

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 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).

1.1 Historical background of *Toxoplasmosis gondii* infection:

The history of *Toxoplasmosis 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 (*Ctenodactylus gondii*). As the organism resembled *Leishmania*, they

tentatively named it *Leishmaniagondii*. The next year, Nichol and Maceaux decided on the basis criteria, that it was not a *Leishmania* organism and proposed the name *Toxoplasmosis 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 with a possibility of transplacental route of infection (Al-Hindy, 1994).

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 faeces. Later identified the faecal stages of cats as coccidian oocysts.

1.2 Classification of *Toxoplasmosis gondii*:

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

Phylum	Apicomplexa
Class	Sporozoa
Order	Eucoccidida
Suborder	Eimerina
Family	Sarcocystidae
Subfamily	Toxoplasmatinae
Genus	<i>Toxoplasma</i>
Species	<i>Toxoplasma gondii</i>

1.3 Life cycle (figure 1):

The activity multiplying asexual form in the human host is an obligate intracellular parasite, pyriform in shape and approximately 36×6mm. 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 thousands organisms and measure from 10 to 100 µm in diameter. Within epithelial cells of the cat, a variety of morphological forms has been described, ultimately leading to male and female gametocytes. The fertilized macrogametes develop into nearly spherical oocysts that are released by rupture of intestinal epithelial cells.

When passed in cats faeces, the oocysts measure about 10 to 13 µm in diameter, the wall has 2 layers and contains undifferentiated material, but the content develop into 2 sporocysts within several days after being passed. Sporocyst in turn, contains 4 sporozoites (Brown and Neva, 1994).

Toxoplasmosis gondii tachyzoites multiply intracellularly by specialized fission 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 (enteroepithelial):

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 *Toxoplasmosis 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.

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 predilection 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. Tissue 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 trypsin 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).

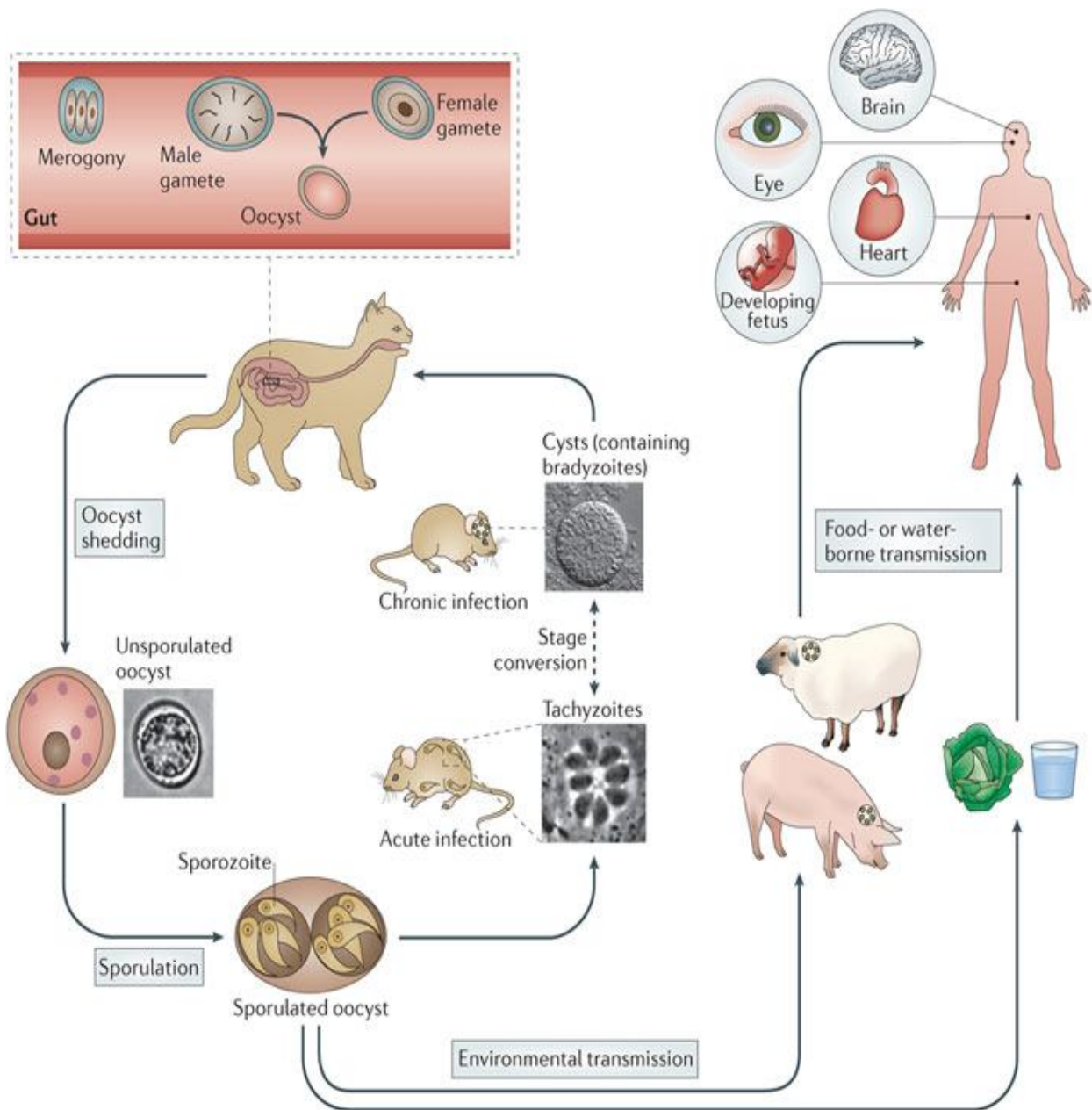


Figure 1: Life cycle of *Toxoplasmosis gondii*

Redrawn from Lynfield R, Guerina NG. *Toxoplasmosis*. *Pediatr Rev* 1997; 18 (3): 75-83.

1.4 Pathology and symptomatology of *Toxoplasmosis gonii* Infection:

Ordinary *Toxoplasmosis 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 Congenital *Toxoplasmosis*:

According to Congenital disease center (CDC) (2008), congenital toxoplasmosis is group of symptoms caused by infection of the unborn baby (fetus) with the parasite *Toxoplasmosis gondii*. The report showed that in the United Kingdom (UK), approximately 2,000 women a year get *Toxoplasmosis* while they are pregnant. If *Toxoplasmosis* occurred during pregnancy, there may be no or just a few mild symptoms, such as a sore throat and a mild fever. However, there is a chance that *Toxoplasmosis* infections will be passed on to the baby; this is known as congenital *Toxoplasmosis*. Usually, the incubation period ranges between 5-23 days and it can take between 4-8 weeks to pass the infection to the baby. The risk of *Toxoplasmosis* being passed to the baby varies; depending on which trimester of pregnancy the infection occur, for example, the risk is estimated to be:

- Between 10-15% if the mother is infected during the first trimester of pregnancy (weeks 0-13).
- As high as 70-80% if the mother catch *Toxoplasmosis* during the third trimester of pregnancy (weeks 27 to birth).

The infection is acquired from the mother and *Toxoplasmosis gondii* may be isolated from the placenta or vaginal discharge (Brown and Neva, 1994).

An intrauterine transmission of *Toxoplasmosis gondii* to the fetus takes place only during the acute phase of infection in the mother i. e. in the index pregnancy (Saxon et al, 1973). It has been shown that if *Toxoplasmosis* infection is acquired during pregnancy, the rate of transplacental transmission to the fetus occurs in about 46 to 65% of cases (Dubey and Frenkel, 1974; Stray-Pederson, 1980) and 50% of mothers who acquired the infection during pregnancy, if not will give birth to infected infants (Russo and Galanti, 1990).

The symptomatology and pathology depend upon gestational period at which the fetus is infected and may be classified as acute, sub-acute and chronic (Klapper and Morris, 1990). In jaundice, skin rashes, hepatomegaly, lymph adenopathy, and meningoencephalitis (Buxton, 1990). This type is rarely seen and pathology of the organs is similar to that described in the adult, varying in degree of severity with of the age of the fetus at the time of infection and the antibody protection from the mother (Vas et al; 1990). Another study reports only 30% (Pratlong *et al.*, 1996).

The sub-acute form is present in about 75% of cases at birth and remains normal for months or years, but they present with anomalies in childhood (Desmont and Couvreur, 1978; Wilson et al., 1980; Pratlong et al., 1996).

In the sub-acute form, there has been subsidence of the acute lesions and the child presents with cerebral calcification, chorioretinitis, which is usually bilateral with contrast to the acquired form,

hydrocephaly or microcephaly and various signs of central nervous system involvement (Desmont and Couvreur, 1978).

The chronic form may be asymptomatic or exhibit mild symptoms of the sub-acute stage (Remington and Kelein, 1990). Relapses may occur and meningo-encephalitis or chorioretinitis may be seen in older children (Wilson *et al.*, 1980). In the brain, miliary granuloma that may be scattered throughout the lesion become calcified in the cortical layers and may be seen radiologically as bilateral round shadow 1-3/mm in diameter. Signs of hydrocephalus and thinning of the skull may occur. The protein in cerebrospinal fluid is increased and thinning of the skull may occur (Wilson *et al.*, 1980).

Toxoplasmosis is found to have an association with prematurity. Laboratory personnel at Taif children's hospital in Saudi Arabia had measured the antibodies level to *Toxoplasmosis gondii* in blood samples from Saudi Arabia premature infants exhibiting symptoms of congenital toxoplasmosis. They found that 32.1% of infants tested were positive to antibodies, which indicates recent infection (Abdalla *et al.*, 1994).

The typical congenital *Toxoplasmosis* triad includes hydrocephaly, chorioretinitis and intracerebral calcification. The lung, liver, spleen and lymph nodes are monocytes affected. Anaemia and leukocytosis with an absolute increase in of severe congenital lesions are not pathognomic (Walter and Israel, 1987). Focal necrosis may be found in the liver, spleen and lungs, and this is surrounded by nonspecific small round cell infiltrations that include polymorphs.

Haemorrhagic extravasations are common, and the fibrinous pleurisy that may also be haemorrhagic. The brain, likewise, contains necrotic foci.

surrounded by small round cells and proliferating tissues. Dystrophic calcification is a prominent sequel. The lymph nodes show no necrosis, but only a reactive change similar to that of the adult lesions (Walter and Israel, 1987).

The pathological examination of the placenta in human cases of toxoplasmosis revealed that about 63.3% of placenta were macroscopically normal, 9% were hydropic, 21% were of mixed or hydropic pattern, 6% had areas of infarction and 0.7% shows areas of calcification. In hydropic placenta, a villous edema was of very variable degree from one placenta to another and from one area to another in same placenta (Abdel- Salam, 1990).

Toxoplasma gondii can be demonstrated in the products of conception, the placenta or the amniotic fluid by the mouse inoculation method (Stray-Pederson, 1980; Pratlong et al. 1996).

Most probably, therefore, intrauterine transmission takes place by a direct invasion from the blood stream into the placenta tissue or into the amniotic fluid, and not indirectly through an invasion of the deciduas (Carrington, 1990). If there, really, should exist an infection route through the uterine wall, some parasites would probably persist in the endometrial, causing a chronic infection, which again may possibly lead to spontaneous abortion, or the theoretical occurrence of congenital toxoplasmosis in the subsequent pregnancies (Stray-Pederson, 1980). Some infants are not infected (during intrauterine life, but are infected during labour from an infected placenta (Reimington and Kelelin, 1990).

Occasionally, the infection is milder and there may be complete recovery, it is estimated that of congenital infection, 5 to 15% of babies will die, 8 to 10% will have severe brain and eye damage, 10 to 13% will

have moderate to severe visual handicaps, and 58 to 75% will be asymptomatic at birth with small proportion developing active chorioretinitis or mental retardation as children or young adults (Stray Pederson, 1980). The incidence of congenital toxoplasmosis in general varies between 0.5 to 6.5 cases per 1000 live birth (Russo and Galanti, 1990).

1.4.1.2 Acquired adult toxoplasmosis:

Most infection as shown by population surveys must have been 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.

The parasite may be proliferating in the cells of the reticuloendothelial system and parenchymal cells of partially any organ. The most severe lesions are seen in striated muscles, the central nervous system and the heart. The lungs, liver, pancreas, spleen, testes, kidneys, hypophysis and the adrenal. In the chronic stage, chronic myocarditis and chronic lesions in the muscle, and a local hypersensitivity to release *Toxoplasma gondii* from ruptured cells may be found. A chronic local lesion with localized lymphadenitis has occurred in laboratory workers following a finger prick with infected material (Gallalan et al. 1964; Botros and Fairchilb, 1972).

1.4.1.3 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 deficiency 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 lymphocytic 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).

1.4.1.4 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.

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 (Jacobs, 1976).

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, 1FAT, IHA, 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 Flourescent 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):

It 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 (Valkoum 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, 1986). 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 Hammed (1991), investigated the disease in Gezira 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 ALHindy. He collected sample 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, across sectional survey was carried out in Khartoum hospital and Omdurman maternity hospital by Alhadi. 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 sero positive prevalence rate was found to be 34.1%. 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 splenomegaly, 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 13.4% 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 IgM 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 (IgM), 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%.

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 females (pregnant and non pregnant) in Dallwat hospital, Gezira State.

Specific objectives:

1. To detect the rate of anti- *Toxoplasma* antibodies using two different serological techniques (latex agglutination test and enzyme linked immune sorbent assay (ELISA).

2. To assess risk factors associated with toxoplasmosis (age, meat and milk consumption, pregnancy stage and history of previous abortions).

Chapter two

Materials and methods

2.1 Study design:

It is across-sectional hospital-based study.

2.2 Study area:

The study was conducted in Dallwat Hospital. It is located in the Gezira state, 156 kilos far away from Khartoum center.

2.3 Study population:

The study was conducted on pregnant and non-pregnant females. They were divided according to the following age groups:

Group	Age
A	15-25
B	26-35
C	above 35

2.4 Sample Size:

Samples were taken from 100 females (50 pregnant and 50 non pregnant).

2.5 Ethical Considerations:

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

2.6 Samples Collection:

Five ml of blood was drawn from each female. The blood was centrifuged at 2000 rpm and sera were extracted and stored separately at -20 °C. 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.

2.7 Data Collection:

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

2.8 Techniques:

2.8.1 Direct Agglutination Test:

Commercial kit (Spine react) was used.

2.8.1.1 Principles of the Test:

Toxo-latex test is a rapid slide agglutination procedure, developed for the direct detection of anti-*Toxoplasma* antibodies in human serum.

The assay is performed by testing a suspension of latex particles coated with antigenic extract of *Toxoplasma gondii* against unknown samples.

The presence or absence of a visible agglutination indicates the presence or absence of anti-*Toxoplasma* antibodies in the sample tested.

2.8.1.2 Procedure:

Before using the kit, components were allowed to reach room temperature. They were gently shaken (R. toxo-latex) to disperse the latex particles. The reagent was checked against the positive and negative

controls. 50 μl of the sample serum were placed into one of the circles on the card. One drop of positive control and one drop of negative control were dispensed into two additional circles. 25 μl of toxo -latex were added next to the serum. Both drops were mixed by spreading them over the surface of the circle.

The slide was then rotated by means of a mechanical rotator at 100 r.p.m for a period of 4 minutes. The presence or absence of visible agglutination was read.

2.8 1.3 Interpretation of the Results:

A homogeneous appearance (negative reaction) should be interpreted as the absence of *Toxoplasma* antibodies or titers lower than 10 IU/ml.

A clear agglutination (positive reaction) should be interpreted as presence of *Toxoplasma* antibodies, which may reflect either a past infection or an evolving *Toxoplasma* infection.

2.8.2 Enzyme linked immune sorbent assay:

2.8.2.1 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 toxo IOM EIA kit and IgG EIA kit.

2.8.2.1.1 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 IgM are added and will in turn bind to any human IgM present and IgG present. The microwells were 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.

2.8.2.1.2 Components:

The components of the commercial kit used are described in appendix 2.

2.8.2.1.3 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 1 μl 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 for 30 minutes at room temperature. 100 μl of negative control, low positive standard (cut-off), high positive standard and 5 μl serum specimens were added to subsequent wells, then, microwells were incubated at room temperature for 30 minutes. They were washed by inverting and flicking into a sink, completely filled with wash buffer and washing was repeated three times, refilled with wash buffer and soaked for 5 minutes. Wells and blot were emptied with absorbent paper using an automatic washer, the wells were filled and spired five times without soak. 100 μl 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. 50 μ l of substrate A, and 50 μ l of substrate B were added to each well and incubated in dark place at room temperature for 10 minutes. The reaction was stopped by adding 50 μ l 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 30 minute.

2.9 Calculation and interpretation of 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 0.9 indicated negative sample, A ratio greater than 1.1 indicated a positive sample. A ratio between 0.9 and 1.1 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.

2.10 Statistical analysis:

Data were analyzed using Statistical Package social science (SPSS) software programme.

Chapter three

Results

One hundred serum samples were collected from pregnant and non pregnant women (50 pregnant and 50 non pregnant women). All samples were examined by latex agglutination test, ELISA IgM and IgG for the presence of anti *Toxoplasma* antibodies. Positive results were detected in 49%, 2% and 37% for latex agglutination, ELISA IgM and ELISA IgG respectively (tables 1, 2, 3).

The results showed that the highest rate 46.9% was reported among women between the 26-35 years age group when examined by latex agglutination test. For ELISA, the highest rate (36.7%) was reported among women in the 26-35 age group. The lowest rate (4.1%) for anti-*Toxoplasma* antibodies was reported among women in the 26-35 age group (table 4, figure 3). The difference in rates among the age groups was found to be statistically insignificant for ELISA IgM and IgG ($P=0.346$ and 0.993 respectively). However, the difference was found to be statistically insignificant at $P=0.100$ for the latex test.

Out of the 25 samples of women drinking cow milk, 1 sample (4.0%) was found to be positive for anti-*Toxoplasma* IgG antibodies by ELISA test (table 5, figure 4) and out of 3 samples of women drinking cow and sheep milk, two samples (66.7%) were found to be positive for anti-*Toxoplasma* IgG antibodies and IgM antibodies.

Out of 5 samples of women drinking cow and goat milk, 2 samples (40%) was found to be positive for anti-*Toxoplasma* IgG antibodies by ELISA test and no IgM antibodies were detected. Out of 9 women who did not consume milk, no sample (0%) and 3 samples (33.3%) were

found to be positive when examined by ELISA IgM and IgG respectively (table 5, figure 4) and out of the 58 samples of women who consumed all types of milk, 1 sample (1.7%) was positive when examined by ELISA IgM and 22 samples (37.9%) were positive when examined by ELISA IgG. When the same samples were examined by latex agglutination test for women drinking cow milk, 12 samples (48%) were found to be positive, and out of the three women who consumed cow and sheep milk, 1 sample (33.3%) was found to be positive. Out of the 5 women who consumed cow and goat milk, 2 samples (50%) was found to be positive.

For those who consumed all types of milk, 29 (50%) were found to be positive by latex agglutination test, while among those who did not consume milk, 4 samples (44.4%) were found to be positive by latex agglutination test (table 5, figure 4). The difference in rates among those who consumed different types of milk was found to be statistically insignificant at $p=0.927$ for the ELISA IgM, 0.828 for the ELISA IgG and 0.958 for the latex.

Among the 18 sera of women who consume cow meat using ELISA IgG test, 8 samples (44.4%) were found positive. 1 sample (5.6%) was detected using IgM antibodies. 7 samples (38.9%) were found to be positive by latex agglutination test. Among the 12 women who consumed sheep meat, 6 samples (50%) were found to be positive by ELISA IgG and no IgM antibodies were detected and 6 samples (50%) were found to be positive by latex agglutination test and out of the 6 samples of women who consumed cow and goat meat, 2 samples (50%) was found to be positive by ELISA IgG and no IgM antibodies were detected and 2 samples (50%) were found to be positive by latex agglutination test.

Out of the 14 samples of women who consumed cow and sheep meat, 2 (50%) were found to be positive by ELISA IgG and no samples (0%) were detected by ELISA IgM and 5 samples (35%) were found to be positive by latex agglutination test and out of 50 samples of women who consumed all types of meat, 1 sample (2%) and 17 samples (34%) and 27 samples (54%) were found to be positive for ELISA IgM, IgG and latex agglutination test respectively. For those women who did not consume any type of meat, 2 samples (100%) were found positive by using latex agglutination test (table 6, figure 5). The difference in rates among those who consumed different types of meat was found to be statistically significant for all groups at $p=0.0121$ for the ELISA IgM, 0.023 for ELISA IgG and 0.041 for the latex test.

The result showed that the highest rate (3.8%) was reported in the first trimester when using ELISA IgM and 46.2% when using ELISA IgG and the lowest rate 0.0% for ELISA IgM and 26.7% for ELISA IgG was reported in the second trimester. For latex agglutination test, the highest rate was reported in non pregnant women (54%) and the lowest (33.3%) was reported in third trimester (table 7, figure 6). These differences in rates were found to be statistically insignificant at $P=0.815$ for ELISA IgM, 0.566 for ELISA IgG and 0.580 for latex test.

The result revealed that anti-*Toxoplasma* antibodies appeared in 3.1% of those who had contact with cats and 0.0% of those who had no contact with cats when using ELISA IgM and when using ELISA IgG, the rate was 42.2% among those who had contact with cats and 27.8% among those who had no contact with cats.

When using latex agglutination test, anti-*Toxoplasma* antibodies appeared in 53.1% of those who had contact with cats and in 41.7% of

those who had no contact with cats (table 8, figure 7). The difference in rates was found to be statistically significant at $P=0.034$ for ELISA IgM, 0.025 for IgG and 0.046 for latex test.

The history of previous abortions revealed that the highest detection rate of anti-*Toxoplasma* antibodies when using ELISA IgM (8.3)% was reported in the two abortion group while the group with a history of one and no abortion showed 53.8% and 32.9% IgG antibodies respectively (table 9, figure 8). When using latex agglutination test, the rate was higher in the one time abortion 69.2%, two and three times abortion 66.7%, 80% respectively (table 9, figure 8). These differences in rates were found to be statistically insignificant at $p=0.105$ for ELISA IgM, 0.245 for ELISA IgG and 0.181 for latex test.

Table1:

The rate of anti-*Toxoplasma* antibodies in study group obtained by latex agglutination test .

Latex test	Frequency	Percentage
Positive	49	49%
Negative	51	51%
Total	100	100%

Table 2:

The rate of anti-*Toxoplasma* IgM in the study group obtained by ELISA test .

Elisa-IgM	Frequency	Percentage
Positive	2	2%
Negative	98	98%
Total	100	100%

Table 3:

The rate of anti-*Toxoplasma* IgG in the study group obtained by ELISA test .

Elisa-IgG	Frequency	Percentage
Positive	37	37%
Negative	63	63%
Total	100	100%

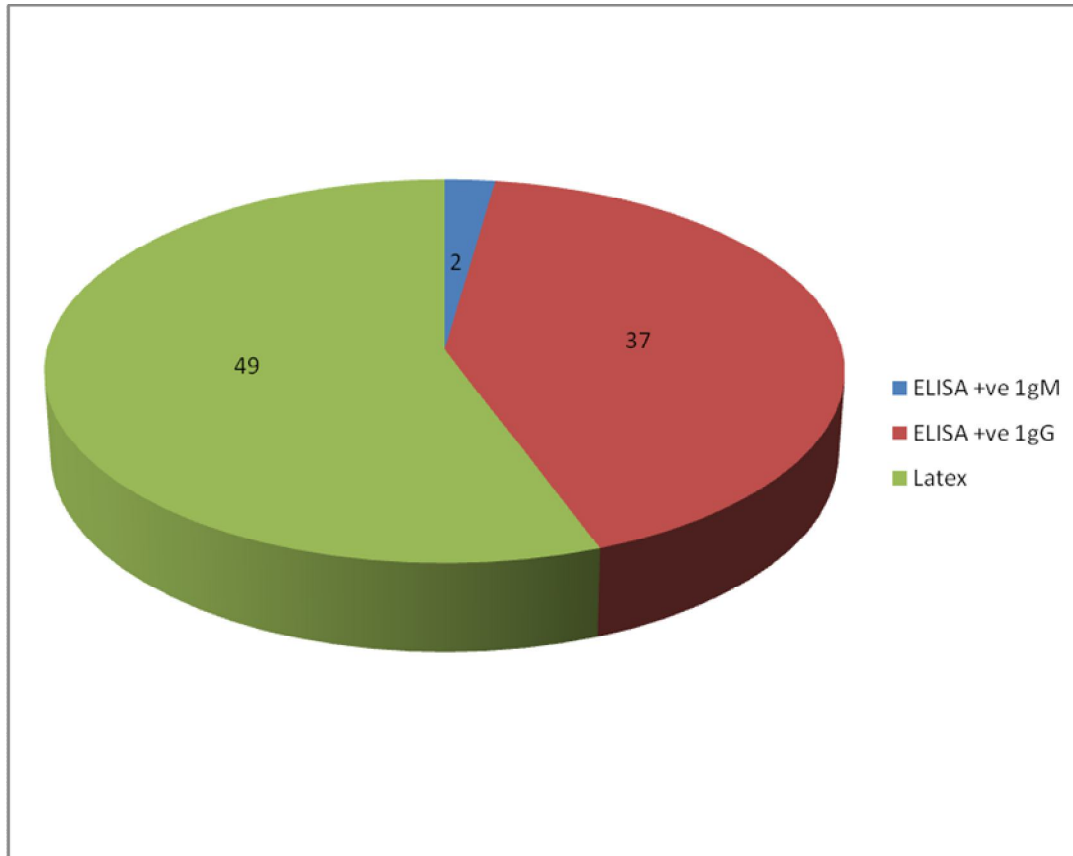


Figure 2:

The rate of anti-*Toxoplasma* antibodies in study group obtained by latex agglutination test and ELISA test.

Table 4:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to age group .

Age group	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
15 – 25	21	0(0%)	8(38.1%)	7(33.3%)
26 – 35	49	2(4.1%)	18(36.7%)	23(46.9%)
Above 35	30	0(0.0%)	11(36.7%)	19(63.3%)
p.value		0.346	0.993	0.100

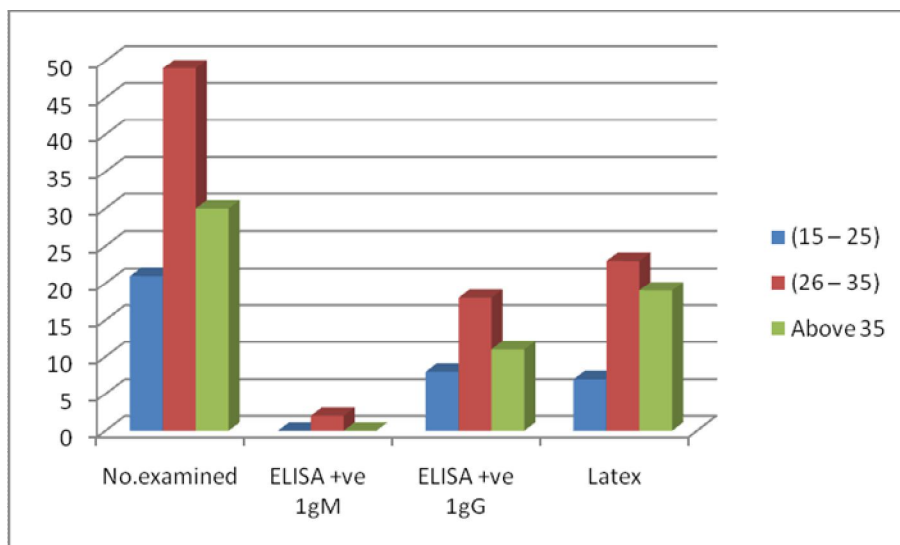


Figure 3:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to age group .

Table 5:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to the type of milk consumed .

Type of milk	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
Don't drink milk	9	0(0.0%)	3(33.3%)	4(44.4%)
Cow milk	25	1(4.0%)	8(32.0%)	12(48.0%)
Cow+ goat	5	0(0.0%)	2(40.0%)	3(60.0%)
Cow+ sheep	3	0(0.0%)	2(66.7%)	1(33.3%)
Drink all type	58	1(1.7%)	22(37.9%)	29(50.0%)
p.value		.927	.828	.958

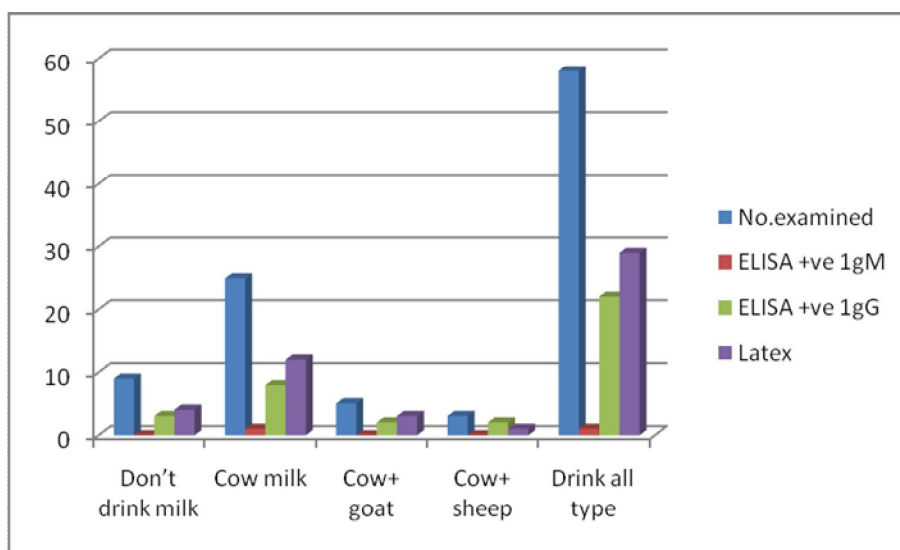


Figure 4:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to the type of milk consumed.

Table 6:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to the type of meat consumed .

Type of meat	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
Cow	18	1(5.6%)	8(44.4%)	7(38.9%)
Sheep	12	0(0.0%)	6(50%)	6(50%)
Cow+ goat	4	0(0.0%)	2(50%)	2(50%)
Cow+ sheep	14	0(0.0%)	4(28.6%)	5(35.7%)
all types	50	1(2%)	17(34%)	27(54%)
Not consumed	2	0(0.0%)	0(0.0%)	2(100%)
p.value		0.012	0.023	0.041

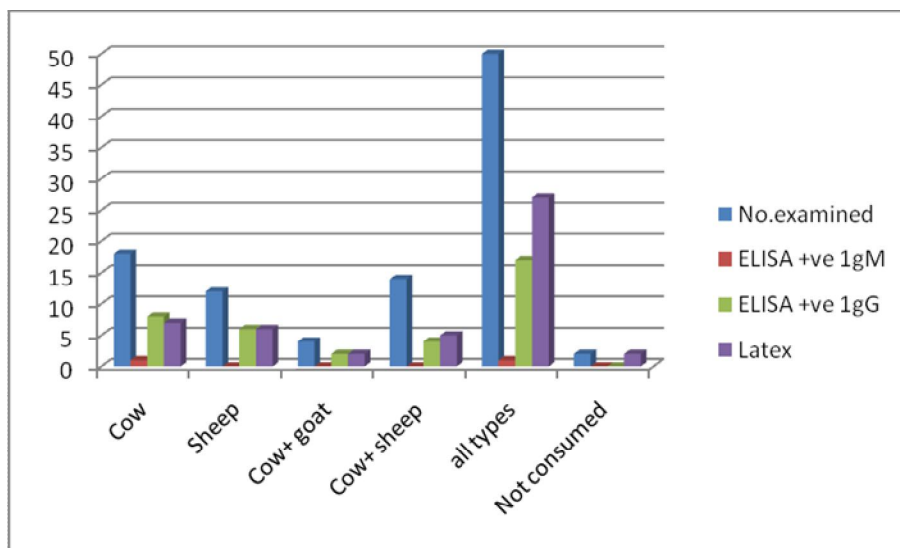


Figure 5:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to the type of meat consumed .

Table7:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to pregnancy stage .

Stage group	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
Non pregnant	50	1(2%)	17(34%)	27(54%)
1 st trimester	26	1(3.8%)	12(46.2%)	11(42.3%)
2 nd trimester	15	0(0.0%)	4(26.7%)	8(53.3%)
3 rd trimester	9	0(0.0%)	4(44.4%)	3(33.3%)
p.value		0.815	0.566	0.580

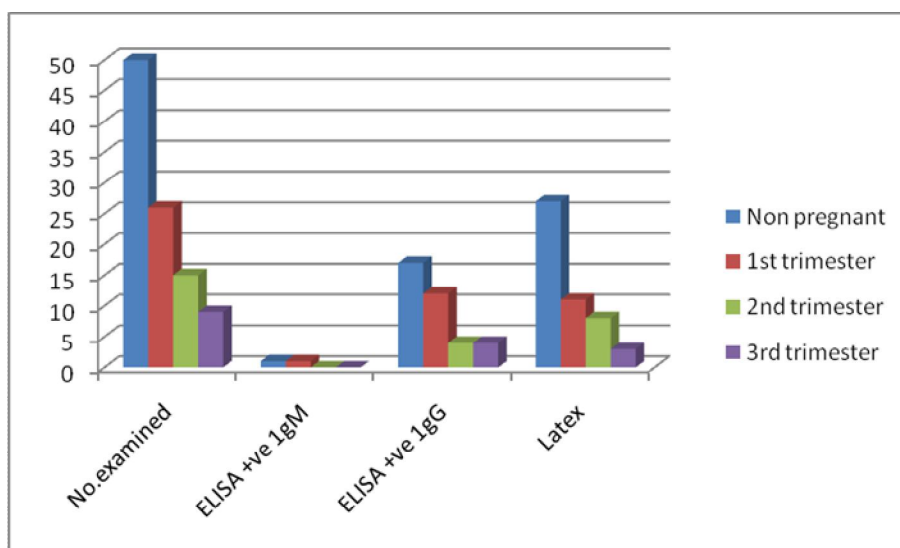


Figure 6:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to pregnancy stage .

Table 8:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to contact with cats .

	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
No contact	36	0(0.0%)	10(27.8%)	15(41.7%)
Contact with cats	64	2(3.1%)	27(42.2%)	34(53.1%)
p.value		0.034	0.0251	0.0463

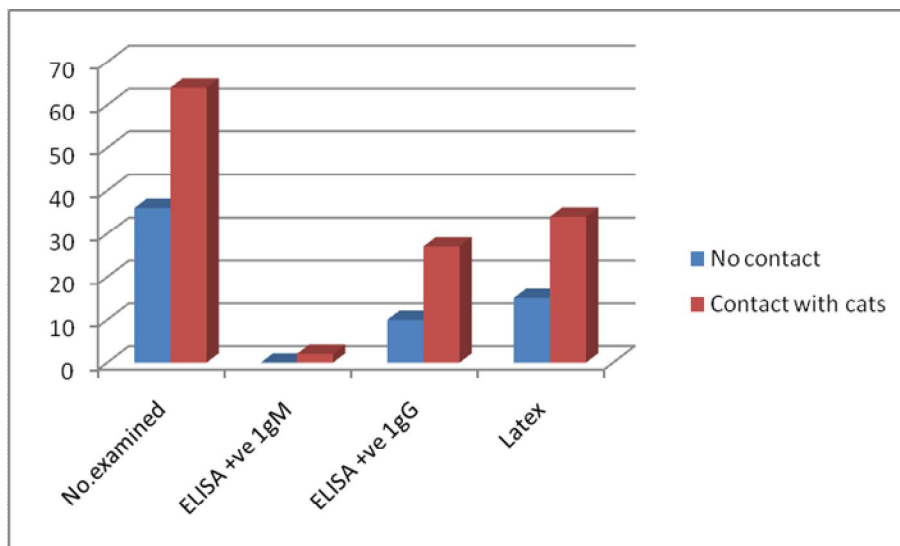


Figure 7:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to contact with cats.

Table 9:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to previous History of abortion .

History of abortion	No.examined	ELISA +ve 1gM	ELISA +ve 1gG	Latex
No abortion	73	0(0.0%)	24(32.9%)	31(42.5%)
1 time	13	1(7.7%)	7(53.8%)	9(69.2%)
2 time	12	1(8.3%)	6(50%)	8(66.7%)
3 time	2	0(0.0%)	0(0.0%)	1(50%)
p.value		0.105	0.245	0.181

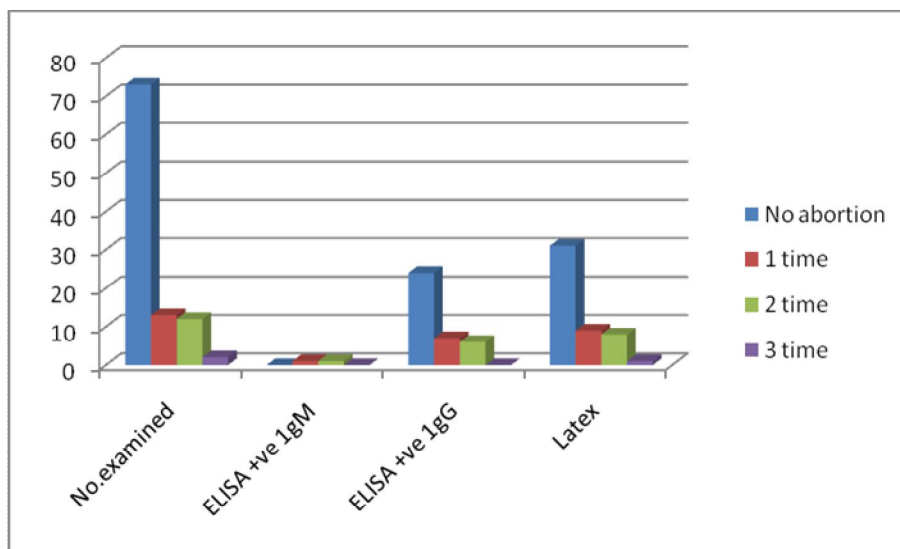


Figure 8:

The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination test according to previous History of abortion .

Chapter four

Discussion

The overall prevalence of positive anti-*Toxoplasma* antibodies using latex agglutination and ELISA tests was found to be 40 and 39% respectively. These rates were found to be higher than the rates reported by Abdel-Hameed (1991) and Bushra (2006) (41.7% and 36%) respectively and higher than the rates reported by Abdel-Raouf (2001) and Eman and Saad (2011) who reported rates of 13.4% and 22.5% respectively.

The highest prevalence rate of toxoplasmosis was reported among the 26-35 years age group (36.7%) by using the ELISA. In a previous study, Frenkel and Ruiz, (1980) reported high rate, but among the 15-25 years age group (61.4%). In this study, another high rate (36.7%) was reported among the above 35 years age group. This rate was lower than the rate reported above by Frenkel and Ruiz (1980), however, it was higher than the rate reported by Eman and Saad (2011) for the same age group (20%). The rate obtained from present study, however, was closer to that reported by Abdal-Raouf (36.3%) for the same age group which, in our opinion is very risky as it is the most fertile period. It has been mentioned that seroprevalence of toxoplasmosis increases with age (Shin et al. 2009). In their study, they observed that the lowest rate was reported in those aged ≤ 25 years, and the rates slowly increased with age with the peak level in the 26-35 age group.

As far as the milk consumption is concerned and its anociation with the occurrence of toxoplasmosis, the rate of anti-toxoplasmosis antibodies in women drinking all types of milk were the highest by both tests (37.9 and 50%) for ELISA and latex respectively.

Eman and Saad (2011) reported 7.1% as the highest rate by ELISA test and 50% by the latex test, but this was for the women who did not drink milk. Abdal-Raouf (2001) using ELISA IgG test found that there is no significant difference between cow milk and other types of milk. This factor might probably be neglected as it has not that much significance in the transmission cycle.

Concerning meat consumption, the rates reported among those who consumed cow meat were 44.4% and 38.9% for ELISA and latex respectively. This was different from the rates reported by Eman and Saad (2011) as their rates were 14.3% and 28.6% by ELISA and latex respectively. Also, they found the rates in women who eat sheep meat to be 7.7% and 24.4% using the same tests. In this study, the rates in women who consumed sheep meat were found to be 50% for both ELISA and latex tests. Abdal-Raouf (2001) revealed that the result of IgG was 17.9% and 17.08% for cow and sheep respectively and as shown above, the role of eating raw or under cooked beef or mutton is of great importance in the transmission of cysts containing bradyzoites. This concept was highlighted by Basalamah and Serebour (1981) in France where raw meat is a government item, who not surprisingly reported *Toxoplasma* serology rate as high as 60-87%. Our finding is also strengthened by Abdel-Hameed (1991) who related the high rate of toxoplasmosis to the consumption of raw or partially cooked liver, marrara, under cooked meat, shaya and abu dammam (spleen) in Sudan. Our conclusion was also supported by the finding of Elsheikha et al. (2009) that showed a significant association between *Toxoplasma gondii* seropositivity and eating meat by-products (Lunceon/Shawerma) in Egypt.

This investigation revealed that there statistically significant difference between those who are in contact with cats and those who are

not. This was supported by that found by Eman and Saad (2012), although, it is contradicting the result of Feldman (1982) who did not find antibodies to *Toxoplasma gondii* in the pacific island where cats are absent suggesting strong evidence that cats are very important in the transmission cycle.

For pregnant ladies, the study showed that the highest prevalence rate (42.3% and 46.2%) for latex and ELISA tests respectively were reported in women in their 1st trimester. These results agree with Eman and Saad (2011) who reported high prevalence rates of 40% and 16% for latex agglutination and ELISA tests respectively. Concerning ELISA IgG, Abdel-Gader (2008) found that the highest rate (18.2%) was in the 2nd trimester. In this study, and for those pregnant women in their 3rd trimester, the relatively high prevalence rates were reported as 26.7% respectively by the ELISA test which is lower than that reported by Eman and Saad (2011) (28.9%) for the same stage of pregnancy. In this study, and for those pregnant women in their 3rd trimester, the relatively high prevalence rates were reported as 44.4% respectively by the ELISA test which is higher than that reported by Eman and Saad (2011) (29.7% for the same stage of pregnancy). This result might probably suggest the possibility of materno foetal transmission. This type of transmission as suggested by Klapper and Morris (1990) increases in the 2nd trimester, it reaches the 65% and up to 80% at term as the highest incidence of congenital toxoplasmosis.

As far as the number of abortions is concerned, the difference was found to be statistically insignificant suggesting that *Toxoplasma* infection may have not contributed to the cause of abortion. This finding agreed with the finding of Eman and Saad (2011), however, it contradicted the finding of Griffin and Williams (1983) who reported a

prevalence of 42.3% in patients who have history of abortion indicating that *Toxoplasma* infection may in this case have contributed to the cause of abortion.

1.5 Conclusion:

From the study, one can conclude the following:

1. Toxoplasmosis is existing in Dallwat as detected by the latex agglutination and the ELISA test.
2. Detection of IgM in pregnant women might possibly suggest the risk of occurrence of congenital toxoplasmosis.
3. There is no correlation between abortion and toxoplasmosis.
4. The most possible source of infections to the human host is handling or eating under cooked meat of infected animal.
5. Cat the most possible source of infection .

2.5 Recommendations

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

1. Serological screening and follow up programme:

a. Pregnant woman should be screened for anti- *Toxoplasma* antibodies.

b. The children of recently infected mothers must be examined and followed up carefully for serological and clinical evidence of congenital infection if they were normal at birth and in early infancy.

2. Health education:

Health education campaign 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 eating raw or insufficiently cooked meat.

c. Avoiding drinking unboiled milk.

d. Avoiding contact with cats faeces or material that may be contaminated with it by either getting rid of cats or by keeping them away from the reach of rodents.

e. Prevention access of flies to vegetables, fruits or food that ordinarily consumed without washing.

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Appendix (1)

The Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Department of Parasitology and Medical Entomology

Name:..... **No.:**.....

Age:.....

Contact with cat: Yes:..... No:.....

Type of milk: Don't drink milk:..... Cow milk:.....

Cow/goat:..... Cow/sheep:.....

All types:..... Not consumed:.....

Stage group Non pregnant:..... 1st trimester:.....

2nd trimester:..... 3rd trimester:.....

No abortion:..... 1 time:.....

2 times:..... 3 times:.....

Appendix (2)

Components of 2 commercial kit (IgM and IgG).

- Microtiter strips -ToxoIgM,IgG negative control.
- Toxo IgM and IgG positive control.
- Dilution buffer IgM and IgG.
- Anti- IgM and anti-IgG conjugate -Washing solution -Substrate reagent.
- Stop solution.
- Adhesive strips.