Sudan University of Science and Technology

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Seroprevalence of Toxoplasmosis in Blood Donors in Alrebat Hospital- Khartoum state

الانتشار المصلي لداء المقوسات في متبرعي الدم في مستشفى الرباط الجامعي –ولاية الخرطوم

A dissertation submitted in partial fulfillment of the degree ofM.Sc. inMedical Laboratory Science (Parasitology and Medical Entomology)

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بسم الله الرحمن الرحيم

الآيـــــــة

قــال تعــالى : (رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ)

صدق الله العظيم

Dedication

I dedicate this research to

My Mother

My Father

My Brother, My Sisters

Acknowledgement

First of all my thanks to Allah.

I wish to express my thanks and gratitude to my supervisor Dr.Mohammed Baha Eldin Ahmed for his close supervision; assistance and continuous support during this work without his help this work could not have been accomplished.

My gratitude is also extended to my colleagues in Alrebat Hospital and all staff in blood bank.

All thanks to everyone who taught me and all those who

stood behind me and gave me personal, close and distant support.

Abstract

The present study was carried out during March to August 2016 at Alrebat hospital in Khartoum state. Serum samples were taken from 100 blood donors with age range between 10-50 years old. The sera were examined for anti - *Toxoplasma* antibodies by the ELISA test. Theoverall rate of anti-*Toxoplasma* antibodies determined by ELISA was32 %(IgG) and 3% (IgM) . The results showed that the highest prevalence rate was reported among the 31-40 age group (47.8%) when examined by ELISA test.

Drinking milk and meat consuming were found to be of no significance in the transmission cycle.

Contacts with cats have been shown to be of great importance inthe transmission cycle.

The present study indicates that prevalence of toxoplasmosis ishigh in the study area.

ملخص الأطروحة

أجريت هذه الدراسة بمستشفى الرباط الجامعي بولاية الخرطوم في الفترة من مارس وحتى اغسطس 2016م حيث جمعت 100عينة مصل من متبرعي الدم .

تراوحت اعمارهم ما بين 10–50 سنة وذلك لتشخيص الإصابة بداء المقوسات . تم اختبار هذه العينات لمعرفة الأجسام المضادة لداء المقوسات باختبار الاليزا .

كانت النسبة للأجسام الخاصة بالطفيل 3% بالاليزاللأجسام المضادة من الفصيلةIgMو 32% بالاليزا للأجسام المضادة من الفصيلة IgG.

أوضحت النتائج ان اعلى معدل لانتشار الطفيل قد تم تسجيله في متبرعي الدم في الفئة العمرية 31-40 سنة حيث بلغت 47.8% باختبارالاليزا .

أوضحت الدراسة ان تناول الحليب واكل اللحم ليس لهما دور في دورة انتقال الطفيل كذلك اوضحت الدراسة ان التعامل مع القطط له اهمية قصوى في دورة انتقال الطفيل.

خلصت الدراسة ان معدل انتشار المقوسات القندية عالي في المنطقة .

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Chapter One

Introduction and Literature Review

Chapter one

Introduction and literature review

Toxoplasmosis is universal zoonotic disease caused by protozoan *Toxoplasma gondii* which was first isolated from the gondii (*Ctenodactylus gondii*), and later in rabbits and dogs many years before its discovery in man (WHO, 1969).

The parasite infects most warm-blooded animals e.g. humans, cattle, sheep, goats, camels, cats, rats, mice, pigeons and chickens (Acha and Szyfres, 1981), but the primary host is the cat in which all the stages of this coccidian including the highly resistant and infective oocyst, have been positively identified (Nichol *et al.*, 1981). Animals are infected through both direct and indirect contact with cat faces or by transmission from mother to fetus. The consumption of unwashed vegetables or undercooked meat and unpasteurized milk from infected animals are potential sources of infection in man (Frenkel and Rize, 1980).

Between 30-60% of the world population is estimated to carry toxoplasmosis. After the first weeks of infection (where it typically causes mild or no illness, or a flu-like illness) have passed, the parasite rarely causes any symptoms in otherwise healthy adults. However,' people with a weakened immune system, such as those infected with human immunity diffiency virus (HIV), may become seriously ill, it can occasionally be fatal. The parasite can cause encephalitis and neurologic diseases and can affect the heart, liver and eyes (chorioretinitis) (Frenkel and Rize, 1980).

1.1 Historical background of *Toxoplasma gondii* infection:

The history of *Toxoplasma gondii* began in the Pasteur institute in Tunisia when Nichol and Maceaux in 1908 observed, a unicellular parasite in the mononuclear cell of the North African rodent (*Ctendactylus gondii*). As the

organism resembled *Leishmania*, they tentatively named it *Leishmania gondii.* The next year, Nichol and Maceaux decided on the basis criteria, that it was not a *Leishmania* organism and proposed the name *Toxoplasma gondii* upon these findings. The parasite was re-described retrospectively by Laveran (1900) in the Japanese paddy bird in JAve and in a rabbit by Splender (1980) in Brazil. The first report of a human infection was made by Jankue in 1923 in Prague when he described toxoplasmosis chorioretinitis in an eleven months child who died of this infection. That was the first evidence that the organism is related to human illness the possibility of transplacental route of infection was reported by Al-Hindy, (1994) in Sudan. In 1939, Wolf *et al*, in New York, isolated the parasite and established it as the cause of neonatal disease in a fatal case infantile encephalitis. In 1948, Sabin and Feldman introduced a serological test (the dye test) which allowed numerous investigators to study the epidemiological and clinical aspect of toxoplasmosis and to demonstrate that toxoplasmosis is the cause of a highly prevalent and wide spread (most often asymptomatic) human infection and to define the spectrum of the disease. He described the oocyst in cat feaces. Later identified the faecal stages of cats as coccidian oocysts.

1.2 Classification of *Toxoplasma gondii*:
According to Levine (1973), the classification of *Toxoplasma gondii* is as follows:

Kindom	protesta
Sub-kindom	protozoa
Phylum	Apicomplexa
Class	Sporozoa
Order	Eucoccidida
Suborder	Eimerina
	Sarcocystideae
Subfamily	Toxoplasmatinae
Genus	*Toxoplasma*
Specie	*Toxoplasma gondii*

1.3 Life cycle (figure 1):

The actively multiplying asexual form in the human host is an obligate intracellular parasite, pyriform in shape and approxirpately 6x6 mm. This stage which is called the tachyzoite, has a cell membrane, nucleus and various organelles. A collection of tachyzoites can fill up a host cell, develop a parasite membrane around themselves, and become cyst. The cyst contains 50 to several thousand organisms and measure from 10 to 100 nm in diameter. Within epithelial cells of the cat, a variety of morphological forms has been described, ultimately leading to male and female garnetocytes. The fertilized macro gametes develop into nearly spherical oocysts that are released by rupture of intestinal epithelial cells (Brown and Neva, 1994).

When passed in cats faeces, the oocysts measure about 10 to 13 nm in diameter, the wall has 2 layers and contains undifferential material, but the content develop into 2 sporocysts within several days after being passed. Sporocyst in turn, contains 4 sporozoites .

Toxoplasma gondii tachyzoitics multiply intracellular by specialized from within a mother cell. As the distended host cells -fill up with parasite, they rupture, releasing parasites that enter new cells. Toxoplasmosis can grow in any mammalian or avian organ or tissue, developing in brain, eye and skeletal muscles (Brown and Neva, 1994).

According to Hutchison *et al.*, (1969), two stages are described in the life cycle:

1.3.1 Direct stage (enterepithelial):

Mice containing the infective cysts are eaten by the cat, which serves as the definitive host of the parasite. The cyst wall is digested, releasing organism that penetrate epithelial cells of the small intestine.

Several generations of intercellular multiplication occur and finally culminating in development of oocysts that are digested into the intestine lumen by rupture of infected intestinal epithelial cells.

After eating cysts, cat excretes *Toxoplasma gondii* oocysts as early as 4 days later. These increased and then taper off by 141 days. Oocysts require 1 to 5 days depending on aeration and temperature, after passage to sporulate (Hutchison *et al.*, 1969).

1.3.2 Indirect stage (extra intestinal):

Ingestion of the sporulated oocysts initiates infection by sporozoites in the intermediate host, which can be virtually any warm- blooded vertebrate including man (Hutchison *et al.*, 1969). The oocyst inducted infection also begin in the intestinal epithelium of the intermediate host, liberate sporozoites, penetrate intestinal epithelium and spread via blood more distant organs of the body where they multiply intercellular in various cell types including brain, skeletal muscles, heart muscles and cells of the reticuloendothelial system. But they have prediction for the retina. Eventually, infected cells rupture and ones are infected by these rapidly multiplying organisms (tachyzoites). After sometimes, multiplication slows down and chronic oocysts containing thousands of slowly multiplying organisms (bradyzoites) are found.

Infected cats may be completely or partially immune, after primary exposure. However, 1-4% of the domestic cats populations are usually found

to be shedding oocysts (Dubey and Frenkel, 1974). Thus, although only the domestic cat or some wild species of the felidea can produce several millions of oocysts, in turn, harbor infective cysts within their tissues. Tissues cysts may persist almost for life in heart, brain, muscles, and development of immunity to new infection which is usually long lasting. Moreover, sometimes, a tissue cyst may burst and the released bradyzoites boost host immunity to a higher level (Hutchison *et al.*, 1971).

Bradyzoites are resistant to digestion by pepsin and trypsine and when ingested in meat they cause infection (Frenkel, 1973). Sporozoites in oocysts may remain viable potentially infective for as long 13-18 months depending upon climatic conditions (Yamaura, 1976; Fayer, 1981).
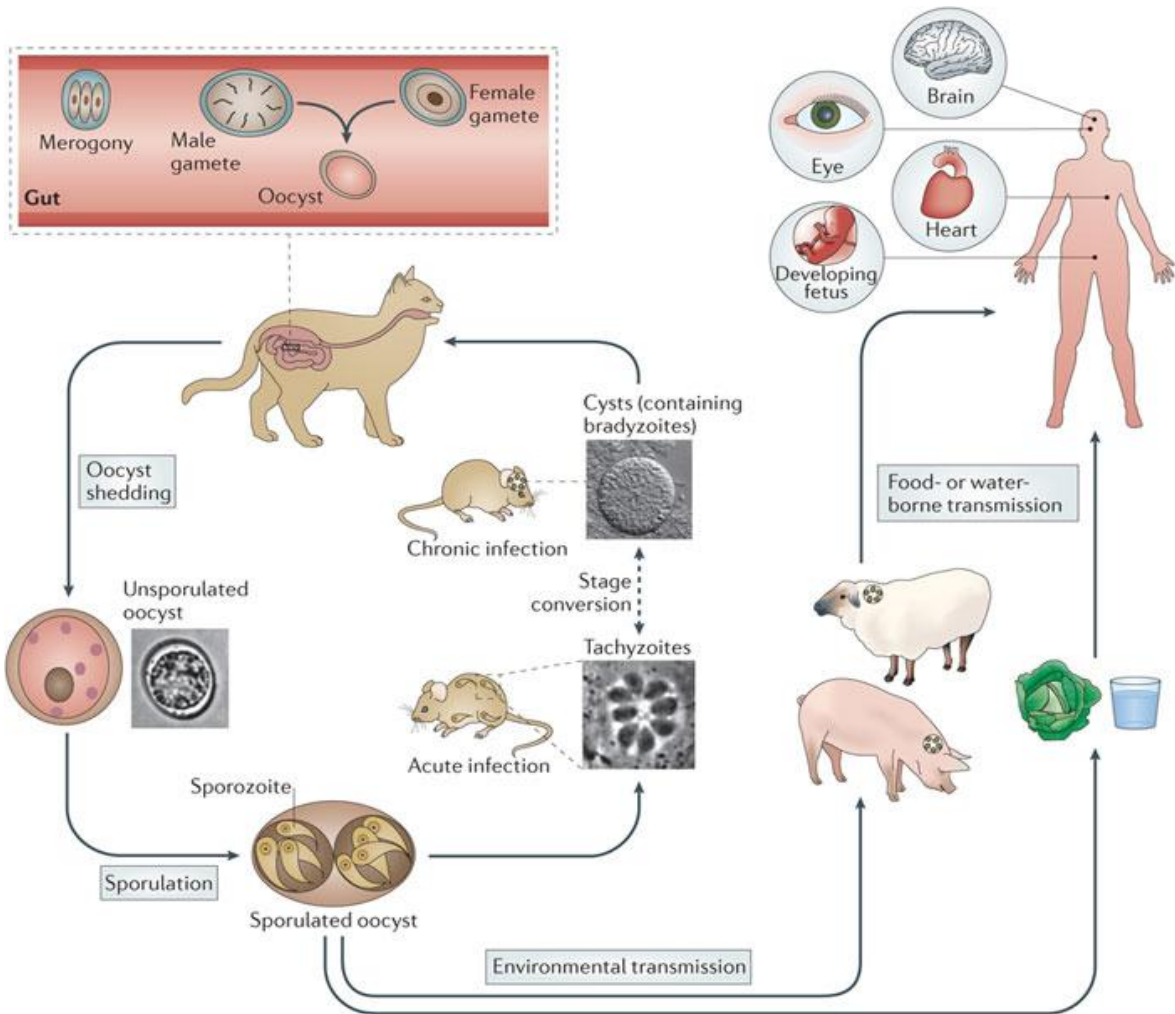
Figure 1: Life cycle of *Toxoplasmosis gondi*

1.4 Pathology and symptomatology of *Toxoplasma gondii* infection:

Ordinary *Toxoplasma gondii* is relatively benign and well adapted parasite and its disease producing properties have been attributed to virulent strains especially susceptible hosts, or the site of the parasite (Brown and Neva, 1994).

1.4.1 Infection in human:

1.4.1.1 Acquired adult toxoplasmosis:

Most infection as shown by population surveys must have beeu asymptomatic as a large number of healthy people have antibodies to *Toxoplasma gondii* in their sera (Krick, 1978; Rernington,1974). According to Schmidt and Roberts (1985), the types of acquired toxoplasmosis can be classified as follows:

1. Asymptomatic.

2. Acute.

3. Glandular.

4. Chronic.

In addition to the fever, erythematous or petechial skin rashes and signs of involvement of the central nervous system are observed. Excluding the asymptomatic type, the glandular type is the most common and is difficult to distinguish from infectious mononucleosis.

The symptoms in the chronic form are difficult to diagnose. Vague gastrointestinal symptoms, muscular and joint pains, and signs of generalized or local central nervous system involvement, pain in the eye, blurring of vision and even blindness may be complained of the parasites.

1.4.1.2 Toxoplasmosis as an opportunistic infection:

Toxoplasmosis has been shown to occur as opportunistic infection complicating immunocompromized patients (Wong *et al.*,1982; Colon, 1988).

Fatal outcome due to unsuspected toxoplasmosis has been recognized in recipients of kidney transplants; patients with neoplastic disease treated with immunosuppressive drugs and acquired immune deffiency virus (AIDS) patients. This probably represents reactivation of previously acquired toxoplasmosis (Feron *et al*., 1990). The presence of persistence parasitaemia observed in humans and animals .can be explained by the existence of extracellular parasite in the circulation (Miller *et al.,*1969). Organisms that are intracellular or encysted are apparently protected from the action of antibodies and perhaps from cell- mediated immunity, although changes in the host cell membrane that may occur at the time of infection may lead to disruption by lmphocytic factors or by macrophages. Data showed that peritoneal alveolar macrophages can kill *Toxoplasma gondii* organisms (Catteral *et al.*, 1987).

Organisms released from ruptured cysts into areas deficient in antibody (e.g. brain and retina) may cause significant tissue damage (Remington and Kelein, 1990). In immune-deficient patients, areas of necrosis may be , widespread, myocardial and skeletal muscles are mostly involved. Rarely, deposition of *Toxoplasma gondii* antigens and antibodies complex in the kidney results in glomerulonephritis (Remington, 1974).

1.4.1.3 Ocular toxoplasmosis:

Toxoplasma gondii is a common zoonotic infection of the retina and the diagnosis of ocular toxoplasmosis is made when there is evidence of chorioretinitis, positive serum antibodies to *Toxoplasma gondii* and when

other causes of chorioretinitis are excluded (Tabbara, 1990; Omer and Tabbara, 1993). Ocular involvement usually arises, as a late manifestation of congenital infection, in childhood or adult life, and the damage is probably immunologically mediated (Tabbara, 1990). This was first reported in 1923 by Jankue who isolated *Toxoplasma gondii* cysts from the retina of a dead eleven months old child in Prague. Ocular toxoplasmosis in adults may be due to reactivation of a congenital infection (Duton, 1989).

The characteristic lesion is a focal retinochoroiditis that is so characteristic and it is possible to diagnose the condition immediately (Tabbara, 1990).

Lesions in the acute and sub acute stage of inflammation appear as yellowish white cotton-like patches in the fundus. The acute lesions has indistinct borders, whereas the older ones are white-gray sharply outlined and spotted by accumulation of choroidal pigment (O'Connor, 1974).

Only, the retinal tissue is invaded (intra-retinal cyst) and the sub Adjacent choroids are usually involved in the inflammatory process (O'Connor, 1974). Retinochoroiditis is symptomatic and may be discovered by chance. Active lesions can cause blurred vision, and ocular pain (Duton 1989).

1.5 Diagnosis of *Toxoplasma gondii* in human:

In addition to clinical findings, the diagnosis of toxoplasmosis depends upon the demonstration of *Toxoplasma gondii* directly or indirectly(Jacobs, 1976).

1.5.1 Direct methods:

Demonstration of the parasite in biopsy material taken from liver, lymph node, spleen, or cerebrospinal fluid in case of adults and in case of suspected congenital infection, biopsy material is taken from the placenta,blood or amniotic fluid. Parasitological detection is made possible by intra peritoneal inoculation of mice by the biopsy material and detection of parasites three weeks later in peritoneal macrophage.

1.5.2 Indirect methods:

There are serological tests for the detection of antibodies in the serum of the infected host. As the direct method is difficult and frequently unrewarding, the serological tests are more frequently used (Sabin and Feldman, 1948). Serological tests are very important in the diagnosis of toxoplasmosis.

Because of the common occurrence of antibodies to the parasite in the general population, diagnosis by serological means requires demonstration of a significant increase in *Toxoplasma* specific antibodies titers in the serum or other body fluids (Jacobs, 1976).

The oldest serological method in use is the Sabin-Feldman dye test which was developed in 1948 (Remington *et al.*, 1970). At that time, it was a great accomplishment as it allowed extensive research in the field of toxoplasmosis.

However, at present, it is not so popular as it utilizes live *Toxoplasma gondii* organisms as antigen and this involves a considerable risk of infection (Jacobs, 1976). The dye test is reserved for cases with indirect heamoagglutination in the presence of strong suspicion of toxoplasmosis (Eissa *et al.*, 1990). Other serological tests which utilize safe soluble antigens of *Toxoplasma* infection are: CF, IFAT, IRA, ELISA, latex etc.

1.5.2.1 Complement fixation test (CF):

It is helpful in distinguishing recent from old toxoplasmosis.The reason for this is that, complement fixation test antibodies develop much more than those detected by the dye test, indirect heamagglutination or indirect florescent antibody test (Choi, 1990). Long-term investigations revealed a marked complement fixation test antibodies within two years after infection in the majority of cases in contrast to persisting antibodies demonstrated by

the other tests. Thus, it is least sensitive than older tests (Fruhbauer *et al*., 1990).

1.5.2 .2 indirect fluorescent antibody test (IFA):

It is the most widely used serological procedure because of its safety, relative eases of performance and economy. It can be performed to detect IgG antibodies within 8-10 days post infection (Omer and Tabbara, 1993). Indirect fluorescent antibody test seropositivity is not lifelong .

1.5.2.3 Indirect heamagglutination tests (IHA):

Is a laboratory test suitable for sero-epidemiological surveys and for routine works (Eissa *et al.*, 1990).

1.5.2.4 Latex slide agglutination:

It is widely used as a satisfactory screening test for toxoplasmosis (Beverley and Freeman, 1973). It is reported to give 96.6% agreement with the dye test in qualitative comparison (Michael and Flamed, 1975). The only disadvantage of this test is the non-specific reaction e.g in Britain, false positives occurred in 1.3%) (Holliman *et al*.,1989). In Sudan, 96.0% agreement between latex agglutination test and ELISA is satisfactory for several purposes (Abdel-Hameed, 1991).

1.5.2.5 Immune sorbent agglutination assay:

Immune sorbent agglutination assay is another more sensitive and specific method for the detection of IgM *Toxoplasma* specific antibodies (Dannemann *et al.*, 1990). It combines the advantages of both the direct agglutination test and double sandwich IgM ELISA (Plantz *et al*., 1987).

The combination of IgM immune sorbent agglutination assay with IgM indirect fluorescent antibody test is proved satisfactory for the diagnosis of acquired acute toxoplasmosis, and can be recommended for laboratories with lower capacity (Valkoun and Stefanik, 1990).

1.5.2.6 Enzyme-linked immune sorbent assay (ELISA):

ELISA has been adopted to replace the older tests in serodiagnosis of toxoplasmosis (Gallalan *et al.*, 1964). It is an enzyme immune assay for quantities detection of IgM and IgG antibodies to *Toxoplasma gondii* in serum and plasma. ELISA is a sensitive test and is highly suitable for the screening of large amounts of samples (Hirvela-Kosti, 1990). The presence of *Toxoplasma* IgM is an indication of a recent or ongoing active *Toxoplasma gondii* infection and is probably the test best parameter for early diagnosis of acute *Toxoplasma gondii* infection.

1.5.3 The role of IgM and IgG *Toxoplasma* antibodies in the diagnosis of toxoplasmosis:

To detect *Toxoplasma gondii* infection in adult, emphasis is placed on rising IgG titre at two weeks interval. Low IgG antibody titre indicates past infection where as high titre indicate an active recently acquired infection. This is then confirmed by the presence of *Toxoplasma gondii* specific IgG. Congenital *Toxoplasma* infections may be difficult to diagnose serologically because maternal IgG crosses the placental barrier and will appear and persist for several months.

1.6 Toxoplasmosis in the Sudan:

In Sudan, the first report of human toxoplasmosis dates back to 1966 when Carter and Fleck, using the dye test carried out a survey in Khartoum and Gezira. They reported prevalence of 27.8% in the general population excluding children less than 10 years of age. Later, Abdel-Hameed (1991), investigated the disease in Geizira where he reported prevalence of 41.7 % with females showing a higher prevalence rate than male. He observed that there was no correlation with an animal contact and on cases of active toxoplasmosis where detected as indicated by the negative IgM test.

In 1994, a cross-sectional survey was carried out in Khartoum by AL-Hindy. He collected samples from 5 sources and examined them for *Toxoplasma* specific IgG and IgM antibodies by ELISA. He reported that 17.5% of males and 30.1 % of pregnant women had positive IgG reaction. The difference in prevalence rate between the two sexes was not significant.

During the period June to December 1996, a cross sectional survey was carried out in Khartoum hospital and Omdurman maternity hospital by Al-hadi. In this study, serum samples were collected from 487 pregnant women. Screening for *Toxoplasma* specific IgM antibodies was made using an enzyme linked immune sorbent assay (ELISA). IgG sera positive prevalence rate was found to be 34.l%. Also, 35 subjects with IgG were re-examined by ELISA for IgM antibodies. He found that 14.3% has positive IgM antibodies indicating active recent infection.

In 2001, a study was conducted by Abdel-Rauof in Khartoum where serum samples were taken from different groups including males, pregnant women, aborters, patients with spleenomegaly, patients with vision defects and mentally retarded patients. Screening of anti- *Toxoplasma* antibodies was made using latex agglutination and specific IgG and IgM using an enzyme linked immune sorbent assay (ELISA).The overall rate of anti- *Toxoplasma* antibodies was 17.3% by ELISA and l3.4% by latex agglutination test. He found that there was no correlation between abortion and high specific *Toxoplasma* antibodies titers.

In a study carried out by Bushra (2006), the overall rate of anti- *Toxoplasma* antibodies was 5.7% by ELISA 19M and 23.9% by latex agglutination test in pregnant women. He reported that positive cases were more expressed in the age group 20 -40 (36.3%).

Eman and Saad (2011) investigated the prevalence of anti- *Toxoplasma* antibodies among pregnant and non-pregnant ladies. They reported an overall positive rate of 22.5% of anti-*Toxoplasma* antibodies , detected by latex agglutination test out of 200 serum samples. When the same samples were examined by ELISA (lgM), the positive rate was 6%.

Abdel-Gader (2008) investigated the prevalence of anti-*Toxoplasma* antibodies among "pregnant and non-pregnant ladies. He reported an overall positive rate of 6% of anti-*Toxoplasma* antibodies detected by latex agglutination test out of 50 serum samples. When the same samples were examined by ELISA, the positive rate was 10%.

1.7 Toxoplasmosis in blood donors:

Toxoplasma gondii infection in blood donors could represent a risk for transmition in blood recipients.

In Egypt, a cross-sectional study was conducted to evaluate the prevalence and risk factors of *Toxoplasma gondii* antibodies in 260 blood donors seen at blood bank. A blood sample was taken to document the *T. gondii* antibody status by using ELISA. Overall, 155 (59.6%) of 260 blood donors were positive for anti-*T. gondii* IgG antibodies (Elshaka *et al*., 2009).

In Iran, in order to study the prevalence of *T. gondii* in Iranian blood donors, six studies have been reviewed. IgG and IgM antibodies varied between 12.3% to 52.8% and 0% to 5.47%. Some of these studies have suggested to doing the screening for all blood donors. However, based on parasitological and epidemiological evidences, there is little chance for parasite transmission by blood transfusion (Karimi *et al*., 2016).

In Karnataka, south India, the seroprevalence of *Toxoplasma gondii* in healthy adult population of blood donors was investigated a total of 1000 serum samples collected in two batches (500 each) in the years 2004 and 2005 from healthy voluntary blood donors were tested for *T. gondii* antibodies by ELISA method, in addition to the other five mandatory tests. The study showed a high prevalence of *T. gondii* antibodies in healthy voluntary blood population (Sundar *et al.*, 2007).

Four hundred and thirty two blood donors in two public blood banks of Durango, Mexico were examined for *Toxoplasma gondii* infection between August to September 2006 .Tested IgG ,IgM antibodies by using ELISA. showed 32 (7.4%) of 432 blood donors had IgG anti-*T. gondii* antibodies positive. 8 (1.9%) of them had also IgM anti-*T. gondii* antibodies positive (Alvarado-Esquivel *et al.*, 2007).

In Taiwan, the cross-sectional study aimed to survey the seroprevalence of *T. gondii* infection and its risk factors among healthy blood donors in Taiwan. A total of 1,783 healthy blood donors from all six-branch blood service centers participated in this study. The blood samples were tested for the presence of *T. gondii* antibodies using ELISA. Of the 1,783 participants, 166 (9.3%) tested positive for anti-*Toxoplasma* IgG, while 5 (0.28%) tested positive for anti-*Toxoplasma* IgM (Chaing *et al.*, 2012).

 Thousands samples of sera taken from healthy blood donors in the Asir Central Hospital, Saudi Arabia tested for specific IgM titres by micro ELISA technique and 4.1% were found positive, indicating possible acute or recent infection.These studies indicate a higher prevalence of toxoplasmosis among healthy blood donors in the Asir region (AL-Amari ,1994).

In Kayseri-Turkey, a total samples from 385 healthy blood donors from Kayseri, examined for anti- *T. gondii* antibodies (IgG-IgM) by enzyme-linked immunosorbent assay (ELISA). The seroprevalence of the anti- *T. gondii* IgG is 20.25%, and IgM antibodies is 2.33% .They mentioned that all blood donors should be screened for toxoplasmosis before transfusion (Eser *et al.*, 2006).

A cross-sectional study was carried out in University of Malaya Medical Centre, Kuala Lumpur. Blood samples from 203 Healthy blood donors were collected and anti-*Toxoplasma* antibodies were detected by using conventional ELISA. 28.1% were positive. There was no significant association between the seroprevalence of toxoplasmosis and various possible risk factors i.e. contact with cat, consumption of undercooked meat and history of blood transfusion. (Nissapatorn *et al.*, 2002).

In China, 864 blood samples (422 males and 442 females) were collected from the students in 4 universities and 95 healthy adults in Shijiazhuang City, using ELISA to detect IgG antibodies specific to *T. gondii*. The positive rates of IgG antibody specific to *T. gondii* were 5.1% and 7.4% in college student blood donors and healthy adults respectively. The positive rates were not significantly different between the sexes and among the different universities (Xin and Song, 2013).

In Loei Province, Northeast Thailand; 345 blood sample were collected from blood donors and examined for anti-*Toxoplasma gondii* antibodies by ELISA.The seroprevelance of the anti-*Toxoplasma* Ig,IgG and IgM antibodies was 4.9%,4.1%,4.3% respectively. The negative results were found in age group that less than 20 years old and more than 51years and the highest seropositive result were found in two age groups (21-30 and 31-40 years old) (Pinlaor *et al.*,2000)

Rationale:

Toxoplasma gondii has a worldwide distribution in human population infecting up to one third of the global population and a wide range of other mammalian and avian species. Toxoplasmosis is a major public health problem, with a high socioeconomic impact in terms of human suffering including the cost of caring for sick, mentally retarded and blind children. The parasite is an extremely successful pathogen, responsible for significant morbidity and mortality, especially in congenitally infected and immuno-compromised individuals, although some subjects experience infection without overt disease or with mild symptoms.

Objectives:

General objective:

To determine the seroprevalence of toxoplasmosis among blood donors in Alribat hospital, in Khartoum State.

Specific objectives:

1. To detect the rate of anti- *Toxoplasma* antibodies using the enzyme linked immune sorbent assay "ELISA".

2. To assess risk factors associated with toxoplasmosis (age, meat and milk consumption).

Chapter two
Materials and Methods

Chapter two

Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area:

The study was conducted in Alribat hospital which is located 2 kilos far away from Khartoum center.

2.3 Study population:

The study was conducted on 100 individuals who visited the hospital to donate blood .They were of different age groups as follows:

G1   10 - 20

G2   21 - 30

G3   31 - 40

G4   41 - 50

2.4 Sample size:

Samples were taken from 100 blood donors.

2.5 Samples collection:

Five ml of blood was drawn from each blood donors. The blood was centrifuged at 2000 rprn and sera were extracted and stored separately at -20ºC. Each sample was aliquoted to smaller volumes to avoid the effect of repeated freeze thawing.

When required, aliquots were thawed to room temperature by using a water bath.

2.6 Data Collection:

A questionnaire was designed for data collection (appendix 1).

2.7 Techniques:

2.7.1 Enzyme linked immune sorbent assay:

2.7.1.1 Determination of IgM EIA and IgG EIA kit:

Enzyme immunoassay (EIA) procedure for the determination of IgM and IgG antibodies to *Toxoplasma gondii* was conducted using index toxolgM EIA kit and IgG EIA kit.

2.7.1.1.1 Principle of the test:

Toxoplasma gondii antigens were fixed to the interior surface of microwells. Patient's serum was added and any antibody specific to *Toxoplasma* will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with horseradish peroxidase enzyme and directed against human 1gM are added and will in turn bind to any human 19M present and IgG present. The microwells are washed to remove unbound conjugate and then chromogen/substrate is added. In the presence of peroxidase enzyme, the colorless substrate is hydrolysed to a colored end-product. The color intensity is proportional to the amount of antibodies present in the patient's serum.

2.7.1.1.2 Assay procedure:

The required numbers of microwells were placed in the microwell holder. One end of each strip was marked for orientation. The sample dilutions were prepared by mixing 1/100 using the serum diluents (10 μl serum to 1μl serum diluents). Prepared working wash buffer by diluting the concentrated wash buffer 1:25 (1 washing 24 distilled water). The calibrators were not diluted as they were ready for use, then, diluted samples were incubated at room temperature. 100 of negative control, low positive standard (cut-off), high positive standard and 5 serum specimens were added to subsequent wells, then, microwells were incubated at room temperature for 60 minutes.

They were washed by inverting and flicking into a sink, completely filled with wash buffer and washing was repeated four times, refilled with wash buffer and soaked for 5 minutes. Wells and blot were emptied with absorbent paper. The wells were filled five times without soak. 100 of enzyme conjugate were dispensed into each well except for the blank well and incubated at room temperature for 30 minutes. At the end of the incubation period, the contents of the well were discarded and washed as outlined above. 100 of substrate added to each well and incubated in dark place at room temperature for 30 minutes. The reaction was stopped by adding 100μl stop solution to each well. This produced color change. Immediately, the absorbance of each well was measured by ELISA reader (mark) at 450 nm filter within 10 minute.

2.8 Calculation and interpretation of the result:

For each test and control serum, the average optical density (OD) obtained during the test run was determined.

The average OD of the low positive control was calculated. This was the cut off value of the assay.

The sample OD was divided by the value obtained in the step above. A ratio less than O.6 indicated negative sample, a ratio greater than 0.9 indicated a positive sample. A ratio between 0.6 and 0.9 indicated equivocal result. For equivocal results, the specimen should be retested.

Specimens that are repeatedly equivocal after retested should be confirmed using an alternate method. If the results remain equivocal, collect a new specimen in two weeks. If the new specimen is positive, the specimen is presumed to be positive.

2.9 Statistical analysis:

Data were analyzed using Statistical Package of social science (SPSS) software programme. They were presented in tables and figures.

2.10 Ethical considerations:

Verbal consent was taken from blood donors after explaining the nature of the study.

Chapter three

Results

Chapter Three

Results

Out of the 100 sera examined from blood donors, IgM was detected in 3 and 32 had IgG. This constitute an overall detection rate of IgM and IgG 3% and 32% respectively (table 1, figure 2).

The results showed that a high rate of IgM (4.3%) was detected among the age group 31-40 years old (table 2, figure 3). The difference in rates among age groups was found to be insignificant at p=0.942 .

The results showed that a high rate of IgG (47.8%) was detected among the age group 31-40 years old (table 2, figure 3). The difference in rates among age groups was found to be insignificant at p=0.026 .

The results revealed that a rate of 7.3% for IgM was reported among those who had contact with cats while it was zero for those who had no contact with cats (table 3, figure 4). The difference in rates between them was found to be ststistically insignificant at p=0.066 .

The results revealed that a rate of 34.1% for IgG was reported among those who had contact with cats while it was 30.5% for those who had no contact with cats (table 3, figure 4). The difference in rates between them was found to be ststistically insignificant at p=0.701 .

The results demonstrated that the highest rate of IgM (11.8%) was detected among those who do not consume milk (table 4, figure 5). The difference in rates was found to be statistically insignificant at p=0.074 .

The results demonstrated that the highest rate of IgG (41.2%) was detected among those who do not consume milk (table 4, figure 5). The difference in rates was found to be statistically insignificant at p=0.074 .

From the results , a rate of IgM (4.3%) was reported among those who consume sheep and beef meat (table 5, figure 6) The difference in rates was found to be statistically insignificant at p=0.515. The rate of IgG (30.0%) was reported among those who consume sheep and beef meat (table 5, figure 6). The difference in rate was found to be statistically insignificant at p=0.515 .

Table 1: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test.

	ELISA IgM	ELISA IgG
Positive	3%	32%
Negative	97%	68%
Total	**100%**	**100%**
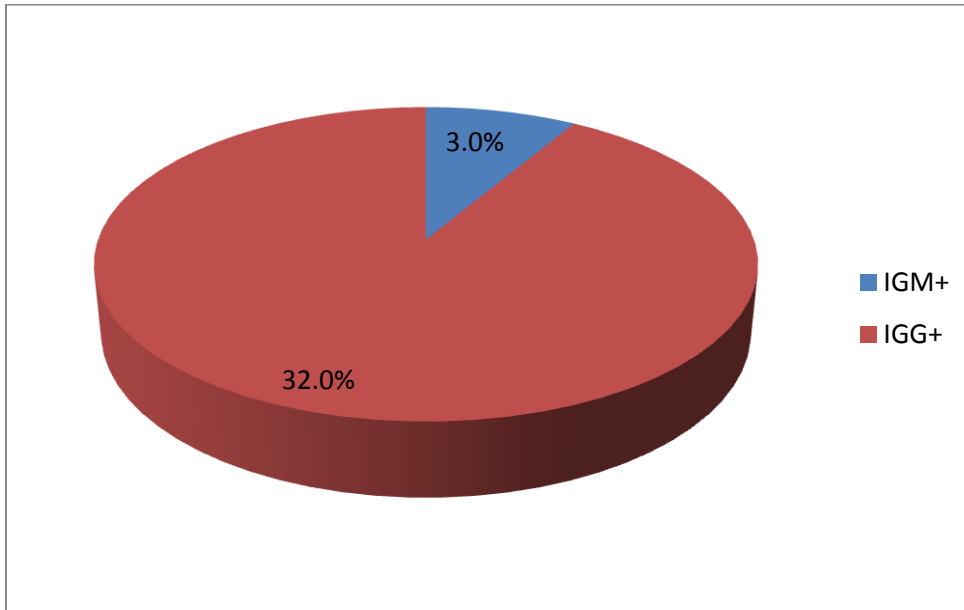
Figure 2: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test.

Table 2: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to age groups.

Age groups	No. examined	IgM +	IgG+
10 - 20	1	0 (0.0%)	1 (100.0%)
21 - 30	69	2 (2.9%)	16 (23.2%)
31 – 40	23	1 (4.3%)	11 (47.8%)
41 – 50	7	0 (0.0%)	4 (57.1%)
Total	100	3 (3.0%)	32 (32.0%)
P-value		0.942	0.026
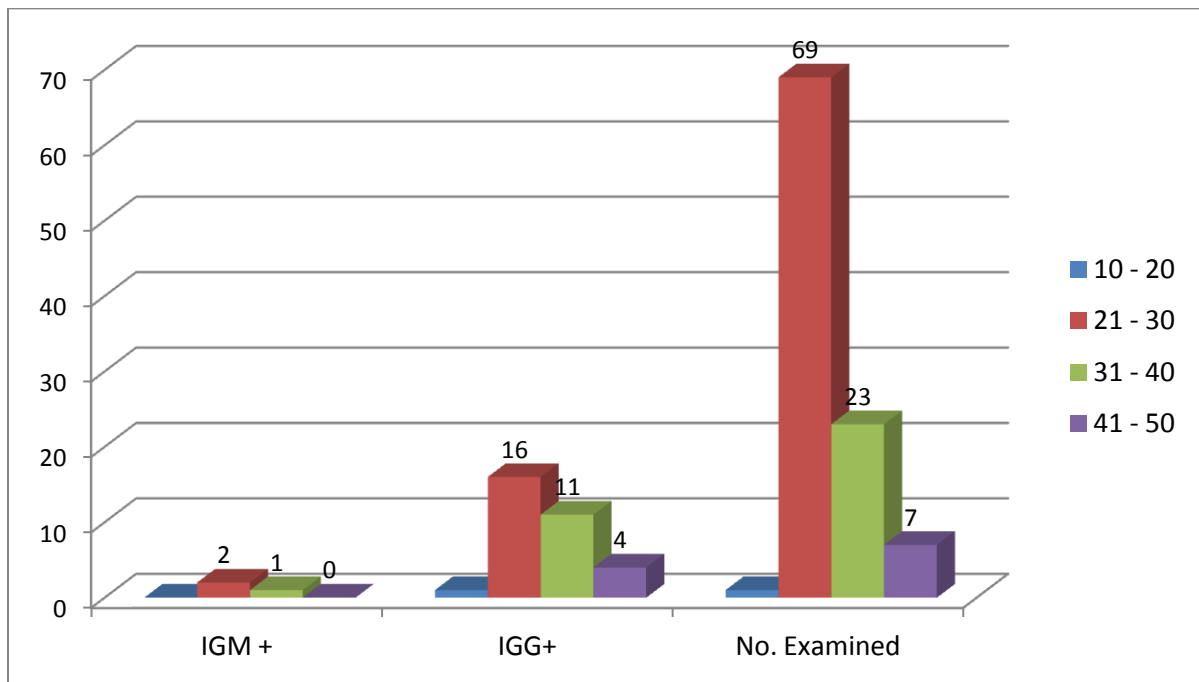
Figure 3: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to age groups.

Table 3: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to contact with cats.

Contact with cats	No. examined	IgM+	IgG+
Contact	41 (100.0%)	3 (7.3%)	14 (34.1%)
No contact	59 (1.0%)	0 (0.0%)	18 (30.5%)
Total	100 (100.0%)	3 (3.0%)	32 (32.0%)
P-value		0.066	0.701
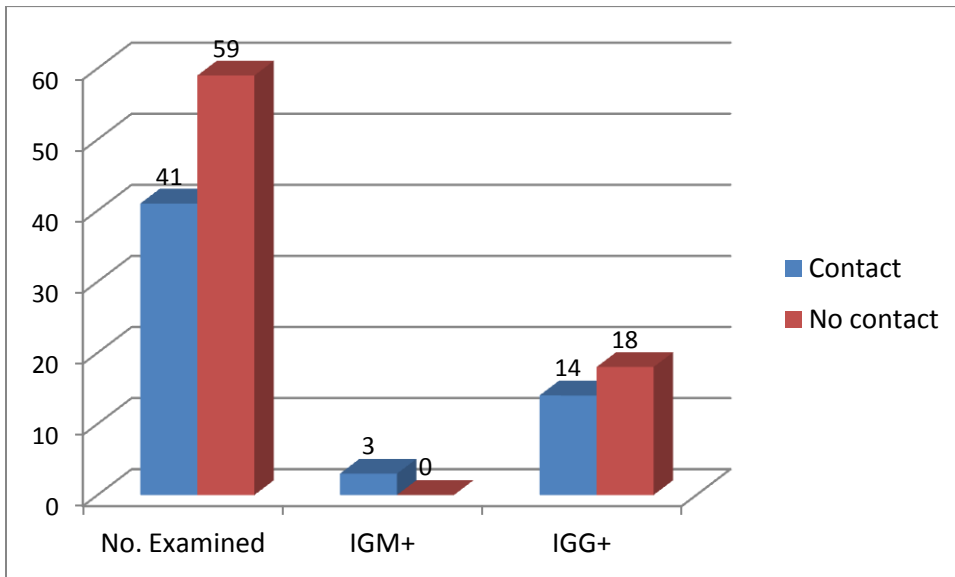
Figure 4: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to contact with cats.

Table 4: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to consumed consumption of milk.

	No. examined	IgM+	IgG+
Milk	83 (100.0%)	1 (1.2%)	25 (30.1%)
No milk	17 (100.0%)	2 (11.8%)	7 (41.2%)
Total	100 (100.0%)	3 (3.0%)	32 (32.0%)
P-value		0.074	0.401
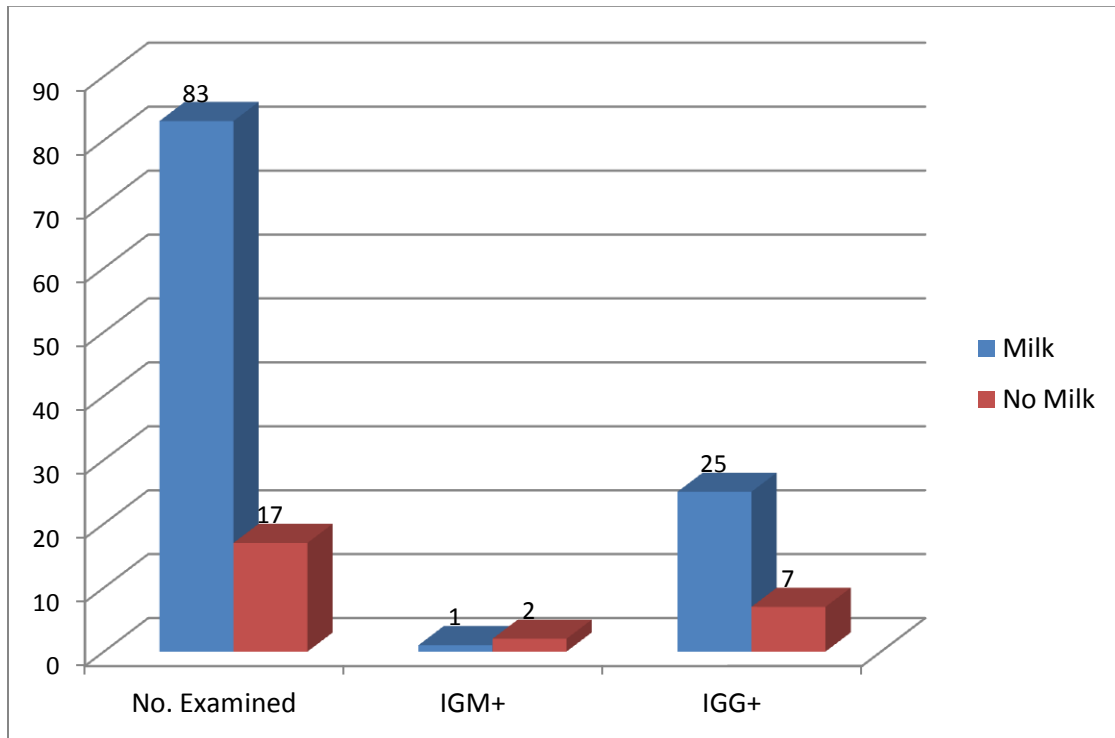
Figure 5: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to consumption of unpastrized milk.

Table 5: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to type of meat consumed .

	No. Examined	IgM+	IgG+
Sheep only	14 (100.0%)	0 (00.0%)	5 (35.7%)
Beef only	16(100.0%)	0 (0.0%)	6 (37.5%)
Sheep and Beef	70 (100.0%)	3(4.3%)	21(30.0%)
P-value		0.515	0.803

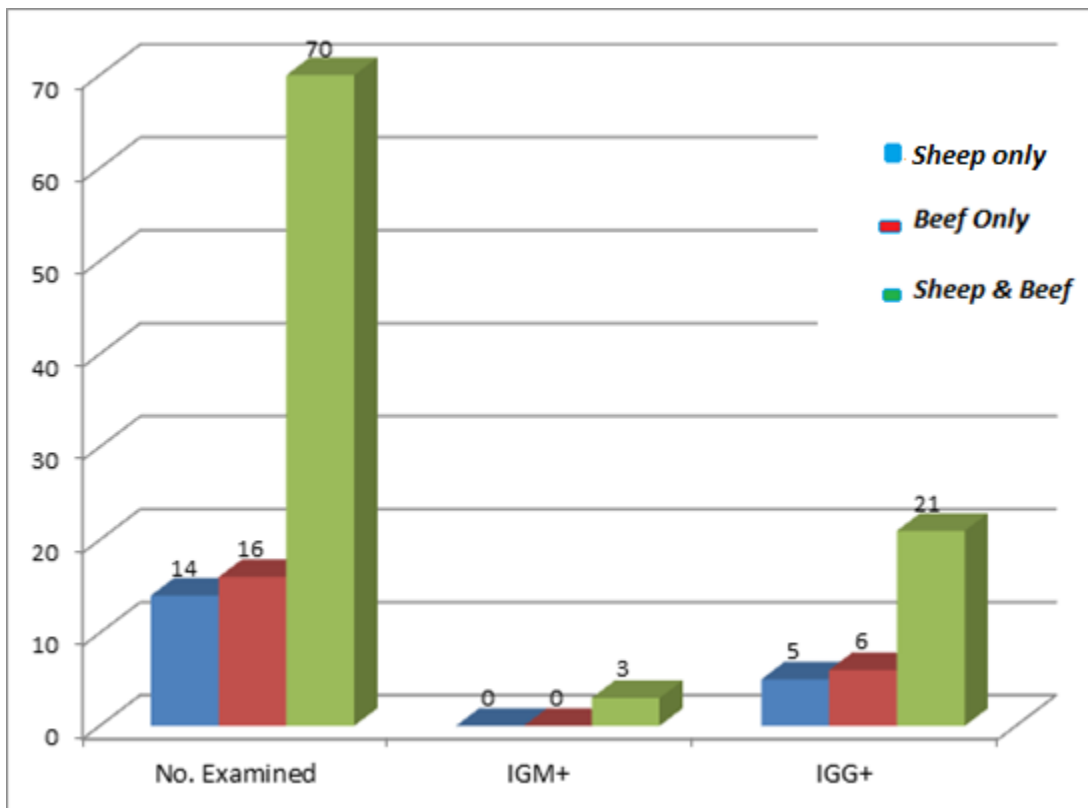**Figure 6:** The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA test according to type of meat consumed.

Chapter four

DISCUSSION

CHAPTER FOUR

DISCUSSION

The overall prevalence of positive anti-*Toxoplasma* antibodies using ELISA test for IgG and IgM was found to be 32% and 3% respectively .These rates were found to be higher than the rates reported by Pinlaor *et al.* (2000) in Loei Province,Northeast Thialand and Alvarado-Esquivel *et al.* (2007) in Durango, Mexico (4.1%,4.3%),(7.4%,1.9%) respectively.

In Durango, Mexico, Alvarado-Esquivel *et al.* (2007) showed that the highest prevalence rates of toxoplasmosis reported (11%) was found in the age group 35–60 years while the lowest rate (4.3%) was in the age group 25–34 years. The difference among these rates (11% vs 4.3%) was statistically significant (p = 0.02). In this study, high rate (47.8%) for IgG was reported among the age group 31-40 .This rate was higher than the rate reported above by Alvarado-Esquivel et al (2007) and lower than the rate reported by Davami et al (2014) in the same age group .In our opinion , this might probably indicates that the infection increases by age .

Contact with cats has not always been associated with *T. gondii* seropositivity

in epidemiology studies . As shown in this study, 30.5% positive IgG was reported in those who had no contact with cats while it was 34.1% for those who had contact with cats and also showed that 7.3% positive IgM was reported in those who had contact with cats . Our result questions the existence of an association between contact with cats and toxoplasmosis in humans.

The study showed a high prevalence of *T. gondii* antibodies in healthy blood donors. It may be appropriate to include screening for *T. gondii* also in the pretransfusion blood testing schedule.

Regional variations have been attributed to climate cultural differences in the amount and type of raw meat consumed and the variable consumption of meat from animal's farmed indoors and frozen meat.

Ingestion of undercooked meat is responsible for the majority of toxoplasmosis cases in France and in The United States as it accounts for half of the cases. In six European countries eating undercooked, raw or cured meat contributed to between 30% and 63% of infections, with soil contact contributing to up to 17% of infection (Birgisdóttir *et al*., 2006).

This study , we reported a rate of 4.3% IgM among those who consume sheep and beef meat and a rate of 30.0% was reported for IgG among those who consume sheep and beef meat .This confirms the association between meat consumption and occurrence of toxoplasmosis .

A large family cluster of acute toxoplasmosis was identified in northern California . IgM antibody tests showed that 10 of 24 members of an extended family had serological evidence of acute *Toxoplasma* infection. All ten seropositive persons had recently consumed raw goat's milk from the family herd as compared with no consumption of raw milk by the 14 persons with negative results. The data suggest that drinking raw milk from infected goats might be another possible vehicle for the transmission of toxoplasmosis (Sacks *et al.,* 1982).

On the country, in this study, highest rates of 11.8% and 41.2% were reported for IgM and IgG respectively among those who do not consume milk. This might also probably raise the questions of the existence of the association between drinking milk and the occurrence of toxoplasmosis.

Chapter Five
Conclusions and
Recommendations

Chapter five

Conclusions and Recommendations

5.1 Conclusions:

1. Toxoplasmosis is existing in Khartoum state as detected by the ELISA test .

2. Infection increases with age.

3. The most possiple source of infection to the human host is consuming beef and sheep meat (mutton) .

5.1 Recommendations:

Appropriate and most effective measures for prevention and control or even eradication of *Toxoplasma* infection should be adopted.These should include:

1. Serological screening :
    All blood donors should be screened for toxoplasmosis before transfusion.
2. Health education:
    Health education camping may be started and concentrated on the risk of contamination by *Toxoplasma* and people are to be advised to follow the preventive measures below:
    a. Handling meat with care, avoid tasting raw meat and wash hands with soap and water after handling meat.
    b. Avoiding drinking unboiled milk.
    c. Avoid contact with cats feaces or material that may be contaminated with it by either getting rid of cats or by keeping them away from the reach of rodents.
    d. Prevention of access of flies to vegetables, fruits or food that ordinarily consumed without washing.

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Appendix

Appendix (1)

The Questionnaire

Sudan University of Science and Technology

Collage of Graduate Studies

Department of Parasitology and Medical Entomology

Seroprvelance of *Toxoplasma* infection in Blood Donors in Khartoum state

- **Name: ………………………….**
- **Patient ID: …………………….**
- **Age: …………… Years**
- **Residence: …………………….**
- **Educational level: …………….**
- **Contact with cats: Yes (   )   No (   )**
- **Type of meat consumption: Beef (   ) Sheep (   ) others (   )**
- **Milk or milk products consumption : Yes (   ) No (   )**

Date: ………………………….   Sig: ………………………..