University of Khartoum

Graduate College

Medical and Health Studies Board

Detection of the Rate of anti-Toxoplasma gondii Antibodies among the Humans, Cattle and Sheep Population in Khartoum State using the latex Agglutination and ELISA Tests.

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(B.Sc. Al Azhari University, 2007)

A thesis submitted for fulfillment of the requirement of Master Degree in parasitology and Entomology

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2012
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الإيّة

تُ دُ

صِدَقُ الله العظَمِيم

سورة الإسراء
Dedication

This thesis is dedicated to:
My Mother Fatmah.
My Father Alnour.
My wife Mona.
Acknowledgement

First of all, I thank Allah.

I am greatly indebted to my supervisor, Dr. Mohammed Baha Eldin Ahmed for his meticulous supervision, guidance and help.

I would like, also to thank my co-supervisor Dr. Ahmed Mohamed Abdel Halim for his help during the period of study.

My gratitude is also extended to members of the parasitology department, university of Khartoum.

My thanks are also to Khartoum teaching hospital staff who helped in the collection of samples and separation of the serum.

I am grateful to the working staff of the Kadaro slaughter house for helping me in collecting blood samples from slaughtered sheep and cattle.
Abstract

Background
Toxoplasmosis is a zoonotic infection of humans and animals, caused by the protozoan parasite *Toxoplasma gondii*. Toxoplasmosis is frequently asymptomatic, but in pregnant women may lead to abortion, stillbirth or other serious consequences in newborns. Transmission occurs through ingestion of *Toxoplasma gondii* oocysts shed into the environment by cats, or by eating meat of infected animals. This study aimed to investigate the sero-prevalance of anti-*Toxoplasma* antibodies among humans and animals their relationship to each other in Khartoum state using Latex agglutination and ELISA.

Methods

This descriptive, cross-sectional study was conducted among 200 males and 300 females (150 pregnant and 150 non pregnant) and 400 domestic animals (200 sheep and 200 cattle). Blood samples from males and non pregnant ladies were taken randomly at Khartoum Teaching Hospital. Blood samples from pregnant ladies were taken at Omdurman Maternity Hospital (Aldayat). Blood samples from animals were taken after slaughter in Alkadaro slaughter house.
ELISA (IgG and IgM) and Latex agglutination test were used for males and females population the Latex agglutination test was used for animals.

Results
The overall detection rate of antibodies to *Toxoplasma gondii* among females was 24.6% by ELISA test and 18% by latex test, 30.5% by ELISA in males and 11%, 18% in sheep and cattle respectively.
The highest prevalence rate among females was reported in the over 51 years age group (37.5%) and the lowest (19.35%) was reported among the 21-30 years age group when using the ELISA.
For males, the highest detection rate (38.50%) was reported among the 1-10 years age group while the lowest detection rate (25%) was reported among the 31-40 years age group when using the ELISA.
The results revealed that the rates of anti-*Toxoplasma* antibodies in sheep and cattle were 11% and 18% respectively when using latex agglutination test. Consumption of animal milk and contact with cat were found to be of no significance.

Conclusion
Toxoplasmosis, in Sudan is prevalent among females (pregnant and non pregnant), males and animals e.g. sheep and
cattle. There is risk of congenital toxoplasmosis occurrence in pregnant ladies with detected IgM antibodies.

المستخلص

خلفية البحث

داء المقوسات هو مرض مشترك بين الإنسان والحيوان يسببه طفيلي التوكسوبلازم ما جوندياي وينتج عن الإصابة بطفيل أحادي الخلية يسمى التوكسوبلازم ما جوندياي، تكاثرها يحدث في القطط، لذا سمي بداء القطط، مرض التوكسوبلازم ما في كثير من الأحيان ليس له أي أعراض إكلينيكية عند الإصابة بالمرض، لا يوجد سن معين للأصابة بالمرض ولكن في النساء الحوامل قد يؤدي إلي الإجهاض، أو ولادة جنين ميت أو عواقب أخرى في المواليد الجديد أما في مرضى نقص المناعة يمكن أن تكون قاتلة إذا لم تعالج. يحدث انتقال المرض إلى الإنسان عن طريق إبتلاع حوصلة الطفيلي أو تناول لحوم الحيوانات المصابة غير مجمدة الطهي. تهدف الدراسة لتحديدеннымية ما عدد حالات انتشار الأجسام المضادة للتوكسوبلازم ما وعوامل الخطر المحتملة لداء المقوسات بين الإنسان (ذكور والإناث) والحيوان في ولاية الخرطوم.

طريقة البحث

أجريت دراسة وصفية مستعرضة لتقييم المصلي في الأجسام المضادة للتوكسوبلازم ما جوندياي في منطقة غير مستوطنة من ولاية الخرطوم ل200 من الذكور و300 من الإناث و400من الحيوان (200اضان و200أبقار). عينة الدم الذكور والإناث غير الحوامل أخذت عشوائيا من مستشفى الخرطوم التعليمي، وعينة الدم للإناث الحوامل أخذت من مستشفى للدائيات أدرمان، وعينة الدم بالنسبة للحيوانات أخذت من مسلخ الكدرو بعد ذبح الحيوان مباشرة.

تم اختيار هذه العينات لمعرفة الأجسام المضادة لطفيلي المقوسات المعوية باختبار التلازمن واختبار المناعة المرتبطة بالخمائر.

نتائج البحث
كانت النسبة الكلية للأجسام المضادة الخاصة بالطفل 24.6% باختبار المناعة المرتبط بالخمائر، و% 18 بإختبار التلالزن من النساء، 30.5% بإختبار المناعة المرتبط بالخمائر لأجسام المضادة من الذكور، 11% و 18% بالتلالزن من الأغهام الأبقار علي التوالي.

أوضحت النتائج أن أعلى معدل للانتشار الأجسام المضادة للطفل قد تم تسجيله في الإناث في الفئة العمرية فوق 51 سنة، حيث بلغت 37.5% وأدنى معدل سجل في الفئة 21 -30 سنة بإختبار المناعة المرتبط بالخمائر.

بالنسبة للذكور، كان أعلى معدل للانتشار الأجسام المضادة قد تم تسجيله في الفئة العمرية 1-10 سنة، حيث بلغت (38.5%) وأدنى معدل في الفئة العمرية 31-40 سنة، حيث بلغت (25%) بإختبار المناعة المرتبط بالخمائر.

وأظهرت النتائج أن معدلات الأجسام المضادة للتوكسوبلازم في الأغهام والأبقار كانت 11% و 18% علي التوالي بإختبار التلالزن.

**إستنتاجات البحث**

داء المقواسات في السودان هو السائد بين الإناث بين الإناث (الحوامل وغير الحوامل) والذكور والحيوانات مثل (الضأن والأبقار)، يكمن خطر حدوث داء المقواسات الخلقي للسيدات الحوامل عند إكتشاف الغلوبولين المناعي للأجسام المضادة (IgM) بهم.
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Chapter One

Introduction and literature review
Chapter 1
Introduction and literature review
Toxoplasmosis is a universal zoonotic disease caused by the 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. human being, 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 coccidium, including the highly resistant and infective oocyst, have been positively identified (Nichol et. al., 1981). Animals are infected by eating infected meat, through both direct and indirect contact with cat faeces or by transmission from mother to foetus. The consumption of unwashed vegetables or undercooked meat and unpasteurized milk from infected animals are potential sources of infection (Frenkle and Ruiz, 1980).

Between 30 and 60 percent of the world population is estimated to carry a *Toxoplasma* infection. After the first few 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 HIV, may become seriously ill, and it can occasionally be fatal. The
parasite can cause encephalitis (inflammation of brain) and neurologic diseases and can affect the heart, liver and eyes (chorioretinitis).

1.1 Historical background of *Toxoplasma gondii* infections:
The history of *Toxoplasma gondii* began in the Pasteur institute in Tunisia when Nicholle and Manceaux in 1990 observed, a unicellular parasite in the mononuclear cell of the North African rodent (*Ctenodactylus gondii*). The organism also resembled *Leishmania* that they tentatively named it *Leishmania gondii*. The next year, Nicholle and Manceaux decided on the basis of morphological criteria, that it was not a *Leishmania* organism and proposed the name *Toxoplasma gondii*. Upon these findings, the parasite was redescribed retrospectively by Laveran (1900) in the Japanese paddy bird in Java and in a rabbit by Splendore (1908) in Brazil. The first report of a human infection was made by Janku in 1923 in Prague when he described toxoplastic 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 congenital neonatal disease in a fatal case of infantile encephalitis. In 1948, Sabin and Feldman introduced a serological test (the dye test) which allowed numerous investigators to study the epidemiological and clinical
aspects of toxoplasmosis and to demonstrate that *Toxoplasma* is the cause of a highly prevalent and wide spread (most often asymptomatic) human infection and to define the spectrum of the disease in human.

Hutchison et. al., (1969) discovered the role of cats in the transmission of the disease. He described the oocyst in cat faeces. Later on, Frenkel et. al., (1970) 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:

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</tr>
<tr>
<td>Order</td>
<td>Eucoccidida</td>
</tr>
<tr>
<td>Suborder</td>
<td>Eimerina</td>
</tr>
<tr>
<td>Family</td>
<td>Sarcocystidae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Toxoplasmataine</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Toxoplasma</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>Toxoplasma gondii</em></td>
</tr>
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</table>

**1.3 Life cycle (figure 1):**

The actively multiplying asexual form in the human host is an obligate, intracellular parasite, pyriform in shape and approximately 36x6 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 thousands organisms and measure
from 10 to 100 um 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 microgametes develop into nearly spherical oocysts that are released by rupture of intestinal epithelial cells (Frenkel et. al., 1970).

When passed in cat’s faeces, the oocysts measure 10 to 13 m in diameter, the wall has 2 layers and contains undifferentiated material, but the contents develop into 2 sporocysts, within several days after being passed. Each sporocyst in turn, contains 4 sporozoites (Brown and Neva, 1961).

Toxoplasma gondii tachyzoites multiply intracellularly by specialized form of division called endodyogeny, in which two daughter cells are formed within a mother cell. As the distended host cells fill up with parasite, they rupture, releasing parasites that enter new cells. Toxoplasma can grow in any mammalian or avian organ or tissue, developing in brain, eye and skeletal muscles (Brown and Neva, 1961).

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

1.3.1 Direct stage (enteroepithelial):

Mice and rats containing the infective cysts are eaten by the cat, which serves as the definitive host of the parasite. The cyst wall is digested, releasing organisms that penetrate epithelial cells of the small intestine. Several generations of intracellular
multiplication occur and finally culminating in development of oocysts that are discharged into the intestinal lumen by rupture of infected intestinal epithelial cells. After eating cysts, cat excretes *Toxoplasma* oocysts as early as 4 days later. These increased and then taper off by 14 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 induced 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 intracellularly in various cell types including brain, skeletal and heart muscle and cells of the reticuloendothelial system. But they have predication for the retina. Eventually, infected cells rupture and new ones are infected by these rapidly multiplying organisms (tachyzoites). After sometime, multiplication slows down and chronic cysts containing thousands of slowly multiplying organisms (bradyzoites) are formed.

Infected cats may be completely or partially immune, after primary exposure. However, 1-4% of the domestic cat population are usually found to be shedding oocysts (Dubey and
Frenkel, 1974). Thus, although only the domestic cat or some wild species of the felidae can produce several millions of oocysts, a wild variety of animals including sheep, cattle and pigs can become infected by ingestion of the oocysts. These infected animals, in turn, harbor infective cysts within their tissues. Tissue cysts may persist almost for life in heart, brain, muscles, and particularly in nervous tissues. Cysts formation coincides with time of development of immunity to new infection, which is usually long lasting. Moreover, somtimes, 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 as 13-18 months depending upon climatic condition (Yamaura, 1976; Fayer, 1981).
Figuer 1: Life cycle of *Toxoplasma gondii*.
1.4 Epidemiology:

*Toxoplasma gondii* is one of the most widely spread parasites found in nature (Aboul-Maged et al., 1987).

There was no significant difference between the prevalence in man or in women, but significantly higher antibodies titers were found in the sera of veterinarians and abattoirs workers (Fisher and Reid, 1973).

A still higher prevalence was noted in the individuals who handle rabbits, and highest of all was the group who were concerned with the trapping of rabbits (Brown and Neva, 1961). The organism is found in nature in herbivores, omnivores and carnivores among warm-blooded vertebrates (Hendrex and Blagum, 1983). The infection is initiated by a sporulated oocyst in cat faeces or tissue cyst in raw meat or organism in raw milk or in raw chicken eggs (Bunxton, 1990). The organism is rapidly destroyed by heating (the tissue cysts are destroyed at 100°C for 30 minutes), drying and freezing. Since 1996, both commercial and domestic freezing of meat before consumption has become common (Fleck and Kwantes, 1980). Therefore, in areas where cats are numerous, sanitation is poor, and the climate is humid, the presence of antibodies to *Toxoplasma* is high (Kelen and Roffsky, 1962).

Nevertheless, in areas where meat is eaten partially cooked or raw, the rate is also high. Raw meat is a government item in
France and “health food” for young children, so it is not surprising that the *Toxoplasma* serology rate is as high as 60 to 87% (Basalamah and Serebour, 1981).

On certain pacific Islands where cats are absent, antibodies to *Toxoplasma gondii* in humans are also absent (Feldman, 1982). Similarly, observation in and around New Delhi suggests low prevalence of *Toxoplasma*, which is consistent with the food habits of local people and their infrequent contact and/or association with cats (Mittal et al., 1990).

Americans eat raw ground beef as steak tartaries. It has been found that 10% of beef meat contains tissue cysts of *Toxoplasma gondii* (Brown and Neva, 1961). Outbreaks of toxoplasmosis has been reported in five medical students in New York City who patronized a snack bar for their hamburger lunches. Presumably, the hamburger was not well cooked (Brown and Neva, 1961). Therefore, serological studies of toxoplasmosis during pregnancy are mandatory in some American States, where the incidence is as high as 30% (Basalamah and Serebour, 1981). Additional important sources of human infection are via blood transfusion (Siegel et al., 1983). Transplantation of infected organs or cut in the skin while handling meat infected with tissue cysts may also be a route of transmission (Frenkel, 1974).

There is considerable difference in the prevalence of infection among population e.g a high prevalence in adults in Central
America may be related to the frequency of stray cats in a climate which favors survival of oocysts and also the type of dwellings (Basalamah and Serebour, 1981). A high prevalence in France may be due to the common habit of eating raw meat (Frenkel, 1973). The percentage of pregnant women with Toxoplasma antibodies in their blood in various parts of the world ranges from 5 to 45%. In the United States, the prevalence rate of Toxoplasma gondii infection among pregnant women was 31.7% (Basalamah and Serebour, 1981).

In Paris, the incidence reached 83% (Basalamah and Serebour 1981; Foulon et. al., 1984). In Mexico, a prevalence rate of 25% has been reported by Desmont and Couvreur (1978). In Brussels, Foulon et. al., (1984) estimated a prevalence rate of 47% among neonatal patients. The high prevalence of Toxoplasma antibodies (61.4%) among Costercians people aged 15-25 years may be due to inhalation of oocysts from infected cats faeces (Frenkel and Ruiz, 1980). Toxoplasma positive reactors (15%) were found among Russian women in Kiev who used to consume raw mice meat (Melvk et. al., 1975). Similarly, in Hawaii and Philippine, a high prevalence of infection due to eating raw meat was reported by Wallace in 1976. A prevalence rate of 9.8% in Hong Kong was reported by Khadre and El-Nageh In 1978. In Scandinavian countries, the prevalence rate of Toxoplasma infection is considerably lower than that reported
from other European countries being 5-45% among adults (Stray Pederson et. al., 1979).

As for Africa, the prevalence rate of *Toxoplasma* specific antibodies have been reported by various workers: In Somalia (43.6%) in the capital and 61% in rural areas using the dye test (Zarid et. al., 1981; Van-Druten et. al., 1990), 20% in Mouritania using indirect fluorescent antibodies test (IFAT), and 42% in Kenya using indirect heamagglutination and dye test respectively (Dumas et. al., 1990), 52% in males and 43% in females in Libya using latex agglutination test, and 8.2% in Niger using indirect florescent antibodies test (Excler et. al., 1988). In Somalia, the prevalence was 42.3% in patients who had a history of abortions versus 18.5% in patients who had no abortions history, indicating that *Toxoplasma* infection may have contributed to the cause of the abortions (Griffin and Williams, 1983).

The prevalence of toxoplasmosis has also been reported in several Arab countries: In Saudi Arabia, the prevalence is about 32.2% (Basalamahas and Serebour, 1981). In Lebanon, a prevalence rate of 30% was reported (Matossian, 1973). In Egypt, 26% also by dye test (Al-Meshari et. al., 1989).

1.5 Pathology and symptomatology of *Toxoplasma* infections:

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, 1961).

1.5.1 Infection in human:

1.5.1.1 Congenital toxoplasmosis:

According to CDC (2008), congenital toxoplasmosis is a group of symptoms caused by infection of the unborn baby (foetus) with the parasite *Toxoplasma gondii*. In the 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 infection will be passed on to the baby; this is known as congenital toxoplasmosis. The time between catching toxoplasmosis and showing symptoms (the incubation period) is 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 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 (week 27 to birth).
The infection is acquired from the mother and *Toxoplasma* may be isolated from the placenta or vaginal discharge (Brown and Neva, 1961).

An intrauterine transmission of *Toxoplasma gondii* to the foetus takes place only during the acute stage 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 foetus occurs in about 46% to 65% of cases (Dubey and Frenkel, 1974; Stray-Pederson, 1979) and 50% of mothers, who acquired the infection during pregnancy, if not treated, will give birth to infected infants (Russo and Galanti, 1990).

The symptomatology and pathology depend upon the gestational period at which the fetus is infected and may be classified as acute, sub acute and chronic (Kilpper and Morris, 1990). In the acute form, death may occur in uterus or the neonate may suffer from jaundice, skin rashes, hepatomegaly, lymphadenopathy, and meningoencephalitis (Bunxto, 1990). This type is rarely seen and pathology in the organs is similar to that described in the adult, varying in degree of severity with the age of the fetus at the time of the infection and the antibody protection from the mother (Vas et. al., 1990).

Materno-foetal transmission of *Toxoplasma gondii* in the first trimester of pregnancy occurs in about 4-25% (Ban and Khan, 1990). Recent studies by Pratlong et. al., in 1996 showed that
the risk of foetal involvement is about 11-15%. Disease is classically more severe when maternal infection occurs early in pregnancy because this is a period of organogenesis, thus, spontaneous in up to 10% of the cases or they may end up by being stillborn (Frenkel, 1974). Clinical congenital *Toxoplasma* infection occurs in about 10-20% of infants (Lee, 1975). The foetal risk in the second trimester is about 25-54% (Pratlong et. al., 1996). Conversely, *Toxoplasma* infection during the third trimester leads to about 65-80% of materno-foetal transmission (Russo and Galanti, 1990; Kilpper and Morris, 1990) although the foetal risk of developing clinical congenital toxoplasmosis is about 20-45%.

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 in 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 granulomata may be scattered throughout. The lesions become calcified in the cortical layers and may be seen radiologically as bilateral round shadow 1-3mm in diameter. Signs of hydrocephalus and thinning of the skull may occur. The protein in cerebrospinal fluid is increased and CSF may be xanthochromic with an increase of lymphocytes and monocytes and organism may be isolated (Wilson et. al., 1980).

Bilateral chorioretinitis is usually present in the eye and shows yellowish foci in the fundus. They may heal with residual scarring and optic neuritis may occur. Lesion may be present in the myocardium, skeletal muscle, and abdominal viscera (Pratlong et. al., 1996).

Toxoplasmosis is found to have an association with prematurity. Laboratory personnel at Taif children’s hospital in Saudia Arabia had measured the antibodies level to *Toxoplasma gondii* in blood samples from Saudia Arabia premature infants exhibiting symptoms of congenital toxoplasmosis. They found that 32.1% of infants tested were positive to *Toxoplasma gondii* antibodies, 46.2% positive to ELISA IgM antibodies which indicate recent infection (Abdalla et. al., 1994).

The typical congenital *Toxoplasma* triad includes hydrocephaly, chorioretinitis and intracerebral calcification. The lungs, liver, spleen and lymphnodes are severely affected. Anaemia and leukocytosis with an absolute increase in monocytes are present in about half of the patients. Histological
features of severe congenital lesions are not pathognomonic (Walter and Israel, 1987). Focal necrosis may be found in the liver, spleen and lungs, and this is surrounded by non-specific small round cell infiltration that includes 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 vilours odema was of very variable degree from one placenta to another and from one area to another in the 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, 1979; Pratlong et. al., 1996).

1.5.1.2 Acquired adult toxoplasmosis:

Most infections 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; Remington, 1974). According to Schmidt and Roberts (1985), The types of acquired toxoplasmosis can be classified as follows:

- Asymptomatic, Acute, Glandular and 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 focal central nervous system involvement, pain in the eye, blurring of vision and even blindness may be complained of the parasites being proliferating in the cells of the reticuloendothelial system and parenchymal cells of practically every 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 may also be affected and generalized lymphoadenopathy is a feature of the more subacute form of the disease. The essential lesion in the acute form of the disease is a small area of focal necrosis surrounded by a variable cellular inflammatory reaction depending upon the tissue affected.
The glandular form is most frequent form of clinical presentation in the acquired type of infection. Focal necrotic lesions with inflammatory infiltrate may also present in the liver, pancreas, kidneys, testes, hypophysis and the adrenal. In the chronic stage, there are chronic myocarditis and chronic lesion in the muscle, and a local hypersensitivity to release *Toxoplasma* from ruptured cells. A chronic local lesion with localized lymphadentitis has occured in laboratory worker following a finger prick with infected material (Gallalan et. al., 1946; Botres and Fairchilb, 1972).

The first two fatal cases providing the original clinical description of an acute febrile exanthornatous disease in adult were reported in 1941 by Penkerton and Henderson.

1.5.1.3 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 AIDS patients. This probably represents reactivation of previously acquired toxoplasmosis (Feron et. al., 1990). The presence of presistence parasitaemia observed in humans and animals can be explined 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 memain that may occur at the time of infection may lead to disruption by lymphocytic factors or by macrophages. Recent data show that preitoneal alveolar macrophages can kill *Toxoplasma* 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 Kelen, 1990). In immunodeficient patients, areas of necrosis may be widespread, myocardial and skeletal muscles are mostly involved. Rarely, deposition of *Toxoplasma* antigens and antibodies complex in the kidney results in glomerulonephritis (Remington, 1974).

1.5.1.4 **Ocular toxoplasmosis:**

*Toxoplasma* 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* 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 meditated (Tabbara, 1990). This was first reported in 1923 by Jankue who isolated *Toxoplasma* cysts from the retina of a dead eleven months old child in prague. Occular
toxoplasmosis in adults may be due to reactivation of a congenital infection (Dutton, 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 lesion has indistinct borders, whereas the older ones are white-gray, sharply outlined and spotted by accumulation of choroidal pigment (O’connor, 1974). Only, the retineal tissue is invaded (intra-retinal cyst) and the sub adjacent choroids, is usually involved in the inflammatory process (O’connor, 1974). Retinochoroiditis is asymptomatic and may be discovered by chance. Active lesions can cause blurred vision, and occular pain (Dutton, 1989).
The inflammation eventually resolves in a few months leaving a scar. Strabismus may be an early symptom in children. Macular involvement causes loss of central vision. Recurrent flare up results in progressive destruction of retinal tissue and some time glucoma. On fundoscopy, acute lesions are yellowish white and elevated older lesion with black areas of choroidal pigmentation (O’connor, 1974). Toxoplasmosis has been estimated to be the cause of 30 percent of chorioretinitis in children (Tabbara, 1990). Studies show that specific IgM antibodies reappear at the time of reactivation of congenital toxoplasmosis later in life, or possibly persist of an extra-ordinary long period up to ten years.
(Sibalic, et. al., 1990). So, old scars are found near active lesions. The process may end in blindness in early childhood or may interfere with the development of the eye resulting in optic atrophy or microphthalmia (Tabbara, 1990).

1.6 Infection in animals:
All animals’ species act as intermediate hosts, except feline species which acts as a definitive host (Innes 1997). In small ruminants (sheep and goats), economical losses occur due to prenatal death and abortion (Bunxton, 1998). *Toxoplasma gondii* infection, however, is the major cause of abortion and perinatal mortality in sheep and goats (Bunxton and Brebner, 1998). In Sudan, *Toxoplasma gondii* was investigated in animals by khalil and Elrayah (2011). They examined 200 animals (70 camels, 50 cattle and 80 sheep) using latex agglutination test (LAT). They showed detection rates of 20%, 32% and 57.5% in animals investigated respectively.

Sheep are considered important in the epidemiology of *Toxoplasma gondii* infection worldwide, especially in Europe (Cook et. al., 2000; Bunxton et. al., 2007). Ingestion of infected lamb serves as a direct source of infection for humans. Moreover, *Toxoplasma gondii* is also an important cause of abortion in sheep worldwide. Abortion and neonatal mortality occur when sheep and goats suffer a primary infection during
pregnancy. Coinciding with the parasitaemia, the ewe displays a febrile response which can exceed 41°C around day six or seven. The cessation of the parasitaemia coincides with the onset of an effective maternal immune response. With the exception of the gravid uterus, the infection then persists as bradyzoites within tissue cysts.

Cattle have high natural resistance to the parasite. *Toxoplasma gondii* causes subclinical infection in cattle (Dubey and Thulliez, 1994).

### 1.7 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 a prevalence of 27.8% in the general population excluding children less than 10 years of age. Later, Abdel-Hameed (1991), investigated the disease in Gezira where he reported a prevalence of 41.7% with females showing a higher prevalence rate than males. He observed that there was no correlation with animal contact and no cases of active toxoplasmosis were 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 rates between the two sexes was not significant.

During the period from June to December 1996, a cross 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 IgG antibodies was done 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 1gM antibodies. He found that 14.3% has positive 1gM 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 for anti-*Toxoplasma* antibodies was done using latex agglutination and specific IgG and 1gM 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 1gM 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. She 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 (1gM) 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%.

1.8 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.

1.8.1 Direct methods:
Demonstration of the parasite in biopsy material taken from liver, lymphnode, 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).

1.8.2 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 titres in sera 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 as popular as it utilizes live *Toxoplasma* 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:

1.8.2.1 Complement fixation test:
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 fluorescent 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).

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

1.8.2.3 Indirect haemagglutination test:
It is a laboratory test suitable for sero-epidemiological surveys and for routine works (Eissa et. al., 1990).

1.8.2.4 Latex slide agglutination test:
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 Harned, 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-Hammed, 1991).

1.8.2.5 Immuno sorbent agglutination assay:
Immuno 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 Immuno 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 (Valkourn and Stefanik, 1990).

1.8.2.6 Enzyme-linked immune sorbent assay “ELISA”:
ELISA has been adopted to replace the older tests in serodiagnosis of toxoplasmosis (Gallalan et. al., 1946). It is an enzyme immune assay for quantitave 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 on going active *Toxoplasma* infection and is probably the best parameter for early diagnosis of acute *Toxoplasma* infection.
Rationale

Justification

There is an increasing awareness of the occurrence of toxoplasmosis in developed and under developed countries as it is cosmopolitan in distribution. It became a necessity to investigate thoroughly the epidemiological factors that influence the transmission of the parasite, putting into consideration that it is a completely zoonotic disease.

Investigating its distribution among the population (humans and animals) is of great importance.

As a completely zoonotic parasite, this requires putting emphasis on the role of domestic animals in the transmission cycle of the parasite.

Parasitological methods of toxoplasmosis is more sensitive and specific but more difficult and require invasive samples there for looking for serodiagnosis simple, specific and sensitive are alternative tools urgently needed and highly applicable so that the
evaluation of the validity of LAT and ELISA can add a new knowledge to the diagnostic accuracy of toxoplasmosis.
Objectives

General objective:
To determine the sero-prevalence of anti-\textit{Toxoplasma} antibodies among the humans population and animals population as well in Khartoum State using the latex agglutination (LA) and enzyme linked immunosorbent assay (ELISA) tests.

Specific objectives:
- To investigate the occurrence of \textit{Toxoplasma gondii} antibodies among females of different ages putting emphasis on those who are pregnant reflecting the possibility of the occurrence of congenital toxoplasmosis.
- To investigate, the occurrence of \textit{Toxoplasma gondii} antibodies among males of different ages.
- To investigate seroprevalence of the disease in domestic animals (sheep and cattle) to elucidate their role in the transmission cycle of the parasite.
Chapter Two
Materials and methods
Chapter 2

Materials and methods

2.1 Study design:
This study is a descriptive cross sectional study.

2.2 Study area:
The study was conducted in Khartoum state (Khartoum, Khartoum North and Omdurman).

2.3 Study population:
The study was carried out on 200 males and 300 females (150 pregnant and 150 non pregnant) and 400 domestic animals (200 sheep and 200 cattle). The human population was categorized according to their age groups as follows:

Group A: 1----------10
Group B: 11--------20
Group C: 21--------30
Group D: 31--------40
Group E: 41--------50
Group F: Over 51

Pregnant ladies were dealt with as a separate entity.

2.4 Samples size:

500 venous blood samples were collected from 200 males and 300 females.

400 blood samples were collected from domestic animals (200 sheep and 200 cattle).
2.5 **Samples collection:**
5ml of blood were collected from each individual and were stored separately at -20°C.
When required, an aliquots was thawed to room temperature by using a water bath.
The labeled container was checked to ensure that the number of the container corresponds to the serial number on the individuals request form.
For animals, the blood was collected from the jugular vein.

2.6 **Data collection:**
A questionnaire was designed for data collection ([appendix](#)).

2.7 **Techniques:**

2.7.1 **Direct agglutination test:**
Commercial Kits produced by linear chemicals, s. I, were used.

2.7.1.1 **Principles of the test:**
Toxo-latex test is a rapid slide agglutination procedure, developed for the direct detection of anti-*Toxoplasma* antibodies in the 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.
R: Toxo-latex reagent: Suspension of polystyrene latex particles coated with antigenic extract of *Toxoplasma gondii* in a buffered saline solution, containing 0.95 g/L of sodium azide.

Control +ve: Human serum with an anti-*Toxoplasma* antibodies concetration >10 IU/ml, containing 0.95 g/L of sodium azide.

Control –ve: Animal serum, containing 0.95 g/L of sodium azide.

**2.7.1.2 Procedure:**

- Before using the kit, components were allowed to reach room temperature.
- Components were gently shaked (R.toxo-latex), to disperse the latex particles.
- The reagent was checked against the positive and negative controls.
- 50μl 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 was dispensed into two additional circles.
- 25μl 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 rotater at 100 r.p.m. for a period of 5 minutes. The presence or absences of visible agglutination were read.
2.7.1.3 Interpretation of the results:
- A homogeneous appearance (negative reaction) was interpreted as the absence of Toxoplasma antibodies or titers lower than 10 IU/mi.
- A clear agglutination (positive reaction) was interpreted as presence of Toxoplasma antibodies which may reflect either a past infection or an evolving Toxoplasma infection.

2.7.2 Enzyme linked immune sorbent assay:

2.7.2.1 Index toxo 1gM ELISA kit:

Enzyme immunoassay (ETA) procedure for the determination of 1gM antibodies to Toxoplasma gondii, for in vitro diagnostic use only.

2.7.2.1.1 Principle of the test:

Toxoplasma gondii antigens were fixed to the interior surface of microwells. Serum was added and any antibody present to Toxoplasma was bound to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with horseradish peroxidase enzyme and directed against 1gM are added and will in turn bind to 1gM 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.
2.7.2.1.2 Components:
1- Antigen coated microwells.
2- Negative control.
3- Low positive standard.
4- High positive standard.
5- Serum diluents.
6- Wash buffer concentrate.
7- Enzyme conjugates.
8- TMB substrate solution (tetramethylbenzidine).
9- Stop solution (1 N Phosphoric and 1N Hydrochloric acids).
10- Instruction manual.
11- Graph paper to prepare standard curve.

2.7.2.1.3 Assay procedure:
1- The required number of microwells were placed in the microwell holder. One end of each strip was marked for orientation.
2- The sample dilutions were prepared by making 1/100 mixture using the serum diluent (10^μl serum to 1 ml serum diluent). The calibrators were not diluted as they were ready for use. The diluted samples were incubated for 30 minutes at room temperature.
3- 100μl of negative control, low positive standard (cut-off), high positive standard and serum specimens were added to the subsequent wells.
4- The microwells were incubated at room temperature for 15 minutes.
5- The microwells 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 aspired five times without soak.
6- 100μl of enzyme conjugate were dispensed into each well and incubated at room temperature for 15 minutes.
7- At the end of the incubation period, the contents of the well were discarded and washed as outlined in step five.
8- 100μl TMB substrate were added to each well and incubated at room temperature for 10 minutes.
9- The reaction was stopped by adding 100μl stop solution to each well. This will produce a colour change from blue to yellow. Immediately the absorbance of each well was measured at 450nm within 10 minutes.

2.7.2.1.4 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 will be the cut-off value of the assay.
- The sample OD was divided by the value obtained in 1 above.
-A ratio greater than 1.0 indicates a positive sample. A ratio lower than 0.9 indicates negative sample. A ratio between 0.9 and 1.1 indicates equivocal result. An equivocal sample was retested with a fresh new sample.

2.7.2.2 Index toxo IgG ELISA kit:
Enzyme immunoassay (EIA) procedure for the determination of IgG antibodies to *Toxoplasma gondii*, for invitro diagnostic use only.

2.7.2.2.1 Principle of the test:
Specific, inactivated, *Toxoplasma* antigens were prepared, purified and coated on micro titration wells. Test serum diluted 1/20 was applied.

Specific antibodies to *Toxoplasma gondii* bound to the antigen in the wells. Unbound material was washed away and anti-human IgG conjugated to horseradish peroxides, was applied. The conjugate binds to the human antibodies bound to the antigen. Unbound material was again washed away. On addition of the substrate (TMB), a colour will develop only in those a well in which enzyme is present, indicating the presence of human anti-*Toxoplasma* antibody. The enzyme reaction was stopped by the addition of stop solution and the absorbance was measured at 450 nm. The concentration of specific IgG antibody is directly proportional to the colour intensity of the test sample.
2.7.2.2 Components:
1- Antigen coated microwells.
2- Negative control.
3- Low positive standard.
4- High positive standard.
5- Serum diluent.
6- Wash buffer concentrate.
7- Enzyme conjugates.
8- TMB substrate solution (tetramethylbenzidine).
9- Stop solution (1 N Phosphoric and 1N Hydrochloric acids).
10- Instruction manual.
11- Graph paper to prepare standard curve.

2.7.2.2.3 Assay procedure:
1-The required number of microwells were placed in the microwell holder. One end of each strip was marked for orientation.
2-The sample dilutions were prepared by making 1/100 mixture using the serum diluent (10 μl serum to 1 ml serum diluents). The calibrators were not diluted as they will be ready for use. The diluted samples were incubated for 30 minutes at room temperature.
3-100 μl of negative control, low positive standard (cut-off), high positive standard and serum specimens were added to subsequent wells.
4-The microwells were incubated at room temperature for 15 minutes.
5-The microwells 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 aspired five times without soak.
6- 100 μl of enzyme conjugate were dispensed into each well and incubated at room temperature for 15 minutes.
7-At the end of the incubation period, the contents of the well were discarded and washed as outlined in step five.
8-100 μl TMB substrate were added to each well and incubated at room temperature for 10 minutes.
9-The reaction was stopped by adding 100 μl stop solution to each well. This will produce a colour change immediately, the absorbance of each well was measured at 450 nm filter.

2.8 Calculation and Interpretation of result;
For each test and control serum, the average optical density (O.D) obtained during the test run was determined.

2.9 Statistical analysis:
All data were computerized and checked for correct entry. It was analyzed using statistical package for social science (SPSS) computer program.
CHAPTER THREE

Results
Chapter 3

Results

Out of the three-hundred serum samples collected from females (150 pregnant and 150 non pregnant) and examined for anti-\textit{Toxoplasma} antibodies, 74 (24.6\%) were found to be positive for anti-\textit{Toxoplasma} antibodies by ELISA test. When using latex agglutination test, 54 (18\%) were found positive for anti-\textit{Toxoplasma} antibodies. This difference was found to be statistically significant ($P=0.00$) (table 1, figure 2).

Out of the 74 serum samples positive by ELISA, 14 samples showed IgM antibodies. This represents a rate of 4.6\%. Out of these, 8 (5.33\%) were positive among pregnant women and 6 (4\%) were positive among non pregnant women (table 2). Similarly, \textbf{IgG} was detected in 60 (20\%) of the women. Out of these, 36 (24\%) were detected among pregnant women and 24 (16\%) were detected among non pregnant women (table 2).

Anti-\textit{Toxoplasma} antibodies monitored by ELISA showed a prevalence rate of 25\%, 26.7\% 19.35\%, 25\%, 19.35\% and 37.5\% in the age groups A, B, C, D, E and F. This difference in rates was found to be statistically insignificant ($P=0.512$). The percentage of positive anti-\textit{Toxoplasma} antibodies using latex agglutination test ranged between 4.83\% in \textbf{C} group and 35\% in \textbf{a} group. This difference in rates was found to be statistically insignificant ($P= 0.120$) (table 3, figure 3).
Out of the 220 samples of women drinking cow milk, 48 samples (21.8\%) were found to be positive for anti-\textit{Toxoplasma} antibodies by using ELISA test. When the same samples were examined by latex agglutination test, 29 samples (13.18\%) were found positive. Out of the 48 samples of women drinking cow and goat milk, 19 samples (39.58\%) were found to be positive for anti-\textit{Toxoplasma} antibodies by using ELISA test. When the same samples were examined by latex agglutination test, 17 samples (35.4\%) were positive. In the 28 women who did not consume milk, 6 samples (21.42\%) were found to be positive for anti-\textit{Toxoplasma} antibodies using ELISA test. When the same samples were examined by latex agglutination test, 8 samples (28.57\%) were positive, and out of 4 samples of women drinking goat milk, one sample (25\%) was found positive using ELISA test, however, not a single case was reported by the latex test. There was insignificant difference between cow milk and other types of milk (P.values for ELISA and latex were 0.270, and 0.323 respectively) (table 4, figure 4).

Among 71 sera of women who used to eat beef, ELISA test showed 19 positive samples (26.76\%) and the latex showed 13 positive samples (18.3\%). \textbf{Out} of the 24 women who consumed mutton, the percentage of positive cases was 25\% when using ELISA test, and 37.5\% when using latex agglutination test. There was insignificant difference between the types of meat consumed using ELISA or latex agglutination test (P.values for
ELISA and latex were 0.278, and 0.165 respectively) (table 5, figure 5).

When using ELISA test, the result showed that the highest rate (42.8%), was reported in pregnant women in their 1st trimester while the lowest rate (20%) was among the non pregnant women. In the 2nd trimester and the 3rd trimester, the rates were 29.41% and 22.2% respectively. There was no significant difference between stages of pregnancy and rates of anti-Toxoplasma antibodies when using ELISA test (P=0.203). For latex agglutination test, the highest rate was also reported in the 1st trimester (47.42%) and the lowest rate (12.96%) was reported in the 3rd trimester. The 2nd and non pregnant women reported rates of 16.17% and 15.33% respectively. This difference in rates was found to be statistically insignificant (P=0.172) (table 6, figure 6).

The result revealed that anti-Toxoplasma antibodies appeared in 28.45% of those who had contact with cats and in 20.8% of those who had no contact with cats when using ELISA. This difference was found to be statistically insignificant (P=428). When using latex agglutination test, anti-Toxoplasma antibodies appeared in 24.39% of those who had contact with cats and 12.8% of those who had no contact with cats (table 7, figure 7). This difference was found to be statistically insignificant (P=0.217).
The history of previous abortions revealed that the highest detection rate of anti-\textit{Toxoplasma} antibodies when using ELISA test (69.7\%) was reported in those with no history of abortion while in the group with a history of one, two and three abortions, rates of 26.3\% 35.7\% and 33.3\% were reported respectively. The difference in rates was found to be statistically insignificant (P=0.401). When using latex agglutination test the rate was 39.47\% in women with no history of abortion and no antibodies were detected in women who aborted three times. The rate was 42.8\% in women who aborted twice and 31.57\% in those who aborted once. The difference in rates was found to be statistically significant (P=0.050) (table 8, figure 8).

Out of the two-hundred serum samples collected from males and examined for anti-\textit{Toxoplasma} antibodies using ELISA (IgG), 61 samples showed IgG antibodies. This constituted a rate of 30.5\% (table 9, figure 9).

Anti-\textit{Toxoplasma} antibodies monitored by ELISA showed a prevalence rate of 38.5\%, 31\%, 29\%, 25\%, 30\% and 30\% in groups A, B, C, D, E and F respectively. This difference in rates was found to be statistically insignificant (P=0.340) (table 10, figure 10).

Out of 180 samples of men drinking cow milk, 53 samples (29.4\%) were found to be positive for anti-\textit{Toxoplasma} antibodies by ELISA (IgG) test, and out of 17 samples of men drinking cow and goat milk, 7 samples (41.2\%) were found to
be positive for anti-*Toxoplasma* antibodies. In 3 men who did not consume milk, 1 sample (33.3%) was positive. There was no significant difference between cow milk and other types of milk (P= 0.421) (table 11, figure 11).

Among 197 sera of men who used to eat beef, 22 samples (11.2%) were found positive when using ELISA. One serum was found positive among those who used to eat goat (50%). Among the 110 sera of men who used to eat beef and mutton, 34 samples (31%) were found positive and among 20 sera of men who used to eat beef, mutton and goat, 4 samples (20%) were found positive. The difference between rates was found to be statistically insignificant (P= 0.517) (table 12, figure 12).

The result revealed that anti-*Toxoplasma* antibodies appeared in 68% of those who had contact with cats and in 25% of those who had no contact with cats when using ELISA (IgG). This difference was found to be statistically significant (P=0.212) (table 13, figure 13).

The result of the four-hundred serum samples collected from animals (200 sheep and 200 cattle) and examined using latex agglutination test, showed that 22 sheep samples and 36 cattle samples were found positive for anti-*Toxoplasma* antibodies. This constituted detection rates of 11% and 18% respectively. This difference in rates was found to be statistically significant (P=0.034) (table 14, figure 14).
**Table 1:** Overall detection rate of anti-*Toxoplasma* antibodies among females (pregnant and non pregnant) using ELISA and latex agglutination tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>No. examined</th>
<th>Number detected</th>
<th>Prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>300</td>
<td>74</td>
<td>24.6</td>
</tr>
<tr>
<td>Latex</td>
<td>300</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2:** Overall detection rate of anti-*Toxoplasma* antibodies among females (pregnant and non pregnant) using ELISA and latex agglutination tests.
Table 2: Detection rate of anti-\textit{Toxoplasma} antibodies among females (pregnant and non pregnant) using ELISA (IgM + IgG).

<table>
<thead>
<tr>
<th></th>
<th>No. examined</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ve IgM</td>
</tr>
<tr>
<td>Pregnant</td>
<td>150</td>
<td>8(5.33%)</td>
</tr>
<tr>
<td>Non Pregnant</td>
<td>150</td>
<td>6(4%)</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>14(4.6%)</td>
</tr>
</tbody>
</table>
Table 3: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. examined</th>
<th>Number detected by ELISA (%)</th>
<th>Number detected by latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40</td>
<td>10(25%)</td>
<td>14(35%)</td>
</tr>
<tr>
<td>B</td>
<td>75</td>
<td>20(26.7%)</td>
<td>13(17.33%)</td>
</tr>
<tr>
<td>C</td>
<td>62</td>
<td>12(19.35%)</td>
<td>3(4.83%)</td>
</tr>
<tr>
<td>D</td>
<td>68</td>
<td>17(25%)</td>
<td>15(22%)</td>
</tr>
<tr>
<td>E</td>
<td>31</td>
<td>6(19.35%)</td>
<td>2(6.45%)</td>
</tr>
<tr>
<td>F</td>
<td>24</td>
<td>9(37.5%)</td>
<td>7(29.16%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.512</td>
<td>0.120</td>
</tr>
</tbody>
</table>

Figure 3: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to age groups.
Table 4: The rate of anti-Toxoplasma antibodies in the study group as obtained by ELISA and latex agglutination tests according to the type of milk consumed.

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>No.examined</th>
<th>ELISA</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t drink milk</td>
<td>28</td>
<td>6(21.42%)</td>
<td>8(28.57%)</td>
</tr>
<tr>
<td>Cow</td>
<td>220</td>
<td>48(21.8%)</td>
<td>29(13.18%)</td>
</tr>
<tr>
<td>Goat</td>
<td>4</td>
<td>1(25%)</td>
<td>0(00%)</td>
</tr>
<tr>
<td>Cow + goat</td>
<td>48</td>
<td>19(39.58%)</td>
<td>17(35.4%)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td><strong>0.270</strong></td>
<td><strong>0.323</strong></td>
</tr>
</tbody>
</table>

Figure 4: The rate of anti-Toxoplasma antibodies in the study group as obtained by ELISA and latex agglutination tests according to the type of milk consumed.
Table 5: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to the type of meat consumed.

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>No. examined</th>
<th>ELISA</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>71</td>
<td>19(26.76%)</td>
<td>13(18.3%)</td>
</tr>
<tr>
<td>Goat</td>
<td>3</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Mutton</td>
<td>24</td>
<td>6(25%)</td>
<td>9(37.5%)</td>
</tr>
<tr>
<td>Beef + mutton</td>
<td>157</td>
<td>34(21.65%)</td>
<td>16(10.19%)</td>
</tr>
<tr>
<td>Beef + goat + mutton</td>
<td>42</td>
<td>15(35.7%)</td>
<td>16(38.09%)</td>
</tr>
<tr>
<td>Beef+ goat</td>
<td>3</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.278</td>
<td>0.165</td>
</tr>
</tbody>
</table>

Figure 5: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to the type of meat consumed.
Table 6: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to pregnancy stage.

<table>
<thead>
<tr>
<th>Stage group</th>
<th>No. examined</th>
<th>ELISA</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non pregnant</td>
<td>150</td>
<td>30(20%)</td>
<td>23(15.33%)</td>
</tr>
<tr>
<td>1\textsuperscript{st} trimester</td>
<td>28</td>
<td>12(42.85%)</td>
<td>13(47.42%)</td>
</tr>
<tr>
<td>2\textsuperscript{nd} trimester</td>
<td>68</td>
<td>20(29.4%)</td>
<td>11(16.17%)</td>
</tr>
<tr>
<td>3\textsuperscript{rd} trimester</td>
<td>54</td>
<td>12(22.2%)</td>
<td>7(12.96%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>\textbf{0.203}</td>
<td>\textbf{0.172}</td>
</tr>
</tbody>
</table>

Figure 6: The rate of anti-Toxoplasma antibodies among females as obtained by ELISA and latex agglutination tests according to pregnancy stage.
**Table 7**: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination tests according to contact with cats.

<table>
<thead>
<tr>
<th>Contact with cats</th>
<th>No.examined</th>
<th>ELISA</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>123</td>
<td>35(28.45%)</td>
<td>30(24.39%)</td>
</tr>
<tr>
<td>No</td>
<td>187</td>
<td>39(20.8%)</td>
<td>24(12.8%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.428</td>
<td>0.217</td>
</tr>
</tbody>
</table>

**Figure 7**: The rate of anti-*Toxoplasma* antibodies in the study group as obtained by ELISA and latex agglutination tests according to contact with cats.
**Table 8:** The rate of anti-*Toxoplasma* antibodies among pregnant females as obtained by ELISA and latex agglutination tests according to previous history of abortion.

<table>
<thead>
<tr>
<th>Number of abortions</th>
<th>Number examined</th>
<th>ELISA</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abortion</td>
<td>76</td>
<td>53 (69.7%)</td>
<td>30 (39.47%)</td>
</tr>
<tr>
<td>1 time</td>
<td>57</td>
<td>15 (26.3%)</td>
<td>18 (31.57%)</td>
</tr>
<tr>
<td>2 times</td>
<td>14</td>
<td>5 (35.7%)</td>
<td>6 (42.8%)</td>
</tr>
<tr>
<td>3 times</td>
<td>3</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.401</td>
<td>0.050</td>
</tr>
</tbody>
</table>

**Figure 8:** The rate of anti-*Toxoplasma* antibodies among pregnant females as obtained by ELISA and latex agglutination tests according to previous history of abortion.
**Table 9:** The rate of anti-*Toxoplasma* antibodies among males using ELISA (IgG) test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Number examined</th>
<th>Number detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA (IgG)</td>
<td>200</td>
<td>61 (30.5%)</td>
</tr>
</tbody>
</table>

**Figure 9:** The rate of anti-*Toxoplasma* antibodies among males using ELISA (IgG) test.
Table 10: The rate of anti-*Toxoplasma* antibodies among males as obtained by ELISA (IgG) test according to age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. examined</th>
<th>No. detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>26</td>
<td>10 (38.5%)</td>
</tr>
<tr>
<td>B</td>
<td>74</td>
<td>23 (31.0%)</td>
</tr>
<tr>
<td>C</td>
<td>52</td>
<td>15 (29.0%)</td>
</tr>
<tr>
<td>D</td>
<td>28</td>
<td>7 (25.0%)</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>3 (30.0%)</td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>3 (30.0%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td><strong>0.340</strong></td>
</tr>
</tbody>
</table>

Figure 10: The rate of anti-*Toxoplasma* antibodies among males as obtained by ELISA (IgG) test according to age groups.
Table 11: The rate of anti-Toxoplasma antibodies among males as obtained by ELISA (IgG) test according to the type of milk.

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>No. examined</th>
<th>No. detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t drink milk</td>
<td>3</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Cow</td>
<td>180</td>
<td>53 (29.4%)</td>
</tr>
<tr>
<td>Goat</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cow + goat</td>
<td>17</td>
<td>7 (41.2%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.421</td>
</tr>
</tbody>
</table>

Figure 11: The rate of anti-Toxoplasma antibodies among males as obtained by ELISA (IgG) test according to the type of milk.
**Table 12:** The rate of anti-\textit{Toxoplasma} antibodies among males as obtained by ELISA (IgG) test according to the type of meat consumed.

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>No. examined</th>
<th>No. detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow (Beef)</td>
<td>197</td>
<td>22 (11.2%)</td>
</tr>
<tr>
<td>Goat</td>
<td>2</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>Sheep (mutton)</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cow + sheep</td>
<td>110</td>
<td>34 (31.0%)</td>
</tr>
<tr>
<td>Goat + sheep</td>
<td>0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cow + goat + sheep</td>
<td>20</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.517</td>
</tr>
</tbody>
</table>

**Figure 12:** The rate of anti-\textit{Toxoplasma} antibodies among males as obtained by ELISA test according to the type of meat consumed.
Table 13: The rate of anti-*Toxoplasma* antibodies among males as obtained by ELISA (IgG) test according to contact with cats.

<table>
<thead>
<tr>
<th>Contact with cats</th>
<th>No.examined</th>
<th>No. detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>25</td>
<td>17 (68.0%)</td>
</tr>
<tr>
<td>No</td>
<td>175</td>
<td>44 (25.0%)</td>
</tr>
<tr>
<td>P.value</td>
<td></td>
<td>0.212</td>
</tr>
</tbody>
</table>

Figure 13: The rate of anti-*Toxoplasma* antibodies among males as obtained by ELISA (IgG) test according to contact with cats.
**Table 14:** The rate of anti-*Toxoplasma* antibodies among animals (Sheep and Cattle) using latex agglutination test.

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. examined</th>
<th>Number detected (rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>200</td>
<td>22 (11.0%)</td>
</tr>
<tr>
<td>Cattle</td>
<td>200</td>
<td>36 (18%)</td>
</tr>
<tr>
<td><strong>P.value</strong></td>
<td></td>
<td><strong>0.034</strong></td>
</tr>
</tbody>
</table>

**Figure 14:** The rate of anti-*Toxoplasma* antibodies among animals (Sheep and Cattle) using latex agglutination test.
CHAPTER FOUR

Discussion
Chapter 4

Discussion

The overall prevalence rate of positive anti-\textit{Toxoplasma} antibodies using latex agglutination and ELISA tests was found to be 18\% and 24.6\% respectively. These rates were found to be lower than the rates reported by Abdel-Hameed (1991) and Bushra (2006) (41.7\% and 36\% respectively) and higher than the rate 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 15-25 years age group (25\%) by using the ELISA. This was far less from the rate reported by Frenkel and Ruiz (1980) who reported 61.4\% prevalence rate among the 15-25 years age group, but close to that reported by Eman and Saad (2011) for the same group although her peak rate was 20\% in the 41-45 years age group. In this study, the result revealed that the highest rate (37.3\%) was in women aged from 41-45 years. This might indicate their prolonged exposure to the parasite and their habits such as eating raw meat, and far from what Abdel-Raouf (2001) where the highest rate (36.3\%) was in the age group 21-29 years which is very risky as it is the most fertile period. It has been mentioned that seroprevalence of toxoplasmosis is known to increase with age (Shin et. al., 2009). In their study, they observed that the lowest rate was reported in
those aged < 20 years, and the rates slowly increased with age with the peak level being in 40-49 age groups.

As far as the milk consumption is concerned and its association with the occurrence of toxoplasmosis, the rates of anti-\textit{Toxoplasma} antibodies in women drinking both cow and goat milk were the highest by all tests 39.58\% and 35.4\% (ELISA and latex test respectively). Eman and Saad (2011) reported 7.1\% by ELISA test as the highest rate and 50\% by latex test, but this was in women who did not drink milk. Abdel-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 beef were 26.76\% and 18.3\% by using ELISA and latex tests respectively. This was different from that found by Eman and Saad (2011) as their rates were 14.3\% and 28.6\% by ELISA (IgM) and latex tests respectively. Also, they found the rates in women who eat mutton to be 7.7\% and 24.4\% using the same tests. In this study, the rates in women who consumed mutton were found to be 25\% and 37.5\% by using ELISA and latex tests respectively. Using ELISA, the result was 25.5\% in women who consumed beef and 26.7\% in those who consumed mutton. Abdel-Raouf (2001) revealed that the result of IgG was 17.9\% and 17.08\% for beef and mutton respectively and as shown
above, the role of eating raw or undercooked beef or mutton is of great importance in the transmission of cysts containing bradyzoites. This concept was high lightened 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 strengthened by Abdel-Hameed (1991) who related the high rate of toxoplasmosis to the consumption of raw or partially cooked liver, marrara, undercooked meat, shaya and abu-dammam (spleen) in Sudan. Our conclusion was also supported by the finding of Elsheikha et. al., (2009) who showed a significant association between *Toxoplasma gondii* seropositivity and eating meat by products (luncheon / shawerma) in Egypt.

This investigation revealed that there is no 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 (2011), 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 rates (42.85% and 47.42%) for latex agglutination and ELISA tests respectively were reported in women in their 1st trimester. These results agree with Eman and Saad (2011), who
reported highest prevalence rates of 40% and 16% for latex agglutination and ELISA testes 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 2nd trimester and 3rd trimesters, the relatively high prevalence rates were reported as 16.17% and 12.96% respectively by the latex agglutination test which is lower than that reported by Eman and Saad (2011) (28.9% and 29.7% for the same stage of pregnancy).

This result might probably suggest the possibility of maternofoetal transmission. This type of transmission as suggested by Kilpper and Morris (1990) increases in the 2nd trimester, it reaches 65% and up to 80% at term as the highest incidence of congenital toxoplasmosis.

As far as the number of abortions is concerned, the highest prevalence rate was found to be 35.7% and 42.8% for ELISA and latex agglutination tests respectively. If these rates were compared with those with no history of abortion, the rates reported were 69.7% and 39.47% for ELISA and latex agglutination testes respectively. 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 final result of Eman and Saad (2011) but not in agreement with the finding of Griffin and Williams (1983) who reported prevalence of 42.3% in patients who has a history of
abortion indicating that *Toxoplasma* infection may in this case have contributed to the cause of abortions.

In this investigation, the overall infection rate in males was found to be 30.5% using ELISA (IgG). This rate is by far lower than the rate reported in Brazil by Coelho et. al., (2003) (79%), however, it is greater than the rate reported by Moghaddam and Hafizi (2009) in Iran (6.4%), Kook et. al., (1999) in Korea (7.3%) and Al-Hindy (1994) (17.5%) in Sudan. It is to be mentioned that discussion of tables 4, 5 and 7 are applicable to tables 11, 12 and 13.

From table 14, the rates of anti-*Toxoplasma* antibodies were 11% and 18% in sheep and cattle respectively. These findings were not in agreement with the findings of Khalil and Elrayah (2011) who reported 32% rate in cattle and 57.5% in sheep in Sudan.
Conclusions

1- Toxoplasmosis, in Sudan is prevalent among females (pregnant and non pregnant), males and animals e.g sheep+ cattle.

2- High prevalence rate was reported among the 1-10 years age group in males and over 51 years age group in females.

3- There is risk congenital toxoplasmosis occurrence in those pregnant ladies with detected IgM antibodies.

4- Beef and mutton seem to play an important role in the transmission cycle.

5- No association between toxoplasmosis and previous history of abortion.

6- The high detection rate of the ELISA test compared to the latex agglutination test reflects the high sensitivity rate the ELISA test.
Recommendations

- Selecting pregnant women with IgM antibodies and follow up their babies after delivery as a future research.
- To complete the picture of the transmission cycle of *Toxoplasma gondii*, cats should be surveyed for the presence of oocyst in their faeces.
- People should be aware through health education by the means of transmission of *Toxoplasma gondii*.
- Pregnant ladies should not under any circumstances handle meat.
- Conducting now and then survey to reaching the exact prevalence of *toxoplasmosis* among the different categories of the population in Sudan.
References


- **Elsheikha, H. M; Azab, M. S; Abousamar, N. K; Rabbar, M. H; Elghannam, D. M; and Raafat, D.** (2009).


affecting a protozoan parasite “Toxoplasma”. Science; 108: 660-663.


-Stray-Pedersen, B; Pedersen, J; Omland, T. (1979). Estimations of the incidence of Toxoplasma infections among


Appendix: Questionnaire

Name.................................................................................................
Age........................................................................................................
Residence.........................................................................................
Occupation
Type of meat consumed: Cow Sheep Goat
Type of milk consumed: Cow Goat

Contact with cats .................................................................
Stage of pregnancy ...............................................................
Previous history of abortion ..................................................