TOXOPLASMA GONDII INFECTION AMONG OUTPATIENTS ATTENDING DODOMA REGIONAL REFERRAL HOSPITAL IN CENTRAL TANZANIA: PREVALENCE AND ASSOCIATED RISK FACTORS IN CENTRAL TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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ABSTRACT

Toxoplasmosis is a parasitic zoonotic disease caused by coccidian intracellular protozoan parasite, *Toxoplasma gondii*. The parasite is ubiquitous and infects almost all warm blooded animals including humans. Primary infection in human is usually asymtomatic or manifest with febrile illness characterized by headache, sore throat, muscle pain and swollen lymph nodes. Clinical implication of the disease is more on pregnant women and immunocompromised individuals. The overall objective of this study was to determine the prevalence and associated factors to toxoplasmosis among patients attending Dodoma Regional Referral Hospital (DRRH) in Central Tanzania. In this cross-sectional study, a total of 395 outpatients attending DRRH between December 2019 and February 2020 were enrolled. Blood was collected from consenting patients. A structured questionnaire with simple, open and closed ended questions was administered to study subjects to collect information on knowledge of infectious agent, transmission mode and information regarding risk of exposures. Of 395 subjects enrolled in this study, 2% were infected with *T. gondii* after screening using polymerase chain reaction. There was no statistical relationship between disease diagnosis and risk of exposures. Only 1.3% of the participants had good knowledge towards toxoplasmosis. Majority (88.4%) of the enrolled participants showed good practises towards toxoplasmosis. The results indicate that *T. gondii* is prevalent among individuals in Dodoma Region and a very low proportion of them had knowledge of the disease. The results suggest the need of health education toward toxoplasmosis among residents of Dodoma. It is important that the health care system diagnostic capacity is enhanced to provide routine diagnosis of *T. gondii* and promote an interdisciplinary collaboration in its risk management.

DECLARATION

I, Glory B. Lema, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

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DEDICATION

I dedicate this work to the Almighty God for the gift of life. I dedicate this work to my parents Mr. Brayson Lema and Mrs. Joyce Lema, my siblings Given and Grace Lema.

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LIST OF ABBREVIATION AND ACRONYMS

Ab	antibody
rDNA	ribosomal deoxyribonucleic acid
AIDS	acquired immunodeficiency syndrome
CI	confidence interval
EDTA	ethylene diamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
DRRH	Dodoma Regional Referral Hospital
HIV	human immunodeficiency virus
IgG	immunoglobulin G
IgM	immunoglobulin M
ITS	internal transcribed spacer
MRI	magnetic resonance imaging
PCR	polymerase chain reaction
RPM	revolutions per minute
SAG2	surface antigen 2

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Toxoplasmosis is a parasitic zoonotic disease caused by a coccidian intracellular protozoan parasite, *Toxoplasma gondii* (Aguire *et al.,* 2019). *T. gondii* infection is worldwide prevalent with at least one third of human population infected (Furtado *et al*., 2011; Flegr *et al.,* 2014). Globally, the seroprevalence of toxoplasmosis in certain populations varies between 1% and 100% (Alzaheb, 2018) and the variation is attributed to the environment, socioeconomic and cultural factors (Mboera *et al.,* 2019). Notwithstanding the fact that higher seroprevalence is in areas where stray cats are plentiful and consumption of raw or under cooked meat is common (Rouatbi *et al.,* 2019).

In Africa, toxoplasmosis has been reported in humans, domestics and wild animals. Food animals are the reservoirs of the parasite and act as a source of transmission to humans through their products such as meat and milk. Overall prevalence of *T. gondii* in food animals ranges from 12% to 37.4% (Tonouhewa *et al*., 2017). Studies have reported human toxoplasmosis prevalence of 6.7% in Korea, 12.3% in China, 23.9% in Nigeria, 58.4% in Tunisia, 21% in Mali, 83.5% in Madagascar, 46%, 35% and 30.9% in different parts of Tanzania; though most studies targeted pregnant women and pastoralists (Retmanasari *et al*., 2016; Gashout *et al.,* 2016; Shao *et al.,* 2015; Paul *et al.*, 2018).

The ubiquitous, apicomplexan *T. gondii* parasite intergrades human life either congenitally or post-natally (Shao *et al.,* 2015). Congenital transmission of *T. gondii* in pregnant women with untreated toxoplasmosis increases the risk of infection to foetus

through the placenta (Wallon *et al.*, 2013). Eating of raw meat, vegetables and milk containing tissue cyst (bradyzoites), oocysts or tachyzoites acts as a second epidemiological relevant mode of transmission (Tenter *et al.*, 2000).

In humans, infection is usually asymptomatic or manifest with febrile illness. However, acute infection of adults and children may result in swollen lymph nodes (Saadatnia and Golkar, 2012). Severe form of a disease occurs in immunocompromised patients and pregnant women. Infection in the first trimester is less likely to pass to the foetus, however if transmission occurs severe outcomes like spontaneous abortion, hydrocephalus, and foetal deaths may occur (Flatt and Shetty, 2012; Maenz *et al.*, 2014). Infection at late stages of pregnancy tends to cause less disease severity although serious congenital manifestation including calcifications and neurological disabilities may manifest (Jones *et al.*, 2010).

Awareness of the pathways of infection and transdisciplinary actions may be significant in controlling disease transmission (Ngô *et al.*, 2017; Suvisaari *et al.*, 2017). Hygiene in food preparation and eating habit have a bigger role in preventing toxoplasmosis, although through natural history of *T. gondii*, the suggested approaches that could help protect humans, domestic animals, wildlife and ecosystem health involves better understanding of the disease and promotion of transdisciplinary collaborations between the government agencies, policy makers, physicians, veterinarians and the general public (Aguirre *et al.*, 2019).

Failure in control of *T. gondii* transmission to human may influence the use of folate inhibitors drug which plays a role in treatment of protozoan infections. The use of pyrimethamine and trimethoprim in combination with sulphonamides have remained to

be the most effective drugs that primarily act against tachyzoite form of *T. gondii* (Konstantinovic *et al.*, 2019).

1.2 Problem Statement and Justification

Infections with *T. gondii* are common in humans and animals around the world with about one-third of the human population exposed to the parasite (Flegr *et al.*, 2014). Several studies have reported a wide range of prevalence of *T. gondii* infection in various locations of Tanzania (Khan *et al.*, 2014; Shao *et al.*, 2015). Evidence from a recent study in Tanzania, suggests that *T. gondii* infection accounts for 0.08% of all total hospital deaths; and are associated with a number of co-morbidities including human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), cryptococcosis and pneumocyts pneumonia (Mboera *et al.*, 2019).

Despite evidence of *T. gondii* parasites in human and animals, it has remained among the least prioritized disease in Tanzania healthcare system most likely due to limited diagnostic capacity and lack of awareness. To the best of my knowledge, there are no documented studies on *T. gondii* in central Tanzania, where culture and livelihood practices are likely to put the population at risk of infection. Therefore, the present study was conducted to determine prevalence, associated risk factors of T. *gondii* infection in Dodoma region of central Tanzania.

1.3 Research Objectives

1.3.1 Overall objective

The overall objective of this study was to determine the prevalence and associated risk factors of toxoplasmosis among patients attending DRRH in Central Tanzania.

1.3.2 Specific objectives

The specific objectives of the study were;

i. To determine prevalence of *T. gondii* infection among patients attending DRRH using polymerase chains reaction (PCR).
ii. To determine associated risk factors and to assess knowledge and practises regarding toxoplasmosis among patients attending DRRH.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Morphology

T. gondii has three morphological forms; oocyst, tachyzoites and tissue cysts containing bradyzoites (McGovern and Wilson, 2013). Definitive hosts bring infectious oocyst to the environments which undergo sporulation. Ingestion of sporulated oocysts by human leads to development of tachyzoites which mostly enter the nucleated cells (Elmore *et al.,* 2010). Tachyzoites in the invasive form, multiply rapidly, lead to cell rupture and invade nearby cells then transported to other parts of the body via blood and lymphatic circulation. Tachyzoites are then transformed into tissue cysts which are dormant form containing bradyzoites. Brain, skeletal muscles and cardiac muscles are the predilection sites for cysts formation (Basavaraju, 2016).

2.2 Life Cycle and Transmission

The life cycle of *T. gondii* has two sub-cycles, sexual and asexual which take part in definitive and intermediate hosts. The sexual cycle of the parasite takes place in feline species (Castro, 2019). Amplification of parasite within host gut cell and differentiation of male and female gametes in cat intestinal epithelium leads to formation of unsporulated oocyst that is excreted in felids faeces to environment. Meiosis takes place and produce highly infectious sporulated oocysts which can persist in environment (Dubey, 2007). Asexual cycle occurs through ingestion of sporulated infectious oocysts from the soil, water or plant material by the intermediate hosts. The released sporozoites from oocysts or bradyzoites from tissue cysts undergo differentiation to tachyzoite inside the intestinal epithelium and become distributed through the body via blood or lymph (Halonen and Weiss, 2014). Tachyzoites enter host cells by phagocytosis process and

multiplies within the cell by repeated endodyogeny and spread to lymphnodes and then to distant organs. Division of tachyzoites by endodyogeny process gives rise to bradyzoite; tissues cyst which grows and remain inside the cell throughout the host life (Dubey, 2009).

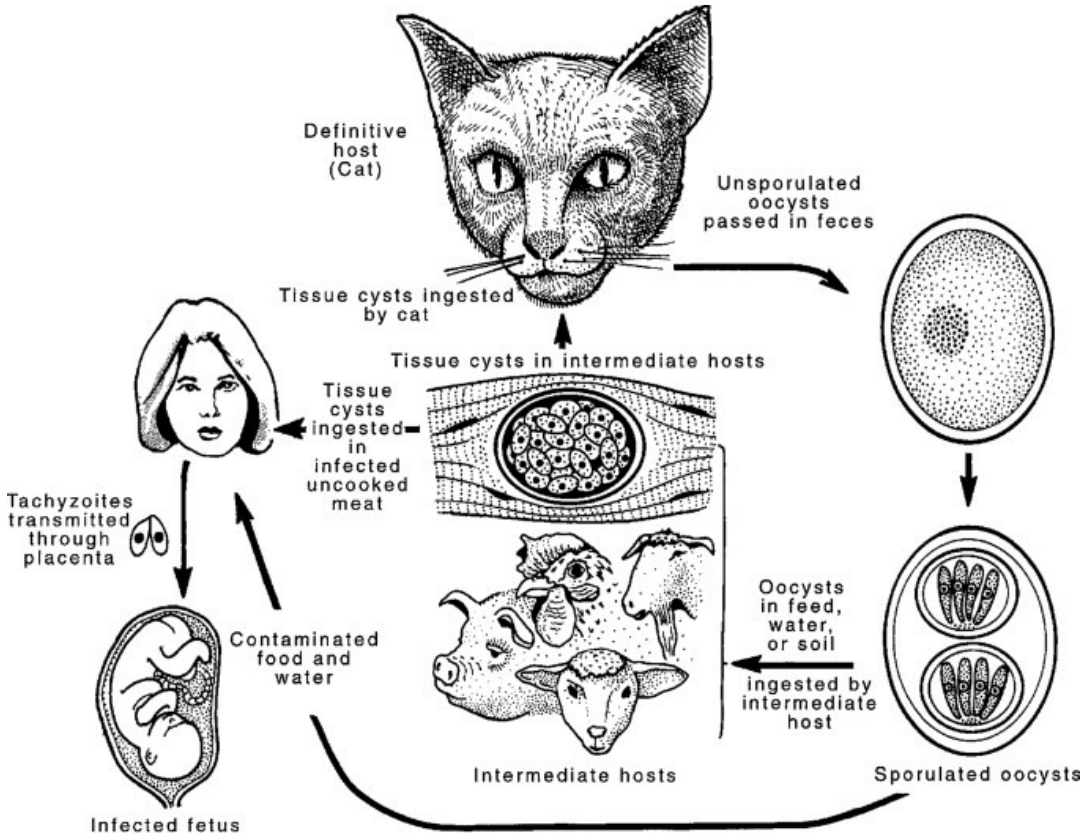

Figure 1: Life cycle of *Toxoplasma gondii*

Source: https://images.app.goo.gl/xcgTxRXpaXkSYKxH7.

2.3 Clinical Manifestations

Primary infection of toxoplasmosis in human is usually asymtomatics or manifest with febrile illness characterized by headache, sore throat, muscle pain and swollen lymph nodes (Paul *et al.*, 2018). Primary infection in pregnant women may lead to congenital toxoplasmosis with complications to foetus such as still birth, miscarriage or complication in central nervous system (Fenta *et al.*, 2019). Immunocompromised

individuals with condition like HIV/AIDS or cancer are at risk of developing severe disease due to immune suppression and reactivation of a latent *T. gondii* infection (Nazari *et al.,* 2018; Bajnok *et al.,* 2019). Predominantly, reactivation of infection has link with diversity of cancers such as ocular tumours and meningioma (Malek-Raafat *et al.,* 2018).

2.4 Epidemiology

The parasite is ubiquitous and infects humans, wild and domestic animals. Food animals can be the reservoirs for *T. gondii* and act as the source of parasite transmission to humans (Guo *et al.,* 2019). The overall worldwide prevalence of *T. gondii* in domestic animals range from 12 to 37.4% (Mboera *et al.,* 2019). The estimated prevalence of anti-*toxoplasma* antibody in most infected ruminants were (36.0%) in camel (26.1%) in sheep and 22.9% in goat, while lowest prevalence was recorded in cattle (12.0%) (Tonouhewa *et al.,* 2017). Chicken and pigs, being the most consumed meat, account for 22% of the *T. gondii* infections to humans (FAO-OMS, 2015). In human, prevalence of toxoplasmosis is mostly associated with culture, geographical locations and climatic conditions. In tropical countries prevalence in human is estimated to be over 50% (Tenter *et al.,* 2000); ranging from 6.7% in Korea to 83.5% in Madagascar (Gashout *et al.,* 2016; Retmanasari *et al*., 2016). Several studies have reported prevalence of toxoplasmosis in Tanzania although most have targeted pregnant women and pastoralists (Khan *et al.,* 2014; Machumi *et al.,*2017; Mirambo *et al.,* 2016; Mwambe *et al.,* 2013; Paul *et al.,* 2018; Shao *et al.,* 2015; Swai Schoonman, 2009). A hospital-based study in northern Tanzania found 41.67% of pregnant women tested positive for anti-*toxoplasma* IgG, indicating chronic infection and 0.69% tested IgM positive indicating acute infection for toxoplasmosis (Shao *et al.,* 2015). Mwambe *et al.*(2013) reported 30.9% of women attending antenatal care in Mwanza were seropositive for *T. gondii* specific antibodies.

2.5 Risk Factors for Infection

Toxoplasmosis is a food-borne disease. Most human infections have been associated with consumption of raw or undercooked meat containing tissue cysts or via environment route by ingestion of oocysts, or from eating contaminated raw seafood, vegetables or fruit (Opsteegh *et al.,* 2014). Different epidemiological studies have identified risk factors for *T. gondii* infection. These include presence of cat(s) in or around the house, cleaning the cat litter box, having 3 or more kittens, gardening in contaminated soil and having poor hand hygiene (Jones *et al.,* 2009). Many studies have showed significant increase in toxoplasmosis prevalence with increasing age, sex, being illiterate, living in remote areas, contact with soil and poor sanitation (Singh *et al.,* 2014; Kamal *et al.,* 2015). A study conducted in India reported, women with age older than 40 years are more at risk of having toxoplasmosis compared with women aged between 18 and 25 years. (Singh *et al.,* 2014). Tilahun *et al.,* (2013) stated that people living in rural areas are more at risk of being exposed to the source of *T. gondii* infection than their counterparts in rural areas.

2.6 Diagnosis

2.6.1 Radiological diagnosis

Magnetic resonance imaging (MRI) is the most sensitive tool for diagnosis of neurotoxoplasmosis. Other techniques such as signal photon emission computerised tomography scan, positron emission tomography can be used to rule out other central nervous system lesions such as lymphoma (Basavaraju *et al.,* 2016; Pillay *et al.,* 2018).

2.6.2 Enzyme immunoassay diagnosis

Detection of antibodies using dye test, indirect immunofluorescence assays, agglutination assays or immunoenzymatic assays have remained to be significant routine screening

techniques in the diagnosis of toxoplasmosis (Ybanez *et al.*, 2020). Enzyme-linked immunosorbent assay (ELISA) formats can be direct, indirect or sandwich assay. Direct ELISA is the simplest and rapid format for detection and quantification of cell surface antigen using enzyme-conjugated antibody while indirect ELISA uses secondary labelled antibody for detection. Sandwich ELISA uses two different antibodies targeting the same antigen (Kohl and Ascoli, 2017).

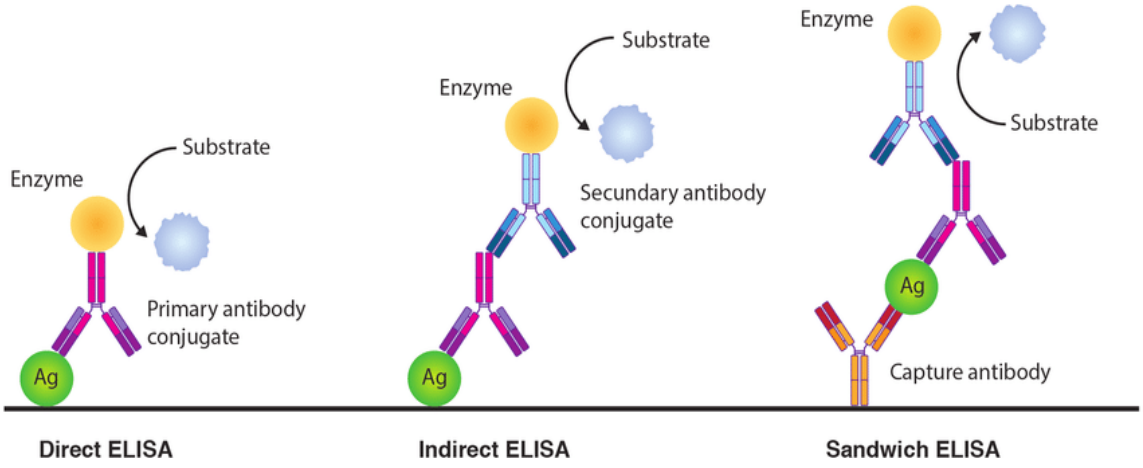

Figure 2: Direct, Indirect and Sandwich ELISA format.

Source: https://www.researchgate.net/figure/ELISA-assays-Direct-ELISA-mostly-used-for-antigen-detection-Indirect-ELISA-mainly-used.

Immunoglobulin M (IgM) and IgG are the common antibodies used to quantitate the specific analytes. Immunoglobulin M is considered to be the earliest antibodies in acute infection and last for a week; therefore, IgM positivity is not interpreted as a sign of acute infection unless complementary biological tests (IgG avidity assessment and/ or testing on a new sample 1–3 weeks later) are made (Murat *et al.*, 2013). Presence of IgG in the body for 2-3 weeks is of primary importance as it establishes the diagnosis of a true beginning infection.

The decrease of days to reach plateau in 2–3 months, marks persistent detectability of the parasite (Gangneux *et al.*, 2012). Though IgA is less used in routine testing, it provides earliest antibody peak in the course of the infection hence useful for the diagnosis of new-born infections (Kodym *et al.*, 2007). Immunoglobulin E is rarely assessed, less sensitive, less informative than other isotypes for diagnosing neonatal toxoplasmosis. Its strength is seen in fine dating of the infection as it is specific in acute infection (Foudrinier *et al.*, 2002).

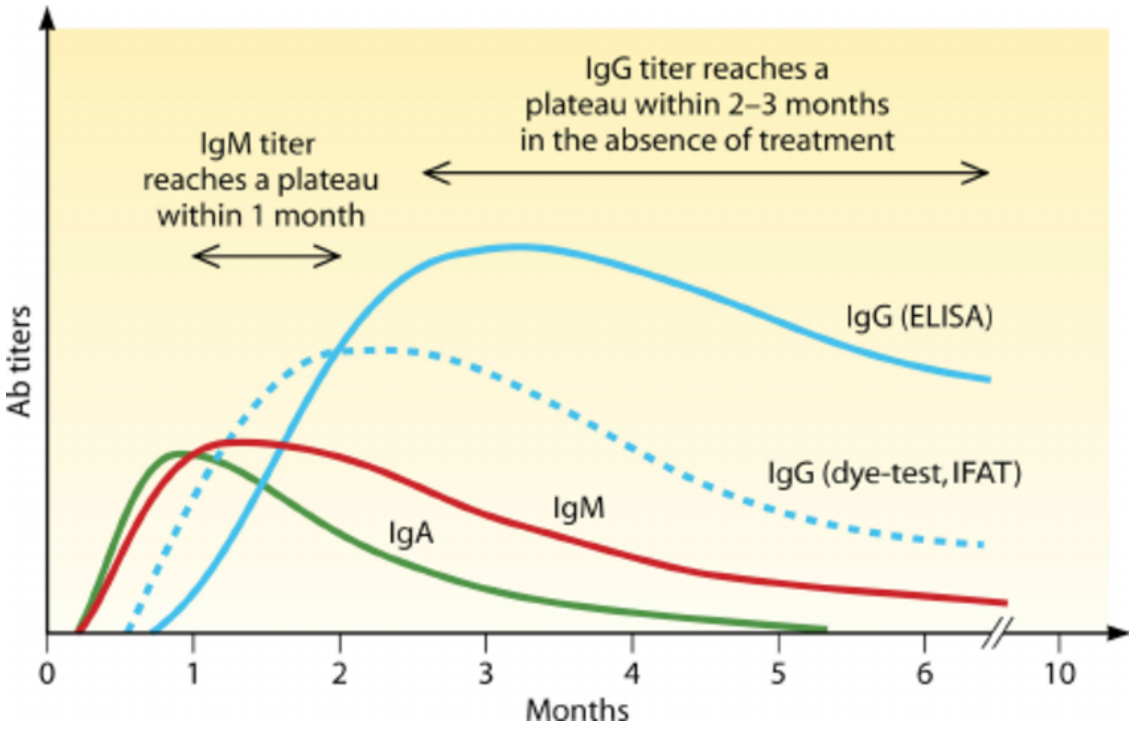

Figure 3: Kinetics of the antibody (Ab) response

Source: https://www.researchgate.net/figure/kinetics-of-the-antibody-Ab-response-The-average-kinetics-of-the-different-isotypes

2.6.3 Molecular diagnosis

2.6.3.1 Detection of *Toxoplasma gondii*

Detection of *T. gondii* in biological samples is more sensitive with the use of real time PCR as it estimates infection intensity (Ramĭrez *et al.*, 2017). Diagnosis of toxoplasmosis is generally based on the detection of DNA sequences and later the amplified product is analysed by electrophoresis in agarose or polyacrylamide gel to see the quality of the DNA. However, the sensitivity and specificity depends on the technique used in the extraction of DNA (Liu *et al.*, 2015). The highly conserved genes i.e. B_1 genes with 35 copies in the genome of the parasite and repeated element (RE) of 529 base pairs (bp) with about 300 copies in the genome are mostly selected for PCR test as diagnostic tool. The B_1 gene is mostly exploited in diagnosis and epidemiological studies as it is a highly repetitive gene with short DNA sequences, although *RE* 529-bp is more sensitive as it increases utility for diagnosis of *T. gondii* by PCR since it demonstrates greater sensibility, based on higher repetition number in the genome of the parasite (Dajem *et al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Setting

The present study was carried at DRRH in Dodoma, central Tanzania. Dodoma region is a semi-arid region, which covers an area of 41 311 km^2, with 2 083 588 inhabitants (NBS, 2012). The average daily temperature of Dodoma is 22.6 ºC, with little rainfall throughout the year (564 mm). The region lies at the heart of Tanzania in the Eastern-Central part of the country and it is bordered by Manyara region to the North, Tanga region to the North East, Singida region to the West, Iringa region to the South and Morogoro region to the East and Southeast. Dodoma region is administratively divided into seven districts, namely Bahi, Chamwino, Chemba, Dodoma Urban, Kondoa, Kongwa and Mpwapwa. The major economic activities include crop agriculture of beans, maize, sorghum, groundnuts, millet, rice, wine grapes and livestock husbandry.

Dodoma Regional Referral Hospital, located within Dodoma city is a 420-bed health facility serving as a referral centre for the whole region of Dodoma and serves about 1.2 million residents. The hospital also receives patients from Manyoni (Singida Region), Kiteto (Manyara Region) and Mtera (Iringa Region) districts. The hospital serves between 300 and 500 outpatients a day and admits between 250 and 280 inpatients.

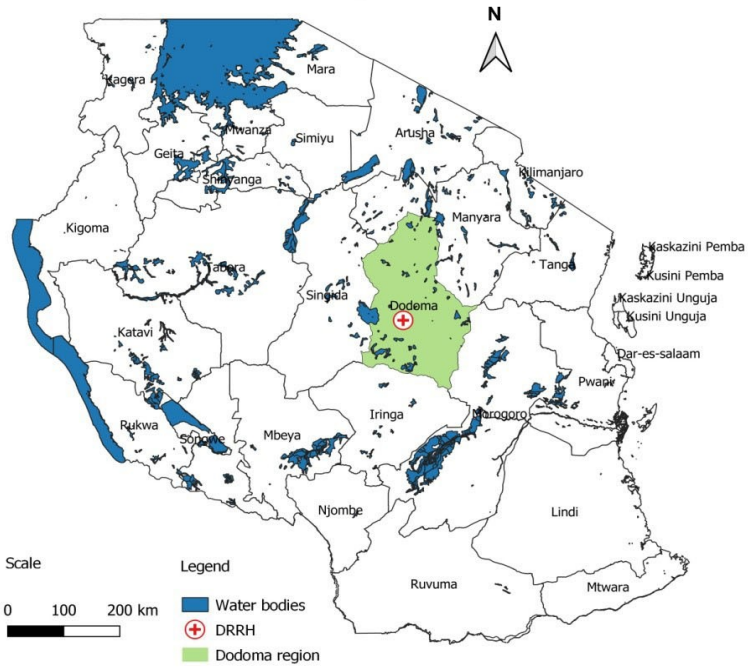

Figure 4: Map of Tanzania showing Dodoma region

3.2 Study Design and Study Population

The present study was a cross sectional hospital based study conducted from December 2019 to February 2020. Purposive sampling technique was used to enroll outpatients with signs and symptoms of muscle pain, fever, headache and swollen lymph nodes who required laboratory blood testing. The study excluded all patients with tiny blood vessels who couldn't provide blood sample in more than one collection tube, patients who refused to consent, patients who needed immediate treatment and pregnant women.

3.3 Sample Size Determination and Sampling Technique

Sample size was computed using the formula $N=Z^2*P\ (1-P)/L^2$. Whereas;

N = minimum sample size required

Z = standard normal deviation of (1.96) corresponding to 95% CI

P =30.9% seroprevalence of toxoplasmosis as reported by (Mwambe *et al.*, 2013)

L = precision of 5%. Based on the formula, the minimum required sample size was 328 patients, which was adjusted to 395 in order to increase precision and take care of accidental losses. Purposive sampling technique was used to recruit 395 outpatients attending the hospital.

3.4 Data Collection

3.4.1 Blood sample collection

A four ml vacuum tube was used to collect venous blood aseptically using sterile disposable syringe for detection of the parasite. The tubes were labelled with a unique identification number. Blood samples collected in EDTA tubes were stored at -20 °C refrigerator until use for genomic DNA extraction.

3.4.2 Interview

Face to face interview using pre-tested structured questionnaire with simple open and closed ended questions was administered to gather information on socio-demographic characteristics (age, sex, residence, marital status, educational level and occupation). Questions on transmission mode and infectious agent were administered to measure knowledge level while questions on food and water hygiene were posed to measure practises towards toxoplasmosis. Closed questions were used with possible answers being yes or no. A correct answer in the knowledge section was assigned score 1, and incorrect answer was assigned score 0. For the open questions, correct answers were assigned a score of 1 and incorrect answers assigned a score of 0.

3.5 DNA extraction

Extraction of deoxyribonucleic acid (DNA) was conducted from 50 µL of serum using Mahook extraction method (Appendix 4). Quality of the extracted DNA was determined using a spectrophotometer and integrity was assessed by performing gel electrophoresis using 1.5% agarose gel.

3.6 Polymerase Chain Reaction

Conventional PCR using the forward (REF: 5 "- CAG GGA GGA AGA CGA AAG TTG -3") and reverse (RER: 5 "-CAG ACC AGT GCA TGC TGG ATT -3") primers were used to identify 529-bp repeat amplicon that exists in 200-300 copies. PCR master mix was prepared (Table 1) and the first cycle took 5 minutes at 95 ºC to activate the enzymes. Thirty repeated cycles were involved, each cycle consisted of 3 steps: denaturation at 95 ºC for 45 seconds, annealing at 60 ºC for 45 seconds, extension at 72 ºC for 45 sec and final extension at 72 ºC for 5 minutes. *T. gondii* DNA was used as a positive control and PCR reaction without DNA template as a negative control.

Contents	**1reaction**	**400 reactions**
2X Enzyme mix	12.5 µL	5 000 µL
Water	8.5µL	3 400 µL
Forward primer	1 µL	400 µL
Reverse primer	1 µL	400 µL
Volume	**23 µL**	**9 200 µL**
DNA template	2 µL	8 000 µL
Total volume	**25 µL**	**10 000 µL**

Table 1: Composition of PCR master mix

3.7 Data Analysis

Data obtained from medical examination records and from questionnaires were entered in a computer, cleaned and analysed using SPSS version 20 software package (IBM Corp,

NY and USA). Descriptive statistics was performed; continuous variables were summarized using measure of central tendency and categorical using cross tabulation to estimate different proportions. Awareness towards toxoplasmosis was described according to the participant's responses to the questions. Association between demographic characteristics, knowledge and practises of the respondents towards toxoplasmosis were measured using chi-square test. The associated risk factors towards toxoplasmosis and the predictor variables were analysed using chi-square test; whereas variables with p-value ≤ 0.05 were considered statistically significant.

3.8 Ethical Consideration

This study was approved by the National Institute for Medical Research (NIMR/HQ/R.8a/Vol. IX/3296). Participation was voluntary and a written consent was obtained before enrollment into the study.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic Characteristics of Participants

A total of 395 participants were included in the study, out of whom 63.0% were females and 37% were males. Age groups of participants ranged from 1-86 years (mean 29.8 years SD± 17.8). Majority (91.6%) of participants were from urban areas. Majority (55.2%) were single and 37.7% had post-secondary education. (Table 2).

Table 2: Socio-demographic characteristics of participants (N= 395)

Variable	Response	Frequency (n)	Percent (%)
Age in years	0-10	53	13.4
	11-17	28	7.1
	18-45	235	59.5
	46-59	50	12.7
	≥60	29	7.3
Gender	Male	146	37.0
	Female	249	63.0
Residence	Urban	362	91.6
	Rural	33	8.4
Marital status	Single	218	55.2
	Married	171	43.3
	Separated	1	0.3
	Widow	5	1.3
Education	None	29	7.3
	Primary education	127	32.2
	Secondary education	81	20.5
	Advance education	9	2.3
	Post-secondary education	149	37.7
Occupation			
	No	52	13.2
	Student	175	44.3
	Employed	75	19.0
	Business	66	16.7
	Farmer	27	6.8

4.2 Prevalence of *T. gondii*

Of all participants, 2% (8/395) were found positive for the presence of *T. gondii* by PCR. Majority of participants infected with the parasite were aged 18-45 years, although

number of participants infected were higher in females than males. Half of the infected participants had post-secondary education. (Table 3).

Table 3: Distribution of *T. gondii* prevalence with demographic characteristics (N=395)

Participant's characteristics	**PCR test for *T. gondii***			
	Negative		**Positive**	
Variables	No	%	No	%
Age (years)				
0-10	53	13.7	0	0.0
11-17	27	7.0	1	12.5
18-45	229	59.2	6	75.0
46-59	49	12.7	1	12.5
60+	29	7.5	0	0.0
Sex				
Male	143	37.0	3	37.5
Female	244	63.0	5	62.5
Residence				
Urban	355	91.7	7	87.5
Rural	32	8.3	1	12.5
Marital status				
Single	213	55.0	5	62.5
Married	168	43.4	3	37.5
Widow	5	1.3	0	0.0
Separated	1	0.3	0	0.0
Education level				
None	28	7.2	1	12.5
Primary education	125	32.3	2	25
Secondary education	80	20.7	1	12.5
Advanced education	9	2.3	0	0.0
Post-secondary education	145	37.5	4	50.0
Occupation				
No	52	13.4	0	0.0
Student	170	43.9	5	62.5
Employed	76	19.4	0	0.0
Business	64	16.5	2	25.0
Farmer	26	6.7	1	12.5

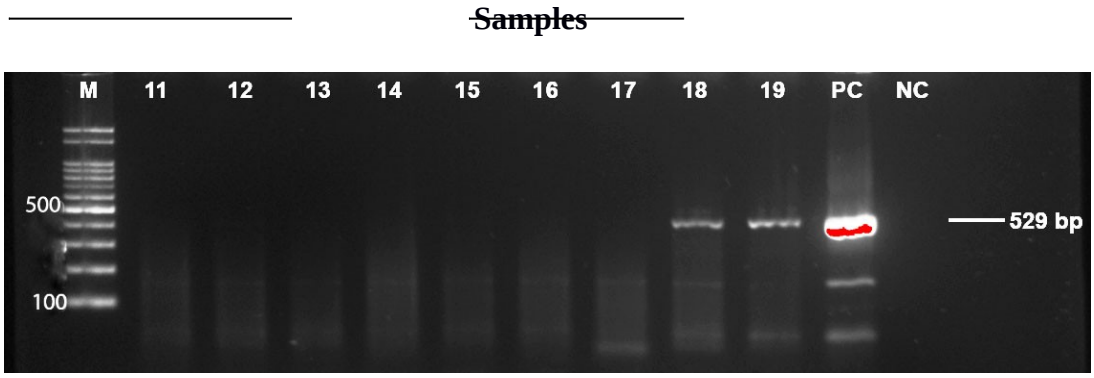

Figure 5: Agarose gel electrophoresis for *T. gondii* parasite. The expected PCR product size is 529 bp. Lane 18 and 19 show samples with *T. gondii* parasite. M= marker, PC= positive control, NC= negative control.

4.3 Distribution of Positive Results with different Exposures

Of the eight infected individuals, seven were those who didn't own cat(s). A considerable proportion of respondents (6/8) reported to wash hands before cooking and knife after cutting raw meat before continuing chopping other vegetables. All of the respondents reported tap water to be the main source of water, whilst (3/8) of the study respondents reported to treat water by boiling method before use. Other details are indicated in Table 4.

Table 4: Distribution of positive results with different exposures (N=395)

Exposure	Total	Positive		p-value
		N	%	
Own cat				1.000
No	349	7	2.0	
Yes	46	1	2.2	
Litter box for cat to sleep				1.000
No	375	8	2.1	
Yes	20	0	100.0	
Cleanliness of litter box				
No	384	8	2.1	1.000
Yes	11	0	0.0	
Cat crossing kitchen				0.548
No	358	7	2.0	
Yes	37	1	2.7	
Eat meat				0.098
No	5	1	20.0	
Yes	390	7	1.8	
Hand wash before cooking				1.000
No	30	0	0.0	
Yes	264	6	2.3	
I don’t know	101	2	2.0	
Knife wash after cutting raw meat				1.000
No	40	0	0.0	
Yes	251	6	2.4	
I don’t know	104	2	1.9	
Drink Milk				0.201
No	79	3	3.8	
Yes	316	5	1.6	
Drink unpasteurized milk				1.000
No	342	7	2.0	
Yes	53	1	1.9	
Eat salad				0.114
No	107	0	0.0	
Yes	288	8	2.8	
Eat unpeeled/unwashed fruits				0.633
No	326	6	1.8	
Yes	69	2	2.9	
Source of drinking/cooking/drinking water				0.727
Tap water	322	8	2.5	
Well water	13	0	0.0	
Spring water	2	0	0.0	
Rain water	1	0	0.0	
Industrial bottled water	56	0	0.0	
Tap and well water	1	0	0.0	
Treat water before use				0.565
No	173	5	2.9	

Yes	165	3	1.8	
Industrial bottled water	57	0	0.0	
Daily activities involve direct contact with soil				0.720
No	259	6	2.3	
Yes	136	2	1.5	
Eat soil				0.527
No	360	7	1.9	
Yes	35	1	2.9	

4.4 Total Score Level of Participants Knowledge Regarding Toxoplasmosis

Amongst 395 of the participants who were asked questions to measure level of knowledge on toxoplasmosis, 1.3% were aware of the disease; i.e. they have heard of the disease, they know how the disease is transmitted. It was found that all of those who have heard of the disease attended school with education level of post-secondary education. (Table 5).

Table 5: Relationship between knowledge regarding toxoplasmosis and socio-demographic characteristics of participants (N=395)

Variables	N	Good knowledge	Poor knowledge	P-value
Residence				
Rural	33	0 (0.0%)	33 (100.0%)	
Urban	362	5 (1.4%)	357 (98.6%)	1.000
Age				
3-10	53	0 (0.0%)	53 (100.0%)	
11-17	28	0 (0.0%)	28 (100.0%)	
18-45	235	4 (1.7%)	231 (98.3%)	
46-59	50	1 (2.0%)	49 (98%)	
60+	29	0 (0.0%)	29 (100%)	0.916
Sex				
Male	146	1 (0.7%)	145 (99.3%)	
Female	249	4 (1.6%)	245 (98.4%)	0.656
Marital status				
Single	218	1 (0.5%)	217 (99.5%)	
Married	171	4 (2.3%)	167 (97.7%)	
Separated	1	0 (0.0%0	1 (100.0%)	
Widow	5	0 (0.0%)	5 (100.0%)	0.235
Education				
None	29	0 (0.0%)	29 (100.0%)	
Primary	127	0 (0.0%)	127 (100.0%)	

Secondary	81	0 (0.0%)	81 (100.0%)	
Advanced	9	0 (0.0%)	9 (100.0%)	
Post-secondary	149	5 (3.4%)	144 (96.6%)	0.139
Occupation				
No	52	0(0.0%)	52 (100.0%)	
Student	175	1(0.6%)	174 (99.4%)	
Employed	75	4 (5.3%)	71 (94.7%)	
Business	66	0 (0.0%)	66 (100.0%)	0.049
Farmers	27	0(0.0)	27(100.0%)	

4.5 Association of Toxoplasmosis Status with Knowledge

The association of *T. gondii* seropositivity with disease awareness is shown in Table 7. All the respondents (8) who were diagnosed positive for the presence of *T. gondii* had poor knowledge on the disease (Table 6).

Table 6: Association of toxoplasmosis diagnosis with knowledge

Diagnosis		Knowledge				
	n	Good	%	Poor	%	P-value
Positive	8	0	0.0	8	100.0	
Negative	387	5	1.3	382	98.7	1.000

4.6 Practises Regarding Prevention of Toxoplasmosis

Majority (88.4%) had good practises towards toxoplasmosis i.e. possessing litter box for cat(s) to sleep and clean it often, hand wash before start cooking, treating cooking/drinking water before use. Majority (91.1%) of respondents who had good practises resided in urban areas. Respondents with age between 18-45 years reported to have good practises (55.6%) on toxoplasmosis compared to others. Females are reported to have good practises (64.2%) towards toxoplasmosis than males (Table 7).

Table 7: Relationship between practises towards toxoplasmosis and socio-demographic characteristics (N=395)

Variables	Good		Poor		P-value
	N	%	N	%	
Residence					**0.403**
Urban	318	87.8	44	12.2	
Rural	31	93.9	2	6.1	
					0.000
Age					
0-10	53	100.0	0	0.0	
11-17	26	92.9	2	7.1	
18-45	194	82.6	41	17.4	
46-59	48	96.0	2	4.0	
60+	28	96.6	1	3.4	
Sex					**0.198**
Male	125	86.6	21	14.4	
Female	224	90.0	25	10.0	
					0.000
Marital status					
Single	177	81.2	41	18.8	
Married	166	97.1	5	2.9	
Widow	5	100.0	0	0.0	
Separated	1	100.0	0	0.0	
					0.000
Education level					
None	29	100.0	0	0.0	
Primary	124	97.6	3	2.4	
Secondary	77	95.1	4	4.9	
Advanced	4	44.4	5	55.6	
Post-secondary	115	77.2	34	22.8	
					0.000
Occupation					
No	51	98.1	1	1.9	
Employed	72	96.0	3	4.0	
Business	65	98.5	1	1.5	
Student	135	77.1	40	22.9	

Farmer	26	96.3	1	3.7	

4.7 Association of Toxoplasmosis Diagnosis with Practises

Regarding practises, 87.5% of the participants who were positive for the disease are reported to have good practises towards toxoplasmosis (Table 8).

Table 8: Association of toxoplasmosis diagnosis with practises (N=395)

Diagnosis	**Practises**					
	n	**Good**	**%**	**Poor**	**%**	**P-value**
Positive	8	7	87.5	1	12.5	
Negative	387	342	88.4	45	11.6	1.000

CHAPTER FIVE

5.0 DISCUSSION

To my knowledge, this is the first study on *T. gondii* to be conducted in central Tanzania to determine prevalence, its associated risk factors, knowledge and practises among patients attending DRRH. A prevalence of 2% of disease among the enrolled subjects was detected by PCR, which indicate active infection by *T. gondii*. Our results revealed lower infection rate compared to other studies in Tanzania (Mwambe *et al.*, 2013; Khan *et al.*, 2014; Shao *et al.*, 2015; Swai and Schoonman, 2009). The low prevalence in this study is likely to be due to the fact that, the study participants enrolled in this study were outpatients coming for different treatment, while most of the other studies targeted pregnant women, livestock keepers and animal health workers who are most at risk of infection (Paul *et al.*, 2018; Mgode *et al.*, 2014). Sporulation of oocysts is more favoured in areas with warm weather, where infection rate is higher compared to hot weather regions. Therefore it is not surprising to find a lower prevalence of disease in Dodoma since it is a semi arid weather.

Distribution of *T. gondii* prevalence in age of respondents was higher in those with 18-45 years compared to other age groups. The number of infection in this group might be explained by the fact that, respondents in this age group are more likely to be active with energy of doing different activities which in one way or another increases the chance of contacting routes of disease infection. A study by Lema *et al*. (2012) reported the seroprevalence of *T. gondii* to be influenced by age.

Females are reported to have higher prevalence of *T. gondii* infection compared to males. The point might be explained by the fact that, it is behavioural that females are more

likely to attend/visit hospitals for checkups/treatments than males. Therefore, a high proportion of attendance justifies why higher number of females were detected with disease. Despite that our study have reported the difference in prevalence between sex groups, a study by Fan *et al.* (2012) reported there is no difference in disease prevalence between sex groups.

Kamal *et al.* (2015) reported statistical relationship between *T. gondii* seropositivity and living in rural area. This study complies with that of Kamal, on relationship between residence and disease prevalence, since those who resided in urban area were more infected compared to those in rural area. Eating habit might explain the point of disease prevalence between areas of residence, whereas respondents in urban are more likely to practise going out and consume grilled meat which might be undercooked, as it is also reported by Muflikhah *et al.* (2018). Also under diagnosis of disease in rural area might be counted as the factor for difference in disease prevalence. Teweldemedhin *et al.* (2019) reported the difficultness of disease diagnosis as it is reported in this study on the unawareness of disease due to under diagnosis.

Exposure to cat faeces, through handling of litter boxes has been found to be associated with *T. gondii* infection. In the present study, a number of participants reported the presence of cat(s) in their homes although there were no significant association between the disease and owning of cat(s). The findings are in agreement with other studies in Tanzania (Mwambe *et al*., 2013) and Palestine (Nijem *et al*., 2009).

The assessment of knowledge on toxoplasmosis revealed low level of awareness (1.3%) among the respondents. The results are in agreement with a study done in Dar es Salaam, Tanzania (Onduru *et al.*, 2019), which reported a small proportion (4.3%) of pregnant

women who were aware of the disease. A study by Velázquez-Hernández *et al.*(2019) reported that only the minority (<10%) of women in Mexico knew about the parasite, the disease, transmission, the clinical manifestations, diagnosis, treatment, and control.

The slight difference in knowledge between the two studies might be attributed by the fact that, pregnant women receive information concerning early identification risk factors, preventive measures and health advice to their pregnancy to encourage healthy lifestyle when attending antenatal clinics (Clinical Practise Guideline, 2019).

The poor knowledge concerning the disease might also be explained by the fact that, the country is having limited diagnostic capacity of the disease, as it is elaborated by Villard *et al.* (2016). Expenses of the diagnostic tools required, automatically causes the disease to remain among the least prioritized diseases by the healthcare system of Tanzania**.** This study complies with that of Onduru *et al.* (2019) on the lack of Kiswahili name to describe the disease. Hence, lack of Kiswahili name might be a contributing factor for low level of disease awareness. Lack of health education might be another factor that influences unawareness of the disease.

The statistical significance of knowledge on toxoplasmosis with occupation might be explained by the fact that, those employed specifically medical personnel's had higher chance of being aware of the disease compared to non-medical ones. A study by Efunshile *et al.*(2017) reported similar score on knowledge of *T. gondii* transmission among medical doctors.

All positive individuals had no knowledge on the disease i.e. didn't know how it is transmitted and had never heard of the disease. Despite the fact that there was no

significance association between disease diagnosis and knowledge, but being unaware of the disease increases the risk of acquiring it (Elsafi *et al.*, 2015).

Majority (88.4%) of participants in our study mentioned prevention measures against toxoplasmosis to include practices related to possession of clean litter boxes, hand washing, treatment of water and washing of knife after use. This study reports the statistical relationship between education and practises towards toxoplasmosis. Regardless of age, gender, residence and education level, health education for prevention of diseases that are foodborne including toxoplasmosis will definitely help to reduce the risk of infection to the community. A study by Pawlowski *et al.*(2001) emphasises on the importance of health education towards the risk of disease infection.

Practicing water and food hygiene automatically become a good practice towards toxoplasmosis. According to Food and Agriculture Organization and the World Health Organization, toxoplasmosis is ranked fourth among the 24 most harmful food-borne pathogens (FAO/WHO, 2014). Hence, poor practice of food and water hygiene may favour transmission of infection due to contamination with felid faeces. Velázquez-Hernández *et al.*(2019) reported 7.6% of the housewives knew about the risk of infection by consumption of contaminated food or water, which reflected poor hygiene practices. They recommended education to respondents so as to improve hygiene of food and water. Thus, these two studies are in agreement with each other that education is important in the prevention of toxoplasmosis in community.

In this study, individuals aged 18-45 years were reported to have good practices towards toxoplasmosis compared to others. The association between age and good practices might be explained by the fact that, respondents within this age group had positive attitude

regarding food and water hygiene. Andiappan *et al.*(2014) reported pregnant women with age between <20 - >40, to significantly avoid stray cats, wash cooking utensils after each use, wash hands after gardening, handling raw meat and eating food.

Despite the fact that, in this study number of singles was higher compared to married, eating food prepared at home reduces the risk of infection. The risk of infection among singles/ bachelors was high since most of them prefer to eat from the restaurants where they might consume undercooked meat. Also the association between marital status and good practices can be explained by the fact that, singles prefer outings where they eat grilled meat, which can be undercooked hence the risk is higher. A study by Elsafi *et al.*(2015) also reported eating outside home as the risk factor for infection.

Significant association was identified between practices of respondents towards toxoplasmosis and occupation. Clearly those employed are reported to have good practises towards toxoplasmosis compared to others. Hence, results give a picture that farmers and business men/women to be the groups at risk of infection than any other group. Nature of work might explain the point of good practises reported by those employed. Mwambe *et al.*(2013) also reported business women to be at risk of *T. gondii* infection than peasants.

CHAPTER SIX

6.0 LIMITATION, RECOMMENDATION AND CONCLUSION

6.1 Conclusion

The study revealed low prevalence of active infection by *T. gondii* among patients attending DRRH. The low prevalence might be attributed by climatic conditions and targeted study participants of this study. There was no statistical association between toxoplasmosis and different exposures. The low level of disease awareness may be due to lack of Kiswahili word to describe the disease, the disease being among the unprioritized disease by the health care system of Tanzania and lack of health education in our community influences the unawareness of the disease. Additionally, this study reports majority of participants to have good practises towards toxoplasmosis.

6.2 Recommendation

The use of larger sample size including both inpatients and outpatients will help to assess the prevalence of the disease, knowledge and practises towards toxoplasmosis. Information derived from this study recommends sensitization of the health care system in the community on diagnosis, treatment and clinical manifestation of toxoplasmosis.

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APPENDICES

Appendix 1: Informed consent to participant age ≥ 18 years

Participant's Consent Statement

The following statement was read to individuals asked to participate in the survey with ≥ 18 years of age

My name is ……, from Sokoine University of Agriculture in Morogoro. I am here carrying out a study on Toxoplasma infection in Dodoma Region of Tanzania. A trained laboratory technologist has already described to you about the collection of a pair of 4mls venous blood for testing the parasite in addition to the diagnosis requested by the clinician you met before coming to the laboratory. In case of any health implications during the time of study, our research team will provide first aid while waiting for further medications. You are free to express any discomfort/pain to the technologist that you might face during the time of sample collection.

I would like to seek your permission to ask some few questions as regards to the transmission of toxoplasma parasite. The results of the survey will lead to a better understanding of the risk of toxoplasma infection in our country. Study participation is voluntary and you have the right to withdraw from the study at any point without negative consequences. However, if you accept to take part in this study, there will be no payment to you. To minimize the risk of personal data disclosure all the facts about you obtained from the questionnaire will be kept confidential and stored in a computer locked with the password known only by the principal investigator.

No names will be used on any of the survey reports, publications or presentations as directed by the Laws of the United Republic of Tanzania. Only we, the researchers, will ever see the surveys with people's names. If you choose not to participate in this study, that is fine too. You will not be treated differently by the health personnel in this area. You may ask the researchers any question you have at any time.

Do you wish to participate? YES/NO (Please circle)

Signature _____ Thumbprint box

If you have any questions regarding this research, you may ask the research staff or contact Ms. Glory B. Lema, Sokoine University of Agriculture, Telephone: +255 672464971; E-mail: glorylema21 @gmail.com

Appendix 2: Informed consent to parents/guardians of participant age 3 month -17 years.

Participant's Consent Statement

The following statement was read to parents/guardians of participants (age 3 month-17 years).

My name is ……, from Sokoine University of Agriculture in Morogoro. I am here carrying out a study on Toxoplasma infection in Dodoma Region of Tanzania. A trained laboratory technologist has already described to you about the collection of a pair of 4mls venous blood for testing the parasite in addition to the diagnosis requested by the clinician you met before coming to the laboratory. In case of any health implications during the time of study, our research team will provide first aid while waiting for further medications. The children's are free to express the discomfort to their parents/guardians/technologist, faced during the time of sample collection.

I would like to ask some few questions on behalf of your child regarding the transmission of toxoplasma parasite. The results of the survey will lead to a better understanding of the risk of toxoplasma infection in our country.

Study participation is voluntary and you have the right to withdraw from the study at any point without negative consequences. However, if you accept to take part in this study, there will be no payment to you. To minimize the risk of personal data disclosure all the facts about you obtained from the questionnaire will be kept confidential and stored in a computer locked with the password known only by the principal investigator.

No names will be used on any of the survey reports, publications or presentations as directed by the Laws of the United Republic of Tanzania. Only we, the researchers, will ever see the surveys with people's names. If you choose not to participate in this study, that is fine too. You will not be treated differently by the health personnel in this area. You may ask the researchers any question you have at any time.

Do you wish to participate? YES/NO (Please circle)

Signature of guardian/parent of participant _____ Thumbprint box

If you have any questions regarding this research, you may ask the research staff or contact Ms. Glory B. Lema, Sokoine University of Agriculture, Telephone: +255 672464971; E-mail: glorylema21 @gmail.com

Appendix 3: Questionnaire assessing knowledge, practices and associated risk factors for *Toxoplasma gondii* infection among patients in Dodoma Regional Referral Hospital in Central Tanzania

Dodoso la kutathimini maarifa, matendo na sababu za hatari zihusuzo ugonjwa unaosababishwa na *Toksoplazima gondii* kwa wagonjwa wanaofika hospitali ya rufaa ya Mkoa wa Dodoma Kanda ya Kati Tanzania.

1. Questionnaire No *Dodoso namba*…….

2. Date (dd/mm/yyyy) *Tarehe*……..

3. Patient ID No *Namba ya mshiriki*…….

4. Area of residence *Eneo la makazi*…….

SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

TAARIFA ZA MSHIRIKI

1.	Age of person being interviewed in years. *Umri wa mshiriki katika miaka iliyokamilika (Andika mwaka wa kuzaliwa kama mshiriki hakumbuki tarehe).*	……
2.	Sex of person being interviewed. *Jinsia ya mshiriki.*	1.Male (Me). 2.Female (Ke).
3.	Marital status. *Hali ya ndoa.*	1.Singe*Sijawahikuolewa/kuoa.* 2.Married*Nimeolewa/Nimeoa.* 3.Cohabiting*Tunaishibilandoa.* 4.Separated*tumetengana*/

		Divorced*Tumeachana.* 5.Widow *Mjane.*
4.	Education level. *Kiwango cha elimu cha mshiriki.*	1. None *Hakusoma.* 2. Primary Education *Elimu ya msingi.* 3.Secondary Education *Elimu ya sekondari.* 4.Advanced Education *Elimu ya sekondari.* 5.Post-Secondary Education (College, University) *Elimu ya juu.* 7. Others……..
5.	Are you currently employed in work which you receive regular month salary? *Kwa sasa umehajiriwa au una kazi inayokupa mshahara kila mwisho wa mwezi*?	1.No *Hapana.* 2.Yes *Ndiyo.*
6.	Occupation. *Je, unafanya kazi gani inayokuingizia kipato*?	…….

KNOWLEDGE, PRACTISES AND ASSOCIATED RISK FACTORS FOR TOXOPLASMOSIS.

MAARIFA, MATENDO NA SABABU ZA HATARI ZA UGONJWA WA TOKSOPLASIMOSISI.

7.	Have you ever heard a disease called Toxoplasmosis? *Umewahi kusikia ugonjwa unaoitwa toksoplasmosisi*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1mark).
8.	a) If YES; Do you know how the disease is transmitted? *Kama ndiyo, unafahamu namnaugonjwa unavyoenezwa*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
9	If NO; Do you know of any disease transmitted from cats to human? *Kama hapana, unafahamu ugonjwa wowote unaoenezwa na paka kwa binadamu*?	Mention. Taja…… Roundworms (1mark). Rabies (1mark). Campylobacteriosis (1 mark). Salmonellosis (1 mark). Giadiasis (1 mark). Scabies's (1 mark).
10.	a) Do you own a cat? *Unamiliki paka*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
	b) If YES, does the cat have a special basket/box/place for sleeping? Kama ndiyo, paka wako ana mahali	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).

	maalumu pa kulala?	
11.	a) Does the basket/box/place cleaned often? *Je sehemu hiyo ya paka kulala inasafishwa mara kwa mara*?	1.No Hapana (0 mark). 2.Yes Ndiyo (1 mark).
	b) If YES, how many times a week and how? *Kama ndiyo, mara ngapi kwa wiki? Na namna gani*?	……..time s a week kwa wiki.
12.	Does the cat come across kitchen area? *Je, paka huja kwenye eneo la jikoni*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
13.	a) Do you eat meat? *Je, unakula nyama*?	1.No *Hapana.* 2.Yes *Ndiyo.*
	b) If YES, how many times a week? *Ikiwa ndiyo, ni mara ngapi kwa wiki*?	……times a week kwa wiki.
14.	The meat you eat is it prepared from home or from other places like groceries? *Nyama unayokula inaandaliwa nyumbani au sehemu nyingine tofauti na nyumbani*?	…….
15.	Who prepares food at home? *Nani anaandaa chakula nyumbani*?	……..
16.	Does the one preparing food at home, wash hands before start cooking? *Je, anayeanda chakula nyumbani, anaosha mikono kabla ya kuanza kupika*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark). 3.I don't know (0 mark).
17.	Does the one preparing food at home, wash the knife used to cut raw meat before	1.No *Hapana* (0 mark).

	continuing chopping vegetables like tomato? *Je, anayeanda chakula nyumbani, anaosha kisu kilichotumika kukata nyama mbichi kabla ya kuendelea kukataa mboga mboga nyingine kama nyanya*?	2.Yes *Ndiyo* (1 mark). 3.I don't know (0 mark).
18.	a) Do you drink milk? Je, wewe una kunywa maziwa?	1.No *Hapana.* 2.Yes *Ndiyo.*
	b) If, YES do you drink unpasteurized milk? *Kama, ndiyo je wewe unakunywa maziwa mabichi?*	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
19.	Do you often eat salad? *Je wewe unakula saladi/mboga mboga mara kwa mara?*	1.No *Hapana.* 2.Yes *Ndiyo.*
20.	Do you eat unwashed or unpeeled fruits? *Je wewe unakula matunda ambayo hayajaoshwa au kumenywa*?	1.No Hapana (0 mark). 2.Yes *Ndiyo* (1 mark).
21.	What is the source of drinking/ washing/cooking water? *Nini chanzo cha maji unayokunywa*?	1.Tap *water Maji ya bombani.* 2.Well *water Maji ya kisima.* 3.River *water Maji ya mtoni.* 4.Spring *well Maji ya*

		chemchem.
22.	a) Do you treat water? *Je, unatibu maji*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
	b) If, YES how? *Kama ndiyo, Elezea*	……..
23.	In your daily activities, do you come into contact with soil? *Katika shughuli zako za kila siku, je unagusa udongo*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).
24.	Do you eat soil? *Unakula udongo*?	1.No *Hapana* (0 mark). 2.Yes *Ndiyo* (1 mark).

Appendix 4: Mahook DNA extraction method.

MATERIALS.

1. TE Buffer
2. Proteinase K
3. Ammonium acetate
4. 70% ethanol
5. Distilled water
6. Eppendorf tubes
7. Vortex machine
8. Centrifuge machine
9. Refrigerator
10. 1000 µL tips
11. 200 µL tips

PROTOCOL

1. Add 300µL of TE (Tris-EDTA) extraction buffer (0.2M Tris-HCL (PH 8), mM EDTA, 0.5M NaCL, 1% SDS) to the 1.5µL tube containing the sample and acid sand wash.

2. Add 200µL of TE extraction buffer containing proteinase K.

3. Vortex to mix and incubate the tube in water bath at 65ºC for 30min.

4. Add 250 µL of 7.5 Ammonium acetate.

5. Mix and incubate the sample on ice or at 5ºC in the refrigerator for 10 min

6. Centrifuge for 15min at 14700rpm.

7. Decant the supernatant and wash DNA pellet with 800 µL of cold 70% ethanol, and then incubate at -20 for 10min.

8. Centrifuge for 5min at 13000rpm, turn the tube upside down on clean sterile. paper towel for 10-15 min to air-dry DNA.

9. Transfer DNA solution to 1.5 µL microcentrifuge tube, add 5 µL of RNase.

10. Incubate at 37ºC for 60min.

11. Suspend the DNA with 60 µL of 1X TE or distilled water.