EVALUATION OF THE LEVEL OF AWARENESS OF CONGENITAL TOXOPLASMOSIS AND ASSOCIATED PRACTICES AMONG PREGNANT WOMEN AND HEALTH WORKERS IN TANZANIA'S TEMEKE DISTRICT DAR ES SALAAM

BY

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the award of the Degree of Master of Science in One Health Analytical Epidemiology.

THE UNIVERSITY OF ZAMBIA

Lusaka

DECLARATION

I, **Onduru Gervas Onduru**, hereby affirm that the contents of this dissertation are my own work, and that they have not previously been submitted for award of a degree, diploma or other qualification at this or any other University.

Name:…….....

Signature: ……Date……..

CERTIFICATE OF APPROVAL

This dissertation submitted by **Onduru Gervas Onduru** has been approved as fulfilling the requirements for the award of degree of Master of Science in One health Analytical Epidemiology by the University of Zambia.

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Supervisor	Signature	Date
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Examiner 1	Signature	Date
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Chairperson	Signature	Date

(Board of Examiners')

ABSTRACT

Toxoplasmosis is a zoonotic disease caused by an intracellular obligate coccidian protozoan called *Toxoplasma gondii*. The parasite infects all warm blooded animals and usually develops in immune-compromised hosts. The risk groups are pregnant women, children and HIV/AIDS patients. Congenitally infected foetuses and children may develop serious clinical outcomes including psychomotor and ocular disorders. In Tanzania, prevalence studies have shown an increase in the level of infection due to toxoplasmosis in the communities while the information on the level of awareness and practices of pregnant women and health workers in regard to toxoplasmosis has not been established.

A cross-sectional study was carried out to assess the awareness and practices towards congenital toxoplasmosis among 371 pregnant women and 22 health workers from six healthcare facilities in Temeke municipality of Dar es Salaam, Tanzania. A structured questionnaire and review of prenatal screening forms were used to collect information during the study. The questionnaire focused on disease aetiology, signs and symptoms, modes of transmission, treatment and management, the antenatal cards/ the screening forms bearing pregnant women demographics and clinical records were reviewed. Of the pregnant women studied, 96% (95%, C.I 94-98%) were unaware of the diseases, had never heard, read or seen any information regarding toxoplasmosis. Majority of respondents including those who had heard read or seen information concerning toxoplasmosis were unaware of aetiology, signs and symptoms. However, the respondents unknowingly observed preventive practices towards the disease including avoiding eating raw, cured or rare meat 90% (95%, C.I 86–93%). There was a significant statistical relationship between practices towards toxoplasmosis and age of pregnant women, for every increase in age by ten years the practices towards toxoplasmosis increased 1.4 times (OR=1.41, 95%, C.I 1.05-1.90). The odds of risk practices regarding *Toxoplasma* were 0.4 times higher in pregnant women aged between 19-25 years as compared to those who were below 19 years old (OR=0.4, $p<0.01$). Multigravidae was statistically significant associated with the practices towards exposure to *Toxoplasma* (OR=2.65, $p<0.01$). Of the 22 health workers who participated in the study, only 36% (95%, C.I 15–58%) were aware of the congenital toxoplasmosis and its clinical outcomes but none of them had diagnosed the disease before.

Generally, lack of awareness contributed by low level of education and lack of priority for prenatal toxoplasmosis screening services was observed among both health workers and pregnant women in Temeke Municipality. To promote awareness and preventive practices towards toxoplasmosis, health education and health promotion of medical personnel's and vulnerable population of pregnant women on the importance of congenital toxoplasmosis is recommended. This can be done through strengthening the curriculum for training of medical personnel to cover more aspects of congenitally transmitted diseases including toxoplasmosis. Development and provision of prenatal brochures addressing different reproductive and child health issues including maternal behaviours' and practices in conjunction with the diseases of congenital importance and their risks during pregnancy. The government through the health systems and policy should support and facilitate diagnostic services for screening of toxoplasmosis among pregnant women attending prenatal health care, not only HIV/AIDS, Syphilis and malaria which are currently screened. Lastly, more research work including population epidemiology studies of toxoplasmosis through one health approach to establish general exposure status and complications associated with the disease in different risk groups is also recommended.

DEDICATION

To my mother Mereza Adhiambo Gervas Onduru and my daughter Careen Onduru

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ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
CDC	Centres for Disease Control and Prevention
ELISA	Enzyme-linked immunosorbent assay
HIV	Human Immunodeficiency virus
MCH	Maternal and child health
NIMR	National Institute for Medical Research
OR	Odds Ratio
PCR	Polymerase chain reaction
RCH	Reproductive and child health
USA	United States of America

CHAPTER ONE

1.1 Introduction

Toxoplasmosis is a zoonotic disease caused by obligate intracellular coccidian protozoan specie called *Toxoplasma gondii* (*T. gondii*). The parasite belongs to apicompexa family in the genus *Toxoplasma* and is the only known species of the genus *Toxoplasma* (Dubey, 2004). All warm-blooded animals are the intermediate host for *T.gondii* while the only definitive hosts are felids (Fergeson, 2005). The population structure of *T. gondii* has three clonal lineages described as Types I, II and III (Tibayrene & Ayala, 2002). Type I is the highly virulent one; studies suggest that Type I strain has a higher frequency in causing ocular toxoplasmosis and is the most predominant in immunocopromised patients i.e. Human Immune Virus/AIDS victims (Khan *et al.*, 2005). On the other hand, isolates from North America and Europe showed Type II and III to be the most prevalent cause of human toxoplasmosis in congenital infections and in the immuno-compromised individuals (Nowakowska *et al.*, 2006). The disease is usually fatal in immune-compromised host mainly due to encephalitis (Betroli *et al.*, 1995). Recent epidemiological data indicates that 60% of the studied adult population in north-eastern Tanzania is seropositive for *T. gondii* (Swai & Schoonan, 2009) and 30.9% pregnant women in Tanzania lake zone are seropositive for toxoplasmosis (Mwambe *et al.*, 2013).

Toxoplasmosis remains asymptomatic in immunocompetent individuals; pregnant women are at risk of developing the disease because during pregnancy immunity is lowered by modulation of hormones (oestrogens). The parasite may then become infective and cause congenital infections in foetus and new-borns leading to problems of Central nervous system and ocular disorders. Vertical transfer of *Toxoplasma* infection can occur before birth or during delivery.

Previous studies suggested that many pregnant women are not aware of risk practices and consequences of infectious diseases and not practicing preventive strategies (Jones *et al.*,

2003). Vertical transmission of infection and manifestations of toxoplasmosis are responsible for congenital problems and severe illness in foetus and newborns such as central nervous system and ocular diseases (Remington *et al,* 2001). These include retinitis mental retardation, blindness, hydrocephalus, hemiperesis, encephalitis, seizure, disequilibrium, intracranial calcification and death (Wallon *et al.*, 1999).

A Prevalence range of toxoplasmosis of 4-60% in animals and humans including pregnant women in Tanzania has been reported by different studies (Doehring *et al.*, 1995; Swai & Schoonman, 2009) and in Mwanza region it was estimated that 39% of pregnant women are infected with *Toxoplasma* (Mwambe *et al.*, 2013). Previously, Gittle *et al., (*1992) reported 4% human toxoplasmosis prevalence in Nyamisati village in Tanzania Coastal region. On the other hand, serological survey in animals also showed that, 14.2 % of goats and sheep were seropositive for toxoplasmosis in southern Tanzania (Connor & Halliwell, 1985). Even though these studies have shown the increased occurrence of the disease in Tanzania, there is no routine screening for toxoplasmosis in pregnant women in Tanzania (Mwambe *et al.*, 2013*).* Despite the evidence and availability of information about the prevalence of the disease in pregnant women in Tanzania, there is lack of information about the awareness and practices of pregnant women and health workers towards congenital toxoplasmosis.

It was observed that previous toxoplasmosis studies in Tanzania were only based on the determination of the prevalence of the disease in the communities. However, the lack of information about pregnant women and healthcare workers awareness and practices

towards toxoplasmosis undermines and limits the scope of health care for pregnant women with regards to congenital infections. .

1.2 Objectives of the study

The general objective of the study was to assess the awareness and practices towards congenital toxoplasmosis among health workers in Temeke and pregnant women attending antenatal care services at health facilities in Temeke municipality, Dar es Salaam Tanzania.

The specific objectives of this study were;

1. To assess the level of awareness of the health workers and pregnant women towards congenital toxoplasmosis in Temeke municipality, Dar es salaam Tanzania and,
2. To determining practices towards congenital toxoplasmosis among pregnant women attending antenatal care services in Maternal and Child Health /Reproductive and Child Health clinics in Temeke.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aetiology of toxoplasmosis

The causative agent for toxoplasmosis, *T. gondii* was first discovered in 1908 by Nicolle and Manceaux in a rodent (*Ctenodectylus gundi*) found in the foothills and mountains of southern Tunisia (Nicolle and Manceaux, 1908). Toxoplasmosis is one of the most widespread zoonotic parasite infections of human (Tenter *et al*., 2000; Dubey, 2010) and is recognized as the pathogen of the immune-compromised hosts (Jones *et al.*, 2001). However severe cases of toxoplasmosis have also been reported in immune-competent individuals (Vaudaux *et al.*, 2010).

Phylogenetic and statistical analyses indicate a highly unusual structure of *Toxoplasma*. Based on multi locus enzyme electrophoresis analysis (MLEE), microsatellite analysis and PCR-RFLP genetic analysis of six independent single-copy loci, the *T.gondii* isolates have been classified into three widespread predominant clonal lineages types I, II and III (Howe &Sibley, 1995; Knan *et al.*, 2005; Darde *et al.*, 2014). Over the past three decades, *T. gondii* has been considered a single species in the genus *Toxoplasma*. Due to rare sexual recombination between the three clonal lineages, all *T. gondii* strains are morphologically and serologically similar and are designed single species (Howe & Sibley, 1995).

Early survey indicated that both type II and type III strains are common in animals (Howe & Sibley, 1995). Several studies among agricultural animals, mostly from North America and Europe, have shown that the majority of isolates are type II, including pigs in the USA and sheep from Britain (Montoya & Liesenfeld, 2004). But chickens in North America exhibit a higher prevalence of type III strains than type II (Dubey *et al.*,

2003). Though isolating *Toxoplasma* from humans is notoriously difficult to conduct and is often based on clinical cases, type II strains are most commonly associated with human toxoplasmosis, both in congenital infections and in patients with AIDS (Honore, 2000). The reasons for the differences between infections of animals by types II and III and humans largely by type II are unclear. At present, there are no enough data to statistically confirm association of host species with *T. gondii* genotypes.

Virulence of *Toxoplasma* in mice is the most recognized phenotypic marker: type I strain leads to a widespread parasite dissemination and death of mice less than 10 days after inoculation of < 10 tachyzoites. In contrast, mice survive infection with a type II strain (50 percent lethal dose (LD_{50}) > 10^3) and tachyzoite dissemination is much less extensive. Type III is generally considered as avirulent in mice, although progressive deterioration and death of mice, notably with neurological symptoms, can occur a few weeks or months after inoculation (Dardé *et al.,* 2014). Type II and III lineages predominate and readily establish chronic infections in animals and humans (Howe & Sibley, 1995). The higher virulence of type I in mice compared with types II or III has been correlated with *in vitro* biological properties of migration, penetration and transmission. The host response is also essential for expression of virulence. Atypical and naturally recombinant strains are usually more virulent in mice than are types II or III (Dardé *et al.*, 2014).

2.2 Life cycle of *Toxoplasma gondii*

Toxoplasma parasite primarily exists in three infectious forms which are tachyzoites, bradyzoites and oocysts (Khan *et al.*, 2005). Tachyzoites is a feeding and rapidly proliferative form of *Toxoplasma* which is able to invade various host cells including macrophages. Tachyzoites are dominant during acute phase of toxoplasmosis. In

immune-competent individuals, the host immunity prevents multiplication of tachyzoites and the parasite is maintained in a slowly dividing latent form called bradyzoites (tissue cysts). Bradyzoites can stay in the host muscle and brain tissues for the entire life time (Jones *et al.*, 2005). *T. gondii* has a two-stage life cycle, consisting of a sexual phase in the definitive host and an asexual phase occurring in the intermediate host.

2.2.1 Sexual cycle in the definite host

Definitive hosts can be infected by ingestion of parasites tissue cysts through eating small rodents or oocysts in the contaminated environment. Once the felids are infected, asexual phase occur by firstly microgametes fertilizing macrogametes in the enterocyte forming fertilized zygotes (gametogony). A wall is then laid around each zygote forming unsporulated oocysts, which are released into the intestinal lumen when enterocytes rupture. These oocysts are then released into the environment with the faeces approximately three to ten days post infections (Tenter *et al*., 2000; Dubey *et al.*, 2011).

Depending on the environmental conditions, like temperature, aeration and humidity, the oocysts begin to sporulate within five days dividing into two sporocysts, each containing four infectious sporozoites which can remain infectious for months in the environment (Petersen & Liesenfeld, 2007).

2.2.1 Asexual cycle in the intermediate host

The most important source of *Toxoplasma* infection for intermediate host is the resistant oocyst present in cat faeces, undercooked meat, body organs, tissues and soil (Robert-Gangneux and Darde, 2012). Once ingested by an intermediate host, the outer walls of cysts or oocysts are disrupted by enzymatic degradation, releasing either bradyzoites or

sporozoites into the intestinal lumen. Unlike other coccidian, *T.gondii* contains two sporocysts which upon replication produce four sporozoites asexually in intermediate host and sexually in definitive hosts. Oocyst-sporozoites transform into invasive tachyzoites in the intestines. Tachyzoites rapidly divide within the host cell, leading to its rupture and finally release the parasites and spread to distant organs in lymph fluids and blood. Tachyzoites are able to penetrate all nucleated cells and rapidly replicate in intracytopenium vacuole disrupting host tissue cells leading to clinical manifestation of the disease (Robert-Gangneux & Darde, 2012).

Sporozoites then actively invade surrounding cells and transform into tachyzoites into the blood and lymphatic system, where they are carried to other cells which they then invade, repeating this cycle. The life cycle of *T.gondii* is summarized in figure 2.1.

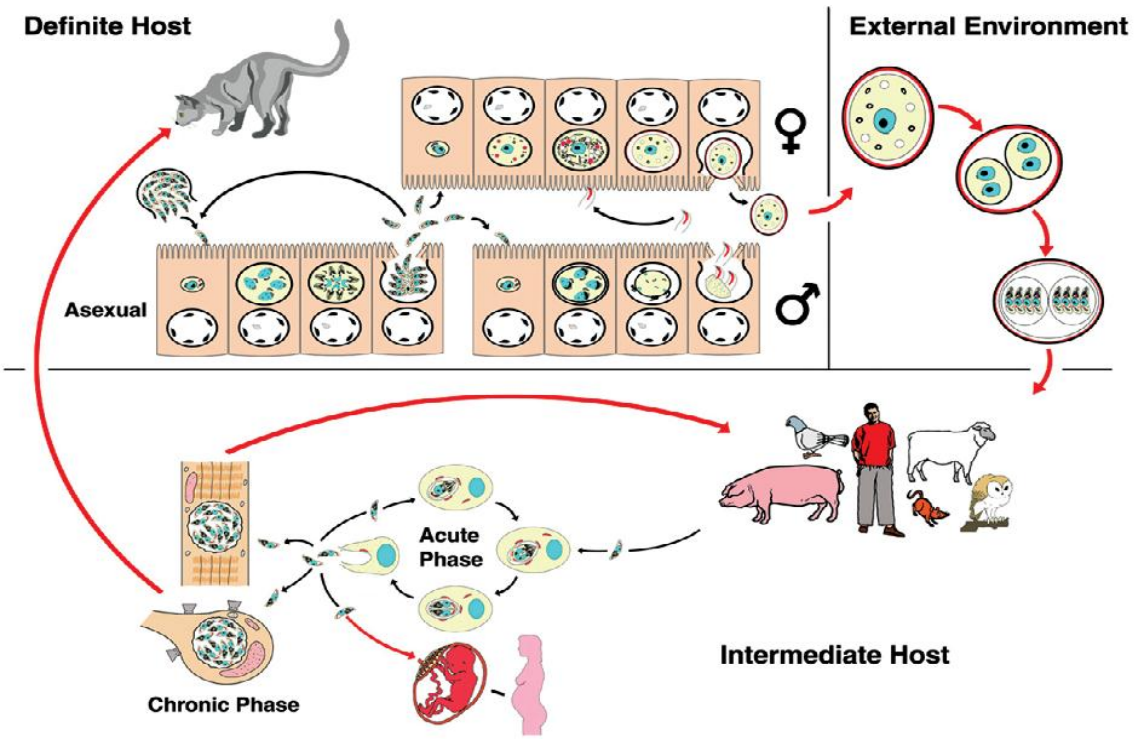

Figure 2.1: The Life cycle of *Toxoplasma gondii* (adapted from Robert-Gangneux and Darde, 2012)

2.3 Susceptible species and host range of *T. gondii*

Toxoplasmosis is a worldwide parasitic disease infecting almost all worm blooded animals with both domestic and wild felids as a definitive host (Bollani *et al.*, 2013). Generally it is estimated that about 30% of the world human population is infected with *T. gondii* (Dubey, 2010).

2.4 Clinical presentation of toxoplasmosis in humans and animals

The infection due to toxoplasmosis presents with a wide range of clinical manifestations in humans and different animals species (Akyar, 2011). Clinical manifestations of toxoplasmosis are caused by cell destruction due to multiplying tachyzoites, which most commonly affect the brain, liver, lungs, skeletal muscles and eyes. Oocyst-induced infection may be more severe than that induced by ingestion of tissue cysts. Signs may persist for one to twelve weeks but more severe disease is very rare in immunocompetent individuals (Tenter *et al.*, 2000). The host immune response to *T. gondii* lead to transformation of the parasite from tachyzoites to bradyzoites (tissue cyst) which remains dormant and can be found in retina, brain, skeletal and cardiac muscles (Lyons *et al.*, 2002).

2.4.1 Clinical manifestation of toxoplasmosis in animals.

Because adult immunocompetent animals efficiently control tachyzoite spread, toxoplasmosis is usually a subclinical illness. However, in young animals, particularly puppies, kittens, and piglets, tachyzoites spread systemically and cause interstitial pneumonia, myocarditis, hepatic necrosis, meningoencephalomyelitis, chorioretinitis, lymphadenopathy, and myositis. The corresponding clinical signs include fever, diarrhoea, cough, dyspnoea, icterus, seizures, and death. *T. gondii* is also an important cause of abortion and stillbirth in sheep and goats and sometimes in pigs. After infection

of a pregnant ewe, tachyzoites spread via the bloodstream to placental cotyledons, causing necrosis. Tachyzoites may also spread to the foetus, causing necrosis in multiple organs. Finally, immunocompromised adult animals (e.g. cats infected with feline immunodeficiency virus) are extremely susceptible to developing acute generalized toxoplasmosis

2.4.2 Clinical manifestation of Toxoplasmosis in humans

Likewise in immunocompetent humans, when symptoms develop, they are nonspecific and febrile. Clinically the parasite has mostly been described in central nervous system and the eye. In congenitally infected children toxoplasmosis presents with retinitis, blindness, anaemia, diarrhoea, rash, strabismus, hydrocephalus, intracerebral calcification and mental retardation (Remington, 2001). In adults the disease is characterized by abortion in pregnant women, lymphadenopath*y associa*ted with fever, fatigue, muscle pain, sore throat and headache (Tenter *et al.*, 2000). Encephalitis is the most significant manifestation associated with disorientations, headache, drowsiness, hemiperesis, reflex changes, convulsions and cause severe damage in immunosuppressed patients (Remington *et al.*, 2006).

2.5 Diagnosis of toxoplasmosis

Most of the zoonotic illness including toxoplasmosis are febrile to be recognized by medical personnel's the fact which undermines the diagnosis of zoonoses. The diagnosis of toxoplasmosis is made by biologic, serologic, histologic methods, or by some combination of the mentioned methods. Clinical signs of toxoplasmosis are nonspecific and are not sufficiently characteristic to be depended upon for a definite diagnosis (Dubey, 2013). *T. gondii* can be diagnosed in the laboratory using various methods

including, microscopy, histopathology, serology, bioassay and molecular methods (Robert-Gangneux and Darde, 2012).

2.5.1 Microscopy

Microscopic examination of Giemsa or Romano*wsky* stained tissue smears from the lesions associated with *Toxoplasma* pathologies can be used to examine the parasite oocyst. Well preserved *T. gondii* are crescent shaped and stain well with any of the Romanowsky stain. Primarily, the light microscopy is extensively used for detection of oocysts in case of highly contaminated samples (i.e. cat faeces). This technique is based upon morphological characteristics of *T. gondii* oocysts observed in a smear prepared directly from faeces (Ferezin *et al.*, 2013). Immunohistochemical staining of Polyclonal and monoclonal antibodies may aid diagnosis of toxoplasmosis. Concentration methods such as flotation have been used to examine parasite in cat faecal samples, however, the examination of concentrated oocyst smear from cat faces cannot practically detect the parasite because the number of oocyst may be too few to detect in direct smears (Hill &Dubey, 2004).

2.5.2 Serological detection of *T. gondii*

Serological detection of antibody against *Toxoplasma* is the primary method used in routine and screening purposes. Different serological tests often measure different antibodies that possess unique patterns of rise and fall with time after infection. A combination of serological tests is frequently required to establish whether an individual has been more likely infected in the distant past or has been recently infected. Unlike immunoglobulin A, D and E, serological determination of exposure status to *T. gondii* mostly targets the immunoglobulin G and M to distinguish between chronic and pre exposure from acute and recent exposure respectively.

Various serological tests including Sabin-Feldman dye test (DT) (reference for IgG test), indirect agglutination assay, immune fluorescent assay test, latex agglutination assay, complement test, immunosorbent agglutination assay, and Enzyme linked immunosorbent assay (ELISA) are considered as the first line method for diagnosis of *Toxoplasma* infection by determining the presence of specific antibodies (Pfaff *et al.*, 2007). Generally, the levels of the circulating IgG and IgM are being considered as important element to diagnose toxoplasmosis (Tekkesin, 2012). The presence of IgM antibody in sera is becoming an inadequate criterion for diagnosis of acute infection while the avidity of IgG in serum antibodies has become a very important diagnostic tool (Liesenfeld *et al.*, 2001). Sensitivity of ELISA for detection of *Toxoplasma* has been reported to range from 70-90% (Naessens *et al.*, 1999).

The DT is considered the gold standard test for diagnosis of toxoplasmosis (Sudan *et al.*, 2013). Modified Agglutination test (MAT) and ELISA test have been developed for most domestic animals species and human (Dubey, 2010). However, the sensitivity and the specificity of most diagnostic tests depend on the host species.

2.5.3 *Toxoplasma gondii* bioassay

Toxoplasma parasite has not been grown in cell-free media, however it can be recovered from laboratory animals, chick embryo and cell culture. Mice, hamsters, guinea pigs, rabbits and kittens are all susceptible to Toxoplasma. Preferably, mice and kittens bioassay are used for isolating *T. gondii* rather than cell line cultures, however mice are more susceptible than others. The possible specimens for inoculums are excretions, secretions, body fluids and /or tissues obtained from the infected hosts by biopsy (Hill & Dubey, 2004), such as lymph nodes or muscle tissues. Induction of either an acute infection with parasite-rich ascites or a chronic infection which is characterized by the

presence of cysts in the brain depending on the virulence of the strain occurs in the inoculated animal.

The parasite mostly colonizes the peritoneal cavity in animal models following intraperitoneal inoculation. Due to a limited number of tissue cyst from food animals to be detected by mouse bioassay, kittens are significantly used because they can be fed a large volume of tissue. Cerebrospinal fluid from a congenially infected child or lymph nodes from a person with lymphadenopathy are good sources of *T. gondii* (Dubey *et al.*, 2013).

2.5.4 Molecular diagnosis

Molecular methods can allow more appropriate diagnosis of toxoplasmosis, especially in cases in which inadequacy of conventional methods is confronted with deteriorating and potentially severe clinical outcome (congenital, ocular toxoplasmosis and cases of immunosuppression) (Su *et al.*, 2010). Molecular tools including allonzymes, DNA cloning and sequencing, PCR, Fragment analyses (RFLPs, Microsatellites, Minisatellites, RAPDS), Loop-mediated isothermal amplification (LAMP), Comparative and functional genomics and Rapid screening methods (DGGE, SSCP and SNPs) are most important for diagnosis of different disease agents when the serology tests are limited. These methods are separated into two groups.

The first group contains techniques aimed at detection of *T. gondii* DNA in biological and clinical samples, such as conventional PCR, nested PCR, real-time PCR and LAMP. The second group includes PCR-RFLP, minisattelites or microsatellites analysis and multilocus sequence typing of a single copy *T. gondii* DNA mainly used for strain typing.

To date, various molecular tools have been developed for direct detection of Toxoplasma parasites in blood and tissue samples. Either Monoplex-PCR, multiplex-PCR, RT-PCR, RT-qPCR and nested PCR may all be used. Currently RT-qPCR has been described and is the most sensitive method for molecular detection of *T. gondii*. Molecular detection of *T. gondii* can be achieved by amplification of different *T. gondii* genetic markers e.g. B1 gene sequence, Surface Antigen protein gene (SAG1, SAG2, SAG3), 529bp (AF146527), ITS-1 or 18 $_{r}$DNA, P30 (single copy gene), Apico and PK1. The B1 gene, although of unknown function, is mostly exploited in a variety of diagnostic and epidemiological studies (Su *et al.*, 2010).

Amplification and sequencing of *T. gondii* 529bp DNA sequence is recently the most usually used molecular approach for the detection of *T. gondii*. This region (529bp) is selected for against other regions because it is small and highly conserved within the *T. gondii* genome than other molecular markers. It is repeated 200-300 times and has a sensitivity of 10-100 times than B1 gene when targeted using qPCR (Calderaro *et al*, 2006). Molecular detection of *T. gondii* in cases of suspected congenital infections could be practiced in the amniotic fluid, and foetal and neonatal blood samples. As well, it is performed in the peripheral blood of immunosuppressed patients and in samples of humour aqueous and cerebrospinal fluid of patients suspected of ocular and cerebral toxoplasmosis, and also in bronchoalveolar lavage fluid (BAL) (Ivovic *et al.*, 2012)

2.6 Prevalence and distribution of toxoplasmosis

Toxoplasmosis is a worldwide parasitic disease most prevalent in Europe, South America and Africa (Bollani *et al.*, 2013). The prevalence of toxoplasmosis varies between countries and within communities in a given region. The prevalence of toxoplasmosis and global coverage according to different studies is as follows;

2.6.1 In European region

Seroprevalence in Europe varies. It is high (up to 54%) in Southern European countries, and decreases with increasing latitude to 5% to 10% in northern Sweden and Norway (Evengard *et al.*, 2001). The age-specific prevalence has been decreasing in Europe over the past three to four decades (Flegir *et al.*, 2014). A study of pregnant women from France over the period 1995 to 2003 found an overall prevalence decreasing by 19% in the investigated period and was 43.8% in 2003, with the highest prevalence in the southwest of France (Berger *et al.*, 2009). The birth-prevalence of congenital toxoplasmosis in France was estimated to be 3.3 infected newborns per 10,000 live births and the birth-prevalence of symptomatic congenital toxoplasmosis was estimated to be ten times lower or 0.34 cases per 10,000 liveborns (Villena *et al.*, 2010). A case series of 139 patients with ocular toxoplasmosis from Switzerland found a mean age of first reported symptoms of 23.9 years. The mean age was 29.6 years in patients reporting only one episode and 17.9 years in patients reporting more than one recurrence and the highest recurrence rate was found in patients below 20 years (Garweg *et al.*, 2008).

A study from the United Kingdom based on active reporting from ophthalmologists identified *Toxoplasma* infection in 83 patients aged 10–54 years, with a mean age of 29 years (Gilbert *et al.*, 1999). In the Netherlands the overall seroprevalence dropped from 40.5% in 1995/1996 to 26.0% in 2006/2007. In women of reproductive age, the seroprevalence went from 35.2% in 1995/1996 to 18.5% in 2006/2007 (Hofhuis *et al.*, 2011). Dolk and colleague in 2008 did surveillance of toxoplasmosis in pregnant women in the Russian Federation found that 4.2% of pregnant women had *T. gondii*-specific IgM-antibodies.

2.6.2 Prevalence in North America

From 1999-2004, data collected by National Health and Nutrition Examination Study (NHANES III), in the United States suggested a toxoplasmosis seroprevalence of 15.8% in the age group 12-49 years. The *T. gondii* seroprevalence was higher among non-Hispanic black persons than among non-Hispanic white persons (age-adjusted prevalence 19.2% vs. 12.1%). The risk factors for acute infection with *T. gondii* in the United States were found to be consumption of raw and undercooked meat, unpasteurized goat's milk and exposure to kittens (Jones *et al.,* 2009). In an outbreak of water-borne toxoplasmosis in the British Columbia, Canada, the rates of symptomatic chorioretinitis were between 0.2 to 0.7%. This was based on the assumption that between 2894 and 7718, individuals acquired infection during the outbreak (Burnett *et al.,* 1998)

2.6.3 Prevalence in South and Central America

In south and Central America, toxoplasmosis is a common infection. A Study from Brazil found that seroprevalence was high in people from poor socio-economic conditions probably due to water borne transmission (Bahia-Oliveira *et al.,* 2003). In slaughterhouse workers, study found a seroprevalence of 73% and suggested that fresh meat was an important source of infection in Brazil (Dias *et al.,* 2005). A study of children from Guatemala found that infection with *T. gondii* often took place before the age of five years at which time 43% were seropositive (Jones *et al.,* 2005). A seroprevalence of nearly 60% has also been found in Amerindians from Venezuela (Chacin-Bonilla *et al.,* 2001). A striking difference in the distribution of *T. gondii* genotypes between different parts of the world with an abundance of so called "atypical strains" has been described (Adjzenberg *et al.,* 2010). A higher prevalence of congenital toxoplasmosis has been found in Brazil compared to Europe followed by a higher rate of

chorioretinitis in newborns and a higher rate of ocular disease in Brazilian infants after birth compared to European infants (Gilber *et al.*, 2008).

Severe, disseminated *T. gondii* infection with multi organ involvement including eye symptoms has been described from French Guiana due to highly virulent strains circulating in a forest-based cycle involving wild felids (Carme *et al.*, 2009). Most patients reported forest-related activities such as ingestion of surface water, consumption of undercooked game meat, and hunting.

2.6.4 Prevalence in Asia

A study of HIV-positive patients from Taiwan found a seroprevalence of 10.2% (Hung *et al.*, 2005) and a study from Korea found an IgG-specific prevalence in pregnant women of 0.8% (Song, 2005). Recent study from India found a seroprevalence of *Toxoplasma*-specific IgG antibodies of 45% (Singh *et al.*, 2004) and a study of HIV-infected patients from Japan found an overall seroprevalence of 44.8%, and the majority of these patients were aged 25 to 34 years (Nissapatorn *et al.*, 2004). Of 640 pregnant women in Thailand a seroprevalence of 28.3% was found (Nissapatorn *et al.*, 2011).

A study from Malaysia showed that people belonging to the Indian ethnic group had a seroprevalence of 55.3% whereas ethnic Chinese had a seroprevalence of 19.4% (Nissapatorn *et al.*, 2004).

Few studies have been published from China, but one study reported a 3.7 times increased risk of being *T. gondii*-IgG positive in patients with posterior uveitis (Wang *et al.*, 1991). A study of the seroprevalence of *T. gondii*-specific IgG-antibodies from northern India found an overall prevalence of 51.6% in males and 89.2% in females (Elhence *et al.*, 2010). In contrast, a study from Chandigarh found an overall

seroprevalence in adults of 15% (Khurana *et al.*, 2010), probably indicating huge differences in the prevalence and incidence of infections with *T. gondii* in India.

In Malaysia, the seroprevalence of toxoplasmosis in different groups was 58% (258/443), with a seroprevalence in the age group 21 to 40 of 28.4%, of which 32 cases were clinically diagnosed with ocular toxoplasmosis (Nissapatorn *et al.*, 2011).

2.6.5 Prevalence of toxoplasmosis in Africa

Toxoplasmosis has not been intensively studied in African region; the present literature from Zambia showed variation in toxoplasmosis prevalence from 4% in HIV positive and negative individuals (Zumla *et al.*, 1991), and in HIV positive alone the prevalence of 23% was reported by Leblebicioud & Hokelek (2006). In Sao Tomé, West Africa, a study found a seroprevalence of 21.5% in children below 5 years of age (Fan .*et al.*, 2005) and a study from Sudan found a seroprevalence in pregnant women from Khartoum of 34.1% (Elnahas *et al.*, 20003). Of the 1,828 HIV positive patients from Bobo-Dioulasso, Burkina Faso, 25.4% had positive *T. gondii* serology (Millogo *et al.*, 2000). *T. gondii* accounted for 43% of patients with uveitis and visual impairment in Sierra Leone (Ronday *et al.*, 1996).

A recent study from Tanzania found a seroprevalence of 60% in adults over the age of sixty years (Swai & Schooman, 2009). A review of toxoplasmosis in some African countries summarized prevalence rates in Egypt (57.9 %), Tunisia (58.4 %), Morocco (50.6 %), Nigeria (20.8 %), Mali (21 %), Benin (3.6 %), Gabon (71.2 %), Madagascar (83.5 %), and Senegal (40.2 %) (Pappas *et al.*, 2009).

2.7 Risk factors and transmission of toxoplasmosis

Toxoplasma gondii is infective in all of its three stages/forms (tachyzoites, bradyzoites and sporozoites) to both definitive and intermediate hosts. The parasite can be transmitted from definitive to intermediate host and vice-versa; toxoplasmosis is conveyed horizontally and vertically between populations. Horizontal transfer is through infection by oocyst while vertical transfer is through tachyzoites which are able to cross the placenta. The main routes of *Toxoplasma* transmission are congenital, feeding (carnivorism) and through faecal- oral route (Robert-Gangneux & Darde, 2012). Definitive hosts (only felids) are mainly infected through carnivorism. In humans; *T. gondii* infection can occur before and after birth and the infection occurring before birth can sometimes not be present at birth but develop later in life. The main factors leading to human infection by *Toxoplasma* are ingestion of the parasite in food such as raw/ undercooked meat, untreated drinking water, eating contaminated fruits or vegetable and handling the soil contaminated with *Toxoplasma* oocysts.

The risk groups are immuno-copromised individuals' i.e. in pregnant women, AIDS patients, people on chemotherapy and children. Toxoplasmosis is a significant cause of death in HIV/AIDS patients, 10% of HIV patients in USA and 30% in Europe die of toxoplasmosis annually (Jones *et al.,* 2001).

2.7.1 Congenital transmission of toxoplasmosis

Congenital toxoplasmosis is documented in human and mice (Pfaff *et al.*, 2007).Vertical transfer of *T. gondii* in human was first proved by Wolf *et al.,* (1939). During parasitemia, parasite from maternal circulation may cross the foetal placenta barrier and proliferate within foetal tissues (Olgica *et al.*, 2006).In large animals and white-tailed deer, congenital toxoplasmosis has also been described (Dubey, 2008). Congenital

toxoplasmosis occurs mainly in infected women with the higher risk of 66.90% during the third trimester (Desmonts & Couvreu, 1994; Remington, *et al.*, 2006) and can lead into severe systemic illness (Dubey, 1977). The life-time risk of acquiring *T. gondii* infection has been calculated to range between 6.4 to 80 per 100 000 population

2.7.2 Horizontal transmission of toxoplasmosis

Horizontal transmission of *T. gondii* may occur in the following ways

2.7.2.1 Feeding (Carnivorism)

Persistence of parasite in tissues of animals (wildlife and domestic) is a key factor for transmission to human. Individuals can be infected by consumption of raw, cured or undercooked meat especially lamb, pork or game meat and acquire the parasite through persisting bradyzoites in the body tissues (Jones *et al.*, 2009; Dubey *et al.*, 2012) (figure 2.2)

2.7.2.2 Faecal-oral route

Toxoplasma shed by felids, mainly cats are the major source of environmental contamination (Dubey, 2004). Cats shed millions of unsporulated oocysts 3-10 days after ingestion of infective parasite. Upon shedding, sporulation of oocyst occurs in 3-5 days (Dubey, 2004). Sporulated oocysts are considered the major source of infection to both human and animals. These oocysts can remain stable in the environment for as long as a year (Dubey, 1998) and contaminate soil, water, vegetable and fruits in the environment posing risks of human and animal infections (figure 2.2)

2.7.3 Other routes of toxoplasmosis transmission

The disease can also be acquired through blood transfusion and during transplantation of heart, kidney, liver and bone marrow (Scheffner, 2001). Flegr *et al., (*2014) suggested

sexual transmission of the disease from infected men to non- infected women during unprotected sex. The transmission mode for *Toxoplasma gondii* is hereby illustrated in figure 2.2. .

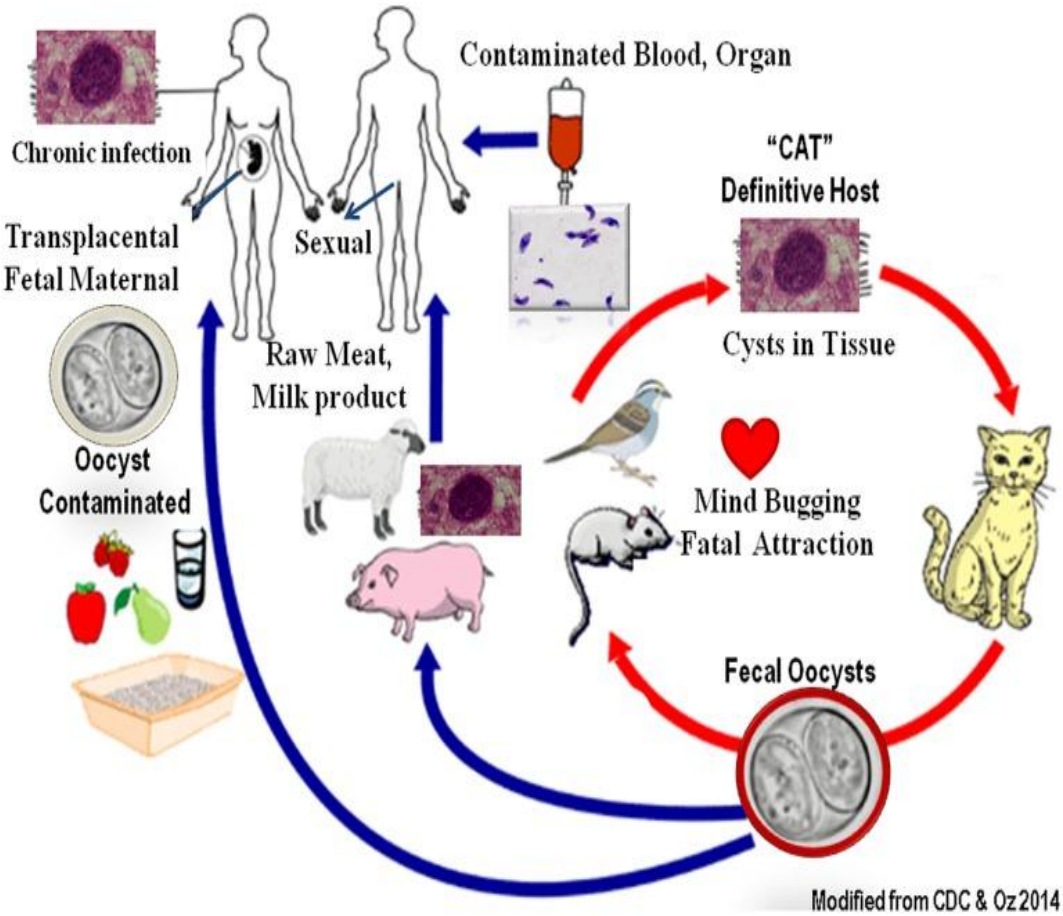

Figure 2.2:*Toxoplasma gondii* transmission model (CDC & OZ, 2014).

2.8 Importance of toxoplasmosis in humans and animals.

2.8.1 In animals

Toxoplasmosis is an economically important disease in animal husbandry, it causes reproductive failure by leading to early embryonic death and resorption, fetal death and mummification (Dubey, 2009), abortion, stillbirths, and neonatal death in small ruminants (Dubey, 2010). The severity of infection is associated with the stage of

gestation at which the ewe becomes infected; the earlier in gestation, the more severe the consequences (Dubey, 2009).

The reported seropositve toxoplasmosis in cattle ranges from 2 to 95%, higher infection by *T. gondii* is observed in calves during their first grazing season. This indicates that pasture is the main source of exposure of calves to *Toxoplasma* (Tenter *et al.*, 2000). Due to variation in production systems; *Toxoplasma* infection has been reported up to 100% prevalence in poultry (Dubey, 2010). Sixty-five percent of *T. gondii* infections were recorded in sheep in Southern Europe and the variation of 4 to 77% prevalence in goats (Tenter *et al*, 2000; Dubey, 2011). In wild animals host species, more than 350 mammals and birds have been described to have *Toxoplasma* infection (Lindsay & Dubey, 2009). Pro-siminas of Madagasca and Marsupials of Australia are the highly susceptible species to *T. gondii* infection (Bermundez *et al*, 2009; Sos *et al*, 2012).

Losses due to toxoplasmosis in animal husbandry vary within and between countries. For example, an estimated annual losses in Uruguay due to toxoplasmosis in ewes during gestation to be 1.4 to 3.9% of the ewes investigated (n=1613), amounting to approximately US$ 1.4 to 1.7 million (Freyre *et al*., 1997). The economic losses due to lamb mortality and missed lactation are estimated at 10 million Euros per year in Italy (Masala *et al.*, 2003). The estimated toxoplasmosis cost to the sheep industry of UK was between £12 million and £24 million each year (http://www.apd.rdg.ac.uk/AgEcon/livestockdisease/index.htm). Overall, toxoplasmosis results in increased production costs, diminished marketability of meat, fewer replacement animals, retardation of genetic progress and a major source of human infection (Freyre *et al.*, 1997).

2.8.2 In humans

The estimated chronic infection due to toxoplasmosis is 1/3 of human population worldwide (Tenter *et al.*, 2000; Dubey, 2004, 2010). In countries like France, where consumption of raw meat is popular, the prevalence is high. Recently, toxoplasmosis is recognized as an emerging food-borne parasitic disease (Dorny *et al.*, 2009) responsible for an estimated 20.7% of food borne deaths due to known infectious agents (Olgica, 2006). However, the disease is still seen as a neglected and underreported disease in many parts of the world, despite having a disease burden similar to that of salmonellosis and campylobacteriosis (Kijlstra & Jongert, 2008a). Toxoplasmosis is estimated to be the third leading cause of food-related deaths in the USA, behind salmonellosis and listeriosis (Mead *et al.*, 1999).

In the public health sector foetal loss from abortions and deaths and the expenditure of large sums of money on health care and special education of children born every year with congenital infection makes toxoplasmosis a disease of economic importance (Frenkel, 1973). Despite the low incidence, the economic impact of congenital toxoplasmosis is high and is estimated up to US$ 1.26 million per case, mainly due to medical costs, annual productivity losses, special education and residential care costs (Roberts *et al.*, 1994). It has been estimated that the U.S society bears an annual burden amounting $400 million to $8.8 billion in caring for affected children (Marquardt *et al.*, 2000). On the other hand Buzby & Roberts (1996) estimated the annual economic impact of toxoplasmosis in the human population of the United States to be about $7.7 billion. In Australia, human toxoplasmosis is estimated to cost $1 billion/year (ACIAR, 2007).

Toxoplasmosis may cause schizophrenia and affect intelligence and personality traits of people (McAllister, 2005), possibly from similar mechanisms that result in altered behaviour of infected mice. These conditions reduce the quality of life and may cause significant economic losses (McAllister, 2005). The costs associated with acquired toxoplasmosis have never been addressed, despite the fact that up to 20% of infected individuals may develop clinical complications, probably leading to one or more days of work missed in mild cases (AFSSA, 2005).

2.9 Prevention and Control of toxoplasmosis

Management of toxoplasmosis is dependent on the backgrounds and the disease settings in the host (Robert-Gangneux & Darde, 2012). Currently there is no human vaccine for toxoplasmosis but in animals, sheep and cat vaccines have been developed. Vaccinating sheep with a live 548 strain of *T. gondii* (Tocovax®) has shown reduction of tissue oocyts development (Garcia *et al.,* 2014). Another live vaccine which is a mutant strain T263 of the parasite has also been used to reduce oocyst shedding in cats (CDC, 2014).

The most effective approach of managing toxoplasmosis in both animal and human is through limiting of exposure to oocysts (Stacey *et al.,* 2010). To minimize exposure, cats should not be fed uncooked meat, viscera, and needs to be kept indoors to prevent hunting. Dead animals should promptly be removed to prevent cannibalism by pigs and other scavenging animals. Foetal membranes and dead foetuses should not be handled with bare hands and also must be buried to avoid infection of other felids and animals. Cats need not to be allowed near pregnant animals and humans. Practices to reduce exposure to parasite in humans includes wearing gloves when gardening or being in contact with soil, changing litter box followed by hand washing with soap, washing fruits and vegetables eaten raw, washing kitchen utensils with soap, freezing, irradiation,

and cooking meat to appropriate temperature (65°C) to kill the parasite and avoid eating undercooked meat (Dubey *et al.,* 1990). Giving health education on meat, soil, and faecal related toxoplasmosis can prevent the exposure to parasite and hence reduce the prevalence of the disease (Lopez *et al.,* 2000). Prevention of infection in new-born can be achieved by serological screening for *T. gondii* in pregnant women in the beginning of pregnancy. Prenatal diagnosis of Toxoplasma was proved beneficial to reduce congenital infection due to toxoplasmosis (Georgiev, 1994).

There are different drug regimen developed for treatment of *Toxoplasma* infections including pyrimethamine, sulfadiazine, folinate, sulfadoxine and spiramycine. Sulfonamides and pyrimethamine (Daraprim®) are two drugs widely used for therapy of toxoplasmosis, the two drugs works synegically by blocking metabolic pathway involving p-Aminobenzoic acid and folic-folinic acid cycle respectively. Immediate therapy with spiramycine before 16th week of gestation and with pyrimethamine plus sulfadiazine after 15thweek of gestation can be used in managing disease in pregnant women (Stacey *et al*, 2010). However, due to drug toxicity in foetus; the drug regimen for managing toxoplasmosis should be scrutinized before being given to pregnant women. Prophylaxis therapy by the above mentioned drugs was proved to reduce incidence of toxoplasmosis in Australia from 50-70% to 1% in 10,000 births (Aspock & Pollack, 1992)

For animals, treatment is seldom warranted. Sulfadiazine (15–25 mg/kg) and pyrimethamine (0.44 mg/kg) act synergistically and are widely used for treatment of toxoplasmosis. Although these drugs are beneficial if given in the acute stage of the disease when there is active multiplication of the parasite, they will not usually eradicate infection. These drugs are believed to have little effect on the bradyzoite stage. Certain

other drugs, including diaminodiphenylsulfone, atovaquone, and spiramycin are also used to treat toxoplasmosis in difficult cases. Clindamycin is the treatment of choice for dogs and cats, at 10–40 mg/kg and 25–50 mg/kg, respectively, for 14–21 days (Dubey, 2013).

2.10 General knowledge level and awareness of pregnant women on toxoplasmosis

Awareness about toxoplasmosis and its transmission can help reduce its prevalence by simple precautions of pregnant women. The level of human exposure to *T. gondii* is mostly influenced by cultural factors and eating habits, such as the consumption of raw or undercooked meat and contaminated herbs and vegetables, the type of meat that is most popular in a particular country, the rate of cat ownership, drinking unfiltered and untreated water, and living in rural areas (Al-Sheyab *et al.*, 2015). *Toxoplasma* exposure during pregnancy is a potential threat to the foetus and can be avoided if pregnant women are aware of the potential sources of infection, mainly contaminated food, water, soil and cat faeces. Literature suggests that pregnant women are not always adequately informed about preventable infectious congenital diseases and most pregnant women have a low level of knowledge regarding these topics (Pereboom *et al.*, 2013). Unlike observation study from Netherlands which recorded high knowledge 75.3% (Pereboom *et al.*, 2013), on average most of the studies have shown lack of awareness of pregnant women on toxoplasmosis, its risk factors, symptoms, and timing of infection, and preventive practices (Ferguson *et al*, 2011; Andiappan *et al.*, 2014; Al-Sheyab *et al.*, 2015; Angesom *et al.*, 2015; Chandrasena *et al.*, 2016). Studies suggest that most women are uneducated about the risks posed by *Toxoplasma gondii* exposure during pregnancy and that there is a clear need for better educational programmes regarding primary prevention of congenital toxoplasmosis if neonatal infection is to be avoided (Ferguson *et al.*, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and the design

A cross-sectional study was conducted in health care facilities in Temeke municipality in Dar es Salaam, Tanzania between January and May 2016.

Temeke district is one of the three districts of Dar es Salaam region. The district has an area of 786.5 km^2, and borders the Indian Ocean to the east, to the north there is Ilala district, south and west of Temeke district is the coastal region. According to 2012 Tanzania population and housing census, Temeke district has a population of approximately 1,368,881 (NBS Tanzania, 2012)

The study was conducted in six health facilities in Temeke municipality with Reproduction and Child Health (RCH) clinics namely Rangi Tatu hospital, Round Table maternity home, Tabukareli dispensary, Kibugumo dispensary, Vijibweni hospital and Kigamboni health centre. Figure3.1 shows the map of Temeke municipality and location of the wards where the study was conducted.

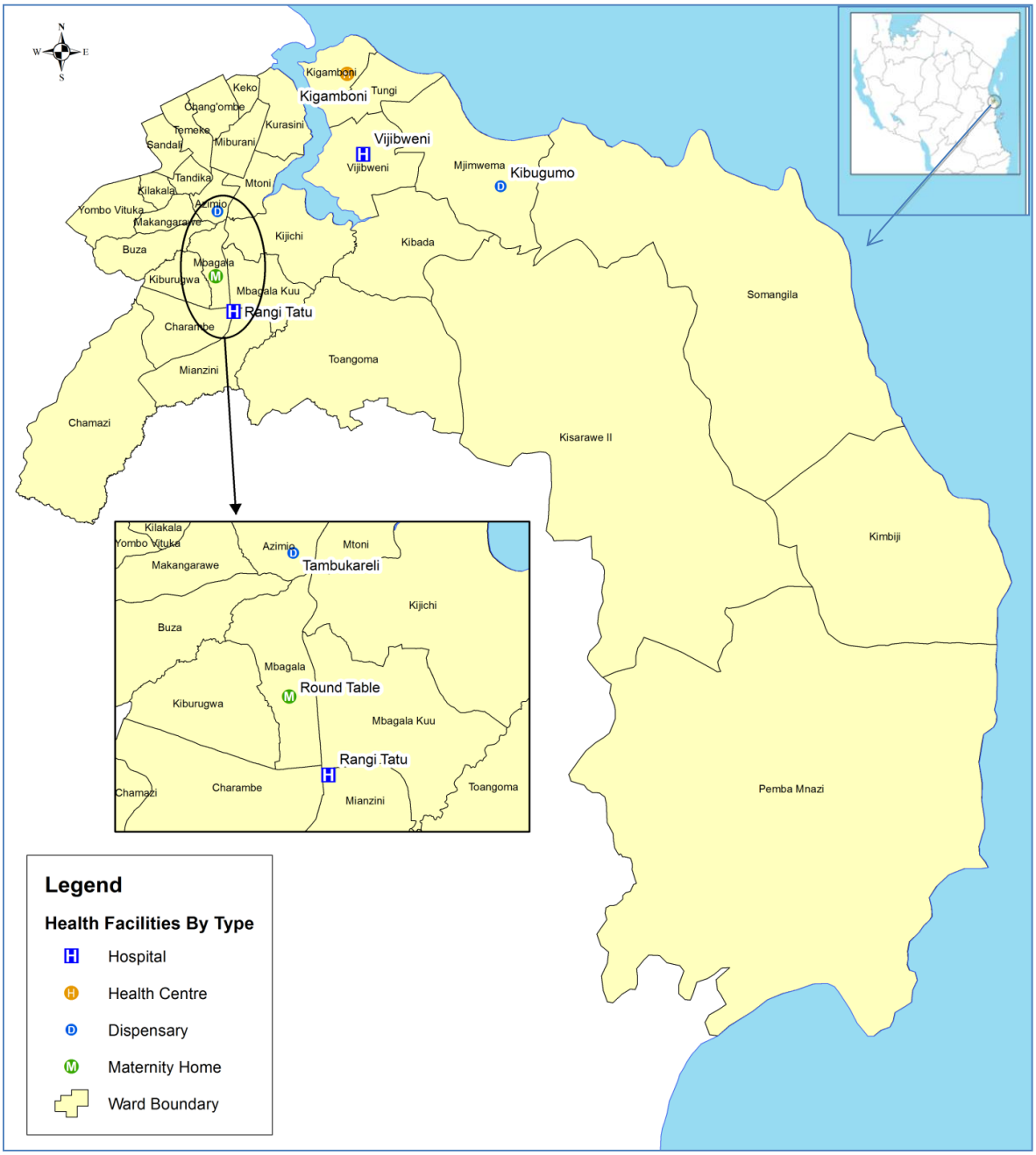

Figure 3.1: Map of Tanzania (top) locating Dar es Salaam region where Temeke municipality. From the map of Temeke municipality, the wards and hospital locations of study sites are shown (Evod, 2016)

3.2 Sample population and respondents

This study involved both health workers providing health care services in the Reproduction and Child Health (RCH) clinics and pregnant women attending those RCH clinics in Temeke Dar es Salaam.

Stratified sampling was used to select wards from among the twenty three wards in Temeke municipality based on the population distribution and where they were either urban or peri-urban settings of Temeke municipality. Strata represented twelve wards from the urban areas and eleven wards from peri-urban areas of Temeke. From the urban area random selection of three wards was done. The selected wards were Mbagala, Mbagala Kuuand Azimio. From the peri-urban areas proximal to the sea (less densely populated) two wards were selected which were Kigamboni and Vijibweni. From each ward, at least one clinic which serves a large number of pregnant women waspurposively chosen (Table 3.1)

Table 3.1: Health facilities selected from the stratified locations

Strata	Name of the ward	Health facility
Urban	Mbagala	Tambukareli Dispensary
	Mbagala kuu	Rangi tatu hospital
	Azimio	Round Table Maternity Home
Peri-urban	Kigamboni	Kigamboni health centre
	Vijibweni	Vijibweni hospital
		Kibugumo dispensary

There were no special criteria for inclusion of the pregnant women; all who attended a clinic on the day of survey had the same chances of being included. To get pregnant women (cases) from among those who attended healthcare in the RCH/MCH clinics, convenience sampling was used by first selecting participant who first came in a queue to get the health service, followed by every other person as per health service provision arrangement used i.e. first come first served. The process was repeated for each visit to the health facility until the desired number (at least 64) of respondents from each clinic was reached. Pregnant women were informed about the study and its aims; consent form (Appendix 2) was signed by those who consented to participate in the study before answering the study questions. Similarly, health workers for this study were purposively obtained among those who attended to pregnant women and present on the day of data collection. The questionnaire was administered by the interviewer and all responses were verbatim.

3.3 Sample size calculation

To get the number of participants, the WHO formula below for Sample Size Determination in Health Studies was used for sample size calculation (Lwanga & Lemeshow, 1991). Actual surveyed participants were 371 pregnant women and 22 health workers (393 participants)

$N = (Z^2_{\alpha/2} * P * (1-P) * D)/E^2$

N = number of respondents (sample population).

P = Expected knowledge level of the disease among pregnant women in Tanzania.

$Z_{\alpha/2}$ is a z-value corresponding to the 5% level of significance.

D is a design effect which = 1, since the random sampling wasto be used.

E is a margin of error (precision), taken as 0.05.Therefore the minimum sample size of 384 was obtained as follows;

N = (1.962 *0.5 (0.5)*1)/ (0.05)2= N~384.

During the study, the actual number of participants surveyed became 393 which was proportionally shared between two categories of study participants. The sample proportion of participants included 94% pregnant women and 5.6% healthcare workers.

3.4 Tools for data collection

The data for this study were collected by structured questionnaire and the review of prenatal screening forms/antenatal clinic cards containing the clinical records of the pregnant women. There were two sets of pretested questionnaires developed for each group of participants, the one for pregnant women was translated in Swahili from English (Appendix 3) and the other for health workers developed and administered in English (Appendix 4). Closed and open structured questionnaires were used to gather information concerning toxoplasmosis awareness and practice in the area. Questions focused on the disease aetiology, signs and symptoms, modes of transmissions, treatment and management. The population characteristics of pregnant women included age, economic activity, education level, marital status, gestational age, abortion history and gravidae.

3.5 Data analysis

Descriptive statistics and logistic regression analysis were computed using STATA version 12 (StataCorp LP, College Station USA).

Population characteristics such as age, education, occupation, history of abortion, gestation age, gravidae and marital status were described as counts and percentages.

Awareness towards the toxoplasmosis was described according to the participant's responses to the questions. The association between demographic data (outcome variables) and risk practices (response variable) of the respondents towards toxoplasmosis was measured using chi-squared test and logistic regression model (bivariate and multivariate). The response variable defining "risk practices" was developed depending on the participant's response on practising either one or more of the risk factors leading to exposure to *Toxoplasma* such as eating raw, cured or rare meat, drinking untreated water, consumption of raw fruit or vegetable, keeping cat and handling of soil. The response variable was coded 0 when the risk practices of pregnant women were below 20% and 1 when the practices of pregnant women were above 20% of all the practices leading to exposure to toxoplasmosis covered by the questionnaires. To adjust for confounding and determine the effect of modification and simultaneous relationships of risk practices to age, abortion history, gravidae, education and marital status, multivariate logistic model was fitted using the variables that had a p-value <0.06 at univariate analysis. During the analysis of health workers data, Maternal and Child Health assistants were considered as nurses because they perform the same role as far as RCH/MCH clinic services are concerned and the sonographer was considered technician along with laboratory technicians

3.6 Ethical Considerations

Ethical clearance to conduct the study was granted by the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol. IX/2127; Appendix 5). The permission from Temeke municipal Research committee was granted for the research to be done in the health facilities under Temeke Municipal council. Voluntary participation consent was sought from each participant by signing the participant informed consent form after the study had been elaborated to them.

CHAPTER FOUR

RESULTS

4.1 Descriptive results

A total of 22 health workers and 371 pregnant women from six health facilities in Temeke Municipality participated in the study. The distribution of pregnant women and health workers varied from one health centre to another. The ages of pregnant women ranged from 12 to 52 years and the mean age was 26.9 years. Participants younger than 19 years accounted for 11% (95%, C.I 7.4-13.6%). The large age group were those in years between 19 and 25 42% (95%, C.I 37-47%), 33% (95%, C.I 28.6-38.4%) of pregnant women were in the age between 26-35 years and those above 35 years were 14% (955, C.I 10.5-17.5%). Majority of pregnant women interviewed were from Round table maternity home in urban area 23.9% (n=89; 95%, C.I 17.4–25.8%), the least representation of pregnant women was from Kibugumo dispensary in peri-urban area 2.4% (n=9; 95%, C.I0.9–3.9%), (Table 4.1).

Table 4.1: Variation in distribution of study participants (pregnant women) from different health facilities (N =371)

Health facility	Health facility type	n	(%)	95% Conf. Interval
Kibugumo	Dispensary	9	2.4%	0.9 - 3.9%
Kigamboni	Health Centre	59	15.9%	14.6 - 24.9%
Rangi tatu	Hospital	77	20.8%	19.6 - 28.3%
Round table	Maternity home	89	23.9%	17.4 - 25.8%
Tambukareli	Dispensary	80	21.7%	11.7 - 19.1%
Vijibweni	Hospital	57	15.3%	12.2 - 19.6%

Half the number of pregnant women who attended the surveyed hospitals were housewives 56.1% (95%, C.I 50.9-61.1%) and 43.9% (95%, C.I 38.8-49.0%) were either employed or involved in small businesses. Occupation was found to have a significant influence on the type of health facility which one attended, (X^2=10.4, P<0.01). Cross tabulations of the type of health facility and occupation showed that business women and those employed were mostly attracted to the dispensaries than hospitals, whilst house wives were more likely to visit hospitals (Table 4.2)

Table 4.2: Type of health facility visited by pregnant women according to their occupation

The type of Health facility attended/Occupation	House wives		Business/employed		Total	
	n	%	n	%	N	%
Hospitals	81	60.5	53	39.5	134	100%
Health centre	34	57.6	25	42.37	59	100%
Dispensary	56	62.9	33	37.1	89	100%
Maternity Home	37	41.6	52	58.4	89	100%

Many of the pregnant women were multigravidae, they had experienced pregnancy more than once 67% (95%, C.I 62.0-71.6%) while the remaining 33% (95%, C.I 28.3-37.9%) were primigravidae. The gravidity of pregnant women varied significantly by age among primigravidae and multigravidae women (X^2 =124, *P<0.0001)*. As the age increases, the pregnant women become multigravidae. At the age below 25 years, most of pregnant women were primigravidae while at the age above 26 years majority were multigravidae (figure 4.1).

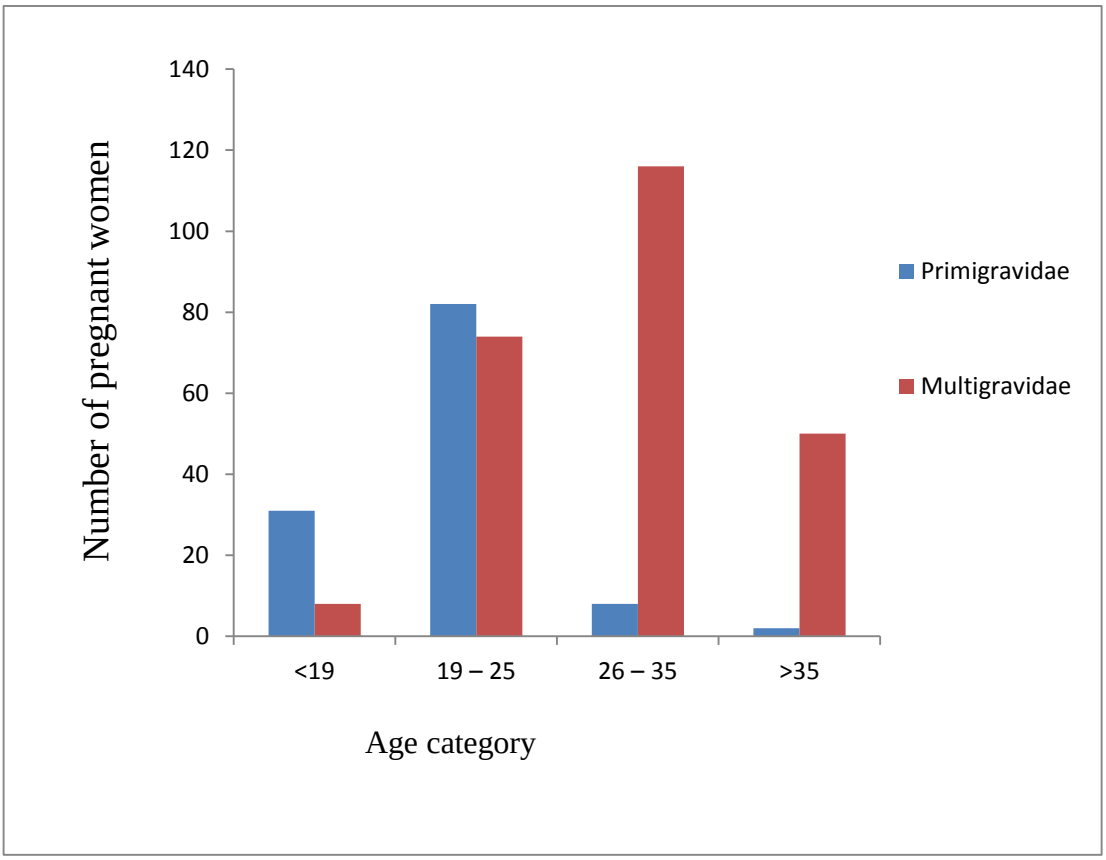

Figure 4.1: Gravidity of pregnant women by age groups.

The majority of the pregnant women had primary education 64% (95%, C.I 58.7-68.5%), 25% (95%, C.I 20.9-29.8%) attended secondary education, 4% (95%, C.I 2.0-6.1%) went through college/university and those who did not attend any formal schooling were 7% (95%, C.I 4.4-9.6%). Most of the pregnant women interviewed 80% (95%, C.I 75.4-83.6%) were married and living with their spouses, this included those cohabiting and living together with partners. Mostly the respondents had no abortion history 77% (95%, C.I72.2-80.9%). The gestation age varied among the pregnant women in which 47% (95%, C.I 41.7-52.0%) were in their 3rd trimester while 39% (955, C.I 34.3-44.3%) were in 2nd trimester, the abortion was highly reported to occur in the second trimester (n=39, Table 4.3)

Table 4.3: The characteristics of the pregnant women (N =371) in the toxoplasmosis awareness study, Temeke, Dares Salaam

Factors	n	%	95%, Conf. Interval
Marital status			
Married	295	80.%	75.4-83.6%
single	51	14%	10.2-17.2%
Separated/divorced	25	6%	4.2-9.3%
Education			
primary	236	64%	58.7-68.5%
secondary	94	25%	20.9-29.8%
college/university	15	4%	2.0-6.1%
Did not attend school	26	7.00%	4.4-9.6%
Gestation age			
1st trimester	51	13.90%	10.2-17.3%
2nd trimester	146	39.40%	34.4-44.3%
3rd trimester	174	44.90%	41.8-52.0%
Abortion history			
yes	87	23.50%	19.1-27.8%
no	284	76.50%	72.2-80.9%

4.2 Awareness level of pregnant women regarding toxoplasmosis

Amongst the pregnant women surveyed, 95.6% (95%, C.I 93.6-97.7%) were unaware that there is a disease called toxoplasmosis i.e. they never heard, read or saw any information regarding toxoplasmosis. The 4.3% (95% C.I 2.2–6.3%) of pregnant women who had heard, read or seen information concerning toxoplasmosis were not aware of

the aetiology, signs and symptoms, modes of transmissions, treatment and managements of the disease.

Of the 16 pregnant women who were aware of the disease, multigravidae women showed high level of awareness 81% (95%, C.I 76.5-84.8%) compared to primigravidae. It was found that none of those who did not attend school had heard, seen, or read about toxoplasmosis. The general awareness level of pregnant women on the clinical manifestation of toxoplasmosis was 3.2% (95%, C.I 1.4-5.0%) for those who said it is associated with encephalitis and4.8% (95%, C.I 2.6-7.0%) for those who said the disease is causing retinitis and mental problems such as hydrocephalus, microcephalus and mental retardation in children. The responses of the pregnant women on the factors associated with toxoplasmosis are presented in Table 4.4, those who did not hear, read or seen any information about toxoplasmosis 96% (95%, C.I .93.6-97.7%) are excluded.

4.3 Practices of pregnant women towards toxoplasmosis

Only 7% (95%, C.I 4.3-9.6%) of pregnant women kept cats of which 5% (95%, C.I 3.1-7.6%) cleaned and changed cat litter. It was found that 90% (95%, C.I 86.6-92.8%) did not eat cured, rare and or raw meat. Similarly, 84% (95%, C.I 79.7–87.3%) thoroughly washed and cleaned fruits/vegetables before eating. Nearly half of pregnant women surveyed 48% (95%, C.I 42.7–52.9%) have been involved in farming/ gardening activities before. However, only 47% (95%, C.I 39.7–54.5%) covered their hands with gloves to avoid any possible exposure to *Toxoplasma* by handling the soil. Table 4.5 provide more details on the women practices.

Table 4.4: Responses of pregnant women on awareness of the factors associated with exposure to toxoplasmosis and its signs and symptoms (n=16)

Risk factors	Response category					
	Yes			No		
	n	%	95%, C.I	n	%	95%, C.I
Changing cat litter	6	37.5	10–64	10	62.5	36–89
Gardening without gloves	8	50%	23–77%	8	50%	23–77%
From pregnant women to foetus(congenital)	7	44%	16–71%	9	56%	28–83%
Eating raw cures/undercooked meat	6	37.5%	10–64%	10	62.5%	36–89%
Blood transfusion	7	44%	16–71%	9	56.3%	29–84%
Sexual intercourse	4	25%	12-49%	12	75%	51–99%
Eating raw fruits/ vegetables	2	12.5%	6–31%	14	87.5%	69–96%
Drinking untreated water	5	31.2%	9–51%	11	68.8%	58–92%
Symptoms & signs						
Swelling of the lymph nodes	4	25%	12-49%	12	75%	51–99%
Chorioretinitis and vision problems	6	37.5%	10–64%	10	62.5%	36–89%
Encephalitis	2	12.5%	6-31%	14	87.5%	69-96%
Hydrocephalus	2	12.5%	6–31%	14	87.5%	69-96%

Table 4.5: Practices of pregnant women towards toxoplasmosis (N =371)

Risk practices and behaviors	Yes		No	
	n(%)	95%, C.I	n(%)	95%, C.I
Have domestic cat(s) at home.	26 (7%)	4.3–9.6%	345 (93%)	90.3–95.6%
Involved in changing and cleaning cat litter.	20 (5.4%)	3.1–7.6%	351 (94.6%)	92.3–96.9%
Ever involved in farming or gardening activities.	177 (48%)	42.7–52.9%	194 (52%)	47.0–57.3%
Wear gloves when farming or gardening.	84 (47%)	39.7–54.5%	93 (53%)	45.4–60.2%
Routinely wash hands with soap after farming /gardening.	133 (75%)	68.2–81.2%	44 (45%)	32.8–49.7%
Do eat raw, cured or rare meat.	38 (10%)	7.1–13.3%	333 (90%)	86.6–92.8%
Eat raw fruits/vegetables.	69 (19%)	14.6–22.5%	302 (81%)	77.4–85.3%
Thoroughly wash and clean fruits/vegetables before eating.	310 (84%)	79.7–87.3%	61 (16%)	12.6-20.2%
Have received blood donation during pregnancy	37 (10)	6.9–13.0%	334 (90%)	86.9–93.0%
The source of water used at home				
Tap	250 (67%)	63.5–72.5%	121 (33%)	14.3-62.6%
well	100 (27%)	22.4–31.4%	271 (73%)	71.7-82.8%
Others	21 (6%)	3.5–8.1%	350 (94%)	93.1-99.4%
Do drink untreated water.	214 (58%)	52.6-63.7%	157 (42%)	37.2-47.3%

4.4 Relationship between demographic data and the practices of pregnant women associated with *Toxoplasma* infection.

Risk practices towards *Toxoplasma* were 0.4 times higher in pregnant women aged between 19-25 years as compared to those who were below 19 years old (OR=0.4, $p<0.01$). Association between practices towards toxoplasmosis and age of pregnant women was statistically significant (*p=0.002)*. For every increase in age by ten years the practices towards toxoplasmosis increased significantly 1.4 times OR=1.41 (95%, C.I 1.05-1.90). When examining link between gravidae and risk practices towards toxoplasmosis, multigravidae was statistically significant associated with the practices towards exposure to *Toxoplasma* (OR=2.65, $p<0.01$). In univariate analysis, education [OR=1.27 (95%, C.I 1.0-1.62)], marital status [OR=1.0 (95%, C.I 0.74-1.3)], gestation age [OR=0.94 (95%, C.I0.69-1.27)] and occupation [OR=0.81 (95%, C.I0.53-1.25)] were not significantly associated with practices towards *Toxoplasma* infection and therefore were excluded in multivariate analysis. When adjusted for education, marital status and gestation age, it was found that age (*p<0.01)* and gravidae OR=2.65 (95%, C.I 1.38-5.08) were statistically significant associated with risk practices towards toxoplasmosis (Table 4.6). Taking into account of the effect of confounders and simultaneous relationships; The practices of pregnant women towards toxoplasmosis for those in the age group between 19 and 25 years versus those aged below 19 years decreased from 0.4 to 0.26 times and odds ratio of age group between 26-35 years versus age below 19 years also decreased from 0.5 times to 0.2. The adjusted association of risk practices towards toxoplasmosis with multigravidae versus primigravidae increased from 1.94 to 2.65.

Table 4.6: Association of risk practices towards toxoplasmosis with the demographic data of pregnant women

Factors	CRUDE OR (95% C.I)	*p*-Value	ADJUSTED OR(95% C.I)	*p*-Value
Age groups				
19-25	0.40 (0.19 -0.82)	0.013	0.26 (0.11 - 0.59)	0.002
26-35	0.50 (0.24 -1.04)	0.064	0.22 (0.09 - 0.59)	0.002
age> 35	1.14 (0.49 - 2.61)	0.762	0.48 (0.17 - 1.34)	0.160
Abortion history				
Yes	1.28 (0.53-3.09)	0.590		
No abortion	0.61 (0.37-0.99)	0.047	0.67 (0.40-1.13)	0.140
Gravidity				
Multigravidae	1.94 (1.19 - 3.13)	0.007	2.65 (1.38 - 5.08)	0.003
Primigravidae	0.17 (0.07 - 0.41)	0.000		

4.5 Observation from the review of prenatal screening forms/prenatal clinical cards

From the review of prenatal screening forms of pregnant women surveyed, it was observed that pregnant women were not screened for toxoplasmosis. The only diseases screened were HIV/AIDS, syphilis and malaria. Pregnant women attending prenatal care in Temeke municipality were being given a combination of sulfadoxine-pyrimethamine (S-P) during their prenatal routine visits to healthcare facilities as intermittent preventive treatment of malaria in pregnancy (IPTp-SP) and also folic acid was provided to prevent anaemia. Sulfadoxine-pyrimethamine dosage was provided in the first trimester while folic acid was given in the early months of pregnancy as possible.

4.6 Awareness of health workers regarding toxoplasmosis

Of the 22 medical health workers, only 36% (95%, C.I 14.5-58.2%) were aware of the disease toxoplasmosis. Awareness of health workers on the risk of exposure to toxoplasmosis through food was low. Only few participants, one clinician, two technicians and one nurse could tell either eating raw, cured and undercooked meat (14%) or drinking untreated water and raw milk (9%) may lead to exposure to *Toxoplasma* parasite. Majority of health workers (95%) were unaware of the possible transmission of *Toxoplasma* through sexual intercourse and through consumption of unwashed and raw fruits or vegetables (86%). Of the 8 participants who knew about toxoplasmosis, all agreed that toxoplasmosis was a congenital disease, and seven health workers said toxoplasmosis could be transmitted through blood transfusion (Figure 4.2)

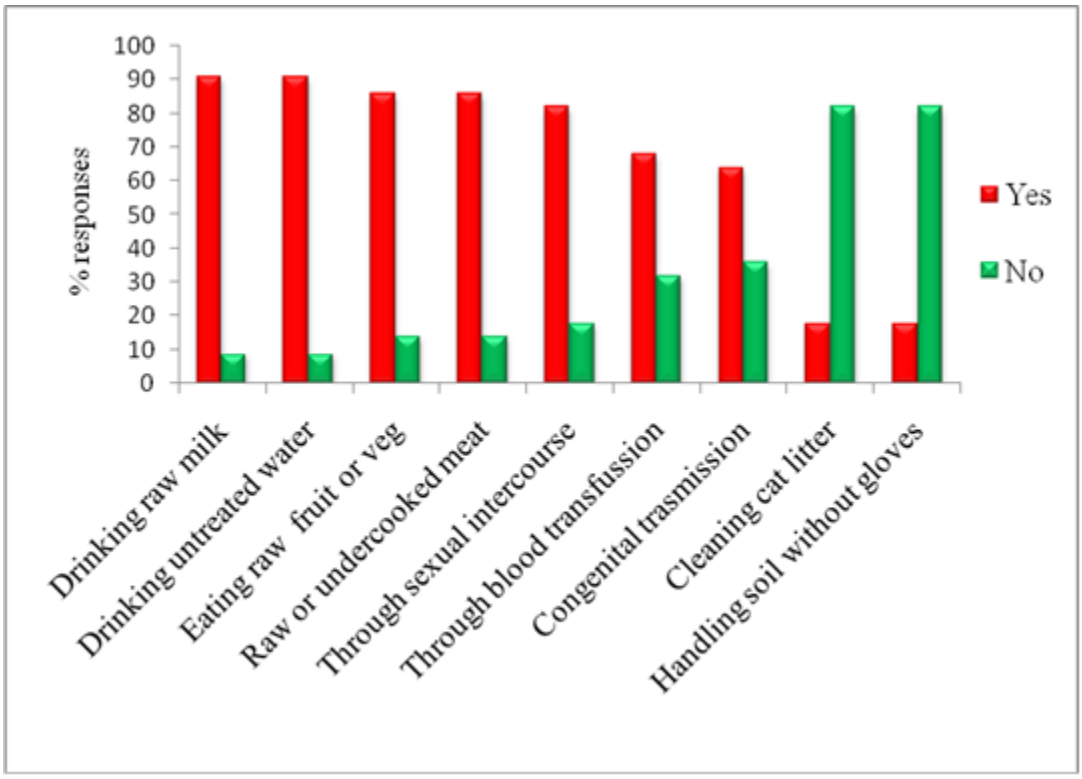

Figure 4.2: Percentages awareness of health workers on risk of exposure to *Toxoplasma* (n =22)

Awareness of health workers on the clinical manifestations of toxoplasmosis in pregnant women and newborn or children was assessed using the key ruling out factors, and the results are summarized in tables 4.7 and 4.8. The study found 64% (95%, C.I 41.8-85.4%) of the health workers were unaware of any clinical manifestation of toxoplasmosis in pregnant women, newborn or children. Twenty seven percent 27% (95%, C.I 7.1-47.5%) were aware of acute toxoplasmosis to cause both swollen lymph nodes or fever and flu in pregnant women, 32% (95%, C.I 10.6-52.9%) said toxoplasmosis could develop serious problems in pregnant women such as encephalitis and abortion and 23% (95%, C.I 3.7-41.7%) responded that toxoplasmosis may not show any symptoms in pregnant women. Table 4.7 is the illustration of health workers responses on the clinical manifestation of *Toxoplasma* in pregnant women.

Of the 22 health workers, 36% (95%, C.I 14.5-58.2%) ascertained that toxoplasmosis in newborn or children can develop serious complications and vision problems such as retinitis and blindness, 32% (95%, C.I 10.6-52.9%) of health workers agreed that a baby with toxoplasmosis can have no signs or symptoms at birth but develop illness later in life time and 23% (95%, C.I 3.7-41.7%)said toxoplasmosis in newborn and children can cause mental problems such as hydrocephalus, microcephalus, intracerebral calcification and mental retardation (Table 4.8).

Table 4.7: Awareness of health workers on the clinical manifestations of toxoplasmosis in pregnant women.

Clinical manifestation of toxoplasmosis in pregnant women	Yes (%)	95% C.I	No (%)	95% C.I	Don't know (%)	95% C.I
Pregnant women can develop serious problems due to toxoplasmosis	32	10.6 - 52.9	4	-4.9-13.9	64	41.8-85.5
Toxoplasmosis in pregnant women can cause fever and flu	27	7.1-47.5	9	-3.1-22.1	64	41.8-85.5
Toxoplasmosis in pregnant women can cause swollen glands(lymphedema)	27	7.1-47.5	9	-3.1-22.1	64	41.8-85.5
Toxoplasmosis in pregnant women can be asymptomatic	23	3.7-41.7	13	-1.9-29.2	64	41.8-85.5

Table 4.8: Awareness of health workers on clinical manifestations of toxoplasmosis in newborn & children.

Clinical manifestation of toxoplasmosis in children	Yes (%)	95% C.I	No (%)	95% C.I	Don't know (%)	95% C.I
Unborn and or newborn children can develop serious complications due to toxoplasmosis	36	14.5-58.2	0	00.0	64	41.8-85.5
A baby with *Toxoplasma* infection can have no signs of illness at birth but develop illness later	32	10.6-52.9	4	-4.9-13.9	64	41.8-85.5
A baby with toxoplasmosis can have vision problems	36	14.5-58.2	0	00.0	64	41.8-85.5
A baby with toxoplasmosis can have mental problems	23	3.7-41.7	13	-1.9-29.2	64	41.8-85.5

CHAPTER FIVE

DISCUSSION AND LIMITATIONS

5.1 Discussion

The assessment of awareness of congenital toxoplasmosis revealed low level of awareness among health workers and pregnant women attending health care facilities in Temeke municipality, Dar es Salaam, Tanzania. According to questionnaire survey results only 4% of pregnant women (95% C.I 2.2-6.3%) and 36% (95%, C.I 14-58%) of health workers were aware of congenital toxoplasmosis in the study area. Pregnant women who were aware of toxoplasmosis didn't have any idea on the aetiology, signs and symptoms of disease i.e. hydrocephaly in children, intracranial calcification, mental retardation and chorioretinitis, mode of transmission, treatment and management of the disease. Most of the pregnant women unknowingly had preventive practices that might limit their exposure to infection due to *Toxoplasma*, such practices and behaviours were avoiding eating raw meat, limiting exposure to cat faeces by not keeping cats, thoroughly washing and cleaning of fruits/vegetables before eating. Even though practices of pregnant women in Temeke are preventive towards the disease, majority of pregnant women drink untreated water from poor sanitary sources which can still put them at a risk of *Toxoplasma* infection.

Of the 371 pregnant women surveyed from Temeke, 96% (95%, C.I 93.6-97.7%) were not aware of toxoplasmosis and its associated clinical outcomes, had never heard, seen or read any information concerning toxoplasmosis. On the contrary, it is likely that pregnant women may have unknowingly experienced the signs or symptoms of the disease without associating toxoplasmosis with those signs and symptoms. For example pregnant women said they have had flu like symptoms and lymph oedema in their gestation period, but they did not know if toxoplasmosis can also present with the same

symptoms. The low awareness of pregnant women in Temeke municipality may be attributed to different factors, one being the lack of common indigenous name (Swahili) used to describe toxoplasmosis in Tanzania. Low level of education also is another factor for low awareness among pregnant women. Most of the pregnant women interviewed63.6% (95%, C.I 58.7-68.5%) had a low education (basic primary education and all pregnant women who did not attend any school 7% (95%, C.I 4.3-9.6), were not aware of toxoplasmosis.

It was observed that toxoplasmosis is not the disease of priority for prenatal screening in Tanzania, a fact which may contribute to low awareness. These findings of low level awareness agree with Angesom (2015) who recorded low level of knowledge (5.8%) among pregnant women in central Afar region in Ethiopia. Angesom (2015) suggested that majority of the community were living a pastoral life, therefore the reason for the low awareness about the disease was illiteracy. Other factors for low awareness in Ethiopia according to Angesom (2015) were the lack of access to media and lack of attention given to the disease in the health centres due to lack of awareness and diagnostic facilities. On the other hand, low knowledge of toxoplasmosis was reported by previous study in Malaysia, Philippines and Thailand, about 11% of the interviewed pregnant women had knowledge about toxoplasmosis (Andiappan *et al.*, 2014). Education has been shown to significantly increase awareness of toxoplasmosis by 80% (Dabritz & Conrad, 2010)

Congenital infection caused by transplacental transmission of *Toxoplasma* infection can lead to a variety of manifestations in the foetus including spontaneous abortion, still-birth, and also an infected newborn may present classic signs of congenial toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcifications and

retinochoroiditis (Berger *et al.*, 2009).Despite the disease being congenially transmitted from pregnant women to their foetus or newborn with a prevalence rate of more than 60% in adults in Tanga Tanzania (Sawai & Schooman, 2009), all pregnant women were unaware of the modes of *Toxoplasma* transmission and only 36% (95%, C.I 14.5-58.2) health workers had the understanding that the disease can be transmitted congenitally. Maternal and child health care in Tanzania does not consider toxoplasmosis important for prenatal screening done for other congenital diseases like HIV/AIDS, syphilis and malaria. The consequences due to lack of toxoplasmosis screening and infection in children in Tanzania remains uncertain. Majority of women tend to be aware of the diseases that they are usually screened for when they become pregnant, however none of the interviewed pregnant women reported to have been tested for the disease. By implication, lack of screening services for *Toxoplasma* in the country may contribute to lack of awareness and knowledge for toxoplasmosis. Since the disease is not given any attention in the Country's health sector and that health workers reported lack of modern tools for routine diagnosis, it is likely that most pregnant women and health workers must have low awareness of the disease. Most of the participants (both pregnant women and health workers) did not know the risks of exposure to Toxoplasma. The lack of awareness of toxoplasmosis was not associated with the practices of pregnant women towards the disease. Practices of pregnant women interviewed in Temeke municipality were positive towards the prevention of disease. This is articulated by some reasons; firstly, 90% (95%, C.I 87–92%) of the pregnant women did not eat raw, cured or rare meat, a factor which account for most of human *Toxoplasma* exposure (Cook *et al.*, 2000). Similar results were obtained by Andiappan *et al.*, (2014) in which only 12.2% pregnant women from Malaysia ate raw or undercooked meat. More than 80% of pregnant women in Temeke washed their hands after gardening/farming, changing cat

litter and after handling raw meat. Secondly, only 19% (95%,C.I 15–23%) of them ate raw fruits and vegetable and 84% (95%, C.I 79-87%)thoroughly washed and cleaned fruits/vegetables before eating. Thirdly, the respondents who kept cats and could be exposed as a result of contamination of food and environment by *Toxoplasma* oocysts in cat faeces were few (only seven percent, 95%, C.I 4–9%) of which 5% cleaned and changed cat litter. Even though some studies (Dubey, 2010) has associated keeping cat with the increased risk of toxoplasmosis, human infection is mostly derived from exposure to *Toxoplasma* oocysts which are shed in cat faeces. Cats usually shed oocyst for few weeks during their life time and this occurs14 days post infection, however cats who are kept indoors and do not hunt for food are unlikely to be infected to shed oocyst in faeces.

The resulting positive preventive practices towards toxoplasmosis among pregnant women in Temeke municipality may be due to the urban lifestyle which promotes good hygienic practices through improved sanitation and standards of living unlike the rural life. Usually eating raw or undercooked meat is a characteristic of pastoralists and hunters who did not participate in this study. Most of the pregnant women were housewives 56 % (95%, C.I 50–61%) and others 44% (95%, C.I 39–49%) doing business or employed. These practices of pregnant women in Temeke may put them at a minimal risk of exposure to toxoplasmosis. However, those practices are not sufficient for limiting exposure to *T. gondii.* This is because, while the majority don't eat raw/undercooked meat, they drink untreated water which may harbour the parasite oocyst. Furthermore the level of presence of *Toxoplasma* in food animals and rodents has not been established. In this case it does not mean *T. gondii* is absent in Tanzania and Temeke municipality in particular.

The current study established the relationship between age and gravidae of pregnant women with risk practices towards *Toxoplasma* infection. Comparably, risk practices of pregnant women aged above 19 years are higher towards toxoplasmosis than those below 19. For every ten years increase in age of the pregnant women, the practices towards toxoplasmosis increased by 41%. These outcomes are supported by a study by Jones *et al.,* (2001) who found the risk of toxoplasmosis to increase at 30 years. The reasons for the increase of risk of exposure to *T. gondii* by age are still unclear. Previous studies suggest that age is associated with lack of knowledge and awareness; hence the negative practices towards toxoplasmosis (Andiappan *et al.*, 2014). In the current study, gravidae was associated with the risk of exposure to toxoplasmosis; pregnant women who were multigravidae were1.93 times higher at risk of exposure to *Toxoplasma* compared to primigravidae. The odds of clinical toxoplasmosis in multigravidae women is either due to unclassified factors such as stress and the effects of female hormones on immunity during gestation (Dubey, 2008)

Although the disease can be transmitted through blood transfusion and organ transplants, in Tanzania, Temeke municipal in particular, blood collected for donation is not screened for toxoplasmosis, perhaps because of underestimated prevalence and cost-effectiveness which limits the possibility and rationale to perform *Toxoplasma* screening of donated blood. High prevalence of *T. gondii* antibodies among blood donors in Dar es Salaam was reported in Tanzania Eastern zone blood bank Lema *et al.,* (2012). Lema *et al.,* (2012) indicated that toxoplasmosis could be a potential hazard for blood transfusion and highlighted the importance of pre-transfusion screening for *T. gondii* among blood donors along with currently scheduled pre-transfusion testing of HIV, Hepatitis B, hepatitis C and Syphilis. Of the pregnant women who participated in this study, 90% (95%, C.I 86.9-93.1%) did not receive blood donation during their gestational period a

fact which keeps them at low risk of exposure to *Toxoplasma* by blood transfusion in their pregnancy period.

In the study area, 58% (95%, C.I 52–63%) of pregnant women drink untreated water which if contaminated with *Toxoplasma* oocysts may serve as a potential source of infection. This is because the main source of water used by the subjects is tapped 67% (95%, C.I 63–72%) and wells 27% (95%, 22–31%). Unlike water supply from the taps which receive treatment though poor and inadequate, most people do not treat water from wells in Tanzania. Water supply system in Temeke municipal is not adequate, poor water sanitation and treatment infrastructure constraints challenges the availability and access to safe and clean water for the communities.

Similar to pregnant women, health workers in Temeke municipality reported unexpected low level of awareness towards toxoplasmosis; only eight 36% (95%, C.I 14.5-58.2%) out of 22 participants knew the disease. This is in agreement with Angesom (2015) who reported only 33.6% (n=68) of health workers who had knowledge of toxoplasmosis. In the current study laboratory technicians and clinicians knew more about toxoplasmosis than nurses, all five technicians and three clinicians knew the disease. The difference in awareness level among health workers may be due to educational level and the profession, all health workers who reported to be aware of toxoplasmosis studied about disease in their medical schools. It was found that most of nurses working in health facilities in Temeke municipality were enrolled nurses who received only basic training in nursing compared to clinicians and laboratory technicians who had a tertiary medical education. It was found that due to shortage of health workers, the only nurse who was aware of the disease serves both as a nurse and laboratory technician in the MCH clinic at Kibugumo dispensary. In Temeke municipality, MCH/RCH clinic health staff

comprises Clinician/medical officer, laboratory technicians and Nurses or MCH/RCH assistants.

Nurses were the dominant health staff in Temeke MCH/RCH clinics and they spent more time with pregnant women than clinicians and laboratory staff. However they displayed an extremely low awareness level towards toxoplasmosis. This could impede the effectiveness of the prenatal care provided in the RCH/MCH clinics in Temeke. Of the 8 health workers who were aware, 62.5% (N=5) knew that toxoplasmosis was a disease of both animals and humans (zoonotic) and could be passed from pregnant mother to the foetus/newborn. These were two clinicians and three technicians. Furthermore, of those who were aware of the disease (36%), all affirmed that the disease could cause serious complications such as eye and mental problems in the foetus and newborn. None of the health workers in the current study had diagnosed the disease in their entire clinical practices. The reasons for not diagnosing the disease were said to be lack of both cases and diagnostic tools to facilitate screening for toxoplasmosis. Pursuant to that, lack of routine screening for toxoplasmosis limits the diagnosis of the disease in pregnant women. Lack of clear and direct cases linked to toxoplasmosis could not be a valid reason for health workers in Temeke not to diagnose *Toxoplasma*. This is because presentation of toxoplasmosis includes fever and sometimes may be similar to other zoonoses and common diseases in Tanzania such as malaria. Therefore there are chances of over diagnosing malaria or unresponsive malaria cases instead of febrile illness of zoonotic diseases including toxoplasmosis. In addition, the observation from prenatal cards showed that toxoplasmosis is seldom diagnosed or reported because most patients with healthy immune systems do not develop signs and symptoms of the disease.

Health workers play an important role in advocating positive preventive practices and behaviours towards diseases. To promote awareness and good practices of pregnant women towards congenital diseases including toxoplasmosis, it is important to invest in periodic updating health worker knowledge through education (nurses in particular) on the common diseases of pregnancy so that they provide enough and comprehensive prenatal care.

5.2 Study limitations

In the current study, the response rate for the medical health workers was seemingly low; this was due to the low staffing level of the RCH clinics in Temeke municipality accounted by the shortage of the medical professionals in the country. The few health staff working in Temeke municipality RCH clinics attended to massive number of patients in a working day, this kept them busy from doing other activities. For this reason it was a challenge to administer the questionnaires to reach the estimated number of staff considering timeframe scheduled and fund allocation for the study. On the other hand, lack of local/indigenous name for toxoplasmosis was a limiting factor to this study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The current study highlights the low level of awareness and practices towards congenital toxoplasmosis among health workers and pregnant women in Temeke municipality. The low level awareness is due to lack of common indigenous name for toxoplasmosis in Tanzania, low level of education and lack of priority for prenatal toxoplasmosis screening services. Lack of awareness is likely to increase the risk of exposure and negative practices towards the disease. This is because people will only care and get attention to protect themselves from the risk of the disease they know.

This calls for population awareness through education on the possible risks and the associated ill health outcomes. At present Maternal and child health care are of greater concern in low and middle income countries with regard to prenatal and neonatal mortality. Prenatal screening for congenital diseases is a vital intervention for improvement of maternal and child health. Health programs should give more attention to most common infectious diseases associated with congenital anomalies including among others HIV/AIDS, rubella, syphilis, hepatitis and toxoplasmosis. While there is evidence of increased prevalence and risk of the disease in pregnant women in Tanzania and lack of awareness, good hygienic practices and behaviours remain important to avoid infection due to *T. gondii*. Pregnant women and health workers seem to practice preventive practices without knowing what they are avoiding.

6.2 Recommendations

To promote preventive practices and raise awareness towards toxoplasmosis among vulnerable populations; also to enhance diagnosis, prevention and treatment of congenital infections including the widespread zoonotic toxoplasmosis;

The following recommendations are made based on the findings of this study.

1. Health education and health promotion of medical personnel's and vulnerable population of pregnant women on the importance of congenital toxoplasmosis. This can be done thorough,
    a) Strengthening of the curriculum for training of enrolled nurses and midwives to cover more aspects of congenitally transmitted diseases including toxoplasmosis, currently most of the enrolled nurses and midwives are unaware of toxoplasmosis.
    b) Development and provision of prenatal brochures addressing different reproductive and child health issues including maternal behaviours' and practices in conjunction with the diseases of congenital importance and their risks during pregnancy.
2. The government through health systems and policy should support and facilitate diagnostic services for screening of toxoplasmosis and other important congenital diseases among pregnant women attending prenatal health care, not only HIV/AIDS, syphilis and malaria which are currently screened.
3. There is also a need for more research to help establish the prevalence and risk factors in both humans and livestock. Population epidemiology studies of toxoplasmosis through one health approach is recommended to establish general exposure status and complications associated with the disease in different risk groups including pregnant women, children and HIV/AIDS patients and livestock.

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APPENDIXES

Appendix 1: The Information Sheet

I am Onduru Onduru Gervas, conducting a research in fulfilment of the requirements for the award of Master of Science degree in One Health Analytical Epidemiology of the University of Zambia.

My study is looking at the awareness and practices towards a disease called toxoplasmosis. This disease is of particular concern as it infects felids and all worm blooded animals. Approximately 30% of world's population is affected; the risk groups are individuals who may be immunosuppressed as in the case of HIV/AIDS patients, children and pregnant women. Since the disease can be congenially transmitted from mother to foetus, makes it of particular concern in pregnant women but has not been investigated extensively for its significance in Tanzania. Information about pregnant women and healthcare workers awareness and practices towards this disease are missing. Therefore your participation in this study will help provide the much needed information which will enhance and further improving the maternal and child health based on the level of awareness and practices towards congenital toxoplasmosis in Tanzania.

To do this, I will ask you a few questions about this disease using the form I have. There is no risk in this study and it has been reviewed and approved by NIMR ethics committee for its suitability to human participants. Participation is entirely voluntary, be assured that the information you provide will be strictly confidential and restricted to the research purpose.

Nevertheless you have the right to seek further clarification or to withdraw. For further information you may contact the following;

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Appendix 2: The Consent Form

I ……have agreed to take part in this research which is studying awareness and practices towards congenital toxoplasmosis among pregnant women and health workers in Temeke-Dar es salaam, Tanzania. I confirm that the study has been adequately explained to me and I understand it to the best of my knowledge.

I attest to participate voluntarily and that I can withdraw at any time without repercussions.

I understand that disguised extracts from my responses may be quoted in the thesis and any subsequent publications.

I agree to provide necessary information needed for this study.

Participant

signature……Date……..

Name of investigator: ……..

Sign……

Appendix 3:Questionnaires for pregnant women.

Ufahamu na matendo juu ya ugongwa wa Toksoplasmosis kwa akina mama wajawazito na wafanyakazi wa afya katika manispaa ya Temeke-Dar es salaam, Tanzania.

SEHEMU A: Taarifa za mshiriki.

Namba ya mshiriki..... Kituo cha afya.....

Na.	Swali		Jibu
A.1	Una umri wa miaka mingapi?	Miaka ……..	
A.2	Unajishugulisha na shuguli gani ya kiuchumi kwa sasa?	……..	
A.3	ipi ni ngazi ya juu ya elimu yako?	Elimu ya msingi	( )
		Elimu ya sekondari	( )
		Elimu ya chuo	( )
		Sikwenda shule	
A.4	Hali ya ndoa	Hujaolewa	( )
		Umeachana/kutengana	( )
		Umeolewa/mnaishi pamoja	( )
A.5	Ni mara ngapi umewahi kuwa mjamzito?	……..	
A.6	Ujauzito wako wa sasa una umri gani?	Miezi mitatu ya kwanza	( )
		Miezi mitatu ya pili	( )
		Miezi mitatu ya tatu	( )
A.7	Kama jibu lako ***A.5*** hapo juu ni zaidi ya mara moja. Je, kwa bahati mbaya au nzuri umewahi kupoteza ujauzito wako?	Ndio	( )
		Hapana	( )

SEHEMU B: Taarifa za uelewa kuhusu ugonjwa wa Toksoplasmosis

Q. No	Swali	Majibu	
B.1	Umeshawahi kusikia kuhusu ugonjwa waToksoplasmosis?	Ndio Hapana (Kama hapana nenda Sehemu C:)	( ) ( )
B.2	Kama jibu lako la **B.1** ni ndio, umepata wapi habari kuhusu ugonjwa wa Toksoplasmosis?	Kutoka kwa mfanyakazi wa huduma za afya Intaneti vitabu / magazeti / filamu / video Radio/ Televisheni Familia / jamaa / rafiki Wengine (taja)…… Sijawahi kupata habari yoyote kuhusu ugonjwa huu	( ) ( ) ( ) ( ) ( ) ( )
B.3	Je, unajua vimelea vinavyosababisha ugonjwa wa Toksoplasmosis?**(KAMA HAPANA NENDA *B.7*)**	Ndiyo Hapana	( ) ( )
B.4	Kama unajua hivyo vimelea , vinaitwaje?	……..	……

B.5	Je, vimelea vinavyosababisha ugonjwa wa Toxopasmosis vinaweza kuwaambukiza binadamu?	Ndiyo Hapana	( ) ( )
B.6	Je, vimelea vinavyosababisha ugonjwa wa Toksopasmosis vinaweza kuwaambukiza wanyama?	Ndiyo Hapana	( ) ( )
B.7	Je, watu wanaweza kuambukizwa Toksoplasma kutokana na kusafisha takataka za paka ?	Ndiyo Hapana	( ) ( )
B.8	Je, watu wanaweza kuambukizwaToksoplasma kwa Kupalilia bila kuvaa kinga (gloves)?	Ndiyo Hapana	( ) ( )
B.9	Je, Toksoplasma inaweza kuambukizwa kutoka kwa mama mjamzito kwenda kwa mtoto?	Ndiyo Hapana	( ) ( )
B.10	Je, watu wanaweza kuambukizwa“Toksoplasma” kwaKula nyama mbichi au isiyoivishwa vizuri?	Ndiyo Hapana	( ) ( )
B.11	Je, watu wanaweza kuambukizwa Toksoplasma wakati wa kuongezewa damu?	Ndiyo Hapana	( ) ( )
B.	Je, Toksoplasma	Ndiyo	( )

12	inaweza kuambukizwa kwa njia ya kujamiiana?	Hapana	( )	
B.13	Je, Kula matunda na mboga zisizooshwa ianaweza kupelekea maambukiziya Toksoplasma?	Ndiyo Hapana	( ) ( )	
B.14	Je, Umewahi kupima ugonjwa wa Toksoplasmosis?	Ndiyo Hapana	( ) ( )	
B.15	Je, Toksoplasmosis inaweza kutibiwa? (**kama hapana nenda *B.17***)	Ndio Hapana	( ) ( )	
B.16	Kama jibu lako katika ***B.15*** juu ni ndio, ni kwa namna gani vinaweza kutibiwa?	……	……	
B.17	Ni dalili zipi za kimatibabu kati ya zifuatazo zinahusishwa na Toksoplasmosis?	Kuharibika kwa retina na kutokuona vizuri	Ndiyo	Hapana
		Mumivu ya kichwa		
		Kuvimba toki (matoki)		
		Kudhoofu kwa mwili na homa		
		Kupoteza kumbukumbu		

SEHEMU C: Maswali juu ya mambo hatari yanayohusiana na maambukizi, njia za kuzuia na dalili za Toksoplasmosis.

Q. Na	Swali	Majibu		
C.1	Je ni mnyama yupi wa nyumbani unafuga kati ya hawa wafuatao? **(kama hakuna nenda c.4)**	Ng'ombe Mbuzi Kondoo Paka Kuku	ndiyo ( ) ( ) ( ) ( ) ( )	hapana ( ) ( ) ( ) ( ) ( )

		Mbwa Punda Njiwa	( ) ( ) ( )	( ) ( ) ( ) ( )
C. 2	Kama unafuga mnyama wa nyumbani kati ya hao. Je, huwa unahusika kusafisha makazi/uchafu utokanao na mnyama huyo?	Ng'ombe Mbuzi Kondoo Paka Kuku Mbwa Punda Njiwa	Ndiyo ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )	Hapa na ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( )
C. 3	Je, huwa unanawa mikono yako kwa saabuni na maji safi kila baada ya kusafisha makazi/uchafu utokanao na wanyama wafugwao?	Ndiyo	( )	
		Hapana	( )	
C. 4	Je, umewahi kushiriki katika shughuli za kilimo au bustani? (**Kama jibu ni hapana nenda*C.7***)	Ndiyo	( )	
		Hapana	( )	
C. 5	Je, huwa unavaa kinga (gloves) wakati wa kulima au kupalilia?	Ndiyo	( )	
		Hapana	( )	
C. 6	Je, huwa unaosha mikono yako na sabuni mara baada ya kulima/kupalilia?	Ndiyo	( )	
		Hapana	( )	
C. 7	Je, huwa unasafishamboga na kumenya vizuri matunda kabla ya kula ?	Ndiyo	( )	
		Hapana	( )	
C. 8	Je, katika kipindi chako cha ujauzito umewahi kupatwa na hali ipi kati ya haya?	Kupoteza kumbukumbu	Ndiyo ( )	Hapa na ( )
		Mumivu ya kichwa		
		Kuvimba toki (matoki)		
		Kudhoofu kwa mwili na homa		
C. 9	Kama jibu lako katia ***A.5*** hapo juu ni zaidi ya moja, Je, umewahi kupata motto mwenye dalili zipi za kimatibabu kati ya hizi?	Mwenye matatizo ya macho		
		Mwenye kichwa kikubwa /kichwa iliyojaa maji		

		Mwenye kupooza/kifafa		
		Mwenye utindio wa ubongo/kudumaa akili		
C. 10	Je, katika kipindi chako cha ujauzito umewahi kutumia dawa ipi kati ya yafuatayo?	Pyrimethamine		
		Sulfadiazine		
		Sulfadoxine		
		Folic acid		
		Spiramycine		
C. 11	Je, katika hali ya kula nyama, umewahi kula nyama mbichi au isiyoiva vizuri?	Ndiyo	( )	
		Hapana	( )	
C. 12	Je, kuna mazingira yanayokusababishia kula matunda au mboga zisizooshwa kwa maji safi?	Ndiyo	( )	
		Hapana	( )	
C. 13	Ipi chanzo cha maji unayotumia nyumbani?	Bomba		
		Kisima		
		Maji ya dukani		
		Chanzo kingine.....		
C. 14	Je unakunywa maji yasiyochemshwa na wala kuwekwa dawa?	Ndiyo ( )	Hapana ( )	
C. 15	Je, Umewahi kuongezwa damu?	( )	( )	

Appendix 4:Questionnaires for Health workers

HEALTH CENTRE: ..... DIVISION: .....

WARD: ..... STREET: .....

S/N: .....

SECTION A: Characteristics of the participant

Q. No	Question	Response	Yes	
A.1	Gender	Male		
		Female		
A.2	How old are you now?	…… ……years		
A.3	Marital status	Single		
		Married/Cohabited		
		Divorced/separated		
A.4	What is your current designation as a health worker?	…… ……		
A.5	What was the highest level of schooling you attended?	Secondary education		
		College/university		
		Postgraduate		

SECTION B: General understanding of *Toxoplasma*

Q. No	Question		
B.1	Have you ever heard about/know toxoplasmosis?	Yes ( ) No ( )	If No go to section "C"
B.2	If your answer to ***B.1*** is yes, where did you get information about toxoplasmosis?	Course of study Internet Books/magazines/films/videos Radio/ Television Family/relative/friend Others (specify)…… I did not see, hear or read anything about this disease	( ) ( ) ( ) ( ) ( ) ( )
B.3	Do you know the parasite causing toxoplasmosis?	Yes ( ) No ( )	If no go to "B.6"
B.4	If your answer in ***B.3*** above is yes, what is the name of the agent?	…… …….	

B .5	Can the parasite infect both human and animal species?	Yes ( )	No ( )	I don't know ( )
B .6	Can people be exposed and acquire Toxoplasma by?	Changing cat litter	Yes	No
		Gardening without gloves		
		From pregnant mother to fetus		
		Eating raw, cured or undercooked meat		
		Drinking raw milk?		
		Drinking untreated water?		
		Receiving blood transfusion		
		Sexual intercourse		
		Eating unwashed fruits and vegetables		
B .7	Have you ever tested for toxoplasmosis?	Yes ( )	No ( )	
B .8	Do you screen pregnant women for toxoplasmosis	Yes ( )	No ( )	
B .9	If your answer to ***B.8*** above is **No** why?	…… ……...		

B.10	Can toxoplasmosis be treated?	Yes ( )	No ( )	
B.11	If your answer in ***B.10*** above is yes, how?	……		
B.12	Which of the following medicine can be used for management of toxoplasmosis?	Pyrimethamine	Yes	No
		Sulfadiazine		
		Sulfadoxine		
		Folic acid		
		Spiramycine		
B.13	Do you give any of the above medicine in ***B.12*** to pregnant women?	Yes ( )	No ( )	
B.14	If your answer in ***B.13***above is yes/no why?	.....		
B.15	Can pregnant women develop serious problems due to Toxoplasma infection?		Yes ( )	No ( )

B.16	Can unborn and/newborn children develop serious complication after infection with toxoplasmosis?		Yes ( )	No ( )
B.17	Can a baby with Toxoplasma have no signs of illness at birth but develop illness later?		Yes ( )	No ( )
B.18	Can a baby with toxoplasmosis have vision and mental problems?		Yes ( )	No ( )
B.19	Which of the following clinical manifestations are associated with toxoplasmosis?	Chorioretinitis	Yes ( )	No ( )
		Hydrocephalus in children		
		Encephalitis		
		Mental retardation		
		Disorientation		
		Intracranial calcification		
B.20	Can Toxoplasma in pregnant women cause fever and feeling like you have “flu”?			
B.21	Can toxoplasmosis in pregnant women cause swollen glands (lymph nodes)?			
B	Can toxoplasmosis in pregnant women cause no symptoms?			

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SECTION C: Questions on Risk factors and practices associated with Toxoplasma infection.

Q.No	Questions	Yes	No
C.1	Do you have domestic cat(s) at home? (**If no, go to *C.3***)		
C.2	Dou you change cat litter/clean cat premises?		
C.3	Have you ever been involved in farming or gardening activities? (**If not, go to *C.5*)**		
C.4	If your answer to **C.3** above is yes, Do you wear gloves when farming or gardening?		
C.5	Do you thoroughly wash and peel fruits and vegetables before eating?		
C.6	Have you ever eaten raw/undercooked or cured meat?		
C.7	Have you ever eaten raw fruits/vegetables?		
C.8	Do you drink raw milk?		
C.9	What is the source of water you use at home?	Tap	( )
		Well	( )
		Mineral bottled water	( )
		Other……..	
C.10	Do you drink untreated water?	Yes ( )	No ( )

Thanks for your participation

Appendix 5: Ethical clearance

THE UNITED REPUBLIC OF
TANZANIA

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23rd February 2016

Mr Onduru O Gervas
National Health Laboratory Quality Assurance and Training Centre
NIMR Complex
C/O Dr Susan Rumisha, NIMR HQ,
DAR ES SALAAM

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Awareness and Practices Towards Congenital Toxoplasmosis Among Pregnant Women and Health Workers in Temeke, Dar es Salaam, Tanzania, (Gervas O O *et al*), whose Local Supervisor is Dr Susan Rumisha, NIMR HQ, Dar es Salaam, has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Health Facilities in Temeke Municipalities in Dar es Salaam

Approval is for one year: 23rd February 2016 to 22nd February 2017.

Name: Dr Mwelecele Malecela

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MIN. OF HEALTH CDGE&CHILDREN

CC: RMO
DED
DMO