age and parity influence the risk of maternal infection. According to studies conducted in several countries, the infection rate by *T. gondii* increases by 7% with every one-year increase in a woman's age [99], meaning that exposure to the parasite and thus the seroprevalence is higher with aged mothers. By comparing *Toxoplasma* infection in first time mothers

and multigravida mothers, it is evident that seroprevalence is higher multiparous women compared to nulliparous women. The difference can be explained by the increase in age of the woman but may also be due to the fact that some mothers are less cautious with their second pregnancies [4, 92, 108, 146].

According to surveys conducted over the last two decades, high seroprevalence for *T. gondii* in women is predominant in Latin America and Africa, whereas moderate seroprevalence is seen in Central, Southern and Eastern Europe and the Gulf countries, while the lowest seroprevalence is found in Northern Europe, North America, and Southeast Asia [148]. Indeed, Central and Eastern Africa are considered to have highest incidence of toxoplasmosis in the world, with high endemic zones and prevalence reaching 80.3% and 85% respectively [3]. Similarly, the seroprevalence in South America is high and can vary from one region to another within the same country. For instance, the average incidence for toxoplasmosis in Brazil is 68.37% [131] with prevalence of 23% in upper-class areas and 84% in poor areas [124]. The high incidence rate in Central/Eastern Africa and South America is explained by the more virulent *T. gondii* strains (atypical strains) found in these regions [7]. Seroprevalence in Europe (excluding Northern Europe) is moderate, ranging between 23% and 46% [123]. In Arab countries (Arabian Peninsula, the Levant, the Nile River strip, North Africa), the average seroprevalence is between 40-50%, except for Egypt and Lebanon (72.61% and 82.95% respectively) [3, 100]. Finally, the lowest incidence of *T. gondii* is found in Northern Europe (between 10-19%), North America (13.2% in the United States of America), and East Asian countries (less than 10%) [12, 67, 123]. On the other hand, in countries where exposure to *T. gondii* is low, the susceptibility of infection for seronegative pregnant women is high. For example, in Northern Europe, susceptibility for toxoplasmosis in women is approximately 90%, and in Ireland where seroprevalence to *T. gondii* is only 25%, the susceptibility to get infected with the parasite during pregnancy is 75% [37]. This is important because unlike other women who have been previously infected with *T. gondii* and have developed antibodies to protect them and their child from congenital toxoplasmosis, seronegative women are more likely to get infected and thus cause fetal infection. Using Monte-Carlo simulations (a mathematical model), the proportion of women who seroconverted during pregnancy was calculated. It is estimated that the global rate of seroconversion for *Toxoplasma* during pregnancy is between 15.4 and 24.3% [53, 140].

The incidence of congenital toxoplasmosis in children is assessed either by confirming seropositivity at birth or by confirmed signs of the disease. According to the meta-analysis published by The Bulletin of the World Health Organization in 2013 (one of the leading public health journals in the world), the global incidence of congenital toxoplasmosis is 190100 cases per year (95% CI: 179 300–206 300). With regards to the global incidence of CT in infants, it is estimated that the approximate incidence rate is 1.5 cases of CT per 1000 live births [140] - Nevertheless, a wide inequality in the incidence of CT exists on a global scale. The incidence rate of congenital toxoplasmosis differs from 0.5 to 10 per 10000 live children in developed countries and varies between 1 to 10 per 1000 live children in developing/poor countries. For example, in the United States, the rate is 0.8/10000 births (500 to 5000 CT cases annually), 2.3/10000 in Australia, 3.4/10000 in the United Kingdom, and 4/10000 in Denmark. In eastern European countries, the incidence of CT is 1.6 per 1000 live births, and in Colombia the incidence rate is as high as 3.8/1000 births [3, 51, 53, 128].

The disease burden by CT in each region was estimated by The Bulletin of the World Health Organization by using the disability-adjusted life year (DALY) metric. DALY is calculated by adding the number of years lost on account of death (YLL) and the number of years lived with the disability (YLD). -These calculations were based on the most recent global burden of disease (GBD), where both sexes in the population were considered to have a life expectancy of 86 years [97]. The duration of all the sequalae caused by CT was considered as permanent, except some cases of chorioretinitis which appeared at 10 years of age on average [74]. Since the severity of sequelae increases according to the virulence of different *T. gondii* genotype (predominant in different parts of the world) [57], the disability weight (DW) factor between 0 and 1 was used to assess the severity of the disease and avoid having the incidence of the sequelae be greater than the total incidence. For example, in South America where a lot of cases of CT are caused by the atypical strain of *T. gondii*, two thirds of infants with CT develop severe chorioretinitis by 4 years of age, compared to the less severe chorioretinitis seen in only 1 out of 6 congenitally infected European children [46]. In addition, the most severe DW was assigned to the infant depending on the most severe sequalae presented from the different symptoms caused by the disease. For example, if a child reported both chorioretinitis and severe central nervous system signs, then the DW was given to the nervous system abnormalities. By making these adjustments when calculating the DALY of each region, and by using the calculated global annual incidence of CT (190100 annual

case, with approximately 1.5 cases of CT per live births), it was possible to determine the global burden of CT. Thus, the global disease burden by CT is 1.20 million DALYs (95% CI: 0.76-1.90) per year [140] (Table 1).

Table 1. Global incidence and burden of congenital toxoplasmosis by region in 2013 according to the World Health Organization.

Region	Incidence cases (95% CI)	Incidence[a] (95% CI)	DALYs (95% CI)
AFR D	26 500 (24 300–30 100)	2.0 (1.8–2.3)	171 500 (92 300–294 500)
AFR E	37 000 (33 900–41 000)	2.4 (2.2–2.5)	235 900 (129 600–379 000)
AMR A	2940 (2360–3540)	0.6 (0.5–0.8)	19 700 (14 100–26 700)
AMR B	15 300 (13 100–17 800)	1.8 (1.5–2.0)	105 300 (82 500–127 500)
AMR C	5077 (4225–6792)	3.4 (2.5–4.1)	35 000 (24 400–41 200)
EMR B	8450 (6950–9530)	2.5 (2.1–2.9)	53 900 (27 800–84 800)
EMR D	26 300 (21 200–31 200)	2.2 (1.7–2.6)	164 900 (84 600–277 800)
EUR A	2170 (1900–2896)	0.5 (0.4–0.6)	13 600 (7 508–23 400)
EUR B	5200 (4500–6090)	1.5 (1.3–1.7)	32 200 (17 500–54 700)
EUR C	4200 (3700–4800)	1.6 (1.4–1.8)	26 400 (14 400–42 700)
SEAR B	6430 (4240–8600)	1.3 (0.9–1.7)	40 300 (18 700–71 800)
SEAR D	25 400 (20 700–30 700)	0.8 (0.7–1.0)	158 300 (85 900–275 400)
WPR A	960 (720–1200)	0.6 (0.5–0.8)	5950 (2900–10 100)
WPR B	24 200 (20 500–28100)	1.1 (0.9–1.3)	154 700 (81 200–253 000)
Total	**190 100 (179 300–206 300)**	**1.5 (1.4–1.6)**	**1 200 000 (760 000–1 900 000)**

Legend: AFR, African Region; AMR, Region of the Americas; CI, confidence interval; DALY, disability-adjusted life year; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, South-East Asia Region; WPR, Western Pacific Region.
[a]Per 1000 live births.

4.2. Mode of infection of *Toxoplasma gondii* in pregnant women

As previously mentioned, a woman who has not been exposed to *T. gondii* and has not developed antibodies to fight against toxoplasmosis is likely to transmit the parasite to the fetus, if the infection occurs during pregnancy or right before conception. Thus, studying the different modes of infection of *T. gondii* in pregnant women helps better understand the occurrence of congenital toxoplasmosis in children. *Toxoplasma* infection in a pregnant woman takes place by oral route, either by ingesting *T. gondii* cysts founds in animal tissue, or through the ingestion of oocysts found in the environment. In both cases, the chance of consumption of the parasite increases with reduced personal hygiene in a woman, the insufficient care dedicated to food hygiene, inadequate pet cat care and the overall lack of knowledge concerning risk factors for

infection. For a long time, the primary cause of the *Toxoplasma* infection in women was thought to be cats, because felids are the only animals capable of shedding *T. gondii* oocysts in the environment. However, more recent studies have since revealed that cat encounter is not the primary cause of toxoplasmosis in pregnant women, but rather consumption of undercooked meat, tainted food, or contaminated water [19, 95].

4.2.1. Ingestion of cysts from raw or inadequately cooked infected meat

The major cause of toxoplasmosis in pregnant women and thus CT in fetuses is the consumption of raw or undercooked meat. Unwashed surfaces and utensils can also be contaminated by cysts if they are in contact with raw infected animal tissue. *Toxoplasma* cysts found in animal muscle tissue are resistant but can be eliminated by freezing meat to – 12°C before cooking and cooking meats until the internal temperature exceeds 67°C [19]. Unfortunately, these recommendations are often overlooked by women while cooking. A study conducted in Ireland in 2011 to determine and assess the risk factor and awareness in mothers with children affected with CT established that the most probable cause of infection of two-thirds of women evaluated was through contaminated food. In fact, none of these mothers had changed their habits of undercooking meat while pregnant. In addition, the study highlighted that many mothers mistook cured packaged meats as cooked, and therefore did not pay special attention while consuming the latter [37]. In accordance, a cross-sectional survey conducted among pregnant women in Saudi Arabia in 2015 confirmed that around 60 % of women continued to consume undercooked meat during their gestation. The same survey also reported that 69% of mothers would dine outside, where undercooked meat was often served (gyros, burgers, fast food) [34].

As previously discussed in section 3.2.2., the parasite can successfully infect all kinds of warm-blooded animals. This makes meat from various farm animals a very important source of food-borne *T. gondii* infection in pregnant women. In fact, the European Food Safety Authority EFSA has stated that the main sources of meat-borne toxoplasmosis are *T. gondii* contaminated small ruminants, pork, and beef meats [75].

Indeed, the largest risk of human infection from *T. gondii* among livestock is from mutton and lamb meat, since seropositive sheep tissue is usually infested with a high number of cysts [9, 29). Goat meat can also be a high source of infection especially in regions where caprine species are often consumed, such as Saudi Arabia, Ethiopia, and some parts of the United States [3, 31].

After small ruminants, the second largest source toxoplasmosis in humans is pork. Even though industrial farming and meat treatment (such as freezing and salting) has decreased the prevalence of *T. gondii* cysts in pigs [17, 66], a study conducted in the United States of America in 2012 estimated that consumption of pork was responsible for 41% of foodborne *Toxoplasma* infections [6]. In addition, it is customary to consume uncooked pork meats in Europe, particularly in Germany, France, and eastern European countries [37, 127]. The latter is a great source of infection in women especially when the source of the raw meats is animal-friendly reared pigs [58].

Although cattle are known to be the most resistant farm animal against *T. gondii* cyst formation, beef remains a frequent source of infection in women because of the higher probability of the meat being eaten raw or not fully cooked (for example in the case of steak and hamburgers), compared to chicken and pork. A quantitative study conducted in the Netherlands to assess the risk of meat-borne toxoplasmosis in humans showed that 67% of *Toxoplasma* infection was indeed from consumption of undercooked beef [75, 107].

The risk of women getting infected by chicken is not directly linked to the seropositivity rate of *T. gondii* in broilers. Raw chicken is often contaminated with several species of bacteria (*Campylobacter, Salmonella, Clostridium*) causing food poisoning, and is therefore cooked thoroughly both at home and in restaurants. This means that the chances of infection through *Toxoplasma* cysts in chicken tissue is low, given that the cysts do not survive high temperatures [54]. Although most poultry products are not consumed without being cooked, some parts of poultry are nowadays being used to produce sausages, which can be unknowingly consumed raw [127].

Other animal species such as horse, game (wild birds, wild boar, deer) and camels can also infect people [8]. In France for example, a severe *T. gondii* infection outbreak in humans was associated with the consumption of horse meat originating from Brazil and North America [115]. According to the EFSA, over 50% of game meat in Europe is contaminated with *T. gondii* cysts, whereas seropositivity for toxoplasmosis in the United States can vary between 9-70% [28, 75]. As a matter of fact, ingestion of improperly cooked venison has been tied to the most recent toxoplasmosis outbreaks worldwide [42, 129]. Finally, camels are a source of *Toxoplasma* infection in Arab countries, especially in Saudi Arabia where a study in Riyadh region in 2013 concluded that 23.6% of camelid produce were infected by the parasite [1].

4.2.2. Ingestion of oocysts from unwashed vegetables and gardening

Toxoplasma gondii oocysts are shed in the environment after passing the gastrointestinal tract of infected cats. Once in contact with oxygen, they sporulate in 1-5 days. The sporulated oocysts are highly resilient to harsh environmental conditions because they possess a very tough outer shell. They can survive in freezing and cold waters and can survive in soil for 18 months with temperatures rising as high as 36°C [65, 66]. Environmental resistance of the oocysts is thus essential because it guarantees their survival on raw fresh produce and soil for extended periods of time. The importance of *T. gondii* infection through soil is confirmed by the fact that true vegans can also become infected. Indeed, in one study done by the European Multicenter on pregnant women, 6-17% of toxoplasmosis cases were attributed to infection through the environment. The women were most likely exposed to the parasite eggs through unwashed vegetables and fruits, and/or thorough gardening [19]. Another study conducted in the United States on pregnant women using oocyst-specific antibody assay revealed that form 76 pregnant women who were recently infected with *T. gondii*, 78% of them had oocyst-specific antibodies [14]. These results are consistent with other studies conducted in China and Arab countries, where increased risk of infection during pregnancy was linked to consuming raw, unwashed vegetables and fruits [3, 79]. In fact, in a recent report on global human toxoplasmosis occurrence, it was noticed that since 2010, more and more outbreaks of *T. gondii* infections were through oocyst ingested by raw vegetables [114]. Furthermore, unsafe practices during gardening and farming increases the risk of toxoplasmosis in pregnant women. These unsafe practices include gardening without gloves followed by unsatisfactory washing of hands, especially in areas with increased number of stray cats [19, 37]. In a cross-sectional survey performed in Saudi Arabia on pregnant mothers, 49.5% of women admitted to being in contact with soil directly while gardening [34].

4.2.3. Ingestion of oocysts from contaminated water

Environmentally resistant oocysts do not only survive in soil, but also survive in water. In fact, the parasite thrives in humid climates because of its oocysts' adaptation to wet environments. The sporulated hard shelled form of the *T. gondii* oocysts ensures its survival in freezing waters of -21°C for 28 days and fresh cold waters for up to of 4°C for 54 months. Unsporulated oocysts can also survive in water in these temperatures. Aside from fresh water, survival of the oocysts has also been confirmed in seawater/salt

water for 24 months [65, 77]. Several outbreaks of toxoplasmosis in the 20th century were linked to contaminated waters, the two major cases being recorded in Panama in 1982 and Canada in 1995. In the Panamian outbreak, the people infected had used water from a pond that was treated with iodine tablets, and in the Canadian outbreak, the municipal water supply had been treated with chloramine disinfectants. In both waterborne incidences however, neither water source was filtered [17, 26]. It is thus evident from these small epidemics of waterborne toxoplasmosis, that unfiltered water, even if treated with chemicals, is a big source of toxoplasmosis with oocysts. This is particularly true in poorer regions, countries with humid climates and areas with high number of *T. gondii* infected stray cats. In Colombia for example, up to 50% recent outbreaks of CT can be explained by contaminated waters. This is also true in other South American countries [12, 50]. In addition, chlorination, and ozone treatment have been proven to be ineffective against oocyst elimination. The only promising treatments of potable water against *T. gondii* oocysts are therefore filtering and ultraviolet ray treatment [32, 127].

4.2.4. Ingestion of oocysts from raw sea mollusks

Even though *T. gondii* is a parasite that infects only warm-blooded animals, it can be found in cold-blooded creatures, such as filter-feeding invertebrates [26]. Clams, mussels, oysters, and other molluscan shellfish are filter-feeders that store phytoplankton in their gills. During this feeding process, these mollusks can trap other waterborne pathogens, such as *T. gondii* oocysts that can survive in both fresh and salt waters. Thus, waterbodies found in areas with high prevalence of *T. gondii* have shellfish with high concentrations of oocysts in their tissues [77]. Given their robust nature, the parasitic eggs can remain viable in the molluscan tissue between 3-85 days, depending on the species [5, 78]. Evidence of sporulation of the oocysts in shellfish was also found in a recent report conducted in New Zealand [22]. This makes filter-feeding Mollusca a great source of infection in aquatic mammals, but also humans. Indeed, if consumed raw or undercooked, contaminated shellfish can increase the risk of toxoplasmosis in women [75]. Although toxoplasmosis from shellfish is not the main mode of transmission of the disease, a case control study done in the United States from 2002-2007 considers consuming undercooked oysters, clams, and mussels as a foodborne risk factor of *T. gondii* infection [64].

4.2.5. Ingestion of oocysts from contaminated milk and cheese

Animals acutely infected by *T. gondii* are capable of shedding *Toxoplasma* tachyzoites in their milk. *T. gondii* have been identified in the raw milk of different intermediate hosts, such as ewes, nannies, and cows [134]. Tachyzoites are usually eliminated by pasteurization, but studies show that they can survive in raw milk for days. *T. gondii* tachyzoites are weak against the low gastric pH, however they can remain viable in gastric fluid for a minimum of an hour [73]. This means that consumption of raw unpasteurized milk and dairy products can cause infection in humans [75]. Although most outbreaks of toxoplasmosis in humans is linked to consumption of unpasteurized goat's milk or goat cheese made from raw milk sold by local farmers [66, 87, 125], sheep and cow milk remain a potential source of infection. In fact, a recent incidence of *T. gondii* disease in Brazil was linked to ingesting contaminated fresh artisan cheese made with cow milk [21].

4.2.6. Ingestion of oocyst through contact with domestic cats

Domestic cats are widely distributed throughout the world and are frequently in contact with humans either as strays or as pets. Indeed, one third of families in the United States own a cat, with a similar estimation in households in the United Kingdom [98, 144]. Cats are epidemiologically significant in toxoplasmosis because they play a pivotal role in transmission of the disease. As definitive hosts, once infected with *Toxoplasma*, they are capable of shedding millions of unsporulated *T. gondii* oocysts in their feces for 1 to 3 weeks. The oocysts sporulate between 1 to 5 days and become infective. After primary infection, cats usually develop an effective immune response which protects the animal from a new *T.* gondii infection. Thus, unless a cat's immunity is compromised, a healthy cat sheds oocysts only once and for a limited period time [25, 33]. It is at this point in the life cycle of the parasite that contact with cat feces can be considered a direct source of infection for women and infants as they can be responsible for congenital toxoplasmosis. Indeed, litter boxes and soil used by cats during defecation offer the conditions necessary for the survival of the oocysts, as they are warm and humid. In optimal settings, the sporulated oocysts can stay infective for over a year [127]. Although stray cats that hunt are predominantly accountable for the transmission of toxoplasmosis through oocyst contamination of soil and water, several studies have revealed that living with a cat is not a primary cause of *T. gondii* infection in women [56]. Indeed, several studies and reviews dating from the past two decades revealed that contact with cats of

all ages or cat litter has little to no effect on the *T. gondii* seroconversion of previously uninfected women [19, 29, 33, 34]. This is explained by the fact that the duration of oocyst excretion is short. The oocysts shed in the feces of the cat are not instantly infective, also significantly decreasing the risk of human infection through fecal matter of the cat, especially if cat litter is changed daily [141]. In addition, the diet and lifestyle of the pet also influences the cat's role in transmission of the disease. In a study done in the United States, North Carolina in 2004, a higher prevalence of *T. gondii* antibodies was found in pet cats having access to the outdoors and being fed raw food compared to cats who were strictly indoors and only ate commercial pet food [103]. Thus, an indoor cat does not pose a risk to pregnant women, especially if the woman takes the proper preventative measures while handling the cat's litter (wearing gloves and washing hands) [64]. In accordance, a study conducted in Ethiopia, where these sanitary measures towards cat feces disposal are not necessarily respected, revealed that the risk of *T. gondii* infection in pregnant women through a cat's litter box was five times higher [101, 147]. Finally, it is important to note that cats are very clean animals and can effectively remove dirt, including *T. gondii* oocysts, from their fur by grooming themselves. It is therefore improbable for pregnant women to get infected by the parasite through petting or touching a cat, whether it is a pet or a stray [25].

4.3. Diagnosis of congenital toxoplasmosis

Although toxoplasmosis in humans is not considered a life-threatening disease in healthy individuals, *T. gondii* infection in seronegative or immunosuppressed pregnant women can lead to the congenital transmission of the pathogen. Congenital toxoplasmosis can affect the fetus with varying degrees of severity and cause lifelong neurological and ocular diseases in infants, such as chorioretinitis, the development of hydrocephalus and intracranial calcification or microcephaly. The wide range of illnesses and fatality caused by CT has motivated public health professionals to develop CT prevention strategies; primary, secondary, and tertiary prevention measures. Primary prevention measures aim to decrease the incidence of CT by educating women and future mothers about the disease and stressing on the different risk factors causing it. Secondary prevention measures aim to diagnose pregnant women during acute *T. gondii* infection and set up a therapeutic regimen either to avoid or treat CT in the fetus. Tertiary prevention measures aim to diagnose and treat infants born with CT [19, 137]. Although these health measures are not uniformly adopted across countries [12], prenatal and neonatal diagnosis of CT

play a pivotal role in the prevention and management of the disease. Indeed, the timely diagnosis of the infection in mother or newborn is essential to ensure the woman or infant receive the proper medical treatment to decrease the burden of the illness.

4.3.1. Prenatal diagnosis

Secondary prevention (prenatal diagnosis) consists of screening either the pregnant woman or the fetus to detect CT prior parturition. Prenatal screening is routinely offered in some European countries like France and Italy where women are considered high risk for toxoplasmosis. In countries like the United States where women are not considered high risk, prenatal screening is possible in some regions but not mandatory [82, 84]. *Toxoplasma gondii* infection can be detected using serological testing, amniocentesis or by ultrasonography.

Serological testing is commonly the first step in diagnosis of CT [109], as it can be used to identify past or current toxoplasmosis in a woman. Serological testing uses the woman's humoral immunity to diagnose *Toxoplasma* infection. After the initiation of the mother's adaptive immune response, the humoral response produces IgG, IgM, IgA and IgE specific antibodies [89]. The serological test is a serum test that uses serum markers to identify the presence or absence of IgG and IgM. Indeed, the presence of both antibodies can indicate acute or chronic infection. Differentiating between acute and chronic infection can be challenging. Therefore, IgG and IgM titers are carefully analyzed to recognize primary infection in the mother. Serum antibodies can be analyzed using different methods such as IFAT, ELISA, and IgG specific Sabin-Feldman dye test. In terms of test sensitivity ELISA presents less false negatives compared to IFAT, whereas IFAT is more specific than ELISA (lower rate of false positives). Sabin-Feldman dye test is highly specific and sensitive but is more expensive, thus the test is used only if acute toxoplasmosis is suspected in the mother [17]. The IgM and IgG levels increase during the first two weeks of acute infection. IgG antibodies peak within 12 weeks to 6 months after infection and are present for life. Since IgG remains in the blood for a lifetime, the presence of the antibody can indicate either a new infection or previous infection, and thus the antibody is responsible for identifying the presence or absence of acquired *Toxoplasma* immunity in the woman. IgM antibodies are detected 1-2 weeks prior to IgG antibodies and indicate recent infection in a pregnant woman because they have a short lifespan. However, IgM antibodies can last for 8 to18 months after acquired infection, thus can persist much longer than the pregnancy [38, 95]. If the pregnant

woman tested is IgG and IgM negative, then she is not infected by *T. gondii* or was infected within the last few days. If she is IgG seropositive but IgM seronegative, then she was infected at least a year ago and is now immune to toxoplasmosis. Seropositive results for both IgG and IgM mean acute infection in the woman. Positive IgM and negative IgG is inconclusive and serological testing must be repeated. If IgG antibodies are detected a few weeks following the first detection of IgM antibodies, then the mother is infected with *T. gondii*. The possible overlap of IgG and IgM seropositivity due to the lifespan of IgM can make it difficult to distinguish infection during pregnancy from past infections. To circumvent the poor diagnostic specificity of serological testing, the serum of the pregnant woman is tested a second time within 2-3 weeks of suspicion of acute *T. gondii* infection. Sabin-Feldman dye test is used in this case, and a powerful increase in serum IgG (at least four-fold compared to initial levels) will confirm acute toxoplasmosis in the mother [95]. France for example, who is very well equipped in terms of serological testing, offers continuous testing for mothers throughout their pregnancy if they are seronegative. By this method, seroconversion can be diagnosed at any point during a woman's life: seropositive IgG and IgM indicates infection during pregnancy and seropositive IgG, but seronegative IgM, indicates infection between two pregnancies [12, 100]. In addition, *T. gondii*-specific IgG avidity test can help determine when the woman got infected with *T. gondii*. Indeed, by narrowing down the time of infection during pregnancy, the risk of fetal transmission can be evaluated (since the severity of CT is time sensitive), and corresponding treatment can be administered. Post exposure to the parasite, IgG antibodies possess a low average affinity. As the humoral immune response progresses, the B-cells will have a higher affinity to the *T. gondii* antigen (stronger binding strength). IgG avidity usually changes from low to high in 4-5 months, meaning that high IgG avidity antibodies indicate the infection happened at least 4 months prior diagnosis [41, 43, 76, 120].

Amniocentesis is usually suggested after confirmation of acute *T. gondii* infection in pregnant women to verify if the fetus is congenitally infected as well. However, amniocentesis can also be used in case serological testing of the mother is inconclusive or if early ultrasonography findings indicate abnormalities in the fetus. Amniotic CT detection in fetus is done by analyzing the amniotic fluid using PCR-assays to detect toxoplasma DNA [109]. Real-time PCR for example has a detection sensitivity of 98%, and a high specificity of almost 100%. Amniotic fluid PCR sensitivity varies depending on time of infection during the pregnancy: 33-75% in the first trimester, 80-

97% in the second trimester and 68-88% in the third [117]. Amniocentesis is offered at the earliest at 18 weeks gestation and is not usually completed before that due to the high rate of false positive results. In parallel, amniocentesis should be completed within the first 4 weeks after suspicion of primary maternal infection. It is important to note that 1% risk of miscarriage is associated with fetal CT diagnosis using amniocentesis. CT can also be diagnosed by detecting *T. gondii* IgM and IgA using cordocentesis (drawing fetal umbilical blood) and was once the gold standard of CT diagnostic testing in fetuses. Cordocentesis is nowadays no longer used because of the higher associated risk of premature termination of pregnancy [47, 56].

Fetal ultrasound is a less invasive diagnostic tool when a pregnant mother is diagnosed or thought to have primary *Toxoplasma* infection. CT can thus be suspected by the abnormal appearance of the fetus. CT infects several organ systems in the fetus, and various abnormalities might be seen on the ultrasound, notably hydrocephalus, ventriculomegaly, intracerebral calcification, enlarged spleen and liver, congenital nephrosis, and ascites [95]. In addition, a lower development rate of the fetus observed on the ultrasound can indicate potential *T. gondii* infection [69]. Placental tissue can also be affected, and placental abnormalities can be detected through ultrasound. Chorial plate and extra-placental membranes inflammation as well as focal villitis seen on the ultrasound can correspond to CT [80]. Although informative, fetal ultrasound does not offer a definitive diagnosis for CT, it remains a great tool for detection of abnormal growth of the fetus. Pregnant women routinely get two ultrasounds done during their gestation. By applying monthly fetal ultrasounds, the accuracy of the diagnostic instrument can increase [93].

4.3.2. Neonatal diagnosis

Neonatal diagnosis also known as tertiary prevention of CT is used to diagnose neonatal *T. gondii* infection by detecting *Toxoplasma* specific antibodies in the blood of the newborn. Neonatal screening is offered in Poland, Denmark, and in several states in the United States. The Guthrie card blood spots method is used to collect blood from the baby and is performed by using the special card to collect capillary blood from the newborn's heel [44]. Through fetal blood, maternal *T. gondii* specific IgG antibodies can be detected. Fetal IgA and IgM antibodies can also be identified, using ELISA and ISAGA respectively [93, 116]. Maternal toxoplasmosis and congenital toxoplasmosis can be discerned by repeating serological testing 10 days after birth, or by using western

blotting. Neonatal screening for *Toxoplasma* specific antibodies in the newborn is especially important if prenatal serological screening or amniocentesis is not performed. In addition, 90% of babies born with CT do not show any pathognomonic clinical signs at birth, and do not develop them until later in life. So, every child born to acutely infected toxoplasmosis should be tested for potential infection [113, 138]. Moreover, the parasite can be directly isolated from the neonate through PCR of the *T. gondii* specific DNA, by testing various biological samples such as urine and cerebrospinal fluid [104]. Finally, clinical examination can diagnose non asymptomatic newborns with CT. Indeed, the classic symptoms chorioretinitis, hydrocephaly, intracranial calcification, and neurological abnormalities exist in 5% of newborns infected with CT [35].

4.4. Treatment of congenital toxoplasmosis

In the previous paragraph, the diagnostic aspect of secondary and tertiary prevention of CT was discussed. Another important aspect of CT prevention is timely treatment. Parents may choose to have the pregnancy terminated or to receive pharmacological therapy if maternal or fetal infection is confirmed. To date, CT is not a curable disease, however it can be treated. The drug therapy protocol and molecules used change according to the presence or absence of fetal infection but also according to the gestational age. Prenatal treatment prescribes either spiramycin or pyrimethamine + sulfadizine combination. Each drug has the potential to kill *T. gondii* tachyzoites but cannot destroy existing bradyzoite cysts [49].

If acute infection in the mother is confirmed but fetal infection is not detected, spiramycin is prescribed to the mother if the pregnancy is below 18 weeks gestation. The dose administered is 1 g PO TID. Sipramycin is a macrolide antibiotic and can reach high concentrations in the placenta without crossing the blood-placental barrier. It is therefore recommended in cases where fetal infection has yet to occur, because in can prevent congenital transmission of *T. gondii* parasite to the fetus. Additionally, spiramycin is not considered toxic and is therefore safe to use during the first trimester. The antibiotic is frequently used as therapy while waiting for the results of the diagnostic tests to confirm or exclude prenatal transmission. In some treatment plans, spiramycin is continued even if CT detection tests show negative results, as prophylaxis to seroconversion in the fetus [49, 95, 118].

If *T. gondii* is confirmed in the fetus or is strongly suspected, then pyrimethamine with folic acid (vitamin B9) and sulfadizine combination is recommended in pregnancies

over 18 weeks gestation. The dose administered for pyrimethamine is 2 mg/kg PO SID the first two days, then 1 mg/kg PO SID for 2-6 weeks, then 1 mg/kg PO SID three times a week. The folic acid treatment is 10 mg PO SID three times a week and is prescribed for a minimal duration of 12 months. The dose for sulfadiazine is 50 mg/kg PO BID. Sulfadiazine is a sulfonamide antibiotic used to amplify the effect of pyrimethamine. Pyrimethamine is an antiparasitic drug capable of crossing the blood-placental barrier and prevents the multiplication of *T. gondii* in the cells of the fetus. Due to its teratogenic properties, pyrimethamine is not used during the first trimester. It also causes a reversible, dose-related suppression of the bone marrow (as well as disorders of the skin and gastrointestinal tract) and thus is coupled with folic acid. The hematological toxicity of pyrimethamine is inhibited by the B9 supplementation. Additionally, complete blood counts must be maintained during therapy due to the serious adverse effects of the pharmaceutical regimen [20, 49, 95].

Neonatal treatment for CT is similar to the protocol recommended for the treatment of congenitally infected fetus. Pyrimethamine and sulfadiazine is administered for at least the first year of the infected infant's life. Steroids may also be helpful in extreme situations to reduce inflammation in the head and eyes [95, 111].

The effectiveness of the treatment protocol for CT is debatable. Several studies conducted on the treatment program in France (screening of the mother, in utero diagnosis and drug administration) have revealed a reduction in the severity of the disease [55]. Other individual reports have uncovered that spiramycin reduces the rate of congenital transmission of *T. gondii* in the first trimester [49], while the pyrimethamine and sulfadiazine together significantly lessen the severity of CT illness [86]. According to long term studies conducted on ill individuals who have been treated for CT, their ability to see compared to sane individuals was not lost [111]. On the other hand, a Cochrane Review of 3332 studies published in the last 30 years and released at the turn of the century concluded that prenatal therapy, when seroconversion is detected during pregnancy, does not lessen the likelihood of transmission but may lessen the severity of congenital toxoplasmosis [112]. Likewise, a low correlation between early treatment and a lower incidence of congenital toxoplasmosis has been established by the SYROCOT (Systematic Review on Congenital Toxoplasmosis) research group in 2007 [135].

5. Primary prevention of congenital toxoplasmosis

Although secondary and tertiary prevention programs have shown some promising results, both prenatal and neonatal diagnosis and treatment of CT have been faced with several challenges. Prenatal screening is often expensive and not always reliable as false positive results are common while detecting *T. gondii* specific IgM antibodies. IgG antibody false positive results are also possible but less frequent [94]. Amniocentesis has a high sensitivity to *Toxoplasma* DNA, but the sensitivity can decrease according to the time of gestation. Moreover, amniotic fluid testing can cause fetal loss. As previously mentioned, there is little proof that prenatal treatment decreases parasite transmission in the fetus or reduces the severity of the clinical signs. Neonatal screening of an infant by detecting either *T. gondii* DNA or *T. gondii* specific antibodies is altogether considered safer and cheaper than prenatal prophylaxis. However, it is proven that PCR testing in the infant through any fluid including serum has low sensitivity to *T. gondii*. The results of neonatal treatment of CT also depends on the severity of the disease in the infant and is thus unsuccessful against very serious clinical manifestations [47, 48].

Primary prevention of congenital toxoplasmosis includes many methods. It involves the general population, especially women, and requires the expertise of both health professionals and veterinarians. Stricter control by official veterinarians on *T. gondii* incidence in food hygiene for example can reduce the risk of acquiring toxoplasmosis during pregnancy [75]. Water quality control by the sanitary department can also eliminate protozoan contamination [83]. With the advancement of genetic engineering technologies, developing a vaccine to combat toxoplasmosis has also become more realistic [89]. Setting guidelines to help women avoid exposure to *T. gondii* during pregnancy through educational programs and during pregnancy checkups can successfully prevent CT in infants [47]. Primary prevention, if effective, can thus be the best strategy to decrease CT, because it will omit the dependency on secondary and tertiary prevention methods, which have not been proven to be 100% effective.

5.1. Prenatal education

Educating women of reproductive age about *T. gondii* can aid them better understand and steer clear of the disease. Indeed, since the sources for toxoplasmosis are well established, teaching mothers to avoid the risk factors during gestation can be the best approach to prevent CT. This education can be offered by certified medical professionals, such as gynecologists, nurses, midwifes and veterinarians. Unfortunately, no universal

program exists today, and prenatal education on CT is not offered in many countries. Ideally, education should begin before the woman gets pregnant and thus be part of preconception care, alongside reproductive history, and genetic screening [109]. In fact, in some countries where prenatal screening for *Toxoplasma*--specific antibodies is offered very early in the pregnancy or even prior to it, the physician can be firmer with the guidelines if the woman is seronegative. The goal is to ensure that women possess the correct behavior towards food, water, personal hygiene, and pet cat care before and during gestation [84].

5.1.1. Dietary, hygienic, and pet cat care recommendations

Foodborne CT is the primary mode of acquisition of the disease. Women should not consume raw or undercooked meat. *Toxoplasma gondii* cysts found in animal tissue are very resistant but can be eliminated with adequate cooking temperatures and rest time. Whole cuts of lamb, pork, veal, and beef should be cooked to internal temperatures of 65.5°C and rest time should be 3 minutes. Wild game and ground meat should be cooked to internal temperatures of 71.1°C or higher and poultry to 73.9°C. Resting time is not necessary for wild game and poultry. [66]. Microwaving meat does not kill cysts. For extra safety, tissue cysts can be killed by freezing meat to internal temperatures of - 12°C for at least 2 days. Woman during pregnancy should be encouraged to buy frozen meat instead of fresh [17]. Cross contamination of cooking surfaces with *T. gondii* cysts can also occur, so cleaning utensils with hot soapy water after each usage is recommended. Woman should be informed that sausages and cold cuts which undergo salting, curing, and smoking do not necessarily mean devoid of cysts and should therefore avoid consuming them raw [90]. Mothers should also be careful while purchasing meat as free-range produce can have a higher incidence of cyst contamination.

Shellfish, such as oysters, mussels, and clams should not be eaten raw because they can be contaminated with oocysts. Raw or unpasteurized milk or dairy products should also not be consumed since tachyzoites can be present. If possible, women should not buy artisanal dairy products as they can cause toxoplasmosis. Vegetables and fruit can also carry *T. gondii* oocysts from the soil and thus should be thoroughly washed before consumption. It is preferable for women not to consume raw vegetables and fruit and to have them peeled or cooked [2]. Although there is no evidence of poultry eggs being contaminated with the parasite, raw eggs should not be consumed [109].

Water and soil are also a great source of infection for pregnant women. Water directly from wells or natural springs especially in areas with high cat density can be contaminated with oocysts and therefore women should not drink from these sources. In addition, only chemically treated water does not eliminate the risk potential of infection. Mothers should either have filtered water or water from plastic bottles. In developing countries, tap water is not recommended. Women should not participate in gardening and farming, or any activity related to soil unless they are wearing gloves and should thoroughly wash their hands afterwards [2, 26].

Finally, pregnant women who live with cats should be given list of guidelines to avoid CT. Although many health professionals advise women to rehome their cats, it is evident that this practice is unnecessary if proper guidance regarding pet care is offered and followed. Domestic cats who live strictly indoors have a very low chance of getting infected by *T. gondii* and thus the probability of them shedding oocysts in their feces during a woman's pregnancy is almost nil. However, to secure the absence of shedding, both gynecologists and veterinarians should advise owners to keep their cat indoors and feed it preserved dry food. Raw foods should not be given to the cat. More importantly, pregnant women cleaning the litter box should wear gloves and wash their hands carefully after disposal of feces. The feces from the litter box should be collected minimum once a day, as oocysts shed require at least 24 hours to become infectious. If possible, it is recommended that someone other than the mother clean the litter box during the pregnancy [33].

5.1.2. Outcome of prenatal education

Since prenatal education is not necessarily offered or uniform across the globe, little evidence exists on its effectivity in reducing CT. Moreover, only a few research have been conducted to assess the influence of education on the quality of life of women during pregnancy [84].

One of the first studies on the usefulness of prenatal education programs for CT prevention was done in 1989, by the Canadian National Health Department. This study concerned 432 pregnant women who were separated into 2 groups: the first group received 10-minute presentation about the effects of proper food, pet cat and personal hygiene on avoiding CT during the first prenatal session, and the second group received prenatal sessions without any information about CT. Before the beginning of the experiment, all women participating were given a questionnaire to fill out about different

important topics concerning pregnancy (including CT). At the end of the experiment (after roughly 6 months), the same questionnaire was refilled. Based on the comparison of the pre-study and post-study tests, a behavior change scoring system was used to evaluate the change in behavior of the women. The group having received information about CT showed better behavior towards pet hygiene compared to the control group. A positive change of behavior in the case class was also seen regarding fully cooking beef products (steak, roast, hamburgers). No significant change was noticed in terms of personal hygiene. Furthermore, as little as 5% of participants recalled where they obtained CT primary prevention from. No data was presented on the impact this study had on the CT seroconversion rate during pregnancy [16].

Another similar but more recent and rigorous study in Belgium was conducted over a period of 23 years (from 1979 to 2001). All women participating in the experiment were serologically tested for *T. gondii* specific antibodies, and seronegative pregnant women were retested during their gestation to detect seroconversion. The duration of the study was divided into 3 chronological periods; the first group (1979-1982) was the control, the second group (1983-1990) received a list of recommendations to avoid CT during every antenatal class, and the third group (1991-2001) was given leaflets educating the women on the parasite and guiding on toxoplasmosis acquisition prevention (these guidelines were repeated at every antenatal class). The control group had a seroconversion rate of 1.43%, whereas groups 2 and 3 had much lower rates, 0.53% and 0,09% respectively [15]. Compared to the previous study conducted in Canada, this research shows the positive impact prenatal education has on not only the behavior of the woman regarding CT prevention, but also in the reduction of the incidence of the disease. In addition, in order to achieve positive behavior change in women, prenatal education should be repeated by specialists and information on CT should be offered in forms of presentations, leaflets or brochures.

5.2. Control of meat borne transmission

Foodborne toxoplasmosis is the primary source of *T. gondii* infection in humans, including pregnant women. Tissue cysts in particular play a great role in the spreading the disease. The incidence and relevance of *T. gondii* infection through the consumption of food producing animals was discussed at length in paragraph 3.2.2. and 4.2.1. Despite not being a notifiable disease in countries, after listeriosis and salmonellosis, toxoplasmosis is the third most common infectious cause of food related death. Although

most the human population is asymptomatic if infected with contaminated meat, immunocompromised individuals and pregnant women are considered high risk, therefore control of the parasite's incidence in meat during inspection by veterinary officials can be very effective in terms of primary prevention.

Currently, there are no control methods for *T. gondii* identification while carcass inspection is performed in slaughterhouses. Tissue cysts in tissues of diseased animals are not visible during visual ante- and post-mortem examination meat inspection. The cysts can be identified by microscopy, but microscopes are rarely used to detect *Toxoplasma* cysts. In fact, the most common techniques for direct detection of tissue cysts are mouse bioassays and PCR. Contrary to PCR-based approaches, the bioassays have the benefit of being able to identify live and infectious T. gondii, but they also have the disadvantage of being costly and requiring the use of experimental animals. However, for detection of cysts through any of these methods to be feasible, high-risk farms should be identified by large-scale screening of food producing animals and thus only focus on those establishments with moderate to high seropositivity. This can be especially useful for free-range farms, or for enclosures with highly susceptible animals like swine and small ruminants [75, 105, 106].

Eliminating the risk of toxoplasmosis in food producing animals can be effective to prevent infection of humans through meat consumption. Several animal hygiene measures can be recommended by the veterinarian, such as: keeping livestock inside; preventing access to unfiltered surface water; preventing cats from being near farms (especially near feed and bedding) and putting in place effective rodent control. It is important to mention that the success of these measures depends on the housing system of the farm, and that outdoor animals will not benefit as much as those indoors. For this reason, vaccination against toxoplasmosis could be a good solution. Unfortunately, only one vaccine is currently commercially available and is not used in reducing tissue cysts in food animals (Toxovax^{TM}) and will be discussed in the following paragraph [105, 126].

5.3. Vaccination against toxoplasmosis

Vaccination is a strategy that might significantly decrease the transmission of *T. gondii* and therefore limit the impact of congenital toxoplasmosis. Most warm-blooded animals, including humans, establish strong immune responses against the parasite after infection, which is generally enough to prevent illness in the future [59].- The impact of

toxoplasmosis is high in immunocompromised patients and fetuses, both in humans and other animals. Since no cure currently exists, developing a vaccine to prevent infection and control the spreading of the disease has become a great center of interest. Indeed, toxoplasmosis is a zoonotic disease and is thus considered a One Health issue. “One Health is a collaborative transdisciplinary approach” [150] between physicians and veterinarians that strives to attain optimal health outcomes for people and animals that are faced with a common problem. The three main goals of a One Health vaccination strategy against toxoplasmosis would be: the prevention of congenital toxoplasmosis in both humans and livestock animals; the prevention of *Toxoplasma gondii* cysts and the prevention of oocyst shedding in cats [61].

Host immunity plays a very important role in the effectivity of the *T. gondii* vaccine. The innate immune response is triggered immediately in response to the parasitic invasion of the host cell, followed by an adaptive immune response that presents an antigen capable of activating T and B cell specific response [89]. Most of the time, the immune response can suppress the acute phase of the infection and prevent reinfection but is unable to remove the tissue cysts from infected animals or oocyst shedding. Over the past three decades, different methods of experimental toxoplasmosis infection and immunity acquisition have been tested on animals, especially mice. Inactivated, live-attenuated, recombinant and subunit vaccines have been researched. Vaccinating mice with a *T. gondii* essential protein (subunit vaccine) initiated immune response and decreased cyst burden while increases survival rate [72]. However, this response was not observed in humans and a similar type of vaccine did not decrease the disease burden in sheep. Consequently, research data acquired by mouse models cannot be effectively applied to food-producing animals, cats, and humans [133, 139]. Several studies proved that phagocytosis of live tachyzoites by phagocytic cells is essential for immune response stimulation in humans, cats, and livestock. Indeed, inactive parasites did not produce inflammatory response [130]. Recombinant live-attenuated vaccines are therefore the most promising in terms of prophylaxis. Unfortunately, there are safety concerns surrounding administration of live vaccines. Hence, developing modified live vaccines that can be phagocytosed by the host but do not cause illness are the new focus of attention [60].

The goal of vaccinating females of reproductive age is to reduce transplacental transmission of tachyzoites in pregnant women and small ruminants. The only commercialized vaccine in the world against *T. gondii* parasite is Toxovax^{TM}. It used to

protect against CT in sheep in some European countries and New Zealand since abortion swarms are common in these animals. Toxovax™ is a live vaccine based on the S48 *Toxoplasma*--strain that is incapable of multiplying in the organism as bradyzoites. Thus, the animal develops an immunity against toxoplasmosis without being a risk for disease spreading. The vaccine is given to ewes 3 weeks prior mating and offers lifelong immunity. The disadvantage of the vaccine is its short shelf life as the tachyzoites in the live vaccine survive for only a few weeks. Administration of Toxovax™ should thus be punctual to be effective [60]. MIC 1-3 KO *T. gondii* is a genetically modified RH-strain of the parasite where the genes coding for the proteins responsible for host cell adhesion (MIC1 and MIC3) are deleted. It is not available as a vaccine but can prevent abortions in ewes as efficiently as Toxovax™ [88]. Regarding human vaccination against CT, administration of attenuated vaccines in women is considered risky. In recent years, immunogenic peptides of T cell and B cell epitopes of *T. gondii* from different phases have piqued interest as they can be used to produce subunit vaccines, also known as immunosense epitope-based vaccines. Thus far, only a handful of epitopes have been identified from *T. gondii* seropositive humans, but the few research conducted on MHC1 – HLA transgenic mice have discovered the activation of protective immunity through the peptides [11].

Vaccinating food-producing animals against *T. gondii* can potentially eliminate cyst formation in animal tissue. Meat would therefore no longer be the main source of toxoplasmosis and would be safer to consume for both humans and other carnivores (especially cats). Data on farm animal vaccination for food hygiene purposes exists mostly for animals at high risk of infection, thus mostly sheep, goats, and pigs. As previously mentioned, the S48 *T. gondii*--strain has a good immune response in sheep and can reduce tissue cysts in the animal through live vaccination but is however only used in ewes to prevent abortions. The S48 vaccine is also a licensed product in goats for abortions but has not been evaluated for its ability to reduce cysts in goat tissue [71]. In swine, both the RH-strain and S48 strain are effective against tissue cyst formation. Live vaccines for pigs against toxoplasmosis are not marketed due to their reduced shelf life, safety issues and the difficulty in obtaining the vaccine in large quantities. An inactive vaccine was therefore developed and tested in pigs in Brazil in 2003 using lysate of the parasite's tachyzoite antigens and immune stimulating complexes ISCOM as an adjuvant. A good humoral response was detected alongside reduction in tissue cyst development after three vaccine administrations [39].

Finally, a vaccine capable of decreasing oocyst shedding in cats would be very beneficial because felines are the only definitive hosts of the parasite. Thus, by reducing environmental contamination with oocysts, the risk of infection in intermediate hosts (food-producing animals and humans) is minimized. The most promising vaccine is the T-263 live bradyzoite vaccine, a chemically engineered mutant *T. gondii*--strain. The live tissue cysts of T-263 administered orally significantly reduced oocyst shedding in feces after one administration and complete protection after two administrations [40]. A 2-year field trial on a pig farm in the United States in 1997-1999 evaluated the effectiveness of T-263 vaccine in cats in terms of reducing the seropositivity in pigs. The *per os* vaccination of cats on the farm considerably decreased the prevalence of toxoplasmosis in the finishing pigs [85]. There are several disadvantages of this vaccination protocol. For instance, the oral vaccine does not guarantee immunity against non-homologous strains of *T. gondii*. Additionally, it is difficult to produce bradyzoite vaccine in large scale because unlike tachyzoites, bradyzoites are tricky to produce in vitro, and require live infected mice for cultivation. Establishing a substitute system for animal models is therefore a significant problem in medicine. Furthermore, conserving cultivated bradyzoites requires freezing in liquid nitrogen. The vaccine needs to be thawed 15 minutes before use. Thus, T-263 is not very practical and has not become commercialized despite the benefits [62]. Another vaccine tested is the FHV-ROP2 subunit vaccine, that uses a genetically modified immune response inducing *T. gondii* rhoptry protein (Rop2). Although the vaccine did not successfully prevent oocyst shedding in the cat [91], a different subunit vaccine using crude rhoptry proteins showed 98% reduction in oocyst excretion after four doses were administered intranasally [149]. More recently, a new strain of *T. gondii* designed by deleting HAP2 protein. The mutant parasite was incapable of producing sporozoites in its oocysts. By orally administering the attenuated vaccine, cats immunized did not shed any oocysts, even when exposed to wild-type *T. gondii*. HAP2-deficient vaccines have thus the potential to immunize felines against more than one type of the parasite [119].

6. Discussion and conclusion

Congenital toxoplasmosis in humans may not be a notifiable illness, but the infection can severely impact the quality of life of infants and can be fatal in fetuses. Even though the disease has been recognized for almost a century and has been studied extensively, congenital toxoplasmosis remains a public health concern since outbreaks are frequent,

especially in developing countries in the Middle East and Latin America where the disease is not managed adequately. Despite its serious nature, toxoplasmosis in pregnant women and infants is preventable. To date, no cure exists to treat infected infants. Health professionals have thus developed prevention protocols to reduce the risk of *T. gondii* acquisition by the fetus. The goal of primary prevention is to educate women about the parasite and to teach them to avoid getting infected while pregnant. Secondary and tertiary prevention is time sensitive and consists of screening both women and fetuses for the presence of *T. gondii*. The sooner the infection is identified, the sooner the mother can begin treatment to reduce the placental transmission of *T. gondii*. However, in many countries, these prevention methods are not obligatory and therefore are not offered. Indeed, in Colombia where the 40% of women are neither tested nor treated for congenital toxoplasmosis, the disease burden is one of the highest in the world [52]. In contrast, in France, a country with moderate incidence of toxoplasmosis, primary and secondary prevention of CT is mandatory, and women receive prenatal screening monthly. As a result, a steady decrease of *T.* gondii seropositivity in women of reproductive age (43.8% in 2003 and 27% in 2020) is observed [102].

It is important to understand that not every country requires equal amount of scrutiny while managing congenital toxoplasmosis because some countries are not high risk. Antenatal screening, amniocentesis, and *T. gondii* detection through PCR (secondary and tertiary prevention), although effective at preventing CT, are expensive and time consuming. The unfortunate reality is that many countries do not possess the resources to combat this parasitosis or choose to spend their resources on more common illnesses. It is for this matter that primary prevention methods should be given more weight, as its cost is lower compared to the other prophylactic methods. While a vaccine against CT in humans can one day be achievable, an enormous amount of data already exists on the mode of infection of toxoplasmosis in humans. Obstetricians and veterinarians, with the right amount of knowledge, can reduce the transmission of the disease by educating their clients of childbearing age about the different risks of contracting toxoplasmosis. A nationwide survey of obstetricians-gynecologists conducted in the United States in 2012 about toxoplasmosis uncovered that many specialists did in fact not know all the different risk factors for infection. Even though most were adequately aware of the measures needed to be taken in pregnant women or infants in case of detection of *T.* gondii (additional diagnostic tests, treatment), preventative guidelines were subpar. In fact, handling cat litter was considered the

greatest source of infection, followed by undercooked meat. Often-missed risk factors were gardening without gloves, consuming unwashed vegetables, drinking unfiltered water, and consuming raw seafood [23]. Therefore, regular training of healthcare workers to update their knowledge on toxoplasmosis is needed so that women receive accurate information. A multidisciplinary team approach when educating patients can also be beneficial. Where physicians' knowledge is lacking, veterinarians can compensate with their deeper understating of *T. gondii* parasite. In fact, the collaboration between human and animal health professionals can begin with inter-professional learning during university. In 2018, the veterinary and medical students at University of California, Davis, participated in Inter-Professional Health Education program about congenital toxoplasmosis. Students had studied about the disease in their respective fields and were asked to solve cases together. While veterinary students had higher understanding of the transmission, risk factors, and pathophysiology of T. gondii infection, medical students were more informed in the medical history and treatment (family history, drugs, etc.). By the end of the study, students from both faculties had improved knowledge on the prevention and control of CT and could offer better guidance in terms of women's habits and cat care [145]. This case-study is interesting because its success opens doors to future larger scale collaborations between physicians and veterinarians in terms of managing congenital toxoplasmosis. Both veterinarians' and obstetricians' clinics are most likely the first location where women will learn about congenital toxoplasmosis. Therefore, a good flow of information between the two practices can offer women more consistent counseling on the prevention of the disease in both humans and pets.

In the future, it would be interesting to conduct more surveys evaluating the knowledge on CT in health care professionals and veterinarians, especially in countries where prenatal education is poor and seroprevalence is of toxoplasmosis in women is high (Latin America, Middle East). The results of these surveys can be utilized to aid in the development of continuous education programs against the risks of congenital toxoplasmosis for both medical workers and the general population. Prenatal education programs and the implementation of a unified approach of patient education can one day hopefully dispel the common misconceptions about owning cats and congenital toxoplasmosis in women of childbearing age.

Summary

Toxoplasmosis is an illness caused by *Toxoplasma gondii*, a ubiquitous parasite capable of infecting all warm-blooded animals through its complex lifecycle. Felids are responsible for the propagation of the parasite as they are the only family capable of passing very resistant oocysts through their feces. Intermediate hosts such as livestock become infected by ingesting the oocysts and develop *T. gondii* cysts in their tissues. Susceptibility is highest in sheep, goats, and pigs, followed by game and poultry. Cattle are considered resistant species. Farm-animals become reservoirs for the disease after infection.

Even though toxoplasmosis is generally asymptomatic, it can cause ocular and neurological diseases in immunocompromised patients and congenital disorders if infection occurs during gestation. Congenital toxoplasmosis in humans may not be a notifiable illness, but the infection can severely impact the quality of life of infants and can be fatal in fetuses. The global annual incidence of congenital toxoplasmosis is 190 100 cases per year, with a rate of 1.5 cases per 1000 live births, and a disease burden of 1.20 million DALYs (disability-adjusted life year). Seronegative pregnant women are at risk of infecting the fetus through vertical transmission. The primary modes of infection of pregnant women are through consumption of contaminated meat, uncooked seafood and water followed by contact with soil and cat feces.

Since congenital toxoplasmosis is not curable, prevention strategies to manage the disease have been developed: primary prevention measures (educating women of childbearing age about the risks of toxoplasmosis acquisition during pregnancy, developing vaccines), secondary prevention measures (prenatal diagnosis and treatment of congenital toxoplasmosis), and tertiary prevention measures (postnatal diagnosis and treatment of congenital toxoplasmosis). Secondary and tertiary prevention methods are useful but face several challenges due to lack of specificity and sensitivity of screenings, contradicting data on drug therapy and lack of availably of prophylactic methods in many countries due to high costs. Primary prevention in the form of education is thus the most reliable method of prophylaxis if taught correctly by health professionals, as vaccines against congenital toxoplasmosis in humans are still not available.

Finally, multidisciplinary team approach between veterinarians and physicians can fortify primary education programs and can become the key to fighting against congenital toxoplasmosis in human infants.

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