Sero-prevalence of *Toxoplasma gondii* Antibodies among Aborted Women in Atbara Area-River Nile State

الانتشار الوصلي للأجسام الوضادة للوقاسيت القنذيت وسط النساء الوجهضاث في منطقة عطبرة- ولاية نهر النيل

A dissertation submitted in partial fulfillment for the requirements of the degree of M.Sc. in Medical Laboratory Science (Parasitology and Medical Entomology)

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سَلَامٌ قَوْلًا مِنْ رَبِّ رَحِيمٍ

صدق الله العظيم

سورة يس الآية : 58
Dedication

To The One Who Hold My Hand Step by Step

My father,

To My Heaven

My mother,

To my brother and sister,

To my teachers,

To my friends,

To my colleagues,

To everyone who supported me

I dedicate this work
Acknowledgement

Thanks firstly and finally to Allah for blessing and giving me the power to complete this research.

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ABSTRACT

This cross-sectional study was conducted in Atbara area- River Nile state during the period from February to November 2017. The aim of this study was to determine the sero-prevalence of toxoplasmosis among aborted women. One hundred and fifty two women with the age ranged between 25-49 years old of mean age was 38±10 were included. Blood samples were taken from all women. All samples were examined to detect *Toxoplasma gondii* antibodies by using latex, ICT and ELISA techniques. The study showed that the overall sero-prevalence of *Toxoplasma* parasite by different techniques was as follows: 35.5% by ELISA technique, 33.6% by Latex agglutination technique and 22.4% by ICT technique. The study revealed that the sero-prevalence of toxoplasmosis antibodies increased gradually with age reaching 54.6% in the age group 41-49 years old followed by 32.9% in age group 31-40 years old and 12.5% in age group 20-30 years old. Assuming ELISA technique as the gold standard method, the sensitivity and specificity of the latex technique were 94 % and 100% respectively and for ICT technique were 64 % and 100 % respectively.
The study found that toxoplasmosis antibodies were more prevalent in women who contact with cats than in women who do not contact with cats.

This study recorded a high sero-prevalence (35.5%) for *Toxopalsmagondii*. The study indicated that toxoplasmosis still exists and considered as one of the risk factors for pregnancy miscarriage. The study recommended that IgG and IgM tests for toxoplasmosis be considered as one of the routine tests for pregnant women and those coming to marry.
مستخلص الدراسة

أجريت هذه الدراسة المستعرضة في منطقة عطرة- ولاية نهر النيل خلال الفترة من فبراير إلى نوفمبر 2017. كان الهدف من هذه الدراسة هو تحديد الانتشار المتصليداء المقوسات وسط النساء المجهشات.

تضمنت الدراسة 152 امرأة كانت عمرهن بين 25 إلى 69 سنة بمتوسط عمر 38± 10 سنة. عينات الدم تم أخذها من جميع النساء. كل العينات تم اختيارها لتعرف على الأجسام المضادة للموسيقية الفردية باستعمال طريقة التجنل بحبيبات اللانكس، شريط الاختبار و طريقة مقاومة الإصصاصية للإنزيم المرتبط.

اظهرت الدراسة أن الانتشار المصلي العام لدى المرضى بالطرق المختلفة كالآتية: 35.5% بطريقة مقاومة الإصصاصية للإنزيم المرتبط، 32% بطريقة التجنل بحبيبات اللانكس، و 22% بطريقة شريط الاختبار.

أوضح الدراسة أن الانتشار المصلي للأجسام المضادة للموسيقات يزيد تدريجياً مع زيادة العمر وصولاً لأعلى نسبة 40.6% في المجموعة العمرية 41-49 سنة، 32.9% في المجموعة العمرية 31-40 سنة و 3.4% في المجموعة العمرية 50-60 سنة.

على إفتراض تقنية مقاومة الإصصاصية للإنزيم المرتبط كالمعيار الذهني، كانت الحساسية و الخصوصية لطريقة التجنل بحبيبات اللانكس 94% و 100% على التوالي وطريقة شريط الاختبار كانت 84% و 100% على التوالي.
وجدت الدراسة أن الأجسام المضادة كانت أكثر انتشارًا في النساء اللاتي لحن احتكاك بالقطط. اللاتي ليس لهن احتكاك بالقطط.

سجلت هذه الدراسة انتشار مصلي عالي (50.5%) للمقاومة القنديّة. دلت هذه الدراسة أن داء المقوسات موجود و يعتبر أحد عوامل الخطر المسببة للأجهاض. توصي الدراسة بأن تعتبر فحوصات القلوبولينات المناعية (IgG,IgM) لداء المقوسات كأحد الفحوصات الروتينية للنساء الحاملين المقبولات على الزواج.
Chapter One

Introduction

&

Literature review
Chapter one
Introduction and literature review

1.1: Introduction:

*Toxoplasma gondii* is a single-cell protozoan that belongs to the family Coccidia. It is an obligatory intracellular protozoan with a heterogeneous life cycle in humans and other vertebrates (Sibley and Boothroid, 1998). Human infection with *T. gondii* causes toxoplasmosis. Postnatal toxoplasmosis is usually an asymptomatic disease, but often takes a severe course in immune compromised hosts (Koskiniemi et al., 1989). Congenital toxoplasmosis is acquired through vertical transmission of *T. gondii* to the fetus by transplacental transfer from the mother usually following acute maternal infection. If congenital toxoplasmosis occurs early in pregnancy, it may lead to severe damage or abortion (Field and Guerina., 1997).

Sporadic abortion is defined as the termination of pregnancy by any means before the fetus is sufficiently developed to survive. While habitual abortion is defined as three or more consecutive spontaneous abortions. Habitual abortion is one of the most distressing problems in obstetrics, particularly in those women who have no successful pregnancies (Howie, 1986). The seroprevalence of toxoplasmosis infection in women with bad obstetric history (including sporadic or habitual abortions) is known to be significantly higher than those without it (Shamaraet et al., 1997). A recent study from India reports a statistical difference between IgG antibody levels against *T. gondii* in habitual abortions as compared to sporadic abortions or normal pregnancies (Kumar et al., 2004). Another study from Egypt revealed that there was a significant IgG and IgMT*T. gondii* antibody level difference between women with no history of
abortion, women with one or two abortions, women with more than three abortions and the control group (Attia et al., 2004).

1.2 Literature review:

1.2.1 Toxoplasma gondii:
Toxoplamosis is a disease which is caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*. The disease is of worldwide zonosis. It’s also estimated by Dubey that up to one third of the world human population is affected by this disease (Dubey, 2009).

In human toxoplasmosis was identified by Nicolle and Manceaux “1908”, when they found a protozoan parasite in tissues of a hamster –like rodent. In Sudan the disease was discovered, when Carter and Fleck in 1966 used the dye test (DT) and reported the prevalence of 61% in 4 different states in the country (Sattiet et al., 2011).

1.2.1.1 Taxonomy:

Based on (Levine, 1980) *Toxoplasma gondii* is classified as follows:

- Kingdom: Animalia
- Sub-kingdom: Protozoa
- Phylum: Apicomplexa
- Class: Sporozoa
- Order: Eucoccididae
- Sub-order: Emeriina
- Family: Sarcocystidae
- Sub-family: Toxoplasmatinae
- Genus: *Toxoplasma*
- Species: *gondii*
1.3 Morphology, transmission and life cycle:

1.3.1 Morphology:

*T. gondii* occurs in three forms; trophozoite, tissue cyst and oocyst. The trophozoite and tissue cyst represent stages in asexual multiplication (Schizogony). While the oocyst is formed by sexual reproduction (gametogony or Sporogony). All three forms occur in the domestic cat and other felines which is the definitive host and which support both schizogony and gametogony. Only the sexual forms, trophozoites and tissue cysts are present in other animals, including humans and birds, which are the intermediate hosts. Both oocyst and tissue cysts are infective by ingestion (Petersen and Duby, 1998).

Tachyzoite:

The tachyzoite (figure 1-1), (previously called trophozoite) is crescent shaped and is approximately 2x6 m in size. The tachyzoite has a pellicle, subpellicular microtubules, a polar ring, a conoid, rhoptries, micronemes, mitochondria, endoplasmatic reticulum, Golgi apparatus, ribosomes, rough surface endoplasmatic reticulum, micropores and a well-defined nucleus. The nucleus is situated in the central or posterior part of the cell. The pellicle consists of three membranes. The inner membrane is discontinuous in three areas: at the polar ring (anterior), at the micropore (lateral) and towards the posterior end. The polar ring is an osmiophilic thickening of the inner membrane at the anterior end of the tachyzoite. The polar ring encircles the conoid, a cylindrical cone which consists of six to eight fibrillar elements arranged like a compressed spring. The 22 subpellicular microtubules originate from the polar ring and run longitudinally for almost the entire length of the cell and probably provide a frame for the parasite. The rhoptries are four to ten club-shaped, gland-like structures with an anterior narrow neck and posterior-sac-like end reaching as
far as the nucleus. The rhoptries contain a honey-combed structure and are thought to have a secretory function associated with host cell penetration. When the parasite has attached to the host cell, the contents of the rhoptries are discharged through the conoid. The micronemes are rice-grain-like structures, usually fewer than 100 in number, situated at the conoidal end of *T. gondii* without any defined function, but they may participate in invasion of the host cell. In addition to the rhoptries and the micronemes, the parasite contains dense granules which also appear to have a secretory function.

In general; horizontal transmission of toxoplasmosis via tachyzoites are not important epidemiologically (Petersen and Duby, 1998).

**Fig 1-1**: Tachyzoites of *Toxoplasma gondii* (CDC).

**Bradyzoite and tissue cysts:**

The bradyzoite (figer 1-2), (brady: slow) is the organism dividing slowly within a tissue cyst and is a synonym of cystozoite. A tissue cyst is a collection of bradyzoites surrounded by a well-defined host cell membrane. The
bradyzoites also multiply by endodyogeny. Tissue cysts are from 5 µm to 60 µm in size in the brain and 100 µm in other tissues and contain four to several hundred bradyzoites. Tissue cysts may develop in any tissue but are most prevalent in neural and muscular organs such as the eye and brain, skeletal and cardiac muscles. The cyst wall is thin (<0.5 µm). The tissue cyst develops in the host cell cytoplasm and its wall is intimately associated with the host cell endoplasmic reticulum (ER); indeed the cyst wall is partly of host origin. Mature cyst walls are lined with a granular material which is also found between the bradyzoites. In older cysts, degenerating bradyzoites may occasionally be found (Petersen and Duby, 1998).

Fig 1-2: Bradyzoite of *Toxoplasma gondii* (CDC).

**1.3.2 Transmission and life cycle:**

**Transmission:**
Toxoplasmosis may be acquired by ingestion of oocysts (figure 1-3) or by ingestion of tissue inhabiting stages of the parasite. Contamination of the environment by oocysts is widespread because oocysts are shed by domestic
cats and other felids. Domestic cats are probably the major source of contamination because oocyst formation is greatest in domestic cats, which are extremely common. Widespread natural infection of the environment is possible because a cat may excrete millions of oocysts after ingesting as few as one bradyzoite or one tissue cyst, and many tissue cysts may be present in one infected mouse. Sporulated oocysts survive for long periods under most ordinary environmental conditions and even in harsh environment for months. They can survive in moist soil, for example, for months and even years. Oocysts in soil can be mechanically transmitted by invertebrates such as flies, cockroaches, dung beetles, and earthworms, which can spread oocysts onto human food and animal feeds. Infection rates in cats are determined by the rate of infection in local avian and rodent populations because cats are thought to become infected by eating these animals (Dubey and Beattie, 1988; Frenkel et al., 1970).

Figure 1-3: Oocyst of Toxoplasma gondii (CDC).

Life cycle:
The definitive hosts of Toxoplasma gondii are domestic cats and other members of the family felidae; while human and other non-feline hosts are intermediate hosts. The sexual multiplication of gametogony (the intestinal cycle) takes place
in the epithelial cells of the small intestine of cats. And the oocyst are based in the unsporulated form in the feces. the asexual multiplication or sporogony (the extra-intestinal cycle) occurs in the extra intestinal tissue (Lu, 1990). Intermediate host, commonly birds and rodent, may then become infected by consuming materials contaminated by *Toxoplasma gondii* sporulated oocysts. After ingestion, oocysts develop into tachyzoites which localize in neural and muscle tissue to develop into cystbradyzoites. Members of the family felidae become infected after consuming intermediate hosts that have these cysts in their tissues. Humans acquire infection with *Toxoplasma gondii* by many routes. The most common ways of infection include consumption of under cooked meat of animals that had tissue cyst, or ingestion of food or water contaminated by infected environmental sample, and infection of fetus transplacentally from the infected mother. As less likely method of infection is receiving a blood transfusion or organ transplant from individuals harboring tissue cyst. *Toxoplasma gondii* commonly forms cysts in the skeletal muscle, brain, eyes and myocardium of the human host (Arora., 2010).

When an oocyst is ingested by a human or other warm-blooded animal, the cyst wall is dissolved by proteolytic enzyme in the stomach and small intestine, freeing sporozoites from within the oocyst. The parasites first invade cells in and surrounding the intestinal epithelium, and inside these cells, the parasite differentiates into tachyzoite, the motile and quickly multiplying cellular stage of *Toxoplasma gondii* (figure 1-4).

Bradyzoites are the slowly dividing stage of the parasite that make up tissue cyst when an uninfected host consumes a tissue cyst, bradyzoites released from the cyst infect intestinal epithelial cells before converting to the proliferative tachyzoite stage. Following the initial period of proliferation throughout the
host body, tachyzoite then convert back to bradyzoites, which reproduce inside host cells to form tissue cysts in the new host (Arora, 2010).

![Diagram of Toxoplasma gondii life cycle](image)

**Figure 1-4:** Life cycle of *Toxoplasma gondii* (CDC).

### 1.4 Pathology:

#### 1.4.1 Toxoplasma in aborted women:

The rate of placental transmission is between 17-25 %, when there is maternal infection during the first and second trimester and 65% when infection occurs during the third trimester from pregnant (Frenkel, 1990). Congenital toxoplasmosis is manifested in classic triad of chorioretinitis, hydrocephalus and cerebral calcification. Other features include microcephaly, neurological
squeal, hepatosplenomegaly, jaundice, anemia and infantialenephrotic syndrome (Moncada and Montoya, 2012).

When the congenital toxoplasmosis occurs early in pregnancy; it may lead to severe damage or abortion (Ghasemi et al., 2015).

1.4.2 Immunocompromised toxoplasmosis:
In immunocompromised human, a previously acquired latent infection can lead to reactive toxoplasmosis with encephalitis. Toxoplasmic encephalitis and disseminated toxoplasmosis have been observed in patient with immunosuppressive therapy because of other malignancies (Astrid et al., 2000).

1.4.3 Ocular toxoplasmosis:
The most commonly detected site of human congenital toxoplasmosis is the eye. Ocular lesions, both clinical and pathological, observed in human infants are well documented (Hutchison and Hay, 1982).

1.4.4 Congenital toxoplasmosis:
If first contacted during pregnancy, Toxoplasma gondii may also be transmitted to the fetus in immunocompetent women. The mechanism of vertical transmission is not yet understood. Congenital toxoplasmosis may cause abortion, neonatal death, or fetal abnormalities with detrimental consequences for the fetus (Remington and Desmonts, 1990; Ebbesen, 2000).

1.5 Diagnosis of Toxoplasma:

1.5.1 Microscopic diagnosis:
Although detection of Toxoplasma gondii in fecal, water, and tissue samples has relied on microscopic examination, but identification by light microscopy alone is less sensitive and unreliable, the oocysts in fecal, water and environment can be enriched from large volumes of samples by filtration or centrifugation for examination, and the time cysts can be stained, to differentiate the parasite from the host cells, also; staining is simple and cost-
effective, and commonly used for this purpose (Da silva et al., 2010). Pwriodic acid Schiff (PAS) can stain amlopection granules in bradyzoites (Gordon et al., 1993) these methods are relatively time consuming and need considerable skill to obtain reliable detection results. Electron microscope is also used to detect tissue cysts in mouse brain and oocysts in the small intestine of infected cats, but it is difficult to be used for routine work (Hutchison et al., 1979).

1.5.2 Serological Assays:

A variety of serological tests, such as LATEX (Toxo latex kit is an agglutination test to detect specific antibodies in serum of toxoplasmic patients. Toxo latex consists of an aqueous suspension of polystyrene particles coated with soluble purified antigens from Toxoplasma gondii. If specific antibodies are present in the sample, a clear agglutination will appear), dye test (DT), modified agglutination test (MAT), enzyme-linked immunosorbent assays (ELISA). Nowadays, ELISA techniques are used for the diagnosis of anti Toxoplasma IgM and IgG antibodies. Since anti Toxoplasma IgG antibodies are generally found in normal population. To diagnose of acute Toxoplasma infection reliably, two separate serum samples taken 10 days apart are used to demonstrate specific anti-Toxoplasma IgG titer rising. Demonstration of specific anti-Toxoplasma IgM antibody in individual sample is the alternative serological technique for diagnosis of acute Toxoplasma infection, immunosorbent agglutination assay (ISAGA), indirect fluorescent antibody test (IFAT) and indirect hemagglutination assays test (IHAT), have been developed to detect different antibody classes or antigens. IgM antibodies are detectable about 1 week post-infection and remain for several months or years. Hence, detection of IgM alone is insufficient for the establishment of acute infection. IgA antibodies are produced earlier than IgM, and may persist for several months therefore, they are considered as markers of
acute infection. The shorter period of IgE provides a greater indication of current infection. Although, the presence of IgG antibodies suggest the occurrence of infection, but it does not provide any information about the timing of infection (Muhammad et al., 2014).

1.6 Treatment:
Most people will spontaneously recover from toxoplasmosis without treatment. Treatment might be needed if the infection occurs in persons with week immune systems or long lasting diseases (AIDS or cancer). Currently recommended drugs in the treatment of toxoplasmosis act primarily against the tachyzoite form of *T. gondii*; thus they do not eradicate the encysted form (bradyzoite). Pyrimethamine is the most effective agent and is included in most drug regimens. Leucovorin (Ie, folinic acid) should be administered concomitantly to prevent bone marrow suppression. Unless circumstances preclude using more than 1 drug; a second drug (eg, sulfadiazine, clindamycin) should be added. The efficacy of azithromycin, clarithromycin, atovaquone, dapsone, and cotrimoxazole is unclear; therefore, they should be used only as alternatives in combination with pyrimethamine plus sulfadiazine or trisulfapyrimidines (eg, a combination of sulfamerazine, sulfamethazine, and sulfapyrazine). These agents are active against tachyzoites and are synergistic when used combination. Careful attention to dosing regimen is necessary because it differs depending on patient variables (eg, immune status, pregnancy), pyrimethamine may be used with sulfonamides, quinine, and other antimalarial and with other antibiotics (Sobrinnet et al., 2007, Soheilianet et al., 2011 ,and Soheilianet et al., 2005).
1.7 Economic importance:

Globally, toxoplasmosis is of great economical importance. It is the main cause of reproductive failure leading to early embryonic death and resorption, fetal death and mummification. These diseases also result in increased production costs, diminished marketability of meat, replacement animals, source of human infection (Dubey, 2009).

It is estimated that one-third of human population had chronic infection and recently recognized as emerging food-borne parasitic disease (Jones and Dubey, 2010).

1.8 Toxoplasma in Sudan:

Many studies have been done to determine the prevalence of toxoplasmosis in Khartoum state (Adnan, 1994) used ELISA technique to detect IgM, IgG prevalence obtained were 23.1%, 16.4% respectively, (Sattiet et al., 2011) found that the seroprevalence was 38.9% of IgG and 12.9% of IgM in pregnant women in Omdurman by ELISA and the highest rate of infection in women aged 21-29 years (26.7%) in Gazera state, (Abdel Hameed, 1991) found the seroprevalence was 41.7% using latex technique in Gazera State. (Khaliilet et al., 2014) studied the relation of some risk factors to the disease in Khartoum State, and found that consuming raw meat, and contact with pets other than cats and dogs were highly significant to infection, (Elsheikh, 2015) in a study in Wad Madani hospital found a strong correlation prevalence of toxoplasmosis and risk factors specially eating undercooked meat (71%), consumption of raw meat (68.1%), and contact with cats (52.1%). (Ahmed, 2016) was recorded that age groups of the participants ranged between 16-40 years. 29% were IgG +ve, while 4.6 were IgM +ve by using ELISA technique in Soba Area.
1.9 Prevention and control:

The risk of infection with *T. gondii* can be reduced by proper food preparation. Meats should be cooked to a temperature sufficient to kill the parasite; the internal temperature of beef, lamb and veal stake or roasts should reach at least 145°F (63°C), and pork, ground meat and wild game should be cooked to 160°F (71°C). Whole poultry should reach a temperature of 180°F (82°C) in the thigh. Freezing, salting, pickling and smoking do not reliably destroy this parasite. Fruits and vegetables should be peeled or washed thoroughly to remove oocyst, hands should be washed after contact with raw meat, soil or sand and before eating or touching the face. Cats should be kept far from house (CFS, 2005).
Rationale:

Atbara area is one of the largest localities, where sheep, goats and cattle are kept in private farms for breeding, milk and meat production. Hence; the intermediate hosts for *Toxoplasma gondii* are present in large population. There are many cases of abortions were reported in many hospitals, also large numbers of cats (definitive host) are found living freely in these farms. The prevalence rate of toxoplasmosis is related to many factors including socio-economic, nutritional habits and contact with cats.
Objectives:

General objective:
- To determine the prevalence of toxoplasmosis among aborted women in Atbara area-River Nile State, Sudan.

Specific objectives:
- To detect the seroprevalence of anti *Toxoplasmagondii* antibodies (IgG, IgM) among the tested aborted women.
- To determine the risk factors for infection with toxoplasmosis.
- To compare the sensitivity between three serological tests (latex, ICT, ELISA) in detection of anti *Toxoplasma* Ab.
- To determine the relation between the seropositivity and the age.
Chapter Two
Materials and Methods
Chapter two
Materials and methods

2.1 Study design:
It is a cross-sectional study.

2.2 Study area:
The study was performed in Atbara area which is located about 350 km South of capital Khartoum, and about 13 km North of Eldamar. Longitude 33,59 East and latitude 17,14 North.

2.3 Study population:
The study was carried out on aborted women (women with history of abortion).

2.4 Study duration:
The study was conducted during February to November, 2017.

2.5 Sample size:
152 blood samples were collected from women under study.

2.6 Sample collection:
A total of 152 venous blood samples were collected from aborted women using 5 ml syringe. The obtained blood samples were collected into plain vacutainer. Sera were separated after centrifugation for 15 minutes in 3000 rpm. Then were stored in -20°C till used.

2.7 Data collection:
A questionnaire was designed to collect data, it including personal information, demographic data, socio-economic data and health data (Appendex 1).
2.8 Methodology:

2.8.1 Latex agglutination test:

Principle of test:

The toxoplasmosis latex reagent is a suspension of polystyrene particles sensitised with *Toxoplasma gondii* antigens. When the reagent is faced against the serum with antibodies anti-toxoplasma, an antigen-antibody reaction takes place being easily visualized because of latex agglutination (Appendix 2).

Test procedure:

- The reagents and samples were brought to room temperature.
- 100µl from sample, positive and negative control was placed into the black circle of the slide.
- Latex reagent was mixed well and added 50µl over serum, positive and negative controls and mixed well with plastic stick and tilt the slide.
- The presence or absences of agglutination were observed within 3 minutes and the results were recorded.

2.8.2 Immuno chromatography test (ICT):

Principle of test:

The toxoIgG/IgM rapid test cassette is a lateral flow of immune chromatographic assay. The test uses anti-human IgM antibody (test line IgM) anti-human IgG antibody (test line IgG) and goat anti-rabbit IgG (control line C) immobilised on a nitrocellulose strip. The burgundy colored conjugate pad contains colloidal gold conjugated to recombinant *T. gondii* antigens conjugated with colloidal gold (*T. gondii* conjugate) and rabbit IgG-gold conjugates. When a specimen followed by assay buffer is added to the sample well, IgM&/or IgG antibodies if present, will binned to *T. gondii* conjugate making antigens antibodies complex. This complex migrates through nitrocellulose membrane by capillary action. When the
complex meets the line of the corresponding immobilized antibody (anti-human IgM&/or anti-human IgG) the complex is trapped forming a burgundy colored band which confirm a reactive test result. Absence of a colored band is the test region indicates a non reactive test result.

The test contain an internal control (C band) which should exhibit a burgundy colored band of the immune complex goat anti-rabbit IgG/rabbit IgG gold conjugate regardless of the color development of any of the test bands. Otherwise, the test result is invalid and the specimen must be retested with another device (Appendix 3).

**Test procedure:**

Specimen and controlled to equilibrate to room temperature 15-30°C prior to testing.

- The test cassette was removed from the foil pouch and used it immediately.
- The test device was placed on a clean and level surface. The dropper was held vertically and transfer 2-3 drops of serum or plasma 60_90µl to the specimen well of the test device, and incubated for 15 minutes.
  - The result was recorded.

### 2.8.3 Enzyme linked Immunosorbent Assay (ELISA) :

**Principle of test :**

Purified *Toxoplasma gondii* antigen is coated on the surface of micro wells. Diluted patient serum is added to wells, and the *Toxoplasma gondii* IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the
sample. The results are read by a micro well reader compared in a parallel manner with calibrator and controls (Appendix 4).

**The procedure:**

- The desired numbers of coated strips were placed into the holder.
- 1:40 dilutions were prepared by adding 5µl of the test samples, negative and positive control, and calibrators to 200 µl of sample diluents and mixed well.
- 100 µl of diluted sera, calibrators, and controls were dispensed into the appropriate wells. For the reagent blank, 100 µl samples diluents were dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well. Incubated for 30 minutes at room temperature.
- Liquid was removed from all wells and repeated washing three times with washing buffer.
- 100 µl of enzyme conjugate were dispensed to each well and incubated for 30 minutes at room temperature.
- Enzyme conjugate was removed from all well. Repeated washing three times with washing buffer.
- 100 µl of TMB chromogenic substrate were dispensed to each well and incubated for 15 minutes at room temperature.
- 100 µl of stop solution was added to stop reaction.
- O.D was read at 450 nm with a micro well reader.
2.9 Sensitivity and Specificity:
The sensitivity was calculated using the formula:
= \frac{\text{True positive}}{\text{True positive + false negative}} \times 100

The specificity was calculated using the formula:
= \frac{\text{True negative}}{\text{True negative + false positive}} \times 100

2.10 Data analysis:
The data were analyzed using statistical package for social science (SPSS) computer program version 15 by using Chi square and T test.

2.11 Ethical consideration:
A verbal approval was taken from women under study.
Chapter Three

Results
Chapter three

Results

3.1 The overall prevalence rate of *Toxoplasma* parasite among aborted women according to the techniques used:

The prevalence of *Toxoplasma* parasite among aborted women by different techniques was as follows: 33.6 % by latex technique, 22.4 % by ICT technique and 35.5 % by ELISA technique. The difference between techniques was found to be statistically significant at p.value=0.00 (table 3.1).

Table 3.1: The overall prevalence rate of *Toxoplasma* parasite among aborted women according to the techniques used.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Number of examined</th>
<th>Number of positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>152</td>
<td>51</td>
<td>33.6 %</td>
</tr>
<tr>
<td>ICT</td>
<td>152</td>
<td>34</td>
<td>22.4 %</td>
</tr>
<tr>
<td>ELISA</td>
<td>152</td>
<td>54</td>
<td>35.5 %</td>
</tr>
</tbody>
</table>

p = 0.00

3.2 General characteristics of the study population:

This study was conducted on 152 aborted women, the age of the subjects ranged between 20-49 years old; the age groups were divided into 20-30 years, 31-40 years and 41-49 years. The frequency of each group as follow 19(12.5%), 50(32.9%) and 83(54.6%) respectively (table 3.2). 3 out of 152 tested women had never been to school (1.9%), 30 had elementary education (19.7%), 54 had ended high school (35.5%) and 65 had university education (42.7%) (Table 3.3). 87 of tested women had contact with cats (57.2%), the difference in note was statistically significant at p=0.001 by using T test (table 3.4).
Table (3.2): Frequency of age groups:

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>19</td>
<td>12.5%</td>
</tr>
<tr>
<td>31-40</td>
<td>50</td>
<td>32.9%</td>
</tr>
<tr>
<td>40-49</td>
<td>83</td>
<td>54.6%</td>
</tr>
</tbody>
</table>

Table (3.3): Frequency of education:

<table>
<thead>
<tr>
<th>Level</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never been to school</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Elementary</td>
<td>30</td>
<td>19.7</td>
</tr>
<tr>
<td>High school</td>
<td>54</td>
<td>35.5%</td>
</tr>
<tr>
<td>University</td>
<td>65</td>
<td>42.7%</td>
</tr>
</tbody>
</table>

Table (3.4): Frequency of contact with cats:

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with cats</td>
<td>87</td>
<td>57.2%</td>
</tr>
<tr>
<td>Women without cats</td>
<td>65</td>
<td>42.8%</td>
</tr>
</tbody>
</table>

P=0.001

3.3 The overall prevalence rate of *Toxoplasma* parasite among aborted women using ELISA technique:

The result showed that out of the 152 blood sample collected from aborted women, 54 were found positive for *Toxoplasma* IgG. This constituted an overall prevalence rate of 35.5% and one IgM positive with prevalence of 0.6% (table 3.5).

Table (3.5): The overall prevalence rate of *Toxoplasma* parasite among aborted women using ELISA technique:
<table>
<thead>
<tr>
<th>Number of examined</th>
<th>Positive IgG, %</th>
<th>Positive IgM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>54 (35.5%)</td>
<td>1 (0.6%)</td>
</tr>
</tbody>
</table>

3.4 The prevalence rate of *Toxoplasma* parasite among aborted women according to age groups using Chi square:
The results showed that the highest prevalence of *Toxoplasma* parasite infection was in age group 41-49 years which comprised 54.6% of all studied population, followed by 32.9% in 31-40 years age group, then 12.5% in age group 20-30 years. Using Chi-square test; the difference in rate was found to be statistically insignificant at p-value 0.06 (table 3.6).

Table (3.6): The prevalence rate of *Toxoplasma* parasite among aborted women according to age groups.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number of examined</th>
<th>Number of positive</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-30</td>
<td>19</td>
<td>7</td>
<td>12.5%</td>
</tr>
<tr>
<td>31-40</td>
<td>50</td>
<td>17</td>
<td>32.9%</td>
</tr>
<tr>
<td>41-49</td>
<td>83</td>
<td>30</td>
<td>54.6%</td>
</tr>
</tbody>
</table>

P=0.06

3.5: Sensitivity and specificity rates of Latex and ICT:
Assuming ELISA as the gold standard, the sensitivity and specificity of the Latex technique were 94% and 100% respectively (table 3.7) and the sensitivity and specificity of the ICT technique were 64% and 100% respectively (table 3.8).
Table 3.7: Sensitivity and specificity rates of Latex:

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>LATEX</td>
<td>37 (24.3%)</td>
<td>14 (9.2%)</td>
<td>51 (33.6%)</td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>17 (11.2%)</td>
<td>84 (55.3%)</td>
<td>101 (66.4%)</td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54 (35.5%)</td>
<td>98 (64.5%)</td>
<td>152 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 94% and specificity = 100%

Table 3.8: Sensitivity and specificity rates of ICT:

<table>
<thead>
<tr>
<th></th>
<th>ELISA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>ICT</td>
<td>28 (18.4%)</td>
<td>7 (4.6%)</td>
<td>35 (23.0%)</td>
<td></td>
</tr>
<tr>
<td>+ve</td>
<td>26 (17.1%)</td>
<td>91 (59.9%)</td>
<td>117 (77.0%)</td>
<td></td>
</tr>
<tr>
<td>-ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54 (35.5%)</td>
<td>98 (64.5%)</td>
<td>152 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 64 % and specificity = 100%
Chapter Four

Discussion
Chapter four

Discussion

Toxoplasma gondii causes severe impairment and death to fetuses or to newborns through congenital infection. In this study, the overall seroprevalence of Toxoplasma infection among aborted women was 35.5% in the tested participants, (35.5% IgG, 0.65% IgM). The study was some extend to consistent with Bahaeldin and Sara (2015) which reported a rate of T.gondii IgG (28.4%) and IgM (5.3%) in Khartoum state.

Also this study was consistent with Musa and Mohamed (2014) in the a lower rate of anti Toxoplasma IgM which was found to be 0% in Khartoum and Omdurman.

This study was inconsistent with those detected by Saja(2012)in Iraq where only 23% were IgG positive, but level of IgM was lower, where saja detected 3.5% IgM positive, and Adnan and Abdel mom’em ., (2009) in Gaza who reported a lower rate of IgG(17.9%) and a higher rate of IgM(12.8%).

The overall prevalence of antibodies increased gradually with age reaching 54.6% in the age group 41-49 years followed by 32.9% in age group 31-40 years, then 12.5% in age group 20-30 years. It's inconsistent with Muna and Nadham(1996) in Basra, Iraq, who found that the antibodies increased gradually with age reaching 23.7% in the age group 35-45 years old.

The present study showed that the rate of seropositivity to T.gondii among women who had contact with cats is higher (57.2%) than those who had no contact with cats (42.8%). This finding is in accordance with those reported by Mohamed et al, (2017) which was 52.1% in contact with cats in Wad Medani and also consistent with khalile et al, (2014) who found that contact with cats was significant P=0.0001.
The prevalence of *Toxoplasma* parasite among aborted women by different techniques was as follows: 33.6 % by latex technique, 22.4 % by ICT technique and 35.5 % by ELISA technique. The difference between technique was found to be statistically significant at p.value=0.00 using Chi square test, ELISA method was found to be the most sensitive, reliable and accurate for the detection of *T. gondii* infection as compared to other methods. It is consistent with study by Muhammadet al., (2014) in Pakistan.
Chapter Five

Conclusion and Recommendations
Chapter five

Conclusion and recommendation

5.1 Conclusion:
This study concluded a high seropositivity for *Toxopalsmagondii* (35.5%) indicating potential for abortion and congenital transmission. Women living in Atbara areas are at higher risks for *T. gondii* infection. The higher prevalent was in age group 41-49 years which was 54.6%. ELISA technique is the most sensitive and specific technique to diagnose *Toxoplasma*.

5.2 Recommendation:-
The result obtained from this study recommended:

- Implementing health education program to the general population, and among pregnant women in health centers in this area, to prevent abortion and infection by *T. gondii*.
- Pre-marital examinations must include Toxoplasmosis.
- Antenatal screening of pregnant women, health educational program and awareness of the disease to women of reproductive age group in general and pregnant women in particular should be created during atental follow up to reduce the risk of *T. gondii* infection in pregnant women.
References
References


Center for Disease Control and Prevention, 2013.


Appendix
Appendix 1

**Questionnaire**

*Sudan University of Science and Technology*

*Collage of Graduate Studies*

*Medical laboratory science (Parasitology and Medical Entomology)*

**Date: ___________________________  Serial No: /**

**Age:**

☐  31-☐  20-☐  ☐

**Location:**

Locality: .................. City: ....................... Village: .........................

**Household breeding:**

☐ No  Yes

If yes:

☐ oth  ☐ ep  goats  ☐ Dogs

**Result:**

<table>
<thead>
<tr>
<th>Test</th>
<th>+ve</th>
<th>-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TOXOPAULOMISO

INSTRUCTIONS FOR USE

STORE AT 2-8°C

10 TETST

50 TESTS

10X0050

10X0100

FOR IN-VITRO DIAGNOSTIC USE ONLY

PRODUCT CODE: 1X0025/1X0050/1X0100

FORTESSA Diagnostics
Principle of the Test

The ELISA test detects the presence of TGF-β in the sample using antibodies specific to TGF-β. The test is based on the competitive inhibition assay principle, where the test sample competes with a labeled standard for binding to the capture antibody immobilized on the microtiter plate. The amount of bound labeled TGF-β is inversely proportional to the amount of TGF-β in the sample.

Materials Provided

- 96-well microtiter plate
- Standard solutions (high, mid, low)
- Antibodies: N-terminal antibody (capture), C-terminal antibody (detection)
- Enzyme-conjugated antibody
- Substrate solution
- Stop solution
- Buffer solutions

Summary of Assay Procedure

1. Prepare microtiter plate by coating wells with N-terminal antibody (capture).
2. Add standard solutions (high, mid, low) and test samples to the plate, along with an equal volume of buffer.
3. Incubate for 1 hour at room temperature.
4. Wash to remove unbound materials.
5. Add enzyme-conjugated antibody and incubate for 30 minutes.
6. Wash again to remove excess antibodies.
7. Add substrate solution and incubate for 10 minutes at room temperature.
8. Stop the reaction with stop solution.
9. Measure absorbance at 450 nm.

Calculation

The absorbance of the sample is compared to the absorbance of the standards to determine the concentration of TGF-β in the sample.