SEROPREVALENCE OF *TOXOPLASMA GONDII* IN SLAUGHTERED PIGS AND RISK OF EXPOSURE FOR ABATTOIR WORKERS IN KIAMBU COUNTY, KENYA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMETS FOR THE AWARD OF THE MASTER OF VETERINARY PUBLIC HEALTH (MVPH), OF UNIVERSITY OF NAIROBI

DR. DORCAS CHEPYATICH

J56/19285/2019 (BVM)

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE

COLLEGE OF AGRICULTURE AND VETERINARY SCIENCE

UNIVERSITY OF NAIROBI

MAY, 2022

DECLARATION

I hereby declare that this thesis is my original work and it has not been submitted for award of a degree in any other University or Institution of higher learning.

Signature……Date…14/4/2022……

Dorcas Chepyatich, (BVM)

J56/19285/2019

SUPERVISOR APPROVAL

This thesis has been submitted for examination with our approval as university supervisors:

DR. PETER GATHURA (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Signature: ……Date: 19/04/2022

DR. LIAN F. THOMAS (BSc, DVM, PhD MRCVS)

University of Liverpool

Signature: ……Date: 26th April 2022

DR. JOSHUA ORUNGO ONONO (BVM, MSc, MBA, PhD)

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Signature: …… Date: 19/04/2022

DEDICATION

I dedicate this work to my family. Special thanks to my mother Christine Cheptanu Chumang'ole and my brothers; Moses Chumang'ole and Michael Chumang'ole for their constant prayers for my wellbeing and financial support throughout this academic journey.

I also dedicate my dissertation to University of Liverpool – Wellcome Trust Institutional Strategic Support Fund, the University of Liverpool Early Career Research Fund, the Soulsby Foundation for funding this field work, ILRI Graduate Fellowships for the financial support and University of Nairobi for providing me with strong foundation for my studies.

ACKNOWLEGMENT

I would like to acknowledge my supervisors, Dr Peter Gathura, Dr Lian F. Thomas and Dr Joshua Onono for their countless hours of reading, reflecting, encouragement and patience throughout the process. Their willingness to provide immediate feedback made this research work easy and of good experience. I would like to acknowledge the chairman of Public Health, Pharmacology and Toxicology Department, Prof James Mucunu Mbaria for allowing me conduct my research work and any assistance requested. Special thanks to the members of Immunology laboratory Dr. Nduhiu Gitahi the principal technologist, Mr Robert Kosgei Ronoh, senior laboratory technologist and Mrs Grace Akinyi Pacho the laboratory technician for their support and participation in this study. Special thanks to Dr. Nicholas Bor, the field coordinator and Dr Derrick Sentamu, research colleague for their countless help and support. I acknowledge the management and workers at the study abattoir for facilitating this work. Finally, special thanks to Nelly. J. Bargoiyet, Mary Mwihaki Kemboi, Denis Kigano Mwangi and Mary Wambui Nduati, DVS interns for their hard work during the fieldwork.

TABLE OF CONTENT

TABLE OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ELISA	Enzyme Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
IHAT	Modified hemagglutination test
IFAT	Immunoflourescent Antibody Test
IgG	Immunoglobin G
IgM	Immunoglobin M
IHA	Indirect Hemagglutination Assay
MAT	Modified Agglutination Test
NACOSTI	National Commission for Science, Technology and Innovation
ODK	Open Data Kit
RPM	Revolution per minute
T. gondii	*Toxoplasma gondii*
WHO	World Health Organization
(χ2)	Chi-square

ABSTRACT

Toxoplasmosis is one of the neglected zoonotic diseases with serious health impact in immunocompromised individuals. Ingestion of infective stages of *Toxoplasma gondii* (*T. gondii*) present either in raw or uncooked meat, contaminated vegetables, fruits and water is the major route for human infections. The main aim of this study was to determine the seroprevalence of *T. gondii* in pigs slaughtered at Ndumbu-ini abattoir and predisposing practices that exposes slaughterhouse workers to this infection across four operational areas within the slaughterhouse: stunning area, scalding and dehairing area, evisceration and cutting and dispatch area.

A cross-sectional study was conducted at a medium-scale pig abattoir in Kiambu County, Kenya where 529 blood samples were collected from pigs slaughtered between the 5th January and 5th March, 2021. Data on the county of origin, farm size, sex and pig liveweight were collected in pigs that were recruited into the study. Sera samples obtained were subjected to Indirect-Enzyme Linked Immunosorbent Assay to detect the presence of *T. gondii* Immunoglobin G (IgG). The overall seroprevalence of *T. gondii* was 34.53% (95% C.I. 30.16 - 39.17%). Pigs' liveweight was used as a rough proxy for age and a statistically significant association was found between increasing liveweight and *T. gondii* status in slaughtered pigs ($p = 0.044$). Farm size also was statistically significant with *T. gondii* seropositivity (<0.001) and when subjected to logistic regression model, farm size with approximately >100 pigs was a significant protective factor towards *T. gondii* seropositivity (OR= 0.17).

Qualitative data were collected on various predisposing practices that would expose slaughterhouse workers to infection across four operational areas within the pig slaughterhouse. Within the slaughterhouse, 100 % of the abattoir workers did not wash their hands and working tools with soap and water at the stunning and scalding and dehairing areas and 95.83% at the evisceration points. Washing of hands and working tools with soap and water was only

practiced at cutting and dispatch section. Abattoir workers were observed adhering to basic personal protective clothing with 100 % wearing their gumboots and white overcoats in three stations with only a small number, 1.04% who did not wear their white overcoats at the cutting and dispatch area. Wearing of gloves was not a common practice with 100 % of workers at stunning, scalding and dehairing and evisceration areas and 95.83% at cutting and dispatch section were observed not wearing gloves. Splashing of blood and raw pork on the faces of the slaughterhouse workers was observed in; 62.50% of workers at the stunning area, 50 % at the scalding and dehairing area, 58.33% at evisceration and 62.50% at cutting and dispatch section. A small number, 8.33% and 4.17 % were observed eating within the slaughterhouse at evisceration and cutting and dispatch sections respectively. About 71.0 % of workers at stunning, 70.83% at scalding and dehairing, 45.83% at evisceration and 66.67% at cutting and dispatch areas were observed not wearing their mask properly despite the ongoing Covid-19 pandemic. This finding calls for training of slaughterhouse workers on the risks for exposure to zoonoses during slaughter process and the need for management to mitigate these risks by establishing standard operating procedures at the abattoirs to minimize risk of infections of workers and also to guarantee food safety.

Keywords: Toxoplasmosis, *Toxoplasma gondii,* foodborne zoonoses, exposure, risk practices, pork.

CHAPTER ONE: BACKGROUND

T. gondii is a zoonotic parasite that affect warm-blooded animals including man (Zhou *et al*., 2018). The parasite exists in three infective stages; the oocysts, tachyzoites and bradyzoites. Oocysts are released through feces of infected cats, the definitive host after completion of the sexual stage of the parasite's lifecycle. Tachyzoites and bradyzoites are involved in the asexual stage of the parasite's lifecycle in man, sheep, goats, pigs, birds, rodents, cattle and poultry, the intermediate hosts (Zhou *et al*., 2018).

People get infected through consumption of oocysts in contaminated water, soil, fruits and vegetables and tissue cysts present in raw or undercooked meat. Humans are majorly infected through consumption of infected raw or undercooked meat and infection rates of between 10% - 30% have been found in most countries (Dubey & Jones, 2008). In pregnant women, the parasite can be passed to the unborn child through the placenta. Globally, *T. gondii* is estimated to infect 30% of human population with over 1 million toxoplasma infection cases being reported in the European nations yearly by the World Health Organization (Demar *et al*., 2007). The infections are unnoticed in persons with strong immune system but it cause serious health complications in people with weak immunity like encephalitis, ocular abnormalities and respiratory infections (Bamba *et al*., 2017). Transplacental infection during the first trimester of pregnancy leads to miscarriages, stillbirths and congenital anomalies such as hydrocephaly and microcephaly (Weiss & Dubey, 2009a)

Pigs are among the major reservoirs of *T. gondii* and they acquire the infections postnatally by consuming sporulated oocysts in dirtied feed, soil and water or by eating bradyzoites in tissue cysts of infected intermediate hosts pigs (Weiss & Dubey, 2009b). Infection through placental route occur also in pigs (Weiss & Dubey, 2009b) . Studies have shown the presence of *T. gondii* in pigs. In Mexico, the status of *T. gondii* in fattening pigs as reported by

Ortega-Pacheco *et al*. (2013) was 95.8% while Sandfoss *et al*. (2011) reported 27.7% Toxoplasma IgG antibodies in feral pigs in Eastern North Carolina . In Africa, a sero-epidemiological study to determine *T. gondii* status in food animals reported 26.0% prevalence in pigs (Tonouhewa *et al*., 2017). In Jos, Plateau State Nigeria, the seropositivity was found to be 46.2% (Ishaku *et al*., 2018) and in Central Ethiopia, the prevalence of *T. gondii* in pigs as reported by Gebremedhin *et al*. (2015) was 32.1%.

Slaughterhouse workers are at risk of contracting zoonotic infections from infected animals they interact with while at work. In developing countries, the slaughtering process has various gaps that can cause transmission of zoonotic pathogens to abattoir workers and these include; Lack of good infrastructure, inadequate training and inability to perform proper ante and carcass inspection (Herenda *et al*., 1994). Some of the studies that has reported zoonotic infections among the slaughterhouse workers include a study in Nigeria where abattoir workers were reported to contract leptospirosis from infected animals brought in for slaughter (Abiayi *et al*., 2015). Studies in Uganda and Tanzania identified brucellosis as an occupational hazard to abattoir workers without personal protective clothing (Nabukenya *et al*., 2013; Swai *et al*, 2009). Studies in Tanzania and Kenya also reported outbreaks of Rift valley fever in slaughterhouse workers (Mohamed *et al*., 2010; Nguku *et al*., 2010). Study by Cook *et al.* (2017) who screened slaughterhouse workers for zoonotic infections in western Kenya found the following prevalence; brucellosis 0.1%, leptospirosis 13.4%, Q fever 4.5%, Rift Valley fever (RVF) 1.2%, taeniasis 1.8% and cysticercosis 2.6% and a study in Thika- Kenya that reported 39.1% human toxoplasmosis in slaughterhouse workers (Thiongo *et al*., 2016a).

Handling of raw meat without protective equipment pose high risk of *T. gondii* infection in man. A study in Mexico on *T. gondii* in workers whose occupation expose them to uncooked meat found that workers who do not wear protective clothing at work have a higher seroprevalence as compared to those who wear them, (Alvarado-Esquivel *et al*., 2011). Eating while

at work without washing hands can also lead to ingestion of infective stages present in the raw meat. Alvarado-Esquivel *et al*. (2011) found that slaughterhouse workers and butchers who eat at work and those who do not eat had *T. gondii* seroprevalence of 9% and 4% respectively. For those undertaking slaughter of animals and preparation of food products, wearing of protective clothing such as, mask, protective glasses, gloves, overalls and gumboots, and avoiding eating while handling raw pork will help to prevent one from the infective stages present in raw meat (Tenter *et al*., 2000) . High standard of hygiene should be maintained by both the slaughterhouse workers and the butchers to avoid infection. Tissue cysts and tachyzoites are both killed by water (Jacobs *et al*., 1960) and therefore, washing hands and working tools with water and soap is highly recommended.

1.1 Problem statement

Toxoplasmosis causes serious health issues in humans especially in immunodeficient people i.e.; those who have undergone tissue transplants, HIV/ AIDS patients, cancer patients, those treated with certain forms of cancer therapy, pregnant women, diabetics, people with chronic illness, the very young and the elderly (Tenter *et al*., 2000). Encephalitis is a common condition associated with toxoplasmosis in immunocompromised patients and it causes great damage to them. Ocular disease may also occur, causing pain, tearing and excessive sensitivity to light (Tenter *et al*., 2000). *Toxoplasma gondii* infections can be associated with mental disorders that may bring about negative impact on human behavior, personality and other phenotypic traits,(Flegr, 2020), Schizophrenia is one of the neurological disorders that is related to *T. gondii* infection (Webster *et al*., 2013). Nisbet *et al*. (2018) stated a prevalence of 32% human toxoplasmosis in a research at a private teaching hospital in Kenya while a study by Wambua & Ndele (2014). on *T. gondii* infection in donated blood reported a seroprevalence of 42.3% via IgG ELISA.

There is scarce information regarding *T. gondii* infection status in food animals in Kenya despite them being the major reservoirs. No study has been published on pigs despite the fact that these animals are commonly raised in Kenya under different production systems including free ranging and backyard production systems (FAO, 2012) that may increase their exposure to infections with *T. gondii* cysts. Therefore, this study investigated the prevalence of *T. gondii* in slaughtered pigs and risk of exposure to abattoir workers at Ndumbu-ini abattoir in Kiambu County, Kenya.

1.2 General Objective

The general objective of this study was to investigate prevalence of *T. gondii* in slaughtered pigs and risk practices that exposes slaughterhouse workers to infection at a medium-throughput pig abattoir in Kiambu County, Kenya

1.3 Specific objectives

The specific objectives included:

1. To estimate the sero-prevalence of *T. gondii* in pigs presented for slaughter in a medium-throughput slaughterhouse in Kiambu County, Kenya.
2. To determine the risk practices that exposes slaughterhouse workers to infection in a medium-throughput slaughterhouse in Kiambu County, Kenya.

1.4 Justification

Toxoplasmosis is one among the neglected zoonotic infections despite the high burden on health systems. Pigs are among the major reservoirs of *T. gondii* and the demand in pork meat in Kenya is anticipated to increase at a rate of 400 tons yearly with the present population rise of approximately 1 million persons and eating rate of 0.4kg for each (Mburugu-Mosoti, 2017). The results from this study will trigger the need for health education, risk communi-

cation and social mobilization of the society towards the success in interrupting the transmission chain of *T. gondii* infections. It will also provide evidence for policy formulation and building of effective inter-sectoral collaboration between the animal and human health sectors and formation of workable zoonotic disease surveillance and control system (one-health platform) that answers to zoonotic disease occurrence in the country. This study also identified research gaps on risk assessments and appropriate zoonoses mitigation plans to be built for Kiambu County and similar production systems where pigs are raised and slaughtered.

CHAPTER TWO: LITERATURE REVIEW

2.1 Aetiology of toxoplasmosis

Toxoplasma gondii, an obligate intracellular protozoan parasite is the causative agent of toxoplasmosis. Toxo is a Greek term for bow or arc in reference to organism's shape. The parasite is zoonotic in nature and can affect warm-blooded animals as well as man *(*Tenter *et al.,* 2000). Household cats and other felids are the definitive host, implying that the parasite is only capable to complete its sexual life cycle in them releasing unsporulated oocysts through their feces (Webster, 2010) . It was first observed by Nicolle and Manceaux in 1908 in blood, spleen and liver of *gondis* a species of a rodent in North Africa *(*Tenter *et al.,* 2000). The arc-shaped protozoan parasite was named after its shape, (Toxon: arc, plasma: form) and its host the gundis (Morrissette & Ajioka, 2009).

2.2 *Toxoplasma gondii* lifecycle

Toxoplasma gondii has 2 distinct life cycles; the sexual and asexual life cycles. The sexual life cycle occurs only in cats, the definitive host while the asexual life cycle occurs in other mammals (including man) and various strains of birds *(*Tenter *et al.,* 2000).

2.2.1 The sexual life cycle

Felines become infected with *T. gondii* by ingestion of tissue cyst bradyzoites in raw meat. Within the cat's gastrointestinal system, the sexual stage begins where the macrogametocytes and microgametocytes develop from ingested bradyzoites and fuse to form zygotes. The zygotes then become encapsulated with rigid wall and shed as oocysts. The zygote sporulate to form sporozoites within the oocyst which become infectious 24 hours or more after the shedding of the feces *(*Tenter *et al.,* 2000).

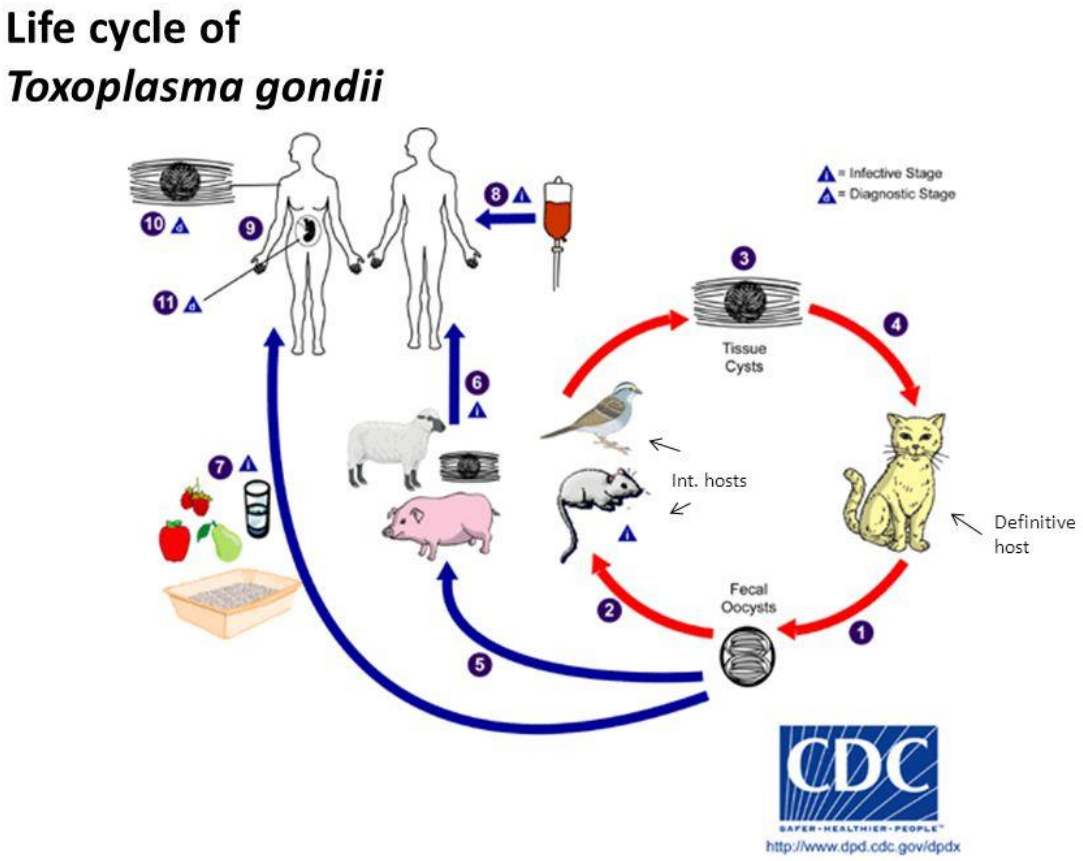


Figure 2. 1: Lifecycle of *T. gondii* (Photo credit; CDC.gov)

2.2.2 The asexual stage

When the intermediate host (Int. hosts) consumes tissue cyst (containing bradyzoites) or sporulated oocysts from the environment, the parasites first invade cells in and surrounding the intestinal epithelium, and in these cells, the parasites differentiate into tachyzoites, the motile and quickly multiplying cellular stage of *T. gondii* (Dubey, 2009). Asexual development occurs in two phases. The first phase occurs during the acute stage where tachyzoites multiply rapidly in many different types of host cells. Inside host cells, the tachyzoites replicate inside parasitophorous vacuoles (a structure produced by apicomplexan parasites that allow the parasite to develop while being protected from phagolysosomes of the host cell).

Tachyzoites multiply inside this vacuole until the host cell dies and ruptures releasing the tachyzoites via the blood stream to all organs and tissues of the body (Dubey, 2009) .

The 2nd phase is the chronic stages of infection, pressure from the host's immune system causes tachyzoites of the last generation stage-convert to bradyzoites to form tissue cysts. Tissue cysts in brain and muscle tissue form approximately 7–10 days after initial infection. Within the tissue cyst, bradyzoite multiply slowly by endodyogeny, they are the terminal life-cycle stage in the intermediate host and are immediately infectious (Dubey, 2009). When a definitive host consumes a tissue cyst (containing bradyzoites), bradyzoites convert into merozoites inside intestinal epithelial cells. The merozoites initiate another asexual phase of proliferation which consists of initial multiplication by endodyogeny followed by repeated endopolygeny in epithelial cells of the small intestine. The terminal stages of this asexual multiplication initiate the sexual phase of the life cycle (Dubey, 2009).

2.3 Review of studies on prevalence of *T. gondii* globally

Toxoplasma gondii infection in pigs has a worldwide distribution with varying prevalence according to sex, age, management system and geography. Low prevalence of < 1% have been found in pigs under confined and controlled management where rodents and cats have limited or no access while high prevalence of >60% have been found in pigs reared in uncontrolled management systems where pigs are allowed to roam freely in search of food (De Berardinis *et al*., 2017)

In a study to estimate *T. gondii* status in slaughtered pigs in Poland, 11.9 % of the sampled pigs were seropositive with pigs from small size farms and those that were extensively managed showing higher seroprevalence. The results highlighted a possible public health risk to pork consumers in the region (Sroka *et al*., 2020). In Northeastern China, serum samples from pigs were collected from 9 counties of Jilin province in a study to investigate the sero-

prevalence of *T. gondii* in the region. Of the sampled pigs 19.1% had *T. gondii* antibodies with the breeding boars and sows having higher seroprevalence. The results showed that the infection is widespread in the region and is a public health concern but there were no significant differences among the piglets, fattening pigs and slaughter pigs (Yang, 2020). Sandfoss *et al*., 2011 reported a seroprevalence of 27.7% in a study to determine the prevalence of *T. gondii* in feral pigs of eastern North Carolina showing their exposure to the parasite and they may be a source of infection to humans and domestic pigs.

In a study to estimate the prevalence of *T. gondii* in pigs from fattening farms in Yucatan Mexico, 95.8% of the sampled pigs had IgG antibodies against the infection indicating a high flow of *T. gondii* in these farms. The risk of infections was higher in small farms and manual feeders were found to be a significant protective factor against *T. gondii* infections in pigs (Ortega-Pacheco *et al*., 2013). Olsen *et al* (2019) conducted a systematic review and meta-analysis to determine the seroprevalence of *T. gondii* in domesticated animals and those that are hunted for food in Nordic-Baltic region in Northern Europe and found that a number of animals have been exposed to *T. gondii* infection with 6% seroprevalence in pigs. In Netherlands, a prevalence of 38% was obtained in a study which showed that animal friendly pig-production system causes re-emergence of *T. gondii* infection in pigs while those from indoor system were all negative (Kijlstra *et a*l., 2004).

2.4 Review of studies on prevalence of *T. gondii* in North Africa

A review study that collected, updated and analyzed epidemiological data on *T. gondii* status in food animals and people of North Africa reported the following; In Morocco, the only study that reported *T. gondii* status was in sheep and goats. The prevalence of *T. gondii* in sheep was found to be less than 30% and there was no significant difference in prevalence of *T. gondii* in sheep from the studies reported in the country. This resemblance can be attribut-

ed to access of cats to the farms and the management system. Studies on *T. gondii* status in goats has only been conducted in Northern Morocco and has reported a low prevalence of 8.5% (Rouatbi *et al*., 2019).

In Algeria, the prevalence of *T. gondii* in sheep, goats and cattle were 11.6%, 13.2% and 3.9% respectively. The prevalence in cattle was lower than in small ruminants showing the low susceptibility of cattle to *T. gondii* infection. Seasons, lack of history of abortion and animal origin were the main risk factors to *T. gondii* infection. Summer, Autumn and spring characteristically favored survival of the parasite's oocysts. The infection rates were reported to be higher on the northern part of Algeria than the southern part due to its high humidity (Rouatbi *et al*., 2019). In Tunisia several studies have been conducted to determine *T. gondii* status in sheep. A high seroprevalence rate of 73.6% was reported by use of modified agglutination test (Rouatbi *et al*., 2019). Boughattas *et al* (2014) also reported a seroprevalence of 38.2% and 73.6% in young and adult sheep respectively by use of modified agglutination test. These reports shows that consumption of sheep meat is a risk factor for *T. gondii* transmission to humans. In a study to assess whether toxoplasmosis can be transmitted through contaminated food, goat milk was tested by molecular method and an infection rate of 7.8% was reported (Amairia *et al*., 2016). In Libya 76.6% of sheep in western part of the country were seropositive with *T. gondii* claiming the presence of cats, climatic conditions and management system as the cause for the high seroprevalence (Azwai *et al*.,1993). In Egypt, serological and molecular methods have been employed to detect the presence of *T. gondii* in sheep and goats. A high seroprevalence of 98.4% and 41.7% and molecular infection rate of 67.6% and 25% has been reported in sheep and goats respectively (Ghoneim *et al*., 2010).

2.5 Review of studies on prevalence of *T. gondii* in West Africa and South Africa

A survey was conducted in Ghana to determine the prevalence of *T. gondii* antibodies in pigs across three regions of the country and an overall seroprevalence of 39% was obtained. This study also found that female pigs, animals raised in semi-intensive systems and older animals had higher levels of *T. gondii* antibodies. The results suggested that pigs in Ghana have been exposed to *T. gondii* and pork with viable cysts is source of infection to its consumers (Arko-Mensah, 1999). Tialla *et al.* (2019) reported *T. gondii* seroprevalence of 49.2% with sex, age, breed and production system showing significant association with *T. gondii* antibody levels in a study to determine *T. gondii* status in pigs in peri-urban and intra-urban areas of Burkina Faso. The study confirmed that pork consumers in the region are at risk of infection upon consumption of undercooked or raw pork. Knowledge on toxoplasmosis in the area will help mitigate the spread of the parasite.

Several studies have been conducted in Nigeria to determine the prevalence of *T. gondii* infection in pigs. Of these studies, Onyiche & Ademola (2015) reported a seroprevalence of 29.14 % with age being statistically significant where older animals as compared to the younger ones had higher exposure rate. Another study to determine *T. gondii* status in pigs slaughtered in Makurdi Nigeria, 4. 4% seroprevalence was obtained from the sampled pigs (Obijiaku *et al*., 2017). On the other hand, Ayinmode & Abiola (2016) while investigating possible sources of *T. gondii* infection for humans in Ibadan Nigeria, serum samples from slaughtered pigs revealed an infection rate of 45.2% , making pork a potential source of human toxoplasmosis .

In South Africa, studies were done to determine *T. gondii* status in domesticated animals and the seroprevalence of *T. gondii* in pigs was 33.96%. Among the risk factors, age, production

system, location, climate, seropositive cats and cats accessing feed stores were statistically significant with *T. gondii* seropositivity (Tagwireyi *et al*., 2019)

2.6 Review of studies on prevalence of *T. gondii* in East Africa

Studies that have reported prevalence of *T. gondii* in animals in Eastern Africa are scarce. In Uganda the first report of *T. gondii* status in pigs was documented in 2016 by Roesel *et al* (2016) who reported a seroprevalence of 28.7% with significant association between the age, presence of cats in the pig premises, source of water and presence of wildlife in the farms. Gebremedhin *et al*. (2015) reported a prevalence of 32.1% in a study to determine the seroprevalence of *T. gondii* in pigs from central Ethiopia. In this study, pigs that were raised from outdoor were at a higher risk of getting infected with *T. gondii* than those from indoor production system. Pigs that were fed with animal byproducts were also at higher risk of acquiring the infection. Similar studies by Mulesa Mugersa in her thesis dissertation reported an infection rate of 32.7% in pigs from selected parts of central Ethiopia (Kebeta, 2014.). The high infection rate in pigs suggested that pigs are a source of infection to man.

The status of *T. gondii* in pigs in Kenya is yet to be reported. The only study that have reported *T. gondii* infection in food animals in Kenya is a study by Thiongo *et al*. (2016b) who reported prevalence of 79.0% in chicken raised under outdoor production in Thika region. The results showed that chicken that are left to roam in search for food are highly exposed to *T. gondii* and are potential source of infection to humans.

2.7 Review of studies on exposure factors for *T. gondii* to abattoir workers

Abattoirs in developing countries are poorly regulated due to scarce resources and inappropriate facilities. Diseases are easily contracted in these abattoirs due to inadequate infrastructure, poor hygiene, lack of proper training and insufficient ante and carcass inspection (Herenda *et al*., 1994). Abattoir workers are at risk of work-associated injuries such as hands

and arms injuries from the sharp working tools (Cai *et al*., 2005) and exposure to disease due to their proximity to animals and animal products (Dorjee *et al*., 2011). These diseases are contracted through accidental ingestion and instillation in wounds (Taylor *et al*., 2001). Exposure is aggravated by opening the carcass, being splashed with body fluids, working without proper personal protective equipment and poor hygiene practices (Cai *et al*., 2005).

2.7.1 Review of studies on exposure factors for *T. gondii* to abattoir workers globally

Handling of raw meat pose high risk of *T. gondii* infection in man, in a study on *T. gondii* infection in workers whose occupation expose them to uncooked meat in Mexico, Alvarado-Esquivel *et al.* (2011) reported the following: Workers who do not wear protective clothing at work have a higher seroprevalence as compared to those who wear them; Slaughterhouse workers and butchers who ate at work and those who did not eat had *T. gondii* seroprevalence of 9% and 4% respectively and finally, that about 7% of the individuals who had splash of blood and raw meat on their faces were seropositive with *T. gondii* as compared to 3% seropositive individuals who did not have splash of blood and raw meat on their faces. Tenter (2009), suggested from a study done to determine the transmission of *T. gondii* from animals to humans in America that wearing of protective clothing such as, mask, protective glasses, gloves, overalls and gumboots will help to prevent one from the infective stages present in raw meat. Jacobs *et al*. (1960), in a study on the resistance of the encysted form of *T. gondii* suggested that high standard of hygiene should be maintained by the slaughterhouse workers and the butchers to avoid infection as tissue cysts and tachyzoites are both killed by water, and therefore, washing hands and working tools with water and soap by the abattoir workers is highly recommended. Youssefi *et al.* (2018) also reported strict rules in industrial abattoirs in Iran where slaughterhouse workers were not allowed to eat and drink while working and they were supposed to change their cloths and wash their hands before eating and drinking in the eateries to avoid transmission of zoonotic infections.

2.7.2 Review of studies on exposure factors for *T. gondii* to abattoir workers in Africa

Ekanem *et al.* (2018) reported a seroprevalence of 55.8% in a study that determined the seroprevalence of *T. gondii* in abattoir workers in Southern Nigeria. Those that had higher risk of infection were workers who were exposed to poultry, those who had worked for more than 5 years and those who sold raw meat. Cook *et al*. (2021) reported an apparent prevalence of 84% in abattoir workers in western Kenya with increase in age, consuming blood and owning poultry being the risk factors for infection. These results highlighted a need for public enlightenment and mitigations plans for at risk population. A study to investigate the prevalence of *T. gondii* in slaughterhouse workers in Thika, Kenya reported an infection rate of 39.1% and an infection rate was higher (100%) in chicken slaughterhouse workers. The high infection rate was an indication for a need to creation of public awareness. Cook *et al. (*2017) reported that some risky practices that could lead to exposure of zoonotic diseases were present in western Kenya, with finding that only 20% of slaughterhouses had hand- washing facilities, around half of the workers wore personal protective clothing; with 49% wearing rubber boots and 53% wearing protective coats and only 7% performed ante mortem inspection. The knowledge of disease transmission from animals to humans was only known by 31% of the slaughterhouse workers. The results of this study indicated a need for appropriate interventions to reduce exposure of various infections to workers.

2.8 Diagnosis of *T. gondii* in porcine host

Detection of *T. gondii* in pigs is achieved through 3 methods, that is; serological assays, bioassays and molecular methods (Polymerase Chain Reaction). Serological assays are rapid and accurate in detecting *T. gondii* antibodies and therefore preferred over the bioassays and PCR. Indirect Fluorescent Antibody Test (IFAT) is regarded as the gold standard test in the serodiagnosis of *T. gondii* in pigs. Modified agglutination test (MAT) and Enzyme linked-Immunosorbent assay (ELISA) are the most common used serological tests (Liu *et al*., 2015).

Modified agglutination test (MAT) is easier, cheaper than the others and doesn't require special equipment. However, the results are evaluated visually and subjectively, and it depends on the skills of the technician. An antibody-ELISA test is used to detect *T. gondii* antibodies indicating that a pig has been exposed to the infection (Liu *et al*., 2015). In cases of active infections, anti-*T. gondii* IgM antibodies will be detected. These antibodies appear fast after an infection and disappear faster than anti-*T. gondii* IgG antibodies after recovery making IgG antibodies detected in latent infections (Remington *et al*., 2004)

All the serological methods used in diagnosis of anti-*T. gondii* antibodies vary in their sensitivity, specificity and predictive values. In a study that compared the performance of MAT and Commercial ELISA for antibodies against *T. gondii* found that out of 70 bioassay positive pigs, 60 were positive by MAT (sensitivity of 85.7%) and 62 were positive in commercial ELISA (sensitivity of 88.6%) while out of 204 bioassay negative samples, 103 were negative in MAT (specificity of 94.6%) and 200 were negative by commercial ELISA (98% specificity) (Gamble *et al*., 2005). Another study done to assess diagnostic accuracy of a commercial antibody ELISA for the detection of *T. gondii* infection in pigs, the sensitivity and specificity of ELISA was found to be 98.9% and 92.7% respectively (Basso *et al*., 2013). ELISA therefore is a useful test for routine screening of pigs. Its automation potential and ability to connect plate readers to laboratory computers reduces transcription errors hence an added advantage of this test.

Mouse bioassay is the main method for detecting cysts in tissues. Biological testing for diagnosis is being reduced or avoided in the current trend, for apparent ethical and animal welfare considerations (Cenci-Goga *et al*., 2011) . However, given the great sensitivity and specificity that make this biological testing the gold standard, the option of inoculating or feeding Toxoplasma-free laboratory animals (mice and cats) to later demonstrate *T. gondii* in organs and tissues should be considered (Cenci-Goga *et al*., 2011) .

Detection of *T. gondii* DNA rely on PCR. This approach has been used in a range of clinical samples from animals and humans and have proven to be simple, sensitive (it can identify DNA from one tachyzoite), reproducible, and cost-effective (Su *et al*., 2010). In a study to determining *T. gondii* distribution in commercial cuts of pork from experimentally infected pigs, *T. gondii* was found in 27/40 (67.5%) and 9/40 (22.5%) of the examined pork samples by mouse bioassay and PCR respectively (Tsutsui *et al*., 2007). Tissue cysts of *T. gondii* were identified by mouse bioassay in 54/98 (55.1%) samples while PCR showed 25/150 (16.6%) positive samples in a study that was detecting *T. gondii* infection in tissues from experimentally infected pigs (Garcia *et al*., 2006)

2.9 Prevention and control of *T. gondii* in pigs and humans.

T. gondii infection in pigs is prevented through good husbandry practices on the farm, these include: 1) adopting an effective rodent control program to minimize mouse populations, 2) creating a level of biosecurity which reduces or eliminates exposure of swine to wildlife, 3) eliminating feral cats or securing feed and swine areas from access by cats, 4) prompt removal of dead pigs to avoid cannibalism, and, 5) changing or thoroughly washing boots before entering barns to avoid tracking in oocysts (Gamble *et al*., 2013).

Human infections are prevented through reduction of exposure from meat, water, vegetables and fruits. *Toxoplasma gondii* organisms are susceptible to extreme heat and cold. Consumption of undercooked meat should be avoided by cooking meat to a safe temperature of 67^{o}C or cooling it to -13^{o}C. A rest time of 3 minutes after the meat has been removed from the heating source is important because the pathogens are being killed at this temperature (Dubey *et al*., 1990). Freezing meat to below zero (0° F) temperatures before cooking also reduces chances of infection (Kotula *et al*., 1991). Drinking water must be treated and fruits and vegetables must be thoroughly washed before consumption (Jacobs, 1961). People handling meat at any stage of its value chain should wash their hands, the surfaces and utensils with water

and soap. Washing is important because the stages of *T. gondii* in meat are killed by water and soap (Jacobs, 1961). Anti- coccidial drugs are used in acute toxoplasmosis as soon as animals are suspected to be infected (Moré *et al*., 2018)**.** However many anti-toxoplasmic drugs are not effective in eradicating the parasite cyst (Hiszczyńska-Sawicka *et al*., 2014). This therefore, has brought about several attempts to develop effective vaccine against *T. gondii* (Burrells *et al*., 2015)

CHAPTER 3: METHODS AND MATERIALS

3.1 Study Site and study design

A cross-sectional study was conducted at Ndumbu-ini abattoir, a medium scale non- integrated pig abattoir which was established in 1972 and is located in Kiambu County. Kiambu County is located in the Central Kenya and is the 2nd most populous, with over 2.4 million people (KNBS 2019). The abattoir receives pigs from different parts of Kenya with the largest number (75%) from Nairobi and Kiambu, 20% from other Central counties and 5% from Western part of Kenya. It has a capacity to slaughter approximately 40 pigs a day and supplies approximately 10% of the pork demand of Nairobi, the capital city of Kenya (Murungi *et al*., 2021).

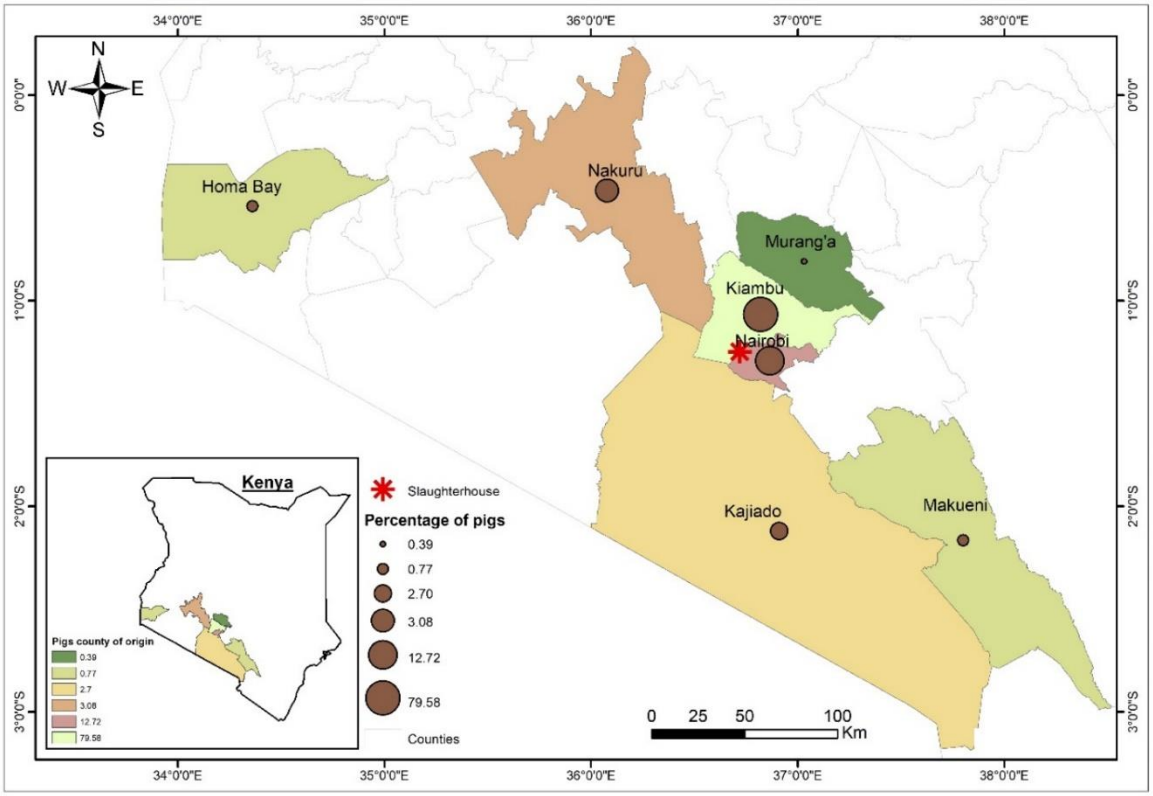


Figure 3. 1**:** Map showing the location of pig slaughterhouse and its catchment for pigs slaughtered in Kiambu, Kenya in 2021.

3.2 Sample size determination for prevalence of *T. gondii*

A minimum sample size was calculated to be 384 considering 50% prevalence due to lack of previous studies of *T. gondii* in pigs in Kenya, utilizing the formula by Dohoo *et al*.(2009).

$$n = \frac{Z_{\alpha}^{2} pq}{L^{2}}$$

Where n= sample size needed; Z= Confident interval of 95% (Standard value is 1.96)

p = prevalence (50%); q = (1-p); and L= Level of precision (5%). The calculated sample size was adjusted to 529 pigs to cater for any unforeseen challenge or loss of samples during processing.

3.3. Selection of study pigs

Pigs were selected using systematic sampling method. After identifying and recruiting the first pig to be presented for slaughter on each sampling day, every 2nd pig presented for slaughter was recruited. To reduce the influence of on-farm clustering on our prevalence estimate, any recruited pig that originated from the same farm as the previously recruited animal was ineligible for sampling and the next eligible pig was recruited, returning thereafter to every 2nd pig. After obtaining informed consent for recruitment from the person presenting the pig to slaughter, the pig was driven using pig board into the weighing crate for the measurement of liveweight using a weighing machine as shown in Figure 3.2a, and data relating to county of origin, husbandry system, farm size and sex were entered in an Open Data Kit (ODK) (Hartung *et al*., 2010) form on a mobile phone and uploaded to the International Livestock Research Institute (ILRI) server. To prevent contracting numerous zoonotic infections and contamination of the pork while collecting data, proper personal protective equipment such as; white overalls, gumboots, masks and gloves were properly worn.

3.4 Biological sample collection and transportation

At exsanguination, blood from the jugular vein was collected to pre-numbered 10ml red-top tube (without anticoagulant) corresponding to the recruitment number as illustrated in Figure 3.2b and the dead pig was tagged with a prenumbered ear-tag. The tubes were tightly closed and wiped down with a virkon and paper towel before being placed upright in to a rack within a cool box for transportation to the University of Nairobi department of Public Health Pharmacology and Toxicology for further processing.

3.5 Sample processing in the laboratory

At the laboratory, samples were centrifuged using NF 200 centrifuge model at 3,000 rpm for 20 minutes at room temperature. The serum samples were separated into two aliquots in 2 mL labeled cryovials as illustrated in Figure 3.2c and stored in a deep freezer at -20 °C before further analysis was carried out to detect the presence of the antibodies against *T. gondii.*

3.6 Detecting presence of antibodies for *T. gondii* using serological test

Serum samples were defrosted in batches and tested using ID Screen® Toxoplasmosis Indirect Multi-species (IDvet, Grabels, France) to determine the presence of *Toxoplasma* specific IgG antibodies. Samples were tested in duplicate according to manufactures directions. Briefly; 150 µl of each sample and one each of positive and negative controls were added to 150 µl of the substrate solution in individual Eppendorf tubes, vortexed and then incubated at room temperature for 20 minutes. After incubation, the samples and controls were centrifuged at 1500 rpm for 10 minutes, 150µl of the supernatant was put in to the Eppendorf tube with neutralization buffer then mixed by vortexing to form a sample of ¼ dilution, and 100 µl each of the positive and negative controls and 100 µl of each sample, in duplicate were added to a 96 well ELISA plate, sealed with microplate sealer then incubated for 15 minutes at 37 ^{o} C while shaking at 800 rpm. Wells were washed 5 times with wash buffer, after which 100 µl

conjugate solution was added to each well and incubated for 15 minutes at 37 ºC while shaking at 800 rpm, washing was repeated five times before 100 µl of substrate solution (TMB) was added in to each well and incubated in the dark for 15 minutes at room temperature before the reaction was stopped with 50 µl stop solution that was added to each well. Plates were read at 450nm with a 655nm filter as shown in Figure 3.2d.

Samples that presented a Sample/positive percentage (SP%) greater than 50% were considered positive, Samples that presented a SP% less than or equal to 40% were considered negative and samples that presented a SP% between 40% and 50% were considered unknown and they were re-tested. The test was considered valid when the positive control mean optical density value was >0.35 and the ratio of the mean value optical density of the positive and negative controls were >3.5. The results were entered in ODK forms and uploaded to ILRI server.

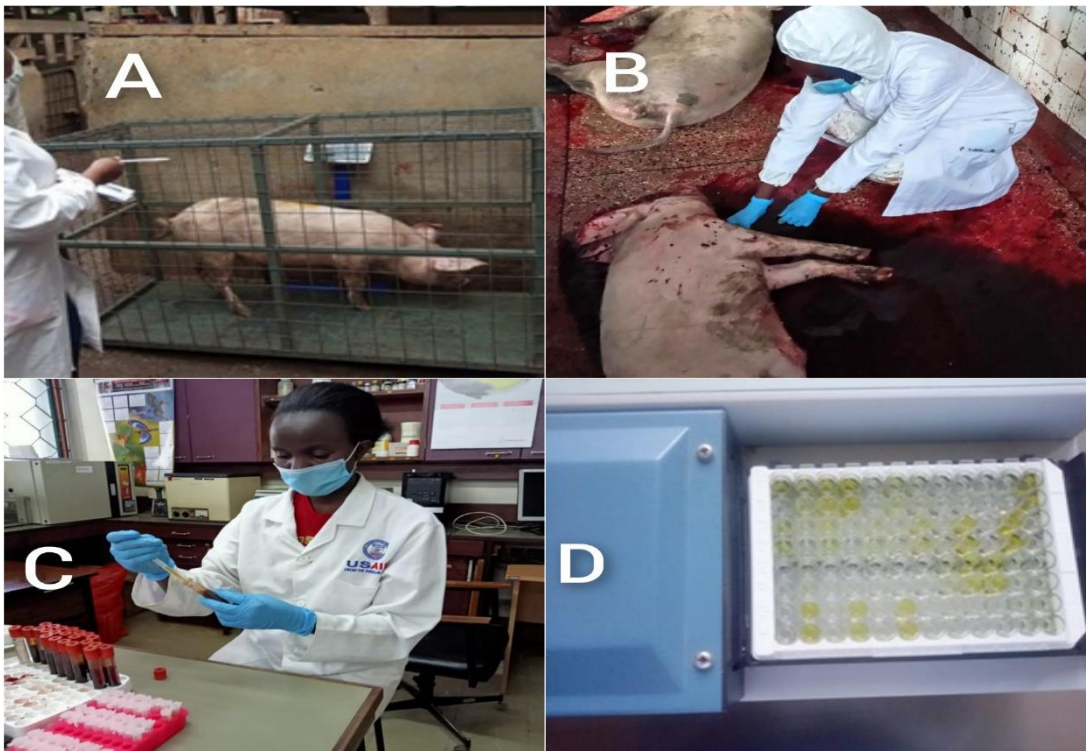
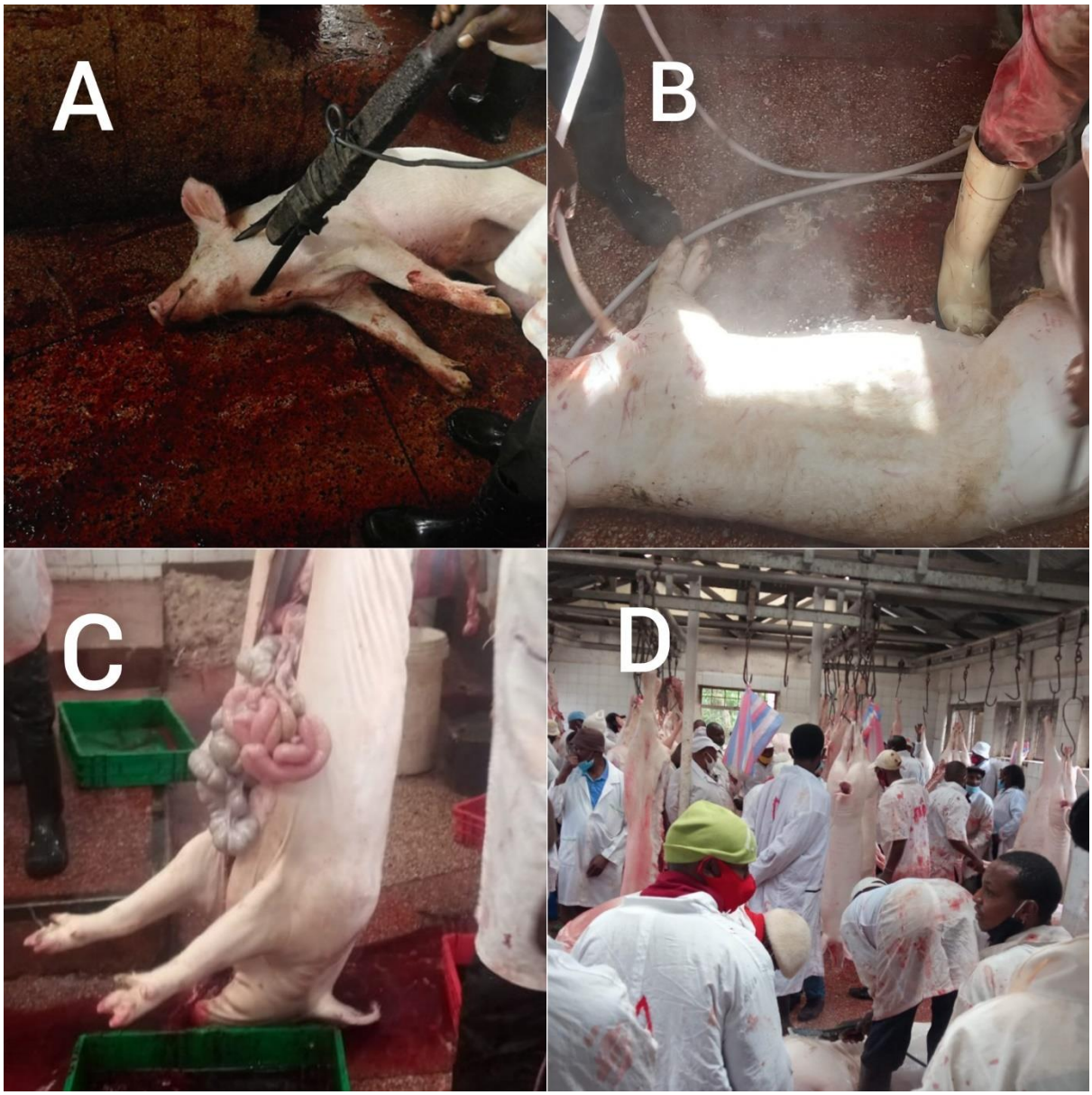


Figure 3.2: Images sho

wing; A: Pig weighing, B: blood collection, C: Serum aliquoting, D: microtiter plate

3.7 Data collection on predisposing factors for *T. gondii* infection by abattoir workers

Qualitative data were collected on various predisposing practices that would expose slaughterhouse workers to infection across four operational areas within the pig slaughterhouse: stunning area, scalding and dehairing area, evisceration and cutting and dispatch area as shown in Figure 3.3. The data that were collected included whether participants wear protective clothing (gumboots, white overalls, gloves and masks), eating while handling raw pork, had splashes of blood and raw pork on their faces and whether they washed their hands and working tools with soap and water. These data were collected by observation of at least 24 personnel per each operational area, and a total of 96 observations were made for the various risk practices. One of these four areas were observed per visit until 24 pigs had passed through the area, data was collected using the observation checklist presented in Supplementary material 1 in an ODK form and uploaded to the ILRI server.


A
B
C
D

3.8 Data Management and Analysis

Data that were collected in ODK forms and uploaded to the ILRI server were downloaded as. csv files, cleaned and merged for statistical analysis in R version 3.6.0 (2019-04-26) (R Core Team 2018). Descriptive statistical measure of proportions was calculated to estimate *T. gondii* seroprevalence and proportions for observed risk practices together with their 95% confidence interval using the DescTools package (Package, 2021). Further tests included computing Chi-square ($\chi 2$) test and students t-test to determine the association between *T. gondii* status and independent variables using Arsenal package (Ethan *et al*., 2021). The variables with significant association ($p < 0.05$) in the univariate analysis were further subjected to a logistic regression, glm () function of MASS package in R (Zeileis *et al*.,2008).

CHAPTER 4. RESULTS

4.1 Demographic characteristics of the sampled pigs

A total of 529 pigs were recruited for the study between 5th January and 5th March 2021, with 53.8% (95% C.I. 49.4-58.1%) female pigs and 46.20% (95% C.I. 41.89-50.57%) male pigs as shown in. The mean liveweight of sampled pigs was 58.8 kg (95% C.I. 56.6-61.1 kg) with a range of 13-230 kilograms. The majority of pigs slaughtered were from Kiambu County 79.58% (95% C.I. 75.7 9- 82.91%) followed by Nairobi County,12.72% (95% C.I. 10.04-15.96%) and the rest were from other central and western Kenyan Counties (Figure 4.1). The majority, 97.68% (95% C.I. 95.87 -98.74%) of recruited pigs had been housed while 2.32% (95% C.I. 1.26 -4.13%) were from outdoor systems. Majority, 63.48% (95% C.I 59.17-67.59%) were from farm size of approximately <10 pigs, followed by those from a farm size of approximately 10<50 pigs, 26.39% (95% C.I 22.70-30.42%) and the rest from other farm sizes as shown in Figure 4.2.

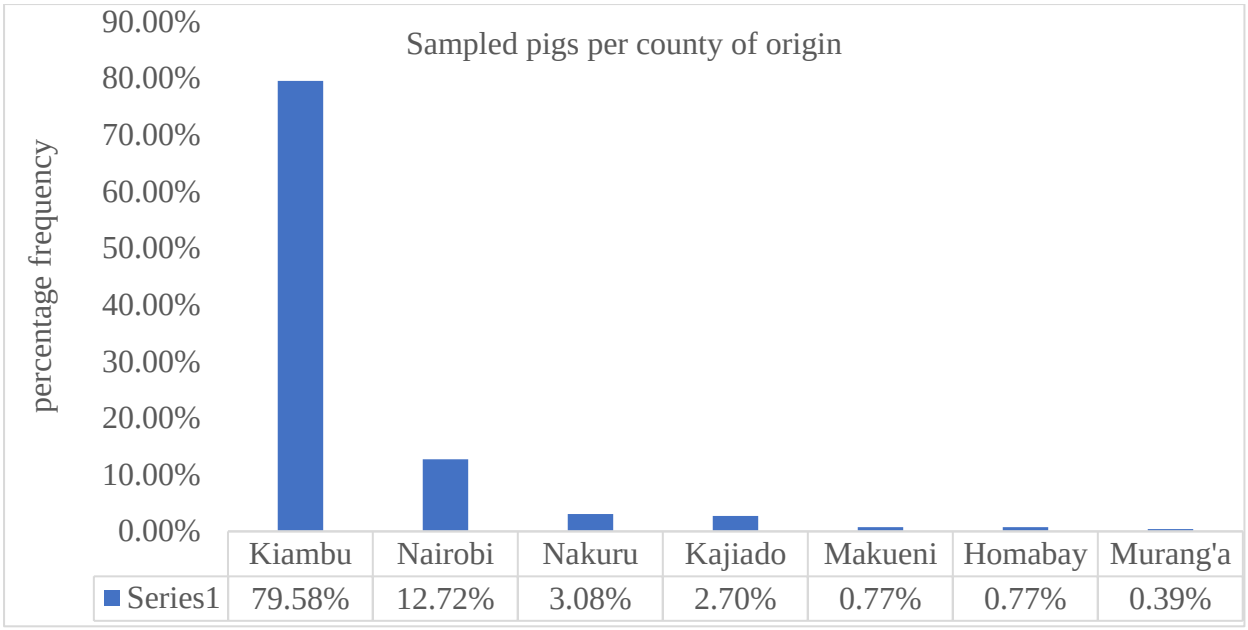


Figure 4. 1: The distribution of pigs by county of origin slaughtered at Ndumbu-ini abattoir Kiambu, Kenya in January-March 2021

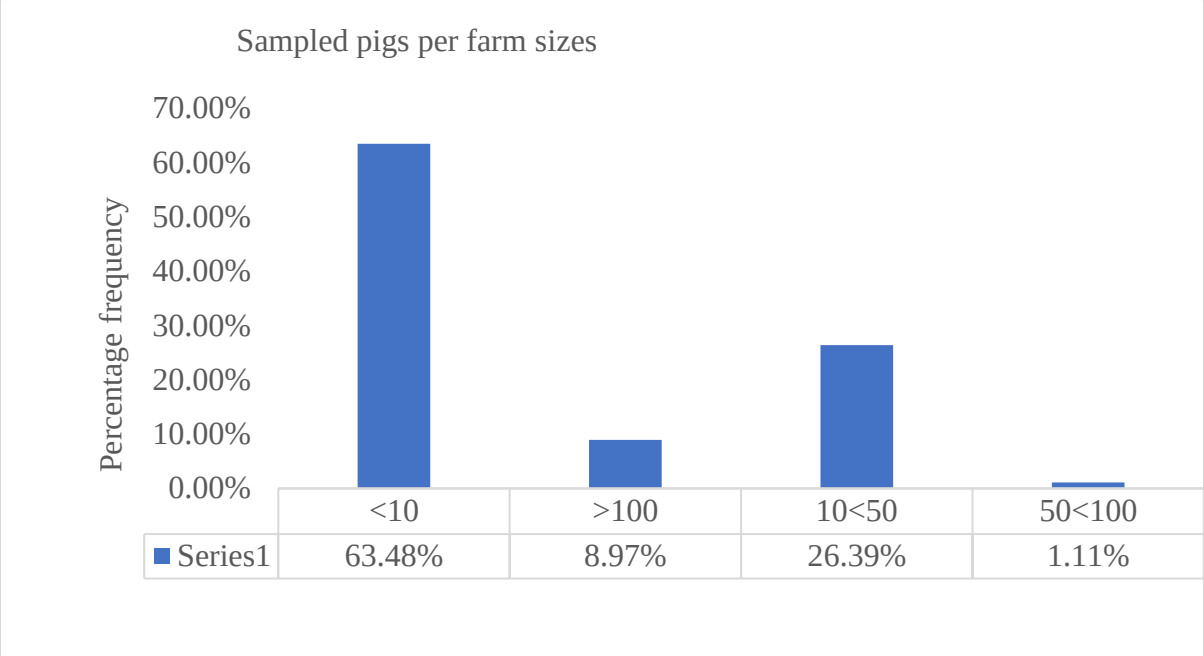


Figure 4. 2: The distribution of pigs by farm size slaughtered at Ndumbu-ini abattoir Kiambu, Kenya in January -March 2021

4.2 Estimated seroprevalence of *T. gondii* in slaughtered pigs

A total of 446 data were available for analysis after testing sera for *T. gondii* IgG antibodies and the overall seroprevalence was estimated to be 34.53% (95% C.I. 30.16 -39.17%). The data that was analyzed were less than the 529 sera obtained from the blood sampled due to an error that occurred while reading a set of sera samples in a microtiter plate reader and the test could not be re-run due to lack of extra ELISA kit. The sero-prevalence of *T. gondii* antibodies by pig variables and their P- values from univariate analysis are shown in Tables 4.1 and 4.2 below. Farm size (p = <0.001***) and mean live weight (p =0.044*) were statistically significantly associated with *T. gondii* status.

Table 4. 1 **The description of categorical variables and their P-values of pigs slaughtered at Ndumbu-ini abattoir Kiambu, Kenya in January - March 2021**

Variable	Category	Frequency	Number positive for *T. gondii* antibodies	Percentage positive for *T. gondii* antibodies	95% C. I	p-values
Sex	Female	243	85	34.98	29.06-41.38	0.744
	Male	200	67	33.5	27.07-40.55	
County of origin for pigs	Nairobi	56	24	42.86	29.97-56.73	0.083
	Kiambu	342	120	35.09	30.08-40.44	
	Makueni	4	1	25.00	1.32-78.06	
	Kajiado	15	3	20.00	5.31-48.44	
	Nakuru	14	1	7.14	0.37-35.83	
	Homabay	4	0	0.00	0.00-60.42	
	Murang'a	2	0	0.00	0.00-60.42	
Husbandry type	Housed	425	146	34.35	29.88-39.11	0.344
	Outdoor	10	2	20.00	3.54-55.78	
Farm size (number of pigs)	<10	270	110	40.74	34.87-46.88	<0.001***
	>100	45	4	8.89	2.89-22.13	
	10<50	114	33	28.95	21.03.38.31	
	50<100	6	1	16.67	0.88-63.52	

4. 2:

Table 4. 2: **Description of continuous variable and its p- value of pigs slaughtered at Ndumbu-ini abattoir Kiambu, Kenya in January- March 2021**

Variable	Range kilograms	mean	S. D	95% C. I	p- Value
Live weight	13-230	58.87	25.54	56.6-61.1	0.044*

***Significant association, *** highly significant association**

4.3 Factors associated with *T. gondii* seroprevalence in logistic regression model

Variables included in full model were; liveweight and farm size. No further model selection was performed. Farms of approximately >100 pigs were a significant protective factor against *T. gondii* seropositivity in pigs, (OR= 0.17, 95% C. I 0.05-0.45) as seen in table 4.3 .

Table 4. 3: **Logistic regression outcome for variables associated with *T. gondii* seropositivity for pigs slaughtered at Ndumbu-ini abattoir Kiambu, Kenya in January-March 2021**

Description of variables	**Estimates**	**SE**	**Z-Value**	**P- value**	**OR**	**95% CI**
Liveweight-kg	0.006	0.004	1.64	0.144	1.006	0.998-1.014
Farm Size>100	-1.734	0.544	-3.19	0.014**	0.177	0.052-0.459
Farm size10<50	-0.474	0.247	-1.92	0.055	0.622	0.379-1.003
Farm size 50<100	-1.111	1.105	-1.005	0.315	0.329	0.017-2.092

4.4 Risk practices that exposes slaughter house workers to *T. gondii* infection

Within the slaughterhouse, 100 % of the abattoir workers did not wash their hands and working tools with soap and water at the stunning, scalding and dehairing areas, and 95.83% (95% C.I.76.88-99.78%) at the evisceration points. Washing of hands and working tools with soap and water was only prevalent at cutting and dispatch section. Abattoir workers were observed adhering to basic personal protective clothing with 100 % wearing their gumboots and white overcoats in three stations with only a small number, 1.04% (95% C.I 0.05- 6.49%) who did

not wear their white overcoats at the cutting and dispatch area. Wearing of gloves was not common practice with 100 % of workers at stunning, scalding and dehairing and evisceration areas and 95.83% (95% C.I 76.88-99.78 %) at cutting and dispatch section observed not wearing gloves. Splash of blood and raw pork on the faces of the slaughterhouse workers was observed in; 62.5% (95% C.I 40.8-80.5 %) of workers at the stunning area, 50.00% (95% C.I 31.4-68.5%) at the scalding and dehairing area, 58.33% (95% C.I 37.0-77.7 %) at evisceration and 62.50% (95% C.I 40.76-80.45 %) at cutting and dispatch section. A small number, 8.33% (95% C.I 1.5-28.5 %) and 4.17 % (95% 0.22-23.12 %) were observed eating within the slaughter house at evisceration and at cutting and dispatch sections respectively. Majority, 70.83% (95% C.I 48.85-86.56 %) of workers at stunning, 70.83% (95% C.I 48.85-86.56 %) at scalding and dehairing, 45.83% (95% C.I 26.17-66.76 %) at evisceration and 66.67% (95% C.I 44.69-83.57 %) at cutting and dispatch areas were observed not wearing their mask properly despite the ongoing Covid-19 pandemic. The prevalence of these risk practices across the slaughter house can be seen in Table 4.4 below

Table 4. 4 **The prevalence of risk practices that expose abattoir workers to *T. gondii* infection as observed across the four areas in the abattoir in Kiambu, Kenya in January -March 2021**

STATIONS	STUNNING		SCALDING AND DEHAIRING		EVISCERA-TION		CUTTING AND DIS-PATCH	
	propor-tion	Percent (C. I)	proportion	Percent (C. I)	Propor-tion	Percent (C. I)	propor-tion	Percent (C. I)
Not wearing gumboots	0/24	0 (0.0-17.2)	0/24	0 (0.0-17.2)	0/24	0 (0.0-17.2)	0/24	0 (0.0-17.2)
Not wearing lab coats	0/24	0 (0.0-17.2)	0/24	0 (0.0-17.2)	0/24	0 (0.0-17.2)	1/24	4.17 (0.2-23.1)
Splash of blood and raw pork on face	15/24	62.5 (40.8-80.5	12/24	50.00 (31.4-68.5)	10/24	58.33 (37.0-77.7)	15/24	62.5 (40.76-80.45)
Incorrect use of masks	17/24	70.83 (48.85-86.56)	17/24	70.83 (48.85- 86.56)	11/24	45.83 (26.17-66.76)	16/24	66.67 (44.69-83.57)
Eating at work	0/24	0 (0.0-17.2)	0/24	0.00 (0.0-17.2)	2/24	8.33 (1.5-28.5)	1/24	4.17 (0.22-23.12)
Not wearing gloves	24/24	100 (82.83-100)	24/24	100 (82.83-100)	24/24	100 (82.83-100)	23/24	95.83 (76.88-99.78)
Not washing hands	24/24	100 (82.83-100)	24/24	100 (82.83-100)	23/24	95.83 (76.88-99.78)	1/24	4.17 (0.22-23.12)
Not washing tools	24/24	100 (82.83-100)	24/24	100 (82.83-100)	23/24	95.83 (76.88-99.78)	0/24	0 (0.0-17.2)

CHAPTER 5. DISCUSSION

The study showed an overall sero-prevalence of *T. gondii* infection using Indirect ELISA antibody test to be 34.53 % confirming the presence of *T. gondii* antibodies in pigs slaughtered at Ndumbui-ni abattoir. This indicates that these animals had been exposed to *T. gondii* infection either through consumption of contaminated feed and water and ingestion of tissue cysts containing bradyzoites. The present study reported higher seroprevalence than studies in; Poland, 11.9% using Direct agglutination Test (DAT) (Sroka *et al*., 2020) , Serbia, 9.2% with Modified Agglutination Test (MAT) (Klun *et al*., 2011) , Northeastern China, 19.1% through an indirect hemagglutination antibody test (IHAT) (Yang, 2020), Nordic-Baltic region, 6% in a systematic review and meta-analysis study (Olsen *et al*., 2019). In contrast higher seroprevalence than the current was reported in Jos Nigeria 46.2% by Ishaku *et al*. (2018). However, the result of this study was comparable with reports from; Central Ethiopia, 32.1% by Gebremedhin *et al*. (2015), South-west China, (30.6%), Northwestern Taiwan, (28.8%) (Fan *et al*., 2004) and in Peru (32.3%) (Suaréz-Aranda *et al*., 2000).

The difference in seroprevalence between the present study and the mentioned reports could be related to differences in; sensitivity and specificities of the diagnostic methods employed (Onyiche *et al*., 2015), sample sizes, cut-off values, sampling methods (Halová *et al*., 2013) and climatic variation (Dubey, 2016).

Dubey, (2016) reported that moist and warm climate is associated with high prevalence as compared to hot and dry. This argument was supported by Villari *et al.* (2009a) who reported that warm and moist environment promote survival and sporulation of *T. gondii* oocysts which is not so in hot and dry conditions. Other possible causes of high seroprevalence in this present study could be environmental and feed contamination with sporulated oocysts from feces of infected cats that gained free access to the pig premises and feed stores and also

feeding pigs with unwashed vegetables, fruits and household leftovers contaminated with the infective stages.

In this study, liveweight was used as a rough proxy for age and their mean had statistically significant association *(*p=0.04) with *T. gondii* seroprevalence in univariate analysis. This is in agreement with studies by Onyiche *et al* (2015) and Halová *et al* (2013), which found statistical association between age and *T. gondii* prevalence. It is therefore expected that the probability of exposure to the parasite would increase with the length of time animals are reared in the farms. Pigs from farms with approximately > 100 pigs had lower likelihood of getting infection as compared to those pigs from farm sizes with <100 pigs. Similar results were reported by Assadi-Rad *et al.* (1995) where sows from small farm size (<29 sows) were 4.5 times seropositive than those from large farm size (>29 sows). Also, Villari *et al.* (2009b) found that *T. gondii* seropositivity decreased with pigs from farms with more than 50 animals. This could likely be that farms with high population of animals have good husbandry practices put in place which include proper hygiene practices, intensive production system, rodent control systems and uncontaminated water and feed that reduced exposure to infective stages of *T. gondii* (Hove *et al*., 2005).

This high prevalence of *T. gondii* in the sampled pigs is a public health threat to pork consumers and people who are occupationally exposed to raw pork. In the recent past, the demand in pork in Kenya has been anticipated to increase at a rate of 400 tons yearly with the population rise of approximately 1 million persons and eating rate of 0.4kg for each (FAO, 2012). The increasing demand for pork suggests that there may be an increased risk of exposure to *T. gondii* through this value chain.

With respect to exposure factors for *T. gondii* infection, abattoir workers may be differently exposed to *T. gondii* infection depending on the activities they are involved in at their various

work stations. Those working at stunning, scalding & dehairing and at evisceration areas were observed not washing their hands and working tools with soap and water as compared to those working at cutting and dispatch area. These practices were likely to be due to the lack of direct provision of piped water and soap for washing hands and working tools at these points, slaughterhouse workers at these areas are predominately paid on a 'per-pig' basis [*communication by slaughterhouse management*], such pay incentives encourage a rapid pace of work and may deter workers from breaking to wash hands and tools.

Hand and tool washing was a prevalent practice only at the cutting and dispatch section of the slaughterhouse. This was possible since the slaughter house management had made provision of two water points within the slaughter house next to cutting and dispatch section and at the entrance which also serves as dispatch point. Presence of these two water points for washing hands and tools demonstrates a greater level of infrastructure investment in this peri-urban slaughterhouse, compared to slaughterhouses in western Kenya where only 3% of all the slaughter houses sampled were able to provide piped water (Cook *et al*., 2017).

Tachyzoites which has been detected in blood and other body fluids of infected animals (Tenter *et al*., 2000) and tissue cysts bradyzoites, the infective stages of *T. gondii* are killed by water (Ambroise-Thomas & Petersen, 2000). Inadequate washing of hands and tools has been previously associated with exposure to *T. gondii*. Kapperud *et al*. (1996) demonstrated that inability to wash kitchen knives after using them on raw meat was independently associated with *T. gondii* seropositivity in pregnant women. Ekanem *et al.* (2018) on the other hand reported in a study in Southern Nigeria that not washing hands before eating at the work place by abattoir workers was significantly associated with *T. gondii* seropositivity. Washing hands and working tools also prevent abattoir workers from zoonotic bacterial infections (Di Ciccio *et al*., 2016) and is one of the method of maintaining meat hygiene for food safety (Cook *et al*., 2017). The provision of accessible tap water for washing hands and working

tools at the four stations and strict rules on adherence on these practices should be encouraged.

Not wearing personal protective clothing such as gumboots and overalls was not a challenge in this current study because of strict management rules enforcement, and without which a worker would not be allowed in. This was the same case with a study by Youssefi *et al* (2018) where 95.6 % of the slaughterhouse workers were using boots and overalls. This was not so in a study in western Kenya where only half of the slaughterhouse workers wore protective clothing; with 53% wearing protective coats and 49% wearing rubber boots (Cook *et al*., 2017). Apart from the strict management rules, the economic status of the slaughterhouse workers in this present study could be much better than for those in western Kenya and they would afford to buy the personal protective clothing (Cook *et al*., 2017). Abattoir workers are at risk of getting injuries on their upper extremities with sharp working tools making it easy for infective stages present in the body fluids and raw pork to penetrate through percutaneous route (Alvarado-Esquivel *et al*., 2011). Wearing of gloves plays a role in preventing the infection through this route. In this present study, slaughterhouse workers in all the four stations were observed not wearing gloves as there were no strict rules on putting on this protective clothing. This makes this results consistent with a study by Alvarado-Esquivel *et al*. (2011), where 71 % of the workers did not wear gloves, the opposite happen in a study by Youssefi *et al*., (2018), where only 3.4% of the slaughter house workers did not wear gloves. Wearing of gloves should be part of the personal protective clothing so as to minimize transmission of zoonotic infections and contamination of meat.

Slaughterhouse workers at stunning, evisceration and at cutting and dispatch areas may be exposed through accidental ingestion of infective stages present in blood, body fluids as they eviscerate and in raw pork respectively (Tenter *et al*., 2000). At the stunning box, stunned pigs were slaughtered and bled by severing the jugular blood vessel (Shimshony & Chaudry,

2005). Since most pigs were not completely unconscious due to poor stunning, their body movements at times made it possible for the splash of blood on the face of the person slaughtering. Delay to clean off blood after slaughtering could cause a pool of blood to accumulate at the stunning box, increasing likelihood of blood splash on the face of a worker who could be bending while slaughtering. At times overcrowding pigs at the stunning box also caused a lot of commotion and in the case where there is a pool of blood, splash of blood on the faces was possible. At the evisceration area, the workers may be exposed to infection through accidental ingestion of tachyzoites present in the body fluids as they eviscerate while at the cutting and dispatch section, cutting of meat also could cause small pieces of pork to get on someone's mouth. Alvarado-Esquivel *et al.* (2011), found that 7% of the individuals that had splash of blood and raw meat on their faces were seropositive with *T. gondii* as compared to 3% seropositive individuals who did not have splash of blood and raw meat on their faces. The splash of blood and raw pork could cause accidental ingestion of infective stages of *T. gondii* present in the raw meat and blood and also a possibility of penetration through mucosal route (Tenter *et al*., 2000). Wearing of mask by the slaughterhouse workers is one of the best preventive methods for *T. gondii* infection through these routes. Wearing of mask by the slaughterhouse workers in this study may have been a result of government directives against Covid-19. This was not the case in a study by Alvarado-Esquivel *et al.* (2011), and Youssefi *et al.* (2018), where masks were part of personal protective clothing. To minimize the risk of infection through these routes, education on proper use of personal protective equipment and its importance on persons health and in meat hygiene should be put in place.

Eating while at work is one of the easiest ways of ingesting the infective stages of *T. gondii* that might have contaminated the unwashed hands. In this study eating within the slaughterhouse structure was not a common practice among the slaughterhouse workers as the slaughterhouse management did not allow selling of cooked food within the slaughterhouse though

8.33 % and 4.17% were observed eating at evisceration and cutting and dispatch sections respectively. These results were unrelated with studies by Alvarado-Esquivel *et al* (2011), where 44% of the slaughterhouse workers were seen eating while at work. On the other hand Youssefi *et al*. (2018), reported strict rules in industrial abattoirs in Iran where slaughterhouse workers were not allowed to eat and drink while working and they were supposed to change their clothes and wash their hands before eating and drinking in the eateries. Adoption of these strict management rules in our abattoirs and continuous training of both the abattoir workers and management team on zoonotic infections and their routes of exposure to humans will help to mitigate these infections.

CHAPTER 6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This study has shown the presence of *T. gondii* infections in pigs slaughtered at Ndumbui-ni abattoir for human consumption in Kiambu County and its environs and the potential risk practices that expose slaughterhouse workers to this Zoonotic disease. The high sero-prevalence is an indication of the presence of infective stages of *T. gondii* either in the environment, pig premises, water or feed. The pork consumers, especially those who consume raw and undercooked pork and abattoir workers who don't observe good basic practices at work are at risk of contracting the infection.

6.2 RECOMMENDATIONS

The following are recommended based on the conclusion above;

1. Strategies that minimize exposure of pigs to the pathogen should be put in place, these include; proper farm sanitation, feeding pigs with uncontaminated feed and water, adopting effective rodent control measures, adopting farm measures that minimize contact with cats.
2. Enhance public awareness on the parasite's transmission chain and the role of every stakeholder in mitigating the infection
3. The abattoir administration should strictly enforce the basic preventive measures on workers to avoid contracting disease
4. Further risk assessments and Zoonoses mitigation strategy should be developed

REFERENCES

Abiayi, E. A., Inabo, H. I., Jatau, E. D., Makinde, A. A., Sar, T. T., Ugbe, D. A., Kumbish, P. R., & Okewole, P. A. (2015). Knowledge, attitudes, risk factors and practices (KARP) that favor leptospira infection among abattoir workers in North Central Nigeria. Asian Journal of Epidemiology, ***8***(4), 104–113. https://doi.org/10.3923/AJE.2015.104.113

Alvarado-Esquivel, C., Liesenfeld, O., Estrada-Martínez, S., & Félix-Huerta, J. (2011). Toxoplasma gondii infection in workers occupationally exposed to raw meat. Occupational Medicine, **61**(4), 265–269. https://doi.org/10.1093/occmed/kqr032

Amairia, S., Rouatbi, M., Rjeibi, M. R., Nouasri, H., Sassi, L., Mhadhbi, M., & Gharbi, M. (2016). Molecular prevalence of Toxoplasma gondii DNA in goats' milk and seroprevalence in Northwest Tunisia. Veterinary Medicine and Science, **2(3)**, 154–160. https://doi.org/10.1002/VMS3.29

Ambroise-Thomas, P., & Petersen, E. (2000). Congenital toxoplasmosis: past, present and future.In congenital toxoplasmosis (pp. 1–7). Springer Paris. https://doi.org/10.1007/978-2-8178-0847-5_1

Arko-Mensah, J. (1999). A Serological Survey of Toxoplasmosis in Pigs in Ghana. http://ugspace.ug.edu.gh/handle/123456789/5119

Assadi-Rad, A. M., New, J. C., & Patton, S. (1995). Risk factors associated with transmission of Toxoplasma gondii to sows kept in different management systems in Tennessee. Veterinary Parasitology, **57**(4), 289–297. https://doi.org/10.1016/0304-4017(94)00677-5*2019 Kenya Population and Housing Census Results - Kenya National Bureau of Statistics*. (n.d.). Retrieved May 18, 2021, from

https://www.knbs.or.ke/?p=5621

Ayinmode, A. B., & Abiola, J. O. (2016). Investigating Potential Sources of Toxoplasmosis for Humans From Slaughtered Food Animals in Ibadan, Nigeria. Folia Veterinaria, **60**(2), 34–40. https://doi.org/10.1515/fv-2016-0016

Azwai, S., El-Gammoudi, F. T., & Gameel, S. E. A. M. (1993). A serological survey of toxoplasmosis in some animal species in Libya. Alex. J. Vet. Sci, **9(3)**, 133-135

Bamba, S., Cissé, M., Sangaré, I., Zida, A., Ouattara, S., & Guiguemdé, R. T. (2017). Seroprevalence and risk factors of Toxoplasma gondii infection in pregnant women from Bobo Dioulasso, Burkina Faso. BMC Infectious Diseases, **17**(1), 1–6. https://doi.org/10.1186/s12879-017-2583-6

Bamba, S., Wenceslas Bazié, W., Lingani, M., Sourabié, Y., Sombié, I., Ndiaye, J. L., & Tarnagda, Z. (2017). Journal of Parasitology and Vector Biology Seroepidemiology of Toxoplasma gondii infection among slaughtered pigs, cattle and goats for human consumption in Bobo-Dioulasso, Burkina Faso. **9**(8), 106–110. https://doi.org/10.5897/JPVB2017.0290

Basso, W., Hartnack, S., Pardini, L., Maksimov, P., Koudela, B., Venturini, M. C., Schares, G., Sidler, X., Lewis, F. I., & Deplazes, P. (2013). Assessment of diagnostic accuracy of a commercial ELISA for the detection of Toxoplasma gondii infection in pigs compared with IFAT, TgSAG1-ELISA and Western blot, using a Bayesian latent class approach. International Journal for Parasitology, ***43*(7)**, 565–570.

Boughattas, S., Ayari, K., Sa, T., Aoun, K., & Bouratbine, A. (2014). Survey of the parasite Toxoplasma gondii in human consumed ovine meat in Tunis City. *PloS One*, **9(**1) https://doi.org/10.1016/J.IJPARA.2013.02.003

Burrells, A., Benavides, J., Cantón, G., Garcia, J. L., Bartley, P. M., Nath, M., Thomson, J., Chianini, F., Innes, E. A., & Katzer, F. (2015). Vaccination of pigs with the S48 strain of Toxoplasma gondii - Safer meat for human consumption. Veterinary Research, **46**(1), 1–12. https://doi.org/10.1186/s13567-015-0177-0

Cai, C., Perry, M. J., Sorock, G. S., Hauser, R., Spanjer, K. J., Mittleman, M. A., & Stentz, T. L. (2005). Laceration injuries among workers at meat packing plants. American Journal of Industrial Medicine, **47**(5), 403–410. https://doi.org/10.1002/AJIM.20157

Cook, E. A. J., De Glanville, W. A., Thomas, L. F., Kariuki, S., Bronsvoort, B. M. de C., & Fèvre, E. M. (2017). Working conditions and public health risks in slaughterhouses in western Kenya. BMC Public Health, **17**(1), 1–12. https://doi.org/10.1186/s12889-016-3923-y

Cook, E. A. J., Gitahi, N., de Glanville, W. A., Thomas, L. F., Kariuki, S., Kang'ethe, E., & Fèvre, E. M. (2021). Prevalence and risk factors for exposure to Toxoplasma gondii in slaughterhouse workers in western Kenya. BMC Infectious Diseases, **21**(1), 1–8. https://doi.org/10.1186/s12879-021-06658-8

De Berardinis, A., Paludi, D., Pennisi, L., & Vergara, A. (2017). Toxoplasma gondii, a Foodborne Pathogen in the Swine Production Chain from a European Perspective. Foodborne Pathogens and Disease, **14**(11), 637–648. https://doi.org/10.1089/FPD.2017.2305

Demar, M., Ajzenberg, D., Maubon, D., Djossou, F., Panchoe, D., Punwasi, W., Valery, N., Peneau, C., Daigre, J. L., Aznar, C., Cottrelle, B., Terzan, L., Dardé, M. L., & Carme, B. (2007). Fatal outbreak of human toxoplasmosis along the Maroni River:

Epidemiological, clinical, and parasitological aspects. Clinical Infectious Diseases, **45**(7). https://doi.org/10.1086/521246

Di Ciccio, P., Ossiprandi, M. C., Zanardi, E., Ghidini, S., Belluzzi, G., Vergara, A., & Ianieri, A. (2016). Microbiological contamination in three large-scale pig slaughterhouses in Northern Italy. Italian Journal of Food Safety, **5**(4). https://doi.org/10.4081/ijfs.2016.6151

Di Ciccio, P., Ossiprandi, M. C., Zanardi, E., Ghidini, S., Belluzzi, G., Vergara, A., & Ianieri, A. (2016). Microbiological contamination in three large-scale pig slaughterhouses in Northern Italy. Italian Journal of Food Safety, **5**(4). https://doi.org/10.4081/ijfs.2016.6151

Dohoo Martin, S. Wayne,, Stryhn, Henrik,, I. R. (2009). Veterinary epidemiologic research. VER, Inc.

Dorjee, S., Heuer, C., Jackson, R., West, D. M., Collins-Emerson, J. M., Midwinter, A. C., & Ridler, A. L. (2011). Assessment of occupational exposure to leptospirosis in a sheep-only abattoir. Epidemiology and Infection, **139**(5), 797–806. https://doi.org/10.1017/S0950268810002049

Dubey, J. P. (2009). History of the discovery of the life cycle of Toxoplasma gondii. International Journal for Parasitology, **39**(8), 877–882. https://doi.org/10.1016/J.IJPARA.2009.01.005

Dubey, J. P. (2016). Toxoplasmosis of Animals and Humans. In Toxoplasmosis of Animals and Humans. CRC Press. https://doi.org/10.1201/9781420092370

Dubey, J. P., & Jones, J. L. (2008). Toxoplasma gondii infection in humans and animals in

the United States. In International Journal for Parasitology (Vol. **38**, Issue 11, pp. 1257–1278). https://doi.org/10.1016/j.ijpara.2008.03.007

Dubey, J. P., Kotula, A. W., Sharar, A., Andrews, C. D., & Lindsay, D. S. (1990). Effect of high temperature on infectivity of Toxoplasma gondii tissue cysts in pork. Journal of Parasitology, **76**(2), 201–204. https://doi.org/10.2307/3283016

Ekanem, U. S., Moses, A. E., Abraham, E. G., Motilewa, O. O., Umo, A. N., Uwah, A. I., & Itina, E. I. (2018). Seroprevalence of anti-Toxoplasma gondii IgG antibody and risk factors among abattoir workers in Uyo, Southern Nigeria. Nigerian Journal of Clinical Practice, **21**(12), 1662–1669. https://doi.org/10.4103/njcp.njcp_44_18

Emmanuel, T., Isaiah, O., & Ademola, O. (2015). Seroprevalence of anti- Toxoplasma gondii antibodies in cattle and pigs in Ibadan , Nigeria. Journal of Parasitic Diseases, **39**(June), 309–314. https://doi.org/10.1007/s12639-013-0350-1

Ethan, A., Atkinson, E., Dougherty, G., Lennon, R., Hanson, A., Goergen, K., Lundt, E., Broderick, B., Mccullough, M., & Heinzen, M. E. (2021). *Package ‘ arsenal ’ R topics documented : 1*.

Fan, C. K., Su, K. E., & Tsai, Y. J. (2004). Serological survey of Toxoplasma gondii infection among slaughtered pigs in northwestern Taiwan. The Journal of Parasitology, **90**(3), 653–654. https://doi.org/10.1645/GE-177R

Flegr, J. (2020). How and why Toxoplasma makes us crazy. https://doi.org/10.1016/j.pt.2013.01.007

FAO. 2012 Pig Sector Kenya. FAO Animal Production and Health Livestock Country Reviews. No. 3

Gamble, H., NAS, & Hill, D. (2013). Toxoplasma - Pork Safety Fact Sheet. National Pork Board, 13–16. http://www.pork.org/wp-content/uploads/2010/04/toxoplasma.pdf

Gamble, H. R., Dubey, J. P., & Lambillotte, D. N. (2005). Comparison of a commercial ELISA with the modified agglutination test for detection of Toxoplasma infection in the domestic pig. **Veterinary Parasitology**, **128(**3–4), 177–181. https://doi.org/10.1016/J.VETPAR.2004.11.019

Garcia, J. L., Gennari, S. M., Machado, R. Z., & Navarro, I. T. (2006). Toxoplasma gondii: detection by mouse bioassay, histopathology, and polymerase chain reaction in tissues from experimentally infected pigs. Experimental Parasitology, **113(**4), 267–271. https://doi.org/10.1016/J.EXPPARA.2006.02.001

Gebremedhin, E. Z., Kebeta, M. M., Asaye, M., Ashenafi, H., Di Marco, V., & Vitale, M. (2015). First report on seroepidemiology of Toxoplasma gondii infection in pigs in Central Ethiopia. BMC Veterinary Research, **11**(1), 1–9. https://doi.org/10.1186/s12917-015-0384-y

Ghoneim, N. H., Shalaby, S. I., Hassanain, N. A., Zeedan, G. S., Soliman, Y. A., & Abdalhamed, A. M. (2010). Comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt. Foodborne Pathogens and Disease, 7(1), 17–22. https://doi.org/10.1089/FPD.2008.0223

Halová, D., Mulcahy, G., Rafter, P., Turčeková, L., Grant, T., & de Waal, T. (2013). Toxoplasma gondii in Ireland: Seroprevalence and Novel Molecular Detection Method in Sheep, Pigs, Deer and Chickens. Zoonoses and Public Health, **60(**2), 168–173. https://doi.org/10.1111/j.1863-2378.2012.01514.x

Hartung, C., Anokwa, Y., Brunette, W., Lerer, A., Tseng, C., & Borriello, G. (2010). Open data kit: Tools to build information services for developing regions. ACM International Conference Proceeding Series. https://doi.org/10.1145/2369220.2369236

Herenda, D., Chambers, P., Ettriqui, A., Seneviratna, P., & Silva, T. P. J. (1994). Manual on meat inspection for developing countries. *Undefined*

Hill, D. E., Chirukandoth, S., Dubey, J. P., Lunney, J. K., & Gamble, H. R.. (2006a). Comparison of detection methods for Toxoplasma gondii in naturally and experimentally infected swine. Veterinary Parasitology, **141**(1–2), 9–17. https://doi.org/10.1016/J.VETPAR.2006.05.008

Hill, D. E., Chirukandoth, S., Dubey, J. P., Lunney, J. K., & Gamble, H. R.. (2006b). Comparison of detection methods for Toxoplasma gondii in naturally and experimentally infected swine. Veterinary Parasitology, **141**(1–2), 9–17. https://doi.org/10.1016/J.VETPAR.2006.05.008

Hiszczyńska-Sawicka, E., Gatkowska, J. M., Grzybowski, M. M., & DŁugońska, H. (2014). Veterinary vaccines against toxoplasmosis. Parasitology**, 141**(11), 1365–1378. https://doi.org/10.1017/S0031182014000481

Hove, T., Lind, P., & Mukaratirwa, S. (2005). Seroprevalence of Toxoplasma gondii infection in domestic pigs reared under different management systems in Zimbabwe. Onderstepoort Journal of Veterinary Research, **72**(3), 231–237. https://doi.org/10.4102/ojvr.v72i3.200

Irveld-Koski, V. H. (1990). Evaluation of ELISA for the Detection of Toxoplasma Antibodies in Swine Sera. Acta Vet. Scand, **31**–413. https://doi.org/10.4102/ojvr.v72i3.200

Ishaku, B. S., Abdullahi, M., Nalong, D., Jonah, R., & Mayowa, O. (2018). Seroprevalence and Risk Factors for Toxoplasma gondii in Pigs, Sheep and Goats at Slaughter in Jos Municipal Abattoir, Nigeria. Veterinary Sciences: Research and Reviews, **4**(2). https://doi.org/10.17582/journal.vsrr/2018/4.2.55.61

Jacobs, L. (1961). Toxoplasmosis in man and animals. New Zealand Veterinary Journal, **9**(5), 85–91. https://doi.org/10.1080/00480169.1961.33433

Jacobs, L., Remington, J. S., & Melton, M. L. (1960). The resistance of the encysted form of Toxoplasma gondii. The Journal of Parasitology, **46**, 11–21. https://doi.org/10.2307/3275325

Kapperud, G., Jenum, P. A., Stray-Pedersen, B., Melby, K. K., Eskild, A., & Eng, J. (1996). Risk factors for Toxoplasma gondii infection in pregnancy: Results of a prospective case-control study in Norway. American Journal of Epidemiology, **144**(4), 405–412. https://doi.org/10.1093/oxfordjournals.aje.a008942

Kebeta, M. M. (n.d.). ***Thesis Ref. No.*** **_____** Toxoplasma gondii in selected sites of central ethiopia: seroprevalence, risk factors and bioassay in pigs**.**

Kijlstra, A., Eissen, O. A., Cornelissen, J., Munniksma, K., Eijck, I., & Kortbeek, T. (2004). Toxoplasma gondii Infection in Animal-Friendly Pig Production Systems.

Klun, I., Vujanić, M., Yera, H., Nikolić, A., Ivović, V., Bobić, B., Bradonji, S., Dupouy-Camet, J., & Djurković-Djaković, O. (2011). Toxoplasma gondii infection in slaughter pigs in Serbia: Seroprevalence and demonstration of parasites in blood. Veterinary Research, **42**(1), 1–6. https://doi.org/10.1186/1297-9716-42-17

Kotula, A. W., Dubey, J. P., Sharar, A. K., Andrews, C. D., Shen, S. K., & Lindsay, D. S.

(1991). Effect of Freezing on Infectivity of Toxoplasma Gondii Tissue Cysts in Pork. Journal of Food Protection, **54**(9), 687–690. https://doi.org/10.4315/0362-028X-54.9.687

KNBS. (2019). 2019 Kenya population and housing census volume i : population by county and Sub-county: Vol. I (Issue November*)*

Liu, Q., Wang, Z.-D., Huang, S.-Y., & Zhu, X.-Q. (2015). Diagnosis of toxoplasmosis and typing of Toxoplasma gondii. Parasites & Vectors 2015 *8:1*, *8*(1), 1–14. https://doi.org/10.1186/S13071-015-0902-6

Mohamed, M., Mosha, F., Mghamba, J., Zaki, S. R., Shieh, W. J., Paweska, J., ... & Njenga, M. K (2010). Epidemiologic and clinical aspects of a Rift Valley fever outbreak in humans in Tanzania, 2007. The American Journal of Tropical Medicine and Hygiene, **83**(2 Suppl), 22–27. https://doi.org/10.4269/AJTMH.2010.09-0318

Mburugu-Mosoti, C. (2017). The pork value chain a review of the actors and the business opportunities in the sector, in Kenya

Moré, G., Venturini, M. C., Pardini, L., & Unzaga, J. M. (2018). Toxoplasma. Parasitic Protozoa of Farm Animals and Pets, 149–168. https://doi.org/10.1007/978-3-319-70132-5_6

Morrissette, N. S., & Ajioka, J. W. (2009). The early years of Toxoplasma research: What's past is prologue. International Journal for Parasitology, **39**(8), 865. https://doi.org/10.1016/J.IJPARA.2009.02.010

Mose, J. M., Kagira, J. M., Karanja, S. M., Ngotho, M., Kamau, D. M., Njuguna, A. N., & Maina, N. W. (2016). Detection of Natural Toxoplasma gondii Infection in Chicken

in Thika Region of Kenya Using Nested Polymerase Chain Reaction. BioMed Research International, 2016. https://doi.org/10.1155/2016/7589278

Murungi, M. K., Muloi, D. M., Muinde, P., Githigia, S. M., Akoko, J., Fèvre, E. M., Rushton, J., & Alarcon, P. (2021). The Nairobi Pork Value Chain: Mapping and Assessment of Governance, Challenges, and Food Safety Issues. Frontiers in Veterinary Science, **8**(February), 1–18. https://doi.org/10.3389/fvets.2021.581376

Nabukenya, I., Kaddu-Mulindwa, D., & Nasinyama, G. W. (2013). Survey of Brucella infection and malaria among Abattoir workers in Kampala and Mbarara Districts, Uganda. BMC Public Health 2013 13:1, **13**(1), 1–6. https://doi.org/10.1186/1471-2458-13-901

Nguku, P. M., Sharif, S. K., Mutonga, D., Amwayi, S., Omolo, J., Mohammed, O., ... & Breiman, R. F. (2010). An investigation of a major outbreak of Rift Valley fever in Kenya: 2006-2007. The American Journal of Tropical Medicine and Hygiene, ***83***(2 Suppl), 5–13. https://doi.org/10.4269/AJTMH.2010.09-0288

Nisbet, A. I., Omuse, G., Revathi, G., & Adam, R. D. (2018). Seroprevalence data at a private teaching hospital in Kenya: An examination of Toxoplasma gondii, cytomegalovirus, rubella, hepatitis A, and Entamoeba histolytica. PLOS ONE, **13**(10), e0204867. https://doi.org/10.1371/JOURNAL.PONE.0204867

Obijiaku, I., Ornguga, N., & Adikwu, A. (2017). Seroprevalence of *Toxoplasma gondii* infection in slaughtered pigs in Makurdi, Nigeria. Sokoto Journal of Veterinary Sciences, **15**(3), 91. https://doi.org/10.4314/sokjvs.v15i3.13

Olsen, A., Berg, R., Tagel, M., Must, K., Deksne, G., Larsen, H., Alban, L., Vang, M., Vedel, H., Sandberg, M., Lundén, A., Rune, C., Pires, S. M., & Jokelainen, P.

(2019). Seroprevalence of Toxoplasma gondii in domestic pigs , sheep , cattle , wild boars , and moose in the Nordic-Baltic region : A systematic review and meta-analysis. Parasite Epidemiology and Control, 3, e00100. https://doi.org/10.1016/j.parepi.2019.e00100

Onyiche, T. G. E., & Ademola, I. O. (2015). Seroprevalence of anti-Toxoplasma gondii antibodies in cattle and pigs in Ibadan, Nigeria. Journal of Parasitic Diseases, **39**(2), 309–314. https://doi.org/10.1007/s12639-013-0350-1

Ortega-Pacheco, A., Acosta Viana, K. Y., Guzmán-Marín, E., Segura-Correa, J. C., Álvarez-Fleites, M., & Jiménez-Coello, M. (2013). Prevalence and risk factors of Toxoplasma gondii in fattening pigs farm from Yucatan, Mexico. BioMed Research International, 2013. https://doi.org/10.1155/2013/231497

Package, T. (2021). *Package ' DescTools .'*

Remington, J. S., Thulliez, P., & Montoya, J. G. (2004). Recent Developments for Diagnosis of Toxoplasmosis. Journal of Clinical Microbiology, **42**(3), 941. https://doi.org/10.1128/JCM.42.3.941-945.2004

R Core Development Team. (2018). R: a language and environment for statistical computing, 3.2.1. Document Freely Available on the Internet at: Http://Www. r-Project. Org. http://www.r-project.org/

Roesel, K., Schares, G., Grace, D., Baumann, M. P. O., Fries, R., Dione, M., & Clausen, P.-H. (2016). The occurrence of porcine Toxoplasma gondii infections in smallholder production systems in Uganda. First Joint Conference of the Association of Institutions for Tropical Veterinary Medicine and the Society of Tropical Veterinary Medicine., 22. https://cgspace.cgiar.org/handle/10568/77110

Rouatbi, M., Amairia, S., Amdouni, Y., Boussaadoun, M. A., Ayadi, O., Al-Hosary, A. A. T., Rekik, M., Abdallah, R. Ben, Aoun, K., Darghouth, M. A., Wieland, B., & Gharbi, M. (2019). Toxoplasma gondii infection and toxoplasmosis in North Africa: a review. Parasite, **26**. https://doi.org/10.1051/PARASITE/2019006

Rougier, S., Montoya, J. G., & Peyron, F. (2017). Lifelong Persistence of Toxoplasma Cysts: A Questionable Dogma?: (Trends in Parasitology **33**, 93–101; 2017). Trends in Parasitology, *33*(5), 414. https://doi.org/10.1016/J.PT.2017.02.001

Sandfoss, M., dePerno, C., Patton, S., Flowers, J., & Kennedy-Stoskopf, S. (2011). Prevalence of antibody to Toxoplasma gondii and Trichinella spp. in feral pigs (Sus Scrofa) of Eastern North Carolina. Journal of Wildlife Diseases, ***47***(2), 338–343. https://doi.org/10.7589/0090-3558-47.2.338

Shimshony, A., & Chaudry, M. M. (2005). Slaughter of animals for human consumption. In Rev. sci. tech. Off. int. Epiz (Vol. **24**, Issue 2).

Sroka, J., Karamon, J., Wójcik-Fatla, A., Piotrowska, W., Dutkiewicz, J., Bilska-Zaja¸c, E., Zaja¸c, V., Kochanowski, M., Da¸browska, J., & Cencek, T. (2020). Toxoplasma gondii infection in slaughtered pigs and cattle in Poland: Seroprevalence, molecular detection and characterization of parasites in meat. Parasites and Vectors, **13**(1), 1–11. https://doi.org/10.1186/s13071-020-04106-1

Su, C., Shwab, E. K., Zhou, P., Zhu, X. Q., & Dubey, J. P. (2010). Moving towards an integrated approach to molecular detection and identification of Toxoplasma gondii. Parasitology, **137**(1), 1–11. https://doi.org/10.1017/S0031182009991065

Suaréz-Aranda, F., Galisteo, A. J., Hiramoto, R. M., Cardoso, R. P. A., Meireles, L. R., Miguel, O., & Andrade, H. F. (2000). The prevalence and avidity of Toxoplasma

gondii IgG antibodies in pigs from Brazil and Peru. ***Veterinary Parasitology*, 91**(1–2), 23–32. https://doi.org/10.1016/S0304-4017(00)00249-1

Swai, E. S., & Schoonman, L. (2009). Human brucellosis: seroprevalence and risk factors related to high risk occupational groups in Tanga Municipality, Tanzania. Zoonoses and Public Health, **56**(4), 183–187. https://doi.org/10.1111/J.1863-2378.2008.01175.X

Tagwireyi, W. M., Etter, E., & Neves, L. (2019). Seroprevalence and associated risk factors of Toxoplasma gondii infection in domestic animals in southeastern South Africa. The Onderstepoort Journal of Veterinary Research, **86**(1). https://doi.org/10.4102/OJVR.V86I1.1688

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. J. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society B: Biological Sciences, **356**(1411), 983–989. https://doi.org/10.1098/RSTB.2001.0888

Tenter, A. M., Heckeroth, A. R., & Weiss, L. M. (2000). Toxoplasma gondii: From animals to humans. International Journal for Parasitology, **30**(12–13), 1217–1258. https://doi.org/10.1016/S0020-7519(00)00124-7

Tenter, A. M., Heckeroth, A. R., & Weiss, L. M. (2000). Toxoplasma gondii: From animals to humans. International Journal for Parasitology, **30**(12–13), 1217–1258. https://doi.org/10.1016/S0020-7519(00)00124-7

Thiongo, S. K., Ichagichu, J. M., Ngotho, M., Aboge, G. O., Kagira, J. M., Karanja, S. M., & Maina, N. N. (2016a). Use of the nested polymerase chain reaction for detection of Toxoplasma gondii in slaughterhouse workers in Thika District, Kenya. South African Medical Journal, **106**(4), 417–419. https://doi.org/10.7196/SAMJ.2016.V106I4.8777

Thiongo, S. K., Ichagichu, J. M., Ngotho, M., Aboge, G. O., Kagira, J. M., Karanja, S. M., & Maina, N. N. (2016b). Use of the nested polymerase chain reaction for detection of Toxoplasma gondii in slaughterhouse workers in Thika District, Kenya. South African Medical Journal, **106**(4), 417–419. https://doi.org/10.7196/SAMJ.2016.v106i4.8777

Tialla, D., Dahourou, L. D., & Gbati, O. B. (2019). Seroprevalence and factors associated with bovine and swine toxoplasmosis in Bobo-Dioulasso, Burkina Faso. https://doi.org/10.14202/vetworld.2019.1519-1523

Tonouhewa, A. B. N., Akpo, Y., Sessou, P., Adoligbe, C., Yessinou, E., Hounmanou, Y. G., Assogba, M. N., Youssao, I., & Farougou, S. (2017). Toxoplasma gondii infection in meat animals from Africa: Systematic review and meta-analysis of sero-epidemiological studies. Veterinary World, **10**(2), 194–208. https://doi.org/10.14202/vetworld.2017.194-208

Tsutsui, V. S., Freire, R. L., Garcia, J. L., Gennari, S. M., Vieira, D. P., Marana, E. R. M., Prudêncio, L. B., & Navarro, I. T. (2007). Detection of Toxoplasma gondii by PCR and mouse bioassay in commercial cuts of pork from experimentally infected pigs. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, **59**(1), 30–34. https://doi.org/10.1590/S0102-09352007000100006

Villari, S., Vesco, G., Petersen, E., Crispo, A., & Buffolano, W. (2009a). Risk factors for toxoplasmosis in pigs bred in Sicily, Southern Italy. Veterinary Parasitology, **161**(1–2), 1–8. https://doi.org/10.1016/j.vetpar.2009.01.019

Villari, S., Vesco, G., Petersen, E., Crispo, A., & Buffolano, W. (2009b). Risk factors for toxoplasmosis in pigs bred in Sicily, Southern Italy. Veterinary Parasitology, **161**(1–2),

1–8. https://doi.org/10.1016/j.vetpar.2009.01.019

Wambua, D. R., & Ndele, D. B. (2014). Toxoplasma gondii infection in donated blood at the national blood transfusion service in kenya.

Webster, J. P. (2010). Dubey, J.P. Toxoplasmosis of Animals and Humans. Parasites & Vectors 2010 3:1, **3**(1), 1–2. https://doi.org/10.1186/1756-3305-3-112

Weigel, R. M., Dubey, J. P., Siegel, A. M., Hoefling, D., Reynolds, D., Herr, L., Kitron, U. D., Shen, S. K., Thulliez, P., & Fayer, R. (1995). Prevalence of antibodies to Toxoplasma gondii in swine in Illinois in 1992. Journal of the American Veterinary Medical Association, **206**(11), 1747–1751. https://europepmc.org/article/med/7782249

Weiss, L. M., & Dubey, J. P. (2009a). Toxoplasmosis: a history of clinical observations. International Journal for Parasitology, **39**(8), 895. https://doi.org/10.1016/J.IJPARA.2009.02.004

Weiss, L. M., & Dubey, J. P. (2009b). Toxoplasmosis: A history of clinical observations. In International Journal for Parasitology (Vol. **39**, Issue 8, pp. 895–901). NIH Public Access. https://doi.org/10.1016/j.ijpara.2009.02.004

Wu, D., Lv, R., Sun, X., Shu, F., Zhou, Z., Nie, K., Duan, G., & Zou, F. (2012). Seroprevalence of Toxoplasma gondii antibodies from slaughter pigs in Chongqing, China. Tropical Animal Health and Production, **44**(4), 685–687. https://doi.org/10.1007/s11250-011-9965-3

Yamamoto, Y. I., Hoshino-Shimizu, S., & Camargo, M. E. (1991). A novel igm-indirect hemagglutination test for the serodiagnosis of acute toxoplasmosis. Journal of Clinical Laboratory Analysis, **5**(2). https://doi.org/10.1002/jcla.1860050210

Yang, G. (2020). Seroprevalence of Toxoplasma gondii infection in pigs in Jilin Province , Seroprevalence of Toxoplasma gondii infection in pigs in Jilin Province , Northeastern China. March 2015.

Youssefi, M., Khadem-rezaiyan, M., Jarahi, L., Shamsian, A., & Moghaddas, E. (2018). Original papers Prevalence of Toxoplasma and Echinococcus IgG antibodies in slaughterhouse workers , a serosurvey in Northeast Iran. **64**(4), 391–397. https://doi.org/10.17420/ap6404.176

Zeileis, A., Kleiber, C., & Jackman, S. (2008). Regression Models for Count Data in R. Retrieved July 15, 2021, from http://cran.r-project.org/package=pscl.

Zhou, J., Wang, W., Song, P., Wang, L., Han, Y., Guo, J., Hao, Z., Zhu, X., Zhou, Q., Du, X., Lu, G., He, S., & Luo, Y. (2018). Structural predication and antigenic analysis of Toxoplasma gondii ROP20. Acta Parasitologica, **63**(2), 244–251. https://doi.org/10.1515/ap-2018-0028

APPENDICES

Observation checklist

	Stunning box		**Scalding and dehairing**		**Evisceration**		**Cutting and dis-patch**	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
1. Protective clothing								
White over-coats								
Mask								
Gumboots								
Gloves								
2. Splash of blood & raw meat on the face								
3. Eating while at work								
4. Washing hands (with soap and water)								
5. cleaning working tools (with soap and water)								