PREVALENCE AND KNOWLEDGE OF RISK FACTORS OF TOXOPLASMOSIS INFECTION AMONG PEOPLE LIVING WITH HIV IN IBADAN, NIGERIA

By

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CERTIFICATION

I certify that this work was carried out by Yusuf Opeyemi OGUNSOLA in the Department of Public Health, Faculty of Basic Medical and Allied Science, Lead City University Ibadan, under my supervision.

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DEDICATION

I dedicate this project to the glory of God Almighty and advancement of Science in humanity.

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ABSTRACT

The causative agent of Toxoplasmosis, *Toxoplasma gondii* still has low mortality rate among adults but its destructive effects on People Living with HIV include Encephalitis, chorioretinitis, mental retardation and eye blindness, and this cannot be overemphasized. Recent information on prevalence among adult and devastating effects of this parasitic infection on the People Living with HIV is still lacking in Ibadan, Nigeria. In view of the above, this study was therefore designed to determine the prevalence of anti-Toxoplama IgG, risk factors of Toxoplasma infection and knowledge of Toxoplasmosis among People Living with HIV in Ibadan, Nigeria.

This descriptive cross-sectional study examined three hundred and sixty eight People Living with HIV selected from PEPFER Clinics in eleven local government areas of Ibadan using multi-stage sampling method. 2ml of blood was collected from each participant. A structured interviewer administered questionnaire was used to collect participants' socio-demographic characteristics, risk factors associated with Toxoplasma infection and knowledge of People Living with HIV about Toxoplasmosis. Individual blood serum was screened for anti-Toxoplasma IgG using Enzyme Linked Immunoassay. Exposures to Toxoplasma infection was defined as a score of ≥1 out of sum two or more factors scoring 1 point for each. Data were analysed using descriptive statistics and Chi square, *P*-value = 0.05.

The mean age of the participant was 39.76 (±12.38) years. Toxoplasma IgG seroprevalence was 22.8%. The seroprevalence was highest (32.3%) among participants age of 41-50 years, with regards to those that were positive 48 (21.2%) were married, 44 (23.4%) had secondary education, 20(16.1%) engaged in market business women, 64(21.3%) are Yorubas, 12 (30.0%) already had more than three children, 64(30.2%) were from monogamous family, 20 (15.6%) practiced Islamic faith, with regards to those that were positive 12 (12%) had habit of eating washed vegetables. Among the People Living with HIV, there was significant association between Toxoplasma IgG seroprevalence and type of family. eating asun, eating vegetable salad, the type of toilet used, contact with cats and the unit of blood ever received for blood transfusion, *P*< 0.05.

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The odds is 0.374 time lower among those that have ever received a unit of blood than those that have ever received more than one unit of blood, and are statistically significant at $p < 0.05$ (OR = 0.374; CI: 0.169 – 0.830).

None of the People Living with HIV had knowledge of Toxoplasmosis: its route of transmission, signs and symptoms, it's effects ontheir lifestyle, effects on immune status and the way to prevent or avoid the infection.

Seroprevalence of Toxoplasma IgG was associated with consumption of vegetable salad, cat possession, family type, serial blood transfusion and consumption of "asun". The participants did not know anything about Toxoplasmosis. Health education and screening for Toxoplasmosis among People Living with HIV is recommended..

Keywords: *Toxoplasma gondii*, people living with HIV, cat possession, vegetable salads, Enzyme Linked ImmunoAssay

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CHAPTER ONE

INTRODUCTION

Toxoplasmosis is an important parasitic infection with a cosmopolitan distribution and significant global impact. It is caused by intracellular protozoa *Toxoplasma gondii (T. gondii)*, which is one of the most common parasites of human being worldwide, having approximately one third of the world's population at risk (Tenter*et al*., 2000). *Toxoplasma gondii* is a zoonotic, obligate intracellular protozoan parasite that has the ability to infect all warm blooded animals, it does not cause clinical illness in the majority of animal species that it infects, but it causes acute life-threatening disease in some, particularly sheep, goats and pig, it manifests itself as a disease of pregnancy by multiplying in the placenta and fetus. Acute, potentially fatal, infections are recorded in New World monkeys (Cunningham *et al*, 1992).

Toxoplasma gondii has a complex life cycle, the asexual reproduction takes place in diverse tissues of mammals and birds (secondary hosts) and sexual reproduction takes place in digestive epithelium of cats (primary host). The genus name '*Toxoplasma*' is derived from the Greek word 'toxon' meaning bow, which describes the crescentric shape of its tachyzoite stage, and the species name '*gondii*' is derived from the rodent, from which it was first isolated in 1908 (Markus, 2003). The existence of *T. gondii* was first established in North African rodents named *Ctenodactylus gondii*. The isolation was done by Nicolle and Manceaux while conducting lieshmaniasis research at the Pasteur Institute in Tunisia, where *T. gondii* merozoites were identified in the

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blood, liver and spleen of the rodents. Around this same time, *T. gondii* was identified in Sao Paulo, Brazil, Spendore in a laboratory rabbit and this suggests its worldwide distribution. This organism was later identified as an agent of infectious disease in 1932 in a case of a congenitally infected infant while another case of toxoplasma encephalitis was reported by Wolf *et al*, in 1939. Moreover, *T. gondii* was later discovered to be a causative agent of severe and potential fatal disease of adult in 1968, after several cases of toxoplasma encephalitis were found in patients with heamatologic cancers. This parasite was widely reported as a cause of morbidity in immunodeficient individuals, in AIDS patients beginning in 1983. Toxoplasmosis continues to be an important disease in the modern world today especially among pregnant women and immunocompromised patients (Sukthana, 2006).

Dubey and Jones, (2008) reported Toxoplasmosis has the third leading infectious cause of food-borne death, after Salmonellosis and Listeriosis (Dubey and Jones, 2008). Seroprevalence varies considerably with high seroprevalence (> 50%) occurring in countries where raw meat is commonly eaten (France, 54%) and in tropical regions of Latin America or Sub-Saharan Africa where cats are numerous and the climate is favourable to oocysts survival (Cook *et al*, 2000; Jones *et al*, 2001; Di Carlo *et al* , 2008). Jones *et al*, (2001) reported that in the United States, 15% of childbearing age women (15 to 44) were infected with *T. gondii*, with the incidence of congenital toxoplasmosis estimated at 400 to 4000 cases per year (Jones *et al*, 2001). The 3 main routes of transmission are ingestion of raw or undercooked meats, exposure to oocyst-infected cat feces, and vertical transmission (Skariah*et al*, 2010; Elmore *et al*, 2010; Jones *et al*, 2010).

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In pregnancy, the most common mechanisms of acquiring infection are through consuming raw or very undercooked meats or contaminated water, or exposure to soil (gardening without gloves) or cat litter (Skariah*et al*, 2010; Elmore *et al*, 2010; Jones *et al*, 2010). Transfusion or organ transplantation from an infected person can also transmit the organism (Dubey and Jones, 2008). Data from a European multicentre case-control study shows that raw or undercooked meat accounts for more than 30% to 63 % of *T. gondii* seroconversions during pregnancy (Cook *et al*, 2008).

Cats are the definitive hosts and since they are the only animals that excrete resistant oocysts into the environment (Silva *et al*; 2001), cohabiting with cats increases the chances of getting infected (Sukthana, 2006).The risk of infection from cats is related to exposure to feces from a cat that is shedding oocysts (Elmore *et al*, 2010). Moreover, several studies have shown that owning a cat poses little risk for human infection (Elmore *et al*, 2010). A study of 24 106 cats in European countries reported a detection rate of *T. gondii* oocysts of 0.11% (Dubey and Jones, 2008). In some animals including humans that serve as intermediate hosts the parasite may cause systemic infection that result in the formation of tissue cysts. Transmission may occur through ingestion of raw or partly cooked meat, especially pork, lamb, or venison containing Toxoplasma cysts. Also, oocysts may be ingested through use knives, utensils, or cutting boards contaminated by raw meat (Joss, 2004) or through ingestion of oocysts shed by cats in the environment, transplacentally, and through organ transplantation ((Nissapatorn*et al*., 2011). Distribution of Toxoplasmosis varies according to geographic location, and pregnant

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women who travel to areas with higher prevalence rates may be at increased risk of infection (Cook *et al*, 2000; Jones *et al*, 2001).

Transmission of *T. gondii* tachyzoites to the fetus can occur via the placenta following primary maternal infection. The incidence of prenatal *T. gondii* infections within the same or similar populations have been estimated to range from about 1 to 120 per 10,000 births (Patton, 1993). Rarely, does infection by tachyzoites occurs from ingestion of unpasteurized milk or by direct entry into the bloodstream through a blood transfusion or laboratory accident; but it does occur through transplantation of an organ that contain tissue cysts.

Toxoplasma gondii is a major cause of economic losses in endemic communities as they are responsible for abortions, still birth and neonatal losses among various classes of livestock (Raeghi*et al*., 2011, Buxton *et al*., 2007, Masala *et al*., 2003). Also, it is associated with congenital defects in humans, and the risk of the infection being passed on to the fetus increases to between 60% and 90% in the third trimester (Tenter, 2000). The severity of congenital infections depends on the stage of pregnancy when the acute infection occurred, and spontaneous abortions or neurological disorders (Black and Boothroyd, 2000).

Toxoplasmosis could be severe and life-threatening during pregnancy, and to fetuses, and new born babies (Robert-Gangneux*et al*., 2009). Occurrence of vertical transmission causes mental retardation, blindness, epilepsy, and death (Petersen, 2007). One of the late sequelae of congenital toxoplasmosis is chorioretinitis (Al-Azawi*et al*.,

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2013). Among the immunocompetent people, toxoplasmosis is usually asymptomatic, subclinical or benign, and can be classified as congenital, acquired or ocular (Oyibo *et al*., 2009). It may precursor spontaneously resolved symptoms such as fever, malaise, and lymphadenopathy, indicating symptomless latent infection (Montoya and Liesenfield, 2004). Among immunocompromised patients, toxoplasmosis can be severe and life-threatening (Robert-Gangneux*et al*., 2009), causing severe encephalitis through acute infection or reactivation of latent infection (Innes, 2010, Hang *et al*., 2007).

Toxoplasmosis is a neglected parasitic infection although it is extremely important economically, medically and epidemiologically (Uttah*et al*., 2013). Compared with other parasitic infections such as malaria and filariasis, it is grossly underreported. Presently, the paucity of research data on various aspects of toxoplasmosis in Nigeria is well noticed. Increase in the prevalence of its complication like stillbirth in some part of the country calls for its research in order to ensure that it does not contribute to this menace.

1.2 Problem Statement

Different studies on Toxoplasmosis, its causative organism and a number of clinical problems where Toxoplasma infection is incriminated call for objective research into assessing its economic and medical implications.

Toxoplasma gondii is a facultative heterogeneous parasite whose definitive hosts are members of the family Felidae, and it is capable of infecting mammals, birds and reptiles as intermediate hosts. Its broad host range, high infection rate, worldwide distribution and the ability to maintain a benign coexistence with its host, are the features which allow *T.*

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gondii to be widely regarded as one of the most successful parasites on Earth (Carruthers, 2002).

Serological studies have indicated incidence of *Toxoplasma* infections ranging from less than 1% in young adults in some areas, to 90% among older persons in other places (Montoya and Remington, 1995). It is estimated that between 30% and 65% of all persons worldwide are infected with *Toxoplasma* (Tenter*et al*., 2000). This infection is widespread biologically as well as geographically. It is widespread probably because of its simple mode of contraction. The infection can occur simply by ingestion of oocysts following the handling of contaminated soil with cat litter or the consumption of contaminated water or food (Walker *et al.,* 2008).

T qondii has been recovered from different locations throughout the world, except Antarctica. Seroprevalence among adults could be as high as 90% in many countries (Akyar, 2011). Some studies have reported incidence of primary maternal infection during pregnancy to range from about 1 to 310 per 10,000 pregnancies in different populations in Europe, Asia, Australia and the Americas (Opsteegh*et al*., 2011). In Brazil, a report shows that 53.03% of pregnant women were positive for IgG and 3.26% were positive for IgM (Vaz*et al.*, 2010). *T. gondii* seropositivity among pregnant women, their fetuses, neonates, and AIDS patients have been investigated in Qatar and its widespread occurrence is confirmed in East Mediteranean (Akyar, 2011). Unfortunately, in many developing countries, the exact prevalence of toxoplasmosis is not well articulated unlike in the developed world (Lindstrom *et a*l., 2006), but large variation was observed between different countries. For example, in France, about 88% of the populations are

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carriers, probably due to a high consumption of raw and lightly cooked meat (Ancha and Szyfres, 2003). High prevalence rates of 68%, 80% and 67% has been reported in Germany, the Netherlands and Brazil respectively (Henriquez *et al*, 2009). In Britain about 22% are carriers, while in South Korea the rate is 4.3% (Tenter*et al*., 2000).

Toxoplasmosis has long been reported to be widespread in West Africa (UNAIDS, 2004). In sub-Saharan Africa, toxoplasmosis often remains undetected and untreated due to insufficient diagnostic procedures (Lindstrom *et al*, 2006). Studies have shown a consistently high *T. gondii*-seroprevalence for this region, ranging from 35% to 84% in different African countries south of Sahara (Tenter*et al*., 2000). Considering that around 30–50% of those coinfected with HIV and *T. gondii* are expected to ultimately develop toxoplasmosis, the high seroprevalence combined with the HIV-pandemic indicates that 2.5–10 million people in this region may be at risk dying from toxoplasmosis (Lindstrom *et al*, 2006). Similarly, high incidences have been found in the Central Africa region (Dubey *et al*., 2005). There is paucity of published work on toxoplasmosis among countries in East and West Africa. However, a serological study carried out among three tribes: Baganda, Masai, and Bondei, has established a widespread distribution of *T. gondii* (Tenter*et al*., 2000).

In Nigeria, toxoplasmosis has been reported both in man and some important animals. In Northern Nigeria, Kamani *et al* (2010) reported 23.9% seroprevalence of toxoplasmosis among adult men and women, with 20% prevalence among women that participated in the study. Among the HIV positive adults in Zaria was 32.4%

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seropositivity and 38.7% among HIV negative adult participants (Ogoina*et al*, 2010). In this country, more work has been reported on veterinary toxoplasmosis, and high seroprevalence of toxoplasmosis have been reported among some animals of economic importance, such as sheep (Okoh*et al*., 1984), chicken in Zaria (Aganga, 1985), pet dogs in Zaria (Aganga and Ortese, 1984), and dogs in Maiduguri (Kamani *et al*., 2010). These studies reveal the high preponderance and spread of veterinary toxoplasmosis in the North and other parts of Nigeria. They also show that these animals reported as having high prevalence may represent possible animal source of infection to humans in the region (Clementino *et al*., 2010). In the Middle belt region of the country, high prevalence of toxoplasmosis has been reported among pregnant women from Benue State. Among women of the 39-42 age brackets, 71.4% presented with serological evidence of toxoplasmosis (Olusi*et al*., 1996). Akinbami*et al* (2013) reported 40.8% seropositivity for toxoplasmosis among pregnant women attending Lagos State University, Lagos while 54% seropositivity among HIV positive adults attending the same hospital was reported by Akanmu*et al* (2010). A work carried out among pregnant women attending Antenatal Clinics at University College Hospital, Ibadan, and St. Mary's Catholic Hospital, Ibadan, showed very high prevalence of *Toxoplasma* antibodies in the sera of both pregnant (75.4%) and postpartum (80.5%) women (Onadeko*et al*., 1996). Moreover, Onadeka *et al*. (1996) observed that polydactylism, a common congenital abnormality, was traced to reinfection or recrudescence of toxoplasmosis which accounted for high antibody levels. The report also showed an association between high prevalence of toxoplasmosis and

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overcrowding with poor environmental sanitation problems, including considerable contamination with cat faeces.

1.3 Justification

Establishing the link between Toxoplasmosis and immune competence is vital, considering the increasing number of immunocompromised patients including HIV positive patients, cancer patients and organ transplant patients. This parasite can lead to life threatening conditions for these individuals, being an opportunistic parasite. Hence the need to generate data that will assist health institution to make policies on whether Toxoplasmosis screening should be included in daily routine laboratory testing or not. In Nigeria, there is no public enlightenment and there is no screening program in PEPFAR for People Living with HIV, toward prevention and control of toxoplasmosis just like Canada and some other developed countries. Therefore, the aim of this study is to determining the seroprevalence of *T. gondii* infection and its associated factors among People Living with HIV. This study will provide basic information that will be used to justify introduction of toxoplasma screening programme and aid the development of guidelines and recommendations to public health regulatory bodies with the aim of reducing the prevalence of toxoplasmosis among the high risk individuals in Nigeria.

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1.4 Research Questions

- What is the prevalence of toxoplasmosis among People Living with HIV in Ibadan, Nigeria?

- What factors are responsible for transmission of toxoplasmosis among People Living with HIV in Ibadan, Nigeria?
- What proportion of the People Living with HIV has knowledge about toxoplasmosis?

1.5 General and Specific Objectives

1.5.1 General Objective

- To determine the prevalence and assess factors associated with transmission of toxoplasmosis among People Living with HIV in Ibadan, Nigeria

- **Specific Objectives**
- To determine the prevalence of toxoplasmosis among People Living with HIV in Ibadan, Nigeria
- To assess the factors associated with the transmission of toxoplasmosis among People Living with HIV in Ibadan, Nigeria
- To determine the level of knowledge among People Living with HIV in Ibadan, Nigeria

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CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background

Toxoplasmosis is an infection of vertebrates caused by an obligate intracellular protozoan parasite, *Toxoplasma gondii* (Tenter*et al.,* 2000). *T. gondii* was first discovered over 100 years ago by Nicolle and Manceaux at the Pasteur Institute in Tunisia, in 1908. When conducting leishmaniasis research on the North African rodent, *Ctenodactylusgondi*, the investigators isolated *T. gondii* merozoites from the rodent's blood, liver and spleen (Frenkel, 1973; Cox, 2002; Sukthana, 2006). Around the same time in São Paulo, Brazil, Splendore independently described *T. gondii* in a laboratory rabbit, and this suggests its worldwide distribution (reviewed by Frenkel, 1973).

The first human case of toxoplasmosis was described by Janků in 1923 (Cox, 2002; Sukthana, 2006). He observed tissue cysts in the retina of an 11-month-old infant in Prague (Sukthana, 2006). In 1939, Wolf *et al*. successfully isolated the parasite from tissue from a neonate with encephalitis, by animal inoculation (Cox, 2002). This served to be the first example of an organism causing disease *in utero*. Adult infection with the parasite was first described by Pinkerton and Henderson in 1941 and childhood infection by Sabin in 1942 (reviewed by Frenkel, 1973).

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2.2 *Toxoplasma gondii*

T. gondii is one of the most common parasites of humans worldwide, it infects not less than one third of the world's population (Tenter*et al.,* 2000). It is a facultative heteroxenous parasite and its definitive hosts are members of the family Felidae, but it is capable of infecting mammals, birds and reptiles as intermediate hosts. Its broad host range, high infection rate, worldwide distribution and the ability to maintain a benign coexistence with its host, are major characteristics of *T. gondii* which allow it to be widely regarded as one of the most successful parasites on Earth (Carruthers, 2002).

Considering its biological name, *T. gondii* belongs to the Phylum Apicomplexa, class Sporozoasida and subclass Coccidiasina. The genus name 'Toxoplasma' is derived from the Greek word 'toxon' meaning bow, which describes the crescentric shape of the tachyzoite stage, and the species name 'gondii' is from the rodent in which it was first isolated (Markus, 2003).

2.3 Life cycle

In 1970, the life cycle of *T. gondii* was described and this is when it was established that its definitive hosts are members of the family Felidae, with the domestic cats inclusive. Various warm-blooded animals serve as its intermediate host, with human inclusive.

Definitive host: This is an organism which supports the adult form of a parasite, it is also known as the organism in which the sexual reproduction of a parasite takes place. It is referred to as the primary host of the parasite.

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Intermediate host: This is defined as the organism which supports the immature or non-reproductive forms of a parasite. It is otherwise called the secondary host.

Toxoplasma gondii has its life cycle in two stages and this consists of a sexual phase in the definitive host, and an asexual phase occurring in the intermediate host. Definitive hosts can be infected by ingestion of parasites within tissue cysts or oocysts. There are five distinct asexual phases that occur in enterocytes before gametogony begins. Microgametes fertilize macrogametes in the enterocyte forming fertilized zygotes. A wall is then laid around each zygote forming unsporulated oocysts, which are released into the intestinal lumen when enterocytes rupture. These oocysts are then released into the environment with the faeces (Peterson and Dubey, 2001). The period between ingestion and shedding of the oocysts is called the prepatent period, this is approximately three to ten days (Tenter*et al*., 2000; Peterson and Dubey, 2001). Cats not previously infected with *T. gondii* shed oocysts after ingestion of infectious stages, whereas cats that have been previously infected are generally immune against renewed oocyst shedding. This immunity does not, however, continue throughout the duration of the cat's life (Petersen, 2003). After the shedding, depending on the temperature, aeration and humidity of the environment, oocysts begin to sporulate within five days, dividing into two sporocysts, each containing four sporozoites. These sporulated oocysts can remain infectious for months in the environment (Peterson and Dubey, 2001). Once ingested by an intermediate host, the outer walls of cysts or oocysts are disrupted by enzymatic activities in the gastrointestinal tract, releasing either bradyzoites or sporozoites into the intestinal lumen. These then actively invade surrounding cells and transform into tachyzoites. Upon

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entering the host cell, the parasite pulls the host cell membrane around itself and is then surrounded by a parasitophorous vacuole (Petersen, 2003;Bhopale, 2003) .Tachyzoites rapidly divide within the host cell, leading to its rupture, releasing the parasites into the blood and lymphatic system, where they are carried to other cells which they then invade, repeating this same process (Bhopale, 2003; Montoya and Rosso, 2005). Their multiplication depends on how the host organism can tolerate the tachyzoites.

Toxoplasma gondii has three infective stages, namely, the sporozoite stage in oocysts in faeces passed by cat, the rapidly dividing tachyzoites found during an acute infection and the slowly dividing bradyzoite stage in cysts during latent infection in the intermediate host (Peterson and Dubey, 2001; Jones *et al*., 2001b).

2.4 Transmission of *Toxoplasma gondii*

There are several ways in which transmission of *Toxoplasma gondii* can occur.

Definitive hosts can be infected by ingestion of oocysts from the environment or cysts in the tissue of its prey. Transmission to humans can occur via accidental ingestion of infectious stages of the parasite through faecal-oral contact, the consumption of contaminated water, fruit or vegetables, contaminated undercooked meat or contact with body fluids of infected animal, via the placenta to the fetus, and rarely via organ transplantation or blood transfusion (Montoya and Liesenfeld, 2004). This is illustrated in Figure 1.1.

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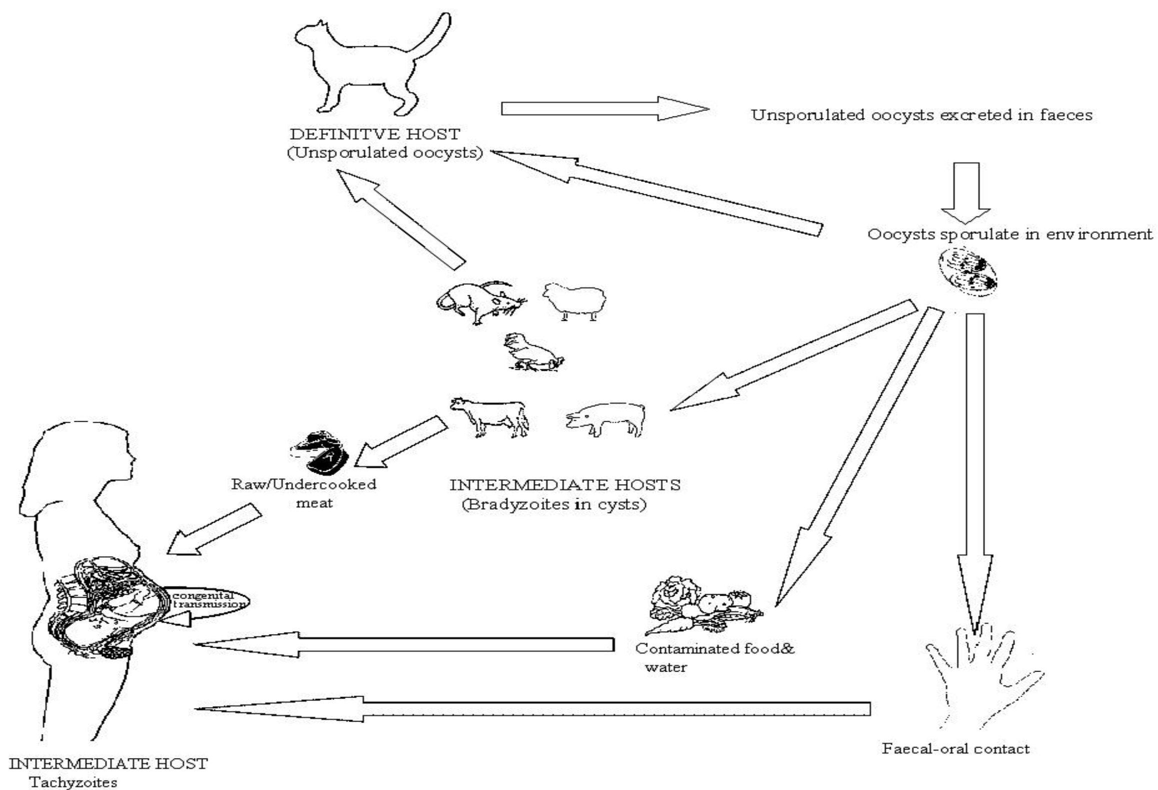

Figure 2.1: An overview of modes of transmission of *T. gondii.*

*(*KesenthriKistiah, 2009)

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In the year 2008, it was reported by Kijlstra and Jongert that in different three large European case-control studies, undercooked meat was shown to be the largest risk factor for *Toxoplasma gondii* infection. The largest of these studies showed that 30-60% of infections in pregnant women were due to the consumption of undercooked contaminated meat.

Ruminants: Experimental infection of cattle, sheep, pigs and goats, all of which are used for human consumption, showed that they are susceptible to *T. gondii* infection via oocysts or tissue cyst intake (Kijlstra and Jongert, 2008). Serological studies on cattle show prevalence that can be as high as 92%, but tissue cysts are rarely found in beef as cattle are known to show a high resistance to *T. gondii* (Tenter*et al*., 2000). Sheep are fully susceptible to *T. gondii* infection and the consumption of mutton or lamb has therefore been identified as a significant risk for *T. gondii* infection (Kijlstra and Jongert, 2008).

Also, pork was generally considered a major source of infection in Europe and the United State of America, but recent studies on fattening pigs in the Netherlands, Austria and Germany have shown that *T. gondii* infections in pigs have dropped to less than 1% over the last ten years (Tenter*et al*., 2000). This is largely due to modern, more hygienic farming systems, as well as the increasing use of frozen meat to feed them (Kijlstra and Jongert, 2008).

Chicken: A number of studies show that free-ranging chickens are increasingly being identified as an important source of *T. gondii* infection, especially in developing countries, including Nigeria. Seroprevalence of up to 65% have been reported and parasite presence was shown in 81% of these seropositive animals (Kijlstra and Jongert, 2008).

Rodents: Rats infected by *Toxoplasma gondii* serve as reservoirs of infection for animals such as pigs, dogs and cats, that feed on the rats and they are therefore epidemiologically important in the transmission of this parasite (Dubey and Frenkel, 1998). Serological data from different countries showed that *T. gondii* prevalence in rats can be as high as 91% in

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the wiska rat, *Hydromyschrysogaster*, in Queensland, Australia. Other areas such as China, Costa Rica, Egypt, Finland, France, Germany, India, Japan, Mexico, Poland, Italy and the USA and UK, have varying prevalence from 1% to 70% in different rodent species. A study in Africa by de Roever-Bonnet in 1972 showed an 8% seroprevalence in *Rattus rattus*(Dubey and Frenkel, 1998).

A rodent control campaign on three organic pig farms over a four-month period showed the disappearance of *T. gondii* from pigs in two of the three farms at the end of the campaign and subsequent reappearance in one of these farms after the rodent control campaign was stopped, thus emphasizing the role of rodents in the transmission of *T. gondii* (Kijlstra*et al.,* 2008).

Water: The transmission of the parasite through contaminated water has generally been considered uncommon, but the widespread infection of marine mammals indicates that contaminated water may be a potential source of infection (Dubey, 2004).

Factors that influence transmission of *Toxoplasma gondii:*

- Carnivorous animals are often infected with *Toxoplasma* through ingestion of bradyzoites from tissue cysts in their infected prey,
- Persons who eat undercooked meat, particularly that of pigs, sheep and goats often infected with *Toxoplasma gondii* through ingestion of bradyzoites from tissue cysts in infected animal
- Infection can also be through the milk of sheep, goats and cattle, and sometimes through chicken eggs

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- The cysts of *Toxoplasma gondii* are less commonly found in poultry and rarely found in beef.
- Its prevalence in commercial farm animals has decreased significantly with the advent of intensive farm management system (Clementino *et al*., 2009).
- Free range poultry, swine, small ruminants, marsupials and some wild game are more likely to harbour cysts unlike those reared with intensive management practices
- Tachyzoites are killed relatively easily by pasteurization, and uncommonly survive gastric digestion but any kind of cooking will definitely kill tachyzoites in an egg.
- Oocysts are only shed by cats but unsporulated oocysts in fresh feces are not uninfective. Appropriate oxygen, humidity, and temperature are necessary for its sporulation to occur. Sporulated oocysts are the most environmentally resistant life stage of the parasite (Halland, 2004).
- Ingestion of as few as ten oocysts may infect an intermediate host, while ingestion of 100 or more oocysts can cause an infection in a cat and this will later shed hundreds of millions of oocysts (Patton, 1993).
- *In utero* transmission of *Toxoplasma* occurs only if primary infection of the mother occurs during pregnancy.
- During pregnancy of an infected woman, parasitemia results in placentitis and infection of the fetus and this occurs in man, sheep and goats, and sometimes in mice, cats and dogs.

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- A woman that has been exposed to *Toxoplasma* 4-6 months prior to pregnancy will develop sufficient immunity to protect herself and the fetus for the rest of her life (Vaz*et al*., 2010).
- If immune activity is suppressed by drug therapy or disease such as AIDS in human, both the mother and the fetus may become susceptible to this infection again (Tenter*et al*., 2000).
- The risk of vertical transmission to the fetus increases from the first trimester (10-24%) to the third trimester (60-90%), and the potential of congenital defect is more severe with earlier infections (Patton, 1993).

2.5 Strains and their characteristics

T. gondii has a highly unusual population structure comprised of three clonal lineages (I, II and III). These differ in virulence and epidemiological pattern of occurrence (Montoya and Liesenfeld, 2004). Studies using murine models show that the type I strain is highly virulent and has a lethal dose of a single parasite regardless of the genetic background of the mouse. Type II and III strains have a 50% lethal dose of more than 103 parasites and the outcome is dependent on the genotype of the host (Mordue*et al.,* 2001). Type I and II strains have been reported in human cases, while type I is often associated with severe congenital and ocular disease, suggesting that it may be more pathogenic in humans (Mordue*et al.,* 2001). Type III has been shown to be more common in animals (Montoya and Liesenfeld, 2004). Until recently, *T. gondii* was considered to have little genetic variability. Recent studies on *T. gondii* isolates from Brazil, however, show that

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they are both genetically and biologically different from those in the USA and Europe (Velmurugan *et al.,* 2008). A recent study on *T. gondii* isolates from chickens in six different African countries (Nigeria, Congo, Egypt, Burkina Faso, Kenya and Mali) revealed four genotypes. Most isolates belonged to the clonal type II and III strains with one Nigerian isolate having an atypical genotype (Velmurugan *et al*., 2008).

2.6 Toxoplasmosis

Toxoplasmosis is a zoonotic infection of animals caused by the protozoan parasite *Toxoplasma gondii*. It has the capacity to infect all warm-blooded animals and, while infection does not cause clinical illness in the majority of animal species, in some it causes acute life-threatening disease and in others, particularly sheep and goats, it may manifest itself as a disease of pregnancy by multiplying in the placenta and fetus.

In human, the majority of horizontal transmissions to humans are caused either by the ingestion of tissue cysts in infected meat or by the ingestion of soil, water, or food contaminated with sporulated oocysts derived from the environment or, less frequently, directly from feline feces. The relative importance of transmissions via tissue cysts versus oocysts in a given population is unknown, except in the case of outbreaks with a well-defined source of infection. Until now, only risk factor studies gave an indication of the predominant route of transmission in a given population (Cook *et al.*, 2000). Persons may be unaware of their exposure or may have difficulty recalling specific risks that occurred. The recent discovery of a sporozoite or oocysts specific protein, which elicited antibody

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production and differentiated oocyst versus tissue cyst induced experimental infection in pigs and mice, may help to solve this problem (Hill*et al*., 2011). Serum antibodies to the sporozoite protein were detected in humans within 6 to 8 months of initial oocysts acquired infection. Therefore, this serological assay could be useful for detecting exposure to oocysts in the early months after *T. gondii* infection and could be useful for epidemiological studies.

2.6.1 Infection through Cysts

2.6.1.1 Consumption of meat

(i) Type of meat. Any meat from warm-blooded animals and birds has been traditionally considered a major source of Toxoplasma infection in Western countries. The risk associated with the type of meat (lamb, pork, and beef, etc.) varies among different countries according to local eating habits and according to the prevalence in meat-producing animals. In a multicenter study in Europe, meat consumption was estimated to be responsible for 30 to 63% of cases of infection, while soil contact represented 6 to 17% of cases (Cook *et al*, 2000). In the United States, in 2009, a case control study showed an elevated risk for *T. gondii* infection in persons eating raw ground beef (adjusted odds ratio [aOR], 6.67; attributable risk [AR], 7%); eating rare lamb (aOR, 8.39; AR, 20%); eating locally produced cured, dried, or smoked meat (aOR, 1.97; AR, 22%); or working ith meat (aOR, 3.15; AR, 5%) (Jones *et al,* 2009). A quantitative assessment of the risk of Toxoplasma in food for consumers is hampered by the lack of data on the

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number of tissue cysts resulting in infection of humans, the distribution and the number of cysts in the different muscle sites in various hosts, as well as their infectivity in commercial meat products. One recent survey of meat from commercial markets (pork, chicken, and beef) in the United States suggested a low risk, perhaps owing to meat treatment processes, which could reduce the viability of cysts (Dubey*et al.,* 2005).

(ii) Cyst resistance. Tissue cysts remain infectious in refrigerated carcasses (1°C to 6°C) or minced meat for up to 3 weeks. Freezing alone is not a reliable means of rendering all tissue cysts noninfective, cysts have remained viable for >11 days at −7°C. However, the deep-freezing of meat at −12°C or lower for at least 3 days is usually efficacious to kill cysts, although it may depend on the thickness of the piece of meat (Dubey, 1988).

Tissue cysts are usually killed immediately by heating to 67°C. The survival of tissue cysts at lower temperatures depends on the duration of cooking. Tissue cysts remain viable at 60°C for about 4 minutes and at 50°C for about 10 minutes (Dubey*et al*., 1990). Cooking for a prolonged period of time may be necessary under household conditions to achieve the temperatures that are required to kill all tissue cysts of *Toxoplasma* in all parts of the meat. Some tissue cysts will remain infectious after cooking in a microwave oven, possibly due to an uneven heating of the meat. However, in a United State case control study by Jones *et al*, (2009), microwave cooking of meat was associated with a reduced risk of recent *T. gondii* infection. This was explained by the fact that microwave cooking is often associated with reheating already-cooked meat or with defrosting or cooking frozen meat (Jones *et al*, 2009).

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Commercial procedures of curing meat with salt, sucrose, or low-temperature smoking may kill tissue cysts, but the survival time of tissue cysts varies greatly with the concentration of the salt solution and the temperature of storage. Salting does not necessarily kill tissue cysts in homemade pork sausages. Under laboratory conditions, solutions containing 2% sodium chloride or 1.4% potassium or sodium lactate are effective within 8 hours of injection for the killing of *T. gondii* tissue cysts in pork loin (Hill *et al*, 2006).

Other food treatment processes, such as gamma irradiation at a dose of 1.0 kGy and high pressure (300 mPa), were found to be efficient for killing tissue cysts in meat, but some treatment procedures are barely applicable for meat prepared for human consumption (Lindsay *et al*, 2006).

2.6.1.2 Infection related to solid organ transplant

As *T. gondii* tachyzoites can invade all nucleated cells, cysts can be found in virtually any organ. Therefore, in solid organ transplantation, Toxoplasma infection can be transmitted through a cyst containing organ from a donor with infection acquired in the distant past to a nonimmunised recipient. However, certain organs are more likely to harbor persistent cysts than others. Muscles commonly sustain parasite encystment; thus, heart transplant patients are at a higher risk for organ related toxoplasmosis than are liver, lung, or kidney transplant patients. Toxoplasmosis was recognized early as an infectious complication in heart transplant patients (Saeij *et al.,* 2005), which motivated the implementation of large

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retrospective studies in several countries from 1980 onwards. However, the incidence of acquired toxoplasmosis in case of a mismatch (D^+/R^-) is variable, since it depends largely on the prevalence of toxoplasmosis in the country of study and on the use of chemoprophylaxis after transplantation. In retrospective studies, the incidence can vary from 9 to 56% when the patients benefit or not from a chemoprophylaxis scheme, respectively, indicating that prevention is efficient (Saeij *et al.,* 2005).

In 2011, a multicenter retrospective study including 22 patients with acquired toxoplasmosis within a median time of 92 days posttransplantation, mismatched transplants were documented for 9 patients and the donor's serology was unknown for 8 other negative recipients (Fernandez-Sabe *et al*. 2011). Twelve of 22 cases were heart transplant patients. The incidence of donor-acquired toxoplasmosis is less frequent in other organ transplant patients, and only 9 and 16 cases were reported for liver and kidney mismatched patients, respectively, supported by solid serologic evidence. A case of disseminated toxoplasmosis following small bowel transplantation was also described, but the serostatus of the donor was unknown, making the source of infection uncertain (Campbell *et al*. 2006).

2.6.2 Infection through Oocysts

2.6.2.1 Survival of oocysts in the environment

Environmental conditions are important for oocyst survival. Moist conditions can increase oocyst survival during long periods of heat, which likely accounts for the high prevalences in tropical countries of South America and Africa. In Colombia, a correlation

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was found between the mean amount of rainfall and the incidence of congenital toxoplasmosis (Gomez-Marin *et al*. 2011). Even in a country with a temperate climate, such as France, the risk of infection in cats was shown to increase when the weather was both warm and moist, or moderate and less moist, reflecting the influence of climatic conditions on the prey population and oocyst survival (Afonso *et al*, 2010).

Despite the low prevalence (<1% in most studies) and short duration of oocyst shedding by cats, the burden in the environment may be very high (Afonso *et al*, 2010). A single cat may shed more than 100 million oocysts, which are nonsporulated. These oocysts need between 1 and 5 days to mature and become infective for other hosts, which explain why direct contact with cats is not thought to be a major risk for human infection. In the United States, an increased risk associated with exposure to kittens was limited to respondents who had 3 or more kittens, thus more likely to be infected through the shedding of oocysts after primary infection (Jones *et al.* 2009). Oocysts are able to sporulate within 2 to 3 days in different types of commercial cat litter and occasionally remain viable for 14 days (Dubey *et al*. 2011). Unsporulated oocysts loose their capacity to sporulate, and, hence, to become infective, after freezing at −6°C during 7 days or after exposure to 37°C for 1 day. Once sporulated, oocysts are resistant to harsh environmental conditions. They remain viable in a moist environment for more than a year. Under laboratory conditions, sporulated oocysts can survive storage at 4°C for up to 54 months. They survive freezing at −10°C for 106 days and heating at 35°C and 40°C for 32 days and 9 days, respectively. However, they are killed within 1 to 2 minutes by heating to 55°C to 60°C (Dubey, 2010), conditions easily obtained when cooking vegetables. The

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wall of sporulated oocysts is highly impermeable and, therefore, very resistant to disinfectants (Dumetre and Darde, 2003).

2.6.2.2 Contamination of water

In water, oocysts can remain viable for long periods of time and it can resist freezing and moderately high water temperatures. They are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment (Dumetre *et al*., 2008). Outbreaks associated with the contamination of reservoirs supplying water, such as those described for the Greater Victoria area of British Columbia, Canada (Aramini *et al*., 1999) in Santa Isabel do Ivai, Brazil (De Moura, *et al.,* 2006); or in Coimbatore, India (Balasundaram *et al.,* 2010), involved a large number of patients. The epidemics were preceded by peaks of heavy rainfall and turbidity in the implicated reservoirs. Smaller epidemics were described after the drinking of raw surface water in remote tropical areas (Benenson, 1982; Demar *et al*., 2007). Freshwater runoff from urban centers next to seashores may contaminate seawater. Toxoplasma oocysts can remain viable for extended periods of time in seawater (Lindsay and Dubey, 2009). Shellfish are filter feeders that concentrate *T. gondii*. Oocysts remained viable and were detected in various species of shellfish under natural conditions (Esmerini, *et al.,* 2011). The consumption of oysters, clams, and mussels has been shown to be a risk factor for acquiring Toxoplasma infection in the United States (Jones*et al.,* 2009).

2.6.2.3 Contamination of soil, vegetables, and fruits

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Contact with soil was identified as a strong risk factor in a European multicenter case-control study, and 6% to 17% of primary infections in humans were attributed to this risk factor (Cook*et al.,* 2000). A United State study showed that the detection of antibodies against Toxoplasma was 2-fold higher in a population with positive Toxocara antibodies, suggesting a common exposure to contaminated soil (Jones*et al*., 2008). The risk of acquiring Toxoplasma infection after soil contact or ingestion is particularly high for children. Toxoplasma oocysts were isolated in as many as 32% of school playgrounds in a Brazilian study in 2010 (Dos Santos *et al.,* 2010).

Contaminated water and soil may act as vehicles for the transfer of oocysts to vegetables and fruit for human consumption, although there are few data available to confirm this. In several risk factor or case-control studies, the eating of unwashed raw vegetables or fruits was associated with an increased risk of primary infection (Berger *et al.,* 2009). Experimentally, *T. gondii* oocysts can adhere to berries, especially raspberries, and can be recovered by bioassays in mice (Kniel *et al.,* 2002), but there has been no report of the detection of Toxoplasma on fruits or vegetables under non experimental conditions.

2.6.3 Infection through Tachyzoites

2.6.3.1 Food-borne contamination

Tachyzoite is a fragile stage outside its host cell; it is easily destroyed by digestive enzymes (10-minutes survival in pepsin-Hydrochloric acid). It is also very sensitive to environmental conditions and is usually killed rapidly outside the host. Therefore, the horizontal transmission of Toxoplasma via tachyzoites is probably not important from an

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epidemiological point of view. However, tachyzoites were suggested to be the cause of rare cases of acquired toxoplasmosis in humans after the consumption of unpasteurized goat's milk (Tenter *et al.,* 2000). The drinking of unpasteurized goat's milk was found to be a risk factor in an epidemiological survey (Jones*et al.,* 2009), suggesting that tachyzoites may enter the host by the penetration of mucosal tissue.

2.6.3.2 Congenital infection

When primary infection is acquired by a pregnant woman, tachyzoites can colonize placental tissues during the dissemination process and from there can gain access to the fetal compartment in about 30% of cases. The frequency of vertical transmission increases with the gestational age at maternal infection. At the beginning of pregnancy, the transplacental passage of tachyzoites is a rare event, but the consequences for the offspring are heavy. The immune control of placental infection is probably a key event in the occurrence of congenital infection but advances in the comprehension of the pathophysiological process remain to be achieved (Pfaff *et al.,* 2007).

Congenital infection is the most important part of the disease burden due to Toxoplasma infection in humans. Clinical manifestations of congenital toxoplasmosis first motivated research on the parasite and its pathophysiology and epidemiology. However, the factors influencing congenital transmission are still poorly known, apart from the term of pregnancy at the time of maternal infection and, of course, the immune status of the mother (Pfaff *et al.,* 2007).

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The observation of a decreasing seroprevalence of toxoplasmosis in industrialized countries has complex consequences for the risk of acquisition of Toxoplasma infection during pregnancy. At first glance, a reduced seroprevalence increases the percentage of pregnant women susceptible to primary infection and, hence, to congenital transmission to their fetuses. However, the lower level of circulation of the parasite in the environment diminishes the global risk of acquiring infection during pregnancy. A national surveillance system was implemented in France in 2007, which aims to collect data from all cases of congenital toxoplasmosis through data transmitted by laboratories certified for prenatal diagnosis or implicated in neonatal serological diagnoses. This network reported 272 cases of congenital toxoplasmosis in 2007 (Villena*et al.,* 2010). If these data can be considered exhaustive, they can allow an evaluation of the overall prevalence of congenital toxoplasmosis in France, 3.3 per 10,000 live births (Villena*et al.,* 2010), which is nearly the prevalence reported in Brazil (1 per 3,000 live births) (Neto *etal.,* 2000) but 3-fold higher than that estimated in a pilot study in Massachusetts (1 per 10,000 live births) (Guerina*et al.,* 1994).

2.6.3.3 Transmission through injection

Fourteen cases of laboratory contamination of a parenteral origin have been reported (Herwaldt, *2001*). Needle-stick by contaminated needle and cat-scratch are potential means of infection.

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2.7 Mechanism of Cell Invasion

Toxoplasma gondii has in its ability to invade a wide variety of host cells. Invasion is an active process relying on parasite motility and the sequential secretion of proteins from secretory organelles, the micronemes, the rhoptries, and the dense granules. Attachment to the host cell membrane is a prerequisite for invasion. It requires the calcium-dependent secretion of adhesins from micronemes, such as the microneme protein MIC2, which recognises host cell receptors and promote parasite reorientation and eventual attachment. Cell invasion relies on a complex interaction between the host cell surface and the parasite, a process called gliding motility, an intricate linear motor system promoted by actin-myosin interactions and dynamic rearrangements of the parasite cytoskeleton (Carruthers and Boothroyd, 2007). Entry is a rapid process (15 to 30 s) distinct from currently known host endocytic events. Toxoplasma forms a tight association between its apical end and the host cell membrane, called the moving junction. This moving junction moves from the apical end to the posterior end of the parasite, leading to the internalisation of the parasite into a parasitophorous vacuole (PV). The establishment of this moving junction around the invading parasite requires the distribution over the entire surface of the parasite of an apical membrane antigen (AMA1), also secreted by micronemes, and the secretion of rhoptry (ROP) neck proteins (RONs) inserted into the host cell membrane (Dubremetz, 2007). The formation of the nascent parasitophorous vacuole membrane (PVM) requires the secretion of proteins from the ROPs. In recent years, a major role for the ROP2 family proteins has been recognized. Of these proteins, ROP18 is associated with the cytosolic face of the PVM and exerts protein kinase activity,

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which has a profound effect on parasite growth and virulence (El Hajj *et al.,*2007), and ROP16 is able to manipulate host gene expression, affecting interleukin secretion (Laliberte and Carruthers, 2008).

Besides ROP proteins, dense granular proteins also contribute to the formation of the parasitophorous vacuole membrane (PVM) during the first hour following invasion. Most host transmembrane proteins are stripped from the PVM during the invasion process; this process modifies biochemical characteristics of the PVM and prevents fusion with lysosomes or any cytoplasmic vesicle. Dense-granule secretions also support the development of a complex network of membrane tubules that develop from the PVM and extend into the vacuolar lumen (Mercier *et al.,* 2005). This network is supposed to have a role in developing exchanges between the parasite and the host cell, bringing in nutrients from the host cell cytosol or exporting proteins or lipids from the parasite toward the PVM or the host cell. The PVM is also closely associated with host cell mitochondria, which contribute to parasite metabolism. Within the parasitophorous vacuole (PV), tachyzoites divide during a 6- to 9-hour cycle, by a process of endodyogeny, leading to the formation of two daughter cells within each mother cell. They exit the cell usually after 64 to 128 parasites have accumulated in the PV (Black and Boothroyd, 2000). Egress from the cell is an active process dependent upon a rise in the calcium concentration after the release from intracellular stores (Sibley, 2010).

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2.8 Clinical Features of Toxoplasmosis in Human

2.8.1 Pathogeny and Development of the Immune Response during the Course of Infection

Following the ingestion of cysts or oocysts, the respective excysted forms, bradyzoites or sporozoites, rapidly invade the small intestinal epithelium, where they convert into tachyzoites. The establishment of infection relies on the intrinsic properties of the parasites. First, the high motility of tachyzoites and cell interactions between the parasite protein MIC2 and the host intercellular adhesion molecules (ICAM-1) are used for paracellular crossing. Moreover, the active invasion of the apical side of the epithelial cell is followed by egress from the basolateral side (transcellular traversal) (Lambert and Barragan, 2010). Whatever the early scenario comprising or not an initial multiplication of tachyzoites in the intestinal epithelium, they further cross the intestinal barrier and invade monocyte cells in contact with the lamina propria, which are key cells for the dissemination of Toxoplasma through the blood flow toward all organs, using them as Trojan horses to cross biological barriers (Bierly*et al.,* 2008), this is as shown with a murine model of infection by intracellular fluorescent parasites (Unno*et al.,* 2008). This peculiar capacity to actively invade all nucleated cells, including professional phagocytic cells, contributes to the complexity of the host-parasite interactions through the direct modulation of the host immune response.

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The cellular and soluble effectors involved in the immune response against *T. gondii* have been extensively studied in the last two decades. It was recognized early that a T helper 1 (Th-1) immune response driven by gamma interferon (IFN-γ) and interleukin-12 (IL-12)

producing cells is essential for the control of the parasite burden. The fine regulation of immune effectors and their signaling pathways were reviewed by Miller *et al*. in 2009 (Miller *et al.,* 2009). Briefly, following the ingestion and transepithelial transfer of parasites, there is a local release of chemokines by infected cells, leading to the attraction of cells of the innate immunity. Neutrophils are attracted to the infected foci early to phagocytose free parasites and contribute to reducing parasite burdens. Other phagocytic cells, such as dendritic cells (DCs) and macrophages, play a pivotal role in the initiation of innate immunity, as they are the major sources of IL-12 as well as IL-18, thus promoting natural killer (NK) and NKT cell activation (Iwasaki and Medzhitov, *2004.*), with both cell types producing IFN-γ in large quantities (French *et al.,* 2006). Moreover, DCs and macrophage cells can present parasite antigens associated with major histocompatibility complex (MHC) class II antigens and costimulatory molecules and further prime T cells (Combe*et al.,* 2006). In addition, DCs and NK cells can also interact directly, with this dialog resulting in the mutual activation and amplification of IL-12 and IFN-γ synthesis, respectively. Classically, the release of IFN-γ can trigger macrophage activation to synthesize tumor necrosis factor alpha (TNF-α), thus being responsible for an amplification loop. The further recognition of parasite antigens by pattern recognition receptors (PRRs) leads to an exacerbation of phagocytic activity with an enhanced production of reactive oxygen species (ROS) and nitric oxide (NO) species and tryptophan starvation through 2-3-indole-amine dioxygenase (IDO) activation (Pfefferkorn, *1984*).

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However, this potent machinery has two limitations. The first limitation resides in the negative counterpart of a strong Th-1 immune response, which may overwhelm its goal and be responsible for severe inflammation, resulting in intestinal tissue damage or even the death of the susceptible host, as shown with a murine C57BL/6 model (Liesenfeld, *2002*). Thus, there is a need for down-regulating effectors, a role devoted at least partially to IL-10 and transforming growth factor β (TGF-β), which modulate macrophage activation (Mosser, *2003*). Such a deleterious effect of an acute Th-1 immune response is also well known in the setting of primary acquired infection during pregnancy and can result in fetal loss, since IFN-γ destabilizes the Th-2 microenvironment necessary for maternal-fetal tolerance. Thus, the complexity of the maternal-fetal interface is magnified by Toxoplasma infection, and the role of the placenta in the immunomodulation process is probably essential for the maintenance of gestation after maternal infection (Pfaff *et al.,* 2007, Robert-Gangneux*et al*., 2011).

On the other hand, despite the powerful host cell effectors described above, some data provided mechanistic details on how Toxoplasma surrounds the host immune system, thus making itself a successful parasite persisting lifelong in host tissues. It is now recognized that the parasite rhoptry protein ROP16 can rapidly process into the host cell nucleus, where it interferes with signaling pathways of host immune responses, particularly through the phosphorylation of the STAT3 and STAT6 transcription factors (Laliberte and Carruther, *2008*), leading to the down-regulation of IL-12 production by macrophages and, subsequently, of IFN-γ (Denkers*et al.,* 2004). Interestingly, this capacity is not shared by all strains but is devoted to type I and III isolates (Saeij*et al.,*

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2007). This could partially explain the greater severity usually observed for infections due to strains harboring type I alleles. Moreover, type II strains, which do not exert this capacity to repress the host response, induce a rapid immune response, limiting parasite growth, thereby ensuring the survival of both the host and parasite and resulting in bradyzoite conversion and the encystment of the parasite for persistence. At the same time, it was shown that Toxoplasma can also inhibit apoptotic mechanisms of the infected cell by antagonizing caspase 8 (Vutova*et al.,* 2007) and interfering with the NF-κB pathway thus ensuring both protection against the rapid clearance of intracellular tachyzoites by macrophages and the long-term survival of bradyzoites in the cysts (Laliberte and Carruthers, 2008).

2.8.2 Toxoplasmosis in Immunocompetent Human

Toxoplasmosis in Immunocompetent Human acquired infection is asymptomatic in more than 80% of cases of immunocompetent subjects (Montoya and Liesenfeld, *2004*). In the remaining cases, patients may experience fever or cervical lymphadenopathy, sometimes associated with myalgia, asthenia, or other nonspecific clinical signs. Lymphadenopathy and asthenia may persist for several weeks, mimicking infectious mononucleosis, especially since monocytosis can be observed on blood smears. A study conducted in the United States showed that only 48% of mothers who gave birth to congenitally infected infants could recall clinical signs suitable with toxoplasmosis during their pregnancy (Boyer *et al.,* 2005). More rarely but not exceptionally, toxoplasmic chorioretinitis with visual impairment may reveal primary infection (Delair E, *et al.* 2008, Montoya and

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Remington, 1996), although it was previously thought that ocular toxoplasmosis was the result of congenital infection. Indeed, in a retrospective study by Delair*et al.,* 100 out of 425 (23.5%) consecutive cases of ocular toxoplasmosis were attributed to acquired toxoplasmosis (Delair*et al., 2008*).

There are indications that the severity of infection depends on the genotype of the strain involved. It is observed that the severity of infection is low in Western European countries and North America, where type II strains predominate (Howe and Sibley, 1995), but much higher severity is reported in other parts of the world, such as South America (Carme *et al.,* 2002; Demar*et al.,* 2007) and Africa, where other genotypes circulate (Mercier *et al.,* 2010). In particular, several studies have shown higher incidences and severities of chorioretinitis in Brazil (Gilbert *et al.,* 2008) or Colombia, both during primary infections of immunocompetent subjects and in congenitally infected infants. The strain genotype could also be an explanation for the high proportion of retinochoroiditis (19 of 100 cases with proven acute infection) in an outbreak in Victoria, British Columbia, Canada, where an atypical cougar isolate was suspected to be the cause and for the 100-fold-higher incidence of ocular toxoplasmosis in patients born in Africa than in patients born in Britain (Gilbert *et al.,* 1995).

2.8.3 Toxoplasmosis in Immunocompromised Patients

Contrasting with the setting of Toxoplasma infection in immunocompetent subjects, toxoplasmosis is always life threatening in immunocompromised patients, whatever the strain, yet the host immune background is of prime importance. Various factors

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responsible for profoundly impaired cellular immunity can lead to severe toxoplasmosis, among which are HIV infection and immunosuppressive therapies. Patients are more commonly at risk for disease reactivation resulting from cyst rupture than for a newly acquired infection, but the risk may differ among categories of patients. In transplant patients, severe or disseminated toxoplasmosis can result from either a reactivation of latent infection in the recipient or infection from a cyst-containing organ from a seropositive donor given to a seronegative recipient (Martina *et al.,* 2011), in this situation heart transplants carry the highest risk (Debrunner*et al.,* 2011). A reactivation of a chronic infection may occur in the recipient irrespective of the type of graft, but the risk is closely related to the duration and degree of immunosuppression, with hematopoietic stem cell transplant (HSCT) patients being most at risk (Derouin and Pelloux, 2008; Roemer *et al.,* 2001). In HIV-infected patients, the incidence of toxoplasmosis is closely related to $CD4^+$ T cell counts, with an increasing risk when the count falls under 100 cells/µl. Toxoplasmic encephalitis (TE) is the most predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache, lethargy, incoordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizures, usually associated with fever. The incidence of TE has decreased since the use of highly active antiretroviral therapy (HAART) (Jones *et al.,* 2002), as was shown with a French cohort, where the risk was divided by 4 and fell from 3.9 to 1.0 cases per 100 person-years (Abgrall*et al.,* 2001). Other organs can be involved, either because they are target organs for encystment and thus are subsequent potential sites for cyst reactivation or because they are secondarily infected following the dissemination of

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parasites from an initial reactivation site. After the brain, the most frequently involved organs are the lungs, the eyes (Rabaud*et al.,* 1996), and the heart, resulting in myocarditis, but the isolation of Toxoplasma from many other sites, such as liver, pancreas (Rabaud*et al.,* 1996), bone marrow, bladder, lymph nodes, kidney, spleen, and skin has been documented. Pulmonary or disseminated toxoplasmosis is seen mostly in transplant patients, who develop rapidly progressive infection and a massive dissemination of parasites (Patrat-Delon *et al.,* 2010).

2.9 Congenital Toxoplasmosis

Classically, congenital infection results from primary acquired maternal infection during gestation. The frequency of vertical transmission and the severity of fetal damage depend on the stage of pregnancy when maternal infection occurs. The placenta plays a main role in the process, as it is both a natural barrier which is supposed to protect the fetus and a target tissue for parasite multiplication (Abbasi *et al.,* 2003). In fact, the placental barrier is more efficient at the beginning of gestation, leading to the passage of parasites in less than 10% of cases during the first trimester, but becomes more permeable throughout pregnancy, allowing parasite transmission in around 30% of cases in the second trimester and 60 to 70% of cases in the third trimester and even more close to the time of deliver. The severity of fetal infection is inversely correlated, since neonates are asymptomatic in more than 80% of cases when infected during the third trimester of gestation (Desmonts and Couvreur,1974). However, when transplacental transmission occurs during the first trimester, the consequences for fetal development are heavy, often leading to severe

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abnormalities or to abortion. Parasite multiplication induces necrosis foci and strong inflammation, leading to major abnormalities in the brain and eye tissues. It can induce the destruction or profound remodeling of the white substance. Infected necrotized foci may block the aqueduct of Sylvius, resulting in hydrocephalus of lateral ventricles. These foci further calcify and can be detected by transfontanellar echography or cranial X ray. Major sequelae include mental retardation, seizures, microcephalus, hydrocephalus, deafness, and psychomotor deficiency. Eye lesions are also more severe in early pregnancy, where microphthalmia, cataract, increased intraocular pressure, strabismus, optic neuritis, and retinal necrosis can be observed (Delair*et al.,* 2011), as can uveitis and retinochoroiditis, possibly leading to blindness if retinal lesions affect the macula. During the second trimester, fetal infection can be of variable severity. Echographical ultrasounds may reveal areas of hyperechogenic mesentery, hepatosplenomegaly, or cerebral calcifications. Clinical manifestations at birth may include epilepsy, anemia, thrombocytopenia-induced petechiae, rash, hepatic disorders, pneumonitis, or retinochoroiditis (Remington *et al.,* 2001). In a prospective European study, intracranial lesions detected at birth were associated with serious neurologic disorders in about 30% of cases (Cortina-Borja *et al.,*2010). Among the data from 272 cases collected in 2007 through the French Surveillance Network (Villena*et al.,* 2010), 11 cases resulted in the termination of pregnancy owing to cerebral lesions or fetal death, and 87% of live-born infants were asymptomatic. The remaining 13% of cases had intracranial calcifications (14 cases), hydrocephalus (3 cases), and/or retinochoroiditis of variable severity (12 cases) (Villena*et al.,* 2010).

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Retinochoroiditis is a common feature that can be observed whatever the time of maternal infection. Its particularity resides in its frequently delayed clinical expression after birth. During a longitudinal United State study including 25 infants who were not treated *in utero* or during their first year of life, Phan *et al*. (Phan *et al.* 2008) observed that 72% of these infants developed new eye lesions during a mean follow-up time of 5.7 years. Another prospective study of 102 infants who benefited from antenatal and postnatal treatment showed that 78% were asymptomatic during a median follow-up time of 7.8 years (Berrebi*et al.,* 2010). An European cohort study showed that the risk of developing eye lesions by 4 years of age was highest for children with serious neurologic sequelae at birth but also significantly increased for those with intracranial lesions or hepatosplenomegaly (Freeman *et al.,* 2008). Conversely, children without retinochoroiditis detected by 4 months were at a low risk of developing eye manifestations by 4 years of age. In any event, the question of long-term pathogenicity may differ according to prevention protocols, and probably according to the strain genotype, as retinal lesions are more extensive in congenitally infected Brazilian infants (Vasconcelos-Santos *et al.,* 2009). Indeed, a comparative prospective cohort study of congenitally infected children in Brazil and Europe showed that, independently of treatment, Brazilian children had a 5-times-higher risk than European children for developing eye lesions, and their lesions were larger, more multiple, more recurrent, and more likely to impair vision (Gilbert *et al.,* 2008).

Although the vast majority of congenital infections results from primary acquired infection during pregnancy, parasite transmission can occur in rare instances in

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immunocompetent, previously immunised women who are reinfected with Toxoplasma during gestation (Elbez-Rubinstein *et al., 2009*). A recent case benefited from genotyping, which revealed that reinfection was due to an atypical strain which was responsible for severe congenital toxoplasmosis, raising the temptation to take primary prevention measures even in previously immunised pregnant women, particularly in cases of travel to areas where atypical genotypes circulate (Elbez-Rubinstein *et al.,* 2009). A reactivation of past infection in HIV-infected women can also lead to congenital transmission, as shown by several case reports (Lindsay and Dubey,2011).

Other exceptional cases of vertical transmission following maternal infection in the 2 months before conception have been described (Marty *et al.,* 1991), but in most cases, the immune background of the mother could explain the prolonged dissemination of the parasite and thus further placental colonization and transmission. Another concept which has emerged from the French experience of the systematic screening of pregnancies for >20 years is that parasite transmission can be delayed, since few asymptomatic neonates born to mothers with periconceptional infection were diagnosed with congenital infection after birth despite negative prenatal screening results (Robert-Gangneux*et al.,* 2009). Indirect arguments also support this hypothesis, since in a study by Romand*et al.*(Romand*et al.,* 2001), the sensitivity of prenatal diagnosis was lower in early pregnancy than in midpregnancy, suggesting that vertical transmission may be delayed for some women infected in early pregnancy (Thulliez,2001). Thus, in rare instances, parasites could persist in the placenta and proceed into the fetal compartment only at the end of gestation, which could explain why neonates may not have any clinical or

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radiological signs *in utero* and at birth. However, such an occurrence is probably rare, and its global frequency is not easily appreciated (Thulliez,2001).

2.10 Prevalence of Toxoplasmosis in Humans

It is generally assumed that approximately 25 to 30% of the world human population is infected by Toxoplasma (Montoya and Liesenfeld, 2004). Actually, the prevalences vary widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region (Pappas *et al*., 2009). Low seroprevalences (10 to 30%) have been observed in North America, in South East Asia, in Northern Europe, and in Sahelian countries of Africa. Moderate prevalences (30 to 50%) have been found in countries of Central and Southern Europe, and high prevalences have been found Latin America and in tropical African countries (Pappas *et al*., 2009).

As for animals, many factors can affect seroprevalence in humans. Climatic factors affect the survival of oocysts in the environment; hence, infection rates in meat-producing animals play a major role. Higher prevalence are classically observed for tropical countries with a humid and warm climate, and conversely, lower prevalence are found for arid countries or for colder countries, but anthropogenic factors explain a large part of the variations in human seroprevalence, including dietary habits (method of cooking meat, hand washing, kinds of meat or vegetables consumed, and vegetable cleaning, etc.); economic, social, or cultural habits; quality of water; and sanitation coverage. Seroprevalence increases with age, but the rate of acquisition of infection in relation to age varies according to the country and socioeconomic level. Near-maximal

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seroprevalence may be reached in childhood in populations living under poor-hygiene conditions, probably linked to telluric or waterborne contamination by oocyst ingestion. This points toward water as an important source of human infection in areas where humans use unfiltered surface water for consumption and probably also in areas where there is contact with freshwater, for instance, for recreation (Bahia-Oliveira, 2003; Ertug, 2005). As an example, in a city located in the northern Rio de Janeiro state (Brazil), the age-adjusted seroprevalence was 84% for the group of the lower socioeconomic level, compared to seroprevalences of 62% and 23% for the groups of the middle and upper socioeconomic levels, respectively (Bahia-Oliveira, 2003). Most persons (up to 84%) in the population of the lower socioeconomic level were infected by the age of 15 years, whereas infection was acquired mostly after the age of 20 years in the population of the upper socioeconomic level (from about 20% for the age group of 20 to 29 years to 70% for the age group of 40 to 49 years). In a multivariate risk factor analysis, this was attributed to differences in water supply, with the poorest populations living in areas supplied with unfiltered water. These different patterns of Toxoplasma acquisition according to socioeconomic levels may be more relevant in underdeveloped tropical countries, but in the United States, Toxoplasma infection was also considered an infection associated with poverty (Hotez, 2008). The overall seroprevalence (U.S. and foreign-born individuals combined) was higher among non-Hispanic black persons and Mexican Americans than among non-Hispanic white persons (Jones, 2007). Logically, increased socioeconomic levels, together with an improvement of hygienic conditions, changes in farming systems, the consumption of frozen meat, and the feeding of cats with

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sterilized food, have led to a continuous decrease of the seroprevalence in most industrialized countries over the last decades. In the United States, a national survey found a decrease in the age-adjusted *T. gondii* prevalence in United State-born persons aged 12 to 49 years, from 14.1% in 1988 to 1994 to 9% in 1999 to 2004 (Jones, 2007). In France, the seroprevalences in pregnant women were about 80% in the early 1960s, around 66% in the 1980s, 54% in 1995, and 44% in 2003, while at the same time, the average age of pregnant women increased (Villenea*et al.,* 2010).

2.11 Diagnostic Structure and Morphological Description of *Toxoplasma gondii*

Tachyzoites are crescentic in shape and approximately 6µm long and 2 µm wide (Bhopale, 2003). They are coated by a three-unit membrane, namely a plasma lemma and an inner membrane consisting of two closely situated membranes, all of which form a structure called the pellicle (Dubey *et al.,* 1998; Bhopale, 2003). Different organelles making up the tachyzoite (Figure 2.2) include apical and polar rings, rhoptries, micronemes, conoid, subpellicular microtubules, mitochondria, micropores, smooth and rough endoplasmic reticula, Golgi complexes, ribosomes and a nucleus consisting of a chromatin mass and a nucleolus (Dubey *et al.,* 1998).

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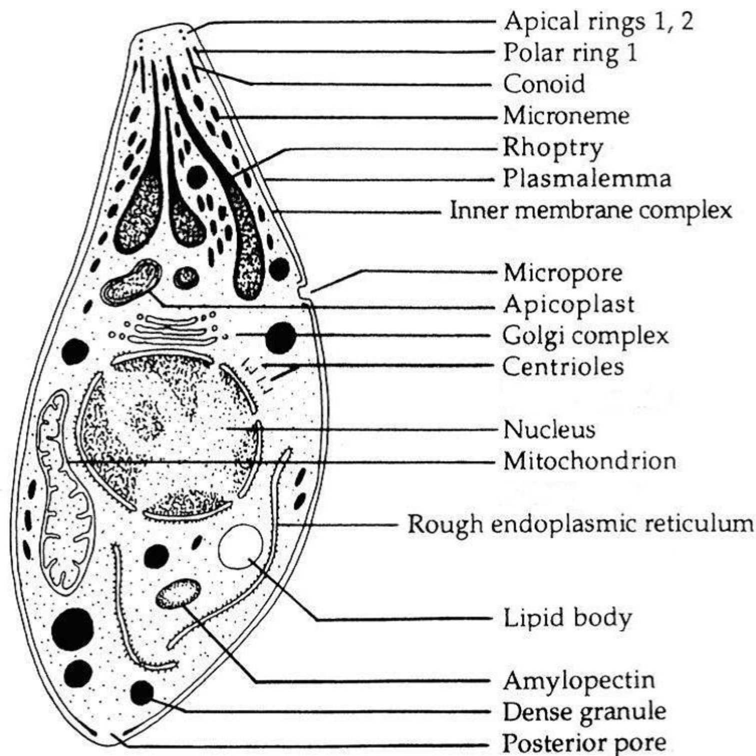

Figure 2.2: Schematic diagram showing organelles of a tachyzoite of *T. gondii*.

(Adapted from Dubey *et al.,* 1998)

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Tachyzoites are spread via the blood system in lymphocytes, macrophages and free in the plasma and are able to infect almost any type of tissue, most especially those in the eye, central nervous system, heart, placenta and skeletal muscle (Montoya and Liesenfeld, 2004). Moreover, tachyzoites are capable of crossing tissue boundaries, such as the blood-brain barrier and the placenta (Carruthers, 2002). They are able to multiply rapidly by a process called endodyogeny and this type of replication leads to cell necrosis when invaded cells can no longer hold these parasites and erupt. Replication of tachyzoites occurs during the first 8-12 days and this accounts for the acute phase of infection

(Carruthers, 2002). This stage is responsible for the clinical manifestations of the disease as it produces a strong inflammatory response (Montoya and Liesenfeld, 2004).

Toxoplasma gondii induces a strong type 1 T-cell-mediated immune response, which promotes a self-limiting infection, ensuring the survival of its host and thus itself (Carruthers, 2002). As the immune response progresses, interferon-γ, secreted by antigen-specific T-cells, restricts tachyzoite replication.

Tachyzoites are sensitive to proteolytic enzymes and can therefore be destroyed during gastric digestion. The pressure of the host's immune system on the parasite stimulates the formation of cysts and causes tachyzoites to be transformed into bradyzoites, marking the beginning of the chronic phase of the infection (Carruthers, 2002; Montoya and Liesenfeld, 2004).

Bradyzoites, the slowly dividing stage of the parasite, are more resistant to proteolytic enzymes and are therefore able to cause infection if ingested as a tissue cyst by a host (Tenter*et al.,* 2000). The structure of bradyzoites is illustrated in Figure 2.3

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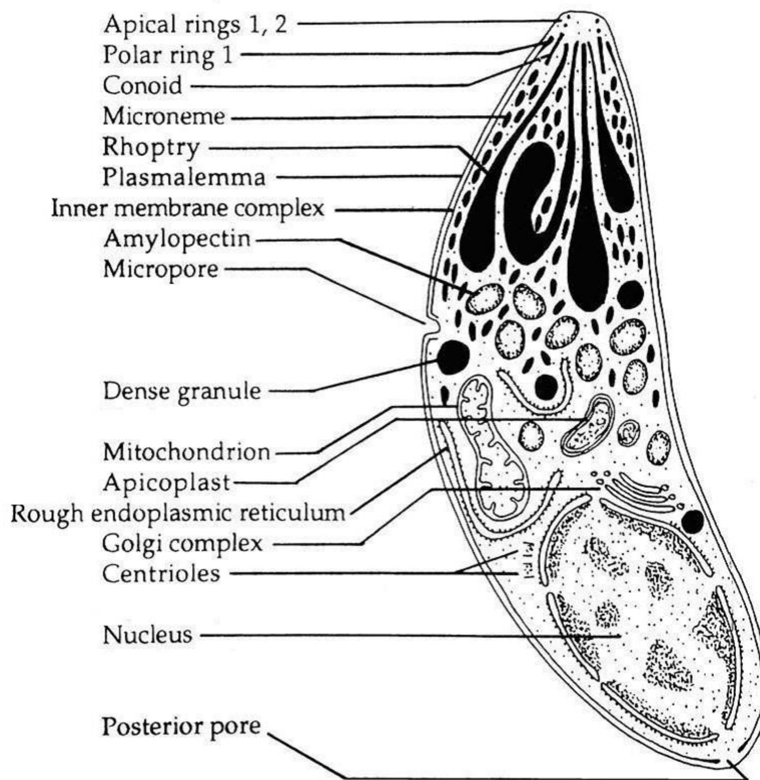

Figure 2.3: Schematic diagram showing organelles of a bradyzoite of *T. gondii* (Adapted from Dubey *et al.,*1998).

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Bradyzoites are found in quiescent tissue cysts, usually in the brain where cysts are spherical in shape, and in muscle tissue where they are elongated (Dubey *et al.,* 1998; Roberts and McLeod, 1999). Tissue cysts are able to persist for the duration of the host's life and bradyzoites can be released from these cysts to form tachyzoites again, causing a reactivated infection in immunocompromised hosts (Montoya and Liesenfeld, 2004).

Oocysts are 10 μm to 12 μm in size and are produced within the intestine of the definitive host. Inside the oocysts are sporozoites found and these are shed in the faeces of the definitive host (Peterson and Dubey, 2001). Characteristically, Oocysts measure approximately 10 by 12 μm. Sporulation occurs outside the body, and the oocyst becomes infectious 1 to 5 days after excretion. Each sporulated oocyst contains two sporocysts and each sporocyst contains four sporozoites. Sporulated oocysts are remarkably resistant and can survive in soil for several months.

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2.12 Diagnosis of Toxoplasmosis

Toxoplasmosis is frequently asymptomatic and clinical manifestations, when present, are usually non-specific and it mimics other infections, making definitive clinical diagnosis very difficult (Hill and Dubey, 2002; Kompalic-Cristo *et al.*, 2004). Diagnosis is usually made by immunological testing, histological identification, isolation in tissue culture, recovery of the parasite DNA by the polymerase chain reaction (PCR) or by a combination of these techniques. Cerebral toxoplasmosis can also be diagnosed using computerized tomography and magnetic resonance imaging (Hill and Dubey, 2002; Markus, 2003; Sukthana, 2006). Serological tests are most widely used, but they have the greatest limitations as they often provide ambiguous results (Markus, 2003; Kompalic-Cristo *et al.*, 2004).

Examples of these diagnostic tools include the Sabin-Feldman dye test, which is the traditional gold standard, indirect fluorescent antibody assay (IFA), complement fixation

test (CFT) and the enzyme-linked immunosorbent assay (ELISA). Serological tests are used to detect increased antibody levels such as IgG, IgM, IgA and IgE (Jones *et al.*, 2003). In a primary *T. gondii* infection, IgM appears a few weeks after infection, followed by IgA and IgE. These acute phase immunoglobulins peak after about two months and are usually undetectable by serological tests by six to nine months but can persist for longer periods of time (Montoya and Rosso, 2005; Sukthana, 2006). IgG, which appears after IgM, peaks after four months and persists at low levels throughout the duration of the host's life (Sukthana, 2006). A problem with serological tests is that the detection of antibodies in immunocompromised individuals may be difficult due to the deterioration of the immune system (Schneider *et al.,* 1992) because it will cause poor immune response to infections. A further problem is that IgM may persist for longer than expected periods and discrimination between recent and older infections may therefore be a problem (Ho-Yen *et al.*, 1992; Remington *et al.*, 2004). This is an important factor when diagnosing toxoplasmosis in immunocompromised individuals as the presence of IgG indicates a risk for the reactivation of a latent infection, and IgM indicates the possibility of an acute infection. Among pregnant women, positive IgM results indicate the likely acquisition of infection during gestation and a positive IgG and negative IgM result indicates a previous infection (Montoya and Rosso, 2005).

However, avidity tests have helped to overcome this problem as they help differentiate between recently and distantly acquired infections (Lappalainen and Hedman, 2004; Montoya and Rosso, 2005). Avidity tests are based on the fact that during acute infections, IgG antibodies bind antigen relatively weakly and therefore have a low avidity.

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Chronic infections, however, have more strongly-binding antibodies and therefore have a high avidity (Lappalainen and Hedman, 2004; Montoya and Rosso, 2005). Some of these problems can be overcome with the use of Polymerase Chain Reaction (PCR). This method has both advantages and disadvantages. Its advantages are that the detection of nucleic acid is not affected by the condition of the immune system, it is generally more sensitive and rapid than serological tests and diagnosis can be made from biopsies, blood, cerebrospinal fluid (CSF) and amniotic fluid. Disadvantages are that false positive results due to contamination may occur; it may be too sensitive in detecting nonviable *T. gondii* remnants that do not cause disease; and may yield false negative results due to inhibition (Johnson *et al.*, 1993).

These problems with PCR can, however, be overcome and more rapid and sensitive methods are regularly being developed. These advances in PCR techniques are helping to make it an invaluable diagnostic tool.

2.12.1 Sabin-Feldman Dye Test

Many serologic tests have been used to detect antibodies to *T gondii*. The most reliable of these is the Sabin-Feldman dye test. Live virulent tachyzoites of *T gondii* are used as antigen and are exposed to dilutions of the test serum and to a complement accessory factor resembling complement that is obtained from *Toxoplasma*-antibody free-human serum. This test is sensitive and so far is the most specific test for toxoplasmosis. But its main disadvantages are its high cost and the human hazard of using live organisms (Johnson *et al.*, 1993).

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2.12.2 Indirect Fluorescent Antibody Test

The indirect fluorescent antibody test (IFAT) overcomes some of the disadvantages of the dye test. In IFAT, killed tachyzoites of *Toxoplasma*, which are available commercially, are used as antigen. Titers obtained by IFAT are similar to those from the dye test. Disadvantages of the IFAT are that a microscope with UV light is needed, fluorescent anti-species globulin is required for each species to be tested, and false-positive titers may occur in hosts with anti-nuclear antibodies. The suitability of IFAT in animal diagnostic work is therefore limited, but it has proved useful in diagnosing acquired human toxoplasmosis (Johnson *et al.*, 1993).

2.12.3 Other serologic tests

Other serologic tests including the indirect haemagglutination test, the latex agglutination test, modified agglutination test, and the enzyme-linked immunoabsorbent assay (ELISA), offer some advantages. For example, agglutination tests are easy to perform. Soluble antigens used for indirect haemagglutination tests are now commercially available in several countries. Although this test is easy to perform, it usually does not detect antibodies during the acute phase of toxoplasmosis. In the modified agglutination test, whole killed tachyzoites are used as antigen, and the test serum is treated with 2-mercaptoethanol to eliminate nonspecific agglutinins. The ELISA test using soluble antigens appears to be specific and may be recommended as the standard test (Johnson *et al.*, 1993).

2.12.4 Interpretation of Results from Serologic Tests

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A single positive serum sample proves only that the host has been infected at some time in the past. Serologic evidence for an acute acquired infection is obtained when antibody titers rise by a factor of 4 to 16 in serum taken 2 to 4 weeks after the initial serum collection, or when specific IgM antibody is detected. The finding of antibody in even undiluted serum is useful in the diagnosis of ocular toxoplasmosis because patients with this disorder usually have low *T. gondii* antibody titers (Johnson *et al.*, 1993).

2.12.5 Histo-Cytology Staining Techniques

Diagnosis can be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy. This procedure is particularly useful in immunosuppressed patients or patients with AIDS, in whom antibody synthesis may be delayed and low. *Toxoplasma gondii* infection can be rapidly diagnosed by making impression smears of lesions on glass slides. After drying for 10 to 30 minutes, the smears are fixed in methyl alcohol and stained with Giemsa stain. Well-preserved *T gondii* organisms are crescent-shaped and stain well with any of the Romanowsky stains, however, degenerating organisms common in lesions usually appear oval and have cytoplasm that stains poorly compared to the nucleus. A diagnosis of toxoplasmosis should not be made unless organisms with the typical structure are seen, as degenerating host cells may resemble degenerating *T gondii* parasites. In thin sections the tachyzoites are oval to round and usually do not stain differently from host cells. Tissue cysts are occasionally encountered in areas with lesions.Tissue cysts are usually spherical and have silver-positive walls; the bradyzoites stain strongly with periodic acid—Schiff stain. Immunohistochemical staining and

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polymerase chain reaction (PCR) can be used to identify *T gondii* tissue cysts or tachyzoites in tissues, even those fixed in formalin. Electron-micrographic examination can aid in diagnosis (Johnson *et al.*, 1993).

2.12.6 Radiologic Test

Computed tomography techniques are also useful in the diagnosis of human cerebral toxoplasmosis.

2.12.7 Animal Inoculation

Inoculation of biopsy materials into laboratory mice and/or cell cultures can help diagnosis.

2.13 Diagnostic Procedure

2.13.1 Identification of Toxoplasma agent

2.13.1.1 Isolation of *Toxoplasma gondii*

Isolation of *T. gondii* from aborted ovine and caprine fetuses and fetal membranes is best made by inoculation of laboratory mice. The best tissues for inoculation are fetal brain and placental cotyledons, and optimum results are obtained with fresh samples free from contamination. Importantly, samples must not be frozen at any stage, as this kills the parasite.

Protocol for the isolation *T. gondii* is outlined below:

i) With aseptic precautions, remove 2–5 g of placental cotyledon or brain tissue from the aborted fetus.

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ii) Homogenise the tissue in an equal volume of 0.3 M sterile phosphate buffered saline (PBS), pH 7.4, with added antibiotics (100 International Units [IU]/ml penicillin and 745 IU/ml streptomycin) in a 'stomacher' (Seward Laboratory, London) or other suitable macerating equipment. Brain tissue may be effectively homogenised by passing it through a 16-gauge needle ten times by means of a syringe.

iii) Inoculate each of three Toxoplasma-free mice intraperitoneally with 0.5 ml of the homogenate.

iv) Kill the mice 6–8 weeks after inoculation and remove the brains. Blood should also be recovered from the mice at this stage and the serum separated and stored at –20°C. Brains from mice that die before 6–8 weeks should also be harvested.

v) Homogenise each mouse brain with an equal volume of sterile PBS by passing through a 16-gauge needle ten times by means of a syringe.

vi) Spread one drop (5 µl) of a given suspension on each of five slides.

vii) Dry and stain with Giemsa, dehydrate and mount under a cover-slip.

viii) Examine slides under a microscope. Tissue cysts appear as circular structures measuring 5–50 µm filled with blue-staining, crescent-shaped bradyzoites.

An alternative method for examining the mouse brain is to take a small portion of forebrain (approximately match-head size) squashed flat with a cover-slip. Tissue cysts should be easily detected under the microscope.

If the tissues inoculated are heavily infected with T. gondii, mice may die at 1–2 weeks.

Failure to demonstrate tissue cysts does not rule out a positive diagnosis. Serum from the mice may be analysed for the presence of antibodies to Toxoplasma (e.g. using an

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indirect fluorescent antibody [IFA] test); if this analysis is also negative, infection with Toxoplasma is unlikely.

2.13.1.2 Histopathology (Tissue section examination)

In animals that die with acute toxoplasmosis, focal mononuclear inflammation with or without focal necrosis may be seen in a number of tissues, including the liver, heart and lungs. The latter may be oedematous. Lymph nodes may have undergone expansion and there may or may not be focal necrosis with or without haemorrhage. Typically Toxoplasma tachyzoites may be demonstrable in association with necrosis and inflammation.

In cases of abortion and stillbirth in sheep and goats, affected placental cotyledons typically contain large foci of coagulative necrosis that may have become mineralised with time. Any associated inflammation is characteristically slight and nonsuppurative. Well preserved samples of placental cotyledons may show moderate oedema of the mesenchyme of the fetal villi with a diffuse hypercellularity due to the presence of large mononuclear cells. Sometimes small numbers of intracellular and extracellular toxoplasms are visible, usually on the periphery of a necrotic area or in a villus that is in the early stages of infection. The Toxoplasma tachyzoites appear ovoid, 2–6 µm long, with nuclei that are moderately basophilic and located centrally or towards the posterior end.

In the fetal brain primary and secondary lesions may develop. Microglial foci, typically with a necrotic and sometimes mineralisedcentre and often associated with a mild focal

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lymphoid meningitis, represent a fetal immune response following direct damage by local parasite multiplication. Toxoplasma tissue cysts are only rarely found, usually at the periphery of these lesions. Focal leukomalacia is also common and is considered to be due to fetal anoxia in late gestation caused by advanced lesions in the placentome preventing sufficient oxygen transfer from mother to fetus. Such foci occur most commonly in the cerebral white matter cores, but
sometimes also in the cerebellar white matter. Focal leukomalacia on its own suggests placental disease or acute insufficiency but the two types of neuropathological change seen together are characteristic of Toxoplasma infection. Confirmation of the identity of *T. gondii*-like structures in tissue sections from such cases, as well as from instances of acute toxoplasmosis, may be achieved by immunohistochemistry that labels intact T. gondii or antigenic debris. The method is both convenient and sensitive and is used with fixed tissues (including archive tissues) that may also exhibit a degree of decomposition, where isolation would not be appropriate or possible. The ABC indirect immunoperoxidase method and the peroxidase–antiperoxidase (PAP) technique are equally good (Uggla *etal.,* 1987).

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2.13.1.3 Nucleic acid recognition methods

Several polymerase chain reaction (PCR)-based assays have been developed for the detection of DNA from *T. gondii*. The main target regions are the B1 repetitive sequence (Burg et al., 1989), the P30 (SAG1) gene (Savva *et al*., 1990) and 18S ribosomal RNA (rRNA) (Ellis, 1998). The sensitivity of the PCR is dependent on the copy number of the

target sequence (P30: 1 copy; B1: 35 copies; rRNA: 110 repeat units). Customised synthetic DNA oligonucleotides are commercially available. Moreover, the method for amplification of the B1 repetitive sequence is used to analyse the lens aspirates of congenitally infected human cataract patients (Mahalakshima et al., 2007) and was found to be more sensitive than the enzyme-linked immunosorbent assay (ELISA). However, although the PCR is extremely sensitive, care should be taken if it is the only test available, as in many situations a more reliable diagnosis will be gained if it is used in combination with other diagnostic data.

Moreover, a real-time PCR has been developed to allow simultaneous quantification and amplification of DNA. It is very similar to existing PCR methods and can be carried out on 96-well microtitre plates. After each round of amplification, fluorogenic dyes intercalate with the double-stranded DNA and the results, shown on an amplification plot, allow quantification of the parasite DNA in the sample. Real-time PCR has been used to amplify and quantify DNA from the T. gondii B1 gene (Costa *et al*., 2000; Lin *et al*, 2000). This quantification of parasite DNA can be used to determine the number of parasites in tissues and fluids, such as the amniotic fluid of patients suspected of being congenitally infected with T. gondii. (Nagy *et al*, 2007). The real-time PCR is a highly sensitive and specific method, however it is expensive and requires specialised detection systems and therefore may only be cost-effective in laboratories where analysis of large numbers of samples is carried out.

The following method is a nested form of the PCR, amplifying the B1 repetitive sequence of DNA (Wastling*et al*., 1993).

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Parasite DNA can be extracted and purified from several tissues, including placenta, the central nervous system, heart and skeletal muscle.

Contaminating red blood cells in tissues are removed by washing in 10 mM Tris/NH_4Cl lysis buffer, pH 7.6, followed by centrifugation at 2000 g for 15 minutes. DNA is then extracted from the resultant pellet and resuspended in 10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ containing proteinase K 100 µg/ml and 0.5% Tween 20.

Samples are incubated at 55°C for at least 1 hour, then the proteinase K is inactivated by boiling. The PCR procedure is performed in 50 µl volumes. The reaction mixture contains 10 mM Tris, pH 8.3, 2.5 mM $MgCl_2$, 40 mM KCl, 0.01% gelatin, 0.1 mM dNTPs, 0.2 µM of each primer, two sense primers P1 and P2 and two antisense primers P3 and P4, and 2.5 units of Taq polymerase.

Primary amplification is performed with primers 1 and 4 to give a 193 bp product over 25 cycles of 93°C for 1 minute, 50°C for 1.5 minutes and 72°C for 3 minutes. The amplified product is then diluted 1/20 in distilled water to reduce amplification of non-specific products.

Secondary amplification using nested primers 2 and 3 and the same reaction conditions, is carried out over 15 cycles to give a 94 bp product. The final product is then visualised on 1% agarose gels. Southern blotting, using a labeled probe, can be used to confirm the identity of the B1 PCR products and to distinguish them from non-specific products.

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2.13.1.4 Oocyst detection in water

Toxoplasma gondii oocysts have been detected in drinking water using the method for the detection of Cryptosporidium oocysts (Isaac-Renton *et al*, 1998). This method relies on the collection of a large-volume sample of water and passing it through a cartridge filter. Identification of Toxoplasma oocysts was by means of inoculation of rodents (Isaac-Renton *et al*, 1998).

2.13.2 Serological tests

There are several serological tests available for the detection of T. gondii antibodies. In one type of test the observer judges the given colour of tachyzoites under a microscope, such as with the dye test (DT) and IFA test.

Another depends on the principle of agglutination of Toxoplasma tachyzoites, red blood cells or latex particles, as with the direct agglutination test (DAT) and indirect haemagglutination test (IHA) and latex agglutination (LA) test, respectively. With the ELISA, the degree of colour change defines the quantity of specific antibody in a given solution. The DT, IFA test, DAT and ELISA are outlined below and the IFA test is given in more detail.

The Dye Test (Sabin and Feldman, 1948) is the so-called ‘gold standard’ serological test for Toxoplasma antibody in humans. Live Toxoplasma tachyzoites are incubated with a complement-like accessory factor and the test serum at 37°C for 1 hour before methylene blue is added. Specific antibody induces membrane permeability in the parasite so that the cytoplasm is able to leak out and the tachyzoite does not incorporate the dye and so appears colourless. Tachyzoites not exposed to specific antibody (i.e. a negative serum

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sample) take up the dye and appear blue. The DT is both specific and sensitive in humans, but may be unreliable in other species. In addition, it is potentially hazardous as live parasite is used. It is expensive and requires a high degree of technical expertise. It should be noted that on animal welfare grounds, tachyzoites should be grown in tissue culture rather than in mouse peritoneum whenever possible.

The IFA test (Munday and Corbould, 1971) is a simple and widely used method. Whole, killed Toxoplasma tachyzoites are incubated with diluted test serum, the appropriate fluorescent antispecies serum is added, and the result is then viewed with a fluorescence microscope. Fluorescent-labelled antibodies are available commercially for a variety of animal species, the method is relatively inexpensive and kits are commercially available. However, the method requires a fluorescence microscope and the results are read by eye, so individual variation may occur. It may be difficult to find some species-specific conjugates and there is a risk of possible cross-reactivity with rheumatoid factor and anti-nuclear antibodies.

The DAT (Desmonts and Remington, 1980) is both sensitive and specific. Formalinised Toxoplasma tachyzoites are added to U-shaped well microtitre plates and dilutions of test sera are then applied. Positive samples will produce agglutination that can be graded, while negative samples will produce a ‘button’ of precipitated tachyzoites at the bottom of the well. The test is simple and easy to perform although relatively large amounts of antigen are required. Kits are commercially available. The method of growth and harvesting of parasites is given below. A commercially available latex agglutination test

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(LAT) is also available. The DAT and LAT are not species specific and are suitable for use in all species.

The original ELISA (Voller*et al*, 1976) uses a soluble antigen preparation made from Toxoplasma RH strain tachyzoites (as described below) and layered into wells in a microtitre plate. Test sera (e.g. ovine in origin) are added, followed by an anti-species enzyme-labelled conjugate such as horseradish peroxidase-labelled anti-ovine-IgG. Any attached conjugate causes a colour change in the substrate that is directly related to the amount of bound antibody, and which can be read with a spectrophotometer at the absorbance specific to the substrate used. The assay is simple, can readily test a large number of samples and is easy to perform with the chosen anti-species conjugate.

Defined anti-species conjugates, substrates and whole kits are commercially available. However, the assay does require a spectrophotometer. The ELISA is well suited to laboratories required to analyse large numbers of samples.

Recently, kinetics ELISA (KELA) has been developed (Werre*et al*, 2002). The KELA system measures the rate of reaction between bound enzyme and the substrate solution that leads to development of colour. Three optical densities (OD) are read at 45-second intervals (using the KELA data management program) and the results are reported in terms of slopes. The correlation between the ELISA and the KELA is very high, and therefore, the two tests are very good diagnostic tools, differing only in their convenience of application.

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To improve the specificity of the conventional ELISA, assays that use recombinant antigens (Johnson and Illana, 1991) and affinity purified Toxoplasma-specific antigens (Lekutis, 2001) have been developed for use (Sager *et al,* 2003 and Tenter*et al*, 1992).

With the conventional ELISA the detection of Toxoplasma-specific IgG and IgM antibodies allows a degree of discrimination between acute and chronic toxoplasmosis. More recently avidity assays have been developed. As the immune response matures, after infection is established, so antibodies of increasing avidity (functional affinity) for the antigen develop. This avidity can be measured and used to indicate active or recent *T. gondii* infection. An assay for the detection of avidity of IgG for the P30 antigen of *T. gondii* in sheep has been developed (Sager *et al*, 2003). This test is a good diagnostic tool for discriminating relatively recent from more established infections.

2.13.3 Preparation of antisera and antigens

Antisera to *T. gondii* and conjugated antisera for use in the IFA test and ELISA, to allow screening of a variety of animal species, may be obtained commercially. International standards for animal sera are not available.

Below are protocols for the preparation of tachyzoite antigen for use in the IFA test and ELISA. Tachyzoites maybe grown in mice or in tissue culture and retained as whole parasites for the IFA test, or prepared as soluble antigen for the ELISA.

• **Production of Toxoplasma tachyzoites in mice**

i) Inject each of six Toxoplasma-free mice intraperitoneally using a 1-ml syringe and a 23-gauge needle, with 0.2 ml of 1 × 107/ml T. gondii tachyzoites of the RH strain, collected fresh from a previous mouse passage or from tissue culture. (For optimum

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recovery of tachyzoites having minimal host mononuclear cells, mice should be more than 6–8 weeks of age and weigh approximately 22–25 g.)

ii) Kill the mice 3 days later by CO_2 inhalation (avoid cervical dislocation as this may cause contamination of peritoneal fluid with red blood cells).

iii) Pin the mouse out on its back on a clean cork mat. Reflect the abdominal skin with aseptic precautions, remove any peritoneal fluid by means of a 21-gauge needle attached to a 1 ml syringe and gently eject the harvested exudate into an equal volume of PBS.

The optimum time to collect tachyzoites is 72 hours after initial inoculation, when there will be sufficient organisms but before there is significant contamination by host cells. It is also important not to delay harvesting peritoneal fluid much past 3 days as the mice will die. (If tachyzoites for mouse inoculation are taken from a frozen stabilate, it may be necessary to harvest mice 4 or 5 days after the initial inoculation and passage the parasite once more through mice before using it as an antigen in the above tests.)

iv) Centrifuge the fluid at 500 g for 5 minutes, aspirate supernatant and resuspend in Hanks' balanced salt solution (HBSS). Alternate between PBS and HBSS washes by centrifugation.

v) Calculate the concentration of tachyzoites and contaminating host cells with an improved Neubauer counting chamber (Count numbers of tachyzoites at 1/1000 dilution and cellular contamination at 1/10).

vi) Carry out further washes (step iv above) as required to reduce cellular contamination to <0.5% host mononuclear cells and <0.25% for red blood cells.

vii) Resuspend the tachyzoites in PBS to give a final concentration of 1 × 107/ml.

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viii) Tachyzoites may be maintained by continual passage in this manner without the addition of penicillin/streptomycin by observing strict aseptic procedures.

• **Preparation of aliquots of a frozen stabilate of *T. gondii* tachyzoites**

i) Produce tachyzoites in mice or tissue culture as described above

ii) Centrifuge at 500 g for 5 minutes and resuspend in Iscove's modified Dulbecco's medium (IMDM) (Gibco BRL, Paisley, UK) approximately three times.

iii) Add the following solutions to give these concentrations: 10% dimethyl sulphoxide; 20% normal horse serum (free from antibody to T. gondii); 70% resuspended tachyzoites to give a final concentration of 1 × 108 tachyzoites/ml.

iv) Allow the preparation to stand on the bench for 1 hour.

v) Dispense into 1-ml aliquots using screw-topped tubes appropriate for liquid nitrogen storage.

vi) Put the tubes into a small container, wrap in thick insulating material and place in –70°C freezer to allow the tachyzoites to freeze gradually.

vii) The next day transfer to liquid nitrogen, keeping well insulated while transferring.

viii) This stabilate may then be used for mouse inoculation or tissue culture growth of the parasite. When removing from storage thaw the sample rapidly in warm water.

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• **Production of Toxoplasma tachyzoites in tissue culture**

i) *Toxoplasma gondii* can be grown and maintained in tissue culture by twice-weekly passage in African green monkey kidney (Vero) cells.

ii) Cells and parasite are grown in IMDM supplemented with 50 IU/ml penicillin, 50 µg/ml streptomycin and 2% fetal bovine serum.

iii) Tachyzoites are harvested from tissue culture flasks by scraping the cell monolayer using a sterile cell scraper.

iv) Using 25 cm2 vented tissue culture flasks that have each been seeded with 1 × 105 Vero cells, add tachyzoites at the rate of two tachyzoites per monolayer cell and incubate at 37°C in a 5% CO_2 humidified chamber. Harvest after 3–4 days.

• Preparation of whole tachyzoites for use in the IFA test

i) Produce 4 × 107/ml suspension of RH strain T. gondii tachyzoites in PBS.

ii) Add formaldehyde (40%) to give a final concentration of 0.2% (v/v).

iii) Incubate at 4°C overnight and divide into aliquots in suitable sealed tubes and store frozen until required.

• Production of soluble antigen for ELISA

i) Produce a suspension of RH strain T. gondii tachyzoites in PBS.

ii) Centrifuge at 2000 g for 15 minutes, retain the pellet and resuspend it in nine times its volume of distilled water.

iii) Rupture the tachyzoites by freezing and thawing three times.

iv) The antigen preparation is then sonicated for 20 seconds at 4°C at an amplitude of 20 microns.

v) Remove any cellular debris by centrifugation at 10,000 g for 30 minutes at 4°C.

vi) Retain the supernatant and store at –20°C until required. (Protein estimation might be expected to give a value of between 2 and 4 µg/ml.)

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• **Protocol for the IFA test**

The following is a protocol for carrying out an IFA test for anti-Toxoplasma IgG antibodies in sheep serum. It only requires minor modifications for testing different species or for measuring IgM antibody.

i) Clean the required number of tissue culture 15-well multitest slides (Flow laboratories) and allow drying.

ii) Layer 5 µl of a whole tachyzoite preparation on to each well and allow to dry.

iii) Fix in methanol for 10 minutes.

iv) Wash twice for 10 minutes each time in 0.3 M PBS, pH 7.4.

v) Add 5 µl of the given test sheep serum (diluted in PBS) to each well. (Prepare serial dilutions of the test sera, e.g. 1/16, 1/32, etc. up to 1/1024.) Ensure that positive and negative control sera are included in each test as well as a 'PBS-only' sample. Incubate for 30 minutes at room temperature.

vi) Wash twice for 10 minutes each time in PBS.

vii) Add 5 µl of an appropriate dilution of rabbit-anti-sheep IgG conjugated to fluorescein isothiocyanate, diluted in 0.2% filtered Evan's blue dye in PBS, to each well and incubate for 30 minutes at room temperature.

viii) Wash three times for 10 minutes each time in PBS.

ix) Mount the slides under cover-slips with buffered glycerol (nine parts PBS one part glycerol) or Citifluor (Citifluor Ltd, London).

x) Examine using a fluorescence microscope, fitted with ×20 and ×40 objective lenses.

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With a negative test serum result the whole parasites will appear red due to the autofluorescence of the Evan's blue dye. They may also present with a green fluorescent cap at the parasite pole (nonspecific polar fluorescence). With a positive test serum the parasites will fluoresce red and at least 80% of them within a given well will be surrounded by an unbroken band of green fluorescence. In an adult sheep/goat a positive titre could be defined as ≥1/64 and a negative titre as ≤1/32. For lamb/kid and fetal sera, respective titres could be defined as ≥1/32 and ≤1/16.

2.14 Prevention and Control Measures for Toxoplasmosis

2.14.1 How to Avoid Toxoplasma Infection

Hygienic measures are paramount to avoiding infection. Health education measures to prevent primary *T. gondii* infection and is drawn directly from the acquired knowledge of the intrinsic resistance and biological characteristics of the infective stages of *T. gondii*. Persons should be advised that they should wash their hands after contact with raw meat, after gardening or other external activity with contact with soil, and after having close contact with a cat. In addition, persons should wash fruits and vegetables (especially those growing in contact with soil) thoroughly before eating them raw. If the person owns a cat, the litter box should be changed every 2 days, preferably by another person, or alternatively, persons should wear a mask and gloves when changing the litter box. Persons should be encouraged to keep their cats inside and feed them only canned or dried commercial food (Cook *et al., 2000*).

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2.14.2 Basic hygienic measures for prevention of toxoplasmosis

The spread of such measures depends on the health care policy applied in a given country. Physicians are at the first line to comprehensively explain the preventive measures to women who are pregnant or plan to be pregnant. Oral counseling should be accompanied by written information for optimal retaining of the information throughout pregnancy. There is evidence to suggest that health education approaches may help reduce the risk of congenital toxoplasmosis, but evaluation studies of educational policies worldwide are lacking (Gollub*et al.,* 2008). It could be deduced from an epidemiological case-control study conducted in France that cat-related prevention measures are probably well known, since cat owners were not more likely to acquire toxoplasmosis than others (Baril*et al.,* 1999). Indeed, cat owners are usually warned of the risk associated with cleaning the litter box or having close contact with cats or kittens and take appropriate measures. Conversely, in that study, the risk for primary acquired infection was highly associated with the consumption of undercooked meat or with rural living, suggesting acquisition through contaminated soil (gardening or working in a rural environment) (Baril*et al., 1999*). Undercooked meat was the main risk factor for infection of pregnant women in another European case-control study, leading those authors to propose the clear labeling of meat at risk according to farming and processing methods (Cook *et al., 2000*). As freezing meat during at least 3 days at −12°C usually allows the killing of cysts from pork meat (Dubey, *1988*), the purchase of frozen meat (at least −20°C) may be recommended to people who cannot think of eating well-done pieces of beef or lamb, on the basis that long periods of freezing at low temperatures should kill all cysts.

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The risk associated with water consumption is less known and was recently reviewed by Jones and Dubey in 2010 (Jones and Dubey, *2010*). More attention is indeed being given to the consumption of water, particularly untreated or unfiltered water, in countries where surface water is the main source of drinking water and where water-filtering systems are malfunctioning or use water filters with a diameter that is too large. Several outbreaks of waterborne toxoplasmosis have been reported both in developing countries (Brazil and India) (Heukelbach*et al., 2007*) and in developed countries (North America and Poland) suggesting that prevention measures should now stress this specific risk and recommend the consumption of mineral water for pregnant women. Moreover, it has not been excluded that the ingestion of contaminated water from lakes or rivers during recreational activities could be a source of Toxoplasma infection, which may explain the large proportions of cases of unexplained toxoplasmosis in pregnant women, as shown in a study in the Northern United States (Boyer *et al., 2011*). From a general point of view, given the potentially huge contamination of the environment by oocyst spreading (Jones and Dubey, *2010*), one must keep in mind all hygienic measures in relation with external activities and should wash hands thoroughly after gardening or other recreation activities.

An introduction of new prevention messages concerning the risk due to more recently recognized sources of contamination (unpasteurized goat's milk and raw shellfish, etc.) is also proposed (Jones *et al., 2009*).

The same guidelines of primary prevention can be given to immunocompromised patients who are seronegative, to avoid Toxoplasma contamination. Recent knowledge of strain

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virulence should be taken into consideration, and these recommendations should now also be provided to travelers who are visiting countries where atypical strains predominate, even if they were previously immunized.

2.14.3 Practical steps for Prevention and Control of Toxoplasmosis

Improper handling of cat litter and not necessarily ownership of cat is accepted as a risk factor of toxoplasmosis (Walker *et al*., 2008). This definitely determines what measures would be effective in preventing or controlling the spread of toxoplasmosis. There are general sanitation and food safety steps needed to be taken to prevent one from becoming infected with *Toxoplasma gondii.* They include the following:

(i)Cats found to be shedding oocysts should be removed from the premises temporarily and treated to eliminate shedding. Since cats are usually meticulous groomers, it is unlikely that oocysts will be found on their fur. This means that regular handling will not be a significant risk.

(ii) Microwave cooking, salting and smoking do not consistently kill all infective *Toxoplasma* stages. So meat should be frozen to –12ºC for at least 24 hours to kill *Toxoplasma* tissue cysts, but it must be noted that sporulated oocysts can survive at –20ºC for up to 28 days.

(iii) Kitchen utensils and surfaces that have come in contact with raw meat should be washed with soap and scalding hot water to kill any bradyzoites or tachyzoites present.

(iv) Individuals should always wash their hands thoroughly after contact with cat stool, litter or litter box.

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(v) Cat feces should be disposed of daily to reduce the risk of transmission. Feces and dirty litter can be disposed of in a septic system if the litter is biodegradable, sealed tightly in a plastic bag and placed in the garbage, or incinerated. Backyard compost units do not produce sufficient heat to destroy oocysts and other pathogens potentially present in fecal material.

(vi) keep cats out of sandboxes and other areas where children play to prevent the cats defecating there (Tenter*et al*., 2000).

(vii) Unwashed fruits or vegetables as well as unpasteurised milk should not be eaten.
(viii) One must ensure that all avenues that could bring one in contact with cat faeces either directly or indirectly are blocked, while the intermediate host population must be properly checked.

2.14.4 Strategic Approaches for Screening, Prevention, and Control of Congenital Toxoplasmosis

2.14.4.1 Screening and treatment of pregnant women to reduce parasite transmission

Serological screening of pregnant women differs among countries according to the prevalence of Toxoplasma and health care policies. The high prevalence (>70%) observed in France in the 1970s motivated the implementation in 1985 of mandatory prenatal serologic screening during the first 3 months of pregnancy, which was strengthened in 1992 by the implementation of monthly repeated testing of seronegative women during pregnancy. In Austria and some other European countries (Belgium,

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Norway, and Italy, at least in some regions), a retesting schedule at 3-month intervals has been implemented, whereas other countries (Poland, Denmark, Sweden, and the United States) have no prenatal screening program. In Switzerland, where the seroprevalence is intermediate (about 25%), the Swiss Working Group on Congenital Toxoplasmosis recently recommended the abandonment of the surveillance and prevention program, arguing for the low incidence and morbidity of congenital toxoplasmosis in this country, although this recommendation is not unanimously shared by all teams (Stricker *et al.,* 2009). In the absence of screening, hygienic measures are the keystone of prevention and should be largely disseminated to pregnant women. In countries with prenatal screening policies, serology should be prescribed as soon as the pregnancy is diagnosed so that the serologic status can be interpreted unambiguously and prevention measures can be given to seronegative patients early to avoid infection. Repeated serologic screening offers the option to start a specific treatment as soon as seroconversion is observed and to propose a prenatal diagnosis. However, independently of any critical consideration about treatment efficacy, there are two limitations to prenatal screening. First, a consequence of screening is the anxiety generated by the diagnosis of Toxoplasma infection in the mother, as highlighted by some authors (Eskild A, and Magnus,.2001). This anxiety may lead the couple to prematurely terminate the pregnancy but, on the other hand, can be greatly lightened in cases of negative amniocentesis results. Second, other authors reported difficulty in applying systematic screening and the possible lack of compliance of patients, limiting the efficacy of health policies (Cornu*et al.,* 2009). In addition, the risk of adverse events associated with amniocentesis must be weighed against the risk of fetal

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transmission associated with the time of pregnancy when maternal infection occurred. A meta-analysis reported prenatal diagnosis-associated risks of fetal loss ranging from 0.33% to 0.74% until 2002 and from 0.3 to 0.4% in recent cohort studies (Khoshnood*et al.* 2007), which are lower than the rate of transmission of parasites during the first trimester of pregnancy but similar to or higher than the rate of transmission of parasites following periconceptional toxoplasmosis. Therefore, the disease burden of congenital toxoplasmosis may be diversely appreciated according to the health care system: the burden of morbidity is expected to be higher in countries without prenatal screening, but the burden of mortality is higher in countries with active prenatal screening (voluntary termination, medical abortion, or fetal loss due to amniocentesis) (Havelaar*et al.,* 2007). However, decisions about the implementation of prenatal screening in a given country should be carefully considered and based on prevalence data, disease burden, technical resources, and diagnostic costs.

As part of primary prevention, the aim of diagnosing maternal infection through serologic screening is to treat the mother to avoid vertical transmission. Spiramycin has been used in France since the 1960s for the primary prevention of congenital toxoplasmosis (Garin*et al*., 1968), but a combination of pyrimethamine-sulfonamide in association with folinic acid can also be used after the first trimester of pregnancy. The rationale for the use of spiramycin relies on reducing the parasite burden in the mother (mainly bloodstream and placenta), since this molecule hardly crosses the placental barrier (Gratzl*et al.,* 2002), whereas pyrimethamine-sulfonamide does so efficiently, as demonstrated by comparative dosages of these drugs in maternal and cord blood sera at

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delivery (Chemla*et al.,* 2005). However, over the last decade, contradictory results on treatment efficacy drawn from several epidemiological studies or meta-analyses of retrospective cohorts have opened a large debate questioning the pertinence of screening. A systematic review by Wallon*etal*. (Wallon*et al.,* 1999) included for analysis 9 out of 2,591 papers on congenital toxoplasmosis and concluded that there was an absence of a clear effect of treatment on reducing vertical transmission. However, some important parameters were lacking in some of those papers, i.e., a prolonged follow-up of newborns for >6 months (one study), the precise delay between maternal infection and the onset of treatment (all 9 studies), and the lack of adequate untreated controls, thus limiting the reliability of general conclusions on treatment efficacy. The delay to treatment is indeed a key point for the evaluation of treatment efficacy, as is an adjustment for the age of gestation at the time of maternal infection. The EMSCOT (European Multicenter Study on Congenital Toxoplasmosis) (Gilbert *et al.,* 2003), a large meta-analysis of 1,208 mother-child pairs from 11 centers, found no effect of treatment on reducing parasite transmission, but those authors did not exclude that their analysis could lack power. Indeed, there are several biases in the interpretations of results from this large-cohort study, such as differences in treatment regimens, the low number of untreated controls (only 106 out of 1,208 patients), and, again, the possible long delay for treatment in cohorts from countries with serologic screening at 3-month intervals. In addition, in that study, the median treatment delay for women first treated with spiramycin was about 4 weeks, whereas it was >8 weeks for those first treated with pyrimethamine-sulfonamide, which could contribute to the decrease in the overall treatment efficacy, since the most

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powerful drug combination was given with a greater delay. Two other retrospective cohort studies failed to detect an effect of treatment on reducing parasite transmission, whatever the delay to treatment (Foulon*et al.,* 1999; Gilbert *et al.,* 2001). Nevertheless, a more recent large meta-analysis from the SYROCOT (Systematic Review on Congenital Toxoplasmosis) study group (Thiebaut*et al*., 2007) including 26 cohorts and 1,745 infected mothers (307 untreated) found weak evidence that treatment that started within 3 weeks of seroconversion reduced mother-to-child transmission compared with treatment started after >8 weeks. All authors agreed to conclude that definitive conclusions on prenatal treatment efficacy should rely on a large randomized controlled trial. However, due to ethical considerations, a trial including an untreated group cannot be undertaken in countries where screening and prevention are routine. To answer, at least partly, the debate on maternal treatment, a 3-year French national clinical trial was started in 2010 to compare the treatment efficacies of spiramycin and pyrimethamine-sulfadiazine and should include 330 patients. The analysis of clinical outcomes in newborns and of transmission rates in both groups should help to answer the question of the role of both the delay of treatment and the therapy used according to the stage of pregnancy.

2.14.4.2 Prenatal screening and treatment to limit fetal damage

Besides decreasing vertical transmission, the associated goal of prenatal treatment is to reduce fetal damage or newborn sequelae, provided that transmission has occurred. The efficacy of prenatal treatment to reduce clinical manifestations at birth can be evaluated with cohorts of patients benefiting from prenatal screening, but as discussed above, the

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evaluation often suffers from the lack of untreated controls and from the necessity to precisely date the maternal infection, since the severity of fetal infection, and, proportionally, the capacity for treatment to reduce sequelae, depends on the term of gestation at seroconversion. Again, different studies have yielded contrasting conclusions. The SYROCOT study found no evidence that prenatal treatment reduced the risk of clinical manifestations (Thiebaut*et al.,* 2007). However, two recent cohort studies showed that (i) any prenatal treatment (spiramycin or pyrimethamine-sulfonamide) reduced the risk of serious neurological sequelae (Cortina-Borja *et al., 2010*) and (ii) any prenatal treatment reduced the risk of intracranial lesions, provided that treatment was given within the first 4 weeks following maternal infection (Gras *et al., 2005*). In addition, in the prospective EMSCOT (Cortina- Borja *et al., 2010*), the number of infected fetuses needed to be treated to prevent 1 case was only 3 for maternal infections at 10 weeks of gestation. These findings hence support prenatal screening and are in agreement with the observation that the systematic screening and treatment of pregnant women have decreased the rate of severe congenital infections in France, where the rate of asymptomatic infected neonates is about 85% at birth (Villena*et al., 2010*). However, due to the lack of a national surveillance system at the onset of the prevention program, it is not possible to quantify precisely the gain in terms of disease burden since then. The impact of treatment on eye sequelae is less clear. Some studies did not find any beneficial effect of prenatal treatment on the number of eye lesions or episodes of recurrence after birth (Freeman K, *et al. 2008*, Thiebaut R *et al*. 2007), whereas others did (Foulon W, et al. *1999*, Kieffer F, *et al. 2008*). Kieffer *et al.* reported on a series of 300 infected infants

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and described that the risk of developing a first retinochoroiditis before the age of 2 years was associated with a delay of >8 weeks between maternal infection and prenatal treatment (Kieffer F, et al. *2008*). Wallon et al. reported the longitudinal follow-up of 327 infected infants (275 of whom were treated antenatally) who were treated with pyrimethamine and sulfadiazine during their first year of life (Wallon M, *et al. 2004*). During a median follow-up time of 6 years, 24% of children developed at least one lesion of chorioretinitis, and 29% had one or more episodes of recurrence before the age of 10 years; 63% had normal visual acuity. However, from the data presented, it cannot be deduced how many of those with visual impairment were not treated antenatally.

2.14.4.3 Postnatal screening of neonates to promote early treatment

The postnatal screening of neonates has been implemented in some countries where prenatal screening was not considered to be a health priority, as is the case in Sweden, Denmark, Poland, and the United States (Massachusetts). The concept of newborn screening for early treatment to reduce long-term eye sequelae has been prompted by American cohort studies, which showed unexpected favorable outcomes for most infants who were treated continuously for 1 year with pyrimethamine-sulfadiazine, despite severe manifestations at birth, compared to untreated infants from previous series (McLeod *et al., 2006).* Phan et al. (Phan *et al., 2008*) reported on 28 children with congenital toxoplasmosis who were diagnosed late and were not treated during their first year of life. Those authors described new chorioretinal lesions in 72% of these children during a mean follow-up time of 5.7 years; 52% of the children developed new eye

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lesions at an age of >10 years. These data are in contrast with data for European cohorts, where eye prognoses seemed better (Wallon M, *et al.* 2004). In the French series described by Kieffer *et al* (2008), only 12% of 300 infected infants treated for 1 year developed a first retinochoroidal lesion until the age of 2 years. However, French series present a confounding factor limiting analyses of the efficacy of postnatal treatment, since most babies also received prenatal treatment. Therefore, the answer regarding the effect of early postnatal treatment should come from countries where decision makers chose the option of neonatal screening only. But how does one obtain an adequate untreated control group? Such a definitive evaluation also needs a randomized controlled trial, with the same ethical limitations as those for prenatal treatment (Kieffer *et al., 2008*).

Freeman *et al*. observed a low incidence of retinochoroiditis among 281 infected infants (18%) identified by prenatal or postnatal screening and proposed a strategy of postnatal therapy based on clinical severity and prognosis (Freeman *et al., 2008*). They suggested a short-course treatment (3 months) or no treatment for asymptomatic newborns who are at a low risk of developing retinochoroiditis but recommended treatment and careful monitoring of children with early eye manifestations and/or severe disease (intracranial lesions, serious neurological sequelae, and hepatosplenomegaly), who have a higher risk for new or worsening lesions. In Denmark, intervention studies since the onset of the national neonatal screening program estimated that the low burden of disease (1.6 per 10,000 live-born infants) and the lack of evidence of postnatal treatment efficacy did not justify the continuation of neonatal screening; hence, the Danish program was stopped in

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2007 (Roser*et al.,* 2010). The Danish protocol was based on a 3-month treatment of infected newborns, which failed to prevent the development of retinochoroidal lesions in children with or without previously detected lesions (Schmidt *et al., 2006*). However, it cannot be excluded that the duration of treatment may have been too short, particularly for symptomatic newborn at birth.

2.15 Treatment of Toxoplasmosis

Traditional drug therapy for clinical toxoplasmosis consists of a combination of pyrimethamine and sulfonamides. This combination can cause dose-related bone marrow suppression with resultant anemia, leucopenia, thrombocytopenia, and reversible acute renal failure. Leucovorin (folinic acid) could be added to the combination to prevent bone marrow suppression and to reduce the severity of congenital infection and increase the proportion of infants asymptomatic at birth (Daffos*et al*; 1988). Spiramycin is one of the current drugs of choice for treatment of infected pregnant women. Treatment may decrease the severity of congenital toxoplasmosis or long term consequences, but possibly not the risk of transmission (Tenter*et al*., 2000).

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CHAPTER THREE

MATERIALS AND METHODS

Study area

The study was done in Ibadan. The city of Ibadan is the capital of Oyo State in the South-Western part of Nigeria. There are eleven Local Government Areas in Ibadan.

Ibadan is located in South-Western Nigeria and in the southeastern part of Oyo state. The ancient city Ibadan has coordinates: 7°23’47’’N 3°55’0’’E and Density: 2,140/sq mi (828/km2). It is at about 119 kilometres northeast of Lagos state. It lies within the tropical forest zone but close to the boundary between the forest zone and the derived savanna. Ibadan ranges in elevation from 150m in the valley area, to 275m above the sea level on the major north-south ridge which crosses the central part of the city. Ibadan covers a total area of 3,080 square kilometres, which is the largest in Nigeria.

Ibadan has a tropical wet and dry climate; it has a lengthy wet season and relatively constant temperature throughout the year. Its wet season runs from March through October while the dry season is from November to February. In the resent years, the mean maximum temperature is 26.46C while the minimum is 21.42C and the relative humidity is 74.55%.

Ibadan is the third largest metropolitan area by population, after Lagos and Kano with population of over 3million. There are eleven Local Governments in the city of Ibadan. Its Metropolitan area consists of five local governments, namely: Ibadan North, Ibadan

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North East, Ibadan North West, Ibadan South East and Ibadan South West local government area, and six non-urban local government area, namely Akinyele, Egbeda, Ido, Lagelu, Ona Ara, and Oluyole local government areas.

Ibadan is relatively peaceful and it accommodates different forms of businesses. People in its neighbouring villages sell their farm produce in Ibadan. Moreover, large number of students is undergoing different studies in different post-secondary institutions in the city.

Along with major tertiary institutions like University College Hospital, State Hospital and a number of well established Private Health institutions, there are one hundred and sixty five Primary Health Centers in Ibadan. People from different wide range of socio-economic status attend the primary health facilities because the cost of the services there is relatively cheap when compared with what is obtainable in other health institutions within the city.

Study design

A descriptive cross sectional study will be employed.

Study population

Only the pregnant women at primary health centers in Ibadan. Only the pregnant women attending selected health care centre and agree with the informed consent form was included in the study.

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Sample size

The sample size of the people living with HIV will be calculated using the equation below:

$$N = \frac{Z^2 pq}{d^2}$$

Where n=sample size, Z=Standard normal deviate for a two tailed test, p=prevalence of Toxoplasmosis among people living with HIV in Ibadan, q=1-p and d=level of precision (5%).

With reference to the prevalence rate of 31.68% of Toxoplasmosis among people living with HIV in Nigeria (Karshima and Karshima, 2020).

The sample size will be 333 people living with HIV in Ibadan.

. This will be used to calculate the sample size:

$Z^2 = 1.96$, p= 31.6%, q= 1- 0.316, d= 5%

$$N = \frac{(1.96)^2 \times (0.316) \times (1 - 0.316)}{(0.05)^2}$$

N= 333.15

The sample size will be 333 people living with HIV

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Sampling Technique

Multi-stage Sampling Technique was adopted:

- Stage 1: The eleven Local Government Areas in Ibadan will be used for the selection of participants from their Primary Health Centers and PEPFAR Clinics
- Stage 2: Two Primary Health Centers will be selected from each of the Local Government Areas using simple random sampling method (use of balloting method). Twenty two Primary Health Centre will be selected in total
- Stage 3: Each of the people living with HIV that attended each of the health centers on the clinic day will be selected for participation.

Data collection

A well structured interviewer's administered questionnaire was used for the pregnant women attending the health centre visited. The questionnaire has in its content:

- Socio-demographic information: age(years), marital status, type of family, religion, level of education, ethnic group, occupation, number of children and age of pregnancy.
- Factors associated with Toxoplasmosis: eating undercooked meat, eating unwashed fruits, eating unwashed vegetables
- Sanitation and Hygeinicpractises: washing of fruits and vegetables before eating

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- Contact with pets like dog and cat
- History of blood transfusion
- Assessment of level of Information and knowledge about Toxoplasmosis

Grading of response to questions on exposure to *T. gondii* infection

i. Grading of response to consumption of undercooked meat - “suya”, “asun”, dried meat (like “ponmo” and “tinko”). Grading score of 1 – 3 point will be used. Anyone who eats any of the meat will be scored 1 point. Scoring 1 and above will be treated as potential contact.

ii. Grading of response to whether participants wash fruits and vegetables before eating. A grading score of 1 - 2 will be used, those that never washed or washed sometimes will be scored 2 and 1 respectively. Scoring 1 and above will be treated as potential contact.

iii. Grading of response to possession of pet – dog and cat. Grading score of 1 – 3 point will be used. Anyone who has any of the pet will be scored 1 point. Scoring 1 and above will be treated as potential contact.

Sample Collection

2ml of intravenous blood sample will be collected through venipuncture after the administration of questionnaire.

Standard Operating Procedure for venipuncture will be strictly followed and each sample will be labeled with a code given to each participant.

The blood samples will be preserved at the temperature between 2°C and 8°C until they will be transported to the laboratory.

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At the laboratory, each blood specimen will be spuned using Bucket Centrifuge at 3000revolution per minutes for five minutes. Serum will then be separated into a sterile plain bottle, labeled and kept frozen at -20°C until the day of serological analysis.

Serological tests

Serum will be tested for anti-*T. gondii* antibodies using Enzyme Linked Immunosorbent Assay (ELISA) test kits for anti-*T. gondii*-specific IgG antibody only.

Data Analysis

Data will be double entered and analyzed using Microsoft Excel and software SPSS application package to determine frequency, proportions, prevalence and level of significance using Chi square test, strength of association will be tested using logistic regression at 95% confidence interval.

Quality Control

- Research assistants will be trained on how to administer questionnaires on the respondents
- Questionnaires will be pretested before field work at Molly Specialist Hospital, and Alakia Primary Health Centre, Ibadan
- Blood specimens will be collected aseptically and preserved between 2°C and 8°C immediately after collection
- Laboratory equipments and procedures will be well standardized, controlled and calibrated

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- Data will be double entered before analysis

Ethical considerations

Written informed consent will be sought from each people living with HIV prior their involvement in the study.

Ethical clearance will be sought and obtained from the Oyo State Ethical Research Committee of the Oyo State Ministry of Health and Lead City University Ethical Research Committee

Information collected from each study participant Will be kept confidential and venous blood specimens collected will be preserved anonymously.

Laboratory Method

Samples will be analysed at the laboratory of Anointed Dynasty Diagnostics, Ibadan, using ELISA microplate washer and ELISA microplate reader.

PROCEDURE

All reagents and specimens will be brought to room temperature prior to testing. The procedure will be strictly followed. The analysis will be processed to completion within short time.

The calibrators will be arranged so that the well A1 will be the Blank well. From well A1, the calibrators will be arranged in a vertical configuration. The procedure below will be followed step by step:

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- Working Wash Buffer will be prepared by diluting the Concentrated Wash Buffer with deinosed water in 1:25. The Concentrated Wash Buffer will be poured into a clean graduated cylinder and will be filled with freshly prepared deinosed water to 1250ml mark for 96well testing

- 100µl of Calibrator 1 will be added to well B1 and C1. 100µl of Calibrator 2 will be added to well D1 and E1. 100µl of Calibrator 3 will added to well F1 and G1. 100µl of Calibrator 4 will be added to well H1 and A2

- 100µl of Specimen Diluent will be added to remaining wells starting from B2

- 5 µl of each specimen will be added to remaining wells starting from B2

- The microwell plates will be gently swirled to mix the content.

- The micowell plates will be covered with Plate sealer and incubated at 37°C for 30minutes in a well monitored incubator

- The plate sealer will be removed

- Using microplate washing machine, each well will be washed 5times with 350 µL of working wash-Buffer per well, the liquid will be removed

- 100 µl of conjugate will be added to each well except for the Blank well

- The microwell plates will be covered with plate sealers and will bde incubated at 37^{0}C for 30minutes in a well monitored incubator

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- The plate sealer will be removed
- Using microplate washing machine, each well will be washed 5times with 350 µL of working wash Buffer per well, the liquid will be removed
- The microwell plate will be turned upside down on absorbent tissue for 3seconds.
- 50 µl of Substrate A will be added to each well
- 50 µl of Substrate B will be added to each well. Then a blue colour will develop in wells containing Positive specimens
- The microwell plates will be covered with plate sealer and incubated in a water bath at 37°C for 10 minutes.
- The Plate Sealer will be removed.
- 50 µl of stop solution will be added to each well.
- A yellow colour will developed in wells containing Positive specimens
- Then it will be read at wavelength of 450nm within 30 minutes of colour development.

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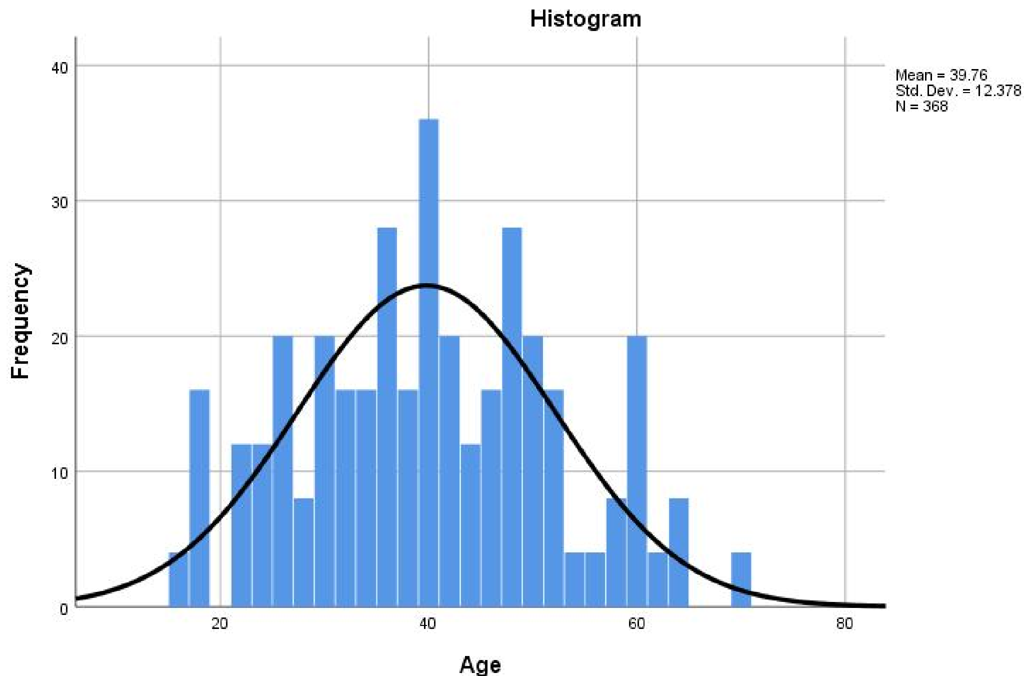
CHAPTER FOUR

RESULTS

4.1 Socio-Demographic Characteristics of People Living with HIV (PLWH)

A total of 368 people living with HIV and enrolled in care at primary health centre in Ibadan in 2022 participated in this study.

As shown in Graph 1, the mean age of the participants was 39.76 (±12.38) years. Table 1 shows that the age group 31 - 40 years among the participants had the highest 112 (30.4%). The majority of the participants 308 (83.7%) were female,228 (62.0%) were married, 200 (54.3%) were from monogamous family settings, 236 (64.1%) were practicingChristian faith, 188 (51.1%) had Secondary education as the highest, 300 (83.3%) were Yorubas, 124 (34.4%) engaged in different market businesses, 88 (23.9%) has never had a child, while the majority 160 (43.5%) were diagnosed with HIV within the last 5years.



Graph 1: Showing the frequency distribution of the age of people living with HIV that participated in this study

Table 1a: Characteristics of people living with HIV at Primary Health Care level in Ibadan, Nigeria, 2022

Characteristics		Frequency	Percent
Age (years):	16 – 20	20	5.4
	21 – 30	72	19.6
	31 – 40	112	30.4
	41 – 50	96	26.1
	51 – 60	52	14.1
	61 – 70	16	4.3
Sex:	Female	308	83.7
	Male	60	16.3
Marital Status:	Never married	84	23.1
	Married	228	62.6
	Living Together	4	1.1
	Separated	8	2.2
	Divorced	8	2.2
	Widowed	32	8.8
Type of Family:	Monogamous	200	54.9
	Polygamous	164	45.1
Religion:	Islam	128	34.8
	Christianity	236	64.1
	Traditional	4	1.1
Educational Level:	No formal Education	12	3.3
	Primary Education	68	18.5
	Secondary Education	188	51.1
	Bachelor/HND	76	20.7
	Post-graduate	24	6.4
Ethnic Group:	Hausa	4	1.1
	Yoruba	300	83.3
	Igbo	48	13.3
	Others	8	2.3

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Occupation:	Full Housewife	20	5.6
	Market Business	124	34.4
	Farming	4	1.1
	Civil Servant	40	11.1
	Student	60	16.7
	Other Occupation	112	31.1

~~Table 1b: Characteristics of people living with HIV at Primary Health Care level in~~ Ibadan, Nigeria, 2022

Characteristics		Frequency	Percent
Number of Child(ren):	0	88	23.9
	1	40	10.9
	2	68	18.5
	3	56	15.2
	4	40	10.9
	>4	76	20.7
Year of HIV Diagnosis:	In last 5 years	160	43.5
	In last 6 to 10 years	108	29.3
	In last 11 to 15 years	68	18.5
	In last 16 to 20 years	28	7.6
	In last 21 to 25 years	4	1.1

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4.2 Prevalence of anti-toxoplasma IgG

As shown in Table 2, the prevalence of anti-toxoplasma IgG among the people living with HIV at Primary Health Centers in Ibadan in 2022 was 22.8% (Table 2).

Table 2:Anti-toxoplasma IgG test results among the people living with HIV attending care at Primary Health Centers in Ibadan, Nigeria, 2022

Result	Frequency	Percent
Positive	84	22.8*
Negative	284	77.2
Total	368	100

*Prevalence of Anti-toxoplasma IgG by ELISA method

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4.3 The distribution of the risk factors for contracting *Toxoplasma gondii* infection

As shown in Table 3, not less than 112(31.1%) of people living with HIV always eat suya while 176(48.9%) of them did eat suya sometimes. Moreover, 88(24.4%) always eat “asun”, 120(32.6%) always eat sundried meat, majority 296(82.2%) of the participants were always eating spinach, 232(66.7%) always eat lettuce while only 100(27.8%) always eat vegetable salad and all the participants reported washing vegetables before eating. Also, the majority of the participants 220(59.8%) reported always eating mango, 236(64.1%) always eat carrot, 108(30%) always eat guava and they reported washing of fruits before eating. All the participants reported sanitation of their hands before and after meal, 360(97.8%) were using soap for washing hand and only 40(10.9%) reported use of ashes for hand washing. Moreover, the majority of the participants 232(63.7%) were using water closet, 240(65.9%) were using government bin for waste disposal, 28(8%), 68(19.5%) and 184(52.9%) were drinking pipe-borne water, water dispenser and pure (satchet) water respectively. The majority of the participants 172(52.5%) were not treating their drinking water. Not less than 204(55.4%) of the participants were not having contact with pet, 184(57.5%) never had contact with dog and 232(74.4%) never

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had contact with cat. The large number of the participants 272(73.9%) have never received blood transfusion, the majority of those that have ever received blood transfusion 56(61%) received the care service at privately owned hospital, and the majority of them 36(45%) have received two units of blood, but 64(72.7%) confirmed that the blood received were actually screened for blood borne infections. Only 24(6.7%) of them had another infection different from HIV infection, 8(50%) were sure of "toilet" disease, 8(40%) treated the infection with prescription obtained directly at a pharmacy store, not all the participants 356(96.7%) confirmed that they were presently taking anti-retroviral medication and 76(20.7%) had family members with HIV infection, majorly 48(63.2%) were spouses. None of the participants was aware of incident of hydrocephaly, polydactylism or blindness in their family.

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Table 3a: Distribution of the risk factors for contracting *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Frequency of eating "Suya"		
Always	112	31.1
Sometimes	176	48.9
Never	72	20.0
Frequency of eating "Asun"		
Always	88	24.4
Sometimes	128	35.6
Never	144	40.0
Frequency of eating Sundried Meat		
Always	120	32.6
Sometimes	140	38.0
Never	108	29.4
Frequency of eating Spinach		
Always	296	82.2
Sometimes	48	13.3
Never	16	4.4
Frequency of eating Lettuce		
Always	232	66.7
Sometimes	76	21.8
Never	40	11.5
Frequency of eating Vegetable Salad		
Always	100	27.8
Sometimes	164	45.6

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Never	96	26.6
Frequency of washing vegetable before eating		
Always	368	100
Sometimes	0	0
Never	0	0
Frequency of eating Mango		
Always	220	59.8
Sometimes	100	27.2
Never	48	13.0
Frequency of eating Carrot		
Always	236	64.1
Sometimes	116	31.5
Never	16	4.4

Table 3b: Distribution of the risk factors for contracting *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Frequency of eating Guava		
Always	108	30.0
Sometimes	96	26.7
Never	156	43.3
Frequency of washing fruits before eating		
Always	364	98.9
Sometimes	4	1.1
Never	0	0
Frequency of sanitation before meal		
Always	368	100
Sometimes	0	0
Never	0	0
Frequency of sanitation after meal		
Always	368	100
Sometimes	0	0
Never	0	0
Use of soap for hand washing		
Yes	360	97.8
No	8	2.2
Use of ashes for hand washing		

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Yes	40	10.9
No	328	89.1
Type of toilet		
Water Closet	232	63.7
Bucket	36	9.9
Pit	68	18.7
Bush	28	7.7
Method of waste disposal		
Government bin	240	65.9
Burning	120	33.0
In water drainages	4	1.1
Source of drinking water		
Pipeborne	28	8.0
Borehole	68	19.5
Water dispenser	4	1.1
Pure(satchet) water	184	52.9
Stream water	20	5.7
Well water	44	12.8

Table 3c: Distribution of the risk factors for contracting *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Type of water treatment		
Chlorine	8	2.4
Waterguard	140	42.7
Other form of treatment	8	2.4
No treatment	172	52.5
Contact with pet		
Yes	164	44.6
No	204	55.4
Frequency of contact with dog		
Always	40	12.5
Sometimes	96	30.0
Never	184	57.5
Frequency of contact with cat		
Always	0	0
Sometimes	80	25.6
Never	232	74.4

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Ever received blood transfusion		
Yes	96	26.1
No	272	73.9
Level of healthcare of blood transfusion		
Private owned	56	61.0
Federal government owned	12	13.0
State government owned	12	13.0
Primary healthcare	12	13.0
Total unit of blood ever received		
One	20	25.0
Two	36	45.0
Three	20	25.0
Four	4	5.0
Did you confirm blood screened of infectious agents		
Yes	64	72.7
No	24	27.3
Have had any other infection		
Yes	24	6.7
No	332	93.3
Name of infection treated		
Toilet disease	8	50.0
Candidiasis	4	25.0
Gonorrhoea	4	25.0

Table 3d: Distribution of the risk factors for contracting *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Place of treatment was received		
Private owned	4	20.0
Federal government owned	4	20.0
Primary healthcare	4	20.0
Pharmacy store	8	40.0
Do you have access to good healthcare		
Yes	300	93.8
No	20	6.2
Are you presently taking anti-retroviral medication		
Yes	356	96.7
No	12	3.3

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Do you have HIV infection among family members		
Yes	76	20.7
No	292	79.3
Family member with HIV infection		
Spouse	48	63.2
Spouse and children	8	10.5
Parents	12	15.8
Distant relative (aunt or uncle)	8	10.5
Report of hydrocephaly in the family		
Yes	0	0
No	368	100
Report of polydactylism in the family		
Yes	0	0
No	368	100
Report of blindness in the family		
Yes	0	0
No	368	100

4.4 Level of knowledge of people living with HIV at primary healthcare level Ibadan, was assessed of toxoplasmosis. Knowledge through awareness, mode of infection, and prevention of toxoplasmosis infection were assessed. As shown in Table 4, only 16(4.4%) of the participants have ever heard of Toxoplasmosis infection, 8(50%) heard about through hospital health talk while others heard about it the reading of books and 8(2.2%) of the participants confirmed of having been tested for Toxoplasmosis infection. Not less than 352(95.6%) of the participants did not know any of the routes through which an individual could contract or prevent the transmission of Toxoplasmosis infection.

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Table 4a: Distribution of the level of knowledge of *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Ever heard of Toxoplasmosis		
Yes	16	4.4
No	236	64.1
Do not know	116	31.5
Source of information about Toxoplasmosis		
Hospital health talk	8	50

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Books	8	50
Tested for Toxoplasmosis before		
Yes	8	2.2
No	100	27.2
Do not know	260	70.6
Transmitted through cat feaces		
Yes	16	4.3
No	0	0
Do not know	348	95.6
Transmitted through eating "suya"		
Yes	4	1.1
No	4	1.1
Do not know	360	97.8
Transmitted through eating "asun"		
Yes	4	1.1
No	4	1.1
Do not know	360	97.8
Transmitted through eating sundried meat		
Yes	0	0
No	12	3.3
Do not know	356	96.7
Transmitted through handling animal manure		
Yes	8	2.2
No	8	2.2
Do not know	352	95.7
Transmitted through transfusion with infected blood		
Yes	8	2.2
No	8	2.2
Do not know	352	95.7

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Table 4b: Distribution of the level of knowledge of *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent
Transmitted through sexual intercourse with infected person		
Yes	0	0
No	4	1.1

Do not know	360	98.9
Know any sign or symptom		
Yes	0	0
No	0	0
Do not know	348	95.6
It infects baby in the womb		
Yes	8	2.2
No	0	0
Do not know	360	97.8
It causes miscarriage in pregnant woman		
Yes	8	2.2
No	0	0
Do not know	360	97.8
Infected baby may have no symptom		
Yes	8	2.2
No	0	0
Do not know	360	97.8
Infected baby may have vision problem		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Infected baby may have many fingers		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Infected baby may have hydrocephaly		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Infected baby may have mental illness		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Infection can be treated with medicine		
Yes	8	2.2
No	0	0
Do not know	356	97.8

Table 4c: Distribution of the level of knowledge of *Toxoplasma gondii* infection among people living with HIV at Primary Health Centre in Ibadan, Nigeria, 2022

Variables	Frequency	Percent

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Avoid contact with cat		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Avoid contact with dog		
Yes	4	1.1
No	0	0
Do not know	360	98.9
Avoid contact with cat feaces		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Avoid contact with dog feaces		
Yes	4	1.1
No	0	0
Do not know	360	98.9
Eating well cooked meat		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Eating properly washed fruits		
Yes	8	2.2
No	0	0
Do not know	356	97.8
Eating properly boiled vegetables		
Yes	8	2.2
No	0	0
Do not know	356	97.8

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4.5 Association between the seropositivity of toxoplasmosis and demographic factors among the people living with HIV at primary healthcare level Ibadan, 2022.

As shown in Table 5, there was statistically significant association between the seropositivity of Toxoplasmosis infection and demographic factors like age groups, types of family and religion ($p < 0.05$). On the other hand, there was no statistically significant association between the seropositivity of Toxoplasmosis infection and other demographic factors like sex, marital status, educational level, ethnic group, occupation, number of children and year of diagnosis of HIV infection ($p > 0.05$).

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Table 5a: Association between seropositivity of Toxoplasmosis and demographic factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics		Seropositivity		χ^2	*P*-value
		Positive(%)	**Negative(%)**		
Age (years):	16 – 20	0(0)	20(100)	18.614	0.002*
	21 – 30	16(22.2)	56(77.8)		
	31 – 40	28(25.0)	84(75.0)		
	41 – 50	32(33.3)	64(66.7)		
	51 – 60	8(15.4)	44(84.6)		
	61 – 70	0(0)	16(100)		
Sex:	Male	8(13.3)	52(86.7)	3.667	0.055
	Female	76(24.7)	232(75.3)		
Marital Status:	Never married	20(23.8)	64(76.2)	10.408	0.064
	Married	48(21.2)	180(78.9)		
	Living Together	0(0)	4(100)		
	Separated	0(0)	8(100)		
	Divorced	0(0)	8(100)		
	Widowed	12(37.5)	20(62.5)		
Type of Family:	Monogamous	64(32.0)	136(68.0)	19.911	0.000*
	Polygamous	20(12.2)	144(87.8)		
Religion:	Islam	20(15.6)	108(84.8)	7.420	0.024*
	Christianity	64(27.1)	172(72.9)		
	Traditional	0(0)	4(100)		
Educational Level:	Non-formal	0(0)	12(100)	8.443	0.077
	Primary	12(17.6)	56(82.4)		
	Secondary	44(23.4)	144(76.6)		
	Bachelor/HND	24(31.6)	52(68.4)		
	Post-graduate	4(16.7)	20(83.3)		
Ethnic Group:	Hausa	0(0)	4(100)	7.752	0.051
	Yoruba	64(21.3)	236(78.7)		
	Igbo	16(33.3)	32(66.7)		
	Others	4(50.0)	4(50.0)		

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* $p < 0.05$

Table 5b: Association between sero-positivity of Toxoplasmosis and demographic factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics		Seropositivity Positive(%)	Negative(%)	χ^2	*P*-value
Occupation:	Full Housewife	8(40.0)	12(60.0)	10.362	0.066
	Market Business	20(16.1)	104(83.9)		
	Farming	0(0)	4(100)		
	Civil Servant	8(20.0)	32(80.0)		
	Student	12((20.0)	48(80.0)		
	Other Occupation	32(28.6)	80(71.4)		
Number of Child(ren):	0	24(27.3)	64(72.7)	3.571	0.613
	1	8(20.0)	32(80.0)		
	2	12(17.6)	56(82.4)		
	3	12(21.4)	44(78.6)		
	4	12(30.0)	28(70.0)		
	>4	16(21.1)	60(78.9)		
Year of HIV Diagnosis:	In last 5 years	48(30)	112(70)	9.190	0.057
	In last 6 to 10 years	20(18.5)	88(81.5)		
	In last 11 to 15 years	12(17.6)	56(82.4)		
	In last 16 to 20 years	4(14.3)	24(85.7)		
	In last 21 to 25 years	0(0)	4(100)		

* $p < 0.05$

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4.6 Association between the seropositivity of toxoplasmosis and the risk factors among the people living with HIV at primary healthcare level Ibadan, 2022.

Table 6 shows that there was statistically significant association between the seropositivity of Toxoplasmosis infection and the risk factors like frequency of eating “asun”, eating spinach, eating vegetable salad, eating carrot, eating guava, the type of toilet, method of waste disposal, source of drinking water, type of water treatment, frequency of contact with dog, contact with cat, total unit of blood transfusion received, type of other infection treated and family members with HIV infection ($p < 0.05$). There was no statistically significant association between the seropositivity of Toxoplasmosis infection and other risk factors like the frequency of eating “suya”, eating sundried meat, eating lettuce, washing vegetable before eating, eating mango, frequency of washing fruits before eating, use of soap for hand washing, use of ashes for hand washing, contact with pet, ever received blood transfusion, level of healthcare for blood transfusion service, confirmation of receiving screened blood, having any other infection, having access to good healthcare, presently taking antiretroviral medication and having HIV infection among family members ($p > 0.05$).

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Table 6a: Association between sero-positivity of Toxoplasmosis and risk factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	Seropositivity Positive(%)	Negative(%)	χ^2	*P*-value
Frequency of eating "Suya"				
Always	28(25)	84(75)	1.784	0.410
Sometimes	36(20.5)	140(79.5)		
Never	20(27.8)	52(72.2)		
Frequency of eating "Asun"				
Always	12(13.6)	76(86.4)	6.492	0.039*
Sometimes	36(28.1)	92(71.9)		
Never	36(25)	108(75)		
Frequency of eating Sundried Meat				
Always	24(20)	96(80)	4.305	0.116
Sometimes	40(28.6)	100(71.4)		
Never	20(18.5)	88(81.5)		
Frequency of eating Spinach				
Always	76(25.7)	220(74.3)	6.970	0.031*
Sometimes	8(16.7)	40(83.3)		
Never	0(0)	16(100)		
Frequency of eating Lettuce				
Always	60(25.9)	172(74.1)	4.940	0.085
Sometimes	20(26.3)	56(73.7)		
Never	4(10)	36(90)		
Frequency of eating Vegetable Salad				
Always	12(12)	88(88)	20.284	0.000*
Sometimes	56(34.1)	108(65.9)		
Never	16(16.7)	80(83.3)		
Frequency of washing vegetable before eating				

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Always	84(23.1)	280(76.9)	1.196	0.274
Sometimes	0(0)	4(100)		
Never	0	0		
Frequency of eating Mango				
Always	52(23.6)	168(76.4)	1.194	0.550
Sometimes	24(24)	76(76)		
Never	8(16.7)	40(83.3)		
Frequency of eating Carrot				
Always	52(22)	184(78)	6.309	0.043*
Sometimes	32(27.6)	84(72.4)		
Never	0(0)	16(100)		

* $p < 0.05$

Table 6b: Association between sero-positivity of Toxoplasmosis and risk factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	**Seropositivity**		χ^2	***P*-value**
	Positive(%)	**Negative(%)**		
Frequency of eating Guava				
Always	24(22.2)	84(77.8)	16.355	0.000*
Sometimes	36(37.5)	60(62.5)		
Never	24(15.4)	132(84.6)		
Frequency of washing fruits before eating				
Always	84(23.1)	280(76.9)	1.196	0.274
Sometimes	0(0)	4(100)		
Never	0	0		
Use of soap for hand washing				
Yes	84(23.3)	276(76.7)	2.419	0.120
No	0(0)	8(100)		
Use of ashes for hand washing				
Yes	8(20)	32(80)	0.203	0.652
No	76(23.2)	252(76.8)		
Type of toilet				
Water Closet	32(13.8)	200(86.2)	50.970	0.000*
Bucket	20(55.6)	16(44.4)		
Pit	16(23.5)	52(76.5)		
Bush	16(57.1)	12(42.9)		
Method of waste disposal				
Government bin	40(16.7)	200(83.3)	19.240	0.000*
Burning	44(36.7)	76(63.3)		
In water drainages	0(0)	4(100)		

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Characteristics	Positive(%)	Negative(%)	χ^2	P-value
Source of drinking water				
Pipeborne	0(0)	28(100)	14.405	0.013*
Borehole	16(23.5)	52(76.5)		
Water dispenser	0(100)	4(100)		
Pure(satchet) water	48(26.1)	136(73.9)		
Stream water	8(40)	12(60)		
Well water	8(18.2)	36(81.8)		
Type of water treatment				
Chlorine	0(0)	8(100)	18.996	0.000*
Waterguard	48(34.3)	92(65.7)		
Other form of treatment	28(16.3)	144(83.7)		
No treatment	4(50)	4(50)		
Contact with pet				
Yes	40(24.4)	124(75.6)	0.411	0.522
No	44(21.6)	160(78.4)		

* $p < 0.05$

Table 6c: Association between sero-positivity of Toxoplasmosis and risk factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	Seropositivity		χ^2	*P*-value
	Positive(%)	Negative(%)		
Frequency of contact with dog				
Always	0(0)	40(100)	21.449	0.000*
Sometimes	36(37.5)	60(62.5)		
Never	44(23.9)	140(76.1)		
Frequency of contact with cat				
Always	0	0	21.147	0.000*
Sometimes	36(45)	44(55)		
Never	44(19)	188(81)		
Ever received blood transfusion				
Yes	16(16.7)	80(83.3)	2.797	0.094
No	68(25)	204(75)		
Level of healthcare of blood transfusion				
Private owned	12(21.4)	44(78.6)	0.050	7.811
Federal government owned	0(0)	12(100)		
State government owned	4(33.3)	8(66.7)		
Primary healthcare	0(0)	12(100)		
Total unit of blood ever received				
One	0(0)	20(100)	14.466	0002*
Two	4(11.1)	32(88.9)		
Three	8(40)	12(60)		

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Four	0(0)	4(100)		
Did you confirm blood screened of infectious agents				
Yes	12(18.8)	52(81.3)	0.051	0.821
No	4(16.7)	20(83.3)		
Have had any other infection				
Yes	4(16.7)	20(83.3)	0.685	0.408
No	80(24.1)	252(75.9)		
Name of infection treated				
Toilet disease	0(0)	8(100)	16.000	0.000*
Candidiasis	0(0)	4(100)		
Gonorrhoea	4(100)	0(0)		
Do you have access to good healthcare				
Yes	64(21.3)	236(78.7)	0.020	0.888
No	4(20)	16(80)		
Are you presently taking anti-retroviral medication				
Yes	80(22.5)	276(77.5)	0.777	0.378
No	4(33.3)	8(66.7)		

* $p < 0.05$

Table 6d: Association between sero-positivity of Toxoplasmosis and risk factors among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	**Seropositivity**		χ^2	***P*-value**
	Positive(%)	**Negative(%)**		
Do you have HIV infection among family members				
Yes	16(21.1)	60(78.9)	0.171	0.679
No	68(23.3)	224(76.7)		
Family member with HIV infection				
Spouse	8(16.7)	40(83.3)	11.822	0.008*
Spouse and children	4(50)	4(50)		
Parents	0(0)	12(100)		
Distant relative (aunt or uncle)	4(50)	4(50)		

* $p < 0.05$

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4.7 Association between the seropositivity of toxoplasmosis and the level of knowledge of Toxoplasmosis among the people living with HIV at primary healthcare level Ibadan, 2022.

As shown in Table 7, there was statistically significant association between the seropositivity of Toxoplasma infection among the people living with HIV and having ever heard of Toxoplasmosis, knowing that Toxoplasmosis is transmitted through contact with the cat feaces ($p < 0.05$). There was no statistically significant association between the seropositivity of Toxoplasma infection among the people living with HIV and the level of knowledge of the means of transmission and prevention of Toxoplasmosis infection ($p > 0.05$).

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Table 7a: Association between seropositivity of Toxoplasmosis and level of knowledge of Toxoplasmosis among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	Seropositivity Positive(%)	Negative(%)	χ^2	*P*-value
Ever heard of Toxoplasmosis				
Yes	0(0)	16(100)	9.255	0.010*
No	64(27.1)	172(72.9)		
Do not know	20(17.2)	96(82.8)		
Transmitted through cat feaces				
Yes	0(0)	16(100)	4.714	0.030*
No	0	0		
Do not know	80(23)	268(77)		
Transmitted through eating “suya”				

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Yes	0(0)	4(100)	3.669	0.160
No	0(0)	8(100)		
Do not know	84(23.6)	272(76.4)		
Transmitted through eating "asun"				
Yes	0(0)	4(100)	2.419	0.298
No	0(0)	4(100)		
Do not know	84(23.3)	276(76.7)		
Transmitted through eating sundried meat				
Yes	0(0)	0(0)	3.669	0.055
No	0(0)	12(100)		
Do not know	84(23.6)	272(76.4)		
Transmitted through handling animal manure				
Yes	0(0)	8(100)	4.948	0.084
No	0(0)	8(100)		
Do not know	84(23.9)	268(76.1)		
Transmitted through transfusion with infected blood				
Yes	0(0)	8(100)	4.948	0.084
No	0(0)	8(100)		
Do not know	84(23.9)	268(76.1)		
Transmitted through sexual intercourse with infected person				
Yes	0	0	1.213	0.271
No	0	4(100)		
Do not know	84(23.3)	276(76.7)		
It infects baby in the womb				
Yes	0(0)	8(100)	2.419	0.120
No	0	0		
Do not know	84(23.3)	276(76.7)		

* $p < 0.05$

Table 7b: Association between sero-positivity of Toxoplasmosis and level of knowledge of Toxoplasmosis among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	**Seropositivity**		χ^2	***P*-value**
	Positive(%)	**Negative(%)**		
It causes miscarriage in pregnant woman				
Yes	0(0)	8(100)	2.419	0.120
No	0	0		
Do not know	84(23.3)	276(76.7)		
Infected baby may have vision problem				
Yes	0(0)	8(100)	2.454	0.117

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No	0	0		
Do not know	84(23.6)	272(76.4)		
Infected baby may have many fingers				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		
Infected baby may have hydrocephaly				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		
Avoid contact with cat				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		
Avoid contact with dog				
Yes	0(0)	4(100)	1.213	0.271
No	0	0		
Do not know	84(23.3)	276(76.7)		
Avoid contact with cat feaces				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		
Avoid contact with dog feaces				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		
Eating well cooked meat				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		

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Table 7c: Association between sero-positivity of Toxoplasmosis and level of knowledge of Toxoplasmosis among people living with HIV at Primary Healthcare level, Ibadan, Nigeria, 2022

Characteristics	**Seropositivity**		χ^2	***P*-value**
	Positive(%)	**Negative(%)**		
Eating properly washed fruits				
Yes	0(0)	8(100)	2.454	0.117

No	0	0		
Do not know	84(23.6)	272(76.4)		
Eating properly boiled vegetables				
Yes	0(0)	8(100)	2.454	0.117
No	0	0		
Do not know	84(23.6)	272(76.4)		

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4.8 The direction and the strength of association between the seropositivity of Toxoplasmosis and factors that are associated with it among the people living with HIV at primary healthcare level Ibadan, 2022 were tested.

As shown in Table 8, the prevalence odds of having Toxoplasmosis infection among people that are living with HIV is 0.295 times lower among those from monogamous

family than from polygamous family, the odds of having toxoplasmosis is 0.573 times lower among those using water closet type of toilet than those not using other types of toilets, the odds is 0.374 time lower among those that have ever received a unit of blood than those that have ever received more than one unit of blood, and are statistically significant at $p < 0.05$. Also, the odds of having Toxoplasmosis among people that are living with HIV is 2.009 times higher among those that practice Islamic religion than any other religion, the odds of toxoplasmosis is 2.348 times higher among those that always eat “asun” than that do not, the odds of having toxoplasmosis is 4.715 times higher among those that always eat vegetable salad than those that do not eat, the odds of having toxoplasmosis is 3.235 times higher among those that always have contact with cat than those that do not, and are statistically significant at $p < 0.05$.

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Table 8: Logistic regression of seroprevalence of Toxoplasma IgG and associated factors among people living with HIV at primary health care level in Ibadan, Nigeria 2022

Variable	*POR	95% Confidence Interval	*p*-value

		Lower	Upper	
Family type	0.295	0.170	0.514	0.000*
Religion	2.009	1.151	3.506	0.014*
Age groups	0.990	0.811	1.208	0.923
Eating "Asun"	2.348	1.140	4.835	0.021*
Eating Spinach	0.523	0.232	1.184	0.120
Eating Vegetable Salad	4.715	2.329	9.547	0.000*
Eating Carrot	1.190	0.669	2.117	0.554
Eating Guava	2.151	0.999	4.634	0.050
Toilet type	0.573	0.457	0.719	0.000*
Source of drinking water	0.854	0.712	1.026	0.092
Method of waste disposal	1.150	0.553	2.392	0.708
Contact with dog	1.305	0.805	2.116	0.280
Contact with cat	3.235	1.873	5.585	0.000*
Unit of blood ever received	0.374	0.169	0.830	0.016*
Heard of Toxoplasmosis	1.166	0.737	1.844	0.511

POR – Prevalence Odds Ratio; $p < 0.05$

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- **Knowledge of People Living with HIV about Toxoplasmosis**

All the People Living with HIV and attending Clinic at Primary Health Care level in Ibadan, Nigeria did not have knowledge of Toxoplasmosis; its route of transmission, its signs and symptoms, its effect on immune system, its effect on their ways of life and the way to prevent or avoid the infection.

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CHAPTER FIVE

DISCUSSION

Various literatures have helped to ascertain that, since detection of *Toxoplasma gondii* over a century ago, it has become established as one of the most successful parasites. This is evident in its global distribution, broad host range and its ability to maintain a passive co-existence with its host. Moreover, the ability to culture *T. gondii*, its genetic manipulative property and its excellent animal model made studying T. *gondii* fairly not difficult.

While it is known that *T. gondii* rarely causes acute disease in healthy individuals, different studies have established its extraordinary high infection rates and this proves it as a serious menace to human health. Presently, very low mortality rate has been observed with Toxoplasmosis worldwide but the most serious form of Toxoplasma infection is congenital. Different studies have shown that Toxoplasmosis is accompanied with serious immunological complication based on immune level of the individual at the time of the infection.

In immunocompetent individuals, *Toxoplasma* infection maybe asymptomatic or self-limiting but in immunocompromised individuals, condition like HIV infection may alter the clinical course of *T. gondii* infection. HIV infection may cause reactivation of the asymptomatic Toxoplasma infection resulting in neurological signs like headache, disorientation, drowsiness, hemiparesis, reflex changes and convulsion. Toxoplasma infection is now a known cause of morbidity and mortality among People Living with HIV and AIDS (PLWHA) Barratt J.L. *et al.,*(2010).

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5.1 Seroprevalence of Toxoplasmosis

According to this study, prevalence of anti- ToxoplasmaIgG antibody among People Living with HIV in Ibadan, Nigeria in 2022 is 22.8%. This is relatively close to seroprevalence of 31.68% reported among People Living with HIV and AIDS across 17 States of Nigeria in 2019 (Karshima and Karshima, 2019). Seroprevalence of 29.1% was reported in Zaria (MukhtaiYola *et al.,* 2015) northern part of Nigeria. Moreover, different prevalence has been reported across the globe, there are other studies that reported higher rates than this finding, 80.3% in Kinglash, 75.2% in Sao Tome – Principe by Hung *et al* in 2007, Gabon 56% (Michito-Equivol*et al*, 2006).

5.2 Seroprevalence and exposure to its risk factors

This study had shown that toxoplasma IgG seroprevalence as 22.8%. The seroprevalence was higher among People Living with HIV and AIDS of age of 41-50 years, which is 33.3%, this could be as a result of high activities, motility and probably decline in immune status found popular among the people of this age range. However, this suggests that people should take appropriate precautions to protect themselves against this infection, also a Seroprevalence of 24.7% was obtained from women as against 13.3% for men, this buttress the fact that women (especiall of reproductive age) are more susceptible to infections because they undergo physiological changes(pregnancies) which ultimately reduces their immune level and affect the foestus, hence screening for women of reproductive ages should be recommended in order not to affect the foetus and this is in consistence with study carried out by (MD Dairo et al., 2018) . With regards to marital status, 48(21.2%) of those that were positive were married, surprisingly, 12(37.5%) of the

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widows were positive to Toxoplasma IgG, possibly their spouses were killed by HIV infection, 44 (23.4%) of them that were positive had secondary education and 184 (16.1%) were market business women. Moreover, 64(21.3%) of those that were positive are Yorubas, 12(30.0%) had more than three children already, majority of those that were positive 64(32.0%), were from monogamous family, 64 (27.1%) of them practiced Christian faith while 48(30%) has their HIV diagnosis in the last five(5) years. With regards to those that were positive 84 (23.1%) had the habit of eating washed fruits, 24(20%) of them had the habit of eating Sundried meat, 40(24.4%) had pet in their houses, 16(16.7) of them had received blood transfusion before and 64 (21.3%)had access to good health care. This shows high occurrence of Toxoplasam infection across across the demographic factors of low education status, monogamy, Christian faith practices, recent HIV infection and poor hygienic practices at the level of individual households among people that are living with HIV.

5.3 Association between Toxoplasma infection and risk factors

There was significant association between Toxoplasma infection among People Living with HIV and religious practice. Following this association with christian religious practices, Islamists do not eat animals that are dirty like pigs that can easily aid transmission of oocysts of *Toxoplasma gondii* to human, but Christian practice do not frown at such.

Consumption of undercooked meat, consumption of unwashed vegetables and unwashed fruits has been shown to be significant risk factors associated with *T. gondii* infection. This was observed in studies done in Lagos (Deji-Agboola *et al*, 2011), (EhisChongs, *et*

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al., 2016), Sudan (Khali *et al*, 2014), Ethiopia (Walle*et al*, 2013), Mexico (Alvarado-Esquival *et al*, 2006) and China (Zhong *et al*, 2016). According to this study, there was association between consumption of unwashed fruits and vegetables, pet possession and receiving blood transfusion. Contaminated water and soil may act as vehicles for transfer of oocysts to vegetables and fruits for human consumption. Liu *et al*, (2009) and Dairo ., *et al(* 2018) reported strong association between Toxoplasma infection during pregnancy and consumption of unwashed raw vegetables and fruits and pet possession in China and Nigeria respectively. Association with consumption of unwashed fruits and vegetables will be product of use of contaminated water and soil contamination by oocysts of *Toxoplasma gondii* in the cultivation and preparation of fruits and vegetables.

Association of Toxoplasma infection with pet possession has been reported in different studies. Possession of cat will expose human to having contact with the oocysts in the feaces of the cat. Also, interaction between any other pet with cat will facilitate such pet to contact the oocysts from cat feaces and contaminated soil, which will eventually infect human.

The risk of transmitting infection through a blood transfusion is theoretically possible if the donor has recently acquired a Toxoplasma infection and is parasitemic at the time of blood sampling. However, this study shown association between Toxoplasma infection and blood transfusion. Presently, screening blood for Toxoplasma antibodies is not available in Nigeria.

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Moreover, there was no significant association between marital status, educational level, occupation, ethnic group, number of children and residence in rural area and consumption of undercooked meat.

From this study, 22.8% of the People Living with HIV were positive with T. *gondii* IgG antibody but due to insufficient fund, recent infection was not confirmed in the People Living with HIV using anti –Toxoplasma IgM which could be useful to detect possible congenital transmission. But, it has been shown by studies that 43 cases of toxoplasmosis acquired during pregnancy would be expected to result in 11 – 21 cases of congenital toxoplasmosis, assuming a 25-50% probability of transmission to the foetus in the uterus (Abu-Madi *et al*, 2010). The possibility of congenital toxoplasmosis in Ibadan may be significantly high among the pregnant women.

In the presence of prevalence of congenital malformation of 5.5/1000 total birth, with postnatal mortality rate of 60.7/1000 total birth reported recently in Kano, Nigeria (MukhtaiYola*et al.,* 2015) while 15.8/1000 birth was reported in Lagos (Iroha *et al*., 2001). It is very imperative to introduce measure that will further monitor and prevent toxoplasmosis and its congenital consequences in Nigeria. Introduction of toxo-screening for pregnant women and health education to inform and orientate the people has started in Italy where 1 – 2 congenital Toxoplasma cases per 10,000 birth was estimated (Stagni*et al*., 2009). Furthermore, Wallen *et al.*, (2013) have demonstrated that monthly prenatal screening and improvements in ante-natal diagnosis may lead to decrease in the congenital infection rate and a better outcome of infected children.

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Presently, the government of Italy, China and Canada provide free voluntary serological screening for Toxoplasmosis in pregnancy.

5.4 Strength of association between seropositivity of Toxoplasma IgG and risk factors

This study has shown statistical significance between the risk factors and seropositivity of Toxoplasma IgG among the People Living with HIV was tested using logistic regression method.But among them the following showed the odd of having Toxoplasma infection among people that are living with HIV is 0.295 times lower among those from monogamous family than from polygamous family, the odds of having toxoplasmosis is 0.573 times lower among those using water closet type of toilet than those not using other types of toilets, the odds is 0.374 time lower among those that have ever received a unit of blood than those that have ever received more than one unit of blood, and are statistically significant at $p < 0.05$ People Living with HIV who has received blood transfusion were 8 times more likely to have Toxoplasma infection compared to others who have not received blood transfusion (OR = 0.374; CI: 0.169 – 0.830).

5.5 Assessment of knowledge of Toxoplasma infection

This study has shown that all the People Living with HIV and AIDS and attending clinic at Primary Health Care level in Ibadan, Nigeria did not have knowledge of Toxoplasmosis; its route of transmission, its signs and symptoms, its effect on immunosuppressed individuals and the way to prevent or avoid the infection. However, it is important to educate the public and most especially the pregnant women and the

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immunosuppressed patients that are more vulnerable about the pathologic effect of Toxoplasma infection.

CONCLUSION

Seroprevalence of Toxoplasma IgG was associated with consumption of unwashed fruits and vegetables, pet possession, blood transfusion and religious practices. People Living with HIV and AIDS who has received blood transfusion were 8 times more likely to have Toxoplasma infection compared to others who have not received blood transfusion.
Blood that is not screened for Toxoplasma infection is not safe for transfusion
People living with HIV and AIDS did not know anything about Toxoplasmosis. More so, Human Immunodeficiency Virus (HIV) does not kill, it is this opportunistic infection that kills just like others when not detected and treated early, since Toxoplasmosis diagnosis is not a routine test. Health Education and Screening for Toxoplasmosis among People Living with HIV and AIDS is essentially recommended.

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RECOMMENDATION

The prevalence of Toxoplasmosis infection is still relatively high among People Living with HIV and AIDS at Primary Health Care level in Ibadan, Nigeria. Following these findings adequate measures should be put in place to control the complications of this infection and curb its gradual increase, the following measures are therefore recommended:

- Government at the Federal, State and Local levels should sponsor Toxoplasma screening in order to have a reliable data for proper planning by Health authorities at different level of governance.
- This effort should be geared towards having a comprehensive nation-wide Health Education incorporated, so that the entire populace can be empowered with its knowledge.
- The National Policy Makers should include in the national guideline, the screening of Toxoplasma infection for HIV patients among other opportunistic infection which may breakdown their immune system
- There should be a definite policy of ensuring that Toxoplasmosis screening and monitoring is included in the tests protocols in the HIV management most especially among those showing acute symptoms of the diseases.
- Ministry of Environment and other relevant Government Agencies should enact a law which should be enforced by the Environmental Health Officers that will ensure “suya”, “asun” and “sun dried meat” are well prepared, processed and packaged in an hygienic environment to forestall and or minimize possibility of contamination with *T. gondii.*
- The test results should facilitate specific treatment and monitoring, in order to reduce the possibility of foetal infection and consequent damages to the foetus.

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- There should be Public Health Campaign for general improvement in personal hygiene, control of stray animals, better standard for animal farming and abattoir inspection should be mandatory within communities
- Use of well treated water for cultivation and preparation of vegetables and fruits being sold in the market should be encouraged.
- Blood donors should be screened for Toxoplasma infection before being qualified to donate blood for blood transfusion.

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