UNIVERSITY OF CAPE COAST

EPIDEMIOLOGY OF SYSTEMIC AND OCULAR TOXOPLASMOSIS AND THEIR ASSOCIATIONS WITH POLYMORPHISMS IN HUMAN INTERFERON GAMMA AND TUMOUR NECROSIS FACTOR CYTOKINE GENES

BY

EMMANUEL KWASI ABU

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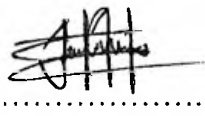MAY 2016

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:................................ Date:.24/05/2016

Name: Emmanuel Kwasi Abu

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:...................... Date:.24/05/2016

Name: Prof. Johnson Nyarko Boampong

Co-supervisor's Signature:........................... Date....24/05/2016

Name: Dr. Irene Ayi

ABSTRACT

The aim was to determine the associations between interferon gamma/tumour necrosis factor gene polymorphisms and *Toxoplama* infection in a community-based epidemiological survey. Sera were tested for IgG and IgM antibodies using ELISA test kits. Ophthalmic examination included visual acuity, slit lamp biomicroscopy and dilated funduscopy. A serologic criterion was a positive test result for either IgG or IgM antibodies or both. Ocular toxoplasmosis was diagnosed based on characteristic retinal lesions. Individuals with ocular infection served as cases and seropositive individuals without ocular disease as controls. There were 390 participants (mean age = 47.0 years), 30.3% males and 69.7% females. Seroprevalence of IgG and/or IgM antibodies was 85%. The study found a statistically significant relationship between *Toxoplasma* seropositivity and the following factors: contact with soil, presence of cats, older age, sources of drinking water, low levels of education, socioeconomic status, rural dwelling, and occupation (farmers and fishers/fish mongers). Ten (3%) subjects had toxoplasmic ocular lesions, contributing to 5.8% of visual impairment. Blindness occurred in 50% episodes of eye infections. The risk for developing *Toxoplasma* ocular lesions was old age (p = 0.028). Low prevalence of ocular infection was found in a population of high seropositivity. The results suggested contamination by sporulated oocysts as the major source of transmission. IFN-γ +874T allele seemed to increase the risk of developing ocular lesions. Also, the presence of the less common TNF−308A allelic form was found protective against the development of ocular infection in the present study.

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DEDICATION

To my wife and daughter

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LIST OF ACRONYMS

AAKD	Asebu Abura Kwamankese District
AIDS	Acquired Immunodeficiency Syndrome
AMA1	Apical Membrane Antigen 1
ARMS	Amplified Refractory Mutation System
CD	Cluster of Differentiation
CF	Count Fingers
CHPS	Community-Based Health Planning and Services
CI	Confidence Interval
CNS	Central Nervous System
CT	Computerized Tomography
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EU/ml	Enzyme Unit/ml
GHS	Ghana Health Service
GHS-ERC	Ghana Health Service's Ethical Review Committee
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
GRA	Dense Granule
H_2O_2	Hydrogen Peroxide
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
HWE	Hardy-Weinberg Equilibrium
ICD	International Classification of Diseases
IFAT	Indirect Fluorescent Antibody Test

IFN-γ	Interferon Gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ISAGA	Immunosorbent Agglutination Assay
LE	Left Eye
MHC	Major Histocompatibility Complex
MIC2	Microneme Protein 2
MRI	Magnetic Resonance Imaging
NaCl	Sodium Chloride
NFkB	Nuclear Factor Kappa Beta
NK	Natural Killer
NO	Nitrogen Monoxide
OD	Optical Density
OR	Odds Ratio
OT	Ocular Toxoplasmosis
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
RE	Right Eye
RONs	Rhoptry Neck Proteins
RPM	Revolutions per Minute
SAG1	Surface Antigen 1
SAG2	Surface Antigen Two

SAG3	Surface Antigen Three
SES	Socioeconomic Status
SNP	Single Nucleotide Polymorphism
SRS	SAG1- Related *Sequence*
STAT-1	Signal Transducer and Activator of Transcription 1
T. gondii	*Toxoplasma gondii*
TAE	Tris-Acetate-EDTA
TGF-β	Transforming Growth Factor Beta
TMB	Tetramethylbenzidine
TNF-α	Tumour Necrosis Factor-Alpha
UK	United Kingdom
USA	United States of America
VA	Visual Acuity
VI	Visual Impairment
WHO	World Health Organization
μl	Microlitre
μm	Micrometre
μM	Micromolar

CHAPTER ONE

INTRODUCTION

Background to the Study

Toxoplasmosis is a zoonotic parasitic infection caused by an obligate intracellular protozoan, *Toxoplasma gondii*, which is found worldwide (Tenter, Heckeroth & Weiss, 2000). The definitive host of the parasite belongs to members of the family *felidae*, but several warm-blooded animals including humans serve as intermediate hosts for the parasite (Lau et al., 2010). It is estimated that about 20 to 90 per cent of the world's population has been exposed to the parasite. The disease is important for its serious implications in immunosuppressed individuals such as HIV patients as well as its severe consequences on fetuses in congenital transmission (Jones et al., 2001). Transmission of *Toxoplasma gondii* infection in humans occurs by eating raw or undercooked meat containing *T. gondii* cysts. Infection can also occur by ingesting oocysts from soil, or by acquiring congenital infection through the placenta, and through blood and organ transplant from infected persons (Jones & Holland, 2010).

Serological surveys demonstrate a wide variation in the prevalence of the infection in various geographic locations. Prevalence is known to be influenced by climate and socioeconomis status (UK Food Standards Agency, 2012). Higher prevalence is observed for tropical countries with humid and warm climate, while

lower prevalence is found for arid and colder countries (Robert-Gangneux & Darde, 2012). The presence of cats and ingestion of raw meat or meat from animals kept outdoors may also account for these variations (UK Food Standards Agency, 2012). Thus, in the moist and tropical areas of Latin America and sub-Saharan Africa, where cats are abundant and the climate favours the survival of *Toxoplasma* oocysts, seroprevalence is higher in these regions (Stephen & Richard, 2001). Under laboratory conditions for instance, oocysts kept at 55°C and 60°C were rendered non-infective (Dubey, 1997). Again, the survival time of *T. gondii* tissue cysts has been found to vary greatly with the concentration of salt solution and at variable temperatures. In the laboratory, tissue cysts have been found to die in 6% NaCl solution at all temperatures examined (4 to 20°C), but survived in aqueous solutions with lower concentration of salt for several weeks (Dubey, 1997; Jamira, Martins, & Vieira, 1991; Navarro, Vidotto, Giraldi, & Mitsuka, 1992). These facts suggest that the concentration of salt in a particular soil may influence the survival of *T.gondii* parasites and subsequently influence disease transmission. Sea water with an average salinity of 3.5% (Sharqawy, Lienhard, & Zubair, 2010), when it makes enough contact with the soil can alter the survival time of the parasites. Some other studies have, however, detected *T. gondii* parasites in sea animals (Rengifo-Herrera et al., 2012; Conrad et al., 2005; Arkush et al., 2003; Lindsay et al., 2001), indicating that the transmission of *T. gondii* infection is perhaps more complex than anticipated.

Higher prevalence in France during the 1960s was believed to be related to preferences for consuming raw or poorly cooked lamb (Stephen & Richard,

2001). *Toxoplasma gondii* prevalence rates of 10 to 80% have been reported between countries and often within a given country or between different communities in the same region (Pappas, Roussos & Falagas, 2009). Testing of all pregnant women for *T. gondii* infection is routine in some countries, including France and Austria (Roberts, Murrell & Marks, 1994). In France, for instance, the existence of a national programme for the prevention of congenital toxoplasmosis since 1978 has seen seroprevalence among pregnant women fall from 84% in the 1960s to 54% in 1995 to 44% in 2003 (Berger, Goulet, le Strat, & Desenclos, 2009).

The infection is known to result in neonatal death, central nervous system (CNS) abnormalities, and uveitis (inflammation of the uvea of the eye) in both immunocompetent and immunosuppressed persons in many parts of the world (UK Food Standards Agency, 2012). Uveitis is a vision-threatening inflammatory ocular disease that affects humans of all age groups. It accounts for about 10–15% of blindness worldwide and *Toxoplasma gondii* is the single most common cause of posterior eye infection in humans (Hooper & McCluskey, 2008; McCannel et al., 1996). The infection involves intraocular inflammation and can lead to progressive loss of functional vision. Ocular toxoplasmosis is endemic in Southern Brazil but in Europe and the USA the prevalence is low. Ocular toxoplasmosis has not been studied in much detail in Africa even though the prevalence is thought to be high (Holland, 2003) and in Ghana there are no documented prevalence data at all.

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Different outcomes of the disease in different populations depend on the interaction of many factors, including the functions of host immune system and parasite factors such as parasite strains (Holland, 2009). There are basically three types of *T. gondii* parasite strains: types I, II and III and each of these or recombinants have been implicated in different populations. Not enough information is, however, available on parasite strains responsible for both systemic and ocular cases in Africa and for Ghana as well.

Immune mechanisms against *T. gondii* infection are associated with a strong T helper 1 response, and the susceptibility to the development of ocular lesions in toxoplasmosis seems to be associated with the production of pro-inflammatory cytokines, including interferon gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) that contribute to the inflammatory responses responsible for damaging the choroid and retina (Vallochi et al., 2005). Functional polymorphisms in cytokine genes interfere with the expression of these molecules and thus have an important role in the genetic regulation of inflammatory response and resistance or susceptibility to infectious diseases. Polymorphisms of IFN-γ and TNF-α genes at positions +874 and -308 respectively have been associated with several diseases (Hashemi et al., 2011; Sen et al., 2011; Hussein et al., 2009; Yu, Zhu, Gu & Fei, 2006). Globally, associations between these gene polymorphisms and the outcome of ocular toxoplasmosis have not been explored in much detail.

Treatment regimens for systemic toxoplasmosis include the use of pyrimethamine and sulfadiazine. In the case of ocular involvement, addition of

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corticosteroids is recommended. Hygiene remains the best preventive measure for *T. gondii* infection because currently there is no vaccine to prevent toxoplasmosis in humans.

Statement of the Problem

Pathogenesis of parasitic infections is highly dependent on the host genetic factors such as cytokine gene polymorphisms. Polymorphisms in the promoter region of TNF-α and IFN-γ genes govern the outcome of infections in terms of disease severity. Thus, an A to T point mutation of IFN-γ at +874 and a G to A single nucleotide polymorphism (SNP) of TNF-α at position -308, have been implicated in many disease outcomes (Zambon et al., 2005; Amim et al., 2008; Hashemi et al., 2011; El-Shabrawi et al., 2006; Kuo et al., 2005). Associations between these gene polymorphisms and the outcome of ocular toxoplasmosis have not only been barely studied but have also been contradictory and inconclusive. While previous studies in Brazilian patients did not associate TNF-α-308G/A polymorphism with the outcome of *Toxoplasma* retinochoroiditis (Cordeiro et al., 2008c), the IFN-γ +874T allelic form seemed to confer some protection against the disease (Albuquerque et al., 2009; Neves et al., 2012). A closer observation also gives a trend of diverse genotypic distribution profile of these cytokine gene polymorphisms in different populations, which could determine the disease outcomes in such populations. The important roles these cytokines play in the pathogenesis of the infection demands further investigations in different populations.

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There is a well documented literature about the distribution, risk factors, disease burden and the economic impact of *Toxoplasma* infection worldwide. However, in Ghana little information is available on seroprevalence of the infection. Again, the few studies in Ghana so far did not employ population-based studies to provide comparable data on *Toxoplasma* infection in the country (Ayeh-Kumi et al., 2010; Ayi et al., 2009). Moreover, documented information on the epidemiology of ocular toxoplasmosis in Ghana does not exist at all. It is therefore necessary to conduct a population-based survey to determine the prevalence levels of both systemic and ocular *T. gondii* infections and their associated risk factors in the population. The current study, apart from seeking to provide the basic epidemiological data on systemic and ocular toxoplasmosis, also seeks to investigate the associations between these outcomes of the infection and TNF-α and IFN-γ gene polymorphisms.

Purpose of the Study

The purpose of the study was to determine the epidemiology of systemic and ocular toxoplasmosis and their associations with TNF-α and IFN-γ gene polymorphisms among the population of three communities in Central Region of Ghana.

Objectives of the Study

The study was intended to achieve the following objectives.

1. To determine the levels of antibodies to *Toxoplasma* infection among the population of three communities in Central Region of Ghana.

2. To determine the prevalence of ocular toxoplasmosis in the study population.

3. To find out the risk factors associated with both systemic and ocular toxoplasmosis infection in the study population.

4. To ascertain the visual outcome in *Toxoplasma* ocular infection in the study population.

5. To determine the distribution profile of IFN-γ +874 and TNF-α 308 genotypes in the study population.

6. To investigate the association between single nucleotide polymorphism of IFN-γ +874T/A and ocular toxoplasmosis in the participants.

7. To determine the associations between ocular toxoplasmosis and TNF-α gene polymorphism at positions -308.

Significance of the Study

In Ghana, seroprevalence of *Toxoplasma* infection has not been determined in a population based study. Again, there is no documented evidence of the status of ocular toxoplasmosis in the country. With the current burden of toxoplasmosis in many parts of the world, a population based epidemiological data will provide important information regarding the status of *Toxoplasma* infection and its associated risk factors in the country. This will help formulate policies to deal with the infection as has been done elsewhere. For instance, since 1978 France has made it mandatory for pregnant women to screen for and treat *Toxoplasma* infection during pregnancy (Berger et al., 2009). The study on cytokine gene polymorphisms will be valuable to help elucidate the complex

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network of genes associated with the manifestation of ocular toxoplasmosis generally and in this study population in particular. Since this is the first epidemiological study on ocular toxoplasmosis in the country, it will provide a comparable data to facilitate the planning of further studies into this area of parasitic infection and add to the existing stock of knowledge.

Delimitations of the Study

The epidemiological study was delimited to the Central Region of Ghana and specifically to three communities, namely Nkanfoa in the Cape Coast metropolis, Moree in the Asebu Abura Kwamankese District and Jukwa in Twifo Heman Lower Denkyira District. The genetic aspect included only patients with retinochoroidal lesions and those who tested positive for anti-*Toxoplasma* IgG or IgM antibodies. The cytokines investigated were limited to only IFN- γ +874 and TNF-α -308.

Limitations of the Study

The following limitations on the study were identified. By the use of a cross sectional study design, the study only measured prevalence rates and therefore information on temporal sequence between the exposure and disease as well as prognosis and natural history of the infection among the study population could not be established in this study. Moreover, the study could not establish causality in respect of cytokine gene polymorphism and the occurrence of ocular lesions; only associations could be determined.

Because ocular infection among the study population was not common, the use of only 30 cases in the case-control aspect made it difficult for the analysis

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to attain statistical significance. The use of logistic regression model, however, made it possible to make some inferences from the data.

The study was done to determine the exposure of the individual participants to *Toxiplasma gondii* by detection of specific IgG and/or IgM antibodies. Thus, the findings were on seroprevalence and risk factors, and did not include clinical findings such symptomatology.

Definition of Terms

Blindness refers to visual acuity of "No Light Perception" (NLP) in the better eye. Technically, it is defined as presenting visual acuity < 3/60 in the better eye.

Cytokines are substances secreted by certain cells of the immune system that have an effect on cellular activity and controls inflammation

Epidemiology is the science that involves the study of the incidence and distribution of diseases in large populations.

Low vision refers to a vision that is so bad that even after treatment and/or standard refractive correction the person's vision does not improve so much. That is, the visual acuity still remains between 6/18 to light perception in the better eye.

Parasite is an organism which lives in or on another organism (its host) and benefits by deriving nutrients at the other's expense

Prevalence refers to the total number of existing cases among the whole population.

Refractive error is a defect of the eye in which parallel rays from a distance object are not brought to a focus on the retina when accommodation is relaxed

and /or when rays from a near object are not focused on the retina when accommodation is stimulated.

Toxoplasmosis is the infection caused by the parasite *Toxoplasma*

Visual acuity is the resolving power of the eye or a measurement of the ability to see two separate objects as separate. Normal vision is designated a visual acuity of 6/6.

Visual impairment is a limitation or reduction in the ability of the eye or the visual system to perform.

Organisation of the Study

The study is organised into six chapters. Chapter one which is the introduction deals with background to the study, statement of the problem, purpose of the study, objectives, significance of the study, delimitation, limitations, definition of terms and organization of the study. Chapter two is the review of literature related to the study. Chapter three is the research methodology. It encompasses description of the study sites, study design, sampling procedure, data collection procedure and data analysis. Chapter four covers the results. Chapter five is the discussion. Summary of findings, conclusion and recommendations are captured in chapter six.

CHAPTER TWO

LITERATURE REVIEW

The History of *Toxoplasma gondii*

Toxoplasma parasite was first described in Africa and Latin America at the same time. Nicolle and Manceaux (1908) found a protozoan in tissues of a hamster-like rodent, *Ctenodactylus gundi*, which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis. Nicolle initially mistook the parasite for *Leishmania*, but soon realized that he had discovered a new organism and named it *T. gondii* based on the morphology and the host. Thus, the correct name for the parasite should have been *T. gundii*. Nicolle and Manceaux (1908) had incorrectly identified the host as *Ctenodactylus gondi* (Dubey, 2007). At the same time Splendore (1908) discovered the same parasite in a rabbit in Brazil, also erroneously identifying it as *Leishmania*, but he did not name it. Knowledge and appreciation of the spectrum of diseases that the parasite can cause then developed gradually. Wolf, Cowen and Paige (1939) at Columbia University identified the parasite in central nervous lesions in infants that had been diagnosed with meningoencephalitis.

Protection to *T. gondii* turned out to be complex involving innate and specific immunity. The high prevalence of the infection in various populations was first shown by the serological test developed by Sabin and Feldmann (1948). This

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procedure relied on the ability of human serum to induce leakage of extracellular dye into live tachyzoites in the presence of complement. Antibodies were found to kill extracellular but not intracellular tachyzoites (Sabin & Feldman 1948; Sabin & Olitsky 1937). Understanding of the role of chronic infection came with the identification by Wilder (1952) of the parasite in necrotic lesions of the retina of eyes previously thought to have been involved with tuberculosis or syphilis. In the next 50 years, protective immunity was found to be mediated largely by immune lymphoid cells (Frenkel 1967; Gazzinelli, Hakim, Hieny, Shearer & Sher, 1991; Suzuki, Orellana, Schreiber & Remington, 1988). During the 1980s and 1990s methods were developed to recognize genetic differences among *T. gondii* isolates from humans and animals (Darde, Bouteille & Pestre-Alexandre, 1987; Howe & Sibley, 1995; Pfefferkorn & Pfefferkorn 1980; Sibley, Leblanc, Pfefferkorn & Boothroyd, 1992)

Population Structure of *T. gondii*

The population structure of *T. gondii* indicates that despite the existence of a well-described sexual phase in its life cycle, the parasite appears to reproduce in nature largely clonally, with sexual recombination occurring rarely (Grigg, Ganatra, Boothroyd, & Margolis, 2001). Demonstration of the sexual cycle in the cat intestine indicates that sexual recombination is possible, and it can be shown to occur in experimental infections (Stephen & Richard, 2001). This must, however, be relatively infrequent in nature, probably because it would require a cat to ingest two separate strains of *T. gondii* in close temporal proximity, so that the initial

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intestinal infection produces gametes that could cross-fertilize (Stephen &
Richard, 2001).

Most strains of *T. gondii* belong to one of three clonal lineages known as
types I, II, and III (Howe & Sibley, 1995; Howe, Honore, Derouin, & Sibley,
1997). A fourth clonal lineage, referred to as type 12, has been described in North
America where it is commonly found in wildlife (Khan et al., 2011). Genotypes of
T. gondii strains isolated from places like Brazil and France have been found to be
highly divergent when compared to the previously described clonal lineages.
These findings show that the genetic makeup of *T. gondii* is more complex than
was previously thought and that unique or divergent genotypes may contribute to
different clinical outcomes of toxoplasmosis in different localities (Khan et al.,
2006). Limited genetic diversity between and within clonal lines indicated that
they have quite recently evolved from a common ancestor (Su et al., 2003). Type
II strains have most commonly been associated with human toxoplasmosis, with
type III being associated with animal infection. Type I strains, however, have
been implicated in several outbreaks associated with a high frequency of ocular
toxoplasmosis (Grigg et al., 2001). Significant global differences, however, exist.
Thus, in Spain, type I and II strains have been found to exist in the proportions of
75 and 52% respectively (Fuentes, Rubio, Ramirez, & Alvar, 2001), while
genotyping of isolates from Crete and Cyprus has shown the presence of type II
(20%) and type III (80%) (Messaritakis, Detsika, Koliou, Sifakis, & Antoniou,
2008). Isolates from France have shown 85% of type II strains with the rest being
type I and atypical strains (Ajzenberg et al., 2002). A predominance of the same

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type II strains has also been indicated in Poland (Nowakowska et al., 2006) and Serbia (Djurkovic-Djakovic et al., 2006). As in France, type II strains appear to be responsible for the majority of symptomatic human cases in the United States (Nowakowska et al., 2006), with types I and III only occurring in 10% and 9% of *Toxoplasma* isolates from patients, respectively (Howe et al., 1997). In Brazil, type I strains appear to be responsible for ocular infections in human patients (Vallochi et al., 2005). It has been shown that parasites isolated from contaminated water in the Brazillian Southern state of Paraná were type I (de Moura et al., 2006). Again, some data on Brazilian patients suggest a cloning diversity when compared with strains from North America and Europe (Khan et al., 2006). A study found that in the Southern Brazilian city of Erechim, type I *T. gondii* predominates (Khan et al., 2006). The genotypes of *T. gondii* strains isolated from São Paulo and Erechim (Brazil) were highly atypical when compared with the previously described cloning lineages reported in the literature. Genotyping of *T. gondii* in 24 chickens from the Amazon, Brazil, indicated that 14 were type I and 10 were type III, confirming that strains in Brazil are divergent from European or North American strains (Lehmann, Marcet, Graham, Dahl, & Dubey, 2006).

Unlike Europe and the Americas, there is a limited available data on the genetic diversity and population structure of *T. gondii* from Africa. Genetic data regarding isolates from Africa are scarce. Genotyping based on *T. gondii* strains isolated from chickens from diverse African countries - Egypt, Kenya, Nigeria, Congo, Mali, and Burkina Faso (Lindstrom et al., 2008; Velmurugan, Dubey, &

Su, 2008) have suggested that like in Europe and the USA, the same three main lineages predominate in Africa with one strain considered to be a recombinant between Type II and III strains (Bontell et al., 2009). Nonetheless, non classical genotypes of the parasite, called Africa 1 and Africa 2, have been isolated from immunocompromised patients with toxoplasmosis acquired in Western and Central Africa (Ajzenberg et al., 2009). Genotyping of animal isolates from Gabon indicated the predominance of type III and the presence of the haplogroups Africa 2 and Africa 3 (Mercier et al., 2010). A study of human samples from Tunisia suggested the predominance of recombinant strains (I/III and I/II) (Boughattas et al., 2010).

Virulence in *Toxoplasma gondii* is influenced by parasite genotype. Studies with mice have demonstrated that infections with the different strains of *T. gondii* result in different outcomes. Type I strains were found capable of causing lethal infection in mice regardless of the host genotype, whereas type II and type III were relatively nonvirulent (Sibley, & Boothroyd, 1992). The enhanced virulence of type I strains is in part due to overstimulation of a Th1 immune response leading to a lethal cascade of proinflammatory cytokines (Mordue, Monroy, La Regina, Dinarello, & Sibley, 2001). Some studies have suggested that type I strains are more pathogenic in immunocompromised patients. Some studies among AIDS patients showed an increased frequency of type I strains (Howe & Sibley, 1995; Khan et al., 2005). Type II strains are the most abundant, while type I strains are relatively rare in agricultural and wild animals from North America (Dubey et al., 2003; Howe, & Sibley, 1995). The

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virulent trait in type I strains is linked to a parasite gene on chromosome *VIIa* that seems to be conserved in all type I strains that have been examined so far (Su, Howe, Dubey, Ajioka, & Sibley, 2002).

Description of *T. gondii* Parasite

Toxoplasma gondii has three infective stages. These are tachyzoites (in groups or clones), bradyzoites (in tissue cysts), and sporozoites (contained in oocysts). The tachyzoite and bradtzoite stages are asexual forms while the oocyst is the product of sexual reproduction (Stephen & Richard, 2001). These infective stages are cells of crescent-shaped, about 5µm in length and 2µm in width. They have a pointed apical end and a rounded posterior end. They are limited by a complex outer membrane, the pellicle, closely associated with a cytoskeleton involved in the structural integrity and motility of the cell. These cells possess many organelles including the apicoplast, the result of a possible acquisition by the parasite via a secondary endosymbiosis of a free-living red alga (Robert-Gangneux & Dardé, 2012).

The term "tachyzoite" (tachos means speed in Greek) was coined by Frenkel (1973) to refer to the stage that rapidly multiplied in any cell type of the intermediate host and in non-intestinal epithelial cells of the definitive host. They can practically invade all categories of vertebrate cells, where they multiply rapidly in a parasitophorous vacuole, leading to cell death. Tachyzoite replication causes acute infection. Tachyzoites enter host cells by actively penetrating through the host cell plasmalemma or by phagocytosis (Morisaki, Heuser, & Sibley, 1995). Upon penetrating the host cell, the tachyzoite assumes an ovoid

shape and becomes surrounded by a parasitophorous vacuole, which appears to be derived from both the parasite and the host cell. Tachyzoites multiply asexually within the host cell by repeated endodyogeny, a specialized form of reproduction where two progeny form within the parent parasite. The progeny continue to grow until they reach the surface of the parent. The inner membrane complex of the parent disappears, and its outer membrane becomes the plasmalemma of the progeny cells (Dubey, Lindsay, & Speer, 1998; Robert-Gangneux & Dardé, 2012).

The term "bradyzoite" (brady means slow in Greek) was again coined by Frenkel (1973) to describe the organism multiplying slowly within a tissue cyst. They result from the conversion of tachyzoites into a slow-dividing stage and form tissue cysts. They grow and remain intracellular as the bradyzoites divide by endodyogeny. They vary in size from 10 μm for the younger cysts, containing only two bradyzoites, to up to 100 μm for the older ones, containing hundreds or thousands of densely packed bradyzoites. The cyst wall consists of a limiting membrane presenting numerous invaginations and an underlying layer of electron-dense granular material (Ferguson, 2002). Although tissue cysts may develop in visceral organs, including the lungs, liver, and kidneys, they are more prevalent in the neural and muscular tissues, such the brain, eyes, and skeletal and cardiac muscles (Dubey et al., 1998). Bradyzoites have a low metabolic activity and well adapted to long-term survival. The death of the host cell may trigger the disruption of the cyst wall and the consequent liberation of bradyzoites.

The resistance of bradyzoites to the acid pepsin allows their transmission through ingestion. Bradyzoites are less susceptible to destruction by proteolytic enzymes than tachyzoites but less resistant to environmental conditions than oocysts, remaining infectious for up to three weeks at 1-4°C, whereas tissue cysts are normally killed by freezing at -12°C. Again, they may survive curing, depending on the conditions used. However, they are killed by temperatures above 67°C and by gamma irradiation (UK Food Standards Agency, 2012)

Sporozoites are located in mature oocysts. Oocysts are 12- to 13-μm ovoid structures that after sporulation contain two sporocysts, each containing four sporozoites. The oocyst wall is an extremely robust multilayer structure protecting the parasite from mechanical and chemical damages. It enables the parasite to survive for long periods, up to more than a year, in a moist environment (Mai et al., 2009).

Life Cycle of *T. gondii*

Toxoplasma gondii has a complex life cycle that includes both sexual and asexual cycles. The asexual cycle occurs in a wide range of intermediate hosts while the sexual cycle occurs exclusively in feline hosts. Within intermediate hosts, the parasite undergoes only asexual development. After the ingestion of cysts present in tissues of an intermediate host, the cyst wall is destroyed by gastric enzymes with the release of bradyzoites. They settle within enterocytes, where they undergo a self-limiting number of asexual multiplications, characterized by the development of merozoites within schizonts (Dubey, 1998). This ends the asexual development.

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Sexual reproduction begins with the formation of male and female gametes from the merozoites (Ferguson, 2002). After fertilization of the female gamete by the male gamete, oocysts are formed within the enterocytes of small intestines of the feline host. The oocysts are liberated by the disruption of the cell and excreted as unsporulated forms in the cat (feline) feces. The process of sporogony occurs after a few days in the external environment. Oocysts require exposure to air, after cat feces are deposited in the soil, for at least twelve hours but up to several days in order to complete sporulation, after which they are infectious by mouth. This information is useful in the management of cat litter boxes, which have a lower chance of harboring infectious oocysts if the feces are removed daily (Stephen & Richard, 2001). Unsporulated oocysts undergo meiotic reduction and morphological changes leading to the formation of sporulated forms with two sporocysts, each containing four haploid sporozoites. After ingestion of tissue cysts, shedding of oocysts begins 3 to 7 days and may continue for up to 20 days. Infected cats can shed more than 100 million oocysts in their feces (Ferguson, 2002; Jones & Dubey, 2010). They can infect a wide range of intermediate hosts, virtually all warm-blooded animals, from mammals to birds.

Following ingestion of oocysts, sporozoites are liberated and penetrate the intestinal epithelium, where they differentiate into tachyzoites (Figure 1). Tachyzoites rapidly replicate by endodyogeny inside any kind of cell and disseminate throughout the organism. As a result of the conversion from tachyzoite to bradyzoite, tissue cysts arise as early as 7 to 10 days post infection and can remain as long as the life of the host. Upon ingestion of these tissue cysts

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by any intermediate host through raw or undercooked meat, cysts are ruptured as they pass through the digestive tract, causing the release of bradyzoites (Figure 1). Bradyzoites in turn will infect the intestinal epithelium of the new host and differentiate back into the rapidly dividing tachyzoite stage for dissemination throughout the body. If the acute phase occurs during pregnancy, the parasite can cross the placenta and infect the fetus ((Robert-Gangneux & Dardé, 2012).
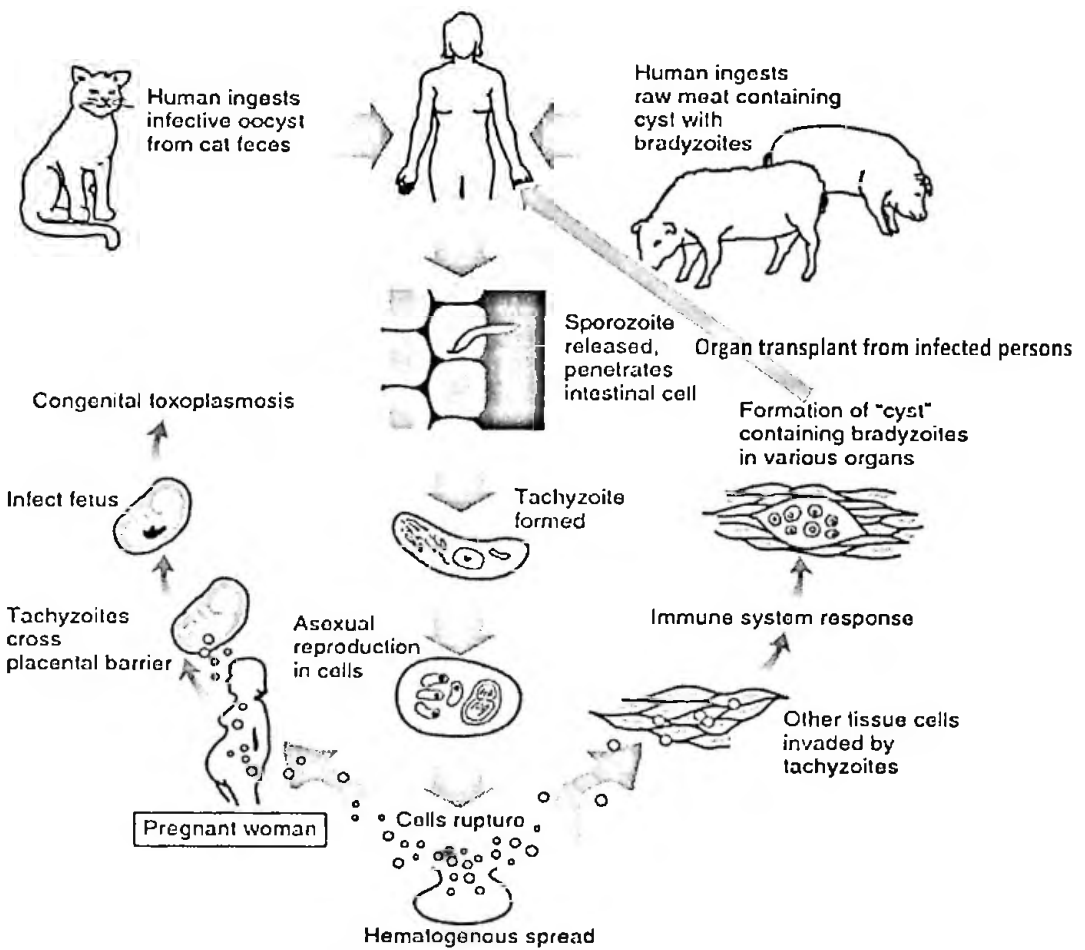
Figure 1: *Toxoplasma gondii* **life and transmission cycle**

Source: Smith, 2000.

Mechanism of Cell Invasion by *T. gondii* Parasites

Parasite invasion is an active process relying on parasite motility and the sequential secretion of proteins from secretory organelles. The parasite has three secretory organelles, the rhoptries and micronemes that secrete their contents at the anterior pole, and dense granules that secrete along the lateral surface and posterior pole of the parasite. Active forward movement of the parasite is an absolute requirement for host cell penetration (Stephen & Richard, 2001). Attachment to the host cell membrane is a prerequisite for invasion. The mechanical events involved in parasite attachment and penetration include (i) gliding of parasite, (ii) probing of the host cell with the conoidal tip of parasite, (iii) indenting the host cell plasmalemma, (iv) forming a moving junction that moves posteriorly along the parasite as it penetrates into the host cell, and (v) partially exocytosing micronemes, rhoptries, and dense granules (Dubey et al., 1998).

Parasite gliding refers to the interaction between the host cell surface and the parasite. It is an intricate linear motor system promoted by actin-myosin interactions and dynamic rearrangements of the parasite cytoskeleton (Carruthers, & Boothroyd, 2007). It requires calcium-dependent secretion of adhesins from micronemes, such as the microneme protein 2 (MIC2), which recognizes host cell receptors and promotes parasite reorientation and attachment. *Toxoplasma* forms a tight association between its apical end and the host cell membrane, called the moving junction. This moving junction complex which defines the parasite entry site on the host cell plasma membrane moves from the apical end to the posterior end of the parasite, leading to internalization of the parasite into a parasitophorous

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vacuole. 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 1 (AMA1), secreted by micronemes, and the secretion of rhoptry neck proteins inserted into the host cell membrane.

Though *T. gondii* is able to invade almost all types of nucleated cells from its vertebrate hosts, very little is known about specific host cell receptors that are involved. Invasion rather occurs when motile parasites contact the host cell with their apical end and this often occurs as a direct consequence of active gliding motility and without specific receptor interaction (Sibley, Charron, Hakansson, & Mordue, 2007; Hakansson, Morisaki, Heuser, & Sibley, 1999).

Parasite penetration occurs by the parasite propelling itself forward, via acto-myosin-dependent motility, into the host plasma membrane (Suss-Toby, Zimmerberg, & Ward, 1996). Entry is a rapid process, about 15 to 30 seconds. This causes an invagination of the plasma membrane resulting in the formation of the parasitophorous vacuole, which is the compartment that the parasite resides in throughout its time in the host cell (Sweeney, Morrissette, LaChapelle & Blader, 2010). The formation of the nascent parasitophorous vacuole membrane requires the secretion of proteins from the rhoptries (Robert-Gangneux & Dardé, 2012). Proteins from dense granules also contribute to the formation of the parasitophorous vacuole membrane during the first hour following invasion. Secretions from dense granule also support the development of a tubulovesicular membranous network within the parasitophorous vacuole. This network supports exchanges between the parasite and the host cell, bringing in nutrients from the

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host cell cytosol and exporting substances from the parasite to the host cell (Mercier, Adjogble, & Daubener, 2005). The parasitophorous vacuole membrane is connected to the host cell mitochondria, contributing to parasite metabolism. Within the parasitophorous vacuole, tachyzoites divide by 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 parasitophorous vacuole (Black & 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).

Toxoplasma gondii Surface Proteins

In the human intermediate host, the parasite switches between two different developmental forms: the tachyzoite and the bradyzoite. One hallmark of the *Toxoplasma* developmental switch is the differential expression of numerous, closely related glycophosphatidylinositol-anchored surface proteins belonging to the surface antigen 1 (*SAG1*) related *s*equence (SRS) superfamily, numbering about 161 (Jung, Lee & Grigg, 2004). Most characterized members of this family are found exclusively on the surfaces of either tachyzoites or bradyzoites, such that the tachyzoite surface is dominated by SAG1, SAG2A, SAG3, SRS1, SRS2, SRS3, and several less highly expressed SRSs (Manger, Hehl, & Boothroyd, 1998), while the bradyzoite surface is dominated by SAG2C, SRS9, and SAG4 (Jung et al., 2004).

In addition to serving as cell adhesion molecules in host invasion (Robinson, Smith & Millner, 2004; Jacquet et al., 2001; Mineo & Kasper, 1994),

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SRS antigens are also important targets of the adaptive immune response. For instance, SAG1 and SAG2A which are immunodominant within the superfamily induce a high antibody response early after infection (Bessieres, Breton & Seguela, 1992). The numerous SAG proteins could also be responsible for allowing *T. gondii* to invade a wide variety of host cells (Lekutis, Ferguson, Grigg, Camps, & Boothroyd, 2001). It is also thought that these SRS molecules play an important role in modulating the immune response. Surface antigen 1 has been found to play a critical role in driving a proinflammatory response (Rachinel et al., 2004). It is speculated that bradyzoite specific SRSs evolved just to be different from their tachyzoite counterparts and hence not recognizable by the strong immune response generated against the tachyzoite SRSs. It is also possible that bradyzoite SRSs have a role in attachment to cells in the brain, as this is the site where many *Toxoplasma* cysts are found in chronic infections (Pollard, Onatolu, Hiller, Haldar & Knoll, 2008; Kim, Karasov & Boothroyd, 2007).

Other highly immunogenic *Toxoplasma* proteins are the dense granule (GRA) proteins (Mercier, Cesbron-Delauw, & Ferguson, 2007). These proteins, which make up most of the circulating antigens in the blood stream of an infected host, can be detected in the acute phase few hours after infection (Hughes, & van Knapen, 1982). They are found in the tachyzoite and bradyzoite stages of the parasite (Cesbron-Delauw, 1994). About 12 different dense granule proteins have been identified (Ching, Lau, Fong, Nissapatorn, & Andiappan, 2014). Those such as GRA2, GRA6, GRA7, and GRA8 have been used via enzyme-linked immunosorbent assay (ELISA) to discriminate acute from chronic *Toxoplasma*

infections (Redlich & Müller, 1998). Dense granule GRA5 antigen aids in cell invasion (Lecordier et al., 1993).

Classification

Toxoplasma gondii is a member of the phylum Apicomplexa, class Sporozoa, sublcass Coccidia, order Eucoccidia, suborder Eimeria, Family: Sarcocystidae; these group members carry out a life cycle that requires more than one obligatory host. The hosts themselves usually participate in a predatory-prey relationship. Oocysts are passed between them through feces. The genus *Toxoplasma* requires transmission between a member of the *felidae* and the rodents to carry out its sexual life cycle. The species *Toxoplasma gondii* is the only species in the genus *Toxoplasma*.

Pathogenesis of *Toxoplasma* Infection

Toxoplasma infection is classified into acute and chronic stages. The acute or early stage is mostly associated with the tachyzoite stage while the tissue cyst is the predominant form during chronic infection, although tachyzoites have been found outside of cysts at this stage (Waree, 2008). Tissue cysts may be formed as early as 3 days post infection but are usually not numerous until 7 weeks and probably persist as viable parasites throughout the life of the host (Derouin et al., 1989; Dubey & Frenkel, 1976). During acute infection, the tachyzoites invade every kind of host cell except non-nucleated red blood cells. The host cell invasion is a major step in its biological cycle and in pathogenesis. They multiply intracellular, causing host cell disruption. The liberated parasites invade and destroy adjacent cells, producing progressively larger focal lesions. If the initial

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infection occurs when the host is pregnant, the tachyzoites associated with the acute phase can cross the placenta and infect the fetus. However, with the onset of the host immune response, a subpopulation of tachyzoites in the brain, undergo stage conversion to bradyzoites which multiply slowly to form large tissue cysts with numerous of bradyzoites (Montoya & Liesenfeld, 2004; Mordue et al., 2001). During acute and chronic toxoplasmosis, lesions or tissue necrosis may be found in many organs of the body such as intestines, liver, spleen, pancreas, lung, heart, eye and the brain.

Pathogenesis in the eye

The immune privileged status of the eye and cytokine responses are key factors in toxoplasmic retinochoroiditis. The pathogenesis of ocular toxoplasmosis is directly linked to the anatomical characteristics of the eye resulting in an immune privileged status. The presence of the blood-retinal barrier and the absence of lymphatic vessels limit the passage of inflammatory cells and lymphocytes and of antibodies and complement components (Hori, Vega, & Masli, 2010). In addition, the ocular characteristics of the distribution and the functions of antigen presenting cells are also of importance. For example, corneal epithelium is deprived of Langerhans cells and the dendritic cells of the ciliary epithelium are not activated by granulocyte macrophage colony-stimulating factor (GM-CSF) and do not stimulate T lymphocytes (Streilein, 1993; Forrester, 2009). There is a low expression of classical major histocompatibility complex (MHC) class I molecules which reduce the lytic activity of CD8+ lymphocytes. MHC II molecules are not expressed in the eye, which limits CD4+ lymphocyte activation.

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An increased expression of surface molecules like CD46, CD55 and CD59 will also inhibit complement activation (Dupouy-Camet et al., 2012; Bora, Gobleman, Atkinson, Pepose, & Kaplan, 1993).

One hallmark of this immune privilege status of the eye is the local production of immunosuppressive cytokines, such as transforming growth factor beta (TGF-β), which seeks not only to reduce the ability of natural killer (NK) cells, a major source of IFN- γ, but also down-regulate macrophage function to produce nitric oxide and indolamine. Indolamine has been shown to prevent parasite multiplication in human cells (Hori et al., 2010; Denniston et al., 2011; Zhou, Horai, Mattapallil, & Caspi, 2011). There is also high constitutive expression of Fas ligand on ocular cells which serves to induce apoptosis and down-regulate the function of T cells and NK cells (Ferguson & Griffith, 2007; Roychoudhury, Herndon, Yin, Apte, & Ferguson, 2010). It is believed that immune responses directly affect the pathogenesis of toxoplasmic retinochoroiditis and some cytokines have been shown to be fundamental to either control or block a protective response against *T. gondii* in experimental models. Thus, the clinical manifestations of toxoplasmosis results from direct tissue destruction by the parasite, while inflammatory cytokine mediated immunopathologic changes may also contribute to disease progression.

In humans, the participation of inflammatory mediators in the pathophysiology of ocular toxoplasmosis (OT) is not yet fully understood. However, a study by Yamamoto et al. (2000) showed that asymptomatic patients secreted significantly more Interleukin 12 (IL-12) and IFN-γ in response to *T.*

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gondii antigens than patients with ocular damage. A decreased production of IL-2 and IFN-γ by peripheral blood mononuclear cells in response to *T. gondii* was observed in subjects with congenital infection, suggesting a status of parasite tolerance (Yamamoto et al., 2000). A possible association between polymorphisms in cytokine genes and OT has been investigated in patients and specific IL-1, IL-10 and IFN-γ alleles were preferentially found in patients with OT (Cordeiro, Moreira, Andrade, et al., 2008; Cordeiro et al., 2008a, 2008b; Albuquerque et al., 2009).

Immune Responses to *T. gondii* Infection

Non-specific immune responses

Toxoplasma gondii is able to trigger the nonspecific activation of macrophage, NK cells and other cells such as fibroblasts, epithelial or endothelial cells during the earliest stages of infection. This activation is for limiting parasite proliferation because of its direct or indirect cytotoxic action and to activate a specific immune response. This non-specific immune response reacts immediately after the first contact between the parasite and the host. It peaks at the end of the first week, and then slowly reduces until it is absent in the second week (Waree, 2008). In mice, the activation of macrophages by interferon gamma in the presence of co-signals, such as tumour necrosis factor-α, is necessary to trigger the cytotoxic activity of macrophages against *T. gondii* (Filisetti & Candolfi, 2004).The innate immune response occurs in the early induction of proinflammatory cytokines during infection of naive animals, and studies have shown that dendritic cells, neutrophils and macrophages respond directly to

parasite antigens by producing IL-12, IFN-γ and TNF-α in the acute stage of toxoplasmosis (Johnson, 1992; Denkers & Gazzinelli, 1998). If the parasite is not destroyed the process of the immune system continues.

Specific immune responses

As an intracellular parasite, specific protective immunity against *T. gondii* is predominantly a cell-mediated response (Ronday, Ongkosuwito, Rothova, & Kijlstra, 1999; Waree, 2008; Joynson & Wreghitt, 2001; Lang, Gross, & Luder, 2007). Studies have shown that normally avirulent strains of *T. gondii* become highly virulent in T-lymphocyte-deficient animals (Waree, 2008; Lindberg & Frenkel, 1977; Gazzinelli, Hieny, Wynn, Wolf, & Sher, 1993). The macrophages and NK cells are primary cells of defense against the parasite during the early infection stage (Sher, 1998; Gazzinelli et al., 1996). Interleukin 12, which is the major cytokine produced by the macrophages and dendritic cells during antigen stimulation, appears to play a major anti-*Toxoplasma* role during the acute phase of the infection. IL-12 activates the production of IFN-γ by NK cells and T-lymphocyte (CD4+ and CD8+) cells. The CD4+ and CD8+ T-lymphocytes are the main players involved in resistance of the host to *Toxoplasma* infection. TNF-α is also essential to activate macrophage to inhibit parasite replication (Bhopale, 2003). IFN-γ activates the macrophages by enhancing their oxidative metabolism, releasing hydrogen peroxide (H_2O_2) that kills the parasites.

Nonoxidative mechanisms, represented mainly by the production of nitrogen monoxide (NO) by macrophages during the chronic phase are involved in inhibition of intracerebral parasite proliferation (Schluter, Lohler, Deckert, Hof,

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& Schwendemann, 1991). IFN-γ also increases the activity of indoleamine 2, 3-dioxygenase (Figure 2), resulting in the breakdown of tryptophan, required for growth of the parasite (Pfefferkorn, Rebhun, & Eckel, 1986). *Toxoplasma* infection induces rapid activation of transcription factors such as signal transducer and activator of transcription 1 (STAT-1) and (Nuclear Factor Kappa Beta) NFkB (Figure 2). The parasite can block nuclear translocation of both factors, disabling macrophages to produce IL-12 or TNF-α, and the parasite is able to evade or subvert the immune response of its host (Denkers, Kim, & Butcher, 2003; Buzoni-Gatel & Werts, 2006).

The antibodies in humoral immune response act as a minor role but still important for diagnosis of toxoplasmosis in humans. In addition, these antibodies have been found to be able to kill extracellular *T. gondii*. They limit multiplication of *Toxoplasma* by engaging in lysing the parasites in the presence of the complement. They are also involved in opsonization and increase in phagocytosis by the macrophages (Sibley, Weidner, & Krahenbuhl, 1985; Bhopale, 2003).
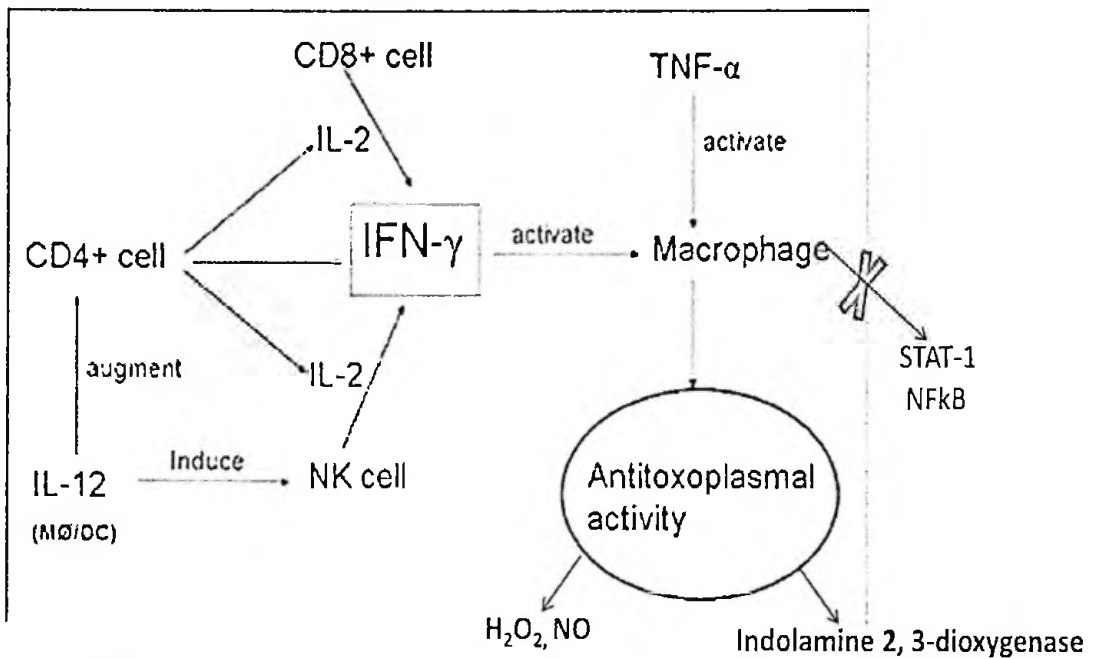
Figure 2: Immune responses to *Toxoplasma* infection

Source: Waree, 2008.

Tumour necrosis factor-alpha cytokine gene

Tumour necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that protects the host against *Toxoplasma gondii* infection by increasing the microbicidal activity of macrophages and by inducing the secretion of interferon-gama (IFN-γ) by NK cells (Filisetti & Candolfi, 2004).This cytokine is involved in several biological processes such as tissue remodelling, epithelial cell barrier permeability, macrophage activation, recruitment of inflammatory cells, effectiveness of the local and systemic inflammation, and amplification of other proinflammatory cytokine actions (Bradley, 2008; Kollias, Douni, Kassiotis & Kontoyiannis, 1999). The biological functions of TNF-α have been demonstrated to be related to the concentration and the duration of exposure to TNF-α molecule. In the acute situation, local production of TNF-α has a clear positive

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action increasing the expression of adhesion molecules on the vascular endothelium to allow immune cells, as macrophages and neutrophils, to reach the sites of tissue damage or infection. Specifically, the release of some chemokines leads to recruitment of different populations of leukocytes independent of antigen recognition.

Many of the classical features of inflammation can be produced by local effects of TNF on endothelial cells. TNF-induced expression of cyclooxygenase-2 results in the production of vasodilatation (Mark, Trickler, & Miller, 2001), causing rubor (redness) and calor (heat) through increased local blood flow. Tumor (swelling) can result from TNF mediated increased vascular permeability, allowing the increased trans-endothelial passage of fluid and macromolecules to create oedema. In addition, TNF-induced expression of pro-coagulant proteins down-regulation of anticoagulant protein can cause intravascular thrombosis (Bevilacqua et al., 1986). Furthermore, TNF-α activates phagocytes to clear infectious agents and cellular debris (Barbara, Ostade & Lopez, 1996; Elahi, Asotra, Matata, & Mastana, 2009). On the other hand, systemic or protracted exposure to TNF-α may be harmful. The TNF-α acts by binding to TNF cellular receptor present on all cells in the body. Generally, higher TNF-α levels are related to the severity of inflammatory response (Scardapane, Breda, Lucantoni & Chiarelli, 2012). It is however, not clear if greater TNF-α production causes more severe inflammation or, conversely, if more severe inflammation elicits greater TNF-α synthesis.

The TNF-α gene is located on human chromosome 6 within the major histocompatibility complex (MHC) (Hajeer & Hutchinson, 2000). It lies in the class III region, between the genes encoding the MHC class human leukocyte antigen class II cell surface molecules and the MHC class I antigen (Figure 3).
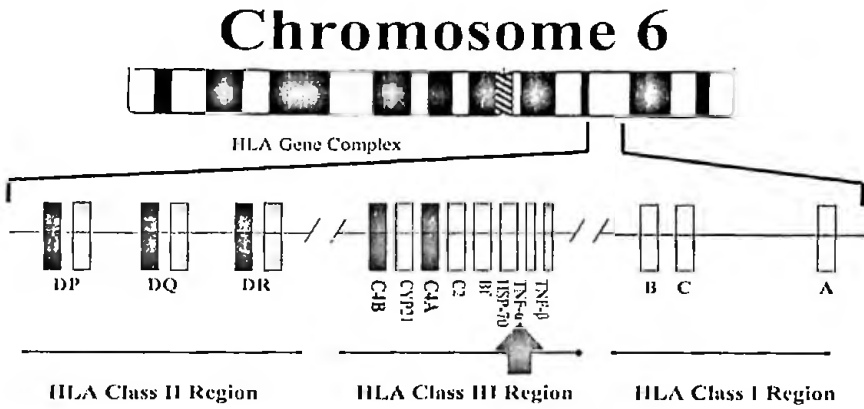
Chromosome 6

Figure 3: Location of TNF-α gene on human chromosome 6p21

Cells of the monocyte/macrophage lineage are the main source of TNF in inflammatory diseases. However, a wide range of cells such mast cells, T and B lymphocytes, natural killer cells, neutrophils, endothelial cells, smooth and cardiac muscle cells, fibroblasts and osteoclasts can produce TNF (Bradley, 2008). Genetic factors are known to affect TNF-α levels as showed by *in vitro* and *in vivo* studies. Differences in cytokine production are also known to be partly attributed to the presence of single nucleotide polymorphisms (SNP) within its corresponding gene. Several SNPs have been identified in the TNF-α locus, some of which have also been shown to influence the rate of transcription and production of TNF-α cytokine (Warlé et al., 2003; Wilson, Symons, Mcdowell, Mcdevitt, & Duff, 1997).

The most commonly studied TNF-α polymorphism is in the locus -308, representing a transition from the guanine (G) to adenine (A) (Figure 4). The presence of the less common -308A allelic form has been found to be correlated with a two- to threefold higher transcriptional activity of TNF-α, leading to higher production levels (Wilson et al., 1997; Mira et al., 1999). This polymorphism has been associated with a higher risk of pulmonary tuberculosis (Bikmaeva, Sibiriak, Valiakhmetova & Khusnutdinova, 2001), with the severity of vivax malaria in Indian patients (Sohail et al., 2008) and with reduced survival in Brazilian patients with severe Chagas disease cardiomyopathy (Drigo et al., 2006).

Another possible functional promoter SNP is the -238G/A that is located within the TNF-α repressor site (Figure 4), which has shown contradicting functions (Fong, Siddiqui & Mark, 1994). While some works demonstrated that the -238A allele is associated with higher TNF-α production with respect to the -238G allele (Scardapane et al., 2012) other data have suggested to the contrary (Pociot, D'Alfonso, Compasso, Scorza, & Richiardi, 1995). Thus, this polymorphic site was associated with severe malarial anemia in The Gambia (McGuire et al., 1999) but with protection from cerebral malaria in Kenya (Knight et al., 1999). Moreover, the GA genotype was found to confer some protection in rheumatoid arthritis (Brinkman et al., 1997).

The TNF-α -376G/A is a rare polymorphic site. The promoter containing the -376A allele demonstrated a promoter activity superior of 35% compared to the G allele in a monocyte cell line (Hajeer & Hutchinson, 2000; Scardapane, et al., 2012). Studies regarding -863C/A and -857C/T genotypes showed that the

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rarer A and T alleles provide increased promoter activity and high production of TNF-α (Date et al., 1999). Many other promoter variants have been described such as +489, +386, −1301, −857, −419, −376, and −244, but these SNPs are rare, with conflicting and inconsistent data (Scardapane et al., 2012).

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T TCCTG CATCCTGTCTG G AAGT TAGAAG G AAACAGACCACAG ACCTG GTCCCCAAAAGAA-337



                                 A
ATG G AG GCAATAGGT T T TG AGG GG CATGGG GACG GGGT TCAGCCTCCAG GGTCCTACAC-278
                                     ▼
                                   -308
                               A        A
ACAAATCAGTCAGTGGCCCAGAAGACCCCCCT CGGAATCGGAGC AGGGAGGATGG-223
                                 ▼        ▼
                              - 244    -238

GGAGTGTGA [GGGGTATCCTTGATGCTTGTGTGTCCCCAACTTTCCAAA TCCCCGCCC-166
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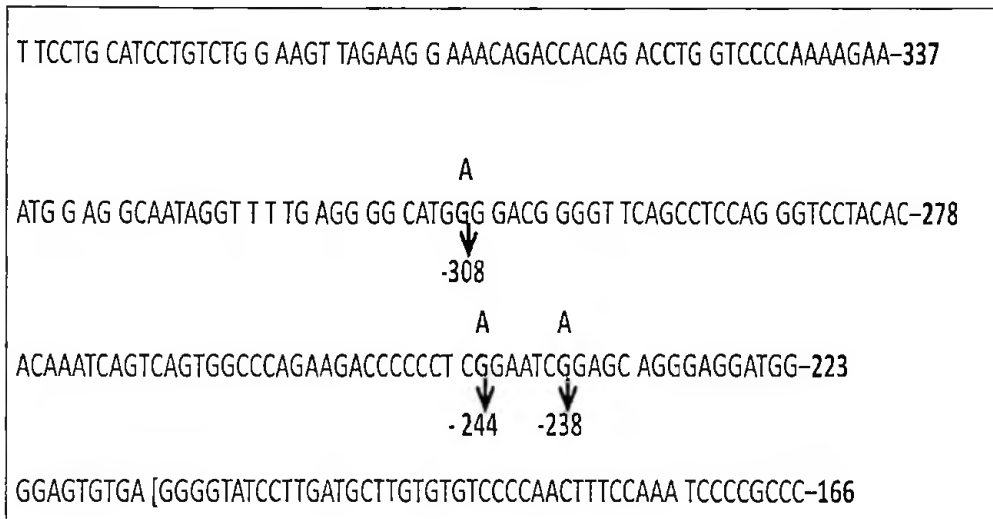
Figure 4: A 230bp strand of TNF-α gene showing 3 SNPs

Interferon gamma cytokine gene

Interferon gamma (IFN-γ) is a highly conserved cytokine, with few allelic variations in its gene which is located on chromosome 12q14 (Figure 5) (Zimonjic et al., 1995). The IFN-γ gene is a polymorphic gene having four exons with three introns. The first intron of the gene contains a CA microsatellite repeat that is highly polymorphic, with up to 8 alleles. Allele 2, with 12 CA repeats, is associated with constitutively high IFN- γ production *in vitro* (Pravica et al., 1999). Directly adjacent to the CA repeat region in the first intron of IFN-γ gene is located a single nucleotide polymorphism (+874 A/T). There is a reported correlation between the presence of the +874 T allele and allele 2 (Pravica,

Perrey, Stevens, Lee, & Hutchinson, 2000). This IFN-γ polymorphic site lies within a binding site for a transcription factor which specifically binds to the allelic sequence containing the T allele. Thus, the +874T allele correlates with high IFN-γ expression. The association of +874 alleles T to A with a low (AA), medium (AT) and high (TT) cytokine production has been shown *in vitro* (Daher et al., 2003; Dabora et al., 2002).
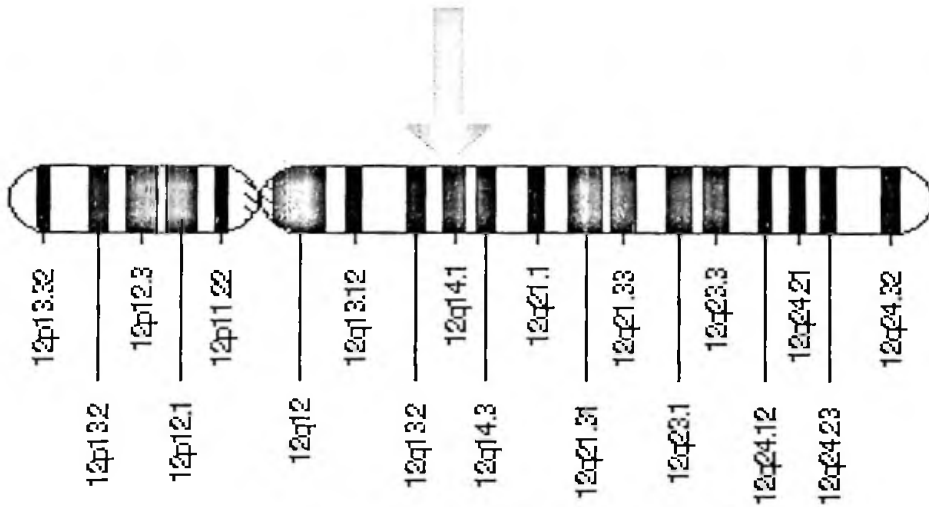
Figure 5: Location of IFN-γ gene on human chromosome 12q14

Transmission of the Infection

The major ways of *T. gondii* transmission are ingestion of oocysts or tissue cysts, and transplacental (maternal or congenital) transmission. Infection from oocysts may occur by accidentally ingesting contaminated soil (e.g. not washing hands after gardening or eating unwashed vegetables or fruits), drinking contaminated water, or eating shellfish grown in contaminated water. Infection with tissue cysts may occur by consuming raw or undercooked meat, by accidentally ingesting tissue cysts after handling raw meat and not washing hands

thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat. Maternal transmission occurs through the transfer of tachyzoites from an infected mother to the unborn child (Abu-Madi, Behnke, & Dabritz, 2010; Elmore et al., 2010; Pereira, Franco, & Leal, 2010).

Toxoplasma gondii transmission may also occur through organ transplantation. Organ transplant recipients can develop toxoplasmosis due to transmission of the parasite with the transplanted organ from a *Toxoplasma* seropositive donor to a seronegative recipient. Heart transplantation has been found to be the most common type of organ transplantation procedure when this occurs, as cysts form in the cardiac muscles (Martina et al. 2011; Derouin & Pelloux, 2008). However, toxoplasmosis is an uncommon outcome from organ transplantation as only 5% of human pathogenic parasites have reportedly caused significant illness in transplant recipients (Barsoum, 2006). It is also possible that parasite transmission could occur as the result of blood transfusion or haematopoietic stem cell transplantation. The chances of either of these occurring are very low and could only occur if the donor was recently infected with *T. gondii* and so had tachyzoites present in the blood and bone marrow (Derouin & Pelloux, 2008).

Congenital transmission

Worldwide, it is estimated that maternal infection of *T. gondii* occurs in 0.1 – 0.8% of pregnancies with another estimated one-third of all the women who acquire the infection during pregnancy transmitting the parasite to the fetus, while

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the rest give birth to normal uninfected babies (UK Food Standards Agency, 2012). The rate of congenital transmission varies considerably depending on the time of gestation that the mother becomes infected. Congenital infection occurs only when a woman becomes infected during pregnancy. Early maternal infection (during the first and second trimesters) may result in severe congenital toxoplasmosis and can result in death of the fetus in utero and spontaneous abortion. On the other hand, late maternal infection during the third trimester would usually result in normal newborns. On the contrary, the risk of transmission from mother to the unborn child is greatest if the mother is infected during the third trimester (Montoya & Liesenfeld, 2004; Mittal & Ichhpujani, 2011). A study in the Netherlands showed 9% transmission from mother to child in the first trimester, increasing to 59% in the third trimester. In France, transmission rates as high as 80% have been found at the end of the third trimester. The overall frequency of subclinical infection in newborns with congenital toxoplasmosis is as high as 85%. (UK Food Standards Agency, 2012)

Transmission via tachyzoites

Toxoplasma tachyzoites have been found in many body fluids, such as saliva, semen, sputum, tears and urine. They have also been detected in the milk of several intermediate hosts, including sheep, goats, and cows (Tenter et al., 2000). A study found experimentally infected male dogs sexually transmitting *T. gondii* parasites to uninfected female dogs and subsequently onto their new born puppies (Arantes et al., 2009). A more recent study demonstrated that *T. gondii* can be sexually transmitted in rats (Dass et al., 2011). *T. gondii* tachyzoites were

detected in human semen (Disko, Braveny, & Vogel, 1971) and the role of semen as a potential source of *Toxoplasma* infection in humans has been hypothesised (Flegr, Klapilová, & Kaňková, 2014). Infrequently, consumption of unpasteurised goat milk has also been associated with acquired clinical toxoplasmosis in humans (Tenter et al., 2000). Tachyzoites are sensitive to proteolytic enzymes and are usually destroyed by gastric digestion. They have been found to be inactivated at pH below 4. It has been suggested, however, that on rare occasions, tachyzoites may enter the host by penetration of mucosal tissue and thereby gain access to the host's circulation or lymphatic system before reaching the stomach (The European Food Safety Authority, 2007; Johnson, 1997). Drinking of any kind of raw milk is a potential risk factor for transmission to humans (The European Food Safety Authority, 2007). It is advisable that milk and eggs should be pasteurised or boiled before human consumption, as these procedures will inevitably kill any potentially present tachyzoites.

Transmission via tissue cysts

Toxoplasma tissue cysts contained in meat or meat-derived products have been shown to serve as important sources of infection for humans. It has been demonstrated that over 50% of all human toxoplasmosis cases are related to cyst containing food sources (Muñoz-Zanzi, Fry, Lesina, & Hill, 2010; Cook et al., 2000). It should be highlighted, however, that the risk of acquiring a *Toxoplasma* infection via food varies with cultural and eating habits in different human populations. It should be kept in mind also that most *Toxoplasma* infections in immunocompetent humans are asymptomatic and, thus, will not be recorded

unless systematic screening programs are carried out in the population under study. While consumption of raw or undercooked meat has been consistently identified as a risk factor in all of these studies, the relative importance of the risk factor and the type of meat associated with it varies among different countries (Cook et al., 2000). In France and Norway for example, consumption of undercooked lamb was a stronger risk factor than consumption of undercooked pork (Kapperud et al., 1996; Baril et al., 1999), whereas in Poland consumption of undercooked pork was identified as the principal risk factor (The European Food Safety Authority, 2007). The consumption of beef has also been identified as a risk factor in some European countries (Cook et al., 2000).

Toxoplasma bradyzoites are more resistant to digestive enzymes than tachyzoites. Ingestion of viable tissue cysts will therefore, usually result in an infection with *Toxoplasma*. Tissue cysts are less resistant to environmental conditions than oocysts. But they have been found relatively resistant to changes in temperature and remain infectious in refrigerated (1 to 4°C) and shred meat for up to 3 weeks (Tenter et al., 2000). Although most tissue cysts are killed at temperatures of -12°C or lower, occasionally some tissue cysts may survive deep-freezing (Dubey, 2000). The survival time of tissue cysts varies greatly with the concentration of salt solution and the temperature of storage.

Survival of tissue cysts at lower temperatures may depend on the duration of cooking. It is important to note that 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. To prevent

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food-borne transmission of *Toxoplasma* to humans, meat and other edible parts of animals should not be consumed raw or undercooked (i.e. they should be cooked thoroughly at 67°C or higher) before consumption. Although freezing alone is not a reliable means of rendering all tissue cysts non-infective, deep-freezing meat (-12°C or lower) before cooking can reduce the risk of infection. In addition, meat should not be tasted during preparation or cooking (Cook et al., 2000). Gamma irradiation and high pressure have been shown to inactivate tissue cysts of *Toxoplasma* under laboratory conditions (Dubey, 2000)

Transmission via sporulated oocysts

Sporulated oocysts present in the environment have been potential sources of infection for humans and other intermediate hosts. A study found that 43% of toxoplasmosis infections in humans were associated with direct or indirect exposure to an occyst contaminated environment (Muñoz-Zanzi et al., 2010). Oocysts as important sources of infection explain the high rate of seropositivity of 24 to 47% in some populations of vegetarians (Hall, Pandit, Golwilkar, & Williams, 1999; Roghmann et al., 1999). Again, the risk factors identified to be associated with *Toxoplasma* infection point to the importance of oocysts in the transmission to humans. For example, contact with soil was identified as a strong risk factor contributing to 6-17% of primary infections in humans (Cook et al., 2000), while in a case-control study in Norway, eating unwashed raw vegetables or fruits was associated with an increased risk of primary infection during pregnancy (Kapperud et al., 1996). Soil contamination with sporulated *Toxoplasma* oocysts depends on the presence of cats roaming on the land, the rate

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of infection of these cats, and climate conditions. After primary infection, a single cat may shed more than 100 million oocysts into the environment (Tenter et al., 2000). Under environmental conditions with sufficient aeration, humidity, and warm temperature, oocysts may sporulate and become infective in less than 1 day. Sporulated oocysts of *Toxoplasma* are very resistant to environmental conditions. They survive short periods of cold and dehydration, and remain infective in moist soil or sand for up to 18 months (Frenkel, 2000).

Oocysts are distributed in the environment through wind, rain and surface water, or harvested feeds. Soil contaminated with oocysts can be taken up by pastoral animals, such as sheep and goats, during grazing. Poultry having outdoor access will also take up considerable amounts of soil and can thus become infected with *Toxoplasma*. This is why free range chickens are now used as sources of animal isolates to characterise *Toxoplasma* strains throughout the world (Lehmann et al., 2006). A growing body of evidence points to water as an important source of *Toxoplasma* infection. Surface water is expected to be variably infected with *Toxoplasma* oocysts. Oocysts are considered to be transferred into the surface water following heavy rainfall. They can remain viable for long periods of time in water and can resist freezing and moderately high water temperatures (Dubey, 1998). The largest and best documented outbreak of acute toxoplasmosis in humans to date occurred in 110 individuals in Vancouver, Canada, in 1995. Comprehensive, retrospective epidemiological studies provided strong evidence that this outbreak was caused by contamination

of municipal drinking water with oocysts (Bell et al., 1995; Bowie et al., 1997; Aramini et al., 1999).

Clinical Disease

Most human infections in immunocompetent individuals are asymptomatic. The infection is reportedly symptomatic in 10-20% of cases, although up to 50% may experience mild symptoms (UK Food Standards Agency, 2012; Ho-Yen, 1992). Signs at birth include fever, maculopapular rash, hepatosplenomegaly, microcephaly, seizures, jaundice, thrombocytopenia and lymphadenopathy. The classic triad of congenital toxoplasmosis is retinochoroiditis, hydrocephalus and cranial calcifications (Heller, 2005). Toxoplasmic pneumonitis starts with general symptoms and progresses with cough, dyspnea and cyanosis. Death can occur in 24 to 48 hours. Toxoplasmic encephalitis is characterized by fever, headache, confusion and seizures (Heller, 2005; Bonfioli & Ofrefice, 2005).

For immunocompromised hosts, the most common presentation is encephalitis, with focal neurological signs, confusion, personality change, cranial nerve dysfunction, meningismus, protracted headache, seizures, stupor, coma, and death (UK Food Standards Agency, 2012; Bonfioli & Ofrefice, 2005). *Toxoplasma* infections have been estimated to cause 3 to 7% of clinically significant cases of lymphadenopathy. Pulmonary toxoplasmosis (pneumonitis) is commonly associated with patients with AIDS who are not receiving appropriate anti-HIV drugs or primary prophylaxis for toxoplasmosis (UK Food Standards Agency, 2012).

Burden of Disease

The burden of disease depends upon a combination of disease severity, duration, and the number of cases that occur. Cases with congenital toxoplasmosis, encephalitis and chorioretinitis account for most of the disease burden due both to the more severe immediate symptoms and the risk of longer term, and potentially life-long sequelae (UK Food Standards Agency, 2012). In congenital transmission, about 4 -10% result in abortion, stillbirth or neonatal death whilst 65-85% of babies are asymptomatic at birth. The most common effects are CNS abnormalities (3-20%), chorioretinitis (4-15%), intracranial calcifications (10-12%) and hydrocephalus (2%). In the longer term, 1-2% of the babies may suffer from learning difficulties and 4-27% from retinochoroidal lesions and by 20 years of age, 53% may suffer from visual impairment and 73% from mild to severe learning difficulties (UK Food Standards Agency, 2012; Havelaar, Kemmeren, & Kortbeek, 2007; Roberts & Frenkel, 1990). Toxoplasmosis in AIDS is rarely seen in patients with a CD4 count >200/μl and is most common in those with a CD4 count <100/μl (Montoya & Remington, 2000). In the USA, *Toxoplasma* encephalitis develops in up to 7% of AIDS patients annually (Schwartzman, 2001), and in New Zealand it was estimated that 30% of seropositive patients with AIDS developed *Toxoplasma* encephalitis (Lake, Hudson, & Cressey, 2002).

The cost of *Toxoplasma* encephalitis in the USA has been estimated to be $23-106m (Roberts et al., 1994). Toxoplasmosis has been assessed as causing the highest disease burden of seven foodborne pathogens in the Netherlands, mainly

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due to stillbirths and chorioretinitis in congenital toxoplasmosis and chorioretinitis in acquired infection. In France, it was estimated to be the third most common cause of death from foodborne illness (Vaillant et al., 2005). In the USA, it was estimated to cause 0.9% of all cases of foodborne illness, 8.0% of hospitalisations (ranked fourth) and 24% of deaths (ranked second) (Mead et al., 1999; Scallen et al., 2011). The economic costs of congenital infection and infection in AIDS patients has been estimated to be $0.4 − 8.8 billion and $1.2− 12m in the UK (Roberts et al., 1994). In terms of Disability Adjusted Life Years (DALY), *Toxoplasma* was adjudged to cause the highest disease burden amongst foodborne pathogens (Kemmeren, Kortbeek, & Havelaar, 2006).

Diagnosis of *Toxoplasma* Infection

Diagnosis of toxoplasmosis is made by immunological testing, histological identification, isolation of the organism in tissue culture, detection of the parasite DNA by using polymerase chain reaction (PCR), antigen detection or by a combination of these techniques (Ayi et al., 2005; Montoya, 2002). Cerebral toxoplasmosis can also be diagnosed using computerized tomography (CT) scan and magnetic resonance imaging (MRI) (Hill & Dubey, 2002; Markus, 2003; Sukthana, 2006). Serological tests are the most widely used tools for toxoplasmosis diagnosis (Bonfioli & Orefice, 2005). They are used to detect increased antibody levels such as IgG, IgM, IgA and IgE. In a primary *T. gondii* infection, IgM generally appears in the first week after infection, peaks at 1 month, and disappears after 9 months. This is followed by IgA and IgE which generally appear 2 to 4 weeks after primary infection, peak around the second or

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third month, and disappear within 7 to 9 months (Bonfioli and Orefice, 2005). However, these acute phase immunoglobulins have been found to persist for longer periods of time (Montoya & Rosso, 2005; Sukthana, 2006). IgG antibodies appear in the second week of infection, peak in six to eight weeks and persist at low levels throughout the duration of the host's life (Bonfioli and Orefice, 2005; 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, Schutte, & Bommer, 1992). Another 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, Thulliez, & Montoya, 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 (Montoya and Rosso, 2005). Avidity tests have helped to overcome this problem as they help differentiate between recently and distantly acquired infections (Lappalainen & Hedman, 2004; Montoya & 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. Chronic infections, however, have more strongly-binding antibodies and therefore have a high avidity (Lappalainen & Hedman, 2004; Montoya & Rosso, 2005).

There are several serological tests for toxoplasmosis diagnosis. Sabin-Feldmann dye test is the gold standard. It is a neutralization assay in which live

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organisms are lysed in the presence of complement and IgG *T. gondii*-specific antibody. It is only used in research centers and reference laboratories. Enzyme-linked immunosorbent assay (ELISA) is the standard test used by most laboratories to detect IgG, IgM, IgA and IgE. Indirect fluorescent antibody test (IFAT) is available. Immunosorbent agglutination assay (ISAGA) detects antibodies by causing agglutination of acetone or formalin-preserved whole organisms (Bonfioli & Orefice, 2005; Hill & Dubey, 2002).

Some of these problems associated with serological testing may be overcome with the use of PCR. However, this technique has both advantages and disadvantages. 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 and amniotic fluid. Its 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, Butcher, Savva, & Holliman, 1993).

Enzyme-linked Immunosorbent Assay (ELISA)

Serological tests have been proven to be most appropriate for epidemiological seroprevalence surveys of *Toxoplasma* infection because of their ability to detect past and present infection by the presence of specific antibodies (Bonfioli & Orefice, 2005). Although the PCR is most sensitive, it can detect infection only when parasite DNA is present in the particular human fluid. Between the serological tests, the ELISA tool is found most viable under field

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conditions for mass screening. The procedure is simple to perform, cost-effective, can readily test a large number of samples and with high levels of specificity and sensitivity (Sudan, Jaiswal, & Shanker, 2013). The Sabin-Feldmann dye test, which was once considered as the gold standard is hardly ever used nowadays because of its potentially hazardous effects owing to the use of virulent live parasites (Udonsom, Buddhirongawatr, & Sukthana, 2010). The indirect immunofluorescent test, on the other hand, requires a fluorescence microscope, the use of expensive reagents and the results are read by eye, thus introducing individual variations (Sudan et al., 2013).

ELISA uses the basic immunology concept of an antigen binding to its specific antibody, which allows detection of very small quantities of antigens such as proteins, peptides, hormones, or antibody in a fluid sample (Gan & Patel, 2013). ELISA uses enzyme-labeled antigens and antibodies to detect the biological molecules. Different types of ELISAs have been employed with modification to their basic steps.

Indirect ELISA

The sample to be analyzed for a specific antigen is coated on the wells of a microtiter plate. This is followed by a solution of non-reacting protein such as bovine serum albumin to block any areas of the wells not coated with the antigen. A primary antibody, which binds specifically to the antigen, is then added, and followed by an enzyme-conjugated secondary antibody. A substrate for the enzyme is added to quantify the primary antibody through a color change. The

concentration of primary antibody present in the serum directly correlates with the intensity of the color (Gan & Patel, 2013; Haapakoski et al., 2013).

Direct ELISA

The direct ELISA principle is similar to the indirect method discussed above. The only difference is that the enzyme-conjugated antibody is directly bound to the antigen, followed by addition of the substrate (Gan & Patel, 2013).

Sandwich ELISA

The sandwich protocol is used to determine a specific sample antigen. The well surface is prepared with a known quantity of bound antibody to capture the desired antigen. After nonspecific binding sites are blocked using bovine serum albumin, the antigen-containing sample is added to the plate. A specific primary antibody is then introduced which "sandwiches" the antigen. Enzyme-linked secondary antibodies are then applied that bind to the primary antibody. After the unbound antibody–enzyme conjugates are washed off, a substrate is added and is enzymatically converted to a color that can be quantified (Gan & Patel, 2013; Canady, Arndt, Karrer, & Bosserhoff, 2013).

Competitive ELISA

A distinguishing feature of competitive ELISA is the process of competitive reaction between the sample antigen and antigen bound to the wells of a microtiter plate with the primary antibody. First, the primary antibody is incubated with the sample antigen and the resulting antibody–antigen complexes are added to wells that have been coated with the same antigen. After an incubation period, any unbound antibody is washed off. The more antigen in the

sample, the more primary antibody will bind to the sample antigen. Therefore, there will be a smaller amount of primary antibody available to bind to the antigen coated on the well. Secondary antibody conjugated to an enzyme is then added, followed by a substrate to elicit a chromogenic or fluorescent signal. Absence of color indicates the presence of antigen in the sample (Dobrovolskaia, Gam, & Slater, 2006).

Ocular Toxoplasmosis

Toxoplasmic retinochoroiditis is identified as the commonest cause of posterior uveitis (intraocular infection) in both immunocompetent and immunosuppressed persons in many parts of the world, accounting for some 30%–50% of all cases of posterior uveitis (Holland, 1999, 2003; Bonfioli & Orefice, 2005; Montoya & Liesenfeld, 2004). The prevalence of ocular toxoplasmosis has been reported to range from about 2% in the United States to 31.8% in southern Brazil (Peixe et al., 2014; Holland, 2003; Bonfioli & Orefice, 2005). The macula is frequently involved (Atmaca, Simsek, & Batioglu, 2004; Bosch-Driessen, Berendschot, Ongkosuwito & Rothova, 2002; Mets et al., 1997), leading to considerable compromise in functional vision (Kadarisman, Marsetio, & Simangunsong, 1991; Brézin et al., 2003). Brazil has a disproportionately high incidence of ocular toxoplasmosis which is in contrast to Europe and the USA where the occurrence of ocular toxoplasmosis is much lower, ranging from 2 to 5% (Holland, 2003). The outcome of ocular toxoplasmosis depends on the interaction of many factors, including functions of the immune system and parasite factors.

In the Middle East, ocular toxoplasmosis accounts for about 33.6% of all cases of posterior uveitis (Nashtaei, Soheilian, Herbort, & Yaseri, 2011). This includes 54.5% in Iran (Soheilian et al., 2004), 27.5% in Ankara, Turkey (Sengun et al., 2005), 44.4% in Saudi Arabia (Hamade, Elkum, & Tabbara, 2009) and 13.8% in Israel (Nashtaei et al., 2011). In Europe, it accounts for an overall of 49.2% in all posterior uveitis cases, consisting of 60.2% in Italy (Mercanti, Parolini, Bonora, Lequaglie & Tomazzoli 2001), 40% in Finland, 39% in Belgium, 43.2% in Switzerland and 48.6% in Amsterdam (Nashtaei et al., 2011; Rothova et al., 1992). The occurrence of ocular toxoplasmosis in Africa may be high though the infection has not been studied extensively (Holland, 2003). It is reported that in Sierra Leone, ocular toxoplasmosis was the most common cause of uveitis, accounting for 43% (Ronday et al., 1996). In Nigeria, toxoplasmosis was probably the single greatest factor in the causation of uveitis in the forest areas of Nigeria, with a prevalence rate of 70% (Ayanru, 1997).

Recent studies seem to contradict the traditional assumption that ocular toxoplasmosis is commonly associated with congenital rather than acquired infection. Epidemiological studies support the fact that acquired infection can lead to late development of ocular lesions in humans. A demonstration of this fact is where an outbreak of an acute toxoplasmosis associated with municipal drinking water in Victoria, Canada, occurred and 19% of the 100 infected persons who experienced overt clinical symptoms subsequently developed acute ocular disease (Bowie et al., 1997). Ocular involvement is usually associated with inflammation of the retina and the uveal tract. Due to its nature of recurrence and progression,

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ocular toxoplasmosis can result in the destruction of the retina and lead to loss of sight (Lyons et al., 2001). The infection has a preference for the posterior pole of the ocular globe (Lihteh, Teodoro, Rafael, 2013; Holland et al., 1999), and is commonly accompanied by anterior uveitis, vitreous inflammatory reactions, retinal vasculitis, and macular edema (Holland et al., 1999).

Recurrent disease is believed to be caused by reactivation of live tissue cysts located at the borders of old scars. It is also believed that proliferating parasites are responsible for tissue destruction while hypersensitivity reactions to the parasite are responsible for associated inflammatory signs, including retinal vasculitis, anterior uveitis, vitreous inflammatory reactions, and retinal edema (Holland, 1999). The most distinguishing feature of ocular toxoplasmosis is a necrotizing retinochoroiditis and the area of necrosis usually involves the inner layers of the retina usually described as a whitish, fluffy lesion surrounded by retinal edema. The retina is the primary site for the multiplying parasites, while the choroid and the sclera may be the sites of contiguous inflammation (Cordeiro et al., 2008).

Optic nerve involvement is associated with the typical manifestation of optic neuritis or papillitis accompanied by papilledema. In this case the optic nerve sheath will usually serve as a conduit for direct spread of *Toxoplasma* parasites into the optic nerve from an adjacent cerebral infection. This also results in optic neuritis or papillitis. Inflammatory cells are seen in the vitreous overlying the retinochoroidal or papillary lesion. In most cases, the inflammatory reaction is severe, and fundus details are not visible. The characteristic "headlight in the fog"

feature is apparent when the retinal inflammation can just be seen through the dense vitreous (Bonfioli & Orefice, 2005). Vitreous detachment may follow, with patients developing precipitates of inflammatory cells on the posterior vitreous face. Posterior synechiae may complicate the course of anterior uveitis, and keratic precipitates seen (Lihteh et al., 2013).

Diagnosis of ocular toxoplasmosis is typically clinical. It is based on a characteristic retinal lesion comprising the presence of a necrotizing focal retinal lesion eventually combined with hyperpigmented retinochoroidal scars in either eye (Holland, O'Connor, Belfort, & Remington, 1996; Bosch-Driessen et al., 2002). Ocular toxoplasmosis patients usually present with low serum titles of IgG and negative IgM. Though positive IgG does not necessarily confirm the diagnosis given the high rate of seropositive individuals in many populations, a negative serology can eliminate the disease from the differential diagnosis (Bonfioli & Orefice, 2005).

Visual Outcome in Ocular Toxoplasmosis

Ocular toxoplasmosis is one of the most devastating causes of visual impairment throughout the whole world (Holland, 2003; de-la-Torre, González, Díaz-Ramirez, & Gómez-Marín, 2007; Lahmar et al., 2009). The most common complications in ocular toxoplasmosis are macular scars, dragging of the macula secondary to peripheral lesions, strabismus, retinal detachment, nystagmus, optic atrophy, cataract, glaucoma, opacification of the media, amblyopia and bilateral involvement (Scherrer, Iliev, Halberstadt, Kodjikian, & Garweg, 2007; Suhardjo & Agni, 2003; Da Mata & Orefice, 2002). Bilateral ocular involvement is more

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common in congenital infections than in postnatally acquired infections (de-la-Torre, López-Castillo, & Gomez-Marin, 2009). Severe visual impairment and blindness is always associated with lesions involving the macular area, papillomacular bundle area, and paramacular areas.

A study by Kadarisman et al. (1991) in Indonesia found 56.7% of visual impairment and 20.1% of blindness among 41 ocular toxoplasmosis patients. This extreme case of high visual impairment was due to correspondingly higher findings of bilateral involvement, occurring in 50% of the cases. They reported complications of strabismus and nystagmus, both occurring in 50% of the cases. Another study in Indonesia found bilateral involvement in 32.4%, blindness in 13.9%, macular lesions in 22.4%, strabismus in 6.4%, congenital cataract in 2.8%, nystagmus in 6.4% and optic nerve atrophy in 2.8% (Suhardjo & Agni, 2003). With the theory that bilateral ocular involvement is more prevalent in congenital cases, it may be concluded that acquired infection was more common in those Indonesian populations.

In Brazil, retinochoroidal lesions were bilateral in 75.8% of patients and unilateral in 24.2%. The major cause of visual disability in that study was papillomacular bundle area lesions, occurring in 76.3%. Other complications were cataracts, microphthalmia, and strabismus (Melamed, Eckert, Spadoni, Lago, & Uberti, 2010). On the contrary, another study from Brazil rather found that a higher proportion of 95.9% were unilateral cases (Eckert, Melamed, & Menegaz, 2007).

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Larger retinochoroidal lesions lead to much more devastating visual disability and interestingly, larger lesions seem to be associated with elder patients (Arantes et al., 2015). Dodds et al. (2008) have reported that signs of increased inflammation leading to marked reduction of visual acuity in 210 patients with toxoplasmic retinochoroiditis were related to older patient age and larger retinal lesions. From Iran, Tabatabaei et al. (2011) also found a relationship between the size of retinal lesions and age of patient, with older patients having larger lesions. This observation may be explained by the fact of a possible multiple recurrences of ocular toxoplasmosis that makes the scars larger in elder patients. Holland (2004) has, however, explained that older patients who are recently infected with the parasite would have a higher chance of ocular involvement and more severe ocular disease occurring with larger lesions. The explanation for this observation is by reason of declining immunity with advancing age (Holland, 2004). Other studies have attributed development of larger retinal lesions to exposure to more virulent *Toxoplasma gondii* strains (Shwab et al., 2014; Arevalo, Belfort Jr, Muccioli, & Espinoza, 2010; Gilbert et al., 2008; Gomez, 2007).

Toxoplasma retinochoroidal lesions occur mostly at the retinal posterior pole. Several reasons have been argued for this finding. It has been argued, for instance, that anatomic and microvascular differences between the macula and the peripheral retina might create a microenvironment that can influence the location of lesions (Holland, 2004). Correspondingly, lesions at the posterior pole are known to cause the most severe forms of visual impairment. Kodjikian et al.

(2006) reported visual impairment occurring in 23% of 130 ocular toxoplasmosis cases in which all but three eyes had marcular lesions. Tabatabaei et al. (2011) reported that most of their ocular toxoplasmosis patients (87.5%) had lesions in the posterior pole. Other studies have reported similar findings (Arevalo et al., 2010; London, Hovakimyan, Cubillan, Siverio & Cunningham, 2010; Accorinti, Bruscolini, Pirraglia, Liverani, & Caggiano, 2008).

Complications of posterior pole (or macular) lesions are the development of strabismus and nystagmus which seem to be the most common signs in patients with long standing lesions and in congenital cases as a result of disturbances of macular function during the developmental age. Strabismus develops as a result of poor central vision and extra foveal fixation due to macular lesions. If fixation becomes continuously poor, nystagmus will be evident as a result of sensory deprivation (Brézin et al., 2003; Kadarisman et al., 1991).

Epidemiology of Human Toxoplasmosis

Prevalence of *Toxoplasma* is known to vary geographically. Seroprevalence is found to be higher in Central Europe, South America and sub-Saharan Africa (Zemene et al., 2012; Negash, Tilahun, & Medhin, 2008; Deniau et al., 1990).

Prevalence in Europe

In Europe, seroprevalence is reportedly high in Southern Europe, and decreases with increasing latitude (Petersen, Kijlstra, & Stanford, 2012). Lower rates are reported in northern Sweden and Norway (Evengård et al., 2001; Jenum

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& Stray-Pedersen, 1998). The age-specific prevalence has been decreasing in Europe over the past three to four decades (Pleyer, Schlüter, & Mänz, 2014; Petersen et al., 2012). A nationwide survey in the Netherlands between 1995/1996 and 2006/2007 demonstrated a considerable decrease in seroprevalence from 40.5% to 26.0% respectively. In women of reproductive age the seroprevalence dropped from 35.2% in 1995/1996 to 18.5% in 2006/2007. An improvement in the preparation of meat for human consumption was suggested as a major reason for this decline (Hofhuis et al., 2011). Studies among pregnant women from France have observed a decline in seroprevalence from 84% in the 1960s to 54% in 1995 and to 44% in 2003, with the highest prevalence in southwestern France (Nogareda, le Strat, Villena, de Valk, & Goulet, 2014; Berger et al., 2009).

A study in Spain by Ramos et al. (2011) to compare the prevalence of *T. gondii* IgG in immigrant and non-immigrant female populations found a higher prevalence of IgG antibodies in immigrants (41%) than non-immigrants (12%). Seroprevalence was highest in women from Eastern Europe (47%), Africa (43%) and Latin America (43%). A report from the Czech Republic by Kolbekova, Kourbatova, Novotna, Kodym, & Flegr, (2007) described a 23% seroprevalence in 3250 military personnel who were tested by complement fixation and ELISA for specific IgG and IgM. A systematic sample of 1,157 women of reproductive age (between 15 and 49 years) in Belgrade, Serbia, found a seroprevalence of 77% (Bobić, Jevremović, Marinković, Šibalić, & Djurković-Djaković, 1998). A survey among antenatal women in London gave a seroprevalence for *T. gondii* as 17.3% in the 2610 samples tested. Seroprevalence was 11.9% in women of UK

origin, 34.8% in women of African/Afro-Caribbean decent and 23.3% among Middle Eastern immigrants (Flatt & Shetty, 2013).

Prevalence in North America

According to the National Health and Nutrition Examination Study (NHANES) III conducted between 1999 and 2004, *T. gondii* seroprevalence in the United States was 15.8% in the age group 12-49 years. Seroprevalence was higher among non-Hispanic black persons (19.2%) than among non-Hispanic white persons (12.1%). No statistically significant differences were found between *T. gondii* antibody prevalence in NHANES II (1999-2000) and NHANES I (1988-94) (Jones, Kruszon-Moran, Sanders-Lewis, & Wilson, 2007).

Prevalence in Latin America

Epidemiological studies suggest that Latin America may have a particularly high seroprevalence of toxoplasmosis, particularly Southern Brazil. In the late 1980s, a seroprevalence rate of 98% was found among 100 children aged 10–15 years in a rural area of Southern Brazil (Nussenblatt & Belfort, 1994). This appears to be the highest seroprevalence of toxoplasmosis reported in the medical literature. In another region of Brazil, Campos dos Goytacazes in Rio de Janeiro state, testing of serum from 1436 residents by ELISA for *T. gondii* IgG revealed seroprevalence values of 84%, 62%, and 23% for the lower, middle, and upper socioeconomic populations respectively. Age, socioeconomic status, and consumption of unfiltered water were the strongest predictors of *T. gondii* seropositivity (Bahia-Oliveira et al., 2003). Another study found a seroprevalence of 73% in slaughterhouse workers and suggested that fresh meat was an important

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source of infection in Brazil (Dias et al., 2005). A study of blood samples from over 1000 indigenous Brazilians of three tribes gave the seroprevalence of toxoplasmosis ranging from 55% to 80% (Sobral, Amendoeira, Teva, Patel, & Klein, 2005).

In a poor neighbourhood of Maracaibo, Venezuela, a reported 33% of women aged 10 to 45 years tested positive for *T. gondii* (Diaz-Suarez & Estevez, 2009). In Chile, a study of over 75 000 blood samples from 13 regions of the country, examined by indirect hemagglutination test, found the seroprevalence of toxoplasmosis of almost 37% (Contreras et al., 1996). A study of children from Guatemala revealed that infection with *T.gondii* often took place before the age of five years at which time 43% were seropositive (Jones et al., 2005). In Cali, Colombia, enzyme immunoassay detected specific IgG and/or IgM in 45.8% of 955 pregnant women (Rosso et al., 2008). One study undertaken in Northern Mexico found only 7.4% of subjects with IgG antibodies and 1.9% with IgM against *T. gondii* (Alvarado-Esquivel et al., 2011).

Prevalence in Asia

Variable seroprevalence rates for toxoplasmosis have been recorded across different Asian nations. A seroprevalence study in Jakarta, Indonesia, involving 1693 blood samples recorded a prevalence of 70% (Terazawa, Muljono, Susanto, Margono, & Konishi, 2003). Consistent reports indicate that seroprevalence is lower in China. A Chinese study of 2634 healthy individuals aged 15–65 years found 12.5% positivity for IgG to *T. gondii* (Xiao et al., 2010). On the other hand, prevalence rates from India, particularly the Southern parts, have been relatively

high. Because many Indians are vegetarians, it is suggested that ingestion of contaminated water or vegetables is a major source of infection there (Dhumne, Sengupta, Kadival, Rathinaswamy, & Velumani, 2007). A study from India found a seroprevalence of *Toxoplasma*-specific IgG antibodies of 45% (Singh & Pandit, 2004) whiles a study of HIV-infected patients from Japan found an overall seroprevalence of 44.8% (Nissapatorn et al., 2004). Similarly, a study from Malaysia showed that people belonging to the Indian ethnic group had a seroprevalence of 55.3% whereas ethnic Chinese had a seroprevalence of 19.4% (Nissapatorn & Abdullah, 2004). Again, a study of the seroprevalence of *T.gondii*-specific IgG-antibodies from northern India found an overall prevalence of 51.6% in males and 89.2% in females (Elhence, Agarwal, Prasad, & Chaudhary, 2010). In contrast, a study from Chandigarh, Northern India, found a seroprevalence in adults of 15% (Khurana et al., 2010). From Korea, a study found an IgG-specific prevalence in pregnant women of 0.8% (Song, Shin, Shin, & Nam, 2005).

Status of Infection in Africa

Seroprevalence of the infection varies greatly across the African continent. Prevalence rates vary according to climatic conditions and the population being studied, whether immune competent individuals or among immunocompromised persons such as HIV patients and pregnant women.

Toxoplasmosis in immunocompetent individuals

Studies among immunocompetent persons have reported seroprevalence rates of 6.4 to 74.5%. Lower rates are reported in South Africa while higher rates

are reported in West Africa. From the Gauteng province of South Africa, Kistiah, Barragan, Winiecka-Krusnell, Karstaedt, & Frean (2011) reported a 6.4% seroprevalence in a generalised cohort population. From East Africa, Swai and Schoonman (2009) reported seroprevalence of 45.7% in a Tanzanian cohort predominantly comprising immunocompetent individuals. A prevalence rate of 74.4% was reported in Ethiopia (Guebre-Xabier et al., 1993) while Griffin and Williams (1983) reported seroprevalence rate of 42% in Kenya. In North Africa, a seroprevalence of 41.7% in Sudan was reported by Abdel-Hameed (1991), while Elsheikha et al. (2009) and Bouratbine, Siala, Chahed, Aoun, & Ben (2001) reported seroprevalence of 59.6% and 58.4%, in Egypt and Tunisia, respectively. In West Africa, the highest seroprevalence rate has been reported of 74.5% in the Republic of Sao Tome and Principe (Fan et al, 2007). Prevalence rates in northern Africa seem to follow a decreasing trend over time from 58.4% in 2001 to 35.2 % in 2013. In southern Africa, there is also an observed decreasing pattern from 37% in 1974 to 20% in 1978 to 11% in 1991 and finally to 6.4% in 2011. In West Africa, however, there is an increasing trend from 21% in 2001 to 27% in 2012. There is also an increasing trend in Eastern Africa from 43% in 1980 to 45.7% in 2009 (Hammond-Aryee, Esser, & van Helden, 2014).

Toxoplasmosis in women of reproductive age and pregnant women

A number of studies have been conducted on the risk group of women of reproductive age and pregnant women. These studies report seroprevalence rates ranging from 12.8% to 92.5%. Higher rates of seroprevalence were reported in West Africa, where Ndumbe, Andela, Nkemnkeng-Asong, Watonsi, & Nyambi

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(1992) reported prevalence of 77.1% in Cameroon, and Hung et al. (2007), 75.2% prevalence in the Republic of Sao Tome and Principe. A seroprevalence of 83.5% was recorded in the southern part of Madagascar (Lelong et al., 1995). The highest rate on the continent was recorded in Accra, Ghana with a prevalence rate of 92.5% (Ayi et al., 2009). The lowest seroprevalence was reported in South Africa, where Kistiah et al. (2011) reported prevalence of 12.8%. Seroprevalence rates in North Africa have also been generally high, with the highest being 58.1% in Egypt as reported by Hussein, Ali, Saleh, Nagaty, and Rezk (2001). Seroprevalence rates in northern Africa have shown a decreasing trend over the years from 58.1% in 2001 to 39.3% in 2012. There is an increasing trend in southern Africa from 3% in 1975 to 12.8% in 2011. In West Africa there is also an observed increasing trend in seroprevalence from 70% in 1989 to 75% in 2007 and 92.5% in 2009.

Toxoplasmosis in Human Immunodeficiency Virus (HIV) patients

The advent of HIV-AIDS has resulted in an increase in the number of *Toxoplasma* seroprevalence investigations in HIV-positive versus HIV-negative populations. Many of such studies have shown higher prevalence rates among HIV-patients as compared to their non-HIV counterparts. A study by Maiga, Kiemtore, & Tounkara (2001) among patients with acquired immunodeficiency syndrome and blood donors in Bamako, Mali, indicated that toxoplasmosis prevalence was 60% in AIDS patients, 22.6% from HIV-seropositive blood donors and 21% from HIV-seronegative blood donors. Results from Zaria, northern Nigeria, gave the seroprevalence of toxoplasma infection as 32.4% in

HIV-negative healthy adults and 38.7% in HIV-infected adults (Ogoina, Onyemelukwe, Musa, & Obiako, 2013). In Africa, seroprevalence of HIV-AIDS related toxoplasmosis ranges from 4-80%, with an average of 36.35%. The highest seroprevalence of *T. gondii* in HIV patients in Africa was reported in Ethiopia by Woldemichael et al. (1998) with 80% seroprevalence in 170 patients tested. The average seroprevalence rate was 52% in West Africa, ranging from 62.5% in Burkina Faso to 21% in Ivory Coast (Ledru et al., 1995; Lucas et al., 1993). The lowest rate on the continent was 4%, recorded in Zambia (Zumla et al., 1991). In North Africa, the highest seroprevalence rate was reported by Addebbous et al. (2012) in Morocco, where a seroprevalence of 62.5% was found. From Southern Africa, a seroprevalence rate of 24.6% was reported in KwaZulu-Natal province (Sonnenberg, Silber, & Jentsch, 1998). Lower rates of 8% and 4% have been reported in the Gauteng province of South Africa (Hari, Modi, Mochan, & Modi, 2007; Zumla et al., 1991).

Current Status of the Infection in Ghana

No population-based study on human *Toxoplasma gondii* has been conducted in Ghana. Data on the only two human *Toxoplasma gondii* surveys conducted in Ghana give widely different seroprevalence rates. One study was conducted to determine the prevalence of *Toxoplasma gondii* infection among patients visiting the Korle-Bu Teaching Hospital in Accra. Of the 165 patients studied, the overall seroprevalence was 49.7%. Among these, 32.7% was seropositive for IgG antibodies. Both IgA and IgM antibodies were found in 29.7% of the patients. This study found a significant association between

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seroprevalence of *Toxoplasma gondii* antibodies and gender, with the male sex being at increased risk of *Toxoplasma gondii* seropositivity (Ayeh-Kumi et al., 2010). The other study was to investigate the seroepidemiology of toxoplasmosis amongst pregnant women in the greater Accra region. Exposure to contact with cats' faeces was found to be the major *T. gondii* infection risk factor. The 159 women aged 15-40 years in their first, second and third trimesters respectively had an overall anti-*T. gondii* antibodies IgG, IgA and IgM seroprevalence of 92.5%, with 4.1% of them having anti-IgG only. Of the seropositive women 17.7% were in their first, 44.0% in their second, and 38.3% in their third, trimesters respectively (Ayi et al., 2009).

Studies on toxoplasmosis in farm animals in Ghana have indicated seropositivity of 39% in pigs, 26.8% in goats, and 33.2% in sheep (Arko-Mensah, Bosompem, Canacoo, Wastling, & Akanmori, 2000; van der Puije, Bosompem, Canacoo, Wastling, & Akanmori, 2000). *T. gondii* prevalence in free-range chickens may serve as a good indicator of the prevalence of parasite oocysts in soil because these chicken feed directly from the ground. Dubey et al. (2008) found the prevalence of *T. gondii* in free-range chickens from Ghana to be higher when compared to those from elsewhere. The chickens were recruited from Ghana, Indonesia, Italy, Poland, and Vietnam. Antibodies to *T. gondii* were found in 64% of chickens from Ghana, 24.4% from Indonesia, 12.5% from Italy, 30% from Poland, and 24.2% from Vietnam.

Risk Factors for *Toxoplasma gondii* Infection

Sources of *T. gondii* infection include the ingestion of cysts in undercooked meat and oocysts in soil contaminated with cat feces. Contaminated water sources have been associated with *T.gondii* infection in many epidemiological studies. A waterborne outbreak of infection with *T.gondii* was first described in American soldiers in Panama and was attributed to drinking water from a contaminated stream (Benenson, Takafuji, Lemon, Greenup, & Sulzer, 1982). An outbreak in the town of Santa Isabel do Ivai, in Parana state, Brazil, was also linked to a contaminated drinking water cistern where nearly 10% of the diagnosed cases developed ocular symptoms (de Moura et al., 2006).

A United States national survey by Jones et al. (2001) associated *T. gondii* seropositivity in the US with the following risk factors: less than college level education, being in a soil-related occupation, and living in a crowded environment. Studies conducted in Mexico have indicated that persons who sorted waste were at increased risk (Alvarado-Esquivel, Liesenfeld, Márquez-Conde, Estrada-Martínez, & Dubey, 2010; Alvarado-Esquivel et al., 2008). A case-control survey to determine the risk factors associated toxoplasmosis in some European cities found that eating undercooked meat (except pork), tasting meat while cooking, contact with soil, and travel outside Europe or North America were all significantly associated with the infection (Cook et al., 2000). Again, a case-control study conducted in the United States, from 2002 to 2007, consistently identified the consumption of and working with raw meat as risk factors for *T. gondii* seropositivity (Jones et al., 2009).

On the contrary, a case-control study conducted among Mexican butchers from 2009 to 2010 found no difference in seroprevalence of *T. gondii* between subjects and controls (Alvarado-Esquivel, Liesenfeld, Estrada-Martínez, & Félix-Huerta, 2011). Most meat handled by the butchers was beef, and this may explain such an observation, because cows are poor hosts for *T. gondii* (Furtado, Winthrop, Butler, & Smith, 2013). Neither the cross-sectional study from the United States by Jones et al. (2001) nor the case-control study conducted in Europe by Cook et al., (2000) did associate feline ownership with *T. gondii* infection. However, the case-control study by Jones et al. (2009) that compared infected subjects with non-infected controls found that ownership of at least three cats was a strong risk factor for infection with the parasite. Again, a case-control study from France found the following risk factors: poor hand hygiene, consumption of undercooked beef, having a cat as pet, frequent consumption of raw vegetables outside the home, and consumption of undercooked lamb (Baril et al., 1999).

Prevention of *T. gondii* Infection

There is currently no vaccine for the prevention of toxoplasmosis. The only way to prevent the infection is for individuals to practice good hygiene. Hands should be washed thoroughly after handling of meat or contact with soil before beginning any other tasks. All fruits and vegetables must also be washed thoroughly before they are consumed. All meat should be well cooked to a minimum temperature of 67 °C so as to kill tissue cysts before consumption. Tissue cysts can also be killed by freezing to -13 °C. Pregnant women should be

especially careful, and should avoid contact with cats, cat litter, soil and raw meat (Tenter et al., 2000; Hill & Dubey, 2002).

Management of *Toxoplasma* Infection

For immunocompetent adults and children with only lymphadenopathy, specific therapy may not be required unless they have persistent, severe symptoms. Prenatal antibiotic therapy is recommended to reduce the number of infants severely affected with *Toxoplasma* infection (Kasper, 2005). Prenatal treatment with pyrimethamine and sulfadiazine in women having fetal infection reduces the squeal of the disease in the new born (Foulon et al., 1999). Prenatal anti-*Toxoplasma* treatment is recommended throughout the pregnancy. Folinic acid is added to regimens to reduce bone marrow suppression. It is also recommended that treatment of the fetus is followed by treatment of the new born throughout the first year of life. Pre-emptive, anti-parasitic treatment should be considered for all symptomatic, seropositive, immunocompromised patients such as AIDS patients suspected to have toxoplasmosis. The most typically used and successful regimen for treatment of toxoplasmosis in immunocompromised patients is the combination of pyrimethamine, sulfadiazine and folinic acid (Montoya & Remington, 2000).

Treatment is recommended for four to six weeks after resolution of all signs and symptoms (sometimes for several months or longer). After treatment of the acute phase in immunocompromised patients, maintenance therapy should be started, usually with the same regimen that was used in the acute phase, but at half doses. At present, maintenance treatment is continued for the life of the patient or

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until the underlying immunosuppression has ceased. In patients with AIDS, primary and secondary prophylaxis are generally discontinued when the patients' CD4 count has returned to more than 200 cells per µl and the peripheral blood viral load has been reasonably controlled for at least six months (Mittal & Ichhpujani, 2011).

Three combination regimens are considered to be the first choices for the treatment of ocular toxoplasmosis: Pyrimethamine, sulfadiazine, folinic acid and prednisone; pyrimethamine, clindamycin, folinic acid and prednisone; pyrimetamine, sulfadiazine, clindamycin, folinic acid and prednisone. In patients who are intolerant to sulfonamides, clindamycin, azithromycin or spiramycin is recommended. Trimethoprim –sulfamethoxazole (Septrin) is a good replacement for pyrimethamine plus sulfadiazine, again in patients with sulfonamides intolerance. Corticosteroids are contraindicated in immunocompromised persons such as AIDS patients. Adult dose for Pyrimethamine is 100mg per day and then decreased to 50mg per day. For children, the recommended dose is 1 mg/kg/day for 6 to 12 months. Sulfadiazine is given at 75mg/kg/day in 4 divided doses for adults and 50mg/kg/day in 2 doses for children. Azythromycin is recommended at 500 mg/day for adults and 250 mg/day for children. Treatment takes 3 to 6 weeks or even more, depending on progress. Folinic acid is recommended at 15 mg every 3 days (Yazici, Ozdal, Taskintuna, Kavuncu, & Koklu, 2009; Bosch-Driessen & Rothova, 1998).

Hardy-Weinberg Equilibrium (HWE)

The unifying concept of population genetics is the Hardy-Weinberg Law. The Hardy-Weinberg Equilibrium relates allelic and genotypic frequencies in population of diploid, sexually reproducing individuals if that population has random mating, large size, no mutation or migration, and no selection. The rule has three assumptions:

1. The allelic frequencies at an autosomal locus in a population will not change from one generation to the next (allelic frequency equilibrium).

2. The genotypic frequencies of the population are determined in a predictable way by the allelic frequencies (genotypic frequency equilibrium).

3. The equilibrium is neutral. That is, if it is perturbed, it will be reestablished within one generation of random mating at the new allelic frequencies (if all the other requirements are maintained) (Tamarin, 2002).

Conforming to HWE for a locus suggests that several conditions are met including absence of recent mutations and genetic drift and conformance with random mating. A non-significant test result for HWE is equivalent to 'non-rejection' of the HWE assumption. Deviation from HWE tests may indicate failure in one or more assumptions. For example nonrandom mating may occur with loci related to some special characteristics as deafness and epilepsy. Other explanations as population stratification and selection bias are possible. Finally, a probable explanation for deviation from HWE is genotyping error. HWE deviation may be the strongest and most straightforward hint that genotyping may

need to be repeated and double-checked. Thus, the key inferences from a genetic-association study may be compromised if HWE is violated (Xu, Turner, Little, Bleecker, & Meyers, 2002). The validity of genetic association studies depends considerably on the use of appropriate controls. Theoretically, disease-free control groups from out-bred populations should follow the HWE. The same applies to the combined group of cases and controls from studies where all subjects have a specific disease or when the disease risk per se is not influenced by the evaluated polymorphism (Salanti, Amountza, Ntzani, & Ioannidis, 2005).

CHAPTER THREE

MATERIALS AND METHODS

The Study Sites

The study was conducted at the University of Cape Coast Eye Unit at the Department of Optometry and in three communities in the Central Region of Ghana. The communities were Nkanfoa in the Cape Coast metropolis, Moree in the Asebu Abura Kwamankese District and Jukwa in Twifo Heman Lower Denkyira District.

The Central Region is one of Ghana's ten administrative regions. It is bordered by the Ashanti and Eastern regions to the north, Western region to the west, Greater Accra region to the east, and to the south by the Atlantic Ocean. It is located between latitudes $5°1'$ and $6°18'$ N and longitudes $0°22'$ and $2°10'$ W (Ghana Statistical Service, 2010). The region consists of 20 administrative districts and has an estimated population of 2,201,863 of which 1,050,112 (47.7%) are males and 1,151,751 (52.3%) being females. The annual growth rate is 3.1%. The Region occupies an area of 9,826 square kilometers, which is about 6.6% of the total land area of Ghana. The region has in all 193 health facilities comprising of 77 public, 100 privates and 16 mission hospitals. Most of these private health institutions, however, are located in the district capitals and other

big towns. In addition, there are 15 functional Community-based Health Planning and Services (CHPS) compounds in six districts (Ghana Statistical Service, 2010).

The region can be broadly divided into two ecological zones: the coastal savannah with grassland along the coast and a semi-deciduous forest, predominating the inland areas. The three communities were selected to represent all the ecological zones of the region. Thus, Moree, represented the coastal communities, Nkanfoa for those located midway between the coastal and the forest zones while Jukwa represented the forest zone within the region. These communities were randomly selected from those of similar characteristics.

Nkanfoa

Nkanfoa is a suburban cosmopolitan community within the Cape Coast Metropolis, located between latitude 5°6' N and Longitude 1°14' W. It is only five kilometers from Cape Coast, the capital city of the Central Region. The community has a population of about 3,870. The occupation of the people ranges from petty trading to farming/gardening to formal sector employment and artisans (masonry, carpentry, and fitting). The main water sources for the community are pipe-borne water, bore holes and water from wells. There is one Community-based Health Planning and Services (CHPS) compound in Nkanfoa which provides basic health needs of the community, with the Central Regional Hospital and the Cape Coast Metropolitan Hospital as referral centres.

Moree

Moree is located in the Asebu Abura Kwamankese District (AAKD),
occupying an area of about 9 square kilometers, and geographically located
between latitudes 5°5′ and 25°5′ N and longitudes 5°1′ and 20°1′ W. With a
population density of 2,522 people/km^2, Moree is by far the most densely
populated settlement in the District. The District shares political and
administrative boundaries with Cape Coast on the South West, Twifo-Hermang
Lower Denkyira District on the North West, Assin South District in the North,
Mfantseman District on the East and the Gulf of Guinea in the South. It is a
coastal savanna with a generally low rainfall pattern of between 100cm and
110cm. The current population of Moree is about 23,845. It has one health center.
The only hospital in the district is located in Abura Dunkwa, the District capital of
AAKD. The main occupation of the people is fishing. Moree is considered one of
the major centers for marine fishing along the West African coast, and one of the
largest fishing communities in the Central Region and probably the whole of
Ghana. Located in the town is the Moree beach resort which serves as a major
recreational center for the district. A huge expanse of the community, about
3.4km, is along the coastal stretch of the area.

Jukwa

Jukwa is mostly a farming community located in the Twifo Heman Lower
Denkyira District. The District is bounded on the north by the Upper Denkyira
East Municipality, to the south by the Abura Asebu Kwamankese district, Cape
Coast Metropolis and Komenda-Edina-Eguafo-Abirem Municipality, to the west

by the Wassa Mpohor East District and to the east by Assin North Municipal and Assin South District. The community is located between latitude 16° 5' N and longitude 19° 1' W with an altitude of 75 meters above sea level. The district lies within the semi-equatorial zone marked by double maximal rainfall in June and October, with the mean annual rainfall being 175cm. The District's vegetation consists basically of semi-deciduous forest that has been largely disturbed by the activities of man through farming, logging and mining. There are large areas of forest reserves including the famous Kakum National park, Bimpong Forest Reserve, Pra Suhyen Forest Reserve, Minta Forest Reserve and Bunsaben Forest Reserve, all of which together cover about 288km (24.0%) of the entire surface area of the district. The community has one health centre. Figure 6 below represents a map of Central Region showing the study communities.
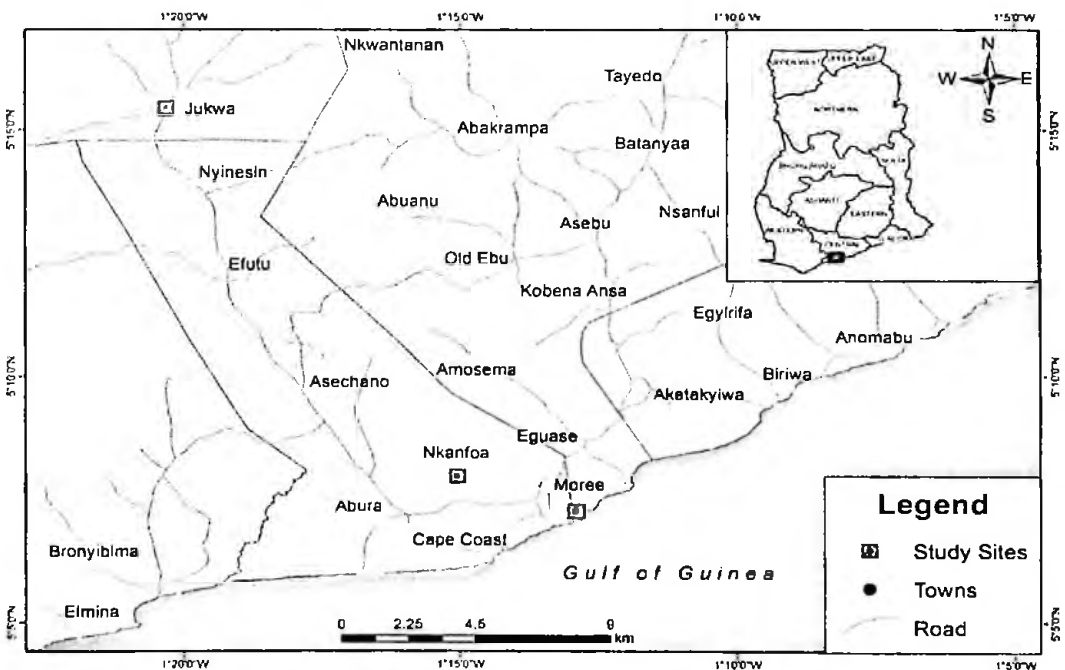
Figure 6: Map of Central Region showing the study communities

Source: Remote Sensing and Cartographic Unit, University of Cape Coast, 2015.

University of Cape Coast Eye Unit

The University of Cape Coast Eye Unit, sited within the Department of Optometry was established in 2003. The purpose of establishing the clinic was to serve as a teaching clinic for Optometry students at the University of Cape Coast and also to provide comprehensive eye care services to the University community and the general public. The clinic is operated largely by optometrists and visiting ophthalmologists. Patients who were diagnosed of *Toxoplasma* retinochoroiditis within the study period were recruited as cases for genotyping.

Study Design

The research employed both a population-based cross-sectional and a population-based case-control study designs. The cross-sectional study design was used in the epidemiological studies of systemic and ocular toxoplasmosis while the study of associations between ocular toxoplasmosis and cytokine gene polymorphisms employed the case-control study design. Ocular toxoplasmosis patients served as cases while a proportionate number of gender and age-matched seropositive individuals without ocular lesions were recruited as controls.

Sample Size Determination

A minimum sample size of 384 participants was calculated for the study. Sample size was determined as described elsewhere (Cochran, 1963; Ovenseri-Ogbomo & Omuemu, 2010). Thus, for a population of more than 10,000,

Sample size, $N = z^2 (1-p) (p)/b^2$, where

N = minimum sample size,

z = the standard normal deviation, usually set at 1.96 which corresponds to the 95% confidence level interval

p = the proportion in the target population estimated to have the infection. With no reasonable estimate, it is set at 50%.

b = degree of accuracy desired, usually set at 5%

Sampling Method

A systematic random sampling as described earlier (Bisika et al., 2009; Ocansey, Kyei Gyedu, & Awuah, 2014) was used to recruit participants by households. In each community, households were systematically selected by first randomly selecting the first house from the center of the community (based on the number of houses counted to the edge of the community). Each subsequent household was then selected by counting the next 5 houses in a random direction until the number needed for that community was obtained. All individuals in the selected households were included in the study provided they met the inclusion/exclusion criteria.

Ethical Approval

The study was conducted according to the Helsinki Declaration on research regarding human subjects. The protocol for this study was reviewed and approved by the Ghana Health Service's Ethical Review Committee (GHS-ERC). Ethical clearance certificate with identification number GHS-ERC: 21/11/12 was issued by the GHS-ERC before commencement of the study (See appendix A for copy of certificate). Approval was also given by the respective District Health Directorates and the Chiefs and Elders of the selected communities. The rational

of the study was explained to the participants in their own language after which chance was given to them to ask questions. Individuals who agreed to participate then signed consent forms attached to the questionnaires after the procedures had been explained to them in their own language. For participants aged <18 years whose assent was sought, their parents/guardians signed the consent forms on their behalf. Confidentiality and safety were assured at all times.

Inclusion/Exclusion Criteria

An inclusion criterion was for a participant to have lived continuously in the particular community for at least 2 years (Portela et al., 2004). A pilot study had indicated that parents were reluctant in allowing their young children to donate blood samples; hence children under 10 years were not included. Participants should also have clear ocular media in both eyes to permit a clear ophthalmoscopic view of both fundi. Thus, individuals who had dense cataracts and corneal opacities in one eye or both eyes were excluded.

Data Collection Procedure

The data collection consisted of four aspects. First, structured questionnaires were administered to all participants to collect their personal information as well as *Toxoplasma* infection risk practices. Second, all participants underwent serology testing using commercial ELISA kits to determine the individuals' exposure to the parasite. Third, all participants underwent ophthalmic examination to determine ocular manifestation of the infection (retinochoroiditis). Fourth, a proportionate number of gender and age-matched seropositive participants without ocular lesions were genotyped to

determine the associations between cytokine gene polymorphisms and susceptibility to ocular lesions caused by the parasite.

Questionnaires

All the participants answered questionnaires that sought information on: a) demographic characteristics: gender, age, educational level, occupation; b) possible exposure to and consumption of *T. gondii* oocysts: i) contact with soil such as working on a garden; ii) contact with cat faeces: owning cats, presence of cats around the house, disposing cat litter; iii) source of drinking water c) possible consumption of *T. gondii* cysts: type of meat consumed, state of meat consumed (undercooked meat or well cooked d) hygiene habits: hand washing, washing of vegetables/fruits or not before consumption. Socioeconomic statuses of participants were determined as was previously done elsewhere by using levels of formal education and occupation/employment status. Individuals in highly skilled professions like engineering, medicine, mechanized farmers, etc were rated as having high socioeconomic status (El-Bayoumy, Saad, & Choudhury, 2007; Ovenseri-Ogbomo & Assien, 2010).

Blood sample collection and serum preparation

Venous blood samples were collected by trained laboratory technologists. About 3 ml of venous blood was collected from each participant using a sterile disposable hypodermal syringe fitted with a 23 gauge needle and dispensed from the syringe barrel into a sterile tube and allowed to clot. Sample tubes were centrifuged at 500 x *g* to precipitate red blood cells. Clear sera were collected into

eppendorf tubes and stored at -20°C until tested. All sample tubes were appropriately labeled with code numbers.

Detection of anti-*T. gondii* antibodies by commercial ELISA kits

Each serum sample was tested for the presence of anti-*Toxoplasma* antibodies IgG and IgM using commercial ELISA kit (VEDALAB-France) and following the manufacturer's instructions. For IgG assay procedure, 100μl of diluted (1:41) test sample, calibrator, positive control, and negative control were transferred into the precoated wells, covered and incubated at room temperature for 30 minutes. Wells were then washed 5 times with diluted wash solution after which 100μl of horseradish peroxidase (HRP) conjugate was added to each well, covered and incubated for 30 minutes. The wells were again thoroughly washed 5 times with the diluted wash solution and after that 100μl of Tetramethylbenzidine (TMB) substrate was added to each well. The plate was covered, incubated at room temperature for 30 minutes and 100μl of stop solution added to each well. Finally, a 450nm set wavelength microplate reader was used to measure the Optical Density (OD) of each well. IgG cut-off values were determined in Enzyme Unit/ml (EU/ml) and calculated using the formula:

EU/ml of test sample = [(EU/ml of calibrator) / (absorbance of calibrator)] X (absorbance of test sample), and interpreted as follows:

A value of less than 40 EU/ml was considered negative for IgG antibody to *Toxoplasma gondii*. A value between 40 and 50 EU/ml meant that the test result was equivocal and had to be retested. An EU/ml value greater than 50 was positive for IgG antibody to *Toxoplasma gondii*.

A similar assay procedure was carried out for IgM antibody detection except that the plate was incubated at 37°C and index calculations were determined by dividing the optical density value of test sample by the optical density value of the calibrator, and interpreted as:

A calculated index less than 0.90 was negative for IgM antibody to *Toxoplasma gondii*. An index value between 0.90 and 0.99 was equivocal and needed to be retested while an index value of 1.00 or greater was positive for IgM antibody to *Toxoplasma gondii*.

Ophthalmic Examination

Ophthalmic examination was conducted by trained eye care personnel (ophthalmologists and optometrists). Distance presenting visual acuity measurements followed by dilated funduscopy was performed on all participants. Pinhole examination was performed to rule out refractive error as the cause of reduced vision. Dilated fundus examination was performed with 2.5% phenylephrine ophthalmic solution. Two drops of the phenylephrine were administered at an interval of 5 minutes and waited for one hour to ensure maximum papillary dilation. Ophthalmoscopy was then performed on each participant and fundus photographs were taken for cases of retinochoroiditis. Ophthalmic examination forms for each participant were correspondingly labeled with the same code as the tubes for the serum samples. Categories of visual impairment (VI) were determined based on the International Classification of Diseases, where ''low vision'' is defined as presenting visual acuity < 6/18 in the better eye, and "blindness" defined as presenting visual acuity < 3/60 in the better

eye. Correspondingly, the following visual acuity categories were determined: normal vision (VA > 6/9), mild visual impairment (VA 6/9 to 6/18), moderate visual impairment (VA < 6/18 to 6/60), severe visual impairment (VA 6/60 to 3/60), and blindness (VA < 3/60) (World Health Organization, 2005).

Diagnostic Criteria

A serologic criterion for systemic infection was a positive test result for any of the two anti-*Toxoplasma* IgG or IgM antibodies or a combination of both. Clinical diagnosis of ocular toxoplasmosis was based on a characteristic necrotizing retinochoroiditis adjacent to variably pigmented chorioretinal scar (Bosch-Driessen et al., 2002) and supported by a positive serologic result.

DNA Extraction

Genomic DNA was extracted from whole blood by strictly following the manufacturer's protocol in the QIAamp DNA Mini and Blood Mini Handbook (2012). Each DNA extraction involved pipeting 20 μl QIAGEN Protease (or proteinase K) and 200 μl of sample into the bottom of a 1.5 ml microcentrifuge tube. 200 μl of AL Buffer was added, followed by pulse-vortexing for 15 s. The mixture was incubated at 56°C for 10 minutes and then briefly centrifuged. Ethanol (96–100%) of 200 μl was added and mixed by pulse-vortexing for 15 s, followed by a brief centrifuge. The mixture was applied to the QIAamp Mini spin column (in a 2 ml collection tube) and centrifuged at 6000 x g (8000 rpm) for 1 min. the QIAamp Mini spin column was placed in a new 2 ml collection tube, 500 μl Buffer AW1 was added, and centrifuged at 6000 x g (8000 rpm) for 1 min. QIAamp Mini spin column was then placed in a clean 2 ml collection tube and

the tube containing the filtrate discarded, 500 µl Buffer AW2 was added and centrifuged at full speed (20,000 x g or 14,000 rpm) for 3 min. QIAamp Mini spin column was later placed in a 1.5 ml microcentrifuge tube, 150 µl Buffer AE was added and incubated at room temperature (15–25°C) for 1min, and then centrifuged at 6000 x g (8000 rpm) for 1 min. Extracted DNA was stored at -20 °C awaiting further analysis.

IFN-γ +874T/A Polymorphism Genotyping by ARMS-PCR

Polymorphism of IFN-γ +874 was determined using an amplification refractory mutation system by polymerase chain reaction (ARMS-PCR) protocol as described elsewhere (Pravica et al., 2000). ARMS-PCR, also called allele-specific PCR (AS-PCR) or PCR amplification of specific alleles (PCR-ASA), is a PCR-based method of detecting single base mutations (Newton et al., 1989).

Two sets of allele-specific primers were designed to amplify wild-type and mutant sequence. The difference between these two primers was the 3' base, where one matched the wild-type sequence and the other the mutant. A primer common to both reactions was present, and both reactions were run in parallel. The reaction containing the mutant primer would only extend mutant template and wild-type template would not be extended by this primer. Detection of a single nucleotide mutation at a predetermined point in a target sequence was achieved by running the ARMS-PCR with a single primer pair and then scoring the production of amplicon as positive or negative (Punia, & Saunders, 2009).

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The ARMS-PCR was carried out in a total volume of 25 μL containing 12.5 μL of GoTaq® Green Master Mix (Promega Corporation, USA), 5 μM of generic primer (100 pmol/μL; 5′ -TCA ACA AAG CTG ATA CTC CA -3′), 5 μM of specific A primer (100 pmol/μL, 5′ - TTC TTA CAA CAC AAA ATC AAA TCA -3′), or 5 μM of specific T primer (100 pmol/μL, 5′ - TTC TTA CAA CAC AAA ATC AAA TCT -3′), 0.5 μM of internal control 1 (10 pmol/μL, 5′ - GCC TTC CCA ACC ATT CCC TTA -3′), 0.5 μM of internal control 2 (10 pmol/μL, 5′ - TCA CGG ATT TCT GTT GTG TTTC -3′), 3μL of genomic DNA and nuclease free water. The T and A polymorphism sequences were identified using sequence specific single stranded oligonucleotides to cover a 24 base pair region surrounding the T to A polymorphism. These sequences are as follows: 1874*T, 5'-cacaaaatcaaatcTcacacaca-3'; and 1874*A, 5'-cacaaaat caaatcAcacacaca-3'.

ARMS-PCR for IFN-γ T/A polymorphism was performed in a thermo cycler (GeneAmp® PCR System 9700, USA) consisting of an initial denaturation step (95°C for 3 min); 10 cycles of 95°C (15 s), 65°C (50 s) and 72°C (40 s), followed by 20 cycles of 95°C (20 s), 55°C (50 s) and 72°C (50 s), with a final extension time of 7 min at 72°C. For each reaction a negative control which contained no DNA but nuclease free water was added in the amplification.

TNF-α 308G/A Polymorphism Genotyping by ARMS-PCR

The ARMS-PCR protocol was based on an earlier one by Gupta and Sehajpal, (2003), where two complementary reactions were established for each allele consisting of target DNA, allele specific ARMS primer (AP1:

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ATAGGTTTTGAGGGGCATCG or AP2: ATAGGTTTTGAGGGGCATCA) and a common primer (CP: AAGAATCATTCAACCAGCGG). This ARMS– PCR method was developed specifically to study the TNF-α-308 SNP. The method is based on the principle that under optimized conditions, the primers with 3′ end mismatch with the complementary template DNA will not result in the amplification of targeted DNA fragment. To assist in the genotyping of -308 polymorphism, the penultimate base in the primer AP1 and AP2 was also mutated from G to C. ARMS primer (AP1) and CP primer pair specifically amplified DNA from GG/GA individuals and not from AA ones. Similarly, A allele-specific ARMS primer (AP2) and CP primer pair gave specific product with A allele and failed to amplify DNA from GG individuals.

The optimized reaction conditions consisted of 3μl of genomic DNA in a total volume of 25μl of reaction mixture containing 0.16 μM of each primer, 12.5 μl of GoTaq® Green Master Mix (Promega Corporation, USA). The reaction was amplified for 35 cycles using a thermo cycler (GeneAmp® PCR System 9700, USA). Each cycle consisted of denaturation at 94°C for 30 s, annealing at 57°C for 20 s, extension at 72°C for 20 s and finally a 3 min extension at 72°C. A negative control which contained no DNA was added in the amplification.

Agarose Gel Electrophoresis

Following amplification, 2% agarose was prepared by melting 2g of agarose (Agarose Type PGP, Park Scientific.U.K) in 100mL of 1X TAE (Tris-Acetate-EDTA) buffer. It was melted in a microwave oven for approximately 3 minutes, allowed to cool for a couple of minutes at room temperature and 1 drop

of ethidium bromide added to the molten gel and swirled to mix uniformly. A gel was cast using the supplied tray and comb. Ten microlitres of PCR product was electrophoresed in 1X TAE buffer at 100V for 45 minutes and the gel exposed to UV light and photographed. DNA quality was confirmed when there was the presence of a highly resolved high molecular weight bands indicating good quality DNA, presence of a smeared band indicating DNA degradation. Images were captured with a digital camera, individual bands compared, and the different genotypes determined (see appendix D, Plates C and D)

Data Analysis

All the data obtained were analysed using the Statistical Package for Social Sciences (version 16; SPSS Inc., Chicago, IL). The descriptive data was analysed in terms of frequencies and percentages using tables and bar charts. Means were computed and recorded with standard deviations. Chi square test was used to test associations between categorical variables while the multivariate logistic regression analysis test (with 95% confidence intervals) was used to predict the associations between dependent and independent variables such as *T. gondii* seropositivity and risks of infection. Chi-square was also used to assess whether or not the data followed Hardy Weinberg equilibrium by comparing the observed values with expected values. The Fisher's exact test was used if any expected frequency was lower than five. Student's *t*-test was used to compare the means of continuous variables between groups, such as age. A two-tailed p-value less or equal to 0.05 was considered statistically significant.

Hardy Weinberg Equilibrium Determination

For a population in Hardy Weinberg equilibrium (HWE), the observed genotype frequencies will conform to $p^2 + 2pq + q^2 = 1$, where p2 = freq (GG), 2pq = freq (GC), and q2 = freq (CC). To determine whether the population was in HWE the p and q values were calculated as, p = GG + ½GC, and q = CC + ½GC. Because there were only two alleles in this case, the frequency of one plus the frequency of the other must equal 100%. That is, $p + q = 1$, where GG = wild type, GC = heterozygote mutant type and CC = homozygote mutant type. Evaluation of the Hardy-Weinberg equilibrium was performed using the control group by comparing the observed and expected frequencies of the genotypes using the chi-square analysis.

Allele Frequency Calculation

Allele frequency is the proportion of all copies of a gene that is made up of a particular gene variant (allele). That is, it is the number of copies of a particular allele divided by the number of copies of all alleles at the genetic place (locus) in a population. Frequency of G (p) = 2(GG) + (GC) / 2 (GG + GC +CC) and frequency of C (q) = 2(CC) + (GC) / 2 (GG + GC +CC). Thus, 1- p = q. Allelic frequencies of the studied SNPs were compared between cases and controls using the logistic regression analysis at a significance level of 0.05.

CHAPTER FOUR

RESULTS

Toxoplasma gondii Seropositivity in the Study Population

A total of 390 participants of mean age 47.0 years (SD = +/- 20.35, range = 10 – 100 years) were studied. There were 118(30.3%) males and 272(69.7%) females. The overall seroprevalence of *Toxoplasma gondii* in the study population was 85% (333/390) (Table 1). Of this, IgG antibodies were detected in 84% (329/390) while IgM antibodies were present in 6% (25/390) of the study population. Twenty-one (5%) participants tested positive for both IgG and IgM antibodies. Four (1%) participants tested positive for only IgM antibodies while 79% (308/390) participants tested positive for only IgG antibodies. The remaining 15% (57/390) of the participants tested negative for all the antibodies.

In terms of the study sites, Jukwa recorded the highest seropositivity of 92% followed by Moree 89% and Nkanfoa 75%. A significant difference (p < 0.001) in the seropositivity of participants from the differenct communities was observed.

Table 1: *Toxoplasma gondii* seropositivity rates according to the study communities

Community	Number tested	*Toxoplasma gondii* seropositivity n (%)			
		IgG only	IgM only	Both IgG and IgM	IgG and /or IgM
Moree	181	146(81)	2(1)	13(7)	161(89)
Nkanfoa	119	82(69)	1(0.8)	6(5)	89(75)
Jukwa	90	80(89)	1(1)	2(2)	83(92)
Total	390	308 (79)	4(1)	21(5)	333 (85)

Toxoplasma gondii Seropositivity according to Age

The ages of participants were categorized into groups (Portela et al., 2004). Seropositivity seemed to increase with age group where age group 10 -19 years had lowest prevalence of 55.8% (29/52) rising to 100% (50/50) in age group 60 -69 years, and then declining slightly to 97% (62/64) among participants aged 70 years or more (Figure 7). The differences in seropositivity among the age groups were statistically significant (p < 0.001).

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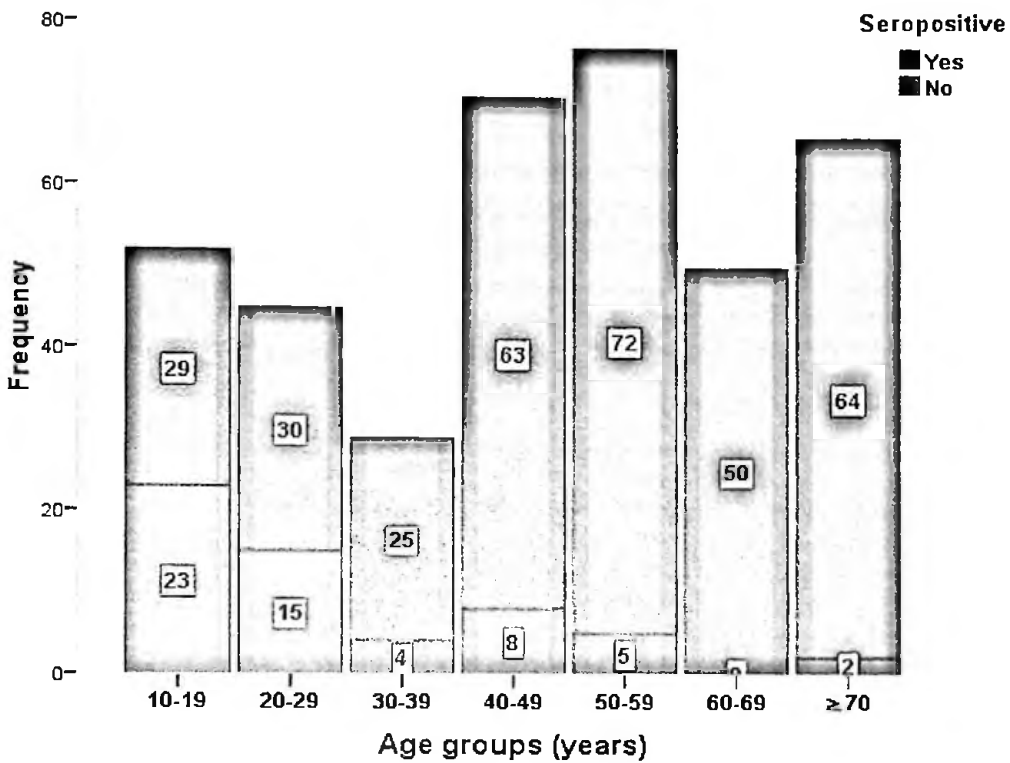
Figure 7: *Toxoplasma gondii* seropositivity according to age groups

Toxoplasma gondii Seropositivity according to Gender

According to gender, 102(86.4%) out of the 118 male participants tested positive for IgG and/or IgM antibodies while the overall seroprevalence of *T. gondii* to IgG and/or IgM antibodies among the female population was 84.9% (231/272) (Figure 8). One hundred and one (85.6%) and 5 (4.2%) of the males tested positive for only IgG and only IgM antibodies respectively. On the other hand, 228 (83.8%) and 20 (7.4%) of the female participants respectively tested positive for only IgG and only IgM antibodies. Though there were more female participants than males there was no significant association between seropositivity and gender ($p = 0.7$)

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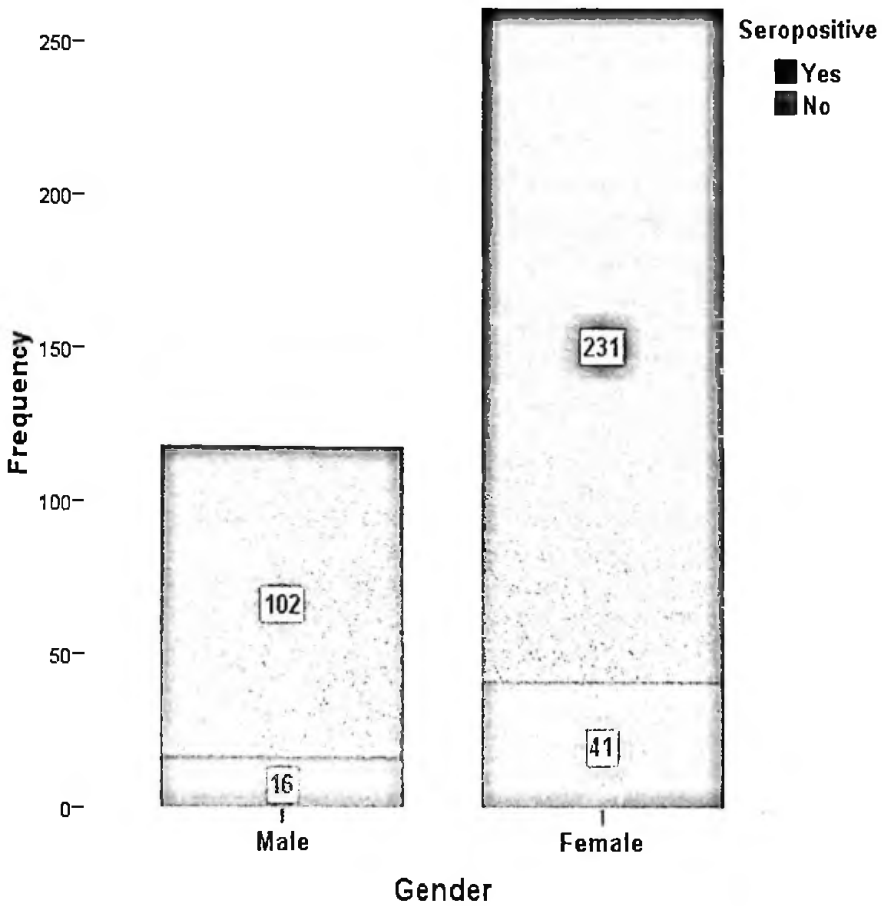
Figure 8: *Toxoplasma gondii* seropositivity according to gender

Seroprevalence of *Toxoplasma gondii* according to Formal Education

The study participants were categorized according to the highest level of formal education attained. The highest seroprevalence was recorded by those with no formal education 96.3% (155/161), followed by basic school level 81% (132/163), then those with secondary level education (secondary/technical/commercial school) of 74.5% (35/47) and finally by participants with tertiary education 57.9% (11/19) (Figure 9). Test of association between seropositivity and educational level was statistically significant ($p < 0.001$)
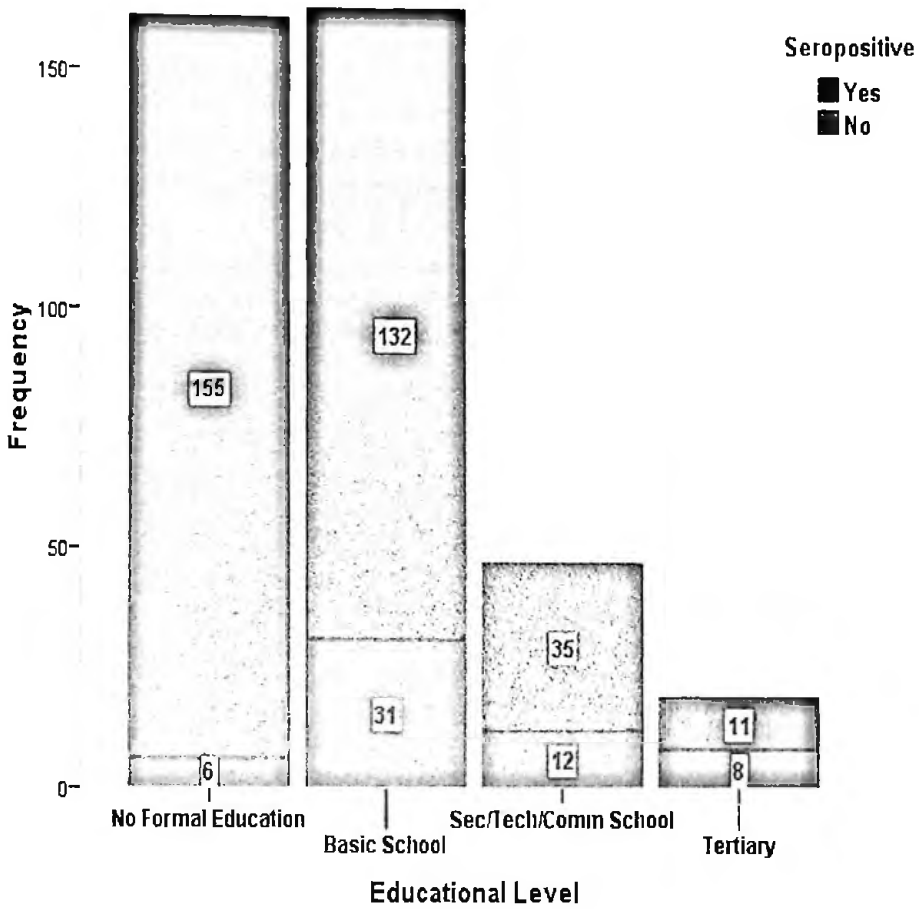
Figure 9: Seroprevalence according to formal education

Seroprevalence according to Occupation of Participants

Seropositivity of the participants was determined according to their occupations (Figure 10). The highest seroprevalence rates were respectively recorded for fish mongers 97.8% (45/46), farmers 97.7% (42/43) and fishermen 94.3% (33/35) while the least seropositivity was detected among school pupils or students 59.3% (38/64). There was a statistically significant association ($p < 0.001$) between seropositivity and the various occupations of participants.

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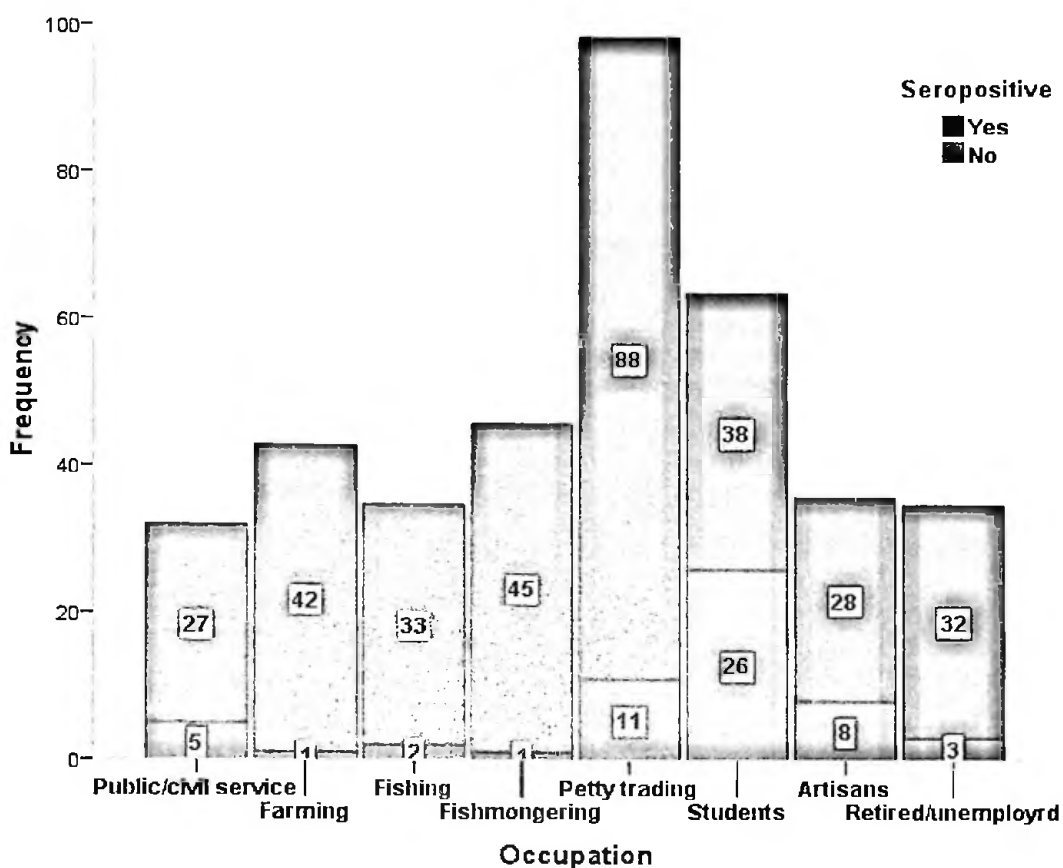
Figure 10: Seroprevalence according to occupation

Seroprevalence according to Sources of Drinking Water

Participants were categorized based on their sources of drinking water supply into sachet or bottled water, pipe borne water, borehole, well-water, and other sources such as river water and ponds. Participants who reported having borehole, well-water and other sources recorded 100% seropositivity rates respectively while those who had sachet or bottled water as their sources of drinking water supply recorded the lowest seropositivity rate of 73.5% (Figure 11). The difference in seropositivity according to sources of drinking water was statistically significant ($p = 0.019$).
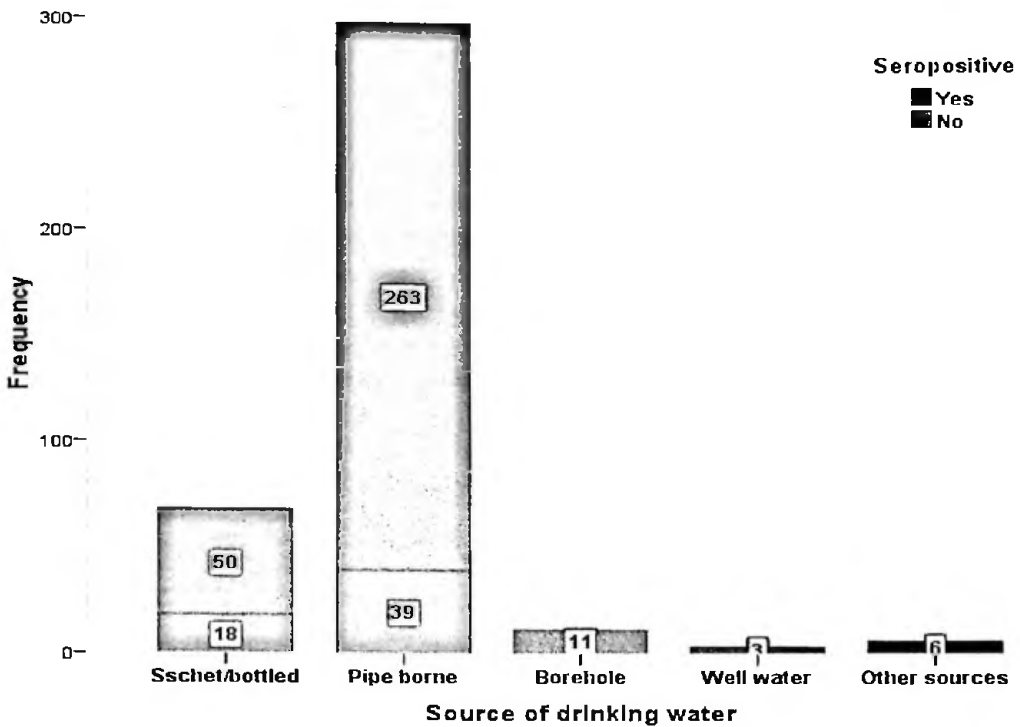
Figure 11: Seroprevalence based on sources of drinking water

Seroprevalence of *T. gondii* according to Socioeconomic Status

In terms of socioeconomic status, 332(85%), 52(13%) and 6(2%) of the participants were in the lower, medium and upper socioeconomic statuses respectively. The highest seroprevalence rate of 89.2% (296/332) was registered by the lower socioeconomic group while the least seroprevalence of 50% (3/6) was recorded for the higher socioeconomic group. A statistically significant association (p < 0.001) between seropositivity and the socioeconomic status of the participants was observed.

Test of Association between *T. gondii* Seropositivity and Variables Assessing Risk of Infection

Associations between *T. gondii* seropositivity and all variables assessing the risk of infection were determined using the Chi square (χ^2) statistical tool. All

variables associated with the presence of the cat (owning a cat, disposal of cat litter, and having cats around) had a significant relationship with seropositivity. However, *Toxoplasma* seropositivity had no association with the presence of dogs in the surroundings (p = 0.25). Again, there was no significant association between the infection and washing of hands or washing of vegetables and fruits before consumption (p = 0.48; p = 0.07 respectively).

Table 2: Test of association between *T. gondii* seropositivity and variables assessing risk of infection

Factor	No. tested	Positive n(%)	χ^2	p-value
Own Cat				
Yes	127	123(96.9)	19.84	<0.001
No	263	210(79.8)		
Dispose cat litter				
Yes	170	163(95.9)	26.613	<0.001
No	220	170(72.3)		
Exposed to the soil				
Yes	244	241(98.8)	93.59	<0.001
No	146	92(63.0)		
Cats around the house				
Yes	356	308(86.5)	4.20	0.04
No	34	25(73.5)		
Dogs around the house				
Yes	329	278(85.5)	1.324	0.25
No	61	55(90.2)		
Wash fruits/vegetables before consumption			3.42	0.07
Yes	314	263(83.8)		
No	76	70(92.1)		
Wash hands with soap before eating				
Always	126	107(84.9)	1.47	0.48
Sometimes	210	177(84.3)		
Never	54	49(90.7)		

Test of Association between Seropositivity and Consumption of Meat

Three hundred and sixty (92.3%) of the participants responded "yes" to consumption meat while 30 (7.7%) responded in the negative. Again, over 94% (340/360) of participants who reported to consume meat also reported that they always cooked the meat till at least it is soft before consumption. No item relating to meat consumption was significantly associated with *T. gondii* seropositivity (Table 3).

Table 3: Test of association between *T. gondii* seropositivity and consumption of meat

Factor	No. tested	Positive n(%)	χ^2	*p*-value
Consumption of chicken			3.13	0.08
Yes	351	296(84.3)		
No	39	37(94.9)		
Consumption of sheep			1.80	0.18
Yes	294	247(84.0)		
No	96	86(89.6)		
Consumption of goat			2.79	0.10
Yes	318	267(84.0)		
No	72	66(91.7)		
Consumption of pork			0.41	0.52
Yes	116	97(83.6)		
No	274	236(86.1)		
Way of cooking meat			3.37	0.19
Very soft	162	138(85.19)		
Soft	178	152(85.39)		
Tough	20	14(70.0)		
Consumption of meat			3.32	0.07
Yes	360	304(84.44)		
No	30	29(96.67)		

Variables with significant differences between groups, by the chi-square test, were selected for a multiple logistic regression analysis to identify independent risk factors for seropositivity.

Multivariate Logistic Regression Analysis between *T. gondii* Seropositivity and Risk of Infection

A multivariate logistic regression analysis was determined for all variables associated with the presence of the cat. The highest risk factor was to have contact with the soil (OR = 38.4, $p < 0.001$), followed by owning a cat (OR = 7.76, $p = <$ 0.001), disposing of cat litter (OR = 6.85, $p < 0.001$) and having cats around ones house (OR = 2.31, $p = 0.04$). Participants who had their sources of drinking water as borehole, well-water, and river water/ponds were at higher risks than those who used pipe borne water or sachet and bottled water (Table 4).

Table 4: Multivariate logistic regression analysis between *T. gondii* seropositivity and risk of infection

Factor	No. tested	Positive n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Own/keep Cat				
No	263	210(79.8)	Reference	–
Yes	127	123(96.9)	7.76(2.7-22.0)	<0.001
Dispose cat litter				
No	220	170(72.3)	Reference	–
Yes	170	163(95.9)	6.85(3.0-15.4)	<0.001
Contact with soil				
No	146	92(63.0)	Reference	–
Yes	244	241(98.8)	38.39(14.4-154.6)	<0.001

OR: ODDs Ratio

CI: Confidence Interval

Table 4 continued: Multivariate logistic regression analysis between *T. gondii* seropositivity and risk of infection

Factor	No. tested	Positive n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Cats around the house				
No	34	25(73.5)	Reference	–
Yes	356	308(86.5)	2.31(1.0-5.2)	0.04
Source of drinking water				
Sachet/bottled	68	50(73.53)	Reference	–
Pipe borne	302	263(87.09)	2.428(1.9-4.9)	0.007
Borehole	11	11(100.0)	6.646E7	<0.001
Well water	3	3(100.0)	6.646E7	<0.001
River water	6	6(100.0)	6.646E7	<0.001

Multivariate Logistic Regression Analysis between *Toxoplasma gondii* Seropositivity and Demographic Characteristics of Participants

Respondents from Jukwa were at the highest risk for the infection (OR = 4.0; p = 0.002) as compared to those from Nkanfoa. The infection was found to increase with increasing age (10-19years, 55.8%; 20-29years, 66.7%, OR = 1.59, p = 0.3; 30-39years, 86.2%, OR = 4.95, p = 0.008; 40-49years, 88.7%, OR = 6.25, p < 0.001; 50-59years, 93.5%, OR = 11.42, p < 0.001; 60-69years, 100%, OR = 9.9×10^7, p < 0.001; >70years, 97.0%, OR = 25.38, p < 0.001). With respect to level of formal education, participants who had "no formal education", "basic school education" and "second cycle education" were respectively 19, 3 and 2 times more likely to be seropositive compared to those who had tertiary education.

Table 5: Multivariate logistic regression analysis between *T. gondii* seropositivity and demographic characteristics of participants

Characteristic	No. tested	Positive n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Community				
Nkanfoa	119	89(75)	Reference	–
Moree	181	161(89)	2.7(1.5-5.1)	0.002
Jukwa	90	83(92)	4.0(1.7-9.6)	0.002
Age group				
10-19	52	29(55.77)	Reference	–
20-29	45	30(66.67)	1.586(0.7-3.6)	0.27
30-39	29	25(86.21)	4.957(1.5-16.3)	0.008
40-49	71	63(88.73)	6.246(2.5-15.6)	<0.001
50-59	77	72(93.51)	11.421(4.0-33.0)	<0.001
60-69	50	50(100.0)	9.969E7(9.9E7-9.9E7)	<0.001
$\geq$70	66	64(96.97)	25.379(5.6-114.9)	<0.001
Education				
Tertiary	19	11(57.89)	Reference	–
Second cycle	47	35(74.47)	2.121(0.7-6.5)	0.189
Basic School	163	132(80.98)	3.097(1.1-8.3)	0.025
No formal education	161	155(96.27)	18.788(5.5-64.0)	<0.001

Table 5 continued: Multivariate logistic regression analysis between *Toxoplasma gondii* seropositivity and demographic characteristics of participants

Characteristic	No. tested	Positive n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Socioeconomic status				
High SES	6	3(50.0)	Reference	–
Med. SES	52	34(65.38)	1.889(0.3-10.3)	0.463
Low SES	332	296(89.16)	8.222(1.6-42.3)	0.012
Occupation				
Students/pupils	64	38(59.38)	Reference	–
Artisans	36	28(77.78)	2.395(0.9-6.1)	0.06
Public/ civil service	32	27(84.38)	3.695(1.3-10.8)	0.017
Petty trading	99	88(88.89)	5.474(2.5-12.2)	<0.001
Retired/ unemployed	35	32(91.43)	7.298(2.0-26.4)	0.002
Fishermen	35	33(94.29)	11.289(2.5-51.0)	0.002
Farmers	43	42(97.67)	28.737(3.7-222.1)	<0.001
Fishmongers	46	45(97.83)	30.789(4.0-237.6)	<0.001

Med. SES: Medium socioeconomic status; SES: socioeconomic status

Socioeconomic status was significantly associated with seropositivity, with participants in the low socioeconomic group being 8 times more likely to be seropositive than those in the high socioeconomic group (OR = 8.2; p = 0.012). In terms of occupation, there was a 2.4 to 30.8 fold increased risk where higher seroprevalence values were observed for fish mongering (OR = 30.8; p <0.001), farming (OR = 28.8; p <0.001), and fishing (OR = 11.3; p = 0.002) (Table 5).

Multivariate Logistic Regression Analysis between Occupations of Participants and Exposure to the Soil

A multivariate logistic regression analysis was performed to determine the relationship between various occupations and their exposure to the soil. It revealed that occupations that recorded higher seropositivity values also had the highest ODDs Ratios for exposure to the soil: fishing (OR = 21.8; p = <0.001); farming (OR = 20.0; p <0.001); fish mongering (OR = 5.8; p <0.001) (Table 6).

Table 6: Multivariate logistic regression analysis between occupations of participants and exposure to the soil

Occupation	No. tested	No. exposed to soil n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Students/pupils	64	21(32.8)	Reference	–
Public/civil service	32	16(50)	2.05(0.9-4.9)	0.11
Artisans	36	19(52.7)	2.29(0.99-5.29)	0.53
Retired/unemployed	35	20(57.1)	2.73(1.2-6.4)	0.20
Petty trading	99	63(63.6)	3.58(1.8-7.0)	<0.001
Fish mongering	46	34(73.9)	5.80(2.5-13.4)	<0.001
Farming	43	39(90.7)	19.96(6.3-63.3)	<0.001
Fishing	35	32(91.4)	21.84(6.0-79.6)	<0.001

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Test of Association between Study Communities and Risk Factors

The finding of significant differences (p < 0.001) in seropositivity between the study communities led to the investigation of association between risk factors for the infection and these study communities. Table 7 represents an analysis of a multivariate logistic regression between the study communities and the risk factor "contact with the soil" (the highest risk factor for the infection). Participants from Jukwa were most likely to have contact with the soil, followed by Moree and Nkanfoa.

Table 7: Multivariate logistic regression analysis between the study communities and contact with the soil

Community	No. tested	No. exposed to soil n(%)	Multivariate adjusted OR (95% CI)	*p*-value
Nkanfoa	119	54(45.4)	Reference	–
Moree	181	124(68.5)	2.6(1.6-4.2)	< 0.001
Jukwa	90	66(73.3)	3.3(1.8-6.0)	< 0.001

In terms of occupation, those that were significantly associated with *T. gondii* seropositivity (that is, fishing, farming/gardening, and fishmongering) were mostly from Jukwa and Moree communities (p < 0.001). Majority 25(55.1%) of participants who were involved in farming/ gardening were from

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Jukwa. All 35 respondents who were engaged in fishing came from Moree and at the same time almost all the fishmongers 44(95.7%) were from Moree.

With respect to age, majority 24(46.2%) of participants within the minimum and the least associated age group (10-19 years) were from Nkanfoa while 19(36.5%) and 9(17.3%) were from Moree and Jukwa respectively. Regarding sources of drinking water, all participants who responded to having river/ pond water and well water as sources of drinking water were residents of Jukwa.

Visual Acuities of Participants

Figure 12 shows the presenting visual acuities of respondents. Using presenting visual acuity (VA) in the better eye, 205(52.6%) of the participants had normal vision (VA > 6/9), 99 (25.4%) had mild visual impairment (VA 6/9 to 6/18), 72 (18.5%) had moderate visual impairment (VA < 6/18 to 6/60), while 9(2.3%) persons were legally blind (VA worse than 3/60). It must be mentioned, however, that by the inclusion/exclusion criteria, many individuals with visual impairment may have been excluded from the study because they had dense cataracts or corneal opacities.

Assessment of Low Vision among Study Participants

Low vision occurrence was assessed in the study participants. All together, 86(22.1%) participants had low vision (VA < 6/18) while 304 (77.9%) had normal vision (Figures 13).
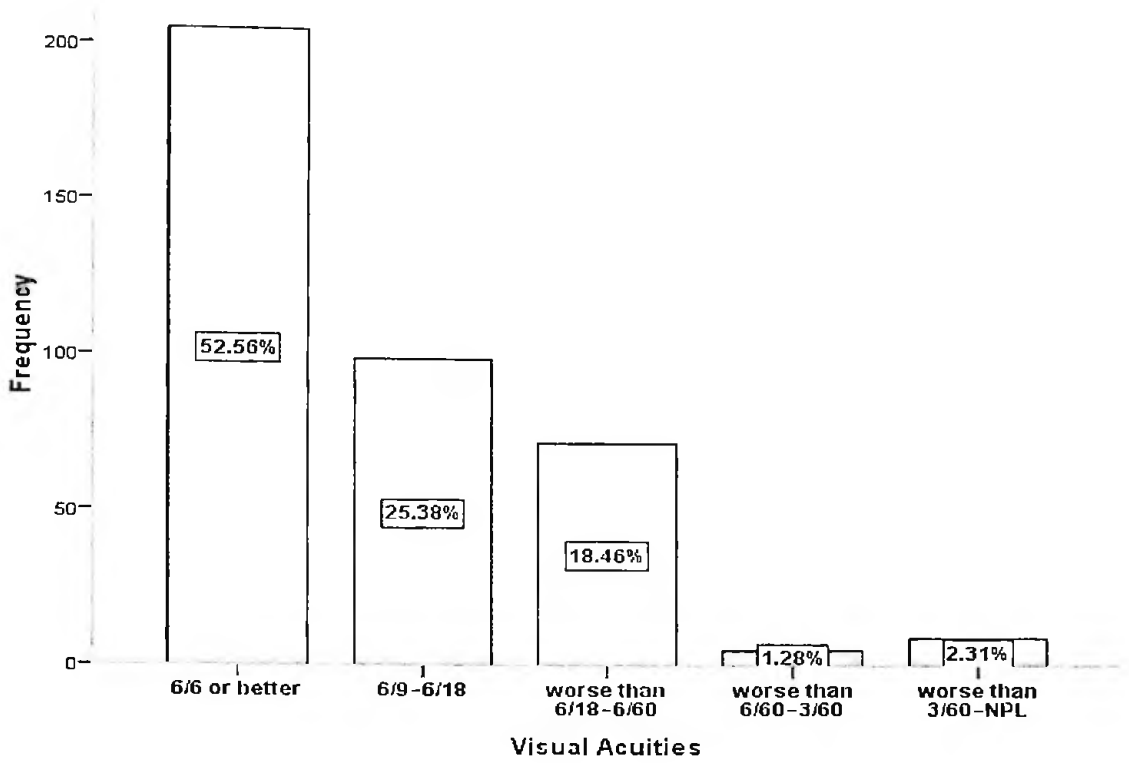
Figure 12: Visual acuities of participants
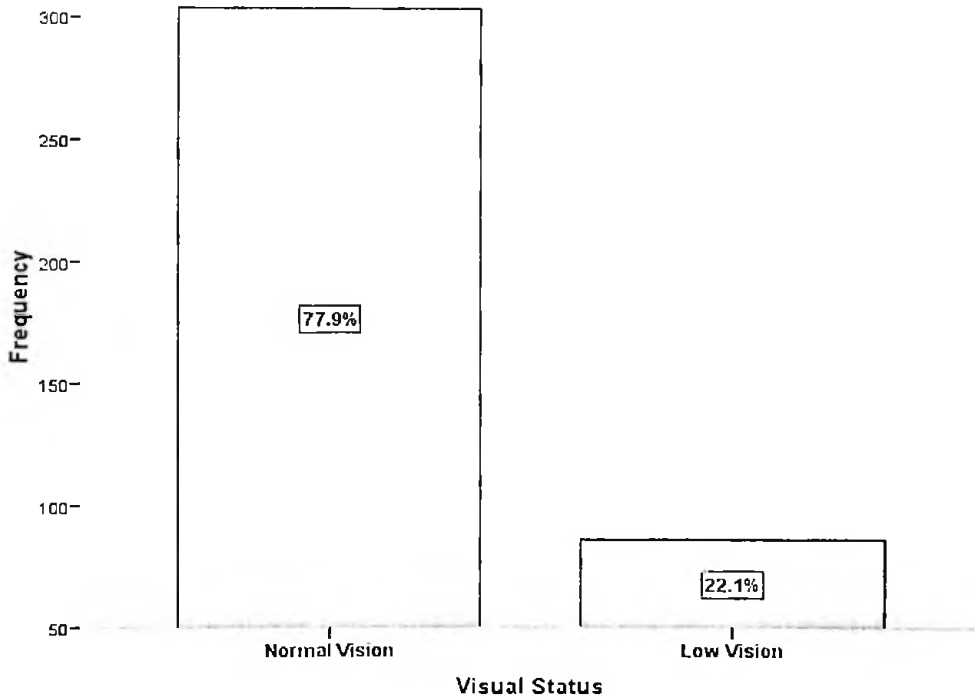
Figure 13: Assessment of low vision in the study participants

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Multivariate Logistic Regression Analysis between Participants' Age Groups and Occurrence of Low Vision

The occurrence of low vision was found to increase significantly with age such that among the elderly of 60 years and above, 51.7% (60/116) exhibited low vision of VA worse than 6/18 (Table 8).

Table 8: Multivariate logistic regression analysis between participants' age group and occurrence of low vision

Age group	No. examined	No. with Low Vision n(%)	Multivariate adjusted OR (95% CI)	p-value
10-19	52	1(1.9)	Reference	-
20-29	45	2(4.4)	2.372(0.2-27.1)	0.49
30-39	29	1(3.4)	1.821(0.1-30.1)	0.01
40-49	71	7(9.9)	5.578(0.7-46.8)	0.11
50-59	77	15(19.5)	12.34(1.6-96.6)	0.02
60-69	50	17(34.0)	26.27(3.3-207.0)	0.002
≥70	66	43(65.2)	95.35(12-735)	< 0.001
Total	390	86(22.1)		.

Assessment of Retinochoroidal Lesions in the Study Population

Toxoplasmic ocular lesions were found in 10 (2.6%) participants, 6 females and 4 males. Among the seropositive population, the prevalence of ocular toxoplasmosis was 3% (10/333). All cases of ocular toxoplasmosis tested positive

for IgG but none had IgM antibodies. Of the 10 ocular cases, one patient was within the age range of 30-39 years, three patients were within age range 50-59 years, and another three patients were within age range 60-69 years while the other three were above 70 years. The mean age of participants with ocular lesions (61.0 +/- 13.9 years) was significantly higher than that of those without ocular lesions (46.64 +/- 20.35 years) [F (1, 388) = 4.90, p = 0.028]. Two persons (all males) had bilateral ocular lesions while the other 8 had unilateral lesions. Figures 14 to 18 show the fundus photographs of 5 participants with their corresponding visual acuities.

Following the convention for assigning the principal cause of vision loss to the primary or the more preventable cause, toxoplasmosis related vision impairment was explored. Out of the 12 eyes with lesions, 1 (8.3%) eye had normal vision (VA of 6/5), another 1 (8.3%) eye had mild visual impairment of 6/12, 2 (16.7%) eyes exhibited moderate visual impairment (VA < 6/18 to 6/60), 2(16.7%%) other eyes had severe visual impairment (VA < 6/60 to 3/60), while 6(50%) eyes were legally blind (VA worse than 3/60). Again, of the 12 eyes, macular lesions occurred in 8 (66.7%) eyes. Low vision (VA worse than 6/18) occurred in 10 (83%) eyes with toxoplasmic ocular lesions.
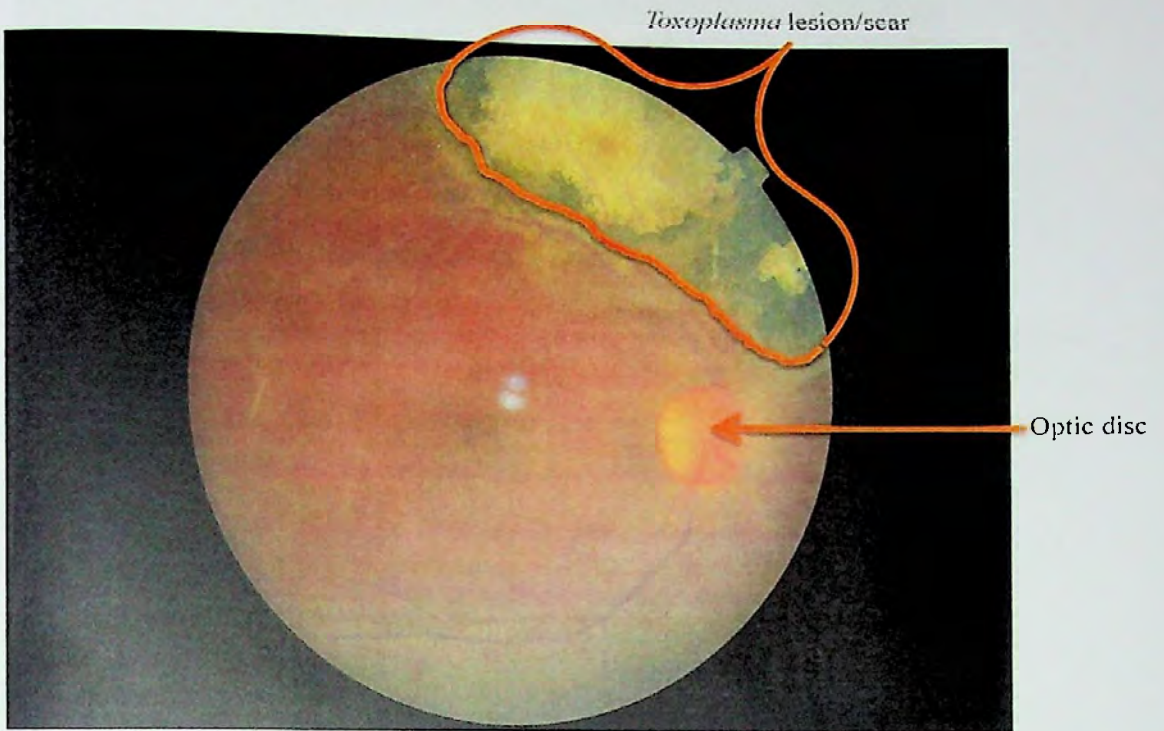
Figure 14: A large peripheral lesion, VA = 6/12 [mild visual impairment (VI)]
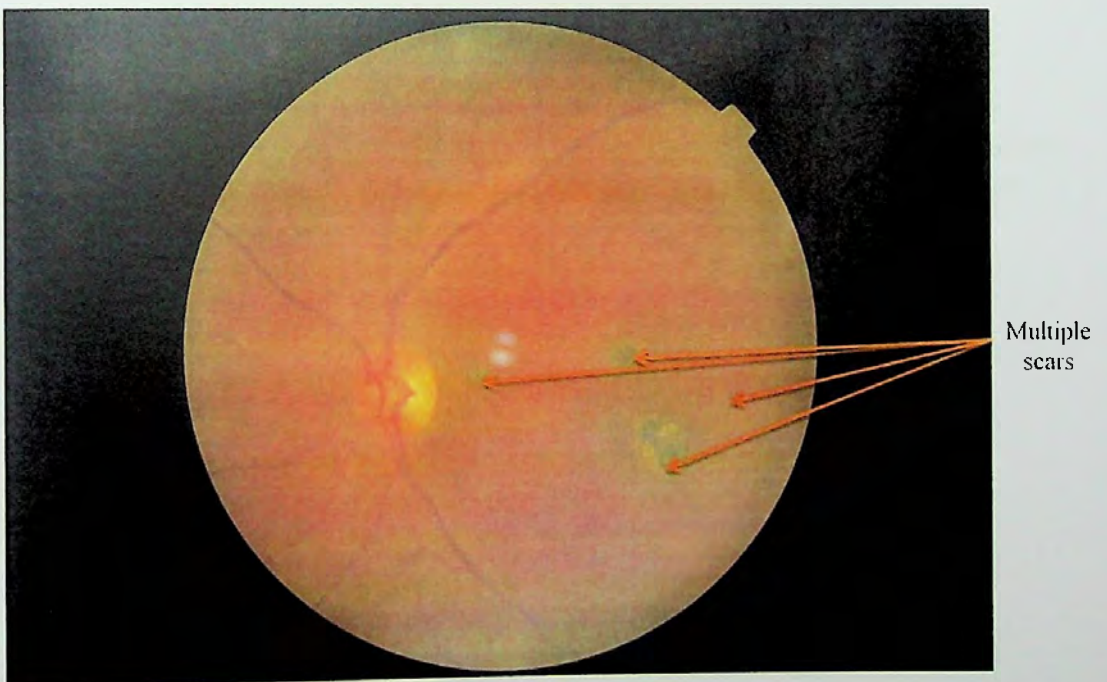
Figure 15: Multiple scars around macular area, VA = 6/36 (moderate VI)

Toxoplasma scar
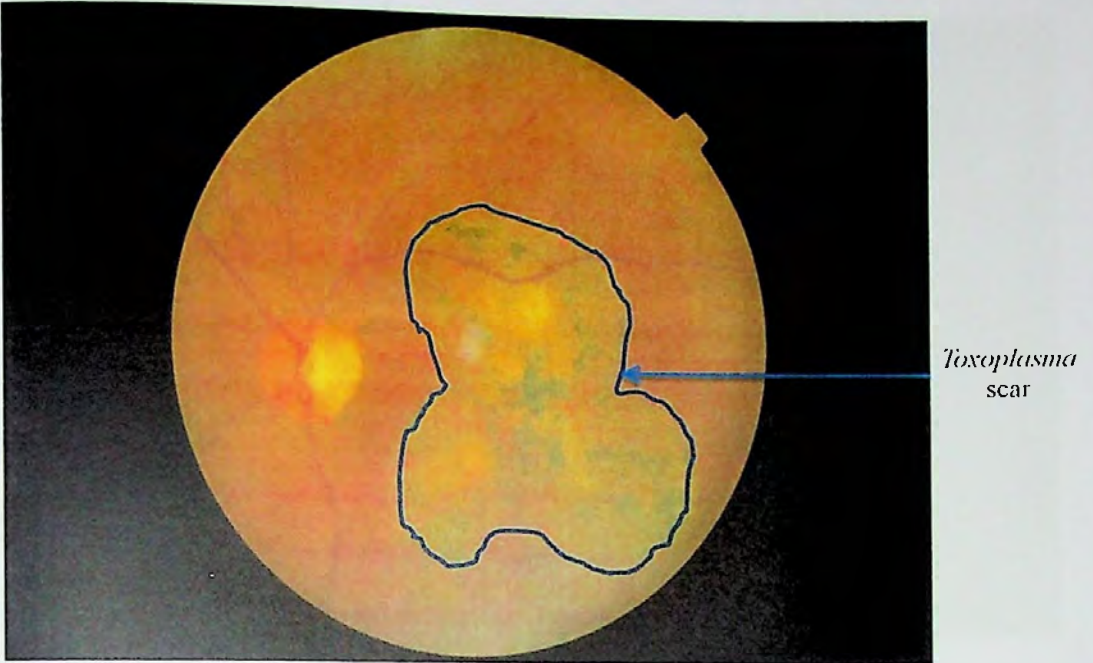
Figure 16: A large macular scar, VA = CF @2m (blind)

Toxoplasma scar
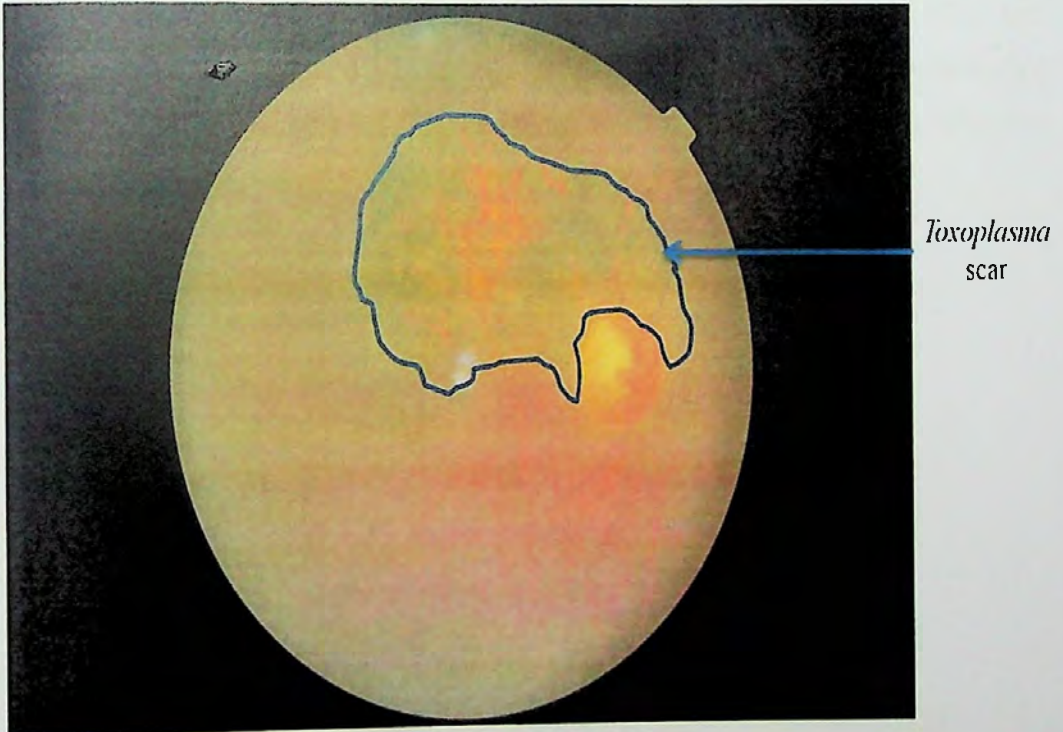
Figure 17: A large juxtapapillary lesion, VA = 6/36 (moderate VI)
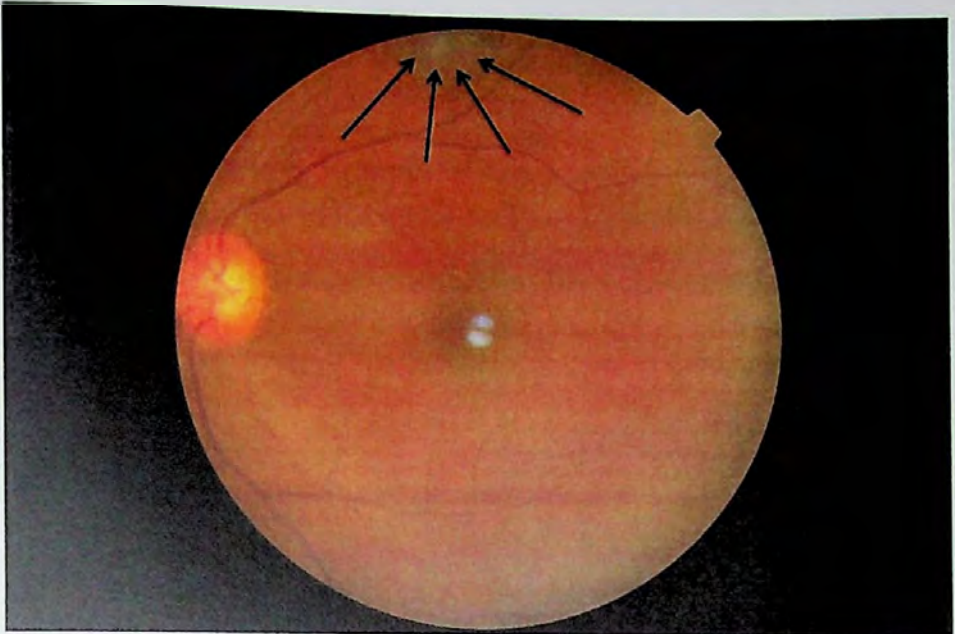
Figure 18: A small peripheral scar, VA = 6/6 (normal vision)

In terms of cause of visual impairment (VA<6/18), cataract was the leading cause 53(61.6%) followed by refractive error 16(18.6%), age-related macular degeneration 6(7.0%), *Toxoplasma* retinochoroiditis 5(5.8%), glaucoma 5(5.8%) and hypertensive retinopathy 1(1.2%) (Fig.19).
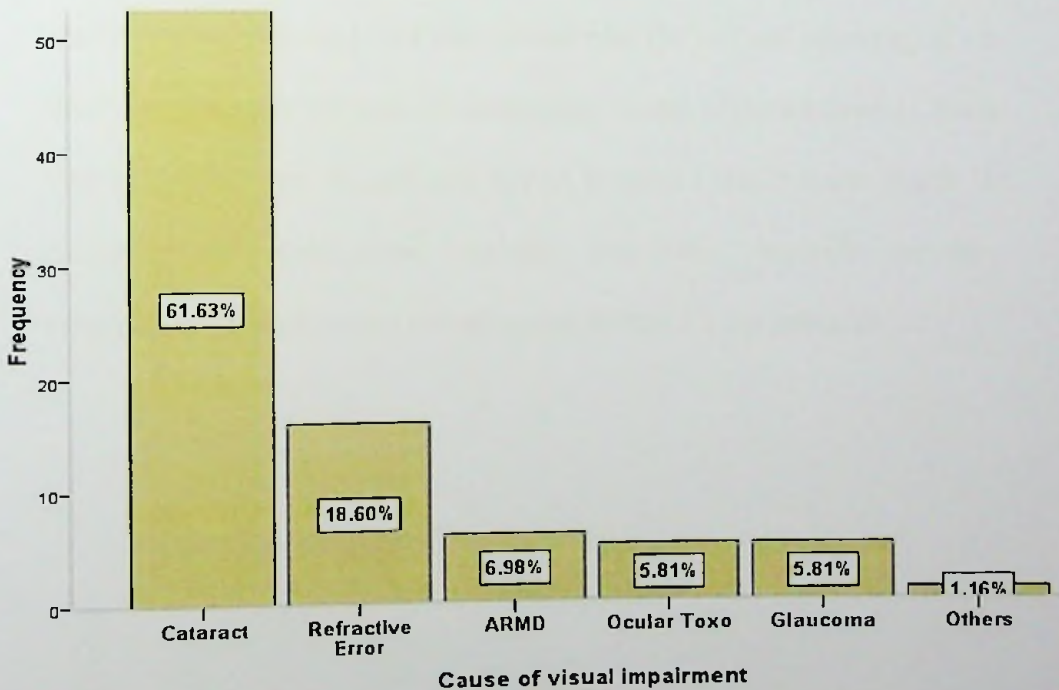
Figure 19: Cause of visual impairment in the study population

Test of Association between Ocular Toxoplasmosis and Variables Assessing Risk of Infection

While seroprevalence rates were higher in the more rural communities (Jukwa and Moree respectively) there was no significant association between the occurrence of ocular toxoplasmosis and the rural communities (Fisher's Exact Test = 3.395, p = 0.199). Apart from age, there was no significant association between ocular toxoplasmosis and other demographic data like gender and socioeconomic status. Again, meat consumption did not have any association with the development of ocular toxoplasmosis. There was also no significant association between ocular toxoplasmosis and any variable assessing contact with cat faeces (owning/keeping a cat, disposing cat litter and contact with the soil). Test of association between ocular toxoplasmosis and variables assessing risk of infection is presented on Table 9. However, the multivariate logistic regression analysis suggested that contact with the soil and disposing of cat litter may increase the risk of developing ocular toxoplasmosis as those who responded 'yes' in each case had an increased ODDs Ratio. Table 10 presents the multivariate logistic regression analysis between seroprevalence/ocular cases and some risk factors for the infection.

Table 9: Test of association between ocular toxoplasmosis and variables assessing risk of infection

Factor	No. examined	Ocular cases n(%)	Fisher's Exact p-value
Gender			
Male	118	4(3.4)	0.50
Female	272	6(2.2)	
Socioeconomic status			
High	6	0(0)	0.27
Medium	52	3(5.8)	
Low	332	7(2.1)	
Own Cat			
Yes	127	1(0.8)	0.18
No	263	9(3.4)	
Dispose cat litter			
Yes	170	6(3.5)	0.34
No	220	4(1.8)	
Contact with the soil			
Yes	244	8(3.3)	0.33
No	146	2(1.4)	
Consumption of meat			
Yes	360	9(2.5)	0.56
No	30	1(3.3)	

Table 10: Multivariate logistic regression analysis between the study communities and seroprevalence/ocular cases

Community /risk factor	Number examined /tested	Seroprevalence			Ocular cases		
		n (%)	OR	*p*-value	n (%)	OR	*p*-value
Community							
Nkanfoa	119	89(75)	Ref.	–	4(1.2)	Ref.	–
Moree	181	161(89)	2.7	0.002	2(0.6)	0.3	0.194
Jukwa	90	83(92)	4.0	0.002	4(1.2)	1.3	0.687
Dispose cat litter							
No	220	170(72.3)	Ref.	–	4(1.8)	Ref.	–
Yes	170	163(95.9)	6.85	<0.001	6(3.5)	1.98	0.34
Contact with the soil							
No	146	92(63.0)	Ref.	–	2(1.4)	Ref.	–
Yes	244	241(98.8)	38.39	<0.001	8(3.3)	2.44	0.33

Cytokine Gene Polymorphism

Genotyping was performed for 30 individuals with ocular toxoplasmosis (ocular group) and 87 without ocular lesions (control group). The ocular group consisted of 10 cases from the three communities plus 20 cases from the Department of Optometry Eye clinic. The control group also consisted of 87 individuals who were seropositive for *T. gondii* but had no ocular lesions. These were matched by age and sex. Allele and genotype frequencies of the ocular group were compared with these frequencies in the control group to determine the

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association between the 2 investigated SNPs (IFN-γ +874 and TNF-α -308) and the occurrence of ocular toxoplasmosis.

Among the 30 patients with toxoplasmic ocular lesions, 18 were males and 12 females, with a mean age of 38.0 years (SD = ± 19.4; Range = 19 − 80 years). Genotype frequencies of the polymorphisms studied were in Hardy-Weinberg equilibrium (IFN-γ: χ^2 = 0.55, p = 0.46; TNF-α: χ^2 = 199, p = 0.58). Hardy-Weinberg equilibrium was tested using the control group only.

IFN-γ Gene Polymorphism

All 117 individuals (30 cases and 87 controls) whose DNAs were analysed using ARMS–PCR at the IFN-γ +874T/A polymorphic site, 75.2% (88) showed A/A homozygosity, 21.4% (25), A/T heterozygosity and 3.4% (4), T/T homozygosity. The genotype frequency analysis showed that A/A homozygotes were the majority in both the ocular and the control groups [20(67%) and 68 (78%) respectively)]. There was a similar distribution of the A/T heterozygotes between cases and controls (27 and 20% respectively).

By logistic regression analysis, the T/T homozygous individuals had a 3.4-fold increased risk when compared to the A/A homozygotes, though the overall distribution was not statistically significant (Fisher's Exact Test Value = 2.46, p = 0.259). Similarly, the A/T heterozygous individuals had a 1.6-fold increased risk when compared with the A/A genotype. Analysis of the allele carriage indicated that the presence of a T allele in either the homozygous or the heterozygous individuals increased the risk of developing toxoplasmic ocular lesions (Table

11). There was no significant difference in the distribution of alleles between cases and controls (p = 0.18) though the T allele had a higher ODDs Ratio, indicating susceptibility to the infection. The lack of significant association could be due to the small number of cases. Table 11 presents the genotype, allele and allele carriage distributions of the IFN-γ +874T/A polymorphism in ocular toxoplasmosis cases and controls.

Table 11: Distribution of genotype and allele frequencies of IFN-γ +874T/A polymorphism in cases of ocular toxoplasmosis and controls

IFN-γ +874T/A Genotypes	Ocular group n = 30		Control group n = 87		OR(95% CI)	p-value
	n	(f)	n	(f)		
AA	20	0.67	68	0.78	Reference	−
AT	8	0.27	17	0.20	1.60(0.602-4.251)	0.35
TT	2	0.07	2	0.02	3.40(0.450-25.69)	0.24
Allele carriage						
AA	20	0.67	68	0.78	Reference	−
AT+TT	10	0.33	19	0.22	3.04(0.408-22.57)	0.28
Alleles						
A	48	0.80	153	0.88	Reference	−
T	12	0.20	21	0.12	1.60(0.802-3.19)	0.18

Table 12 below compares the distributions of IFN-γ and TNF-α genotypes in different populations

Table 12: Comparison of IFN-γ and TNF-α genotypes in different populations

IFN-γ genotype			Study	Population
AA	AT	TT		
88(75.2%)	25(21.4%)	4(3.4%)	This study	Ghanaian
46.2%	35%	18.8%	Rekha et al., 2006	Indian
27%	63.7%	8.9%	Albuquerque et al., 2009	Brazilian
40%	42.5%	17.5%	Neves et al., 2012	Brazilian
31.6%	54%	14.4%	Visentainer et al., 2005	Brazilian
14%	33%	53%	Hussein et., 2009	Egyptian

TNF-α genotype			Study	Population
GG	GA	AA		
107(91.5%)	9(7.6%)	1(0.9%)	This study	Ghanaian
79%	18%	3%	Cordeiro et al., 2008b	Brazilian
57.7%	42.3%	0%	Pujhari et al., 2012	Japanese
71.4%	28.6%	0%	Mishra et al., 2015	Indian
84.1%	15.9%	0%	Parikh et al., 2004	Ugandan

TNF-α Gene Polymorphism

Genotyping was performed for all 30 cases and 87 controls at TNF-α -308 by ARMS–PCR. Table 13 presents the distribution of TNF-α -308G/A genotype, allele and allele carriage between ocular toxoplasmosis cases and control. The wild type (GG genotype) was the largest group in both cases and controls [29(97%) and 78(90%) respectively]. This was followed by the heterozygous mutant (3% in cases and 9% in controls) and finally the homozygous mutant (0% in cases and 1% in controls). There was no significant difference in the distribution of both genotypes and alleles between the cases and controls ($p = 0.59; 0.16$). However, both GA heterozygous and AA homozygous individuals gave lower ODDs Ratios when compared with the GG homozygotes, indicating that the presence of the A allele conferred some protection against the development of toxoplasmic ocular lesions. The allele carriage analysis (AA vs GA+GG) also indicated that the presence of the G allele increased the risk of developing ocular lesions. Similarly, when compared with the G allele, the A allele gave a lower ODDs Ratio.

Table 13: Distribution of genotype and allele frequencies of TNF-α 308G/A polymorphism in cases of ocular toxoplasmosis and controls

TNF-α 308G/A Genotypes	Ocular group n = 30		Control group n = 87		OR(95% CI)	p-value
	n	(f)	n	(f)		
GG	29	0.97	78	0.90	Reference	–
GA	1	0.03	8	0.09	0.34(0.040–2.807)	0.31
AA	0	0.00	1	0.01	$10^{-8}(10^{-8}–10^{-8})$	
Allele carriage						
AA	0	0.00	1	0.01	Reference	–
GA+GG	30	1.00	86	0.99	3.35(0.406–27.586)	0.26
Alleles						
G	59	0.98	164	0.94	Reference	–
A	1	0.02	10	0.06	0.34 (0.075–1.508)	0.16

CHAPTER FIVE

DISCUSSION

Overall Seroprevalence of *T. gondii* in the Study Population

The overall seroprevalence of 85.4% found in this present study was quite high but lower than 92.5% prevalence observed in an earlier hospital-based study among pregnant women in Accra, reported elsewhere (Ayi et al., 2009). Another hospital-based study among patients of mean age 30.2 years visiting the Korle Bu Teaching Hospital in Accra rather reported a lower prevalence of 49.7% (Ayeh-Kumi et al., 2010). The differences of prevalence in these studies may be due to the different populations studied (pregnant women, sick people and the general population respectively) and the age differences of the study populations (average ages: 28.1, 30.2 and 47 years respectively). Studies on *T. gondii* infection in farm animals in Ghana have indicated seropositivity of 39% in pigs, 26.8% in goats, 33.2% in sheep, and 64% in chickens (Arko-Mensah et al., 2000; van der Puije et al., 2000; Dubey et al., 2008).

The infection rate in this current study was comparable to findings from Southern Brazil and some European countries. As high as 98% seroprevalence has been reported in southern Brazil (Nussenblatt & Belfort, 1994). A seroprevalence of 84% was also found among a lower socioeconomic population in Brazil (Bahia-Oliveira et al., 2003). Other higher seroprevalence data have been reported

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from northern Brazil (Coêlho, Kobayashi, & Carvalho, 2003; Cavalcante et al., 2006). Similar findings have also been reported in Holland and Germany (Kortbeek, 1999). The current finding is also similar to reported seroprevalence rates of 83.7% and 60% in two different studies from the East African country of Ethiopia (Zemene et al., 2012; Negash et al., 2008) and 66% to 88% from Togo in West Africa (Deniau et al., 1990). The finding is, however, higher compared to reported cases from other West African countries of 63.1% in Sao Tome (Fan et al., 2006); 32.4% and 23.9% in Nigeria (Ogoina et al., 2013; Kamani, Mani, Egwu & Kumshe, 2009), 25.4% in Burkina Faso (Millogo et al., 2000); 37.2, 55.6% and 70% in Ivory Coast (Dumas et al., 1989).

The current finding was also similar to a report of 89% among females from a northern Indian population (Khurana et al., 2010). The present finding was, however, lower than all other reported cases from Asian countries, ranging from 70% in Indonesia (Terazawa et al., 2003), 44.8% in Japan (Nissapatorn et al., 2004), 12.5% in China (Xiao et al., 2010), to 0.8% in Korea (Song et al., 2005). *T. gondii* seroprevalence in North America is reportedly low. Seroprevalence in the US is reported at 15.8% (Jones et al., 2007).

Differences in seroprevalence in the different geographic locations are associated with a number of factors that support the survival or otherwise of the parasite either as oocysts in the soil or tissue cysts in meat and meat products. It could depend on the climate, cultural and eating habits or observation/neglect of appropriate sanitary and hygienic practices of the particular population. The possible reason for the higher prevalence values in the current study is associated

with particiapants' exposure to the risk factors for the infection as discussed below.

Seroprevalence according to the Study Communities

Seropositivity was significantly different in the three study communities ($p < 0.001$). In many epidemiological studies, differences in *T. gondii* seropositivity between different communities in the same geographical locations have often been attributed to the rurality or otherwise of those communities. Rural communities have been associated with higher prevalence rates while suburban or cosmopolitan areas have often recorded lower rates. A study to evaluate *Toxoplasma gondii* infection among pregnant women in Northeast Iran found that living in a rural environment was highly associated with the infection ($p < 0.001$), with seropositivity in women living in urban and rural areas being 29.1 and 47.5%, respectively (Babaie et al., 2013). Similar findings have been reported in southern Brazil (Spalding, Amendoeira, Klein, & Ribeiro, 2005), Thailand (Nissapatorn, Suwanrath, Sawangjaroen, Ling & Chandeying, 2011), China (Liu et al., 2009), Mexican city (Alvarado-Esquivel et al., 2006) and Saudi Arabia (Mohammad, Amin, Balaha, & Moghannum, 2010).

The reason for associating the infection with rural dwellers has to do with their exposure to many risk factors such as contact with the soil, drinking from contaminated water sources, lower socioeconomic levels and abundance of roaming cats in the surroundings. In the present study, many of the risk factors associated with the infection were also associated with the rural communities (Jukwa and Moree) that had greater seroprevalence values. For instance, Jukwa

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which had the highest ODDs (OR = 4.0, p < 0.002) for seropositivity also had the highest ODDs (OR = 3.3, p < 0.001) for contact with the soil, followed by Moree (OR = 2.6, p = < 0.001). Again, the risk of having pond, well or river water as the source of drinking water was also associated with participants from Jukwa. The risk of engaging in farming and fishing or fish mongering was also associated with Jukwa and Moree respectively where all the fishermen and fish mongers came from Moree while almost all the farmers came from Jukwa.

Seroprevalence according to Gender

Consistent with many reported cases of *Toxoplasma gondii* infection, the current study found no significant association between gender and seropositivity. A study in Egypt among 250 human samples found no significant difference between males (34.7%) and females (35.8%) (*P*>0.05) (Aboelhadid, Abdel-Ghany, Ibrahim, & Mahran, 2013). Similar results have been reported in Ethiopia, Nigeria, and Tanzania (Negash et al., 2008; Kamani et al., 2009; Swai & Schoonman, 2009). However, a higher risk of infection for men than women was observed for the hospital-based study in Accra, Ghana (Ayeh-Kumi et al., 2010). Similar results have been found in France (Fromont, Riche, & Rabilloud, 2009) and Illinois (Weigel, Dubey, Dyer, & Siegel, 1999). The higher risk of *Toxoplasma* infection for men has been attributed either to contact with soil or to improper hygiene and thus a difference between men and women might appear in populations with high exposure to soil (Fromont et al., 2009). In the current study, however, both men and women were all at risk of being exposed to the soil as indicated by their professions (Table 6). Thus, fish mongering (for women),

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farming and fishing (for men) were the highest risk factors for the infection (Table 5). The Implication is that both men and women in this study had equal chance of being exposed to the soil as indicated by their respective professions.

Seroprevalence according to Age

The study found an increasing pattern of seropositivity with increasing age group which was also observed in Egypt where seropositivity increased from 11% in participants below 20 years to 42% in those above 50 years (Aboelhadid et al., 2013). Similarly, a study from Ethiopia found seropositivity of 64.0% in the 15-19 year group and 94.1% in the 30-35 year group (Zemene et al., 2012). Another report from Tanzania found that *T. gondii* infection increased by 1.4% with one year increase in age (Mwambe et al., 2013). From the city of Maiduguri, Nigeria, there was a positive correlation between the mean antibody titre and the age of the subjects, with seroprevalence being highest among the subjects aged 51-60 years and lowest in the subjects below 21 years (Kamani et al., 2009). The same trend was also observed in the Netherlands and Japan (UK Food Standards Agency, 2012), the Rio de Janeiro state, Brazil (Bahia-Oliveira et al., 2003), and the UK (Allain, Palmer & Pearson, 1998). In France, the estimated seroprevalence increased from about 2.5% at 7 years to about 50% at 40 years and at 95 years old, the estimated seroprevalence was about 75% (Fromont et al., 2009).

The increase in seropositivity with age may be attributed to the cumulative probability of contact with one of the several risk factors for the infection. For instance, since *T. gondii* infection is strongly associated with contact with soil, the longer one lives the more likely he/she will be exposed to the soil. It might also be

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attributed to the reason of decreased cell-mediated immunity with advanced age. Surprisingly, a study in a rural area of the Minas Gerais state of Brazil and another from Iran found no significant difference between seropositivity and age groups (Portela et al., 2004; Babaie et al., 2013). In both findings it was suggested that a significant proportion of the population acquired toxoplasmosis at an early age.

Seroprevalence according to Socioeconomic Status

There was an observed increasing trend of seropositivity in the lower socioeconomic group of the study participants. Such a trend was reported in the northern Rio de Janeiro state of Brazil, where the age-adjusted seroprevalences were 84%, 62% and 23% in the lower, middle and upper socioeconomic levels respectively (Bahia-Oliveira et al., 2003). In the United States, *Toxoplasma* infection was described as an infection associated with poverty (Jones et al., 2001; Munoz-Zanzi, Fry, Lesina, & Hill, 2010). It has been suggested that individuals of lower socioeconomic status may be related to occupations with greater soil exposure and therefore be at higher risk of being infected (Jones et al., 2001).

Seroprevalence according to Occupation

With respect to occupation, it was observed that jobs which tended to expose the individuals to the soil were strongly associated with the infection. Such an observation was also made in the US where soil-related occupations were associated with *T. gondii* seropositivity (Jones et al., 2001). Other reports have associated the infection with certain occupations. For instance, Swai and Schoonman (2009) reported from Tanzania that seroprevalence of *Toxoplasma*

antibodies was significantly higher amongst livestock keepers and abattoir workers. Again, in the Sao Tome study, children whose parents were non-skilled workers showed significantly higher seroprevalence than those of semi-skilled and skilled workers (Fan et al., 2006).

Fishermen after their fishing expedition normally sit at the sea shore to mend their nets, thereby exposing themselves to a greater contact with the soil. This might account for the observation that fishermen were more susceptible to *T. gondii* infection in the current study. Fish mongers were also at a high risk of being exposed to the soil as the analysis showed that fishing, farming and fish mongering were respectively the jobs that had the highest risk of exposing the individuals to the soil (Table 6).

Seroprevalence according to Level of Formal Education

In the current study, it was found that higher level of formal education was significantly associated with consistent reduction in the risk of infection. Lower levels of education have been associated with increased risk for toxoplasmosis in many epidemiological studies. In the US and Chile, for instance, two separate studies found that participants with lower than college education were significantly associated with *T. gondii* infection (Jones et al., 2001; Munoz-Zanzi et al., 2010). Lower levels of education are usually associated with lower socioeconomic status and may be related to employment in jobs with greater soil exposure (Jones et al., 2001).

Seroprevalence according to Meat Consumption

The present findings suggested that meat consumption was not a potential source of *Toxoplasma gondii* infection in the study population. There was no significant association between seropositivity and participants who responded "yes" to consumption of meat (p = 0.10) nor was there any significant association between seropositivity and any variables relating to meat consumption (Table 3). The reasons for this observation may be attributed to the exhaustive way of cooking meat by the participants. Nearly 94% of participants who reported to consume meat also reported that they always cooked the meat till at least it was soft before consumption. Cooking food to 67°C or higher is found to be sufficient enough to immediately kill tissue cysts (Dubey et al., 1990, 2000).

The absence of any factor concerning meat consumption may also reflect the fact that this source of infection has become less important than others. The advent of latest evolutions of the agricultural methods has brought changes in the risk of infection of domesticated animals (Fromont et al., 2009). For instance, reported seropositivity in slaughter pigs in most countries is now under 8%; thus, the importance of pork meat as source of infection is probably declining (Tenter et al., 2000). The frequent consumption of frozen meat may also lower the risk of infection through meat as most tissue cysts are killed at temperatures of -12°C or lower (Dubey, 2000). However, *Toxoplasma* tissue cysts contained in meat or meat-derived products have been shown to serve as important sources of infection for humans (Cook et al., 2000; Munoz-Zanzi et al., 2010; The European Food Safety Agency, 2007; Kapperud et al., 1996) and that the risk of acquiring the

infection via meat sources depends on cultural and eating habits in different human populations (Cook et al., 2000). Many studies around the world have demonstrated that over 50% of all human toxoplasmosis cases are related to cyst containing food sources (Munoz-Zanzi et al., 2010; Cook et al., 2000). Higher prevalences in France were attributed to the consumption of raw or undercooked lamb (Stephen & Richard, 2001). Therefore, the only reason why this study had no association with meat consumption could be the exhaustive way of cooking meat by the participants.

Risk Factors for Seropositivity in the Study

The risk factors associated with *Toxoplasma* infection point to the importance of oocysts in the transmission to humans and that up to 43% of toxoplasmosis infections in humans has been associated with direct or indirect exposure to oocyst contaminated environment (Muñoz-Zanzi et al., 2010). Soil contamination with sporulated *Toxoplasma* oocysts depends on the presence of cats roaming and defecating on the land and that many studies around the world have associated *T. gondii* infection with the cat (Robert-Gangneux & Darde, 2012). In the present study, there was a statistically significant association between *T. gondii* seropositivity and all variables assessing the presence of cats with a 2.3 to 38.4-fold increased risk of infection. Ayi et al. (2009) found exposure to cat faeces as the major risk factor for *T. gondii* infection in the study of pregnant women in Accra, Ghana. A study to assess seroprevalence and risk factors of *T. gondii* among pregnant women in Southwestern Ethiopia significantly associated having cats at home with *T. gondii* antibodies (Zemene et

al., 2012). Another study from Ethiopia observed that individuals with a known history of association with cats were 5.3 times more likely to be seropositive than those with no history of such association (Negash et al., 2008). In the Democratic Republic of São Tomé and Príncipe, children who had a history of raising cats showed significantly higher seroprevalence than those who did not (Fan et al., 2006).

On the contrary, a study among pregnant women in Iran found no association between contact with cats and *Toxoplasma* infection. The reason for this observation was attributed to the fact that few Iranians kept cats at homes and that about only 7% of the population studied had contacts with cats (Babaie et al., 2013). Similarly, a study from a Medical Centre in Kuala Lumpur, Malaysia did not find any significant association between seropositivity and contact with cats (Nissapatorn et al., 2003). The reason could be due to the fact that their study was conducted in an urban community.

Soil is often contaminated with the oocysts that are shed by infected cats, the definite host of *T. gondii*. As a result, individuals with increased soil contact have more chances to be infected (Petersen, Vesco, Villari & Buffolano, 2010). Like the current study, a serological screening and evaluation of exposure factors for *Toxoplasma gondii* transmission among 2126 pregnant women in southern Brazil found contact with soil to be the major factor for infection (Spalding et al., 2005). In Iran, soil contact was highly associated with the infection (p < 0.001) (Babaie et al., 2013). In contrast, some studies found no association between

Toxoplasma infection and contact with soil as reported by Nissapatorn et al. (2003) in Malaysia and Zemene et al. (2012) in Ethiopia.

The habit of dogs eating or rolling in cat excreta makes them possible carriers of *T. gondii* oocysts (Stephen & Richard, 2001). A study from Nigeria found that dog owners were twice as likely to be seropositive when compared with participants who did not own dogs (Kamani et al., 2009). On the contrary, the present study did not find any association between the presence of dogs and *T. gondii* seropositivity.

Contaminated water may act as vehicles for the transfer of oocysts to vegetables and fruits for human consumption. In areas where drinking water sources become contaminated or where humans use untreated surface water for consumption, oocysts transmitted through water may serve as important source of infection. A well documented outbreak of acute toxoplasmosis in humans occurred in 110 individuals in Vancouver, Canada, in 1995. Retrospective epidemiological studies indicated that the outbreak was caused by contamination of municipal drinking water with oocysts (Aramini, Stephen & Dubey, 1998; Aramini et al., 1999; Bell et al., 1995; Bowie et al., 1997). In Brazil, a study attributed the infection to the consumption of untreated water (Bahia-Oliveira et al., 2003). Waterborne transmission to humans was also identified in a study from Turkey (Ertug, Okyay, Tukmen & Yuksel, 2005). Similarly in this study, sources of drinking water were significantly associated with *T. gondii* infection where participants who reported to have relied on river water, well water, or even boreholes as drinking water sources recorded 100 per cent seropositivity as

opposed to seropositivity rates of 73.5% and 87.1% respectively for sachet/bottled water and pipe borne water sources.

T. gondii infection has also been associated with consumption of unwashed vegetables and fruits as reported in Canada by Chaudhry, Gad and Koren (2014) and among pregnant women in China (Liu et al., 2009). A case control study in France also identified eating unwashed vegetables or fruits as a risk factor for the infection (Baril et al., 1999). In another case-control study in Norway, eating unwashed raw vegetables or fruits was also associated with an increased risk of primary infection during pregnancy (Kapperud et al., 1996). The present study did not, however, find any significant association between seropositivity and consumption of unwashed vegetables or fruits. Similarly, a study among pregnant women from southern China found no statistically significant differences between participants who consumed unwashed raw vegetables or fruits and those who washed them (Duan et al., 2012).

Ocular Toxoplasmosis

This is a prevalence report of *Toxoplasma* ocular infection in a community-based epidemiological study delimited to the three communities. The findings, however, provide important information to the broader understanding of ocular toxoplasmosis.

Risk Factors for Ocular Toxoplasmosis in the Study Population

In this study, while seropositivity was significantly associated with the more rural communities, this was not the case with the development of ocular

lesions as there was no association between ocular cases and the rural dwellers. A study by Ferreira et al. (20014) in Brazil did not associate the development of ocular toxoplasmosis with environmental factors. This was similar to the present study where no variable assessing risk of infection was associated with the development of ocular infection (Tables 9 and 10).

The only risk factor for ocular toxoplasmosis in the current study was old age. Aging has been identified as the major risk factor for developing ocular toxoplasmosis (Holland, 2009). Similarly, Portela et al. (2004) found that the frequency of ocular toxoplasmosis increased significantly with advancing age, where almost 50% of individuals older than 60 years of age had ocular lesions. Bosch-Driessen et al. (2002) also found ocular involvement in *Toxoplasma* patients to associate with advanced age at onset. The reasons may be attributed to the possible decrease of cell-mediated immunity with advanced age (Bosch-Driessen et al., 2002; Johnson, Greven, Jaffe, Sudhalkar & Vine, 1997). The study by Ferreira et al. (2014) associated ocular disease with age and literacy.

Prevalence of Ocular Toxoplasmosis in the Study Population

The prevalence of ocular toxoplasmosis in the entire study population was 2.6% and 3% among the seropositive population. These findings were less than reported cases from Southern Brazil where ocular toxoplasmosis is endemic and seroprevalence is also high (Bahia-Oliveira et al., 2003). In the southern city of Erechim, Brazil, 17.7% of adults were found to have retinal lesions caused by toxoplasmosis (Glasner et al., 1992). Again, in Southeastern Brazil 80% prevalence of ocular toxoplasmosis was detected in a cohort study of newborns

with congenital toxoplasmosis (Vasconcelos-Santos et al., 2009). A more recent study in the city of Campos dos Goytacazes, located in the north of Rio de Janeiro, Brazil found a prevalence of ocular infection of 31.8% (Peixe et al., 2014).

The current finding is similar to reports from Northern Brazil where some studies found that between 3.4 and 10.8% of acutely infected adults had retinochoroidal lesions (Neves et al., 2009; Silva, Neves, Benchimol & Moraes, 2008). Another study from the Northeastern part of Brazil found the prevalence of ocular lesions in 1.2% of the study population (de Amorim-Garcia et al., 2004). The prevalence rate from this current study was higher than findings in Europe and North America where reported cases of ocular toxoplasmosis are much lower. In the US, for example, prevalence of ocular toxoplasmosis among the seropositive population was estimated at 2% (Holland, 2003). A study in Canada during an outbreak of toxoplasmosis through water contamination found that between 0.3 and 0.7% of infected individuals developed ocular toxoplasmosis (Burnett et al., 1998). An incidence rate of 0.4 per 100,000 was determined for patients born in Britain cumulating in a life time risk of 18 per 100,000 (Gilbert et al., 1999).

The higher prevalence rates and severe disease outcomes have been attributed to infection with more virulent parasite strains. Type II strains have been found predominantly responsible for human cases in France and North America (Howe et al., 1997; Howe & Sibley, 1995) while type I *Toxoplasma* and atypical strains appear to be responsible for the majority of ocular infections in

Brazil (Vallochi et al., 2005). Not enough information is, however, available on the genetic structure of parasite strains responsible for ocular cases in Africa and for Ghana as well. While some studies of animal isolates from Africa found a predominance of the archetypical clonal lineages (types I, II and III) as in Europe and North America (Velmurugan et al., 2008), a study of human samples from Tunisia suggested the predominance of recombinant strains (I/III and I/II) (Boughattas et al., 2010). A recent genotyping of *Toxoplasma gondii* samples from the West African city of Dakar found only recombinant strains I / III and I / II / III (Ndiaye et al., 2013).

In Ghana, the only study that has reported genotypes of *T. gondii* strains was done on isolates from chicken that suggested unique recombinant strains quite different from those from Brazil and Europe (Dubey et al., 2008). What seems clear from all these studies is the fact that parasite strains found in Africa seem quite distinct from those found in Southern Brazil. Thus, parasite strains responsible for ocular toxoplasmosis in the current study population may be those of less virulence. A further study to characterize parasite strains responsible for human systemic and ocular toxoplasmosis in this area will be interesting. The occurrence and severity of ocular disease may also be attributable to host genetic factors such as the possible occurrence of polymorphisms in the genes encoding IFN-γ and TNF-α in the human host (Albuquerque et al., 2009).

The finding in this study of 50% of eyes with ocular involvement being legally blind was quite high. Kadarisman et al. (1991) and Suhardjo and Agni, (2003) rather reported 20.1 and 13.9% % of blindness in Indonesia respectively.

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The finding in this study was due to the fact that majority (83%) of ocular lesions were located at the macular areas. Other studies have also reported several cases with macular involvement (Silveira et al., 2015; de-la-Torre et al., 2009; Kodjikian et al., 2006). This apparent propensity for macular involvement has been attributed to unique anatomic or immunologic factors in that microenvironment.

Unilateral ocular involvement in ocular toxoplasmosis has been reported to vary between 60% and 83% (Bosch-Driessen et al., 2002), consistent with the 80% found in the current study. Interestingly, some studies from Brazil and Indonesia found as high as 75.8% and 50% of bilateral cases respectively (Melamed et al., 2010; Kadarisman et al., 1991). Bilateral involvement has been associated with congenital transmission and highly divergent, non-archetypal and more virulent *T. gondii* strains (Bottós et al., 2009; de-la-Torre et al., 2009).

In terms of public health implications, the current study provides significant findings about the disease in this population. The blinding potential of the infection was enormous, occurring in 50% episodes and rendering over 80% of infected eyes visually impaired. The two bilateral ocular cases had sufficient visual impairment to disqualify the individuals from obtaining driving license. The burden of disease related to ocular toxoplasmosis is indeed considerable. In terms of Disability Adjusted Life Years (DALY), *Toxoplasma* infection was adjudged to cause the highest disease burden amongst food borne pathogens (The European Food Safety Agency, 2007).

In the case of congenital disease, retinochoroiditis has been found to account for 35% of the burden, second only to stillbirth, and in the case of acquired infection retinochoroiditis accounted for almost the entire burden (Havelaar et al., 2007). Bosch-Driessen et al. (2002) reported vision loss in at least one eye occurring in 24% of patients with ocular toxoplasmosis. A British survey of visual impairment in congenitally infected children reported that 17% presented at least one retinal lesion and 9% suffered from severe bilateral impairment (Tan et al., 2007). There is the need for public health strategies in terms of early detection and treatment as well as routine serological screening of all pregnant women as practiced elsewhere (Roberts et al., 1994).

IFN-γ Gene Polymorphism in the Study Population

Similar to this study which found the normal genotype (AA) as the predominant group (75.2%), a report by Rekha, Ishaq and Valluri (2006) in India, also observed that the AA genotype was the majority (46.2%), followed by the AT genotype (35%), and TT genotype (18.8%). In contrast to the current finding, other studies have described a different profile of genotype distribution in different populations. For instance, the mutant genotypes were found to be the predominant group in Brazilian populations. Albuquerque et al. (2009) reported a profile of 27% of the normal AA genotype, 63.7% of the heterozygous mutant (AT) genotype and 8.9% of the homozygous mutant (TT) genotype. Neves et al. (2012) reported a similar profile of 40% of the AA genotype, 42.5% of the AT genotype and 17.5% of the TT genotype among a population of Rio de Janeiro, Brazil. Two other studies from Brazil found similar patterns of genotype

frequency distribution (Amim et al., 2008; Visentainer et al., 2005). A study in Egypt by Hussein et al. (2009), however, found the homozygous mutant genotype (TT) as the predominant group (53%) followed by the AT genotype (33%) and the AA genotype (14%).

IFN-γ +874T/A polymorphism has been shown to associate with several diseases. For instance, the A/A genotype was found to be associated with tuberculosis in Iran (Hashemi et al., 2011), Brazil (Amim et al., 2008) and Spain (López-Maderuelo et al., 2003); hepatitis B in China (Yu et al., 2006); *Helicobacter pylori* gastritis in Italy (Zambon et al., 2005); type 2 diabetes mellitus in Greece (Tsiavou et al., 2005); Wegener's granulomatosis in Germany (Spriewald et al., 2005); atopic patients in Egypt (Hussein et al., 2009). Similarly, the A/T and T/T genotypes were found to associate with breast cancer in Iran (Kamali-Sarvestani, Merat & Talei, 2005), hepatitis C in Taiwan (Dai et al., 2006), and Hashimoto's disease in Japan (Ito, Watanabe, Okuda, Watanabe & Iwatani, 2006).

The current findings do not seem to corroborate with the only two earlier studies that have investigated the associations between IFNγ +874 T/A polymorphism and the occurrence of ocular lesions caused by *Toxoplasma gondii*. These studies which were all conducted in Brazilian populations suggested that individuals with the A allele in its homozygous form had the tendency to develop ocular lesions. The current study rather suggests that the presence of the T allele either in the homozygous (TT) or heterozygous (AT) forms increases the risk of developing ocular lesions in a 3.4- and 1.6 –folds respectively.

The polymorphism at the IFN-γ +874T/A position has been demonstrated to associate with different levels of IFN-γ production. The T/T genotype is known to associate with high levels of IFN-γ production, the A/T with medium levels and the A/A with lower levels (López-Maderuelo et al., 2003; Daí et al., 2006; Henao, Montes, París & García, 2006). The finding that the TT genotype (higher IFN-γ production) was associated with susceptibility to developing ocular lesions may be related to an exaggerated inflammatory response following the increased cytokine production. An excessive inflammatory reaction may consequently seek to breach the immune privilege status of the eye and result in retinochoroidal destruction. Indeed, this was reported by Neves et al. (2012) regarding the scale of morbidity where the presence of the A allele seemed to confer protection against the development of clinical symptoms, although this did not result in ocular lesions. The physiopathological mechanisms that underlie retinal damage in ocular toxoplasmosis are yet to be fully understood as the role of proinflammatory and immunoregulatory factors remain unclear (Gaddi & Yap, 2007). Lahmar et al. (2009) have reported increased levels of proinflammatory cytokines including IFN-γ and particularly IL-12 in patients with ocular toxoplasmosis than in other ocular diseases. Again, expression of IFN-γ was found to be associated with the development of severe intraocular inflammatory disease in transgenic mice (Geiger et al., 1994). Similarly, Stanford et al. (2005) reported an association between high IFN-γ production and the outcome of ocular disease where the presence of the T allele in either the heterozygous or homozygous forms were significantly higher in cases compared to controls.

The differences in IFN-γ genotype distribution profile in different populations may also determine which allele is associated with susceptibility or otherwise to developing ocular lesions. A careful observation of the pattern of genotype distribution seems to suggest the role of natural selection in relation to the T allele being associated with increased risk in the current study as against the AA genotype in the Brazilian studies. The genotypes occurring in fewer proportions seem to be associated with susceptibility to developing ocular lesions. That is, 24.8% of (AT + TT) in this Ghanaian population and 27% of AA genotype in a Brazilian population (Albuquerque et al., 2009). Neves et al. (2012) also reported a 40% of the AA genotype in another Brazilian population. For instance, if the AA homozygous individuals (75.2%) were to have an increased risk of developing ocular lesions in the current study, the implication would be that the prevalence of *T. gondii* retinochoroiditis in the population would be higher than the current state of 2.6%. Conversely, if the T allele was to be associated with susceptibility to ocular infection in the Brazilian populations (73 and 60% respectively), we would expect a higher prevalence of retinochoroiditis in those populations than is currently reported (31.8 and 5.8% respectively).

TNF-α Gene Polymorphism in the Study Population

Like the current study, many studies have shown that at the TNF alpha −308 polymorphic site, the GG genotype is the most frequent while the AA genotype is the least common in all populations studied. Cordeiro et al. (2008a) reported a profile of TNF alpha −308G/A genotype distribution among a Brazilian population where the GG genotype were the majority (79%) followed

by the AG genotype (18%) and then the AA genotype (3%) similar to the finding of 91.5%, 7.7% and 0.8% respectively in the current study. In a Japanese population, genotype distribution pattern of 57.7% for the wild type, 42.3% for heterozygotes and 0% for homozygous mutant has been reported (Pujhari, Ratho, Prabhakar, Mishra & Modi, 2012). Mishra et al. (2015) also reported a profile of 71.4%, 28.6% and 0% respectively among an Indian population. Similarly in a Ugandan population, the distribution was 84.1% for normal, 15.9% for heterozygous individuals and none for the AA genotype (Parikh, Dorsey & Rosenthal, 2004).

Although the distribution of genotypes and alleles between cases and controls did not attain statistical significance, the presence of the A allele gave lower ODDs Ratios and indicated protective against the development of ocular infection in the present study. The presence of the less common TNF−308A allelic form has been found to correlate with the binding of transcription factor, either by increasing promoter activity or by inhibiting the repressor of transcription and thereby increasing the production of TNF (Wilson et al., 1997; Mira et al., 1999). In contrast to the present study, the only previous study that sought to investigate the association between TNF-α-308 polymorphism and the development of toxoplasmic retinochoroiditis did not find any association (Cordeiro et al., 2008a). Their finding was unexpected as the role of TNF-α in the pathophysiology of ocular toxoplasmosis is very relevant. For instance, pretreatment of rat retinal vascular endothelial cells with TNF-α was found to inhibit the growth of *T. gondii* tachyzoites within these cells. This cytokine

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appeared to restrict parasite replication by starving the parasites of the amino acid L-tryptophan (Brunton, Wallace, Graham, & Stanford, 2000). In a murine model, TNF- α was also found to induce the release of IFN-γ by NK cells leading to the production of nitric oxide by macrophages, resulting in the destruction of *T. gondii* parasites (Mayer & Hemmens, 1997).

Several studies have associated the A allele with a protective effect against many diseases including infectious and non infectious uveitis (intraocular inflammation). From Australia, El-Shabrawi et al. (2006) observed that the A allele frequency at position -308 of the TNF- α gene in patients suffering from a Human Leukocyte Antigen (HLA)-B27–associated uveitis was significantly lower than those in HLA-B27–negative control subjects. Kuo et al. (2005) also described a reduced A allele frequency (6.6%) at position -308 of the TNF-α gene in their HLA-B27–positive uveitis patients in the UK. A similar protective outcome with the minor A allele was associated with the development of ankylosing spondylitis in a population of Taiwan (Shiau et al., 2007). Contrary to the current finding, Sen et al. (2011) found a significant association of the A allele and the AA genotype with the occurrence of Eales' disease. Their explanation for this observation was that high expression of TNF- α could be responsible for the inflammation-associated angiogenesis in the proliferative stage of Eales' disease thus influencing susceptibility of the individual to the disease. The A allele was also associated with susceptibility to a non-infectious intermediate uveitis in the UK (Atan et al., 2013).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary of Findings

This was a community-based cross-sectional/case-control study to investigate the epidemiology of systemic and ocular toxoplasmosis and their associations with human gene polymorphisms among the population of the Central Region of Ghana. The overall seroprevalence of *Toxoplasma gondii* in the study population was 85%. According to the study sites, Jukwa recorded the highest seropositivity of 92% followed by Moree (89%) and Nkanfoa (75%). *Toxoplasma* seropositivity had a significant relationship with being exposed to the soil, occupation, age, sources of drinking water, level of formal education, and socioeconomic status. There was also a statistically significant association between *T. gondii* seropositivity and all variables assessing the presence of cats with a 2.3 to 38.4-fold increased risk of infection. However, there was no association between seropositivity and gender, meat consumption, and washing of hands or washing of vegetables/fruits before consumption. According to occupations, seropositivity was respectively highest among fishermen, farmers, and fish mongers.

The prevalence of ocular toxoplasmosis among the seropositive population was 3%. Individuals who developed ocular lesions had a significantly higher mean age than those without ocular lesions. Unilateral ocular involvement occurred in 80% of all ocular infections. Low vision occurred in 83% eyes with toxoplasmic ocular lesions while 50% eyes were legally blind. Regarding causes of visual impairment *Toxoplasma* retinochoroiditis accounted for 5.81

In terms of interferon gamma polymorphism, 75.2% of the participants were homozygous for the normal AA genotype, 21.4% were A/T heterozygous while 3.4% were T/T homozygous. The T/T homozygous individuals had a 3.4-fold increased risk when compared to the A/A homozygotes though no statistically significant difference was observed in the distribution of genotypes between the cases and controls.

For Tumour necrotic factor alpha gene polymorphism at position 308, the distribution of the wild type (GG genotype), the heterozygous mutant (GA) and homozygous mutant (AA) among cases and controls was respectively 91.5%, 7.7% and 0.8%. Both GA heterozygous and AA homozygous individuals had lower ODDs Ratios when compared with the GG homozygotes.

Conclusions

T. gondii seroprevalence in the current study was high and comparable to other findings in Africa, some earlier findings in Europe, and in Brazilian populations. The major risk factors for *T. gondii* seropositivity were rural dwelling, having contact with the soil, old age, having basic school or lower,

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lower socioeconomic status, and having cats in the surroundings. Occupations that had higher risks were farming, fishing, and fish mongering, suggesting that the seashore could be a good ground for sporulation and survival of *Toxoplasma* oocysts. The findings also suggested that meat consumption was not a potential source of *Toxoplasma gondii* infection in the study population.

The prevalence of ocular toxoplasmosis in this Ghanaian study population was lower than findings from Southern Brazil, where high seropositivity is also associated with high ocular infection. The major risk factor associated with the development of toxoplasmic ocular lesions was old age.

Toxoplasma retinochoroiditis contributed to 5.8% of visual impairment in the entire study population. Blindness occurred in 50% episodes of eye infections while over 80% of infected eyes were rendered visually impaired.

The results suggested that the presence of the IFN-γ +874T allele either in the homozygous (TT) or heterozygous (AT) forms may increase the risk of developing ocular lesions. The idea of natural selection seemed to play a role in determining which genotype or allele is associated with susceptibility or otherwise of developing ocular lesions. Also, the presence of the less common TNF$-$308A allelic form was found protective against the development of ocular infection in the present study.

Recommendations

The higher prevalence rates of both systemic and ocular toxoplasmosis in this study call for public health strategies in terms of early detection and treatment

as well as routine serological screening of all pregnant women as practiced in many countries. Individuals at increased risk should be educated to observe good hygienic practices in their everyday activities. A national prevalence study is needed to inform a stronger public health policy. The revelation that fishers and fish mongers were at increased risk of being infected warrants further studies to verify the viability of the parasites at the seashore. Investigations at other polymorphic loci of IFN-γ and TNF-α is recommended, considering the roles of these cytokines in the pathogenesis of ocular toxoplasmosis and the fact that the associations were not significant. A further study to characterize parasite strains responsible for human systemic and ocular toxoplasmosis in this area is also recommended.

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APPENDICES

APPENDIX – A: ETHICAL CLEARANCE CERTIFICATE

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: nanatuesdaykad@yahoo.com

My Ref. :GHS-ERC: 3
Your Ref. No.

February 28, 2013

EMMANUEL KWASI ABU, Principal Investigator
University of Cape Coast
School of Biological Sciences
Department of Human Biology

ETHICAL CLEARANCE - ID NO: GHS-ERC: 21/11/12

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Toxoplasmosis and its association with IFN-γ +874 polymorphism among coastal and inland farming communities in the central Region, Ghana"

This approval requires that you submit an Inception and Mid-term reports of the study to the Ethical Review Committee (ERC) for continuous review. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol

SIGNED...
PROFESSOR FRED BINKA
(GHS-ERC CHAIRMAN)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

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VOLUNTEER AGREEMENT FORM

The above document describing the benefits, risks and procedures for the research title "Epidemiological study of systemic and ocular toxoplasmosis and their associations with human cytokine gene polymorphisms: a community-based survey of the central region, Ghana" has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____   _____

Date   Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____   _____

Date   Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____   _____

Date   Name and Signature of Person Who Obtained Consent

APPENDIX– C

PREPARATION OF REAGENTS

Preparation of 0.5M EDTA solution at pH 8.0

1. Weigh 186.1g disodium ethylenediamine tetraacetate•2H$_2$O into 800 ml of deionised water.

2. Stir the solution vigorously using a magnetic stirrer.

3. Add NaOH solution to adjust the pH to 8.0.

4. Adjust the volume to 1L with deionised water.

5. Filter the solution through a 0.5 micron filter.

6. Sterilise solution in an autoclave.

7. Store at room temperature for further use.

Preparation of 1 litre of 10X TAE Running Buffer, pH 8

1. Dissolve 48.5g Tris (hydroxymethyl amino methane) in about 800 mL of deionised water.

2. Add 11.4 mL glacial acetic acid and 2 mL 0.5M EDTA

3. Add deionised water to 1L.

4. Store at room temperature.

5. Dilute stock solution 10:1 to make a 1X working solution.

LABORATORY PHOTOS GALLERY
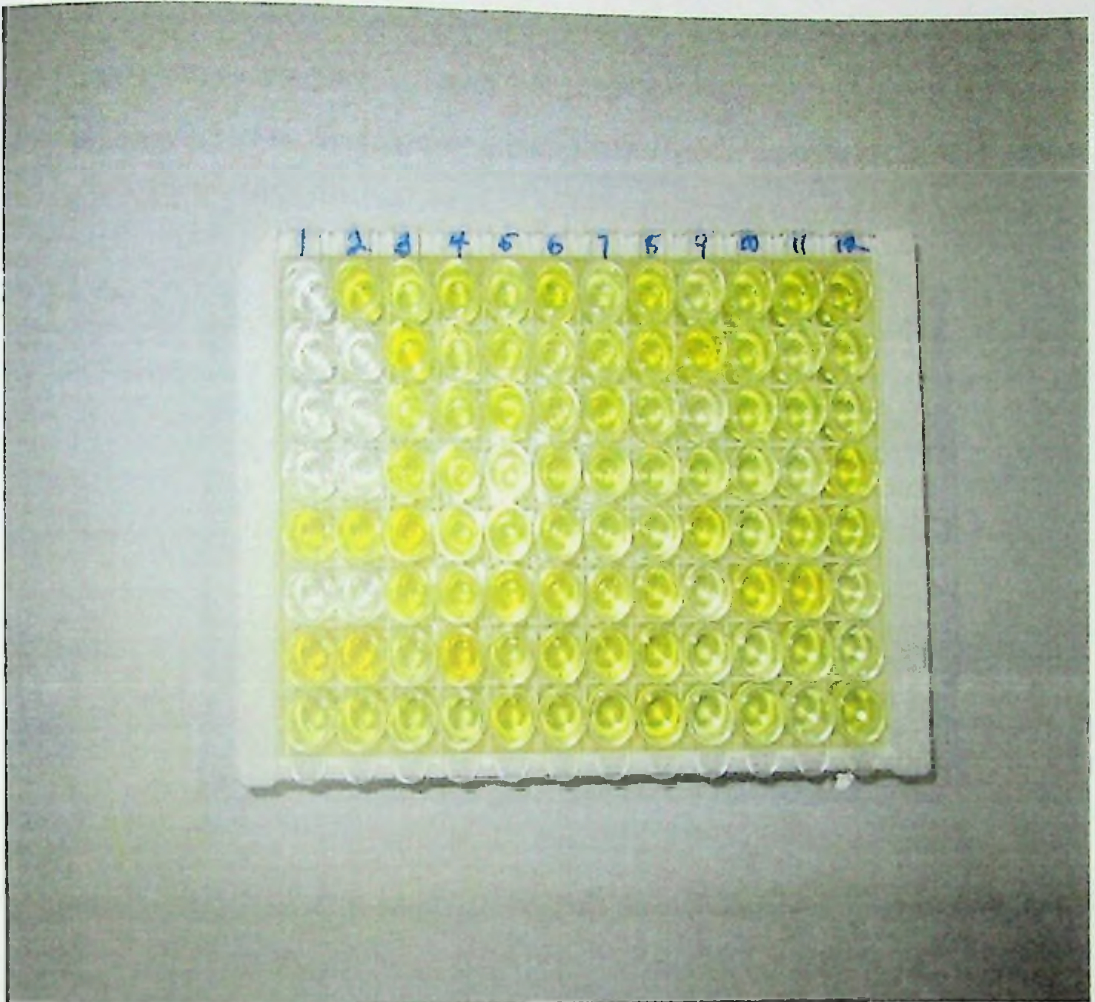
Plate A: IgM microtitre ELISA plate showing test results

Columns 1 and 2 contain cut-off calibrator, positive and negative controls, and blank wells. The rest of the wells contain test samples from study subjects.

Plate B: IgG microtitre ELISA plate showing test results

Columns 1 and 2 contain cut-off calibrators, positive and negative controls, and blank wells. The rest of the wells contain test samples from study subjects.
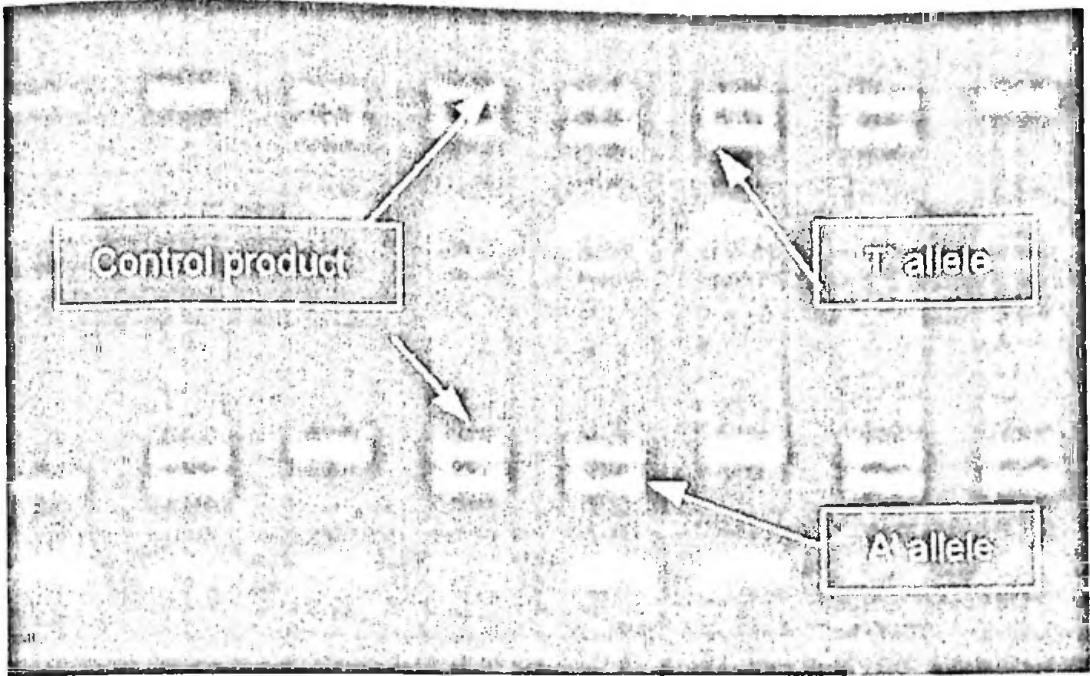
Plate C: Ethidium bromide-stained agarose gel showing the three genotypes of IFN-γ (+874) polymorphism by ARMS-PCR. The upper lane shows products from the T allele specific primer pair and the lower lane products from the A allele specific primer pair.

GG GG GG GA GG GA GG GG AA GG GG GG AA
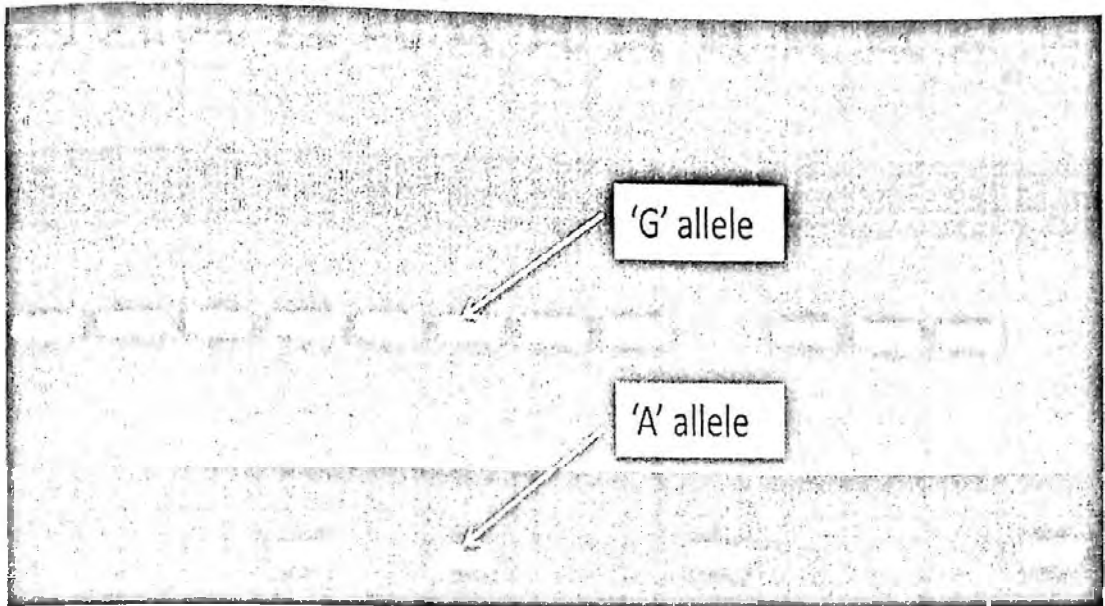
Plate D: A representative ethidium bromide-stained agarose gel showing the three genotypes of the TNF-*a* (-308) polymorphism by ARMS-PCR. The upper lane shows products from the G allele specific primer pair and the lower lane products from the A allele specific primer pair.
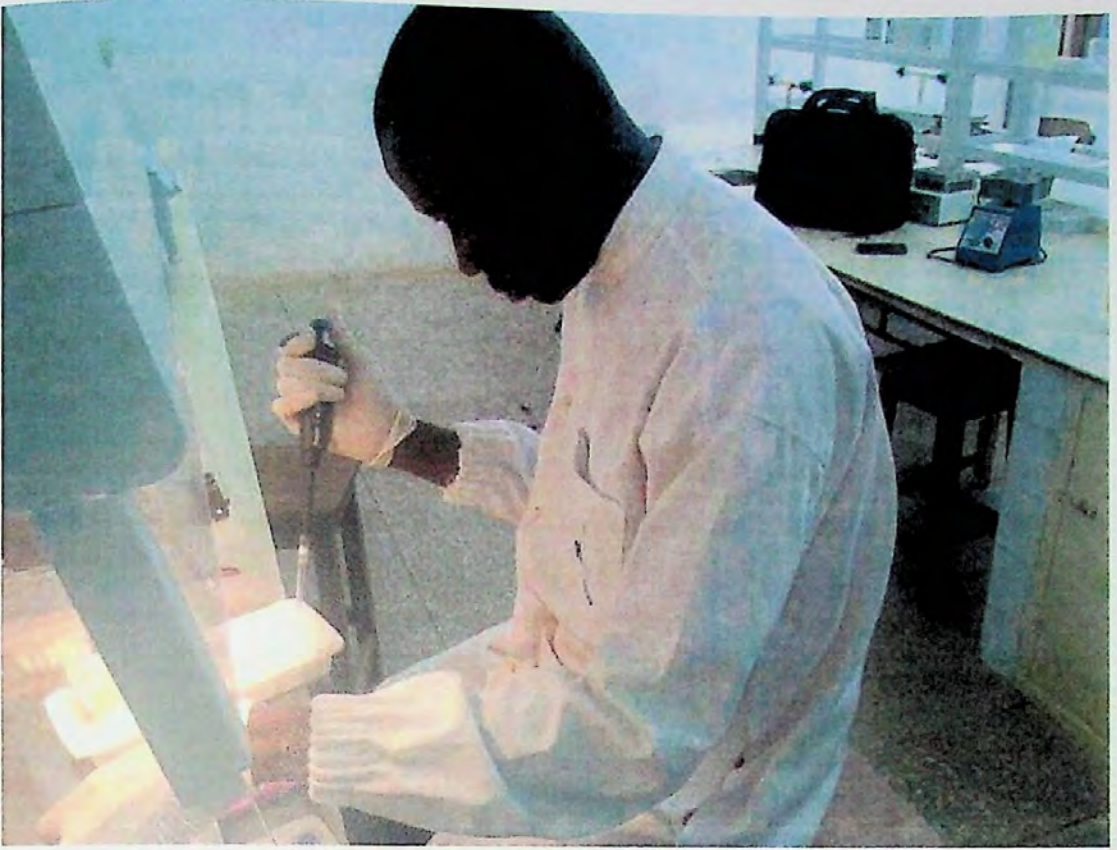
Plate E: Preparing samples and reagents for PCR procedures.

STATISTICAL ANALYSIS OUTPUT

Chi-Square Test for significance association between *T.gondii* seropositivity and the study communities

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	31.335[a]	6	.000
Likelihood Ratio	32.789	6	.000
Linear-by-Linear Association	19.643	1	.000
N of Valid Cases	390		

Chi-Square Test for significance association between *T.gondii* seropositivity and age groups of participants

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	69.561[a]	6	.000
Likelihood Ratio	67.584	6	.000
Linear-by-Linear Association	59.678	1	.000
N of Valid Cases	390		

Chi-Square Test for significance association between *T.gondii* seropositivity and formal education of participants

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	33.822[a]	3	.000
Likelihood Ratio	35.353	3	.000
Linear-by-Linear Association	32.546	1	.000
N of Valid Cases	390		

Chi-Square Test for significance association between *T.gondii* seropositivity and occupation of participants

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	51.521[a]	7	.000
Likelihood Ratio	48.117	7	.000
Linear-by-Linear Association	9.145	1	.002
N of Valid Cases	390		

Chi-Square Test for significance association between *T.gondii* seropositivity and socioeconomic status of participants

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	26.473[a]	2	.000
Likelihood Ratio	21.159	2	.000
Linear-by-Linear Association	26.177	1	.000
N of Valid Cases	390		

Chi-Square Test for significance association between *T.gondii* seropositivity and sources of drinking water

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.782[a]	4	.019
Likelihood Ratio	13.479	4	.009
Linear-by-Linear Association	9.759	1	.002
N of Valid Cases	390		

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Chi-Square Test for significance association between contact with soil and

T.gondii seropositivity

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	93.585[a]	1	.000		
Continuity Correction[b]	90.741	1	.000		
Likelihood Ratio	99.716	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	93.345	1	.000		
N of Valid Cases[b]	390				

Chi-Square Test for significance association between owning cats and *T.gondii*

seropositivity

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	19.839[a]	1	.000		
Continuity Correction[b]	18.500	1	.000		
Likelihood Ratio	24.611	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	19.789	1	.000		
N of Valid Cases[b]	390				

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Chi-Square Test for significance association between disposing cat litter and *T.gondii* seropositivity

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	26.613[a]	1	.000		
Continuity Correction[b]	25.143	1	.000		
Likelihood Ratio	30.275	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	26.545	1	.000		
N of Valid Cases[b]	390				

Chi-Square Test for significance association between gender and *T.gondii* seropositivity

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.151[a]	1	.697		
Continuity Correction[b]	.054	1	.816		
Likelihood Ratio	.153	1	.696		
Fisher's Exact Test				.757	.413
Linear-by-Linear Association	.151	1	.698		
N of Valid Cases[b]	390				

T-test to compare mean ages of participants with ocular lesions and those without

	Levene's Test for Equality of Variances		t-test for Equality of Means							
									95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	3.068	.081	2.213	388	.028	14.355	6.488	1.600	27.111
Equal variances not assumed			3.171	10.042	.010	14.355	4.527	4.274	24.436

Chi-Square Test for significance association between ocular lesions and socioeconomic status

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	2.572[a]	2	.276	.266		
Likelihood Ratio	2.193	2	.334	.461		
Fisher's Exact Test	2.902			.266		
Linear-by-Linear Association	1.126[b]	1	.289	.425	.229	.144
N of Valid Cases	390					

Chi-Square Test for significance association between ocular lesions and study communities

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	3.119[a]	2	.210	.199		
Likelihood Ratio	3.279	2	.194	.217		
Fisher's Exact Test	3.395			.199		
Linear-by-Linear Association	3.002[b]	1	.083	.108	.066	.037
N of Valid Cases	390					

Logistic regression analysis of INF-gamma genotype distribution between cases and controls

INFG[a]	B	Std. Error	Wald	df	Sig.	Exp(B)	95% Confidence Interval for Exp(B) Lower Bound	Upper Bound
AT Intercept	-1.386	.271	26.137	1	.000			
[Case=1.00]	.470	.499	.889	1	.346	1.600	.602	4.251
[Case=2.00]	0[b]	.	.	0	.	.	.	.
TT Intercept	-3.526	.717	24.160	1	.000			
[Case=1.00]	1.224	1.032	1.407	1	.236	3.400	.450	25.691
[Case=2.00]	0[b]	.	.	0	.	.	.	.

a. The reference category is: AA.

b. This parameter is set to zero because it is redundant.

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ORIGINAL ARTICLE

Associations between Polymorphisms within Interferon Gamma and Tumor Necrosis Factor Genes and *Toxoplasma* Retinochoroiditis in Ghanaian Patients

Emmanuel Kwasi Abu, OD[1,2], Johnson N. Boampong, PhD[2], Samuel Kyei, OD[1], Richmond Afoakwah, PhD[2], and Irene Ayi, PhD[3]

[1]Department of Optometry, School of Allied Health Sciences, University of Cape Coast, Ghana, [2]Department of Biomedical and Forensic Sciences, School of Allied Health Sciences, University of Cape Coast, Ghana, and [3]Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Ghana

ABSTRACT

Purpose: To evaluate associations between IFN-γ +874T/A and TNF-α-308G/A polymorphism with the development of *Toxoplasma* retinochoroiditis.

Methods: By ARMS–PCR, a cross-sectional genetic study involving 30 patients with *Toxoplasma* retinochoroiditis and 87 controls was carried out.

Results: IFN-γ +874: by comparing with the AA genotype, individuals with the TT genotype had a 3.4 odds ratio (OR); AT had a 1.6 OR; and the T allele had a higher OR (1.6), indicating a likely susceptibility of IFN-γ +874T to the infection, though the overall distribution was not significant ($p = 0.259$). For TNF-α-308G/A, individuals with both GA and AA genotypes had lower ORs when compared with the GG genotype, implying the A allele could confer protection against *Toxoplasma* ocular lesions.

Conclusions: IFN-γ +874T allele may increase the risk of ocular lesions in *Toxoplasma* infection. The principle of natural selection seems to also play a role. The less common TNF-308A allelic form could be protective against the development of *Toxoplasma* ocular infection.

Keywords: Genes, Ghana, interferon gamma, retinochoroiditis, *Toxoplasma*, tumor necrosis factor

Ocular toxoplasmosis (OT) is a major cause of posterior uveitis and visual impairment in many parts of the world.[1] There is a disproportionate occurrence of ocular toxoplasmosis in different populations with comparable seroprevalence findings. The outcome of OT depends on the interaction of many factors, including functions of the host immune system, as well as host and parasite genetic factors.[2] Immune reactions to *Toxoplasma gondii* infection are associated with a strong T-helper 1 response, though a study among Colombian OT patients showed a T-helper 2 dominant immune response to SAG1 and ROP18 peptides.[3,4] Susceptibility to the development of ocular lesions seems to be associated with the production of proinflammatory cytokines, including interferon

gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) that contribute to the inflammatory responses responsible for damaging the choroid and retina.[4] Functional polymorphisms in the promoter regions of TNF-α and IFN-γ genes regulate their production levels and the outcome of infections.[5–9] Thus, a T to A single nucleotide polymorphism (SNP) of IFN-γ at +874 and a G to A SNP of TNF-α at position -308, have been implicated in many disease outcomes.[10–13] Associations between these gene polymorphisms and the outcome of OT have, however, been contradictory and inconclusive. While previous studies in Brazilian patients did not associate TNF-α-308G/A polymorphism with the outcome of *Toxoplasma* retinochoroiditis,[14] the IFN-γ +874T allelic form seemed

to confer some protection against the disease.[15,16] A closer observation also gives a trend of diverse genotypic distribution profile of these cytokine gene polymorphisms (particularly of IFN-γ +874T/A) in different populations. This could also be a determining factor to the disease outcome in such populations. The aim of the current study was to evaluate the distribution of IFN-γ +874T/A and TNF-α-308G/A genotypes and their associations with the occurrence of *Toxoplasma* retinochoroiditis in patients from Ghana, West Africa.

MATERIALS AND METHODS

Sample Size Determination

A sample size of 39 was estimated, based on the expression $N = Z^2 (1 - p)(p)/b2$, where

N = estimated sample size,
Z = the standard score at 95% confidence interval (1·96),
b = desired error bound taken as 5% and
p = prevalence of ocular toxoplasmosis in this population being 2.6%[17]

Study Participants

The study included 30 patients with *Toxoplasma* retinochoroiditis (ocular group) and 87 individuals without ocular lesions (control group). The ocular group consisted of 20 consecutive patients with *Toxoplasma* retinochoroiditis, who visited the University of Cape Coast Eye Unit, plus 10 patients from an earlier population-based survey, all in the Central Region of Ghana.[17] The control group comprised 87 healthy individuals matched by age and sex, who were positive for anti-*Toxoplasma* IgG antibodies but had no ocular lesions and no history of uveitis. The study was conducted in accordance with the Helsinki Declaration on research regarding human subjects. The protocol for this study was reviewed and approved by the Ghana Health Services' Ethical Review Committee (ID: GHSERC: 21/11/12). Participants signed consent forms after the study protocol had been explained to them.

Ophthalmic examination was conducted by trained ophthalmologists and optometrists. The examination included slit-lamp biomicroscopy and dilated funduscopy by binocular indirect ophthalmoscopy. Fundus photographs were taken for cases of retinochoroiditis. Clinical diagnosis of OT was based on characteristic ocular lesions consistent with *Toxoplasma* retinochoroiditis, in addition to a positive serologic result and in the absence of other identifiable ocular morbidities.

The criterion for positive serologic result was a positive test for any of the two anti-*Toxoplasma* IgG or IgM antibodies, or a combination of both. Serologic results were obtained using the commercial ELISA test kit (VEDALAB, Alencon, France), following the manufacturer's instructions.

DNA Extraction

Genomic DNA was extracted from whole blood using DNA blood mini kit (QIAgen, Hilden, North Rhine-Westphalia, Germany) by strictly following the manufacturer's protocol.

IFN-γ +874T/A Polymorphism Determination

The IFN-γ +874T/A polymorphism was determined using an amplification refractory mutation system by polymerase chain reaction (ARMS-PCR), as described previously.[15,18] The ARMS-PCR was carried out in a total volume of 25 µL containing 12.5 µL of GoTaq® Green Master Mix (Promega Corporation, Fitchburg, WI), 5 µM of generic primer (100 pmol/µL; 5′ - TCA ACA AAG CTG ATA CTC CA -3′), 5 µM of specific A primer (100 pmol/µL, 5′ - TTC TTA CAA CAC AAA ATC AAA TCA -3′), or 5 µM of specific T primer (100 pmol/µL, 5′ - TTC TTA CAA CAC AAA ATC AAA TCT -3′); 0.5 µM of internal control 1 (10 pmol/µL, 5′ - GCC TTC CCA ACC ATT CCC TTA -3′); 0.5 µM of internal control 2 (10 pmol/µL, 5′ - TCA CGG ATT TCT GTT GTG TTTC -3′); 3 µL of genomic DNA, and nuclease free water (Fitchburg, WI, USA). ARMS-PCR was performed in a thermocycler (GeneAmp® PCR System 9700, USA) consisting of an initial denaturation step (95°C for 3 min); 10 cycles of 95°C (15 s), 65°C (50 s), and 72°C (40 s), followed by 20 cycles of 95°C (20 s), 55°C (50 s), and 72°C (50 s), with a final extension time of 7 min at 72°C.

TNF-α-308G/A Polymorphism Determination

The polymorphism for TNF-α-308G/A was also determined using ARMS-PCR as described by Gupta and Sehajpal.[19] The optimized reaction conditions consisted of 3 µL of genomic DNA in a total volume of 25 µL of reaction mixture containing 0.16 µM of each primer, 12.5 µL of GoTaq® Green Master Mix (Promega). The reaction was amplified for 35 cycles, each cycle consisted of denaturation at 94°C for 30 s, annealing at 57°C for 20 s, extension at 72°C for 20 s, and finally a 3 min extension at 72°C.

All PCR products were analyzed in 2% agarose gel electrophoresis stained with ethidium bromide and visualized on UV transillumination.

Data Analysis

All the data obtained were analyzed using the Statistical Package for Social Sciences (version 16; SPSS Inc, Chicago, IL). Allele and genotype frequencies were compared between cases and controls using multivariate logistic regression analysis (with 95% confidence intervals, CI). Hardy–Weinberg equilibrium was tested using the control group by comparing the observed values with expected values. A *p* value ≤0.05 was considered statistically significant. The Fisher's exact test was used when any expected frequency was <5.

RESULTS

Genotype frequencies of the polymorphisms studied were in Hardy–Weinberg equilibrium (IFN-γ: $\chi^2 = 0.55$, $p = 0.46$; TNF-α: $\chi^2 = 199$, $p = 0.58$).

IFN-γ Gene Polymorphism

All 117 individuals (30 cases and 87 controls) were tested by ARMS–PCR at the IFN-γ +874T/A polymorphic site. In total, 88 individuals (75.2%) showed AA homozygosity, 25 (21.4%) were AT heterozygous, while four (3.4%) were TT homozygous. Genotype frequency analysis showed that AA genotypes were the majority in both the ocular and the control groups [20 (67%) and 68 (78%), respectively)]. There was a similar distribution of the AT genotypes between cases and controls (27 and 20%, respectively). By logistic regression analysis, the TT homozygous individuals had ORs of 3.4 when compared with the AA genotype, though the overall distribution was not statistically significant (Fisher's exact test value = 2.46, *p* = 0.259). Similarly, the AT heterozygous individuals had 1.6 OR when compared with the AA genotype. Analysis of allele carriage indicated that the presence of a T allele in either the homozygous or the heterozygous forms gave higher ORs,

indicating a likelihood of developing toxoplasmic ocular lesions. There was no significant difference in the distribution of alleles between cases and controls (*p* = 0.18) though the T allele had a higher OR, also signifying susceptibility to the infection. The lack of significant association could be due to the small number of cases (as *Toxoplasma* ocular infection is rare in Ghana[17]). Table 1 presents the genotype, allele, and allele carriage distributions of IFN-γ +874T/A polymorphism in cases and controls.

TNF-α Gene Polymorphism

After genotyping was performed for all 30 cases and 87 controls at TNF-α-308 by ARMS–PCR, the GG genotype was the largest group in both cases and controls [29 (97%) and 78 (90%), respectively]. This was followed by the GA genotype (3% in cases and 9% in controls) and finally the AA genotype (0% in cases and 1% in controls). There was no significant difference in the distribution of both genotypes and alleles between the cases and controls (*p* = 0.59; 0.16). However, both GA and AA genotypes had lower ORs when compared with GG, indicating that the presence of the A allele could confer some protection against the development of toxoplasmic ocular lesions. Allele carriage analysis (AA vs GA+GG) also indicated that the presence of the G allele may increase susceptibility to ocular lesions. Similarly, when compared with the G allele, the A allele gave a lower OR. Table 2 presents the distribution of TNF-α-308G/A genotype, allele, and allele carriage between cases and controls. Table 3 compares the distributions of IFN-γ and TNF-α genotypes in different populations.

DISCUSSION

The current study sought to evaluate the distribution profile of IFN-γ +874T/A and TNF-α-308G/A

TABLE 1. Distribution of genotype and allele frequencies of IFN-γ +874T/A polymorphism in cases of ocular toxoplasmosis and controls.

IFN-γ +874T/A	Ocular group (*n* = 30)		Control group (*n* = 87)		OR	95% CI	*p* value
	n	(f)	*n*	(f)			
Genotypes							
AA	20	0.67	68	0.78	Reference	Reference	Reference
AT	8	0.27	17	0.20	1.600	0.602–4.251	0.35
TT	2	0.07	2	0.02	3.400	0.450–25.691	0.24
Allele carriage							
AA	20	0.67	68	0.78	Reference	Reference	Reference
AT+TT	10	0.33	19	0.22	3.036	0.408–22.565	0.28
Alleles							
A	48	0.80	153	0.88	Reference	Reference	Reference
T	12	0.20	21	0.12	1.600	0.802–3.193	0.18

TABLE 2. Distribution of genotype and allele frequencies of TNF-α-308G/A polymorphism in cases of ocular toxoplasmosis and controls.

TNF-α-308G/A	Ocular group (n = 30)		Control group (n = 87)		OR	95% CI	p value
	n	(f)	n	(f)			
Genotypes							
GG	29	0.97	78	0.90	Reference	Reference	Reference
GA	1	0.03	8	0.09	0.336	0.040–2.807	0.31
AA	0	0.00	1	0.01	1.0×10^{-8}	1.0×10^{-8} to 1.0×10^{-8}	
Allele carriage							
GA+GG	30	1.00	86	0.99	Reference	Reference	Reference
AA	0	0.00	1	0.01	0.30	0.036–2.464	0.26
Alleles							
G	59	0.98	164	0.94	Reference	Reference	Reference
A	1	0.02	10	0.06	0.336	0.075–1.508	0.16

TABLE 3. Comparison of IFN-γ and TNF-α genotypes in different populations.

Study	Population	IFN-γ genotype		
		AA	AT	TT
This study	Ghanaian	88 (75.2%)	25 (21.4%)	4 (3.4%)
Rekha et al. (2006)[20]	Indian	46.2%	35%	18.8%
Albuquerque et al. (2009)[15]	Brazilian	27%	63.7%	8.9%
Neves et al. (2012)[16]	Brazilian	40%	42.5%	17.5%
Visentainer et al. (2005)[22]	Brazilian	31.6%	54%	14.4%
Hussein et al. (2009)[11]	Egyptian	14%	33%	53%

Study	Population	TNF-α genotype		
		GG	GA	AA
This study	Ghanaian	107 (91.5%)	9 (7.6%)	1 (0.9%)
Cordeiro et al. (2008)[14]	Brazilian	79%	18%	3%
Pujhari et al. (2012)[32]	Japanese	57.7%	42.3%	0%
Mishra et al. (2015)[33]	Indian	71.4%	28.6%	0%
Parikh et al. (2004)[34]	Ugandan	84.1%	15.9%	0%

genotypes and also to determine their associations with *Toxoplasma* retinochoroiditis in Ghanaian patients.

IFN-γ Gene Polymorphism in the Study Population

Similar to this study, which found the AA genotype as the predominant group (75.2%) in the population, a report by Rekha et al.[20] in India also observed that the AA genotype was more frequent (46.2%), followed by the AT genotype (35%), and TT genotype (18.8%). In contrast to the current finding, the AT genotypes were found as the predominant group in Brazilian populations. Albuquerque et al.[15] reported a profile of 27% of AA genotype, 63.7% of AT genotype and 8.9% of the TT genotype. Neves et al.[16] also reported a similar profile of 40% of the AA genotype, 42.5% of the AT genotype, and 17.5% of the TT genotype in a population of Rio de

Janeiro, Brazil. Two other studies from Brazil found similar patterns of genotype frequency distribution.[21,22] A study in Egypt by Hussein et al.[11] however, found the TT genotype as the predominant group (53%) followed by the AT genotype (33%) and the AA genotype (14%). IFN-γ +874T/A polymorphism has been shown to associate with several diseases. For instance, the AA genotype was found to be associated with tuberculosis in Iran,[10] Brazil,[21] and Spain[5]; hepatitis B in China[13]; *Helicobacter pylori* gastritis in Italy[23]; type 2 diabetes mellitus in Greece[24]; Wegener's granulomatosis in Germany[25]; atopic patients in Egypt.[11] Similarly, the AT and TT genotypes were found to associate with breast cancer in Iran;[26] hepatitis C in Taiwan;[6] and Hashimoto's disease in Japan.[27] The current findings do not seem to corroborate the only two earlier studies that investigated associations between IFN-γ +874 T/A polymorphism and the occurrence of ocular lesions caused by *Toxoplasma gondii*. Those studies, which were all conducted in Brazilian

populations, suggested that individuals with the A allele in its homozygous form had the tendency to develop ocular lesions.[15,16] The current study suggests that the T allele is associated with susceptibility to ocular lesions in 3.4- and 1.6-fold, respectively. Polymorphisms at the IFN-γ +874T/A position is known to associate with different levels of IFN-γ production. The TT genotype is associated with high levels of IFN-γ production, the AT with medium levels and the AA with lower levels.[5-7] The finding that the TT genotype (higher IFN-γ production) was associated with susceptibility to developing ocular lesions in this study may be related to severe inflammatory responses following increased cytokine production. Exacerbation of inflammatory reactions is known to result in retinochoroidal tissue destruction.[16] It was reported in the Brazilian patients regarding the scale of morbidity where the presence of the A allele seemed to confer protection against the development of clinical symptoms, although this did not result in ocular lesions.[16] The pathophysiologic mechanisms that underlie retinal damage in ocular toxoplasmosis are not yet fully understood, as the role of proinflammatory and immunoregulatory factors remain unclear.[28] Lahmar et al. reported increased levels of proinflammatory cytokines including IFN-γ and particularly IL-12 in patients with ocular toxoplasmosis than in other ocular diseases.[29] Again, IFN-γ was found to associate with the development of severe intraocular inflammatory disease in transgenic mice.[30] Stanford et al. also reported an association between high IFN-γ production and the outcome of ocular disease, where the presence of the T allele in either the heterozygous or homozygous forms was significantly higher in cases compared with controls.[31] The differences in IFN-γ genotype distribution profile in different populations may also determine which allele is associated with susceptibility or otherwise to developing ocular lesions. A careful observation of the pattern of genotype distribution seems to suggest the role of natural selection in relation to the T allele being associated with susceptibility to ocular infection in the current study as opposed to the AA genotype in the Brazilian studies. The genotypes occurring in fewer proportions seem to be associated with susceptibility to developing ocular lesions. That is, 24.8% of AT + TT in this Ghanaian population and 27% of AA genotype in a Brazilian population.[15] Neves et al. also reported 40% of the AA genotype in another Brazilian population.[16] For instance, if the AA homozygous individuals (75.2%) were to have an increased risk of developing ocular lesions in the current study, the implication would be that the prevalence of *T. gondii* retinochoroiditis in the population would be higher than the current state of 2.6%.[17] Conversely, if the T allele was to be associated with susceptibility to ocular infection in the Brazilian populations (73 and 60%, respectively), we would expect a higher prevalence of retinochoroiditis in those populations than is currently reported (31.8 and 5.8%, respectively).

TNF-α Gene Polymorphism in the Study Population

As in the current study, many studies have shown that at the TNF-α-308 polymorphic site, the GG genotype is the most frequent, while the AA genotype is the least common in all populations studied.[14,32-34] The only previous study that sought to investigate the association between TNF-α-308 polymorphism and the development of toxoplasmic retinochoroiditis did not find any association.[14] That finding was unexpected as the role of TNF-α in the pathophysiology of OT is very relevant. For instance, pretreatment of rat retinal vascular endothelial cells with TNF-α was found to inhibit the growth of *T. gondii* tachyzoites within these cells. This cytokine appeared to restrict parasite replication by starving the parasites of the amino acid L-tryptophan.[35] Similar to the current finding, several studies have associated the A allele with a protective effect against many diseases, including infectious and non-infectious uveitis. From Australia, El-Shabrawi et al. observed that the frequency of A allele in patients suffering from a human leukocyte antigen (HLA)-B27-associated uveitis was significantly lower than those in HLA-B27-negative control subjects.[36] Kuo et al. also described a reduced A allele frequency (6.6%) in their HLA-B27-positive uveitis patients in the UK.[37] A similar protective outcome with the A allele was associated with the development of ankylosing spondylitis in a population of Taiwan.[38] The less common TNF-308A allelic form has been found to associate with the binding of transcription factor, either by increasing promoter activity or by inhibiting the repressor of transcription and thereby increasing the production of TNF.[8,9] Contrary to the current finding, Sen et al. found a significant association of the A allele and the AA genotype with the occurrence of Eales disease.[12] Their explanation for this observation was that high expression of TNF-α could be responsible for the inflammation-associated angiogenesis in the proliferative stage of Eales disease, thus influencing susceptibility of the individual to the disease. The A allele was also associated with susceptibility to a non-infectious intermediate uveitis in the UK.[39]

In conclusion, the results suggested that the presence of the IFN-γ +874T allele either in the homozygous (TT) or heterozygous (AT) forms may increase susceptibility to developing ocular lesions. The concept of natural selection seemed to play a role in determining which genotype or allele of IFN-γ +874 is associated with susceptibility or otherwise of developing ocular lesions. Also, the presence of the less common TNF-308A allelic form could be protective against the development of ocular infection in the present study. It is important to indicate that, due to considerations of unique cytokine genotypic profile for different populations, these results are valid for our study population and may not be applicable to the whole of Africa.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ORIGINAL ARTICLE

Epidemiology of Ocular Toxoplasmosis in Three Community Surveys in the Central Region of Ghana, West Africa

Emmanuel Kwasi Abu[a,b], Johnson Nyarko Boampong[b], Joseph Kwame Amoabeng[a,c], Alex A. Ilechie[a], Samuel Kyei[a,d], Andrew Owusu-Ansah[a,e], Samuel Bert Boadi-Kusi[a,d], Benjamin Amoani[b], and Irene Ayi[f]

[a]Department of Optometry, School of Physical Sciences, University of Cape Coast, Ghana; [b]Department of Biomedical and Forensic Sciences, School of Biological Sciences, University of Cape Coast, Ghana; [c]Department of Ophthalmology, Effia Nkwanta Regional Hospital, Sekondi, Ghana; [d]Discipline of Optometry, School of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa; [e]Department of Ophthalmology, the Second Xiangya Hospital, Central South University, Changsha, Hunan, China; [f]Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana

ABSTRACT

Purpose: To conduct the first ever population-based survey on ocular toxoplasmosis in the Central Region of Ghana.

Methods: A cross-sectional population-based study was conducted in three randomly selected communities in the Central Region, Ghana. Visual acuity (VA) measurement, dilated fundus examination by indirect ophthalmoscopy and serology testing were performed on all participants. Ocular toxoplasmosis was diagnosed based on characteristic retinal lesions and supported by positive serologic testing using commercial enzyme-linked immunosorbent assay (ELISA) kits.

Results: A total of 390 subjects aged 10–100 years (mean age 47 years) were examined; 118 (30.3%) were male and 272 (69.7%) female. Ten subjects (6 females and 4 males) had toxoplasmic ocular lesions (prevalence 2.6%). Of these, two had bilateral lesions and eight had unilateral lesions. Subjects with toxoplasmic ocular lesions were older than those without lesions ($p = 0.028$). The development of ocular toxoplasmosis was not associated with rural dwelling, sex, keeping cats, or consumption of meat.

Conclusion: The prevalence of ocular toxoplasmosis in our Ghanaian study population was lower than findings from Southern Brazil, where there is a similar prevalence of infection in the general population.

Introduction

Toxoplasma retinochoroiditis is a major cause of posterior uveitis in many parts of the world, accounting for some 30–50% of all cases of posterior uveitis.[1,2] The prevalence of ocular toxoplasmosis has been reported to range from about 2% in the United States to 17.7% in Southern Brazil.[1–3] The posterior pole of the eye is frequently involved leading to considerable compromise in functional vision.[4] Brazil has a disproportionately high prevalence of ocular toxoplasmosis, but in Europe and the US the prevalence of ocular toxoplasmosis is much lower.[1] The outcome of ocular toxoplasmosis depends on the interaction of many factors, including functions of the host immune system as well as host and parasite genetic factors. In the Middle East, ocular toxoplasmosis accounts for about 33.6% of all cases of posterior uveitis.[5] This includes 54.5% in Iran,[6] 27.5% in Ankara, Turkey,[7] 44.4% in

Saudi Arabia,[8] and 13.8% in Israel.[5] In Europe, ocular toxoplasmosis accounts for an overall 49.2% of all posterior uveitis cases, consisting of 60.2% in Italy,[9] 40% in Finland, 39% in Belgium, 43.2% in Switzerland and 48.6% in Amsterdam.[5] Ocular toxoplasmosis has not been studied in much detail in Africa even though the prevalence is thought to be high. It is reported that in Sierra Leone, ocular toxoplasmosis was the most common cause of uveitis, accounting for 43%.[10] In Nigeria, toxoplasmosis was probably the single greatest factor in the causation of uveitis in the forest areas, with a prevalence of 70%.[11] A hospital-based study in Nigeria also found a total of 30 out of 10,595 consecutive eye patients had ocular toxoplasmosis (0.28%).[12] Documented information on the prevalence of ocular toxoplasmosis in Ghana does not exist at all. Here we report the first population-based study on ocular toxoplasmosis in Ghana, West Africa.

CONTACT Emmanuel Kwasi Abu, OD ✉ eabu@ucc.edu.gh ⊙ Department of Optometry, School of Physical Sciences, University of Cape Coast, UPO, Ghana, West Africa. Tel: +233244990030.

Materials and methods

The Central Region, with 20 districts and situated in the southern part of the country along the Atlantic Ocean, is one of 10 administrative regions of Ghana. The region can be broadly divided into two ecological zones; the coastal savannah with grassland along the coast, and a semi-deciduous forest predominating in the inland areas. The study was conducted in three communities; Moree, representing the coastal communities, Nkanfoa, for those located midway between the coastal and the forest zones, and Jukwa for the forest zone within the region. These communities were randomly selected from those of similar characteristics.

Moree is predominantly a fishing community located between latitude 5°25′N and longitude 1°20′W. A vast length of this community lies along the coastal stretch of the land. Jukwa is primarily a farming community lying within a district with large areas of forest reserves and located between latitude 5°16′0″N and longitude 1°19′60″W with an altitude of 75 m above sea level. Moree and Jukwa are both rural communities. Nkanfoa is a suburban cosmopolitan community located within the Cape Coast Metropolis. It is situated between latitude 5°06′19″N and longitude 1°14′47″W. Occupations of the people range from petty trading to farming/gardening, artisanship (masonry, carpentry, and fitting) and formal sector employment.

We conducted the study in accordance with the tenets of the Declaration of Helsinki on research regarding human subjects. The protocol for this study was reviewed and approved by the Ghana Health Service Ethical Review Committee (GHS-ERC 21/11/12). Approval was also given by the respective District Health Directorates and the chiefs and elders of the selected communities. Volunteer participants signed consent forms attached to examination sheets after the procedures had been explained to them in their own language. For participants younger than 18 years whose assents were sought, their parents/guardians signed the consent forms on their behalf. Confidentiality and safety were assured at all times.

A community-based cross-sectional design was employed. A minimum sample size of 384 was calculated based on the expression $N = Z^2(1-p)(p)/b^2$, where N = minimum sample size, p = anticipated prevalence of Toxoplasma ocular infection (estimated as 50%), b = desired error bound taken as 5%, and Z = the standard score at 95% confidence interval (1.96). We used a systematic random sampling to select households. Altogether, 103 households were selected from which 425 residents were enumerated. A total of 390 residents who consented to the study and met the inclusion/exclusion criteria were examined, giving a response rate of 91.8%. Inclusion criteria were that a resident had lived continuously in the particular community for at least 2 years and also had clear ocular media in both eyes to permit a clear ophthalmoscopic view of both fundi.[13]

The research team consisted of trained ophthalmologists and optometrists, an ophthalmic nurse, two social workers as field investigators, three laboratory technologists and two community volunteers. The ophthalmic nurse administered dilating drugs while the social workers were involved in explanation of the study purpose and procedures. The laboratory technologists drew blood samples. Prior to examination days, the team of field investigators and community volunteers visited all selected households (on days that residents did not go to farms or go fishing) to explain the purpose of the study and to ask for their consent to participate. The purpose of the study and the examination procedures were explained to residents in their local languages, after which those who met the inclusion criteria and agreed to participate by signing the consent forms were scheduled for examination at the community health centers. At the health centers, participants donated blood samples and underwent ophthalmic examination. A pilot study had indicated that parents were reluctant to allow their young children to donate blood samples; hence children under 10 years were not included. All participants answered questionnaires that sought information on demographic data and infection-related risk factors.

Venous blood samples were collected by trained laboratory technologists. About 3 mL of venous blood was obtained from each participant using a sterile disposable hypodermal syringe fitted with a 23-gauge needle and dispensed from the syringe barrel into a sterile tube and allowed to clot. Sample tubes were centrifuged at 500 g to precipitate red blood cells. Clear sera were collected into Eppendorf tubes and stored at −20°C until tested. All sample tubes were appropriately labeled with code numbers.

Ophthalmic examinations were conducted by trained ophthalmologists and optometrists. Distance presenting visual acuity measurement followed by dilated funduscopy was performed on all participants. Dilated fundus examinations were performed with 2.5% phenylephrine ophthalmic solution. Two drops of phenylephrine were administered at an interval of 5 minutes. We performed binocular indirect ophthalmoscopy on each participant after 1 hour of drug instillation and maximum pupillary dilation. Fundus photographs were taken for cases of retinochoroiditis. Ophthalmic examination forms for each participant were correspondingly labeled with the same code as the tubes for the serum samples.

Each serum sample was tested for the presence of anti-*Toxoplasma* antibodies immunoglobin G (IgG) and immunoglobin M (IgM) using commercial enzyme-linked immunosorbent assay (ELISA) test kits (VEDALAB, Cerisé, France) following the manufacturer's instructions. ELISA results were obtained using a microplate reader as a measure of optical densities of the reaction intensity of *T. gondii* antigen and serum anti-*T. gondii* antibodies at a filter wavelength of 450 nm. Cut-off points and antibody index calculations were performed according to the manufacturer's recommendation to categorize samples as positive or negative. Clinical diagnosis of ocular toxoplasmosis was based on a characteristic necrotizing retinochoroiditis adjacent to variably pigmented chorioretinal scar,[4]in addition to a positive serologic result.

Data were analyzed using SPSS (version 16, SPSS Inc, Chicago, IL, USA). Fisher's exact test was used to determine associations between ocular toxoplasmosis and risk factors while the chi-square test was used in the case of seropositivity. The t-test was used to compare mean ages of individuals with ocular lesions and those without. Risk for ocular infection was determined using the seropositive population as the comparison group. Multivariable-adjusted logistic regression analysis was used to predict the association between dependent and independent variables. The dependent variables were seroprevalence and ocular infection while independent variables were the risk factors. For seroprevalence, all factors with significant differences between groups by the chi-square test were adjusted for in the logistic regression analysis. For ocular infection, age was adjusted for. A p-value ≤ 0.05 was considered statistically significant.

Results

A total of 425 individuals were enumerated from 103 households, of which 390 persons were examined (response rate 91.8%). Ages ranged from 10–100 years (standard deviation 20.35 years), where 118 were male (30.3%) and 272 female (69.7%). Overall, 85% of the study population (333/390) tested positive for *T. gondii*, while toxoplasmic ocular lesions were found in 10 participants (2.6%). Among the seropositive population, the prevalence of ocular toxoplasmosis was 3.0% (10/333). All cases of ocular toxoplasmosis tested positive for IgG, but none had IgM antibodies. Of the 10 ocular cases, one patient was within the age range 30–39 years, three patients were 50–59 years, three were 60–69 years, and the remaining three were older than 70 years. The mean age of participants with ocular lesions (61.0 ± 13.9 years) was significantly higher than that of those without ocular lesions (46.64 ± 20.35 years; $p = 0.028$). Two persons (both male) had bilateral ocular lesions while the other eight had unilateral lesions.

While seroprevalence rates were higher in the more rural communities (Jukwa and Moree), there was no significant association between the occurrence of ocular toxoplasmosis and rural communities ($p = 0.199$). Table 1 compares the risk of *T. gondii* seropositivity and ocular toxoplasmosis. Seroprevalence rate was highly associated with environmental/sanitary conditions; contact with soil ($p < 0.001$), disposal of cat litter ($p < 0.001$), keeping of cats ($p \leq 0.001$), and source of drinking water ($p = 0.02$). However, there were no significant associations between ocular toxoplasmosis and any variable assessing contact with cat feces (owning/keeping a cat, disposing of cat litter, contact with

Table 1. Test of association between systemic/ocular toxoplasmosis and variables assessing risk of infection, Central Region, Ghana.

Factor	Examined, n	Seroprevalence			Ocular cases	
		n (%)	χ^2	p-value	n (%)	Fisher's Exact p-value
Sex			0.15	0.697		0.50
Male	118	102 (86.44)			4 (3.9)	
Female	272	231 (84.92)			6 (2.6)	
Socioeconomic status			26.7	<0.001		0.15
High	6	3 (50.00)			0 (0)	
Medium	52	34 (65.38)			3 (8.8)	
Low	332	296 (89.16)			7 (2.4)	
Cat ownership			19.8	<0.001		0.10
Yes	127	123 (96.90)			1 (0.8)	
No	263	210 (79.80)			9 (4.3)	
Dispose of cat litter			26.6	<0.001		0.54
Yes	170	163 (95.90)			6 (3.7)	
No	220	170 (72.30)			4 (2.4)	
Contact with soil			93.6	<0.001		0.73
Yes	244	241 (98.80)			8 (3.3)	
No	146	92 (63.00)			2 (2.2)	
Consumption of meat			3.3	0.07		0.60
Yes	360	304 (84.44)			9 (3.0)	
No	30	29 (96.67)			1 (3.4)	

Table 2. Multivariable-adjusted logistic regression analysis between the study communities and toxoplasmosis seroprevalence/ocular cases, Central Region, Ghana.

Community/risk factor	Examined/tested, n	Seroprevalence n (%)	OR[a] (95% CI)	p-value	Ocular cases n (%)	OR[b] (95% CI)	p-value
Community							
Nkanfoa	119	89 (75.0)	Reference	–	4 (4.5)	Reference	–
Moree	181	161 (89.0)	2.70 (1.46–5.06)	0.002	2 (1.2)	0.30 (0.05–1.49)	0.132
Jukwa	90	83 (92.0)	4.00 (1.67–9.59)	0.002	4 (4.8)	1.10 (0.26–4.45)	0.919
Dispose of cat litter							
No	220	170 (72.3)	Reference	–	4 (2.4)	Reference	–
Yes	170	163 (95.9)	6.85 (3.02–15.54)	<0.001	6 (3.7)	1.60 (0.44–5.73)	0.481
Contact with soil							
No	146	92 (63.0)	Reference	–	2 (2.2)	Reference	–
Yes	244	241 (98.8)	38.39 (14.39–154.55)	<0.001	8 (3.3)	1.50 (0.32–7.42)	0.587

[a] Adjusted for "contact with soil," "dispose of cat litter," "community," "socioeconomic status," "cat ownership."
[b] Adjusted for age.
OR, odds ratio; CI, confidence interval.

soil and contaminated water sources). However, patients who responded "yes" to contact with soil and disposal of cat litter had higher odds ratios than those who responded in the negative. Table 2 presents the multivariable-adjusted logistic regression analysis between seroprevalence/ocular cases and some risk factors for infection. Apart from age, there were no significant associations between ocular toxoplasmosis and other demographic data like sex and socioeconomic status. Again, meat consumption did not have any association with the development of ocular toxoplasmosis.

Discussion

This is a prevalence report of *Toxoplasma* ocular infection in a community-based epidemiological study delimited to three southern rural communities in Ghana. The findings, however, provide important information to the broader understanding of toxoplasmosis. As per the current study, rural communities have been associated with higher *T. gondii* seroprevalence rates while suburban and urban areas have often recorded lower rates.[14-17] The reasons for associating infection with rural dwellers has to do with their exposure to many risk factors such as contact with soil, drinking from contaminated water sources, lower socioeconomic level and abundance of roaming cats in the surroundings. In this study, while seropositivity was significantly associated with the more rural communities, this was not the case with the development of ocular lesions as there was no association between ocular cases and rural dwellers. A study by Ferreira and colleagues in Brazil did not associate the development of ocular toxoplasmosis with environmental factors.[18] This was similar to the present study, where no variable assessed for risk of infection was associated

with the development of ocular infection. The major risk factor for ocular toxoplasmosis in the current study was older age. Aging has been identified as the major risk factor for developing ocular toxoplasmosis.[19] Similarly, Portela and co-workers found that the frequency of ocular toxoplasmosis increased significantly with advancing age, where almost 50% of individuals older than 60 years had ocular lesions.[13] Bosch-Driessen and co-authors also found ocular involvement in patients with *Toxoplasma* was associated with advanced age at onset.[4] The reasons may be attributed to the possible decrease of cell-mediated immunity with advanced age.[4,20] The study by Ferreira and colleagues associated ocular disease with age and literacy.[18]

The current study found the prevalence of ocular toxoplasmosis in the study population to be 2.6%, and 3.0% among seropositive individuals. These findings are lower than reported cases from Southern Brazil where ocular toxoplasmosis is endemic and seroprevalence is also high.[21] In the southern city of Erechim, Brazil, 17.7% of adults were found to have retinal lesions caused by toxoplasmosis.[3] Again, in Southeastern Brazil, 80% prevalence of ocular toxoplasmosis was detected in a cohort study of newborns with congenital toxoplasmosis.[22] The current findings are similar to a study from Northern Brazil which found that 3.4% of acutely infected adults had retinochoroiditis.[23] Another study from Northeastern Brazil found the prevalence of ocular lesions to be 1.2% of the study population.[24] Seroprevalence data from Northern Brazil are also high.[25,26] The prevalence rate from this current study was higher than findings in Europe and North America where reported cases of ocular toxoplasmosis are much lower. In the US, for example, prevalence of ocular toxoplasmosis among the seropositive population was estimated at 2%.[1] A study in Canada during an outbreak of toxoplasmosis

through water contamination found that between 0.3% and 0.7% of infected individuals developed ocular toxoplasmosis.[27] An incidence rate of 0.4/100,000 was determined for patients born in Britain cumulating in a lifetime risk of 18/100,000.[28] The higher prevalence rates and severe disease outcomes have been attributed to infection with more virulent parasite strains. Type II strains have been found as predominantly responsible for human cases in France and North America[29,30] while type I *Toxoplasma* and atypical strains appear to be responsible for the majority of ocular infections in Brazil.[31] However, not enough information is available on the genetic structure of parasite strains responsible for ocular cases in Africa and for Ghana as well. While some studies of animal isolates from Africa found a predominance of the archetypical clonal lineages (types I, II and III) as in Europe and North America,[32] a study of human samples from Tunisia suggested the predominance of recombinant strains (types I/III and I/II).[33] A more recent genotyping of *T. gondii* samples from the West African city of Dakar found only recombinant strain types I/III and I/II/III.[34] In Ghana, the only study that has genotyped for parasite strains was done on isolates from chickens that suggested unique recombinant strains quite different from those in Brazil and Europe.[35] What seems clear in all these studies thus far is that parasite strains found in Africa seem quite distinct from those found in Southern Brazil. Thus, parasite strains responsible for ocular toxoplasmosis in the current study population may be less virulent. A further study to characterize parasite strains responsible for human systemic and ocular toxoplasmosis in this area would be interesting.

The occurrence and severity of ocular disease may also be attributable to host genetic factors such as the possible occurrence of polymorphisms in the genes encoding interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) in the human host.[36] Unilateral involvement in ocular toxoplasmosis has been reported to vary between 60% and 83%,[2,4] consistent with the 80% found in the current study.

In terms of public health implications, the burden of disease related to ocular toxoplasmosis is indeed considerable. In terms of disability adjusted life years (DALY), *Toxoplasma* infection was deemed to cause the highest disease burden among food borne pathogens.[37] In the case of congenital disease, retinochoroiditis has been found to account for 35% of the burden, second only to stillbirth, and in the case of acquired infection retinochoroiditis accounted for almost the entire burden.[38] Bosch-Driessen and colleagues[4] reported vision loss in at least one eye occurring in 24% of patients with ocular toxoplasmosis.

A British survey of visual impairment in congenitally infected children reported that 17% presented at least one retinal lesion and 9% suffered from severe bilateral impairment.[39] There is a need for public health strategies in terms of early detection and treatment as well as routine serological screening of all pregnant women as practiced elsewhere.[40]

In conclusion, we found ocular toxoplasmosis was associated with older age. There was a low prevalence of toxoplasmic ocular infection in a population with high *T. gondii* seropositivity, a pattern different from Southern Brazil where high seropositivity is also associated with high ocular infection. Further epidemiological studies in other regions of this country are needed to confirm the prevalence rate of ocular toxoplasmosis in Ghana.

Acknowledgments

Declaration of interest

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Research Article

Visual Outcome in Ocular Toxoplasmosis: A Case Series of 30 Patients from Ghana

Emmanuel Kwasi Abu[1]*, Johnson Nyarko Boampong[2], Richmond Afoakwah[2], Elvis Ofori Ameyaw[2], Michael Ntodie[1] and Irene Ayi[3]

[1]Department of Optometry, School of Physical Sciences, University of Cape Coast, Ghana

[2]Department of Biomedical and Forensic Sciences, School of Biological Sciences, University of Cape Coast, Ghana

[3]Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Ghana

*Corresponding author: Dr. Emmanuel Kwasi Abu, Department of Optometry, School of Physical Sciences, University of Cape Coast, Ghana, Tel: +233244990030; E-mail: eabu@ucc.edu.gh

Abstract

Purpose: To determine visual outcome (low vision and blindness) in patients with inactive ocular toxoplasmosis.

Methods: This study employed a cross sectional design involving a series of 30 patients with inactive toxoplasmic ocular lesions. Ophthalmic assessment including best corrected visual acuity (BCVA) measurement, slit lamp biomicroscopy, and dilated fundus examination by indirect ophthalmoscopy was performed on all participants. Ocular toxoplasmosis was diagnosed based on characteristic retinal lesions in addition to a positive serologic testing using commercial ELISA kits. Visual impairment (VI) was determined based on the International Classification of Diseases.

Results: Their ages ranged from 16-59 years (mean age of 34.2 ± 14.19), with 19 (63.3%) males and 11 (36.7%) females. There were 33 infected eyes in all (3 patients had bilateral cases). The most common complaint (77%) was blurred vision in the infected eyes. 11 (33%) eyes had mild or no visual impairment (VI category 1), 22 (67%) eyes had low vision (VA<6/18), and 11 (33%) eyes were blind (VA<3/60). Posterior pole (p<0.001) and larger retinal lesions (p=0.04) were the major causes of visual impairment. However, there was no association between visual impairment and the number of lesions occurring in the infected eyes (x^2=3.52, p=0.11). Older patient age was significantly associated with: posterior pole lesions (0.003), larger retinal lesion sizes (p=0.001) and multiple lesions (p=0.001). Only three cases each of strabismus and bilateral involvement suggest that acquired infection is more common in this Ghanaian population.

Conclusion: Low vision and blindness were common in *Toxoplasma* eye infection in our Ghanaian population and that posterior pole and larger retinal lesions rather than multiple lesions were the major causes of reduced vision.

Keywords: *Toxoplasma*; Visual impairment; Ocular toxoplasmosis; Ghana

Introduction

Ocular toxoplasmosis is caused by an intracellular protozoan parasite *Toxoplasma gondii*. It is responsible for up to 70% of all posterior uveits cases in certain populations [1]. It is also a leading cause of visual impairment throughout the whole world, accounting for up to 57% of visual impairment and 24% of blindness in infected persons [2,3].

About a third of the world's population has been infected with *T. gondii* [4]. A fascinating observation, however, is the disparity in the occurrence of ocular toxoplasmosis in different populations with similar seroprevalence findings. In the United States, for example, where 15.8% of the general population was reported to have been infected with *Toxoplasma*, ocular involvement among the infected individuals was estimated at 2% [5,6]. In Brazil ocular toxoplasmosis is disproportionately high and seroprevalence findings are equally high. In the southern Brazilian city of Erechim, 85% of the population was

infected with *T. gondii* and 17.7% of the infected individuals had retinal lesions caused by the parasite [7]. A more recent study in the city of Campos dos Goytacazes, located in the north of Rio de Janeiro, found 31.8% prevalence of ocular infection in the population where 76.4% of the people were infected with the parasite [8]. In Colombia an estimated 5.5% of the entire population has Toxoplama retinochoroidal scars and 47% of the population has been infected with the parasite [9]. Seroprevalence surveys in Ghana have reported *Toxoplasma* infection rates between 49.7% and 92% [10-12] while toxoplasmic retinochoroidal lesions occur in 3% of the population exposed to the parasite [13]. Higher and severe ocular disease outcomes have been attributed to infection with more virulent parasite strains and human genetic factors.

The most common complications and characteristics of ocular toxoplasmosis that lead to increased visual disability include macular scars, dragging of the macula secondary to peripheral lesions, strabismus, retinal detachment, nystagmus, optic atrophy, cataract, glaucoma, opacification of the media, amblyopia and bilateral involvement [14]. There are no prior reports that describe visual function in ocular toxoplasmosis in Ghana. The purpose of the current

study was to determine the visual outcome in patients with inactive ocular toxoplasmosis in a Ghanaian population.

Materials and Methods

The study participants consisted of 20 consecutive patients with inactive ocular toxoplasmosis, who visited the University of Cape Coast Eye Unit plus 10 patients from an earlier population-based survey in the Central Region [13]. The study was conducted in accordance with the Helsinki Declaration on Research Regarding Human Subjects. The protocol for this study was reviewed and approved by the Ghana Health Service's Ethical Review Committee (ID: GHS-ERC: 21/11/12). Participants signed consent forms after the study protocol had been explained to them.

Ophthalmic examination was conducted by trained ophthalmologists and optometrists. The examination included distance Best Corrected Visual Acuity (BCVA) measurement, slit lamp biomicroscopy and dilated funduscopy by binocular indirect ophthalmoscopy. Fundus photographs were taken for cases of retinochoroiditis. Clinical diagnosis of ocular toxoplasmosis was based on characteristic ocular lesions consistent with *Toxoplasma* retinochoroiditis, in addition to a positive serologic result and in the absence of other identifiable ocular morbidities. The criterion for positive serologic result was a positive test for any of the two anti-*Toxoplasma* IgG or IgM antibodies or a combination of both. For purposes of consistency, description and measurement of toxoplasmic retinal lesions was made by one clinician.

Venous blood samples were collected by trained laboratory technologists. About 3 ml of venous blood was obtained from each participant, clear sera prepared and tested for the presence of anti-*Toxoplasma* antibodies IgG and IgM using commercial ELISA test kit (VEDALAB-France) and following the manufacturer's instructions. ELISA results were obtained using a micro-plate reader as a measure of optical densities of the reaction intensity of *T. gondii* antigen and serum anti-*T. gondii* antibodies at a filter wavelength of 450 nm. Cut-off points and antibody index calculations were done according to the manufacturers' recommendation to categorize samples as positives or negatives. Serology testing was performed at the time of inclusion to this study.

Patients who had other ocular morbidities such as macular degeneration, cataracts and glaucoma that could interfere with visual function were excluded. This was necessary in order that every visual disability would be attributed to the *Toxoplasma* lesions.

Patients' data such as age, gender, and chief complaints were recorded. Description of ocular lesions included size of lesion in disc diameters (DD), location of lesion, and number of lesions. Sizes of lesions were categorized as <2DD and ≥ 2DD; in multiple lesions the largest one was considered [15]. Lesions located within the major vascular arcades were classified as posterior pole lesions and those outside the major arcades as peripheral lesions. Posterior pole lesions were further classified as macular (involving the macula), juxtamacular (around the macular) and juxtapapillary (around the optic disc). In the case of multiple lesions priority was given to macular lesions. Lesions were also classified as single or multiple (2 or more lesions). Categories of visual impairment (VI) were determined based on the International Classification of Diseases criteria [16]. For

purposes of this study, visual impairment was determined after best refractive correction and for each infected eye separately.

Data was analyzed using the Statistical Package for Social Sciences (version 16; SPSS Inc., Chicago, IL). The Chi-square test was used to determine associations between categorical variables and where counts were below 5, the Fisher's Exact Test was used. The t-test was used to compare the mean ages of patients with different characteristics of *Toxoplasma* ocular lesions. Multivariate logistic regression analysis test (with 95% confidence intervals) was used to predict associations between dependent and independent variables. A p-value ≤ 0.05 was considered statistically significant.

Results

A total of 30 cases with inactive *Toxoplasma* ocular scars were included for the current study. Their ages ranged from 16 to 59 years (mean age=34.2; SD=± 14.19), where 19 (63.3%) were males and 11 (36.7%) females. All the patients tested positive for IgG antibodies but none had IgM antibodies. Twenty three (77%) patients complained of blurred vision in the infected eyes, 14(47%) complained of seeing floaters, 5(17%) patients reported of mild pains while 3(10%) reported seeing halos around bright light. Three patients (10%) had complications of strabismus and two (6.7%) patients had developed cataracts in the infected eyes. Twelve (40%) patients had the lesions in their right eyes while in 15(50%) patients lesions occurred in the left eyes and 3(10%) patients had bilateral cases. Altogether, there were 33 infected eyes.

Visual Acuity	N (%)
6/6 or better	6 (18.2)
6/9-6/18	5 (15.2)
worse than 6/18-6/60	10 (30.3)
worse than 6/60-3/60	1 (3.0)
worse than 3/60	11 (33.3)
Total	33 (100.0)

Table 1: Best corrected visual acuities of infected eyes.

Table 1 shows the BCVA of the infected eyes where 11(33%) eyes had visual impairment category 1 (no or mild visual impairment), 22(67%) eyes had low vision (VA<6/18), and 11 (33%) eyes being legally blind (VA<3/60). Majority of the infected eyes 18 (54.5%) had retinal scars/lesions of sizes less than 2DD while in 15 eyes the lesions were of sizes 2DD or bigger. However, the bigger retinal scars ≥ 2DD were more likely to cause low vision and blindness respectively [Low vision:<2DD, (41%); ≥ 2DD (59%, OR=6.50, p=0.04); Blindness: <2DD (27%); ≥ 2DD (73%, OR=5.7, p=0.03). Posterior pole retinal lesions occurred in 24 (72.7%) eyes and in 9 (27.3%) eyes the lesions were at the periphery. Lesions at the posterior pole were: macular 11, juxtamacular 6, and juxtapapillary 7. The cause in all eleven patients with legal blindness was attributed to posterior pole lesions: 10 macular cases and 1 juxtapapillary scar. No lesion outside the posterior pole contributed to any visual impairment.

Characteristic	Number of eyes (n=33)	Patients with low vision (n=22): VA<6/18			Patients with blindness (n=11): VA<3/60		
		N (%)	OR (CI)	p-value	n(%)	OR (CI)	p-value
Size of lesion							
<2 DD	18	9(50)	Reference	Reference	3(17)	Reference	Reference
≥ 2 DD	15	13(87)	6.5 (1.13-37.48)	0.04	8(53)	5.7 (1.15-28.35)	0.03
Location of lesion			χ^2			χ^2	
Posterior pole	24	22(92)	24.75	< 0.001	11(46)	6.2	0.02
Periphery	9	0 (0)			0 (0)		
Number of lesions			3.52	0.11		1.79	0.24
Single	23	13 (57)			6 (26)		
Multiple (2 or more)	10	9 (90)			5 (50)		

Table 2: Relationship between characteristics of *Toxoplasma* retinochoroidal lesions and low vision/blindness in the infected eyes.

Low vision of VA worse than 6/18 occurred in 22 (66.7%) eyes, out of which 11 occurred at the macular, 5 juxtamacular and 6 juxtapapillary. Multiple (two or more) scars occurred in 10(30.3%) eyes; the other 23(69.7) eyes had single lesions. However, there was no association between cause of visual impairment and the number of lesions occurring in the infected eyes (χ^2=3.52, p=0.11). Table 2 gives the relationship between characteristics of *Toxoplasma* ocular lesions and low vision/blindness in the infected eyes where bigger and posterior pole lesions were significantly associated with the causes of low vision and blindness in the infected eyes. Figures 1-3 represent fundus photographs of some of the patients.
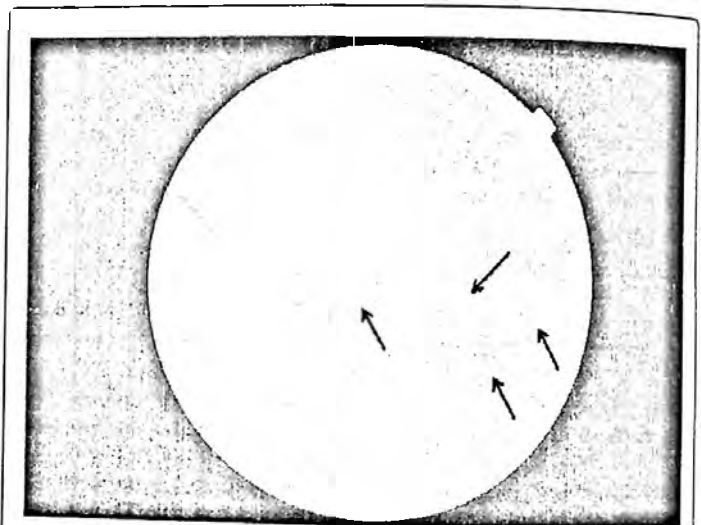
Figure 2: Multiple posterior pole lesions of a 50 year old patient; Size of lesion<1DD; VA=6/36.
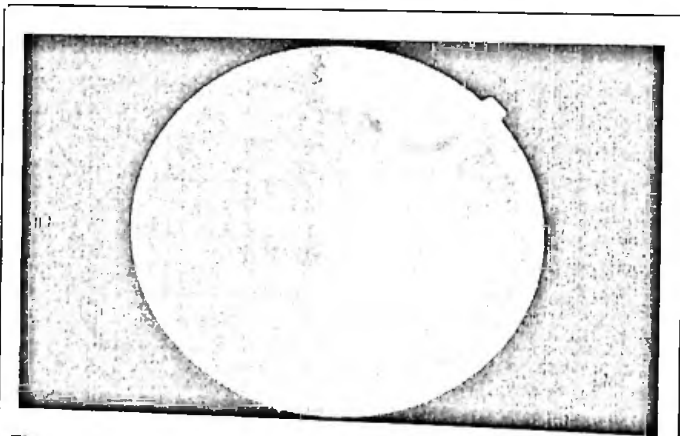
Figure 1: A large peripheral lesion of a 26 year old patient; Size of lesion>2DD; presence of vitreous strands; VA=6/12.

Elder patients had larger retinochoroidal lesions: mean age of patients (43.7 ± 13 years) with lesions ≥ 2DD was significantly higher than the mean age of patients (27.1 ± 10 years) with smaller lesions <2DD F=14.2, p=0.001]. Similarly, multiple lesions occurred significantly (F=13.4, p=0.001) in elder patients (mean age of 47.4 ± 11 years) than in younger patients (mean age of 29.4 ± 12 years).
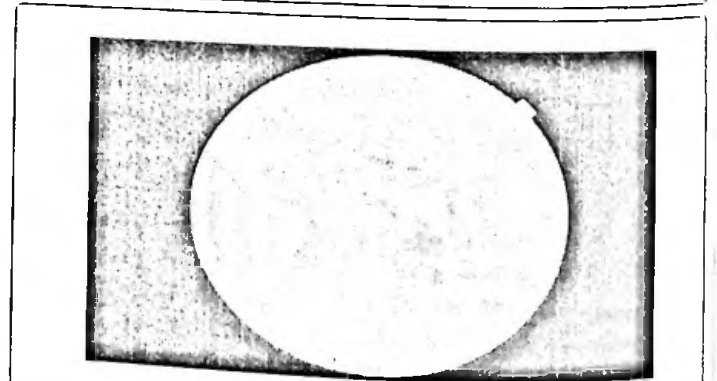
Figure 3: A large macular scar of a 59 year old patient; Size of lesion>2DD; VA=CF @2 m.

Again, elder patients had significantly more posterior pole lesions than younger patients (p=0.003). However, there was no association between gender and location (χ^2=0.6, p=0.67), size (χ^2=2.9, p=0.13), and number (χ^2=0.8, p=0.41) of retinal lesions. Table 3 presents the relationship between characteristics of *Toxoplasma* retinochoroidal lesions and ages/gender of patients.

Description of lesion	Mean age (SD)	F-test	p-value	Gender		χ^2	p-value
				Male	female		
		14.2	0.001			2.9	0.13
Size of lesion				13 (76)	4 (24)		
< 2DD	27.1 (± 10.4)			6 (46)	7 (54)		
≥ 2DD	43.5 (± 13.3)						
		10.5	0.003			0.6	0.67
Location of lesion				13 (59)	9 (41)		
Posterior pole	38.6 (± 14.1)			6 (75)	2 (25)		
Periphery	22.1 (± 3.8)						
		13.4	0.001			0.8	0.41
Number of lesions				15 (68)	7 (32)		
Single	29.4 (± 12.1)			4 (50)	4 (50)		
Multiple	47.4 (± 11.0)						

Table 3: Relationship between characteristics of *Toxoplasma* retinochoroidal lesions and ages/gender of patients.

Discussion

This is a report of characteristic visual findings associated with *Toxoplasma* ocular infection from Ghana. Similar to the current finding, a study by Kadarisman et al. [2] in Indonesia found 56.7% of visual impairment and 20.1% of blindness in 41 eyes infected with toxoplasmosis. Another study from Indonesia found visual impairment and blindness to occur in 70 and 13.9% respectively in patients with ocular toxoplasmosis [14]. A study by Kodjikian et al. [17] reported a lower prevalence (23%) of visual impairment in 130 ocular toxoplasmosis cases. The devastating visual outcome in ocular toxoplasmosis is highly associated with the preponderance of posterior pole lesions. This was particularly the case in the current study where all the eyes with low vision 22 (67%) and blindness 11 (33%) had retinal lesions at the posterior pole. A study from Brazil found that the major cause of visual disability was papillomacular bundle area lesions, occurring in 76.3% [18]. Another study found the main (67.9%) contribution to vision loss in inactive lesions to be macular scars [19]. Tabatabaei et al. [15] reported that most of their ocular toxoplasmosis patients (87.5%) had lesions in the posterior pole. Several reasons have been argued for the preference of toxoplasmic ocular lesions at the macular area. It has been argued, for instance, that anatomic and microvascular differences between the macula and the peripheral retina might create a microenvironment that can influence the location of lesions [20]. Another major cause of visual impairment was larger lesion size (≥ 2DD) which occurred in 45% of eyes and contributed to 87% of low vision. Tabalabei et al. [15] however, reported that only 5 (12.5%) of their patients had lesions larger than 2DD. The reason for this disparity may be related to the younger age of their patients (mean age of 24.3 ± 11.7) as compared to mean age of 34.2 ± 14.19 in the current study. They also found a relationship between the size of retinal lesion and age of patient, with older patients having larger lesions [15]. Dodds et al. [21] have also reported that signs of increased inflammation leading to marked reduction of visual acuity in patients with toxoplasmic retinochoroiditis were related to older patient age and larger retinal lesions. These observations may be explained by the fact of a possible multiple recurrences of ocular toxoplasmosis that makes the scars larger in elder patients and also by reason of declining immunity with advancing age [20]. In the current study, not only were elder patients likely to have larger retinal scars but they were also likely to have multiple (2 or more) and posterior pole lesions (Table 3).

Unlike this study which found only three cases of strabismus and no nystagmus, other studies have found higher cases of strabismus and nystagmus in ocular *Toxoplama* infection [14,17]. The development of strabismus and nystagmus seem to be associated with congenital cases as a result of disturbances of macular function during the developmental age. Strabismus develops as a result of poor central vision and extra foveal fixation due to macular lesions, and if fixation becomes continuously poor nystagmus becomes evident. Another feature of congenitally acquired infection which was absent from this study is the predominance of bilateral involvement. Kodjikian et al. [17] reported bilateral involvement of 96% in their congenital cases compared to 10% in the current study. Kadarisman et al. [2] found bilateral involvement in 50% of their cases where they reported complications of strabismus and nystagmus, both occurring in 50% of the episodes. For these reasons we may speculate that postnatally acquired infection is more common in this Ghanaian population.

In conclusion, we found higher episodes of low vision and blindness in our ocular toxoplasmosis patients. The major causes of visual impairment were posterior pole and larger retinal lesions and not multiplicity of the lesions. Posterior pole, multiple and larger retinal scars were significantly associated with older patient age.

Acknowledgement

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Infection risk factors associated with seropositivity for Toxoplasma gondii in a population-based study in the Central Region, Ghana

E. K. ABU[1,2]*, J. N. BOAMPONG[2], I. AYI[3], G. GHARTEY-KWANSAH[2], R. AFOAKWAH[2], P. NSIAH[4] AND E. BLAY[3]

[1] Department of Optometry, School of Physical Sciences, University of Cape Coast, Ghana
[2] Department of Biomedical and Forensic Sciences, School of Biological Sciences, University of Cape Coast, Ghana
[3] Department of Parasitology, Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Ghana
[4] Department of Chemical Pathology, School of Medical Sciences, University of Cape Coast, Ghana

SUMMARY

About 20–90% of the world's population has had contact with Toxoplasma gondii parasites. The aim of this study was to determine the seroprevalence and risk factors associated with T. gondii infection in the Central Region, Ghana. A community-based cross-sectional study was conducted in three selected communities. Serum samples were tested for the presence of anti-T. gondii IgG and IgM antibodies by ELISA. A serological criterion for seropositivity was a positive test result for any of the two anti-Toxoplasma IgG or IgM antibodies or a combination of both. In all, 390 participants of mean age 47·0 years consisting of 118 (30·3%) males and 272 (69·7%) females were tested. The overall seroprevalence of T. gondii was 85% (333/390) where fishermen, farmers and fishmongers, respectively, had the highest seropositivity. IgG and IgM antibodies were detected in 329 (84%) and 25 (6%), respectively, while both IgG and IgM antibodies were detected in 21 (5%) of the participants. Respectively, 1% (4/390) and 79% (308/390) of participants tested positive for IgM-only and IgG-only antibodies. There was a significant relationship between Toxoplasma seropositivity and contact with soil, presence of a cat in the surrounding area, age, sources of drinking water, level of formal education, and socioeconomic status. The results suggest that the seashore may serve as a good ground for sporulation and survival of Toxoplasma oocysts.

Key words: ELISA, parasite, seropositivity, Toxoplasma gondii.

INTRODUCTION

Toxoplasmosis is a zoonotic parasitic disease caused by an obligate intracellular protozoan, Toxoplasma gondii, which is distributed worldwide. The definitive host of the parasite belongs to members of the family Felidae, but several warm-blooded animals including humans serve as intermediate hosts for the parasite. The disease is important for its serious implications in immunosuppressed individuals such as HIV patients as well as its severe consequences for fetuses in congenital transmission [1]. Serological surveys demonstrate a wide variation in the prevalence of

* Author for correspondence: Dr E. K. Abu, Department of Optometry, School of Physical Sciences, University of Cape Coast, UPO, Ghana, West Africa.
(Email: eabu@ucc.edu.gh)

the infection in various geographical locations. Prevalence is known to vary with age, climate and socioeconomic status (SES) [2]. Higher prevalence has been observed in tropical countries with humid and warm climates, while lower prevalence is found for arid and colder countries [3]. Seroprevalence is higher in Central Europe, South America and Africa [4–6]. In the USA and the UK, it is estimated that 16–40% of the population are infected with *T. gondii*. Lower prevalence rates of 4–39% have been reported in South East Asia, China and Korea and 11–28% in Scandinavia [3, 7]. Seroprevalence in India has been reported to be 24·3% [8]. In Brazil, about 50–80% of the population is infected with *T. gondii* [9]. Testing of all pregnant women for *T. gondii* infection is routine in some European countries, including France and Austria [10]. In France, for instance, the existence of a national programme for the prevention of congenital toxoplasmosis since 1978 has seen seroprevalence in pregnant women fall from 84% in the 1960s to 54% in 1995 and to 44% in 2003 [4]. In Ghana, only two hospital-based data are available on *T. gondii* infection in humans [11, 12]. Here we conducted the first ever population-based study on *T. gondii* seropositivity and its related infection risk factors in Ghana.

METHODS

The study areas

The Central Region, situated at the southern part of the country along the Atlantic Ocean, is one of the ten administrative regions of Ghana. The region can be broadly divided into two ecological zones: the coastal savannah with grassland along the seashore and a semi-deciduous forest, predominating the inland areas. The study was conducted in three communities, Moree, representing the coastal communities, Nkanfoa, located midway between the coastal and the forest zones, and Jukwa, the forest zone within the region. Moree is predominantly a fishing community located between latitude 5° 25′ N and longitude 1° 20′ W. A huge expanse of the community is along the coastal stretch of the area. Jukwa is mostly a farming community lying within a district with large areas of forest reserves located between latitude 5° 16′ 0″ N and longitude 1° 19′ 60″ W with an altitude of 75 m above sea level. Nkanfoa is located between latitude 5° 06′ 19″ N and longitude 1° 14′ 47″ W. The vegetation consists of shrubs, grass and a few scattered trees. The occupation

of the people ranges from petty trading to farming/gardening and artisanship (masonry, carpentry, fitting).

Ethical approval

The study was conducted according to the Helsinki Declaration on Research regarding human subjects. The protocol for this study was reviewed and approved by the Ghana Health Service's Ethical Review Committee (ID: GHS-ERC: 21/11/12). Approval was also given by the respective District Health Directorates and the Chiefs and Elders of the selected communities. Volunteer participants signed consent forms attached to the questionnaires after the procedures had been explained to them in their own language. For participants aged <18 years whose assent was sought, their parents/guardians signed the consent forms on their behalf. Confidentiality and safety were assured at all times.

Selection of study participants

A community-based cross-sectional design was employed. A minimum sample size of 384 was calculated based on the expression

$$N = Z^2(1 - p)(p)/b^2,$$

where N = minimum sample size, p = anticipated prevalence of *Toxoplasma* infection of 50%, b = desired error bound taken as 5% and Z = the standard score at 95% confidence interval (1·96). An inclusion criterion was for a participant to have lived continuously in the particular community for at least 2 years [13]. A pilot study had indicated that parents were reluctant in allowing their young children to donate blood samples; hence children aged <10 years were not included. The research team visited various households (on days that residents did not go to farms or go fishing) to explain the purpose of the study and to request their consent to participate. The purpose of the study and the examination procedures were explained to residents in their local language after which those who met the inclusion criteria and agreed to participate by signing the consent forms were interviewed by questionnaires and asked to donate a blood sample at the community health centres.

Questionnaire

The questionnaire sought information on (*a*) demographic characteristics (gender, age, educational level,

occupation); (*b*) possible exposure to and consumption of *T. gondii* oocysts: (i) contact with soil, working on a garden, (ii) contact with cats (owning cats, presence of cats around the house, disposing of cat litter); (iii) source of drinking water; (*c*) possible consumption of *T. gondii* cysts, type of meat consumed, state of meat consumed (undercooked meat or well cooked); (*d*) hygiene habits (hand washing, washing of vegetables/fruits or not before consumption).

Blood sample collection and serum preparation

Venous blood samples were collected by trained laboratory technologists. About 3 ml of venous blood was obtained from each participant using a sterile disposable hypodermal syringe fitted with a 23-gauge needle and dispensed from the syringe barrel into a sterile tube and allowed to clot. Sample tubes were centrifuged at 500 *g* to precipitate red blood cells. Clear sera were collected into Eppendorf tubes and stored at −20 °C until tested. All sample tubes were appropriately labelled with code numbers.

Detection of anti-*T. gondii* antibodies by commercial ELISA kit

Each serum sample was tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies using a commercial ELISA kit (VEDALAB, France) according to the manufacturer's instructions. For IgG assay procedure, 100 µl of diluted (1:41) test sample, calibrator, positive control, and negative control were transferred into the precoated wells, covered and incubated at room temperature for 30 min. Wells were then washed five times with diluted wash solution after which 100 µl of horseradish peroxidase (HRP) conjugate was added to each well, covered and incubated for 30 min. The wells were again thoroughly washed five times with the diluted wash solution after which 100 µl tetramethylbenzidine (TMB) substrate was added to each well. The plate was covered, incubated at room temperature for 30 min and 100 µl of stop solution added to each well. Finally, a 450 nm set wavelength micro plate reader was used to measure the optical density (OD) of each well. IgG cut-off values were determined in enzyme units (EU)/ml and calculated using the formula:

$$\text{EU/ml of test sample} = \frac{\text{EU/ml of calibrator}}{\text{absorbance of calibrator}} \times \text{absorbance of test sample},$$

and interpreted as follows: a value of <40 EU/ml was considered negative for IgG antibody to *T. gondii*. A value between 40 and 50 EU/ml indicated the test result was equivocal and had to be retested. An EU/ml value >50 was considered positive for IgG antibody to *T. gondii*.

A similar assay procedure was carried out for IgM antibody detection except that the plate was incubated at 37°C and index calculations were determined by dividing the OD value of test sample by the OD value of the calibrator, and interpreted as: a calculated index value <0·90 was negative for IgM antibody to *T. gondii*. An index value between 0·90 and 0·99 was equivocal, and an index value ⩾1·00 was positive for IgM antibody to *T. gondii*. A serological criterion for systemic infection was a positive test result for any of the two anti-*Toxoplasma* IgG or IgM antibodies or a combination of both.

Data analysis

Data were analysed using SPSS version 16 (SPSS Inc., USA). χ^2 test was used to determine associations between categorical variables. $P \leqslant 0·05$ was considered statistically significant. Logistic regression analysis was used to predict the association between *T. gondii* seropositivity and risk factors.

RESULTS

A total of 390 participants of mean age 47·0 years (s.d. ±20·35, range 10–100 years) were studied. There were 118 (30·3%) males and 272 (69·7%) females, while 332 (85%), 52 (13%) and six (2%) of the participants were of lower, medium and upper SES, respectively. Table 1 shows *T. gondii* seropositivity rates in the study communities. The overall seroprevalence of *T. gondii* to IgG and/or IgM antibodies in the study population was 85% (333/390). Of this, IgG antibodies were detected in 84% (329/390) while IgM antibodies were present in 6% (25/390) of the study population. Twenty-one (5%) participants tested positive for both IgG and IgM antibodies. Four (1%) participants tested positive for only IgM antibodies while 79% (308/390) participants tested positive for only IgG antibodies. The remaining 15% (57/390) of the participants tested negative for all antibodies.

Test of association between *T. gondii* seropositivity and variables assessing risk factors is presented in Table 2. Although there were more female participants

Table 1. *Toxoplasma gondii seropositivity rates in the study communities*

Community	Number tested	T. gondii seropositivity, n (%)			
		IgG only	IgM only	Both IgG and IgM	IgG and/or IgM
Moree	181	146 (81)	2 (1)	13 (7)	161 (89)
Nkanfoa	119	82 (69)	1 (0·8)	6 (5)	89 (75)
Jukwa	90	80 (89)	1 (1)	2 (2)	83 (92)
Total	390	308 (79)	4 (1)	21 (5)	333 (85)

than males there was no significant association between seropositivity and gender ($P = 0.7$). Again, no item relating to meat consumption was significantly associated with *T. gondii* seropositivity (Table 2). There was also no significant association between *Toxoplasma* seropositivity and washing of hands or washing of vegetables/fruits before consumption ($P = 0.48$, $P = 0.07$, respectively).

From Table 2, variables with a significant difference between groups by the χ^2 test were included in a multivariate logistic regression analysis to identify independent risk factors for the disease. Table 3 shows a multivariate logistic regression analysis between *T. gondii* seropositivity and demographic characteristics of the participants. *T. gondii* infection was found to increase with increasing age: 10–19 years (55·8%), 20–29 years (66·7%, OR 1·59, $P = 0.3$), 30–39 years (86·2%, OR 4·95, $P = 0.008$), 40–49 years (88·7%, OR 6·25, $P < 0.001$), 50–59 years (93·5%, OR 11·42, $P < 0.001$), 60–69 years (100%, OR $9·9 \times 10^7$, $P < 0.001$), >70 years (97·0%, OR 25·38, $P < 0.001$). With respect to level of formal education, participants who had no formal education, basic school education and second cycle education were respectively 19, three and two times more likely to be seropositive compared to those who had tertiary education. SES was significantly associated with seropositivity, with participants in the low SES group being eight times more likely to be seropositive than those in the high SES group. In terms of occupation, the least seroprevalence value of 59% was observed for students/pupils while the higher seroprevalence values were observed for jobs that exposed the individuals to the soil, e.g. farming, fishing and fishmongering.

Table 4 presents a multivariate logistic regression analysis between *T. gondii* seropositivity and risk factors. There was a statistically significant relationship between *Toxoplasma* seropositivity and responses relating to the presence of cats. The highest risk factor was to have contact with the soil (OR 38·4, $P <$ 0·001), followed by owning a cat (OR 7·76, $P = $ <0·001), disposing of cat litter (OR 6·85, $P < 0.001$) and having cats around one's house (OR 2·31, $P = $ 0·04). Source of drinking water was also found to be significantly associated with *T. gondii* infection.

Table 5 shows a multivariate logistic regression analysis between various occupations of participants and their exposure to the soil. Participants who were engaged in petty trading, fishmongering, farming and fishing were at higher risks of having contact with the soil.

Analysis of soil samples from the study areas indicated that the soils were slightly alkaline for all the areas as follows: Moree (pH = 7·9, Na^+ = 1·69); Nkanfoa (pH = 7·3, Na^+ = 1·36); Jukwa (pH = 7·8, Na^+ = 0·41).

DISCUSSION

This is a report of a population-based study on *T. gondii* from the West African state of Ghana. The overall seroprevalence of 85·4% found in the present study was quite high but lower than an earlier hospital-based study in pregnant women in Accra, Ghana. That study of pregnant women of mean age of 28·1 years had a seroprevalence of 92·5% [11]. However, another hospital-based study in patients of mean age 30·2 years visiting the Korle Bu Teaching Hospital in Accra reported a lower prevalence rate of 49·7% [12]. The differences of prevalence rates in these studies may be due to the different populations studied (pregnant women, sick people and the general population) and the age differences of the study populations. Studies on *T. gondii* infection in farm animals in Ghana have indicated seropositivity of 39% in pigs, 26·8% in goats, 33·2% in sheep, and 64% in chickens [14–16]. The infection rate in the present study was comparable to findings from southern Brazil and some European countries. A seroprevalence of 84% was found in a lower SES populations

Table 2. *Test of association between* Toxoplasma gondii *seropositivity and variables assessing the risk of infection*

Factor	No. tested	Positive, n (%)	x^2	P value
Gender			0·151	0·697
Male	118	102 (86·44)		
Female	272	231 (84·92)		
Own cat			19·84	<0·001
Yes	127	123 (96·9)		
No	263	210 (79·8)		
Dispose cat litter			26·613	<0·001
Yes	170	163 (95·9)		
No	220	170 (72·3)		
Exposed to the soil			93·59	<0·001
Yes	244	241 (98·8)		
No	146	92 (63·0)		
Cats around the house			4·20	0·04
Yes	356	308 (86·5)		
No	34	25 (73·5)		
Dogs around the house			1·324	0·25
Yes	329	278 (85·5)		
No	61	55 (90·2)		
Wash fruits/vegetables before consumption			3·42	0·07
Yes	314	263 (83·8)		
No	76	70 (92·1)		
Wash hands with soap before eating			1·47	0·48
Always	126	107 (84·9)		
Sometimes	210	177 (84·3)		
Never	54	49 (90·7)		
Consumption of chicken			3·13	0·08
Yes	351	296 (84·3)		
No	39	37 (94·9)		
Consumption of mutton			1·80	0·18
Yes	294	247 (84·0)		
No	96	86 (89·6)		
Consumption of goat			2·79	0·10
Yes	318	267 (84·0)		
No	72	66 (91·7)		
Consumption of pork			0·41	0·52
Yes	116	97 (83·6)		
No	274	236 (86·1)		
Way of cooking meat			3·37	0·19
Very soft	162	138 (85·19)		
Soft	178	152 (85·39)		
Tough	20	14 (70·0)		
Consumption of meat			3·32	0·07
Yes	360	304 (84·44)		
No	30	29 (96·67)		

in Brazil [5]. Similar findings have been reported in Holland and Germany [17]. The current finding is also similar to reported seroprevalence rates of 83·7% and 60% in two different studies from the East African country of Ethiopia and 66–88% from Togo in West Africa [6, 18, 19]. The finding is, however, higher compared to reported cases from other West African countries of 63·1% in São Tomé,

32·4% and 23·9% in Nigeria, 25·4% in Burkina Faso, and 37·2%, 55·6% and 70% in the Ivory Coast [20–24].

Consistent with many reported cases of *T. gondii* infection, the current study found no significant association between gender and seropositivity. A study in Egypt in 250 human samples found no significant difference between males (34·7%) and females (35·8%)

Table 3. *Multivariate logistic regression analysis between* Toxoplasma gondii *seropositivity and demographic characteristics of participants*

Characteristic	No. tested	Positive, n (%)	Multivariate aOR (95% CI)	P value
Age group, years				
10–19	52	29 (55·77)	Reference	—
20–29	45	30 (66·67)	1·586	0·27
30–39	29	25 (86·21)	4·957	0·008
40–49	71	63 (88·73)	6·246	<0·001
50–59	77	72 (93·51)	11·421	<0·001
60–69	50	50 (100·0)	$9·969 \times 10^7$	<0·001
>70	66	64 (96·97)	25·379	<0·001
Level of formal education				
Tertiary	19	11 (57·89)	Reference	—
Second cycle	47	35 (74·47)	2·121	0·189
Basic school	163	132 (80·98)	3·097	0·025
No formal education	161	155 (96·27)	18·788	<0·001
Socioeconomic status				
High	6	3 (50·0)	Reference	—
Medium	52	34 (65·38)	1·889	0·463
Low	332	296 (89·16)	8·222	0·012
Occupation				
Student/pupil	64	38 (59·38)	Reference	—
Artisan	36	28 (77·78)	2·395	0·06
Public/civil service	32	27 (84·38)	3·695	0·017
Petty trading	99	88 (88·89)	5·474	<0·001
Retired/unemployed	35	32 (91·43)	7·298	0·002
Fisherman	35	33 (94·29)	11·289	0·002
Farmer	43	42 (97·67)	28·737	<0·001
Fishmonger	46	45 (97·83)	30·789	<0·001

aOR, Adjusted odds ratio; CI, confidence interval.

($P > 0·05$) [25]. Similar results have been reported in Ethiopia, Nigeria, and Tanzania [18, 22, 26]. However, a higher risk of infection for men than women was observed for the hospital-based study in Accra, Ghana [12]. The higher risk of *Toxoplasma* infection for men has been attributed either to contact with soil or to improper hygiene and thus a difference between men and women might appear in populations with high exposure to soil [27]. In the present study, however, both men and women were at risk of being exposed to the soil as indicated by their professions (Table 5). Thus, fishmongering (for women), farming and fishing (for men) were the highest risk factors for the infection (Table 3).

In the present study we found an increasing pattern of seropositivity with increasing age group which was also observed in Egypt where seropositivity increased from 11% in participants aged <20 years to 42% in those aged >50 years [25]. Similarly, a study from Ethiopia found a seropositivity of 64·0% in the 15–19 years age group and 94·1% in the 30–35 years age group [6]. Another report from Tanzania found

that *T. gondii* infection increased by 1·4% for a 1-year increase in age [28]. From the city of Maiduguri, Nigeria, there was a positive correlation between the mean antibody titre and the age of subjects, with seroprevalence being highest in subjects aged 51–60 years and lowest in subjects aged <21 years [22]. The same trend was also observed in The Netherlands and Japan [2], Rio de Janeiro state, Brazil [5], France [27], and the UK [29]. The increase in seropositivity with age may be attributed to the fact that since *T. gondii* infection is strongly associated with contact with soil, the longer an individual lives the more likely they are to be exposed to the soil. It might also be attributed to the reason of decreased cell-mediated immunity with advanced age. Surprisingly, a study in a rural area of the Minas Gerais state of Brazil found no significant difference between seropositivity and age groups. That finding suggested that a significant proportion of the population acquired toxoplasmosis at an early age [13]. We also observed an increasing trend of seropositivity in the lower SES group in the present study.

Table 4. *Multivariate logistic regression analysis between* Toxoplasma gondii *seropositivity and risk of infection*

Factor	No. tested	Positive, n (%)	Multivariate aOR (95% CI)	P value
Own/keep cat				
No	263	210 (79·8)	Reference	—
Yes	127	123 (96·9)	7·76	<0·001
Dispose cat litter				
No	220	170 (72·3)	Reference	—
Yes	170	163 (95·9)	6·85	<0·001
Contact with the soil				
No	146	92 (63·0)	Reference	—
Yes	244	241 (98·8)	38·39	<0·001
Cats around the house				
No	34	25 (73·5)	Reference	—
Yes	356	308 (86·5)	2·31	0·04
Source of drinking water				
Sachet/bottled	68	50 (73·53)	Reference	—
Pipe borne	302	263 (87·09)	2·428	0·007
Borehole	11	11 (100·0)	$6·646 \times 10^7$	<0·001
Well water	3	3 (100·0)	$6·646 \times 10^7$	<0·001
River water	6	6 (100·0)	$6·646 \times 10^7$	<0·001

aOR, Adjusted odds ratio; CI, confidence interval.

Table 5. *Multivariate logistic regression analysis between various occupations of participants and their exposure to the soil*

Occupation	No. tested	No. exposed to soil, n (%)	Multivariate aOR (95% CI)	P value
Student/pupil	64	21 (32·8)	Reference	—
Public/civil service	32	16 (50)	2·05	0·11
Artisan	36	19 (52·7)	2·29	0·53
Retired/unemployed	35	20 (57·1)	2·73	0·20
Petty trading	99	63 (63·6)	3·58	<0·001
Fishmonger	46	34 (73·9)	5·80	<0·001
Farmer	43	39 (90·7)	19·96	<0·001
Fisherman	35	32 (91·4)	21·84	<0·001

aOR, Adjusted odds ratio; CI, confidence interval.

Such a trend was reported in the northern Rio de Janeiro state of Brazil, where the age-adjusted sero-prevalences were 84%, 62% and 23% in the lower, middle and upper SES levels, respectively [5]. In the USA, *Toxoplasma* infection was considered an infection associated with poverty [1, 30]. It has been suggested that individuals of lower SES may be related to occupations with greater soil exposure and therefore be at higher risk of becoming infected [1].

We found a statistically significant association between *T. gondii* seropositivity and all variables assessing the presence of cats with a 2·3- to 38·4-fold increased risk of infection. The role of the cat in the transmission of toxoplasmosis has been established and many studies around the world have associated

T. gondii infection with cats [3]. Ayi *et al.* [11] found exposure to cat faeces to be the major risk factor for *T. gondii* infection in the study of pregnant women in Accra, Ghana. A study to assess seroprevalence and risk factors of *T. gondii* in pregnant women in southwestern Ethiopia significantly associated having cats at home with *T. gondii* antibodies [6]. Another study from Ethiopia observed that individuals with a known history of association with cats were 5·3 times more likely to be seropositive than those with no history of such association [18]. In the Democratic Republic of São Tomé and Principe, children who had a history of raising cats showed significantly higher seroprevalence than those who did not [20]. With respect to occupation, we observed that

jobs which tended to expose the individuals to the soil were strongly associated with the infection. Such an observation was also made in the USA where soil-related occupations were associated with *T. gondii* ser-opositivity [1]. Other reports have associated the infection with certain occupations. Swai & Schoonman [26] reported from Tanzania that seroprevalence of *Toxoplasma* antibodies was significantly higher in livestock keepers and abattoir workers. Again, in the São Tomé study, children whose parents were un-skilled workers showed significantly higher seropreva-lence than those of semi-skilled and skilled workers. Fishermen after their fishing expedition normally sit by the seashore to mend their nets, exposing them-selves to a greater contact with the soil. This might ac-count for the observation that fishermen are more susceptible to *T. gondii* infection in the present study. Fishmongers were also at a high risk of being exposed to the soil as our analysis showed that fishing, farming and fishmongering were, respectively, the jobs that had the highest risk of exposing indivi-duals to the soil (Table 5).

In the present study, we found that higher level of education was significantly associated with consistent reduction in the risk of infection. Lower levels of edu-cation have been associated with increased risk for toxoplasmosis in many epidemiological studies. In the USA and Chile, two separate studies found that participants with less than college education were significantly associated with *T. gondii* infection [1, 30]. Lower levels of education are usually associated with lower SES and may be related to employment in jobs with greater soil exposure. Our finding sug-gested that meat consumption was not a potential source of *T. gondii* infection in this study population. There was no significant association between seroposi-tivity and participants who responded 'yes' to con-sumption of meat ($P = 0.10$) nor was there any significant association between seropositivity and any variables relating to meat consumption (Table 2). The reasons for this observation may be attributed to the exhaustive way of cooking meat by the participants. Nearly 94% of participants who reported consuming meat also reported that they always cook the meat until it is at least soft before consumption. However, *Toxoplasma* tissue cysts con-tained in meat or meat-derived products have been shown to serve as important sources of infection for humans [30–33], and that the risk of acquiring the in-fection via meat sources depends on cultural and eat-ing habits in different human populations [31].

In conclusion, *T. gondii* seroprevalence in the study population was high. The major risk factors asso-ciated with *T. gondii* seropositivity in the present study were old age, contact with soil, presence of a cat, lower level of formal education, and lower SES. Consumption of meat was not associated with the in-fection and seemed to suggest that contamination by sporulated oocysts may be the major source of trans-mission of toxoplasmosis in the study population. Fishing and fishmongering were highly associated with *T. gondii* seropositivity suggesting that the sea-shore may serve as a good ground for sporulation and survival of *Toxoplasma* oocysts. Further studies are needed to verify the viability of the parasites at the seashore.

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DECLARATION OF INTEREST

None.

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