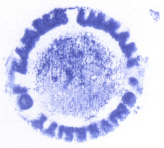
SEROPREVALENCE OF IgG ANTI-TOXOPLASMA ANTIBODIES IN HIV POSITIVE ADULT ZAMBIANS ATTENDING THE ANTIRETROVIRAL THERAPY CLINIC AT THE UNIVERSITY TEACHING HOSPITAL

by

Chomba Sinyangwe

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Medical Parasitology



The University of Zambia
June, 2009

DECLARATION

I declare that this dissertation represents my own work, and that it has not previously been submitted for a degree, diploma or other qualifications at this or another University.

Signature.....

Date.....26/06/09.....

CERTIFICATE OF APPROVAL

This dissertation of Chomba Sinyangwe has been approved as fulfilling the partial requirements for the award of the Master of Science in Medical Parasitology of the University of Zambia.

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Date.....8/07/09.....

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ABSTRACT

Introduction

Toxoplasma gondii is an intracellular protozoan parasite, and causes zoonotic infection. It is estimated that more than one billion people world wide are affected by the parasite. *Toxoplasma* encephalitis has become one of the most frequent opportunistic infection in HIV infected patients. The diagnosis of toxoplasmosis is complicated and involves the use of multiple serological and radiological investigations in combination with the clinical signs and symptoms of the patient. Toxoplasmosis thus poses a serious diagnostic challenge even for health facilities with advanced diagnostic equipment. In resource-poor settings such as Zambia the diagnosis of toxoplasmosis is based mostly on clinical presentation only requiring the clinician to have a high index of suspicion for toxoplasmosis. Despite the importance of this condition, the seroprevalence of toxoplasmosis among HIV positive Zambians had not previously been fully investigated.

Objective

To determine the seroprevalence and factors associated with IgG anti-*toxoplasma* antibodies among HIV positive adult Zambians attending the ART clinic at the UTH.

Method

A Prospective cross-sectional hospital-based study was carried out on 156 HIV positive adult Zambians (18 years and above) attending ART clinic at UTH. 3mls of sera from 156 HIV positive persons attending the ART clinic at UTH were analysed for the presence of IgG anti-*toxoplasma* antibodies using commercial ELISA assays (Genesis Diagnostic Toxoplasma IgG Enzyme Immunoassay, Cambridgeshire, UK and Biotec Toxoplasma IgG Enzyme Immunoassay, Sulffolk, U.K). A questionnaire was used to collect data on sex, age, HIV status, CD4 count, residential area, education level, occupation and work, food habits and socioeconomic variables.

Results

The prevalence of IgG anti-toxoplasma antibodies was 17.9%. Females constituted 55% of the population. The chi-square test showed a statistically significant association between IgG anti-toxoplasma serostatus and blood transfusion (p= 0.0152). This result was upheld with multiple logistic regression (OR=0.307, confidence interval 0.114-0.826). There was also a statistically significant association between age and IgG anti-toxoplasma seropositivity (p=0.0374). However, this result did not hold under multiple logistic regression.

Conclusion

The seroprevalence of IgG anti-toxoplasma antibodies among Adult HIV positive patients at UTH is much higher than previously reported. A large proportion of HIV positive patients attending the ART clinic at UTH and are at risk of toxoplasmosis may not be receiving co-trimoxazole prophylaxis. There was a significant association between blood transfusion and IgG serostatus shown in this study. This suggests that screening of donor blood for toxoplasmosis may be important.

This dissertation is dedicated to the memory of my late father Mr. Benson Ngolwe Sinyangwe. MHSRIP

AKNOWLEDGEMENTS

I would like to acknowledge the following persons without whose contribution this study would not have been possible. My supervisors, Dr Cecilia Shinondo and Dr James Chipeta. Thank you for giving me your time and attention. Your guidance and encouragement through out this study from inception to end is immensely appreciated. Dr. Tendei M'soka without whose generous offer of laboratory space I would not have been able to conduct this study. Mr David Rutagwera, for his assistance with the laboratory component of the study. My family for their support and encouragement. The study participants for giving me the opportunity to learn from them and in my own small way contribute to the body of scientific knowledge. My rock Linda Mpongwe Kangwa for being understanding and helping me to balance my academic and social life. Continue being good always. Lastly but not least, God almighty for the many blessings in my life.

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LIST OF ABREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
BAL	Bronchioaveolar Lavage
CD4	Cluster of Differentiation 4
CSO	Central Statistical Office
ELISA	Enzyme Linked Immunosorbent Assay
HAART	Highly Active Antiretroviral Therapy
HCL	Hydrochloric Acid
HRP	Horseradish Peroxidase
HIV	Human Immunodeficiency Virus
IAAT	Immunosorbent Agglutination Assay Test
IFA	Indirect Fluorecent Antibody Assay
IgG	Immunoglobulin G
OD	Optical Density
PCR	Polymerase Chain Reaction
TMB	Tetramethyl benzidine
UNAIDS	United Nations AIDS Agency
UTH	University Teaching Hospital
VCT	Voluntary Counseling and Testing
ZSBS	Zambia Sexual Behaviour Survey
ZDHS	Zambia Demographic Health Survey

1. INTRODUCTION

Toxoplasmosis is a zoonosis caused by the obligate, intracellular parasite, *Toxoplasma gondii.* It is a coccidian protozoa belonging to the phylum Apicomplexa, class Sporozoasida, order Eucoccidiida that exists in three forms: an oocyst that produces sporozoites, a proliferative form called tachyzoite and a tissue cyst that has an intracystic form termed a bradyzoite (Boyer *et. al*, 2004). *T. gondii* has a ubiquitous distribution, being able to develop in a wide variety of vertebrate hosts but its definitive host is the domestic cat and certain other members of the cat family. The parasite was first observed in 1908 and the first human case recognized in 1923 (John & Petri, 2006). Hutchinson (1965) was the first to demonstrate the role of domestic cats in the transmission of the parasite.

It is estimated that more than one billion people world wide are infected by the parasite (Switaj *et. al*, 2005). Risky behaviour facilitating the transmission of toxoplasmosis include consumption of undercooked cyst-containing meat, consumption of un-pasteurized milk from infected animals, ingestion of *Toxoplasma* oocysts from contaminated vegetables and fruits, soil or drinking water. Oocysts may also be ingested from contaminated hands that have come in direct contact with infected cat feaces (Boyer *et. al*, 2004). Toxoplasmosis may also rarely be acquired through organ transplant and blood transfusion (Holliman, 2003).

Transplacental transmission of toxoplasmosis occurs in humans resulting in congenital disease in the foetus with serious consequences such as

hydrocephalus, microcephaly, blindness and mental retardation (Boyer *et. al*, 2004).

The advent of the Human immunodeficiency Virus (HIV) pandemic brought previously rare or relatively unimportant diseases such as toxoplasmosis to prominence. Toxoplasmic encephalitis has become one of the most frequent opportunistic infection and the most common cause of focal brain lesion complicating the course of acquired immune deficiency syndrome (AIDS). If untreated, toxoplasmic encephalitis is uniformly fatal (Hoffman, 2005). Most cases result from the reactivation of a latent infection associated with the impairment of the individual's immunity. The clinical features of the disease include fever, persistent headache, and deterioration of mental status, focal neurological signs, retinochoroiditis and pulmonary disease. Disseminated disease involving the central nervous system, heart, liver and lungs has been described in postmortem findings (Holliman, 2003).

Although cerebral toxoplasmosis has become rarer as a result of the availability of Highly Active Antiretroviral Therapy (HAART) it still remains the most important neurological opportunistic infection in HIV patients. Typically cerebral toxoplasmosis is now diagnosed in HIV patients who are not aware of their HIV status or in those not under regular routine care (Hoffman, 2005). This is very likely in our setting where health care services are not widely available to all coupled with a low uptake of voluntary counseling and testing (VCT) service in the Zambian society, resulting in a large number of patients first seeking ART services late in the course of HIV infection.

The diagnosis of toxoplasmosis is complicated and involves the use of multiple serological and radiological investigations in combination with the clinical signs and symptoms of the patient. Toxoplasmosis thus poses a serious diagnostic challenge even for health facilities with advanced diagnostic equipment. In resource-poor settings such as Zambia the diagnosis of toxoplasmosis is based mostly on clinical presentation only requiring the clinician to have a high index of suspicion for toxoplasmosis. Despite the importance of this condition, the seroprevalence of toxoplasmosis among HIV positive Zambians had not previously been fully investigated.

This study determined the seroprevalence of IgG anti-toxoplasma antibodies using commercial ELISA assays (Genesis Diagnostic Toxoplasma IgG Enzyme Immunoassay, Cambridgeshire, UK and Biotec Toxoplasma IgG Enzyme Immunoassay, Sulffolk, U.K).

1.1 Background

1.1.1 *HIV Situation*

Zambia is one of the countries in sub-Saharan Africa that is highly impacted by the HIV pandemic. Latest statistics estimate the prevalence of HIV in the Zambian adult population at 14.3% with the urban areas generally showing a higher prevalence than the rural areas (CSO, 2008). It is estimated that over 1 200 000 Zambians are living with the HIV virus the majority of whom do not know their HIV status as the uptake of VCT in the country is low with estimates putting it at between 12% and 15% (CSO, 2005).

Prompted by this high burden of HIV, the Zambian government with support from cooperating partners has since 2005 made ART services in public hospitals and clinics throughout the country accessible to the general population free of charge. This has led to a surge in the numbers of persons enrolled on HAART.

However, despite the widespread availability of ART services, Zambians continue to suffer from AIDS due to the low uptake of VCT thus predisposing this large number of individuals to opportunistic infections such as toxoplasmosis usually as the first manifestation of AIDS.

1.1.2 *Toxoplamosis*

Toxoplasmosis may be acquired mainly through ingestion of tissue cysts from undercooked beef, lamb, or pork, ingestion of oocysts from soil, milk, water, or vegetables. Toxoplasmosis can also be transmitted transplacentaly when primary infection occurs in a pregnant woman resulting in congenital disease in the newborn with serious consequences such as hydrocephalus, microcephaly, convulsions, blindness and mental retardation. Other less common routes include inhalation of oocysts, contaminated blood transfusions, organ transplants, and accidental inoculation acquired in the laboratory. All the three forms of the parasite exist in members of the cat family, who are the definitive hosts. When a cat becomes infected, the organism undergoes sexual reproduction in its intestine. As a consequence, a cat sheds millions of noninfectious unsporulated oocysts in its feces. Sporulation occurs in the environment in the next 3-4 days at room temperature. After sporulation, the oocyst becomes infective (sporozoite) for at least a year. Ingestion of the sporulated oocyst results in an acute infection (Wu and Garcia, 2006).

The acute infection is typified by tachyzoites that invade and proliferate in almost any type of mammalian cell with the exception of non-nucleated erythrocytes. As the tachyzoites enter the cell, they become vacuolated and undergo reproduction via endodyogeny. In this process, two daughter cells are formed within the parent parasite, which becomes destroyed with the host cell as the daughter cells are released. When the organism reaches the eye through the bloodstream, depending on the host's immune status, a clinical or subclinical focus of infection

begins in the retina. As the host's immune system responds and the tachyzoites convert themselves into bradyzoites, the cyst forms. The cyst is extremely resistant to the host's defenses, and a chronic latent infection ensues (Wu and Garcia, 2006).

Cerebral toxoplasmosis is the most common and important manifestation of toxoplamosis. Previously cerebral toxoplasmosis was considered to be a rare disorder that was more common in children than adults, however with the emergence of HIV the incidence of cerebral toxoplasmosis has increased significantly, causing brain abscesses and encephalitic toxoplasmosis. Cerebral toxoplasmosis almost always results from the reactivation of the latent infection (Hoffman, 2005). The clinical manifestations are seen in proportion to declining CD4 count, usually occurring in individuals with CD4 T cell count less than 100 cells/μl (Hoffman *et al.*, 2007). The median number of CD4 T lymphocytes in AIDS patients with acute toxoplasma encephalitis in one study was 60 cell/μl or less, with approximately 80% of the patients having a CD4 count of less than 100 cells/μl (John & Petri, 2006). It has been noted that between 25% and 50% of AIDS patients who are seropositive for *T. gondii* will develop cerebral toxoplasmosis, frequently as the first manifestation of AIDS in areas where seroprevalence rates are high (Wong & Remington, 2005). This clearly demonstrates the importance of this disease in AIDS patients.

Another pathology caused by *Toxoplasma gondii* in AIDS patients is ocular toxoplasmosis accounting for 1–3% of ocular infections in patients with AIDS

(UNAIDS, 2002). Disseminated disease affecting the lungs, heart, the liver and spleen has also been documented (John & Petri, 2006).

2. STATEMENT OF THE PROBLEM

Toxoplasmosis is a common and important opportunistic infection among HIV positive persons world wide (Hoffman, 2005). In AIDS patients, toxoplasmosis can be a life-threatening infection occurring commonly as a reactivation and seen most frequently as encephalitis. Toxoplasmosis is the most frequent cause of focal intra-cerebral lesions in AIDS patients (Hoffman *et al.,* 2007). With an HIV prevalence of 16% (CSO, 2005)b among Zambians, toxoplasmosis therefore poses a serious threat to the approximately 1 200 000 Zambians living with HIV.

In the last few years HAART has become more widely available and accessible to ordinary Zambians through the Zambian Government's free ART policy. However, HIV positive Zambians have continued to suffer from AIDS due to among other factors the low uptake of VCT services. This has led to a large number of patients first seeking ART services late in the course of HIV infection thus making them susceptible to opportunistic infections such as toxoplasmosis.

Despite this serious situation, data on the seroprevalence of toxoplasmosis in the general population and among HIV positive Zambians is scanty. The extent of the problem of toxoplasmosis among HIV positive Zambians has therefore not been fully determined.

The only published study done on toxoplasmosis among HIV positive Zambians found a seroprevalence rate of 4% (Holliman *et al.*, 1991). This however contrasts sharply with the seroprevalence rate of 11% among HIV negative Zambians reported in the same study and 23% reported in another study among pregnant Zambian women (Leblebicioglu and Hokelek, 2006). In their study,

Holliman et al (1991) also reported a seroprevalence of toxoplasmosis among Ugandan HIV positive persons of 34%. However, no explanation was given for the difference in seroprevalence rates between Ugandan and Zambian HIV positive persons. Indeed it was noted that the cultural, traditional practices, feeding habits and climatic conditions in the two countries are alike. Further, the risk factors for the transmission of toxoplasmosis such as consumption of undercooked beef or pork are prevalent in Zambian communities. It is therefore important to undertake a study among HIV positive Zambians taking into consideration factors such as sex, age, social status, feeding habits, clinical diagnosis and principal presenting symptoms of the study participants.

3. STUDY JUSTIFICATION

The diagnosis of toxoplasmosis is complicated and may involve the use of multiple investigative techniques (serological, molecular and radiological) in combination with clinical signs and symptoms of the patient. Diagnosis of this condition therefore poses a serious challenge even in centers with advanced diagnostic equipment. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases (Hill and Dubey, 2002).

Given the difficulty associated with diagnosing cerebral toxoplasmosis and the high risk of developing the disease in HIV positive persons with the resulting serious consequences, in a resource poor setting like Zambia clinicians must have a high index of suspicion for toxoplasmosis in order to make a diagnosis of the condition. A study to determine the seroprevalence of toxoplasmosis among HIV positive Zambians is therefore of absolute importance. This study will assist in the management of HIV positive Zambians by documenting the burden of this infection and thus raise clinicians' index of suspicion for toxoplasmosis. The study will further underscore the need for current Ministry of Health recommendation for co-trimoxazole prophylaxis in AIDS patients in Zambia for the prevention of toxoplasma encephalitis and other susceptible opportunistic infections.

Finally the study will help reduce the widely perceived underdiagnosis of toxoplasmosis (Conlon, 1988; Fleming, 1990; Canning, 1990) and thus improve the treatment of this condition in the Zambian AIDS patient.

4. LITERATURE REVIEW

The incidence of toxoplasmosis in patients infected with HIV depends mainly on the existence of latent anti-*toxoplasma* antibodies in the population affected (Holliman *et. al.*, 1990). In the general population seroprevalence studies on toxoplasmosis have been conducted in various geographical areas and show wide variations. In the United States of America serological studies have demonstrated that 15% to 68% of adults are seropositive for *Toxoplama gondii* (Pauwels *et al.*: 1992 Zufferey *et al.*, 1993). In France, 93% of Parisian women of childbearing age showed latent *Toxoplasma gondii* infection (John, and Petri, 2006). A study of 335 individuals in Venenzuela found a seroprevalence rate of 49.8% similar to the rate in a Brazilian study of 50% (Chacin-Bonilla *et al.*,2003: Ramirez *et al.*, 1997).

Information on the seroprevalence of toxoplasmosis in the general population in Africa is not widely available but also shows variations in the different geographical areas. One paper gives the following prevalence rates of toxoplasmosis among pregnant women in Africa: The rate of positive antibody titers is 81% in the Central African Republic, 48% in Tanzania and more than 75% in Ethiopia. This paper gives the prevalence in Zambia among pregnant women as 23% (Leblebicioglu & Hokelek, 2006). Holliman *et al* (1991) on the other hand, found a seroprevalence of 11% among HIV negative adult Zambians. A study among South African women in Kwa-Zulu Natal province found a seroprevalence rate of 39% (Moonasar *et al.*, 2001). The differences observed in prevalence rates of toxoplasmosis are attributed to geographic differences and

feeding habits but these reasons do not sufficiently explain the wide variations observed.

Seroprevalence studies of anti-*toxoplasma* antibodies among HIV patients have also been conducted in various geographical regions. The seroprevalence rates in this group are closely related to the rates in the general population. In a Malian study the seroprevalence of IgG and IgA *toxoplasma*-specific antibodies was 60% in AIDS patients and 22.6% in HIV positive individuals (Maiga *et al.*, 2001). In Democratic Republic of Congo the Indirect Immunofloresence antibody test showed seroprevalence of toxoplasmosis in AIDS patients of 33% (De Clercq *et al.*, 1986). Toxoplasmosis is also said to account for most cases of intracranial mass lesions among HIV positive individuals in Kwa Zulu Natal, South Africa (Bhigjee, 2005).

The only published study on toxoplasmosis among HIV positive Zambians showed a seroprevalence rate of only 4% in contrast to 34% found among HIV positive Ugandans in the same study (Holliman, 1991). The authors of this study did not propose an explanation for the difference in the seroprevalance rates between HIV positive Zambians and Ugandans and noted that the feeding habits, climate, cultural and traditional practices in the two countries are similar. A major weakness in this study was the non-collection of socioeconomic and demographic variables apart from age and sex. These variables are important as they can help explain the differences in the seroprevalence of toxoplasmosis among populations. It has been shown in some studies that poor socioeconomic

conditions are a risk factor for exposure to toxoplasmosis (Garcia *et al.*, 2004). The CD4 count of the study participants was also not collected in this study.

While the seroprevalence rates of toxoplasmosis in HIV positive persons are closely related to the rates in the general population, the nature of this relationship is unclear. A study in the Czech Republic showed a higher seroprevalence (42.8%) of toxoplasmosis among HIV positive persons than in the general population (30.2%). This study also showed that HIV positive persons are at increased risk of acquiring toxoplasmosis than the general population (Kodym *et al.*, 2006). Other studies also indicate higher Toxoplasma prevalence in HIV positive patients compared to that in HIV negative individuals. They include the following prevalences for HIV positive versus HIV negative persons: 30.9%–36.7% vs. 26.7% in Mexico (Botto de los Bueis *et al.*, 1998), 38.8% vs. 20.8% in Nigeria (Uneke *et al.*, 2003), and 67.8% vs. 30.9% in India (Meisheri *et. al.*,, 1997). Other studies have shown similar seroprevalence rates of toxoplasmosis in HIV positive and the general populations. In another Mexican study, the seropositivity of IgG among 92 HIV positive persons was found to be 50%, corresponding to prevalence rates in the general population (Ramirez *et al.*, 1997). Similar results were obtained in a study in Switzerland (Zuffery *et al.*, 1993). In the Zambian study however a higher seroprevalence rate was found in HIV negative than in HIV positive study participants: 4% vs. 11% (Holliman *et al.*, 1991). This difference was however not statistically significant.

The diagnosis of toxoplasmosis is complicated and involves the use of multiple investigative techniques in combination with clinical signs and symptoms of the

patient. The diagnosis of toxoplasmosis therefore poses a serious challenge even in facilities with advanced diagnostic equipment. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. Toxoplasmosis in fact mimics several other infectious diseases (Hill & Dubey, 2002). The methods used to aid diagnosis include serology, culture, radiology, histology, and trials of therapy, although none of these is absolutely diagnostic. To date, the only means by which definitive diagnosis can accurately be made is by brain biopsy with microscopic identification of tachyzoites (Holliman *et al.,* 1991). In advanced centers the diagnosis of toxoplasmosis is usually made by a combination of Computed Tomography findings, serological findings and the response to empiric anti-*toxoplasma* therapy (Holliman *et al.,* 1991). The Sabin and Feldman dye test is still the "gold standard" serological test. It is however technically demanding and is rarely performed outside reference laboratories. Other serological tests such as the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test (IAAT) are more commonly used.

The diagnosis of toxoplasmosis has also been achieved using polymerase chain reaction (PCR) to amplify toxoplasma deoxyribonucleic acid. PCR has enabled detection of *T. gondii* DNA in brain tissue, cerebrospinal fluid (CSF), vitreous and aqueous fluids, bronchoalveolar lavage (BAL) fluid, and blood in patients with AIDS (Montoya, 2002).

In this study the ELISA method was used to determine the seroprevalence of anti-*toxoplasma* IgG antibodies among HIV positive Zambians. The ELISA method has compared favourably in terms of specificity and sensitivity to the "gold standard" Sabin-Feldman dye test (Asai *et al.*,1992).

5. OBJECTIVES

5.1 General objective

- To determine the seroprevalence and factors associated with of IgG anti-toxoplasma antibodies in HIV positive adult Zambians attending the ART clinic at the UTH in Lusaka.

5.1.1 *Specific objective*

a) To determine the seroprevalence of anti-*toxoplasma* IgG antibodies among adult HIV positive Zambians attending the ART clinic at the UTH in Lusaka

b) To determine whether seroprevalence of IgG anti-toxoplasma among HIV positive adult Zambians attending the ART clinic at UTH is associated with age, gender, CD4 count, meat in diet, socioeconomic variables (level of education, residential area density, employment status, type of house, level of income, and source of drinking water), keeping cats, blood transfusion, handling of dirt and selected clinical symptoms.

6.0 RESEARCH QUESTION

What is the seroprevalence of anti-*toxoplasma* IgG antibodies among HIV positive adult Zambians attending the ART clinic at the UTH and what is it associated with?

7. MATERIALS AND METHODS

7.1 Study site

The University Teaching Hospital at Lusaka is the highest and largest referral hospital in Zambia, admitting referred cases from across the country and providing primary care to the surrounding communities. The Adult Antiretroviral Therapy (ART) clinic at the hospital has over 3000 patients enrolled onto the ART programme with approximately 2000 on Highly Active Antiretroviral Therapy (HAART).

7.2 Study design

Prospective hospital-based cross-sectional study

7.3 Study population and sample size

Study participants were selected from among adult persons attending the antiretroviral therapy clinic at the University Teaching Hospital by the simple random sampling technique. This was done to ensure equal chance of selection of any of the HIV positive adult Zambians attending the Adult ART clinic at UTH in the study sample.

Sample size was estimated by use of a sample size tables as indicated by Browner *et al.* (2001). With the following:

Confidence level = 95% Total Width = 0.15

Expected proportion = 0.11

Sample size was estimated at **136**

The number of subjects enrolled in the study was **156**

7.4 Variables to be measured

The independent variable measured was anti-toxoplasma IgG sero-status. The dependent variables measured were: age, gender, CD4 count, meat in diet, level of education, residential area density, employment status, type of house, level of income, source of drinking water, keeping cats, blood transfusion, handling dirt and selected clinical symptoms (chronic or severe headache, convulsions, weakness of part of the body).

7.5 Inclusion criteria

Only consenting HIV positive adults who were above18 years of age, attending the adult ART clinic at the University Teaching Hospital were included in the study

7.6 Exclusion criteria

Patients not confirmed by laboratory testing for HIV, less than 18 years of age and those not consenting to participate were not included in the study.

7.7Collection of serum

From each study participant, 3 mls of peripheral blood was collected in plain sterile bottle and serum extracted by centrifugation for the analysis of anti-toxoplasma IgG using the commercial ELISA assay (Genesis Diagnostic Toxoplasma IgG Enzyme Immunoassay, Cambridgeshire, UK and Biotec Toxoplasma IgG Enzyme Immunoassay, Sulffolk, U.K). The assay was performed as directed by the manufacturer.

7.8 Principle of the ELISA test

Purified *toxoplasma gondii* antigen was coated on the surface of microwells. Diluted patient serum is added to the microwells, and the anti-*toxoplasma* IgG specific antibody, if present binds to the antigen. All unbound materials were washed away. Horse Radish Peroxidase (HRP) conjugate was added, which bound to the antibody-antigen complex. Excess HRP conjugate was washed off and a solution of TMB reagent was added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the colour generated was proportional to the amount of IgG specific antibody present in the sample. The results were read by a microwell reader compared in a parallel manner with calibrator and controls.

7.9 ELISA assay procedure

The desired number of coated wells is placed into the holder. A 1:100 dilution of test samples was prepared by adding 5 micro liters of the sample to 0.5 milli liters of Sample Diluent and mixed well. 100 micro millliliters of diluted sera and 15, 50, 150 micro liters standards and the positive and negative controls were dispensed into appropriate wells. The preparation was placed in an incubation bag incubated at room temperature Celsius for 20 minutes. Direct sunlight and heat was avoided during incubation. At the end of incubation the micro wells were washed using wash buffer diluted in distilled water 1:9. The washing was done in five cycles using an automatic micro plate washer. The wells were then blotted on absorbent paper. 100 micro liters of Conjugate were dispensed into each well and incubated for 20 minutes at room temperature in an incubation bag. After the

incubation the wells were again washed in five cycles with wash buffer using an automated micro plate washer. Using a repeating dispenser, 100 micro liters of TMB Substrate into each well and the plate incubated for ten minutes at room temperature in an incubation bag. After 10 minutes 100 micro liters of stop solution was added to each micro well. The optical densities were then read immediately in a micro plate reader. The OD was read at 450 nm.

The optical densities of the standards and controls were plotted against their respective concentrations and a line drawn to join the points. The concentrations of the unknown diluted sera were read from this graph. Concentration below 15 IU/milli liters considered negative; concentration above 15IU/micro liters were considered positive for anti-toxoplasma IgG.

7.10 Data collection tools

A questionnaire was administered on all study participants. The following variables were taken into account: age, gender, CD4 count, meat in diet, socioeconomic variables (level of education, residential area density, employment status, type of house, level of income, and source of drinking water), keeping cats, blood transfusion, handling of dirt and selected clinical symptoms. The questionnaire was piloted for one week at UTH prior to commencement of the study. Research assistants were trained in questionnaire administration to ensure standardization.

A laboratory form was used to capture data obtained from laboratory serological testing using ELISA performed on the serum samples obtained from the study participants.

Data on the questionnaires and laboratory forms was entered in EPI info 6.0 by double entry method and cleaned for errors.

7.11 Statistical analysis

Statistical analysis was performed with SAS statistical software package (SAS Institute, Inc., Cary, NC, USA). The prevalence, means and ranges were calculated. The chi square test and multivariate logistic stepwise regression analysis were used to assess the association of toxoplasma infection with the variables of interest. A P value of less than 0.05 was considered statistically significant.

8. RESULTS

A total of 156 HIV positive adult Zambians attending the anti-retroviral therapy clinic at the University teaching Hospital were enrolled in the study. Females constituted 55% of the population. The prevalence of IgG anti-toxoplasma antibodies was 17.9%, with equal contribution from males (8.71%) and females (8.71%). Age among the subjects ranged from 23 to 67 years. The average age was 40.3 years (SD: +/-8.61 years). The majority of subjects (61%) were below 49 years of age.

As shown in figure 1, of the subjects who tested positive for anti-toxoplasma IgG, 13 were 35 years of age and below, 10 were between 35 and 49 years old while 5 were above 49 years old. In contrast, for those who tested negative the majority were above 49 years old

Figure 2 shows that the majority of subjects among those who tested positive for anti-toxoplasma IgG were from high (15/28) and medium (10/28) density areas. Those who tested negative for anti-toxoplasma IgG also showed a similar pattern of distribution.

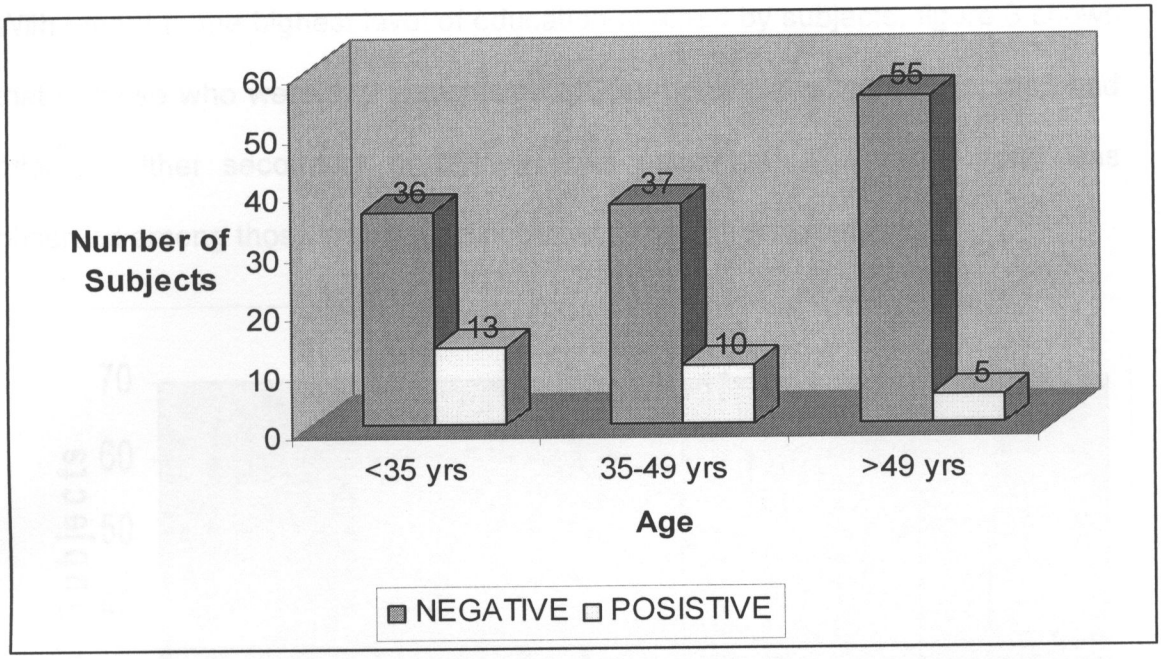

Figure 1 Age distribution of subjects by their IgG anti-toxoplasma serostatus. Anti-toxoplasma IgG serostatus was determined by ELISA.

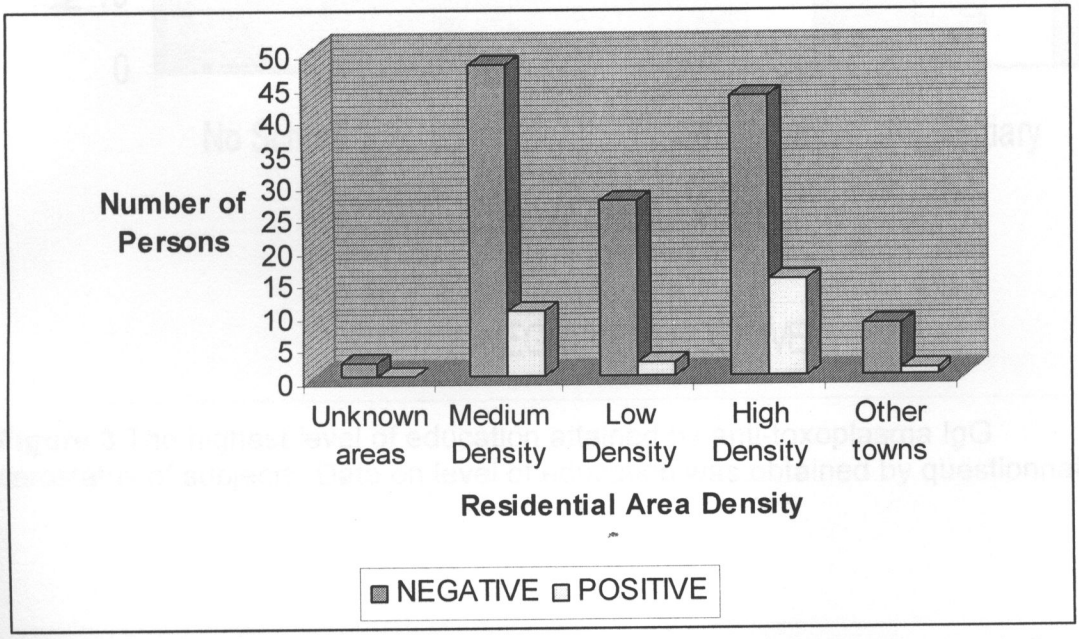

Figure 2 Residential area densities of subject's residences by their IgG anti-toxoplasma serostatus.
Note: Residential area density classification was based on demographic map of Lusaka district (Lusaka City Council, 2002).

With regard to the highest level of education attained by subjects, figure 3 shows that of those who were anti-toxoplasma IgG seropositive, a large proportion had attained either secondary or tertiary level education. The same trend was observed among those who tested negative for IgG anti-toxoplasma.

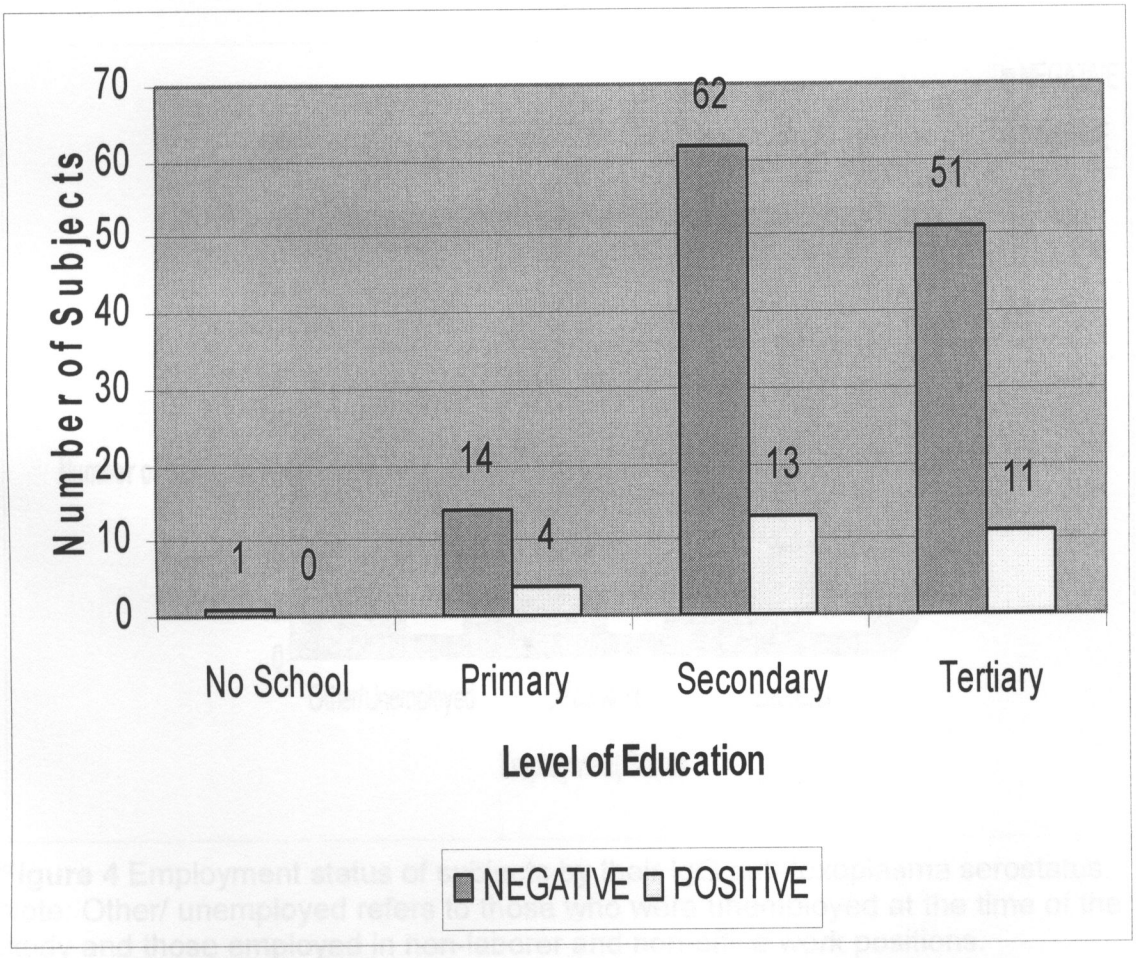

Figure 3 The highest level of education attained by anti-toxoplasma IgG serostatus of subjects. Data on level of education was obtained by questionnaire.

Laborers accounted for only a small proportion (5/28) of subjects who tested positive for anti-toxoplasma IgG as shown in figure 4. The majority of subjects who tested seropositive were either unemployed or had other employment. This pattern was unchanged among those who tested negative.

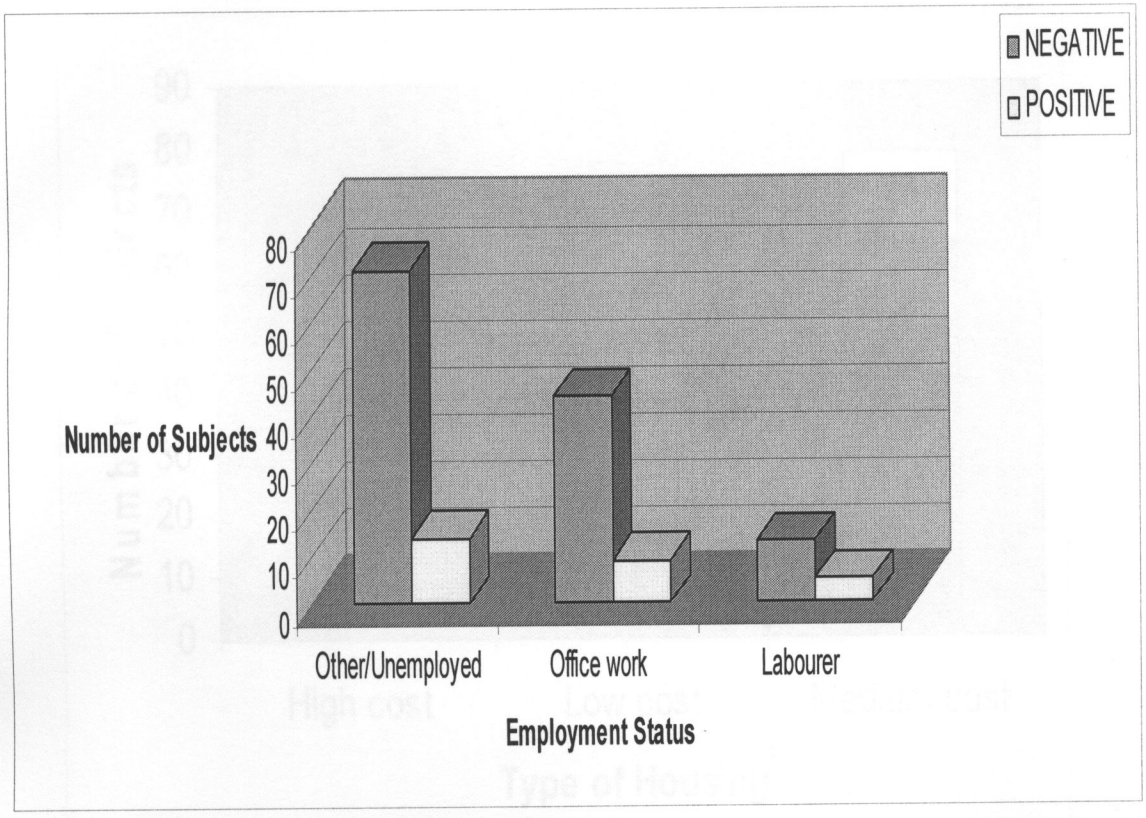

Figure 4 Employment status of subjects by their IgG anti-toxoplasma serostatus. Note: Other/ unemployed refers to those who were unemployed at the time of the study and those employed in non-laborer and non-office work positions.

The majority of subjects among those who tested positive for IgG anti-toxoplasma reported living in medium (15/28) and low cost (8/28) houses. Only a small proportion reported living in high cost houses. The same trend was observed among subjects who tested negative for anti-toxplasma IgG.

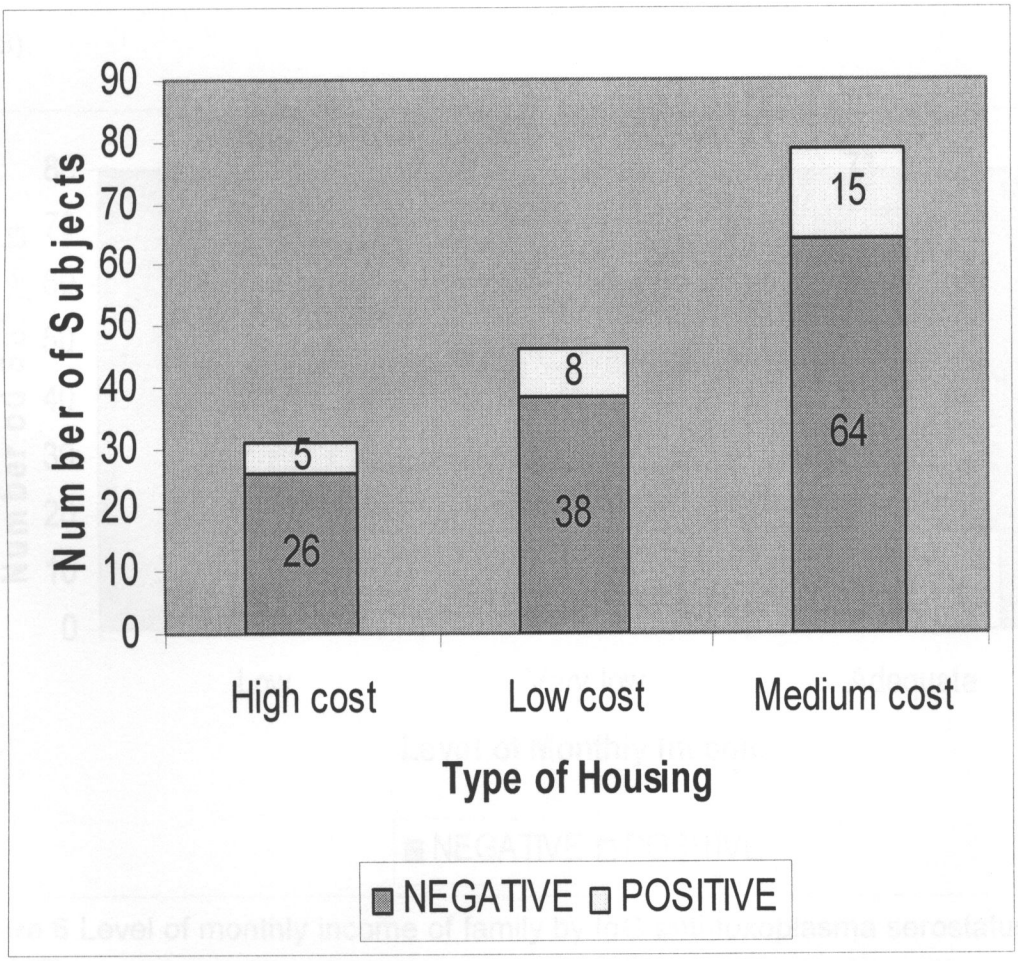

Figure 5 Housing condition by IgG anti-toxoplasma serostatus of subjects. Data on housing condition was obtained by questionnaire.

As shown in figure 6, among subjects who tested positive, the majority (17/28) reported earning an adequate monthly income. A similar trend was also observed among subjects who tested negative for IgG anti-toxoplasma. The dertermination of the adequacy of monthly income was based on the basic needs basket in Lusaka district for basic food items (Jesuit Center for Theological Reflection, 2008).

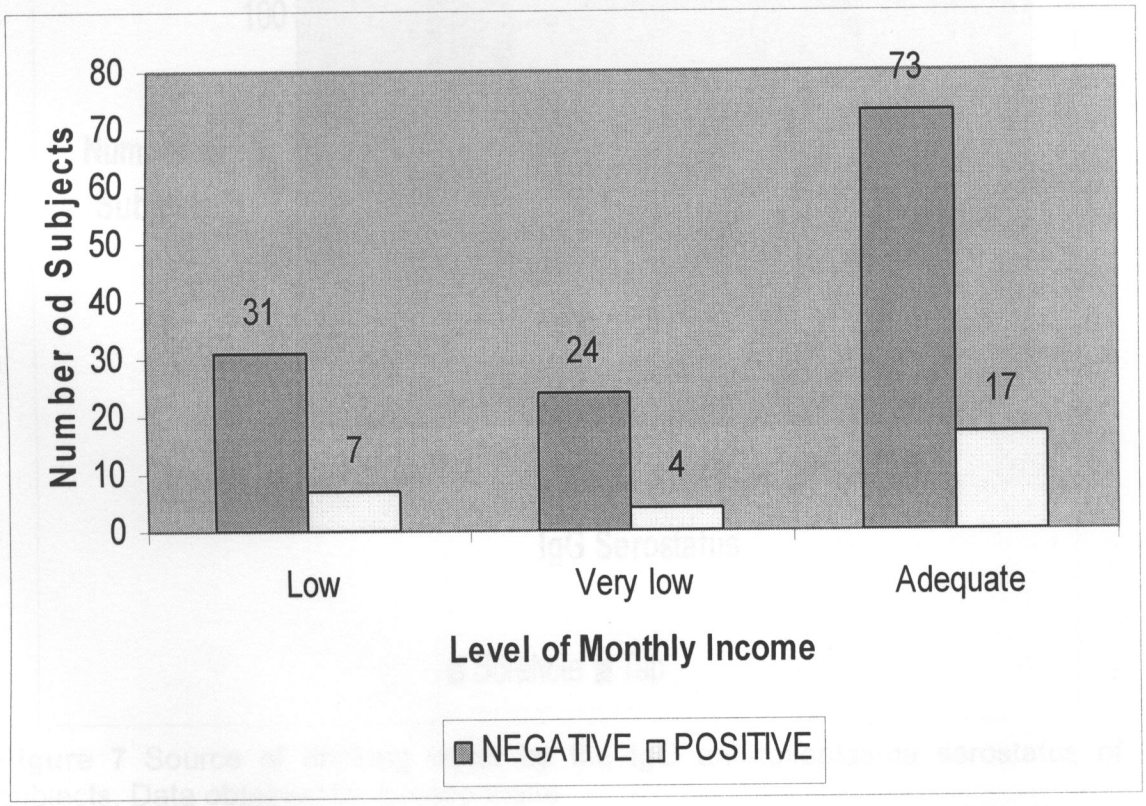

Figure 6 Level of monthly income of family by IgG anti-toxoplasma serostatus. of subjects
Note: Very low= Zambian Kwacha equivalent of less than 30 US Dollar/month, Low= Zambian Kwacha equivalent of above 30 US Dollar/month but less than monthly basic needs basket of basic food items for family of six, Adequate= Greater than income adequate for monthly basic needs basket of basic food items for family six.

Tap water was the source of drinking water for the majority (23/28) of the subjects who tested positive for IgG anti-toxoplasma. Similar trend is shown for subjects who tested negative for IgG anti-toxoplasma.

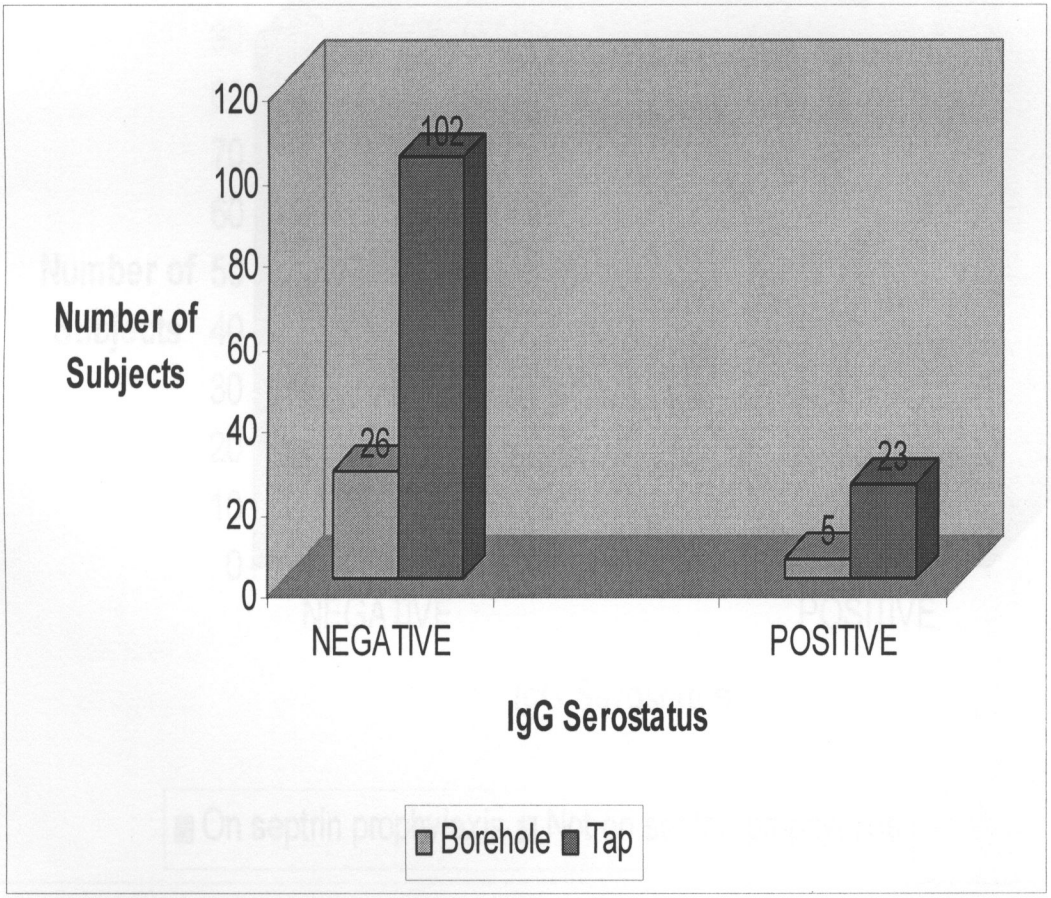

Figure 7 Source of drinking water by the IgG anti-toxoplasma serostatus of subjects. Data obtained by questionnaire

The study showed an alarmingly low septrin use among subjects who tested positive (15/28) and those who tested negative (83/128) for anti-toxoplasma IgG.

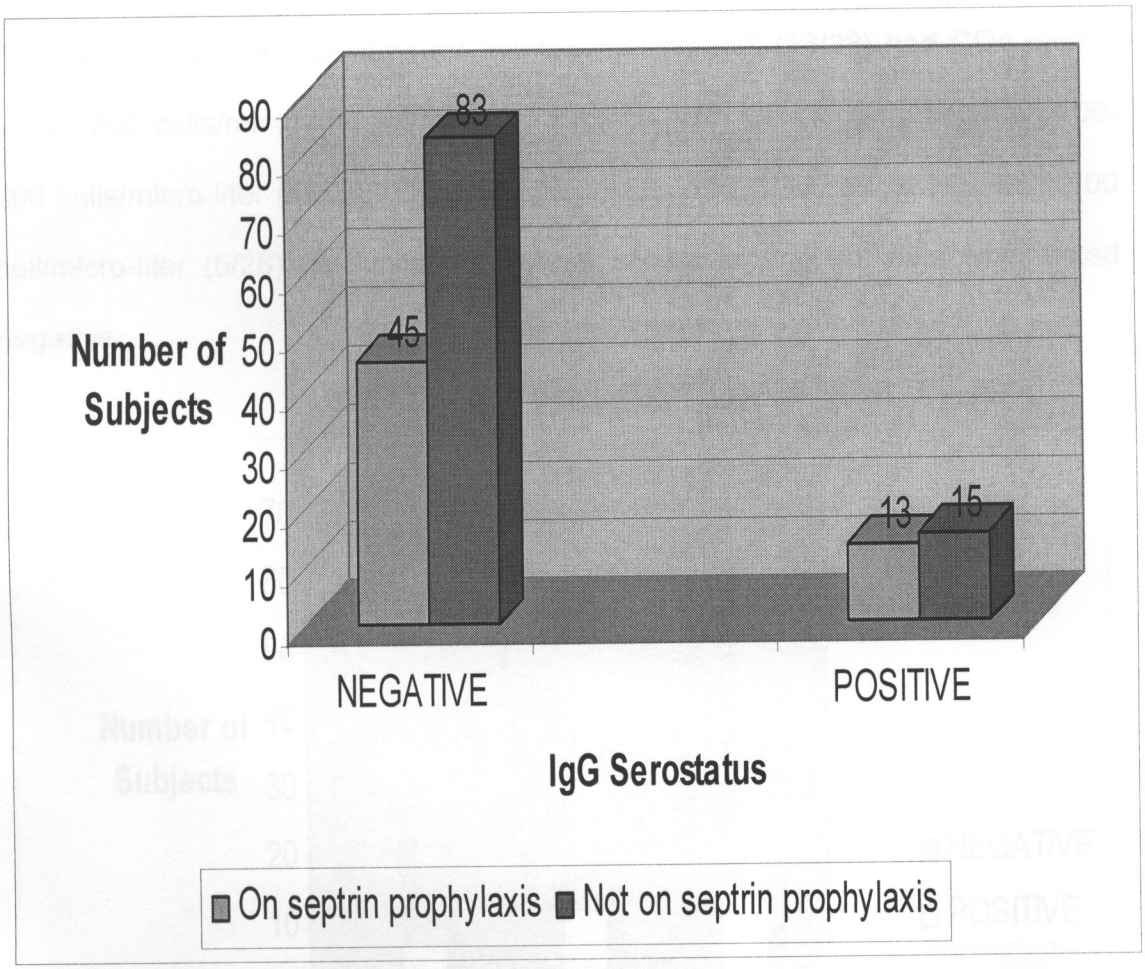

Figure 8 Septrin (co-trimoxazole) use among subjects by IgG anti-toxoplasma serostatus.

Twenty eight individuals had CD4 counts less than 100 cells per micro-liter representing 17.95% of the subjects. The CD4 count ranged from 10 to 867 cells/ micro-liter with mean of 251.4 cells/ micro-liter. The majority of subjects among those who had tested positive for anti-toxoplasma IgG (13/28) had CD4 counts above 200 cells/micro-liter followed by subjects with CD4 counts between 100-200 cells/micro-liter (10/28). Only a few subjects had CD4 counts less than 100 cell/micro-liter (5/28). A similar trend was shown among subjects who tested negative.

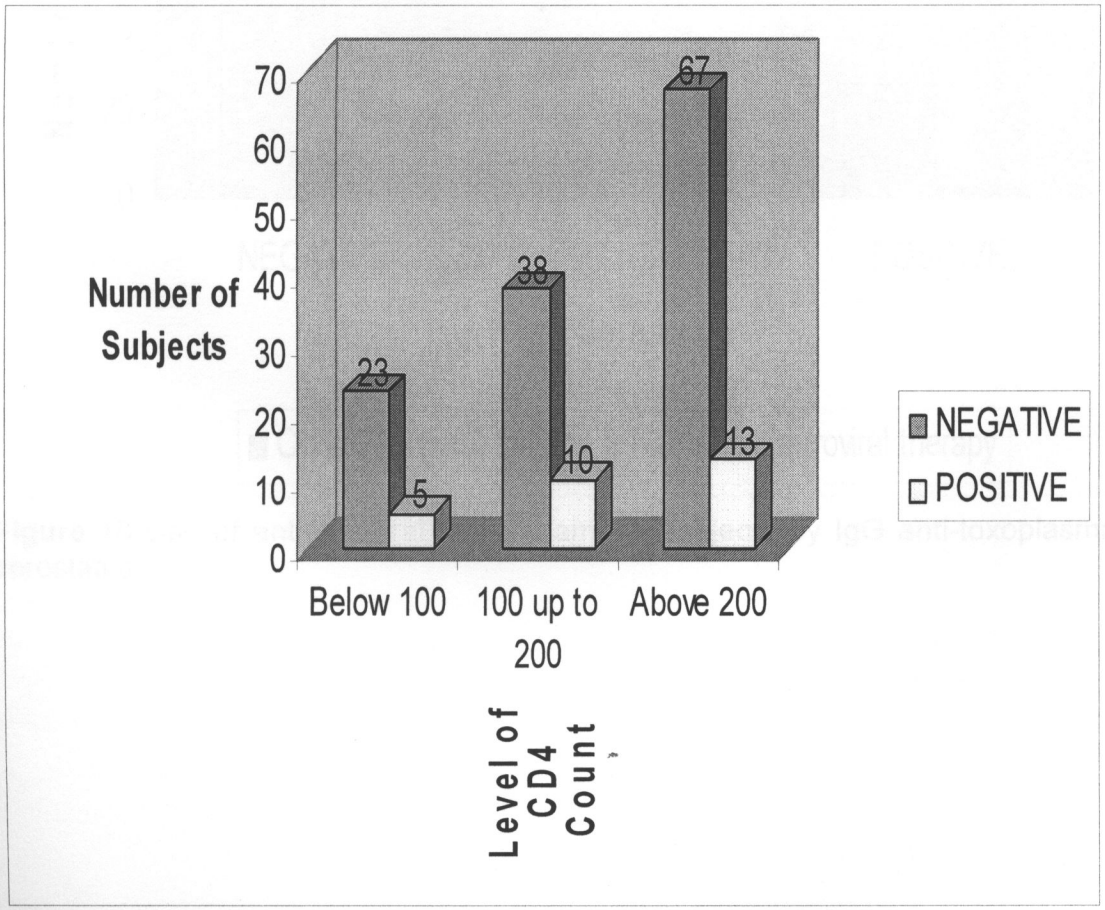

Figure 9 Level of CD4 count by IgG anti-toxoplasma serostatus of subjects. CD4 count data was obtained from subjects' clinical documents.

An overwhelming majority (26/28) of subjects among those who tested positive for anti-toxoplasma IgG were on anti-retroviral therapy as shown in figure 10. A similar result is shown among subjects who tested negative (113/128).

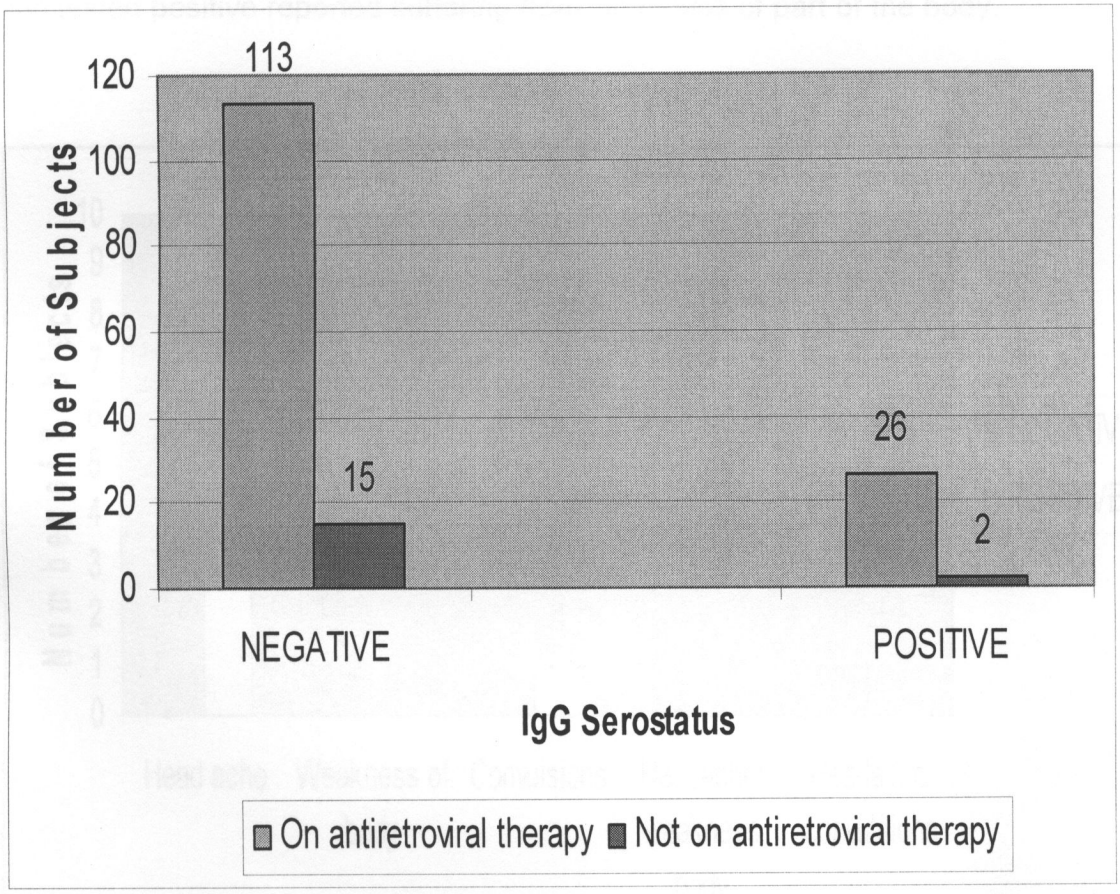

Figure 10 Use of antiretroviral therapy among subjects by IgG anti-toxoplasma serostatus.

Among subjects who tested positive for anti-toxoplasma IgG and reported symptoms of toxoplasmosis, severe or chronic headache was the most frequent symptom reported. One subjects reported convulsions. No subjects among those who tested positive reported suffering from weakness of part of the body.

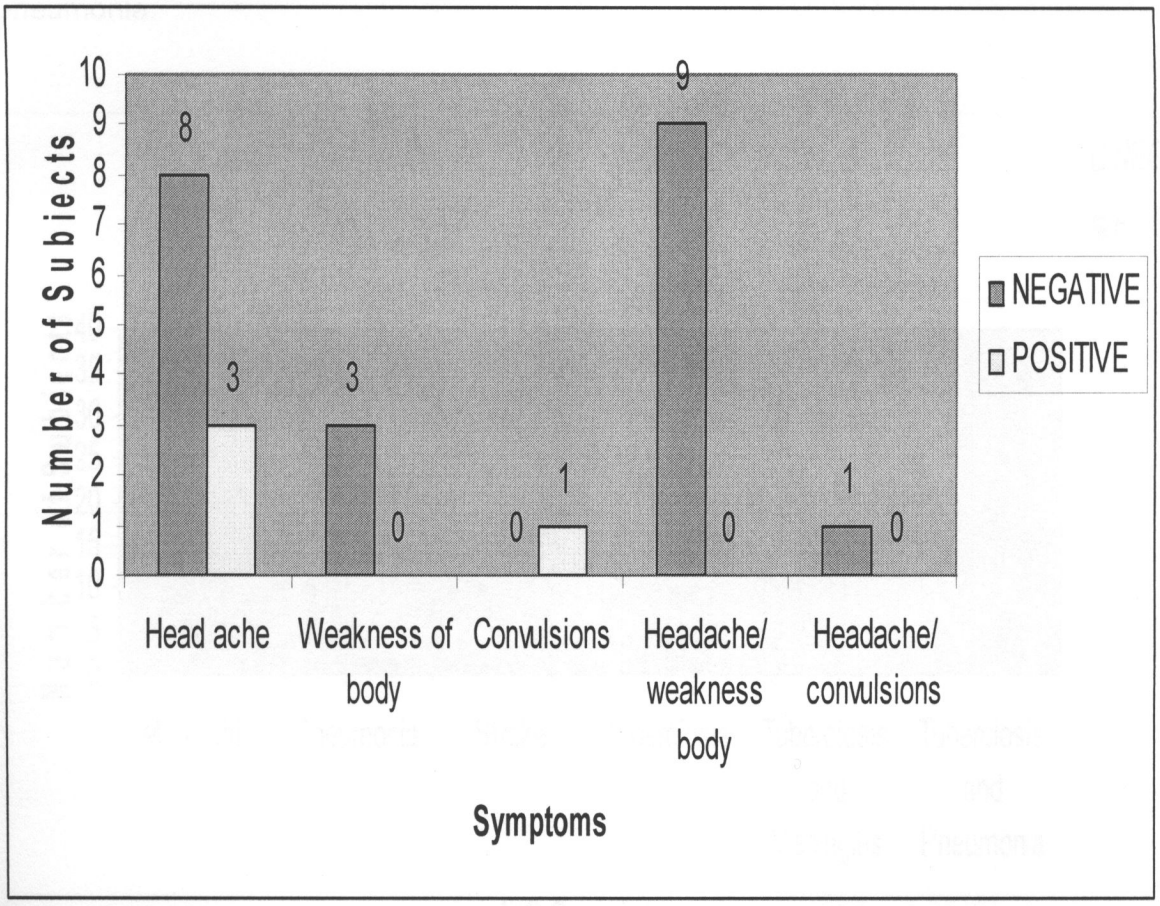

Figure 11 Selected Symptoms suffered by subjects in recent past by IgG anti-toxoplasma serostatus. Data obtained by questionnaire

The majority of subjects reported not ever suffering from opportunistic infections (83/156). The most frequent opportunistic infections among all subjects were tuberculosis, meningitis and pneumonia. Among subjects who tested positive for anti-txoplasma IgG and reported suffering from opportunistic infections, the majority had suffered from tuberculosis (10/12). Two subjects had suffered from pneumonia.

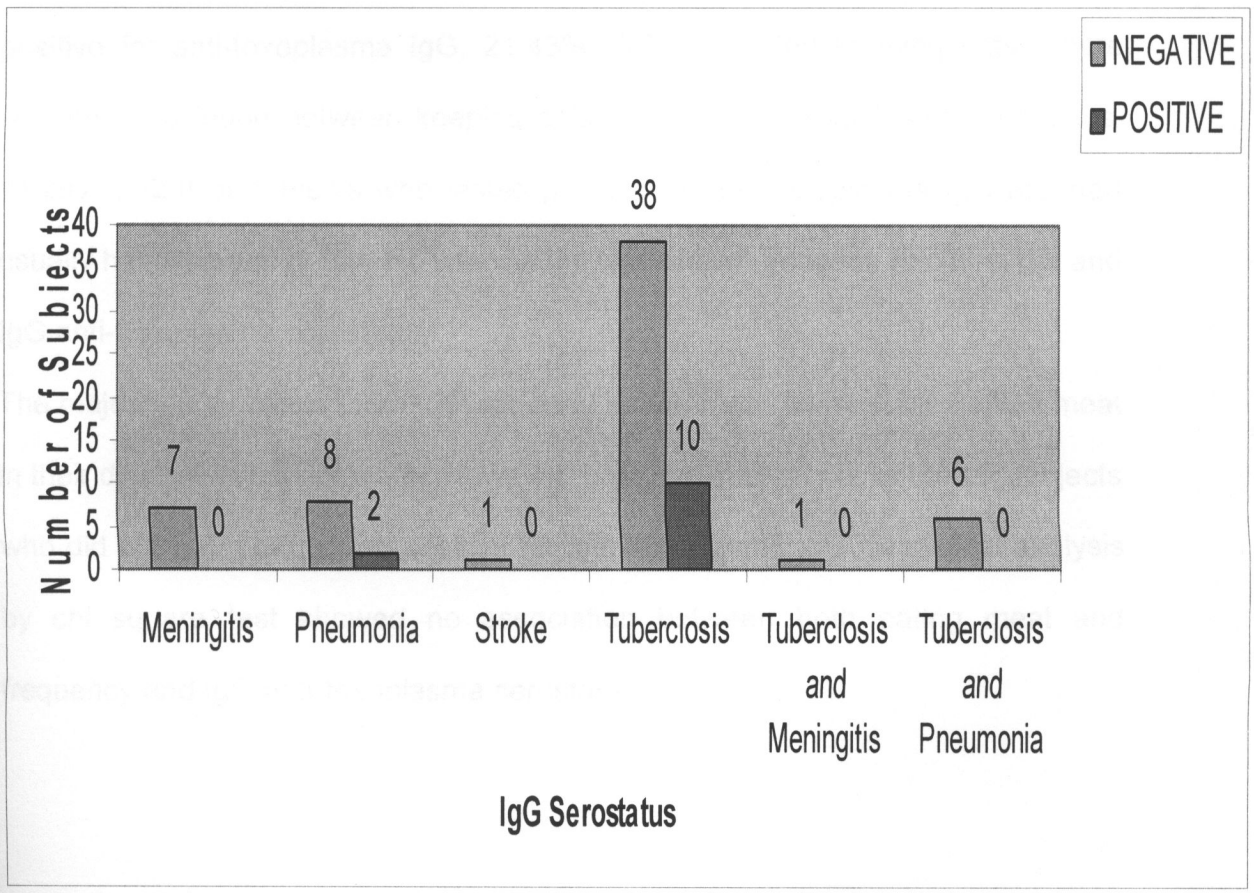

Figure 12 Frequency of opportunistic infections among subjects reporting any opportunistic infection by IgG anti-toxoplasma serostatus. Data was obtained by questionnaire

The table below shows that only 14.1% of subjects reported receiving blood transfusion. However among subjects who tested positive for anti-toxoplasma IgG, 28.57% (8/28) had received blood transfusion. The chi-square test showed a statistically significant association between IgG anti-toxoplasma serostatus and blood transfusion (p= 0.0152). This result was still significant with multiple logistic regression (OR=0.307, confidence interval 0.114-0.826).

13.46% of subjects reported keeping a cat as a pet. Among subjects who tested positive for anti-toxoplasma IgG, 21.43% (6/28) reported keeping cats. There was no association between keeping cats and IgG anti-toxoplasma serostatus. 14.29% (4/28) of subjects who tested positive for anti-toxoplasma IgG reported usually handling soil or dirt. No association was shown between handling dirt and IgG anti-toxoplasma serostatus.

The majority of subjects (150/156) reported eating meat. Most subjects had meat in their diet 1-4 times per week. However, only one subject out of the six subjects who did not eat meat tested positive for anti-toxoplasma IgG. Statistical analysis by chi square test showed no association between both eating meat and frequency and IgG anti-toxoplasma serostatus.

Table Comparison of Known Toxoplasma Risk Factors and their Associations with Anti-toxoplasma IgG Serostatus of 156 HIV Positive Adult Zambians Attending Antiretroviral Therapy Clinic at University Teaching Hospital

Characteristic	Toxoplasma IgG status				TOTAL		P Value (Chi Square)
	Negative		Positive				
	#	%	#	%	#	%	
Received blood transfusion							
Yes	14	8.97	8	5.13	22	14.10	0.0152 (5.8974)*
No	114	73.08	20	12.82	134	85.9	
Keep cats							
Yes	15	9.62	6	3.85	21	13.46	0.1727 (1.8594)
No	113	72.44	22	14.10	135	86.54	
Usually handle dirt/ soil							
Yes	33	21.15	4	2.56	37	23.72	0.1952 (1.6780)
No	95	60.90	24	15.38	119	76.28	
Meat in diet							
Yes	123	78.85	27	17.31	150	96.15	0.9335 (0.0070)
No	5	3.21	1	0.64	6	3.85	
Frequency of eating meat							
>4 times a week (High)	26	17.22	5	3.31	31	20.53	
1 to 4 times (Medium)	76	50.33	16	10.60	92	60.93	0.8522 (0.3198)
< once a week (Low)	22	14.57	6	3.97	28	18.54	

Note: # represents number of subjects, % represents percentage

* Statistically significant result

Chi square test showed a statistically significant association between age and IgG anti-toxoplasma serostatus (chi sq=6.5706 p=0.0374). However, multiple logistic regression showed the association to be non-significant. There was no association between IgG anti-toxoplasma serostatus and, gender, CD4 count, level of education, residential area density, employment status, type of house, level of income, source of drinking water and selected clinical symptoms (chronic or severe headache, convulsions, weakness of part of the body).

9. DISCUSSION

The seroprevalence of IgG anti-toxoplasma antibodies among HIV positive Zambian adults in the present study was 17.9%. This result is much higher than the finding by Holliman et al seventeen years earlier in a study done at the same hospital (Holliman *et al.,* 1991). In their study of the prevalence of IgG anti-toxoplasma antibodies in patients attending the dermatology/sexually transmitted diseases clinic at the University Teaching Hospital, Holliman et al found a seroprevalence of 4% and 11% in HIV positive and HIV negative subjects respectively. They noted that this prevalence was much lower than the prevalence found in adult Ugandans in the East African arm of the same study. They however did not offer any explanation for this observation. Since Holliman et al in their study did not collect social demographic and other clinical data of study participants, the probable effect of these variables on the lower anti-toxoplasma IgG seroprevalence is difficult to ascertain. However the seroprevalence of IgG anti-toxoplasma antibodies in the present study while being higher than previously reported, is still lower than the seroprevalence reported in similar studies conducted in the Sub-Saharan region. The seroprevalence of IgG anti-toxoplasma antibodies in HIV positive adults in this region is in the range of 60% to 33% (Holliman *et al.*, 1991: Maiga *et al.,* 2001: De Clercq *et al.*, 1986: Uneke *et al.*, 2003). The reasons for the lower seroprevalence among HIV positive adult Zambians are not clear. As noted by Holliman et al (1991), the recognized risk factors for transmission of toxoplasmosis such as socio-demographics and food habits are generally similar

among the peoples of the Sub-Saharan region. The higher seroprevalence of IgG anti-toxoplasma among HIV positive adult Zambians in the current study may therefore suggest that clinical toxoplasmosis among HIV positive adults at UTH may be more common than previously thought.

In the present study women constituted 55% of the subjects which may suggest that women account for a higher proportion of patients attending the anti-retroviral therapy clinic at the UTH. The seroprevalence of IgG anti-toxoplasma antibodies among females (16.28%, 14/86) and males (20%, 14/70) was not significantly different. This result was in agreement with the findings of similar studies (Fan *et. al.,* 2007).

In the present study the seroprevalence of anti-toxoplasma IgG among subjects who tested positive tended to reduce with advancing age. The seroprevalence was highest among subjects less than 35 years of age and lowest among those older than 49 years of age. This result is not in agreement with findings from other studies (Davarpanah et al, 2007: Feldman & Miller, 1965). The seroprevalence is expected to rise with increasing age as it is expected that older persons would have had longer exposure to the risk factors such as consumption of under-cooked cyst-containing meat or cyst-containing water or soil than younger persons (Fan *et. al.,* 2007). The finding in the present study is therefore surprising and can not be easily explained. In contrast the majority of the subjects among those who tested negative for anti-toxoplasma IgG, were above 49 years of age.

Among the subjects who tested positive for anti-toxoplasma IgG, a lager proportion had CD4 counts above 100 cells/micro-liter meaning that they were less likely to develop cerebral toxoplasmosis which usually occurs at CD4 counts of less than 100 cells/ micro-liter. However, toxoplasmosis has been known to occur at higher CD4 counts (John & Petri, 2006), therefore HIV positive persons with CD4 counts above 100 cells/ micro-liters need to take co-trimoxazole as prophylaxis for toxoplasmosis. Results from the present study however show that among subjects who tested positive for anti-toxoplasma IgG, 15/28 were not on co-trimoxazole prophylaxis. This result is worrying as 25%-50% of these subjects are prone to suffering from a reactivation of toxoplasmosis usually leading to cerebral toxoplasmosis (Wong & Remington, 2005). The current Ministry of Health (MOH) HIV/AIDS treatment guidelines, recommend that all HIV Positive persons with CD4 count less than 200 cells/micro-liter should receive co-trimoxazole as prophylaxis for common susceptible opportunistic infection (MOH, 2007). These susceptible opportunistic infections include toxoplasmosis. This result therefore suggests that a proportion of patients attending the ART clinic at UTH who are at risk of toxoplasmosis may not be receiving prophylactic medications to prevent them from developing toxoplasmosis.

The study showed a statistically significant association between blood transfusion and positive serostatus for ant-toxoplasma IgG antibodies. Blood transfusion is recognized as a route of transmission of toxoplasmosis (John & Petri, 2006). Case studies have reported transmission of toxoplasmosis following the transfusion of blood or blood-related products (Mele *et. al.*, 2002: Chu, 1999:

Siegel *et. al.,* 1971).However to the best of our knowledge, none of the studies reviewed in the present study reported any association between IgG serostatus and blood transfusion. This result from the present study appears to raise the importance of blood transfusion as a means of toxoplasmosis transmission among patients attending the ART clinic at the UTH. Currently, blood collected from donors for subsequent transfusion is not screened for toxoplasmosis therefore posing a risk of transmission of the disease to recipients of the blood and blood products. Screening of blood for toxoplasmosis before transfusion is therefore of importance especially among high risk groups such as HIV positive persons. Furthermore, individuals that receive frequent blood transfusions such as sickle cell disease patients, hemophiliacs and carcinoma patients may also be at risk of acquiring toxoplasmosis through repeated blood transfusions. These groups of persons may also benefit from implementation of a blood screening programme for toxoplasmosis.

Domesticated cats are known to be definitive hosts and risk factors for transmission of *T. gondi.* However, in the present study, the keeping of cats as pets was not associated with a positive serostatus for IgG anti-toxoplasma antibodies. This result is consistent with findings of other studies (Cook *et al.*, 2000). Other studies have however reported an association between keeping of cats and toxoplasma serostatus (Alvarado-Esquivel *et. al.,* 2007: Avelino, Júnior, de Parada & de Castro, 2004). As such HIV patients who are seronegative for anti-toxoplasma antibodies are advised to avoid keeping cats or cleaning cat litter boxes and to thoroughly wash their hands after contact with cats, cat feaces or

soil in order to avoid the transmission of toxoplasmosis (Center for Disease Control, 1997).

Consumption of meat was not associated with IgG anti-toxoplasma antibodies. It is known that consumption of under-cooked cyst-containing meat is a risk factor for toxoplasmosis (John & Petri, 2006: Roghmann, Faulkner *et. al.*, 1999). In the present study, data on consumption of under-cooked meat was not specifically collected. The result in the present study may therefore suggest that meat products consumed by study participants may be well cooked so as to make transmission through this route improbable. This observation is supported by the result in the present study that showed that increased frequency of meat in diet was also not associated with a positive serostatus for IgG anti-toxoplasma antibodies.

With regard to the symptoms of the subjects who tested positive for anti-toxoplasma IgG and reported experiencing selected symptoms of toxoplama encephalitis, three had suffered from chronic or severe headache while one had had convulsions. However, there was no association between symptoms and IgG serostatus. Demonstrating associations between toxoplasma serostatus and clinical symptoms may be challenging because toxoplasmosis is a salient disease with a myriad of clinical symptoms. The most recognized symptoms of toxoplasmosis are those of toxoplasma encephalitis, which is only one clinical form of toxoplasmosis. The other clinical symptoms of toxoplasmosis may thus go unnoticed and unrecognized. The result from the present study may therefore be expected.

10. CONCLUSION

The seroprevalence of IgG anti-toxoplasma antibodies among Adult HIV positive patients at UTH was determined to be 17.9%. This is much higher than previously reported. A large proportion of HIV positive patients attending the ART clinic at UTH and are at risk of toxoplasmosis may not be receiving co-trimoxazole prophylaxis. There was a significant association between blood transfusion and IgG serostatus shown in this study. This suggests that the screening of donor blood for toxoplasmosis may be of importance. There was no statistically significant association between IgG anti-toxoplasma serostatus and age, gender, CD4 count, meat in diet, level of education, residential area density, employment status, type of house, level of income, source of drinking water, keeping cats, handling of dirt and selected clinical symptoms.

11. RECOMMENDATIONS

Based on the results from the study, the following recommendations are made:

1. That a toxoplasmosis screening programme for all blood collected for subsequent transfusion be implemented in all blood banks in the country.
2. A larger study on the seroprevalence of toxoplasmosis among HIV positive and HIV negative persons should be conducted to better document the epidemiology of this disease among Zambians.
3. All eligible HIV positive persons attending the ART clinic at UTH should receive co-trimoxazole prophylaxis according to MOH guidelines.
4. HIV positive persons who also test positive for toxoplasmosis should receive co-trimoxazole as prophylaxis against toxoplasmosis as chances of disease reactivation are high.
5. Clinicians caring for HIV positive persons should have a high index of suspicion for toxoplasmosis as the disease may be more common than previously assumed.
6. Laboratory diagnosis of toxoplasmosis at the University Teaching Hospital should be included in the range of tests conducted. This can be achieved through acquisition of laboratory equipment for the diagnosis of the disease which are currently lacking.

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APPENDIX 1

Information Sheet

My name is Chomba Sinyangwe. I am a medical doctor by training and studying for a Masters Degree in the School of Medicine at the University of Zambia. The study that I am undertaking seeks to determine how common the antibodies to one of the most important opportunistic infections in HIV positive adult Zambians called *toxoplasmosis* are. In order to investigate this, small amounts of blood (2 mls) will need to be collected from a vein from each study participant and analysed for these antibodies. In addition study participants will be asked to answer questions in this questionnaire. Findings from this study will help clinicians to better treat HIV positive persons and also inform policy makers at the Ministry of Health leading to improved management of this important disease. I am therefore requesting your consent to participate in this study by allowing the collection of this small amount of venous blood and answering the questions asked in this questionnaire as accurately and as honestly as possible. Please be assured that the results of the blood analysis and all the responses to the questions shall be treated with the highest level of confidentiality and shall solely be for the purpose of the research. To this end you will note that the questionnaire is anonymous and therefore does not require your name to be given. No names will be collected or used in any public discussion, report or publication that comes from this study. Further your name won't be written on any of the other forms or sample; the result of the tests will only be known by your code number.

Also note that it is within your right to refuse to participate in this study and even after giving consent, you have the right to withdraw from the study. The care and treatment you are receiving from this clinic will not be negatively affected if you refuse to participate in this study. You will continue to enjoy the usual care and treatment.

I will give you a card today that you can bring to this clinic in two-three weeks if you want your result. We wont ever record your name, you' will get your results

by your ID number on the card. If, at any time, you have questions about the study, your results, or your rights as a study participant, you may contact:

Dr Chomba Sinyangwe
Department of Biomedical Sciences
University of Zambia
School of Medicine
Ridgeway Campus
CELL: 0977-474810
E-mail: csinyangwe@yahoo.com

You may also contact the University of Zambia Research Ethics Committee on the following address:

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Telephone: 260-1-256067
E-mail: unzarec@zamtel.zm

Please indicate whether you have permitted the researcher to proceed with venous blood collection and the questions?

YES ------- NO ------

APPENDIX 2

Consent form

I attest that the information sheet has been read and or/translated to the participant and he consents to the level of participant indicated below.

Dated.....

Signature.....

Name.....

I attest that this form was read and or/translated to me the participant and I consent to the level of participation indicated below.

Dated.....

Signature.....

Thumbprint.....

APPENDIX 3

QUESTIONNARE OF THE STUDY ON SEROPREVALENCE OF ANTI-*TOXOPLASMA* IgG ANTIBODIES AMONG ADULT HIV POSITIVE ZAMBIANS

Instructions: Please marks (X) in the appropriate box corresponding to the respondent's answer or fill in information given by the respondent as requested. ***All spaces must be filled in.***

General Recruitment Particulars

Study No ☐☐☐

Date ☐☐ ☐☐ ☐☐ Recruiting Officer _____

Respondent's Particulars

Date of birth ☐☐ ☐☐ ☐☐ **dd mm yy**

Age ☐☐ Sex ☐ **m/f**

Residencial area _____

Clinical assessment

Body weight ☐☐☐.☐ Height ☐☐.☐

Latest CD4 Count (done in last 6 months) ☐☐☐☐

Current or previous chronic/ severe illness:

☐ Chronic/Severe headache

☐ Weakness of a part one side of the body

☐ Convulsions

_____ Others (specify)

Currently on septrin (co-trimoxazole) prophylaxis? ☐ Yes ☐ No

Currently on antiretroviral therapy? ☐ Yes ☐ No

Ever received blood transfusion? ☐ Yes ☐ No

Socio-economic variables

Highest level of education achieved

☐ No school ☐ Primary ☐ Secondary ☐ Tertiary

Employment

☐ Peasant farmer ☐ Office work ☐ Laborer ☐ Other

Level of income per month

☐ Less than K120 000

☐ K120 000 – K650 000

☐ Above K650 000

Daily activities involve handling/touching soil (dirt)? ☐ Yes ☐ No

Type of house lived in ☐ Low cost ☐ Medium cost ☐ High cost

Has cat as pet? ☐ Yes ☐ No

Source of drinking water ☐ Tap ☐ Borehole ☐ Well

Diet includes meat? ☐ Yes ☐ No

If yes, how often?

☐ More than four times a week

☐ One to four times a week

☐ Less than once a week

The End

APPENDIX 4

LABORATORY DATA CAPTURING FORM FOR ANTI-*TOXOPLASMA* IgG ANTIBODIES ELISA TEST

Instructions: Please indicate (+), (-) and (E) for positive, negative and equivocal results for each specimen respectively. Also indicate the titer value in the appropriate cell

RESPONDENT	*ANTI-TOXOPLASMA SEROSTATUS*	
Number	**Titer**	**Sero-status**
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
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