Screening of Toxoplasmosis Antibodies in Women with Spontaneous Abortion Attending Wad Medani Obstetrics and Gynecology Teaching Hospital, Gezira State, Sudan (2015)

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A Dissertation
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Date : 20 August 2015
DEDICATION

Special thanks ...... to my mother
who can take the place of many
but no one can take her place

ACKNOWLEDGMENTS
All thanks and praise be to Allah. I express my Appreciation to my supervisor for his advice and patience until the research come out to the light. I extend my respect and appreciation to all staff workers of the National Cancer Institute, and Wad Medani Obstetric and Gynecology Teaching Hospital for their supporting effort. Special recognition to Dr. Bakri Yossif and Everyone who contributed to this project.

Screening of Toxoplasmosis Antibodies in Women with Spontaneous Abortion Attending Wad Medani Obstetrics and Gynecology Teaching
Abstract

*Toxoplasma gondii* is commonly associated with congenital infections that are not clinically apparent. Primary *T. gondii* infection in the first trimester pregnancy may cause severe congenital anomalies or even fetal loss, in addition to suspecting in some risk factors that probably aided in the distribution of this disease. This is across sectional hospital-based study aim to determine the anti-*Toxoplasma gondii* antibodies among women with spontaneous abortions attending Wad Medani Obstetrics and Gynecology Teaching Hospital in Gezira State. Ninety six samples (3 ml for each sample) were collected from women with spontaneous abortions. Detection of IgG and IgM were done according to standard methods by using immunoassay analyzers (Cobas e411) in the laboratories of the National Cancer Institute, during January- January 2015. The results showed that 40 (41.6%) were seropositive for (IgG) antitoxoplasma antibodies, while 2 (2.1%) were seropositive for IgM antitoxoplasma antibodies. The highest rate of infection (47.6%) was detected among women aged 26-35 years. No significant associations between seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies and other risk factors considered in this study (including the house – keeping of cats). The presence of high seropositivity among aborted women, emphasizing the importance of an organized educational programs targeting pregnant women in order to prevent the risk of infection during pregnancy without ignoring the other risk factors.
ندى النور ابراهيم شيو
ماجستير العلوم في العلوم والتقنية البيولوجية (تقنية بيولوجية) اغسطس 2015
مركز العلوم والتقنية البيولوجية
كلية الهندسة والتكنولوجيا
جامعة الجزيرة

ملخص الدراسة

القوسية القونيدية عادة مرتبطة بالإصابات الخلقية التي هي غير واضحة سريريا. الأصابة الأولية بالمقوسة القونيدية في الثلث الأول من الحمل قد يؤدي إلى تطورات خلقية خطيرة أو يؤدي إلى فقدان الجنين كما أن هناك عددًا من عوامل الخطر المشتبه في مساهمتها في انتشار هذا المرض. وهذه الدراسة هي عبارة عن دراسة مقطعية تهدف إلى الكشف عن الأجسام المضادة لداء القوسية القونيدية بين النساء اللاتي تردن على مستشفى أمراض النساء والتوليد التعليمي بود مدني بسبب الاحجاض التلقائي. ولقد تم جمع 96 عينة (3 مل لكل عينة) من أولئك النساء. تم استخدام طرق القياسية للكشف عن أضداد الغلوبولين المناعي (M) وأضداد الغلوبولين المناعي (G) باستخدام جهاز تحليل المناعة (Cobas e411) بالمعهد القومي للسرطان بودمدني في الفترة من يناير إلى يونيو 2015. أظهرت النتيجة أن 40 (41.6%) % من العينات كانت إيجابية لمضادات القلوبيليين المناعي (M). تم الكشف عن أعلى معدل للإصابة (47.6%) بين النساء اللاتي تترواح اعمارهم بين 26-35 عاما. لا يوجد ارتباط ملموس بين الانتشار المصلى لمضادات المقوسة القونيدية وعوامل الخطر الأخرى المذكورة في هذه الدراسة (يشمل ذلك تربية القطط في المنازل). يوجد نسبة عالية من الإيجابية المصلية بين النساء المجهضات، يؤكد على أهمية وجود برامج تعليمية تستهدف النساء الحوامل وذلك للوقاية من خطر الإصابة أثناء الحمل مع عدم تجاهل عوامل الخطر الأخرى.
TABLES OF CONTENTS

<table>
<thead>
<tr>
<th>Subject</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>iii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>Arabic Abstract</td>
<td>vi</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>vii</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xi</td>
</tr>
<tr>
<td>List of Plates</td>
<td>xii</td>
</tr>
<tr>
<td>List of Appendices</td>
<td>xiii</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xiv</td>
</tr>
</tbody>
</table>

CHAPTER ONE: INTRODUCTION

CHAPTER TWO: LITERATURE REVIEW

2.1. Toxoplasma. gondii
2.1.1. Definition
2.1.2. History
2.1.3. Classification
2.1.4. Epidemiology
2.1.5. Transmission and Life Cycle
2.1.5.1 Transmission
2.1.5.2. Life Cycle
2.1.5.2.1. Asexual Cycle
2.1.5.2.2. Sexual Cycle
2.1.6. Pathology
2.1.7. Signs and Symptoms
2.1.7.1. Toxoplasmosis in Immunocompetent Adults and Children 8
2.1.7.2. Toxoplasmosis in the Immunodeficient Patient 8
2.1.7.3. Toxoplasmosis in Pregnancy 8
2.1.8. Lab Diagnosis 9
2.1.8.1 Direct Detection 9
2.1.8.1.1. Animal Inoculation 9
2.1.8.1.2. Histopathological Diagnosis 9
2.1.8.1.3. Polymerase Chain Reaction (PCR) 10
2.1.8.2. Serologic Techniques 10
2.1.8.2.1. Sabin Feldman Dye Test (SFDT) 11
2.1.8.2.2. Enzyme-Linked Immunosorbent Assays (ELISA) 11
2.1.8.2.3. IgG Avidity 11
2.1.8.2.4. Indirect Fluorescent Assay (IFA) 12
2.1.8.2.5 Indirect Hemagglutination Test (IHAT) 12
2.1.9. Control 12
2.1.9.1. Treatment 12
2.1.9.2. Prevention 13

CHAPTER THREE: MATERIAL AND METHODS 14
3.1. Study Area and Population 14
3.2. Study Design 14
3.3. Sample Size 14
3.4. Data Collection 14
3.5. Sampling 14
3.6. Ethical Approval 14
3.7. Equipments 15
3.8. Principle 15
3.9. Data Analysis 15
CHAPTER FOUR : RESULT AND DISCUSSION
4.1 Results
4.2 Discussion

CHAPTER FIVE: CONCLUSIONS and RECOMMENDATIONS
5.1 Conclusions
5.2 Recommendations

REFERENCES
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Seropositivity of Anti-\textit{Toxoplasma gondii} IgG and IgM in relation to participant's age</td>
<td>21</td>
</tr>
<tr>
<td>4.2</td>
<td>Seropositivity of Anti-\textit{Toxoplasma gondii} IgG and IgM in relation to studied risk factors</td>
<td>22</td>
</tr>
<tr>
<td>Fig. No</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>Life Cycle of <em>Toxoplasma gondii</em></td>
<td>6</td>
</tr>
<tr>
<td>4.1</td>
<td>Seropositivity of IgG Among Study Group</td>
<td>18</td>
</tr>
<tr>
<td>4.2</td>
<td>Seropositivity of IgM Among study Group</td>
<td>19</td>
</tr>
<tr>
<td>4.3</td>
<td>Seropositivity of Anti- <em>Toxoplasma gondii</em> IgG and IgM in Relation to Participant's Age</td>
<td>20</td>
</tr>
</tbody>
</table>
# LIST OF PLATES

<table>
<thead>
<tr>
<th>Plate No</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Cobas e411</td>
<td>16</td>
</tr>
<tr>
<td>App. No</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Questionaire</td>
<td>32</td>
</tr>
</tbody>
</table>
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Human Immunodeficiency Virus Infection</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECL</td>
<td>Electrochemiluminescence</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
</tbody>
</table>
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CHAPTER ONE
INTRODUCTION

*Toxoplasma gondii* is an obligate, intracellular, parasitic protozoan infecting all warm-blooded animals, with a worldwide distribution (Darde *et al*., 2011). The life cycle of *T. gondii* has two parts and three infectious stages. The two parts of the life cycle are the sexual cycle that occurs within cats and other felines which are the definitive hosts and the asexual cycle that occurs in the intermediate hosts – which are virtually any warm-blooded animal, including humans. The three infectious stages are the sporulated oocysts that contain the sporozoites, the tachyzoites, and the tissue cysts that contain bradyzoites: all three stages are infectious to both the feline definitive hosts and the intermediate hosts (Gunn and Pitt., 2012).

A human may become infected by accidentally ingesting oocysts passed in cat feces through contaminated soil, ingesting tissue cysts within raw or undercooked meat, drinking unpasteurized milk, contaminated water, or unwashed fruits or vegetables. And by direct transmission of tachyzoites from mother to fetus through the placenta (congenital infection) (Schwartzman and Amaguire, 2006; Vasan and Tsuji, 2009).

Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy. Even though, pregnant women are often asymptomatic or have only mild symptoms, infection may cause spontaneous abortion, still birth, or serious health problems in the fetus if the parasites are transmitted (i.e., congenital toxoplasmosis) and cause severe sequelae in the infant including mental retardation, blindness, and epilepsy (Al-Harthi *et al*., 2006).

The diagnosis of *T. gondii* infection or toxoplasmosis may be established by serological tests, polymerase chain reaction (PCR) in body fluids or tissues, histological methods or isolation of the parasite from body fluids or tissues (Montoya, 2002).

A serological survey during pregnancy represents a valuable tool for the diagnosis in the neonate and may bring a rapid and effective treatment of an affected child. Thus, all pregnant women should be tested at booking and sero-negative women followed at intervals for evidence of sero-conversion (Elnahas *et al*., 2003).
1.2. Rationale

Toxoplasmosis is one of the infections that can be transmitted through placenta during pregnancy. The overall rate of transmission of maternal infection to the fetus is about 45%. Of these, 60% are subclinical infections, 9% resulting in death of the fetus, and 30% have severe damage (Singh, 2003). The present study aimed to evaluate the seroprevalence of *T. gondii* in Women with Spontaneous Abortion in Gezira.

1.3. Objective

1.3.1. General objective

The aim of this study to determine the seroprevalence of toxoplasmosis antibodies in women with spontaneous abortion attending Wad Medani Obstetrics and Gynecology teaching Hospital, Gezira State, Sudan.

1.3.2. Specific objective

- To measure IgG antibodies level of *T. gondii* in women with spontaneous abortion using immunoassay analyzer (Cobas e411).
- To measure IgM antibodies level of *T. gondii* in women with spontaneous abortion using immunoassay analyzer (Cobas e411).
CHAPTER TWO
LITERATURE REVIEW

2.1. Toxoplasma. gondii

2.1.1. Definition

Toxoplasma.gondii is an obligate, intracellular, parasitic protozoan that causes the disease toxoplasmosis. (Vasan and Tsuji, 2009).

2.1.2. History

Although T. gondii was first observed in North African rodents ( gondii ) by Nicolle and Manceaux in 1908, it was not identified as an agent of infectious disease until 1923 according to a case occurred in an-11-month-old congenitally infected infant by Janku. It was reported as a cause of encephalitis in 1939 by Wolf and colleagues (Wolf et al., 1939). The high prevalence of the infection in various populations was First shown by the serological test developed by Sabin and Feldman (1948), which is still the standard basis for evaluation new serological test. T. gondii become recognized as a severe and potentially fatal disease of adults in 1968 after several cases of toxoplasma encephalitis were found in patients with hematologic cancers. It then became more widely recorded as a cause of morbidity in immunedeficient patients, including AIDS patients beginning in 1983. T. gondii continues to be an important disease in the modern world, especially in pregnant women and immunocompromised patients (Montoya et al., 2010).

2.1.3. Classification

The parasite is a member of the phylum Apicomplexa, class Sporozoa, subclass Coccidia, order Eucoccidia and suborder Eimeria (Levine et al., 1980). The name Toxoplasma is derived from the Greek word ‘ Toxon’ ( meaning arc or bow) referring to the curved shape of the trophozoite and plasmid mean “form”. The species name “gondii” is the name of a North African desert rodent (Ctenodactylus gondi) from which this parasite was originally found in (Freij and Sever, 1991) and this the only species in the genus Toxoplasma.
2.1.4. Epidemiology

Toxoplasmosis is one of the more common parasitic zoonoses world-wide. Disease in humans caused by *T. gondii* was first recognised in the late 1930s. In 1939, Sabin (Sabin, 1939) first proved that *Toxoplasma* isolates from humans and those previously obtained from animals belonged to the same species. In 1948, the introduction of the methylene blue dye test by Sabin and Feldman (Sabin and Feldman, 1948) enabled seroepidemiological studies in humans as well as a broad range of animal species which provided evidence for a wide distribution and high prevalence of *T. gondii* in many areas of the world. Since then, it has been estimated that up to one third of the world human population has been exposed to the parasite. However, seroprevalence estimates for human populations vary greatly among different countries, among different geographical areas within one country, and among different ethnic groups living in the same area.

The prevalence of the infections depends on local eating habits, environmental conditions, and the presence of definitive hosts. Prevalence also increases with age (Paris, 2013). For women of childbearing age, a survey of 99 studies within 44 countries found the areas of highest prevalence are within Latin America (about 50–80%), parts of Eastern and Central Europe (about 20–60%), the Middle East (about 30-50%), parts of Southeast Asia (about 20–60%), and parts of Africa (about 20–55%) (Pappas et al., 2009).

In Sudan, seroprevalence rates among pregnant women in Khartoum were documented as 34.1% (Elnahas et al., 2003). The seroprevalence of toxoplasmosis in Gezira State, however, was found to be as high as 41.7% (Abd Elhameed, 1991).

2.1.5. Transmission and Life Cycle

2.1.5.1 Transmission

Humans become infected by ingesting tissue cysts when eating raw or undercooked meat of animals harboring tissue cysts, and by ingestion of oocysts in soil-contaminated food (raw vegetables) or drinking water—believed to be the major source of infection in tropical areas. Transmission can also occur when changing the litter box of a pet cat, by blood transfusion or organ transplantation, and transplacentally from mother to fetus, leading to congenital infections (Magill et al., 2013).
Transplacental infection (congenital infection) occurs in approximately 30% of women if they are first infected during pregnancy and thus have no pre-existing immunity (Paris, 2013).

2.1.5.2 Life Cycle

*Toxoplasma gondii* is an obligate intracellular parasite that exists in nature in three forms: the oocyst (which releases sporozoites), the tissue cyst (which contains and may release bradyzoites) and the tachyzoite. Life cycle of *T. gondii* consists of two cycles (Figure, 2.1).

2.1.5.2.1 Asexual Cycle

The asexual cycle consists of two distinct stages of growth depending on whether the infection is in the acute or chronic phase. The tachyzoite stage defines the rapidly growing form of the parasite found during the acute phase of toxoplasmosis. Tachyzoites are approximately 5 μm long and 2 μm wide (Smith, 1995).

They replicate inside a cell with a generation time of 6 to 8 h (in vitro) until they exit the cell to infect neighboring cells, usually after 64 to 128 parasites have accumulated per cell. In the infected animal, tachyzoites differentiate into bradyzoites and form tissue cysts that first appear 7 to 10 days post infection (Radke and White, 1998). These cysts are found predominantly in the central nervous system and muscle tissue, where they may reside for the life of the host. The development of tissue cysts throughout the body defines the chronic stage of the asexual cycle. Cysts that are ingested through eating infected tissue are ruptured as they pass through the digestive tract, causing bradyzoite release. These bradyzoites can then infect the epithelium of the intestinal lumen, where they differentiate back to the rapidly dividing tachyzoite stage for dissemination throughout the body, thereby completing the asexual cycle (Black and Boothroyd, 2000).
Figure (2.1): Life cycle of *Toxoplasma gondii*
2.1.5.2.2. Sexual Cycle

The sexual cycle takes place in intestine of the definitive host. Known definitive hosts are members of the feline family, predominantly domestic cat. When bradyzoites or oocysts are ingested by a feline, formation of oocysts processed in the epithelium of the small intestine (Schwartzman, 2001). Several million unsporulated oocysts may be released in the feces of a single cat over a period 3 – 18 days, depending on the stage of *T. gondii* ingested. Under mild environmental conditions oocysts may sporulate. Within 3 weeks period. Those infecting humans and other intermediate hosts. Oocyst can spread in the environmental and contaminate water, soil, fruits, vegetables and herbivores following consumption of infected plant material. Investigation of outbreak of toxoplasmosis have led to recovery of oocysts from soil, but not from water. Oocysts have been found to be very stable, especially in warm and humid environment, and resistant to many disinfecting agents [but survive poorly in arid, cold climates]. (Rorman *et al.*, 2006).

2.1.6. Pathology

*T. gondii* invades numerous organs, infecting a broad spectrum of cell types. Tachyzoites infect macrophages and are disseminated through the blood to many organs, where they invade, asexually multiply and cause cellular disruption, leading to cell death. The resulting necrosis attracts inflammatory host cells, such as lymphocytes and monocytes. It is this inflammatory response that causes the major pathology in infected individuals. As host resistance develops, usually around 3 weeks post infection, tissue cysts may form in many organs, primarily in brain and muscle. These quiescent cysts enable *T. gondii* to evade the adaptive host immune. As tissue cysts periodically rupture, the released bradyzoites are killed by the host immune system. If immune surveillance becomes compromised, such as due to chemotherapy or AIDS, these bradyzoites develop into tachyzoites, causing active toxoplasmosis (Mandal and Mukhopadhyay, 2013).

2.1.7. Signs and Symptoms

The outcome of *Toxoplasma* infection depends on the immune status of the infected person. In contrast to the relatively benign clinical course of toxoplasmosis in the vast majority of immunocompetent individuals, it is potentially life threatening in both AIDS and other immunocompromised patients (Paniker and Ghosh, 2013).
2.1.7.1. Toxoplasmosis in Immunocompetent Adults and Children

Immunocompetent persons with primary infection are usually asymptomatic, but latent infection can persist for the life of the host. However, there is a risk of reactivating infection at a later time should the individual become immunocompromised, even if infection was asymptomatic or only mildly symptomatic initially. The most common presentation of symptomatic postnatally acquired toxoplasmosis in immunocompetent patients is painless cervical adenopathy. Acute visceral manifestations are associated in rare cases. (Carme et al., 2002).

2.1.7.2. Toxoplasmosis in the Immunodeficient Patient

Toxoplasmosis can be life-threatening for immunocompromised patients, usually due to reactivation of chronic infection. Toxoplasmic encephalitis is the most common presentation of toxoplasmosis in immunocompromised patients (Yan et al., 2013). A wide range of clinical findings, including altered mental state, seizures, weakness, cranial nerve disturbances, sensory abnormalities, cerebellar signs, meningismus, movement disorders, and neuropsychiatric manifestations are observed in patients with Toxoplasmic encephalitis. Other organs commonly involved in immunocompromised patients with Toxoplasmosis are the lungs, eyes, and heart. (Montoya, 2002).

2.1.7.3. Toxoplasmosis in Pregnancy

*T. gondii* is a protozoal parasite that can cause devastating disease in the fetus and newborn yet remain unrecognized in women who acquire the infection during gestation (Remington et al., 2011). If a woman is infected before she becomes pregnant, then her immune system will attack the parasite and make it harmless. Problems only occur if a woman becomes infected for the first time while pregnant. *T. gondii* infection acquired during pregnancy may result in severe damage or death of the fetus and long-term sequelae in offspring, depending on the virulence of the parasite, on the immune response of the mother and on the pregnancy period of the woman when infected. It can also develop during the birth of normal children that later presents retinochoroiditis (Dubey, 1977).

Transmission to the fetus occurs predominantly in women who acquire their primary infection during gestation. In rare cases, congenital transmission has occurred in chronically infected women whose infection was reactivated because of their
immunocompromised state (e.g., from AIDS or treatment with corticosteroids for their underlying disease) (Montoya and Remington, 2008).

Chorioretinitis occurs in over 80% of infected infants. Major sequelae of infection are neurological problems including seizures, mental retardation, and deafness. The risk of severe disease is greater when maternal infection is acquired in the first or second trimester (Couvreur and Desmonts, 1962). Despite higher rates of transmission of maternal infection to the fetus in the third trimester, transmission that occurs later in pregnancy generally results in subclinical infection or milder manifestations of congenital toxoplasmosis at birth. Neonatal features of infection vary and include hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, blindness, epilepsy, developmental delay, thrombocytopenia and anaemia (Stillwaggon et al., 2011).

2.1.8. Lab Diagnosis

2.1.8.1 Direct Detection

2.1.8.1.1 Animal Inoculation

Culture of live parasite definitively predicates the etiology of infection in tissues, but it is relatively insensitive and slow, taking up to several weeks. Many tissues culture lines may be used, but human fibroblasts are the most easily observed for evidence of parasite growth. Peritoneal inoculation of mice is a more sensitive technique, which may kill mice with a single infective parasite (Schwartzman, 2001). The mice should be tested for the presence of toxoplasma organisms after 6-10 days from inoculation, if no organisms are found, serology can be performed on the animals after 4-6 weeks from inoculation (Paris, 2013).

2.1.8.1.2 Histopathological Diagnosis

Tissue biopsies may demonstrate tachyzoites or cysts, which stain with hematoxyline and eosin in routine histopathological preparation. The Romanvsky stains, such as Giemsa and wright’s, also demonstrate T. gondii forms well. The parasite can be found in different cell types, including endothelial cells, fibroblast, hepatocytes, macrophage, various cells of the CNS. This characteristic differentiates T. gondii from other intracellular parasites, which infect only a single cell type. Cytocentrifuge preparation of CSF, amniotic fluid or bronchoalveolar lavage fluid may also demonstrate
tachyzoites. None of this morphological techniques is sensitive, and many lesions attributable to *T. gondii* infection have no identifiable parasite (Schwartzman, 2001).

### 2.1.8.1.3. Polymerase Chain Reaction (PCR)

This test is used in conjugation with standard serological test to assist in the diagnosis of *T. gondii* infection by detection *T. gondii* DNA in tissue, blood and body fluids. Detection of *T. gondii* DNA in blood, CSF, amniotic fluid or fetal / neonatal tissue is suggestive of acute infection. Detection of *T. gondii* DNA using polymerase chain reaction (PCR) minimizes the problems faced when using serodiagnostic or culture–based assays and facilitates diagnosis in difficult cases.

*T. gondii* PCR targets the *T. gondii* B1 gene, 35 copies of which are found in each organism. While exquisitely sensitive, PCR cannot distinguish between latent and acute *T. gondii* infection. Diagnosis of acute toxoplasmosis should not rely solely on the results of a positive PCR assay (Sabri, 2015).

### 2.1.8.2. Serologic Techniques

The use of serologic tests for demonstration of specific antibody to *T. gondii* is the initial and primary method of diagnosis. Different serologic tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serologic tests is usually required to establish whether an individual has been most likely infected in the distant past or has been recently infected. The clinician and clinical laboratories must be familiar with these problems and consult reference laboratories if the need arises (Montoya, 2002).

Acute systemic toxoplasmosis has traditionally been diagnosed by seroconversion. Anti-*Toxoplasma* immunoglobulin G (IgG) titers present a 4-fold increase that peak 6-8 weeks following infection and then decline over the next 2 years, although they remain detectable for life. Anti-*Toxoplasma* IgM appears in the first week of the infection and then declines in the next few months. The presence of anti-*Toxoplasma* IgA has also been shown to be detectable in acute infection; however, since the titers can last for more than 1 year, its value in helping to diagnose an acute phase is limited. Detection of IgG is possible within 2 weeks of infection using the ELISA test, the IgG avidity test, and the agglutination and differential agglutination tests. The presence of IgG indicates a likely past infection, while the presence of IgM usually
indicates acute infection (particularly in the absence of IgG). However, IgM has, in some cases, been documented to persist for months or years. Lack of IgG and IgM may exclude infection. IgM alone that then transitions to IgG without IgM or both IgG and IgM indicates likely acute infection. There is a significant rate of false IgM positivity. The sensitivities and specificities of the commercially available IgM and IgG tests vary substantially (Hökelek et al., 2014).

2.1.8.2.1. Sabin Feldman Dye Test (SFDT)

The Sabin Feldman dye test was the first test system able to detect specific antibodies to *T. gondii* at low levels and to differentiate acute and latent infection (Sabin and Feldman, 1948). The test is based on complement-mediated cytolysis of anti-body-coated live *T. gondii* tachyzoites, which is indicated by their inability to take up methylene blue. Although the Sabin-Feldman dye test is the gold standard for detecting *Toxoplasma* antibodies in human, it is performed only in reference laboratories because live virulent (Udonsom et al., 2010).

2.1.8.2.2. Enzyme-Linked Immunosorbent Assays (ELISA)

Most clinical laboratories use an ELISA for the routine screening of specific IgG and IgM, whereas other techniques are mostly reserved for reference laboratories. Briefly, soluble antigen is coated on a plastic medium (for example 96 well plate) on which a dilution of a serum to test is used. The presence of specific antibodies revealed using a secondary enzyme–linked antibody conjugate and chromophore. The reaction is measured by quantification of the developed colour using an optical density reader. This technique can be automated, making a high throughput of samples possible (De Craeye, 2012). Several ELISA kits commercially available each using its own form (classic ELISA, sandwich, double sandwich, immunocapture), conjugate and antigen or a set of antigens (De Craeye, 2012).

2.1.8.2.3. IgG Avidity

This is auxiliary test to determine if the infection is acute or previously acquired when the IgM serological reaction is positive in an asymptomatic patient. The test is based on the greater strength of the ionic bindings between antigen and antibody produced from old infection when compared to recent ones. Depending on the method used, pregnant women with high avidity antibodies are those who have been infected at
least 3-5 months earlier. This is more useful in Pregnant women in their first months of the gestation who have a positive test for both IgG and IgM Toxoplasma antibodies. When avidity is low or borderline it may be misleading and a more careful interpretation is critical. Low – avidity results may persist for as long as 1 year (Lopes et al., 2007).

2.1.8.2.4. Indirect Fluorescent Assay (IFA)

The IFA was widely used to demonstrate *T. gondii* specific antibodies: Serially diluted serum sample are incubated with live, inactivated *Toxoplasma* fixed to a class slide. *T. gondii* specific antibodies present in the serum would bind to the inactivated parasite, and the complex is then detected using secondary antibody coupled to fluorescent molecule usually fluorescein isothiocyanate –labeled anti human Ig (or anti – IgG or anti IgM). Every dilution has to be read with fluorescent microscope. The reading is not always easy and objective. False positive IgM results are possible due to the eventual presence of Rheumatoid factors (Ambroise- Thomas et al., 1998).

2.1.8.2.5 Indirect Hemagglutination Test (IHAT)

As knowledge of toxoplasmosis has increase, efforts have been made to improve and extend the range of specific diagnostic tests. In the search for alternative methods, attempts have been made to develop a haemagglutination test for the presence of toxoplasma antibodies in serum. Bozdech and jira Using sensitized human group O Rh-negative cells, concluded that the test was less sensitive even than the complement – fixation reaction (Sabri, 2015).

2.1.9. Control

2.1.9.1. Treatment

Most drugs used for the treatment of toxoplasmosis are active only against the tachyzoite forms of the parasite and treatment does not eradicate the infection. Treatment of toxoplasmosis in immunocompetent patients is usually unnecessary. In immunocompromised patients, the recommended treatment is a combination of pyrimethamine given at 25-100 mg daily and trisulfapyrimidines given at 2-6 g daily, both for a month. This drug combination inhibits dihydrofolate reductase in *T. gondii*, preventing synthesis of DNA and protein. Folinic acid can also be administered to reduce bone marrow depression caused by the pyrimethamine. Clindamycin has been found to
be effective at treating toxoplasma encephalitis in AIDS patients. In acutely infected pregnant women, the recommended treatment includes spiramycin if the fetus has not yet acquired toxoplasmosis. Spiramycin is an antibiotic that localizes to the placenta and has been shown to reduce placental infection by 60%. It does have some teratogenic effects, which must be weighed against the risk of congenital infection. If the fetus is infected, the aforementioned drug combination is administered instead of spiramycin. No killed vaccine is currently available to reduce or prevent congenital infections in humans and animals, but research to develop such an agent is under way (Magill et al., 2013).

### 2.1.9.2. Prevention

Hygiene measures, particularly for pregnant women and seronegative immune-compromised patients:

- Wash hands before handling food.
- Thoroughly wash all fruit and vegetables, including ready-prepared salads, before eating.
- Thoroughly cook raw meats and ready-prepared chilled meals.
- Wear gloves and thoroughly wash hands after handling soil and gardening.
- Avoid cat feces in cat litter or in soil. (Patient. info, 2015).
CHAPTER THREE
MATERIALS AND METHODS

3.1. Study Area and Population

Gezira State is located in the middle of Sudan. It has an area of 27,549 km², and a population of 2,796,000 persons. Wad Madani is the capital of the State. This study was conducted in Wad Medani Obstetrics and Gynecology Teaching Hospital. This hospital is a tertiary referral hospital delivery of nearly 18,000 patients a year.

3.2. Study Design

This study was a cross-sectional hospital-based study.

3.3. Sample Size

A total of (96) samples were obtained from women who have been subjected to spontaneous abortion.

3.4. Data Collection

Ninety six samples were collected through four months. All Participants admitted in Wad Medani Obstetrics and Gynecology Teaching Hospital, Gezira State, at the time of data collection of the study and diagnosed as abortion with no obvious cause. The questionnaire surveyed basic data, including age, gender, education and residence. Possible risk factors, including raw meat consumption and Cat contacts.

3.5. Sampling

A single blood sample was taken from each participant. Venous blood sample (3 ml) was collected by standard procedure for each participate in plain container and the sera was separated by centrifugation at 4000 rpm for 10 minutes after allowing the blood samples to clot at room temperature. The clear serum was stored at -20 until used for analysis.

3.6. Ethical Approval

This research was carried out after obtained the ethical approval from the ministry of health, permission from Wad Medani Obstetrics and Gynecological Teaching Hospital, and consent from candidates for the research.
3.7. Equipments

Detection of antibodies' levels was done by immuno-assay using (Cobas e411, serial No:0868-16, manufactured by Hitachi high technologies corporation, Tokyo – Japan) (Plate 3-1)

3.8. Principle

Bridging Principle is designed to detect antibodies (IgM, IgG), not antigen. This is accomplished by including biotinylated and ruthenium-labeled antigens in the reagents for which targeted antibody has affinity.

The sample volume is 10 µL and the total duration of assay is 18 minutes:

- first step, serum antibodies bind with the biotinylated and ruthenium-labeled antigens to form an immune complex.
- immune complex then reacts with streptavidin-coated microbeads through the action of the biotinylated antigen.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, and the unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of analyte in the sample.

The concentration of the antibody is evaluated and calculated by means of a calibration curve that was established using standards of known antibody concentrations (Cobas e411, 2006).

The cutoff values for positive and negative results were determined according to manufacture procedure

\[
\begin{align*}
T. gondii \text{ IgG: } & <1 \text{ (non reactive), } \geq 1.0 \leq 30 \text{ (Indeterminate), } \geq 30 \text{ (Reactive).} \\
T. gondii \text{ IgM: } & <0.8 \text{ (non reactive), } \geq 0.8 \leq 1.0 \text{ (Indeterminate), } \geq 1.0 \text{ (Reactive).}
\end{align*}
\]

3.9. Data Analysis

Computer program (SPSS) was used in data analysis. Chi-square test was used and appropriate p values of <0.05 were considered significant.
Plate ( 3-1 ) Cobas e411
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

About 96 women were included in this study, their ages ranged between 15 to 45 years. 54 of the 96 samples were negative. Of the positive samples (n =42), 40 were positive to IgG, 1 to IgM, 1 to IgG and IgM. Generally 41.6% of women were seropositive for *T. gondii* specific IgG antibodies (Fig. 4-1), and 2.1% were seropositive for *T. gondii* specific IgM antibodies (Fig. 4-2).

The highest percentage of positive results of *T. gondii* IgG were seen between the age group 26-35 (47.6 %), then group 36- 44 (41.6%) , and the last one were age group 15- 25 (35.7%). The positive results of *T. gondii* IgM in age groups 15-25 and 26-35 were equal (2.3 %), and None were positive in group 35-45 (Fig. 4-3). There was no statistical difference found as indicated in table ( 4-1).

Although, cat contact is the leading cause of *Toxoplasma* infection in various parts of the world, in this study, over 77% of women gave history of cat contact, but no statistical difference was shown between the seropositive and seronegative groups.

Results showed that IgG seroprevalence tends to be lower in educated participants (40%) compared to the uneducated group (54.5%). There was no significant associations between seroprevalence of anti- *Toxoplasma* IgG and IgM antibodies and other risk factors considered in the study ( p values >0.05). These factors include: age groups, education background, cat contact, eating raw meat and eating mud table (4-2).
**Figure (4-1):** Seropositivity of IgG among study group
Figure (4-2): Seropositivity of IgM among study group
Figure (4-3): Seropositivity of anti-\textit{Toxoplasma gondii} IgG and IgM in relation to participant's age
Table (4-1): Seropositivity of anti-\textit{Toxoplasma gondii} IgG and IgM in relation to participant's age

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total</th>
<th>IgG</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NEGATIVE</td>
<td>POSTIVE</td>
<td>NEGATIVE</td>
<td>POSTIVE</td>
<td></td>
</tr>
<tr>
<td>15 - 25</td>
<td>42</td>
<td>27</td>
<td>15</td>
<td>41</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>26 - 35</td>
<td>42</td>
<td>22</td>
<td>20</td>
<td>41</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>36 - 45</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>0</td>
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<tr>
<td>Total</td>
<td>96</td>
<td>56</td>
<td>40</td>
<td>94</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

(IgG \( \chi^2 = 1.2; \ p = 0.5 \))

(IgM \( \chi^2 = 0.29 \ p = 0.3 \))
**Table (4-2)**: Seropositivity of anti-*Toxoplasma gondii* IgG and IgM in relation to studied risk factors

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Total</th>
<th>IgG</th>
<th></th>
<th>IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>46</td>
<td>26</td>
<td>20</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.4%</td>
<td></td>
<td>(2.1%)</td>
</tr>
<tr>
<td>Rural</td>
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<td>30</td>
<td>20</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40%</td>
<td></td>
<td>(2.0%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>85</td>
<td>51</td>
<td>34</td>
<td>83</td>
<td>2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>40%</td>
<td></td>
<td>(2.3%)</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>5</td>
<td>6</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>54.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>74</td>
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<td>34</td>
<td>73</td>
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<td></td>
<td></td>
<td>45.9%</td>
<td></td>
<td>(1.3%)</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>16</td>
<td>6</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.2%</td>
<td></td>
<td>(4.5%)</td>
</tr>
<tr>
<td>Eating Raw meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53</td>
<td>28</td>
<td>25</td>
<td>52</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47.1%</td>
<td></td>
<td>(1.8%)</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>28</td>
<td>15</td>
<td>42</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>34.8%</td>
<td></td>
<td>(2.3%)</td>
</tr>
<tr>
<td>Eating Mud</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>86</td>
<td>50</td>
<td>36</td>
<td>84</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41.8%</td>
<td></td>
<td>(2.3%)</td>
</tr>
</tbody>
</table>
4.2 Discussion

The seroprevalence of toxoplasmosis in pregnant women, on worldwide scale, varies from 7 to 51.3% and in women with abnormal pregnancies and abortions the seroprevalence varies from 17.5 to 53.3% (Kumar et al., 2004). The seroprevalence of toxoplasmosis in Gezira State was within the ranges reported previously by other investigators in Sudan. Previous reports showed antibodies against toxoplasmosis in women ranged between 27 to 34% (Abd Elhameed, 1991; Elamin et al., 2012).

In the current study, the seroprevalence of IgG antibodies to *T. gondii* in women with spontaneous abortion was found to be 41.6 % . A previous study from Khartoum State reported a seroprevalence of 58.3% among aborted women, which is higher than our findings (Khalil et al., 2012).

As regards to the other countries; the seropositive of IgG antibodies in the current study is comparable to Senegal (Ndir et al., 2004) in 40% , but it is lower when compared with seroprevalence of toxoplasmosis in Iraq (Aziz and Drueish, 2011) in 77.1% of cases. However, it is higher than the 17.9% found in aborted women in Palestine (Al-Hindi and Lubbad, 2009). **On the other hand, the** IgM antitoxoplasma antibodies seropositive rate in this study (2.1%) was low compared to studies in Egypt 18.4% (Tammam et al., 2013) and Palastine 12.8% (Al-Hindi and Lubbad, 2009).

In this study the consumption of raw meat showed no significant difference. This Finding was inconsistent to the result reported by Abd Elhameed (1991) which suggested that the food habits of the Gezira population suggest ingestion of *Toxoplasma* cysts in meat to be the main mode of transmission of the disease. However Abd Elhameed (1991) found no correlation between prevalence of Toxoplasmosis and residence in the Gezira area which supporting our finding.

Mohamed et al., (2013) reported that, there was a significant difference between women who eating soil during pregnancy due to diet appetite and those did not, which showed no significant difference in our study. This may due to difference in sample size and diagnostic method used. In the present study we did not find statistical association between seroprevalence of anti-*Toxoplasma* antibodies and other risk factors including
education, raw meat and cat contact which is in agreement with result found by Abdel Raouff and Elbasheir (2014) in other study in Sudan.

Many of the risk factors examined, such as cat contact, eating raw meat, and some personal habits have been documented to have an influence on Toxoplasma transmission in different parts of the world. The absence of a statistically significant relationship between the prevalence of Toxoplasma infection among aborted women in Gezira and many of the factors explored in the study, does not indicate that these factors have no influence on the transmission of toxoplasmosis. The variability in prevalence rates between reports could be due to diagnostic test used, along with different population having different susceptibility to infection, and the suitability of the environment for the spread of the infection.
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1- The seropositivity of *T. gondii* IgG and IgM antibodies among aborted women in Wad Medani maternal hospital were 41.6% and 2.1%, respectively.

2- The highest percentage of positive results of *T. gondii* IgG were seen between the age group 26-35 (47.6 %), then group 36- 44 (41.6%), and the last one were age group 15-25 (35.7%), but there was no statistical difference found as indicated in table (4-1).

3- About 74% of women gave history of cat contact, but no statistical difference was shown between the seropositive and seronegative groups.

4- IgG seroprevalence tends to be lower in educated participants (40%) compared to the uneducated group (54.5%).

5- There was no significant associations between seroprevalence of anti-*Toxoplasma* IgG and IgM antibodies and other risk factors considered in the study (age groups, education background, cat contact, eating raw meat and eating mud).

5.2 Recommendations

- The researcher recommended to screen pregnant women for *T. gondii* antibodies as a routine test.

- Organized educational programs targeting this high risk group to prevent infection during pregnancy.

- Further studies must be conducted to determine the impact of this disease and to establish strategies to follow in order to reduce congenital toxoplasmosis in the populations at risk.
REFERENCES


University of Gezira
Faculty of Engineering and Technology
(Département of Biotechnology)

Questionnaire:

Name………………………………………………………………………
Age……………………………Date………………………………………

Residence :
Urban □ rural □

Educated
Yes □ No □

Eating raw meat
Yes □ No □

Contact with Cat
Yes □ No □

Eating Soil
Yes □ No □

Sign……………………………………………………………………